



In Life

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**Dynamics of endometrial macrophage subpopulations
and their relevance in processes associated with
the development of mare endometrial fibrosis
in the course of endometrosis**

A doctoral thesis was completed in
the Team of Molecular Basis of Equine Reproduction
at the Institute of Animal Reproduction and Food Research
Polish Academy of Sciences in Olsztyn,
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Doctoral thesis carried out under the project OPUS 2020/37/B/NZ9/03355
funded by National Science Centre, Poland

Olsztyn 2025

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ABSTRACT

DYNAMICS OF ENDOMETRIAL MACROPHAGE SUBPOPULATIONS AND THEIR RELEVANCE IN PROCESSES ASSOCIATED WITH THE DEVELOPMENT OF MARE ENDOMETRIAL FIBROSIS IN THE COURSE OF ENDOMETROSIS

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A doctoral thesis was completed under the supervision of dr hab. Anna Szóstek-Mioduchowska in the Team of Molecular Basis of Equine Reproduction at the Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn.

Equine endometrial fibrosis is a main hallmark of endometrosis, a degenerative condition of the mare endometrium. It is characterized mainly by excessive deposition of extracellular matrix (ECM) components combined with glandular alterations within fibrotic foci. Endometrosis is considered to be a significant cause of infertility in mares. It is estimated that in its most severe form, it can reduce the foaling rate by up to 90%. To date, no effective treatment for endometrosis has been identified, and the underlying pathophysiology of this condition remains to be fully elucidated. Current research highlights the important role of macrophages (MΦ) in the processes associated with the development of fibrosis. It has been demonstrated that the distinct subpopulations of MΦ, namely pro-inflammatory MΦ1 and anti-inflammatory MΦ2a, play a significant role in the processes associated with the development of fibrosis in vital organs such as the lung, kidney, and liver. Nevertheless, the precise role of distinct MΦ subpopulations in the processes associated with endometrial fibrosis development in the progression of endometrosis remains to be understood. Thus, this dissertation examined (1) the distribution of different types of tissue-resident MΦ in mare endometrium in the course of endometrosis, (2) the effect of the secretome of equine *in vitro*-generated monocyte-derived (eqMD)-MΦ^{IFNγ/LPS} and eqMD-MΦ^{IL-4/IL-13} on the expression of ECM-related components, inflammatory markers, transcriptomic profiles, and functional characteristics of endometrial fibroblasts cultured *in vitro*, and (3) the effect of intrauterine administration of eqMD-MΦ^{IFNγ/LPS} and eqMD-MΦ^{IL-4/IL-13} on the expression of ECM-related components, inflammatory markers, transcriptomic profiles in the mare endometrium.

The percentage and ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells (MΦ1/MΦ2) were determined in mare endometria (cat. I, IIA, IIB, III according to Kenney and Doig classification; in total = 32) using flow cytometry. The proteomic profiles of eqMD-MΦ^{IFNγ/LPS} (n=5) and eqMD-MΦ^{IL-4/IL-13} (n=5) were analyzed using LC-MS. Fibroblasts were isolated from category I endometria of mares (n=6) during the mid-luteal phase of the estrous cycle and treated for 96 hours with conditioned medium (CM) of eqMD-MΦ^{IFNγ/LPS} and eqMD-MΦ^{IL-4/IL-13}. The transcriptomic profiles, expression of ECM-associated components, expression of inflammatory markers, and functional characteristics of treated endometrial fibroblasts were determined using RNA-seq, qPCR, Western blot, ELISA, immunofluorescence staining, MTT assay, BrdU assay,

and scratch assay. In order to determine the transcriptomic profiles and mRNA transcription of ECM-associated components and inflammatory markers, the mare endometrial biopsies were taken six hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} (n=3) or eqMD-M Φ ^{IL-4/IL-13} (n=3). To unravel affected signaling pathways, *in silico* analyses were conducted using ingenuity pathway analysis (IPA) after all transcriptomic studies.

An increase in the percentage of CD68⁺iNOS⁺ARG1⁻ cells and a decrease in the percentage of CD68⁺iNOS⁻ARG1⁺ cells were observed in categories IIA, IIB, and III when compared to category I endometria (p<0.05). Characteristic differences in the molecular profile of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} were demonstrated, emphasizing the potential proinflammatory role of M Φ ^{IFN γ /LPS} and the involvement of M Φ ^{IL-4/IL-13} in tissue remodeling. Moreover, the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM affected the expression of ECM-associated components and inflammatory markers (p<0.05) in endometrial fibroblasts *in vitro*. Additionally, the treatment with eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM altered the expression of 406 and 282 genes in endometrial fibroblasts *in vitro*, respectively. Both eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM increased the viability (p<0.05), proliferation (p<0.05), and migration (p<0.05) of mare endometrial fibroblasts *in vitro*. The intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} increased, while eqMD-M Φ ^{IL-4/IL-13} decreased the mRNA transcription of ECM-associated components (p<0.05). However, only the intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} increased the mRNA transcription of inflammatory markers (p<0.05). Moreover, the intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} altered 127 genes associated with e.g., cachexia signaling and neutrophil degranulation, while eqMD-M Φ ^{IL-4/IL-13} influenced 310 genes involved e.g., in Sheddase and CLEAR pathways.

The findings indicate that the imbalance in the population of M Φ with a predominance of the M Φ 1 affects the inflammatory process and ECM remodeling in the mare's endometrium, leading to the dysregulation of molecular pathways in a way that promotes the development of fibrosis in the progression of endometrosis.

STRESZCZENIE

DYNAMIKA ZMIAN SUBPOPULACJI MAKROFAGÓW W BŁONIE ŚLUZOWEJ MACICY I JEJ ZNACZENIE DLA PROCESÓW ZWIĄZANYCH Z ROZWOJEM WŁÓKNIENIA BŁONY ŚLUZOWEJ MACICY KLACZY W PRZEBIEGU ENDOMETROSIS

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Włóknienie endometrium jest główną cechą charakterystyczną endometrosis, degenerującego zapalenia błony śluzowej macicy klaczy. Charakteryzuje się ono nadmiernym odkładaniem składników macierzy pozakomórkowej (ECM) w połączeniu ze zmianami gruczołów w obrębie obszarów włóknienia. Endometrosis uznawane jest za istotną przyczynę niepłodności u klaczy. Szacuje się, że w najbardziej zaawansowanym stadium może prowadzić do obniżenia wskaźnika wyżrebień nawet o 90%. Dotychczas nie opracowano skutecznej metody leczenia endometrosis, a patofizjologia tego schorzenia pozostaje nie w pełni wyjaśniona. Prowadzone obecnie badania podkreślają istotną rolę makrofagów (MΦ) w procesach związanych z rozwojem włóknienia. Dotychczas wykazano, że różne subpopulacje MΦ, a mianowicie prozapalne MΦ1 i przeciwzapalne MΦ2, odgrywają istotną rolę w procesach związanych z rozwojem włóknienia w płucach, nerkach i wątrobie. Niemniej jednak, dokładna rola poszczególnych subpopulacji MΦ w procesach związanych z rozwojem włóknienia błony śluzowej macicy w przebiegu endometrosis pozostaje nieznana. Stąd też, w niniejszej rozprawie doktorskiej przeanalizowano (1) rozmieszczenie i ilość poszczególnych subpopulacji tkankowych MΦ w błonie śluzowej macicy klaczy w przebiegu endometrosis, (2) wpływ sekretom otrzymanych w hodowli w warunkach *in vitro* z końskich monocytów (eqMD)-MΦ^{IFN γ /LPS} i eqMD-MΦ^{IL-4/IL-13} na ekspresję czynników związanych z ECM, markerów stanu zapalnego, profile transkryptomiczne oraz właściwości fibroblastów błony śluzowej macicy klaczy hodowanych w warunkach *in vitro* oraz (3) wpływ eqMD-MΦ^{IFN γ /LPS} i eqMD-MΦ^{IL-4/IL-13} podanych domacicznie na ekspresję czynników związanych z ECM, markery stanu zapalnego oraz profile transkryptomiczne błony śluzowej macicy klaczy.

Odsetek i stosunek komórek CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ (MΦ1/MΦ2) w błonie śluzowej macicy klaczy (kat. I, IIA, IIB, III, zgodnie z system klasyfikacji Kenneya i Doiga; łącznie = 32) określono przy użyciu cytometrii przepływowej. Profile białkowe uzyskanych subpopulacji eqMD-MΦ^{IFN γ /LPS} (n=5) i eqMD-MΦ^{IL-4/IL-13} (n=5) przeanalizowano za pomocą metody LC-MS. Fibroblasty wyizolowano z błony śluzowej macicy klaczy kategorii I (n=6) w środkowej fazie lutealnej cyklu rujowego i traktowano przez 96 godzin kondycjonowanym medium (CM) pochodzącym z hodowli eqMD-MΦ^{IFN γ /LPS} i eqMD-MΦ^{IL-4/IL-13}. Profile transkryptomiczne, ekspresję czynników związanych z ECM,

ekspresję markerów stanu zapalnego i cechy funkcjonalne traktowanych CM fibroblastów określono za pomocą RNA-seq, qPCR, Western blot, ELISA, barwienia immunofluorescencyjnego, testu MTT, testu BrdU i analizy zarastania szczelin. W celu określenia profili transkryptomicznych i ekspresji na poziomie mRNA czynników związanych z ECM i markerów stanu zapalnego biopsje błony śluzowej macicy kłaczy pobrano sześć godzin po domacicznym podaniu autologicznych eqMD-MΦ^{IFN γ /LPS} (n=3) lub eqMD-MΦ^{IL-4/IL-13} (n=3). Aby zidentyfikować szlaki sygnałowe związane z genami, których ekspresja uległa zmianie, przeprowadzono analizy *in silico* z wykorzystaniem narzędzi Ingenuity Pathway Analysis (IPA), dla wszystkich analiz transkryptomicznych.

Zaobserwowano wzrost procentowego udziału komórek CD68⁺iNOS⁺ARG1⁻ i spadek procentowego udziału komórek CD68⁺iNOS⁻ARG1⁺ w błonie śluzowej macicy w kategoriach IIA, IIB, III w porównaniu do błony śluzowej macicy kategorii I (p<0.05). Wykazano charakterystyczne różnice profili molekularnych pomiędzy eqMD-MΦ^{IFN γ /LPS} i eqMD-MΦ^{IL-4/IL-13}, podkreślając potencjalną prozapalną rolę eqMD-MΦ^{IFN γ /LPS} i zaangażowanie eqMD-MΦ^{IL-4/IL-13} w przebudowę tkanek. Ponadto, eqMD-MΦ^{IFN γ /LPS}-CM i eqMD-MΦ^{IL-4/IL-13}-CM wpłynęły na ekspresję czynników związanych z ECM (p<0.05) oraz na ekspresję markerów stanu zapalnego na poziomie mRNA (p<0.05) w fibroblastach błony śluzowej macicy hodowanych w warunkach *in vitro*. Dodatkowo eqMD-MΦ^{IFN γ /LPS}-CM i eqMD-MΦ^{IL-4/IL-13}-CM wpłynęły na ekspresję odpowiednio 406 i 282 genów w fibroblastach błony śluzowej macicy hodowanych w warunkach *in vitro*. Zarówno eqMD-MΦ^{IFN γ /LPS}-CM jak i eqMD-MΦ^{IL-4/IL-13}-CM zwiększyły żywotność (p<0.05), proliferację (p<0.05) i migrację (p<0.05) fibroblastów błony śluzowej macicy hodowanych w warunkach *in vitro*. Domaciczne podanie eqMD-MΦ^{IFN γ /LPS} zwiększyło, podczas gdy domaciczne podanie eqMD-MΦ^{IL-4/IL-13} obniżyło ekspresję czynników związanych z ECM na poziomie mRNA (p<0.05). Jednocześnie domaciczne podanie eqMD-MΦ^{IFN γ /LPS} zwiększyło ekspresję markerów stanu zapalnego na poziomie mRNA. Co więcej, domaciczne podanie eqMD-MΦ^{IFN γ /LPS} wpłynęło na ekspresję 127 genów, w tym związanych ze ścieżką sygnalizacyjną kacheksji i degranulacją neutrofili, podczas gdy domaciczne podanie eqMD-MΦ^{IL-4/IL-13} wpłynęło na ekspresję 310 genów zaangażowanych m. in. w szlaki Sheddase i CLEAR.

Uzyskane wyniki sugerują, że zachwiany stosunek tkankowych MΦ1/MΦ2 z przewagą MΦ1 wpływa na proces zapalny i przebudowę ECM w błonie śluzowej macicy kłaczy, prowadząc do nieprawidłowej regulacji szlaków molekularnych w sposób sprzyjający rozwojowi włóknienia błony śluzowej macicy w przebiegu endometrosis.

List of abbreviations

AA – antibiotic antimycotic solution

ACN – acetonitrile

ACTA2 – α -smooth muscle actin

ADAM – a disintegrin and metalloproteases

AGEs – advanced glycation end-products

AHR – aryl hydrocarbon receptor

AREG – amphiregulin

ARG – arginine

ARG1 – arginase 1

BrdU – bromodeoxyuridine

BSA – bovine serum albumin

BCA – bicinchoninic acid

BCL-2 – B-cell leukemia/lymphoma 2 protein

BCL-X_L – B-cell lymphoma extra-large

CAT – cathepsin-G

CCL – chemokine (C-C motif) ligand

CD – cluster of differentiation

cDNA – complementary DNA

CL – corpus luteum

CLEAR – Coordinated Lysosomal Expression and Regulation

CM – conditioned medium

COL – collagen

CRP – C-reactive protein

CXCL – chemokine (C-X-C motif) ligand

CX3CL1 – C-X₃-C motif chemokine ligand 1

DAMPs – damage-associated molecular patterns

DAPI – 4',6-diamidino-2-phenylindol

DEGs – differentially expressed genes

DMEM/F-12 – Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham medium

DMSO – dimethyl sulfoxide

ECM – extracellular matrix

EDTA – ethylenediaminetetraacetic acid

ELA – elastase

ELISA – enzyme-linked immunosorbent assay

EMT – epithelial-to-mesenchymal transition

EndMT – endothelial-to-mesenchymal transition

eqMD – equine *in vitro*-generated monocyte-derived

FA – formic acid

FBS – fetal bovine serum

FDR – false discovery rate

FGF – fibroblasts growth factor

FN – fibronectin

GA – gentamycin-amphotericin

GM-CSF – granulocyte-macrophage colony-stimulating factor

GnRH – gonadotropin-releasing hormone

GPR – G protein coupled receptor

HBSS – Hanks' Balanced Salts Solution

HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

hFGF – human fibroblastic growth factor

HIF – hypoxia inducible factor

HRP – horseradish peroxidase

IFN – interferon

IL – interleukin

IL1RN – interleukin 1 receptor antagonist

INHBA – inhibin subunit beta A

iNOS – nitric oxide synthase

IPA – Ingenuity Pathways Analysis

IPF – idiopathic pulmonary fibrosis

LATS – large tumor suppressor

LC-MS – liquid chromatography-mass spectrometry

LL-37 – C-terminus 37 amino acid peptide

LPS – lipopolysaccharides

LTBPs – latent TGF- β binding proteins

M Φ – macrophages

M Φ 1 – macrophages type 1

MΦ2 – macrophages type 2

MCP – monocyte chemoattractant protein

M-CSF – macrophage colony-stimulating factor

MIP – macrophage inflammatory protein

miRNA – microRNA

MMPs – matrix metalloproteinases

MMP – matrix metalloproteinase

MMTS – methyl methanethiosulfonate

MPO – myeloperoxidase

NASH – nonalcoholic steatohepatitis

NETs – neutrophil extracellular traps

NFAT5 – nuclear factor of activated T-cells 5

NF-κB – nuclear factor kappa B

NGS – next generation sequencing

NLRC4 – NOD-like receptor family CARD domain-containing protein 4

NLRP3 – NOD-like receptor family pyrin domain containing 3

NO – nitric oxide

NOX – NADPH oxidase

qPCR – quantitative Polymerase Chain Reaction

PAMPs – pathogen-associated molecular patterns

PBMCs – peripheral blood mononuclear cells

PB – phosphate buffer

PBS – phosphate-buffered saline

PCA – principle component analysis

PDGF – platelet-derived growth factor

PDGFR – platelet-derived growth factor receptor

PG – prostaglandin

PPAR – peroxisome proliferator-activated receptor

PVDF – polyvinylidene fluoride

RIN – RNA integrity number

RIPA – radioimmunoprecipitation assay

ROS – reactive oxygen species

RPMI – Roswell Park Memorial Institute

RT – room temperature

scRNA-seq – single-cell RNA sequencing

SD – standard deviation

SDS – sodium dodecyl sulfate

SDS-PAGE – sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SR – scavenger receptor

STAT – signal transducer and activator of transcription

STRING – Search Tool for the Retrieval of Interacting Genes/Proteins

TBS-T –Tris-buffered saline, containing 0.1% Tween-20

TEAB – triethylammonium bicarbonate

TET – ten-eleven translocation methylcytosine dioxygenase

TFEB – transcription factor EB

TGF – transforming growth factor

Th – T helper lymphocytes

TIMPs – tissue inhibitors of matrix metalloproteinases

TIMP – tissue inhibitor of matrix metalloproteinases

TLRs – toll like receptors

TMT – tandem mass tag

TNF – tumor necrosis factor

TREM – triggering receptor expressed on myeloid cells

VEGF – vascular endothelial growth factor

WNT – Wingless-related integration site

YAP1 – Yes1 associated transcriptional regulator

1. Introduction

Endometriosis is a chronic degenerative condition of the mare endometrium characterized by the gradual accumulation of extracellular matrix (ECM) in the stroma and pathological alterations in the endometrial glands. Of all histological changes that occur in endometriosis, endometrial fibrosis is the most common and significant alteration (Hoffmann et al., 2009b; Kenney, 1978; Kenney & Doig, 1986). Although the exact etiology of endometriosis is unknown, it seems that it can be correlated with the age of the mare and is interconnected with inflammation (Hanada et al., 2014). It is important to specify that endometriosis of mares must not be mistaken for endometriosis in women. Even while endometriosis patients have been shown to exhibit signs of fibrosis inside and around the endometrial lesions (Garcia Garcia et al., 2023), the most distinctive feature of the condition in women is the extrauterine implantation of endometrial tissue, which is not present in the mare during endometriosis (Lamceva et al., 2023).

Fibroblasts and macrophages (MΦ) are cells important in regulating tissue microenvironment and inflammation in the context of fibrosis development. In response to chronic inflammation and persistent tissue damage, fibroblasts play a key role in mediating the pathological fibrotic accumulation of ECM and proliferation of stromal cells (Kendall & Feghali-Bostwick, 2014). Moreover, these cells are highly responsive to cytokines, chemokines, and growth factors that are released in fibrotic tissue (Kendall & Feghali-Bostwick, 2014). A growing number of studies point to MΦ as the master regulators of chronic inflammation and the development of fibrosis (Wynn & Barron, 2010; Wynn & Vannella, 2016). They secrete chemokines that maintain cell activation and recruit other inflammatory cells, as well as regulate the balance between deposition and degradation of ECM (Wynn & Barron, 2010). Importantly, the recruited and resident MΦ proliferate and undergo marked phenotypic and functional changes during classical activation into type 1 macrophages (MΦ1) or alternative activation into type 2 macrophages (MΦ2), depending on the inflammatory condition (Murray & Wynn, 2011). Because of that type of plasticity, MΦ can play a crucial role in the initiation, maintenance, and progression of fibrosis (Wynn & Vannella, 2016).

A recent study of the transcriptomic profile of mare endometrium has already revealed the increased expression of genes associated with cellular infiltration by MΦ at the early stage of endometriosis, pointing toward their potential role in fibrosis development (Szóstek-Mioduchowska et al., 2023). Thus, an in-depth understanding of the dialog

between fibroblasts and MΦ in mare endometrium can bring us closer to understanding the pathogenesis of endometriosis and developing effective therapeutic strategies.

1.1. Endometriosis

Endometriosis is a chronic degenerative condition of the mare endometrium that impairs the histological structure and endometrial function. It was previously referred to as chronic degenerative endometritis or chronic degenerative endometrial disease (Bracher et al., 1996; Kenney, 1978; Kenney & Doig, 1986; Ricketts & Alonso, 1991). Regardless of the terminology, it is characterized by active or inactive stromal and/or periglandular fibrosis of endometrium (Hoffmann et al., 2009a; Kenney, 1978; Kenney & Doig, 1986). The observed periglandular fibrosis can be either destructive, resulting in the degeneration and even necrosis of glandular epithelial cells, or non-destructive, with intact glandular epithelium. Additionally, it may affect one or more glands, leading to the development of glandular nests (Schöniger & Schoon, 2020). Aside from glandular atrophy or hyperplasia, other histological changes in the endometrium include lymphatic lacunae, glandular and lymphatic cyst formation, pleomorphism, and inflammation (Flores et al., 1995; Kenney, 1978; Kenney & Doig, 1986). Furthermore, the alterations in the expression of prostaglandin synthases (Szóstek et al., 2012a) and steroid hormone receptors, as well as variations in endometrial secretory functions, have also been observed (Hoffmann et al., 2009b). Moreover, the fibrotic foci differ significantly regarding glycoconjugates (Walter et al., 2001) and enzyme patterns (Hoffmann et al., 2009a). In addition to the histological alterations in the endometrium, endometriosis also includes alterations in the function and structure of the myometrium (Hanada et al., 2014), with observed changes in the myometrial transcriptome (Drzewiecka et al., 2025) and dysregulated myometrial contractility (Piotrowska-Tomala et al., 2024).

Impaired secretory function of endometrial glands can adversely affect the amount and/or quality of histotroph (exocrine secretions of endometrial glands). As mare trophoblasts are non-invasive, it is considered crucial for conceptus development and survival (McDowell et al., 1987; Perera et al., 2025; Wilsher & Allen, 2012). According to the proteomic analysis of uterine lavage fluid, the release of vital proteins was impaired in mares with endometriosis (Diel de Amorim et al., 2020), which may compromise the development of early conceptus, resulting in pregnancy loss (Allen & Wilsher, 2009; Diel de Amorim et al., 2020). Changes in the uterine microenvironment can also impact migration of the embryo and affect placenta development, which can lead to problems

with maintaining a pregnancy and, consequently, to infertility in mares (Bracher et al., 1996; Lehmann et al., 2011; Wilsher & Allen, 2012).

1.1.1. Classification system of endometria

Since there is a direct correlation between the degree of lesions and the reproductive success of the mare, it was necessary to introduce a system for the categorization of endometrium. To date, the Kenney and Doig classification system, which was first proposed in 1986 and later refined by Schoon et al. (1992), is commonly used to assess the severity of endometrial changes in mares. This approach, which is based on the histological analysis of endometrial sections, takes into consideration fibrotic, inflammatory, atrophic, and lymphatic abnormalities as well as gland density, dilatation, and nesting in the endometrium and correlates these with the foaling rate and general fertility (Kenney, 1978; Kenney & Doig, 1986; Schoon et al., 1992). This classification system facilitates the diagnosis and grading of endometrial pathology in mares by providing a consistent framework for the histological assessment of endometrial samples. Under this classification system, endometria can be divided into four categories:

- **category I** – endometrium, which is devoid of signs of fibrosis and inflammation, with the absence of glandular alterations. The expected foaling rate is above 80%;
- **category IIA** – endometrium with mild changes, like a slight amount of stromal fibrosis and mild inflammatory alterations around observed gland branches. The expected foaling rate is in the 50 – 80% range;
- **category IIB** – endometrium with moderate changes – fibrotic, lymphatic, and inflammatory alterations get more extensive and widespread, up to 60% of glands are altered, and the foaling rate drops to the 10 – 50 % range, which can be associated with at least two years of infertility period;
- **category III** – endometrium with severe changes – observed inflammatory and fibrotic alterations are widespread and severe, occurs severe nesting of the glands and lymphatic lacunae. The foaling rate falls below 10%, and the mare can practically be considered infertile (Kenney & Doig, 1986).

1.1.2. Risk factors for the development of endometriosis

The pathogenesis of endometriosis remains unclear and not well understood. However, over the years, specific risk factors have been identified, which can facilitate the identification of mares with a greater likelihood of developing this pathology. It is essential to comprehend these risk factors to develop management strategies that will lessen the likelihood of endometriosis development in susceptible mares. Among the earliest discussed risk factors of endometriosis are recurring natural difficulties of aging, pregnancy, parturition, and involution of the uterus after parturition (Kenney, 1978; Kenney et al., 1975). Aging appears to be the element most closely linked to other observed factors. Tissue remodeling and immune system dysfunction associated with aging result in compromised homeostasis and increased vulnerability to the formation of fibrotic tissue (Diel de Amorim et al., 2020). In a study of the age-dependent prevalence of endometriosis in mares, Ebert et al. (2014) demonstrated that endometriosis prevalence was 93% in mares over the age of twenty. However, since elderly maiden mares have also been shown to develop even severe endometriosis, it turns out that this condition cannot be directly linked to uterine involution or the number of pregnancies and foaling (Ricketts & Alonso, 1991). Alternatively, periglandular fibrosis and changes in endometrial glands over the lifespan of a mare have been correlated with fluid collection in the uterus (Kenney, 1978; Özgen et al., 2002; Reilas et al., 1997). After breeding, mares accumulate uterine fluid. It leads to post-breeding endometritis, which is essential for the efficient clearance of pathogens introduced into the uterine lumen (Troedsson, 2006). Susceptible mares have problems with this natural cleaning of the uterus and can accumulate intrauterine fluid, which, in consequence, can lead to the development of persistent endometritis – chronic inflammation of the endometrium (Schöniger & Schoon, 2020). Factors responsible for heightening the risk of persistent endometritis include increased vulnerability to infection, bacterial pathogenicity and type (Christoffersen et al., 2015), myometrial contractility defects that restrict uterine fluid clearance (Leblanc et al., 1994), or certain defects such as poor vulvar conformation (Hemberg et al., 2005) or changes in the uterine position (Leblanc et al., 1998). Importantly, even post-breeding endometritis led to an increase of fibrosis in the endometrium (Katila & Ferreira-Dias, 2022; Reilas et al., 2016), which can indicate the correlation between inflammation and endometriosis development. In conclusion, there are no unambiguous risk factors for endometriosis development, hence, the individual obstetrical and gynecological history must also be taken into account.

1.1.3. Diagnosis of endometriosis

The only currently available standardized scientific method for diagnosing endometriosis is histopathological assessment of endometrial biopsies (Kenney, 1978). Although it is considered safe and useful, it has its limitations and disadvantages. Despite the existence of a standardized method for endometrium classification, this evaluation is susceptible to subjective judgment, which may affect the overall assessment. Moreover, judging the condition of the entire uterus from a single section may also raise questions about the result of the examination (Ricketts & Barrelet, 1997; Rojas et al., 2020). It is crucial to look for alternative techniques for diagnosing endometriosis, particularly ones that are less invasive and more dependable. The use of blood biomarkers, particularly serum concentration of collagen (COL) type 3, is one of the recently proposed candidates. Nonetheless, it should be considered more as an aid for histological evaluation rather than a substitute (Alpoim-Moreira et al., 2022b). Meanwhile, for optimal and reliable results, the diagnosis should be based on the correlation of all available data.

1.1.4. Treatment of endometriosis

Endometriosis remains a considerable problem due to the lack of a suitable method of treatment. Effective methods for stopping or reversing observed fibrotic changes have been sought for years with negligible results. Mechanical endometrial curettage was proposed as an easy and safe to perform therapeutic strategy for subfertile mares. Even though 58% of the mares gave birth to live foals during the breeding season after curettage, this treatment had no noticeable impact on the older mares (Ricketts, 1985). An alternative method for mechanical intervention is the use of different chemical agents. Previously, kerosene was proposed as a method for the treatment of mare endometrium. By irritating the uterus and causing uterine edema, kerosene resulted in the expulsion of retained excretions and was demonstrated to increase the fertility rate of mares with severe endometriosis. However, the treatment had relatively short-term benefits, without the intended long-term improvement, and raised questions concerning the animal welfare and safety of the method, thus, it cannot be considered a reliable option (Bracher et al., 1996; Coyle et al., 2017). Another chemical agent used as a possible remedy for endometriosis was dimethyl sulfoxide (DMSO). Treatment with 30% DMSO resulted in alleviated inflammation and reduction of periglandular fibrosis and did not cause any negative changes in the endometrium. However, this effect did not translate into an increase in mare fertility (Ley et al., 1989).

In the context of treating fibrotic diseases, as a result of their unique properties, tremendous interest has been drawn to mesenchymal stem cells. It has already been shown that the transplantation of mesenchymal stem cells into the uterus of mares with endometriosis can benefit the pattern of secretory proteins and influence the proliferation of glandular epithelial cells (Mambelli et al., 2014). Notably, in this context, further studies are still required, as the use of cells leaves a wide margin for improvement in administration route, dosage, and cell preparation. Recently, Wong et al. (2024) showed that preconditioning of mesenchymal stem cells with transforming growth factor (TGF)- β 1 led to the anti-fibrotic secretory phenotype of these cells, which, however, turned pro-fibrotic after longer exposure to this pro-fibrotic factor.

Likewise, further studies are needed in the context of epigenetic regulation during endometriosis. Although data on methylation changes in the course of endometriosis are still scarce, there are first indications that using demethylating agents gives a possibility for a new treatment method (Alpoim-Moreira et al., 2023). Nevertheless, despite several different proposals, there is still no effective treatment method that will alleviate ongoing endometrial fibrosis.

1.2. Fibrosis

Fibrosis is a pathological condition with the predominant characteristic of excessive deposition of ECM components such as different types of COL and fibronectin (FN), which disrupts tissue architecture and raises morbidity and mortality rates (Henderson et al., 2020; Wynn, 2008). Therefore, fibrosis is interrelated with the malfunction of organs, the development and progression of numerous chronic diseases, and one-third of premature deaths worldwide (Zeisberg & Kalluri, 2013). Although fibrosis displays many similarities between tissues, it can also be exceptionally organ-specific. However, unquestionably, fibrosis is a complex and complicated process and generally consists of overlapping stages like inflammation and immune cell infiltration, cell activation and proliferation, and tissue remodeling (Distler et al., 2019). Another common feature is the presence of persistent irritants that sustain the production of profibrotic factors and stimulate overproduction of ECM components, leading to altered tissue architecture. In this regard, fibrosis can be considered a result of chronic inflammation triggered by infection, tissue injury, or any given tissue response (Wynn, 2007, 2008). In consequence, the key molecular and cellular mechanisms of fibrosis development are activated, which can have many similarities with wound healing, with the difference that there is no

resolution of the process and a pathological environment emerges (Henderson et al., 2020; Wynn, 2007).

1.2.1. Fibroblasts

Fibroblasts are the cells that play a central role in the development and progression of fibrosis. These cells are the most abundant in connective tissue and play a critical role in tissue development, growth, and homeostasis (Talbot et al., 2022). Fibroblasts are very diverse and tissue-specific cells with distinct phenotypes. They display functional specialization depending on the organ of origin and spatial location (Lynch & Watt, 2018). These cells are highly heterogeneous, which makes it challenging to characterize them. Their populations do not consistently express vimentin, platelet-derived growth factor receptor (PDGFR)- α , PDGFR- β , or cluster of differentiation (CD) 90, which have so far been proposed as fibroblasts' molecular markers (Davidson et al., 2021). In murine fibroblasts, intra- and inter-organ heterogeneity was discovered by single-cell RNA sequencing (scRNA-seq), in which only around 20% of genes were shared between fibroblasts of different organs, and this heterogeneity was linked with differences in the formation and maintenance of ECM (Muhl et al., 2020). Moreover, fibroblasts were also shown to exhibit positional, mechanical, inflammatory, and metabolic memory, that results from a combination of epigenetic modifications, cell-to-cell and cell-to-ECM interaction, and also as a response of different signaling factors, which further enable fibroblasts to retain information about their previous microenvironment (Kirk et al., 2021).

The fibroblasts are primarily responsible for the formation and remodeling of ECM, however, it has become evident that they have a variety of other functions. Fibroblasts secrete cytokines, growth factors, and signaling factors, generate mechanical forces, and regulate tissue metabolism, creating a specific microenvironment for other cells. At the same time, fibroblasts constitute a source of positional information and remain target cells of adaptive and innate immune responses (Plikus et al., 2021). Moreover, these cells are entitled to the function of sentinel cells due to their dual role, both as structural elements but also as essential immunomodulatory cells (Smith et al., 1997). Fibroblasts can recognize pathogens as a result of the expression of toll-like receptors (TLRs), which recognize pathogen-associated molecular patterns (PAMPs). In response, fibroblasts start the secretion of chemokines such as chemokine (C-C motif) ligand 1 (CCL1), CCL2, CCL5, chemokine (C-X-C motif) ligand 1 (CXCL1), CXCL8, CXCL1, C-X3-C motif

chemokine ligand 1 (CX3CL1) and pro-inflammatory cytokines like tumor necrosis factor (TNF)- α , interferon (IFN)- γ , interleukin (IL)-6, IL-10, IL-12 that collectively can induce the recruitment of specialized inflammatory cells. At the same time, fibroblasts also secrete peptides like the C-terminus 37 amino acid peptide (LL-37) or defensins that directly perform antimicrobial activity (Bautista-Hernández et al., 2017). A graphical summary illustrating fibroblast function within connective tissue is presented in Figure 1. All the complexity surrounding the physiology and role of fibroblasts underscores their critical role in maintaining homeostasis, responding to pathological conditions, and regulating the development of fibrosis.

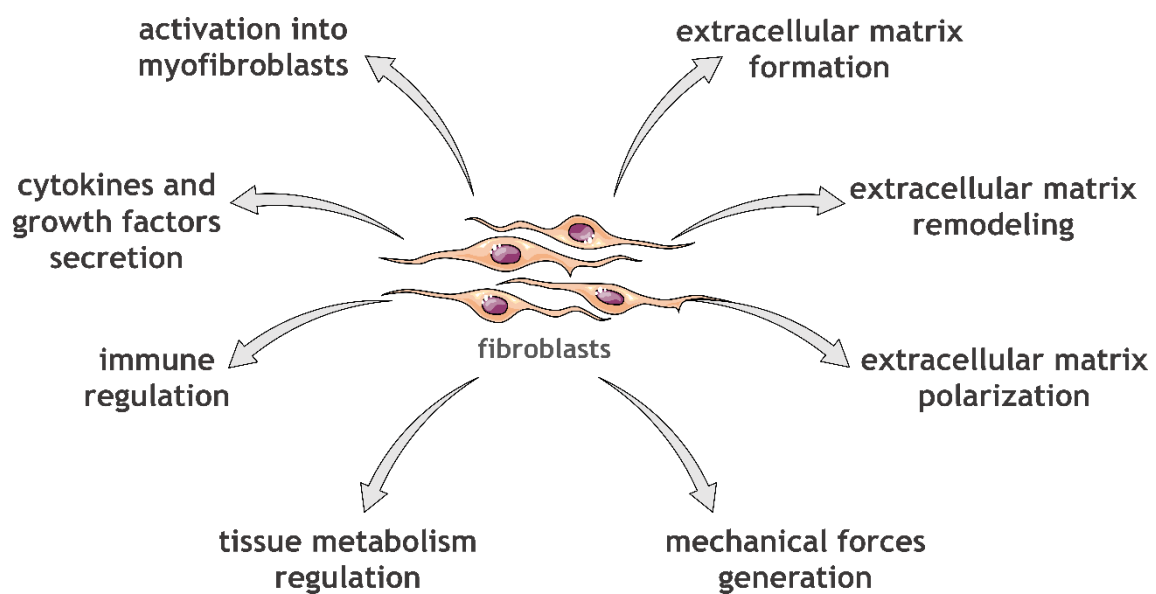


Figure 1. Summary of the main fibroblast functions within connective tissue. The figure was prepared with the use of pictures from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License.

1.2.2. Mechanisms associated with the development of fibrosis

The primary mechanism underlying tissue fibrosis is the activation of fibroblasts into myofibroblasts, which exhibit increased expression of α -smooth muscle actin (ACTA2) and higher deposition of ECM components (Schuster et al., 2023). Among components deposited by myofibroblasts, relevant to the subject of fibrosis, are COL type I, III, IV, V, VI, FN, elastins, fibrillins, latent TGF- β binding proteins (LTBPs), proteoglycans like hyaluronan or syndecan, and tenascins (Klingberg et al., 2013). Notably, myofibroblasts can also originate from other mesenchymal cells and also from endothelial and epithelial cells. The processes by which endothelial and epithelial cells are transformed into

mesenchymal cells are called endothelial-to-mesenchymal transition (EndMT) and epithelial-to-mesenchymal transition (EMT), respectively (Wynn, 2008). There are several ways to activate myofibroblasts, including direct PAMPs-TLRs interaction on fibroblasts, mechanical regulation, autocrine signals from already activated myofibroblasts, or paracrine signals from immune cells, like lymphocytes and MΦ that are present during chronic inflammation (Hinz et al., 2019; Wynn, 2008). Specifically, the known factors of myofibroblast differentiation comprise the following: growth factors and cytokines (TGF-β1, fibroblast growth factor [FGF]-2, IFNγ, IL-1β, IL-4, IL-6, IL-12, IL-13, IL-17, TNF-α), integrins, and factors related to oxidative stress (advanced glycation end-products [AGEs], reactive oxygen species [ROS], NADPH oxidase [NOX]) (Yuan et al., 2019). Moreover, chemokines (monocyte chemoattractant protein [MCP]-1, macrophage inflammatory protein [MIP]-1β), angiogenic factors (vascular endothelial growth factor [VEGF]), and peroxisome proliferator-activated receptors (PPAR) were also indicated as mediators of fibrosis (Henderson et al., 2020). Notably, a lot of these factors are simultaneously known markers of inflammation (Roe, 2021), which further emphasizes the role of chronic inflammation in the development of fibrosis. The consequences of the presence of the aforementioned factors are the activation of specific signaling pathways that are responsible for mediating myofibroblast differentiation and fibrosis. The most well-established pathway is the TGF-β signaling pathway; however, others, including the Wnt/β-catenin signaling pathway, nuclear factor kappa B (NF-κB) signaling pathway, Hedgehog signaling pathway, or Notch signaling pathway, have already emerged as potential targets for fibrosis studies, as they regulate the genes associated with fibrosis development (Yuan et al., 2019).

An important difference between tissue remodeling and the development of fibrosis is the inhibited ability of myofibroblasts to dedifferentiate and undergo apoptosis. During fibrosis, myofibroblasts express numerous biochemical, extracellular, and intracellular survival factors that stop the resolution of remodeling. Changes in the microenvironment triggered by fibrotic ECM, autocrine production of pro-fibrotic factors, metabolic reprogramming, and senescence can all be reasons for extreme myofibroblast resistance to apoptosis. As a result, myofibroblasts expressed anti-apoptotic factors (B-cell leukemia/lymphoma 2 protein (BCL-2), B-cell lymphoma extra-large (BCL-X_L) which help in maintaining of pathological state (Gibb et al., 2020; Hinz & Lagares, 2020).

1.2.3. Matrix metalloproteinases and their tissue inhibitors

Matrix metalloproteinases are a family of zinc-dependent proteinases that are primarily responsible for the degradation of ECM components. About 24 different MMPs have been already identified, which are further subdivided into collagenases (MMP-1, MMP-8), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10), matrilysins (MMP-7, MMP-26), membrane-type MMPs (MMP-14, MMP-15), and other MMPs (MMP-12, MMP-20), based mainly on their structure and substrate specificity (Murphy & Nagase, 2008). The main substrates for MMPs include COL, FN, elastin, and other proteoglycans. As a result of controlled ECM degradation, MMPs not only influence the extracellular space but also influence cell migration, proliferation, and differentiation or exert pro- and anti-inflammatory effects (Visse & Nagase, 2003). Several tissues and cells are capable of secreting MMPs, among them are fibroblasts, MΦ, lymphocytes, neutrophils, and endothelial cells (Cui et al., 2017). Matrix metalloproteinases are expressed as zymogens, which are their inactive precursors. These precursors are targets for other proteolytic enzymes that process the zymogens and generate their active form. Therefore, MMPs activity can be regulated in three stages: during transcription, during proteolytic activation of MMPs, and through control of mature MMPs (Verma & Hansch, 2007).

The contrary to MMPs are tissue inhibitors of matrix metalloproteinases (TIMPs), which are major regulators of their activity. Distinguished are four different TIMPs (TIMP-1, TIMP-2, TIMP-3, TIMP-4), which are similar in the context of their sequence and structure but have specific attributes and characteristics (Coates-Park et al., 2024). The primary role of TIMPs, as their name states, is the inhibition of MMPs activity. However, it has been shown that TIMPs also demonstrated MMPs-independent functions and affected cell growth, cell differentiation, and apoptosis (Ries, 2014).

As the endogenous TIMPs play a major role in controlling the MMPs activity, disorders like rheumatoid and osteoarthritis, atherosclerosis, tumor growth, metastasis, and fibrosis might arise from a disturbance of the MMP/TIMP equilibrium (Bode et al., 1999). The imbalance of MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios was suggested to be a possible contributor to COL deposition in murine skin disorders (Zhou et al., 2021), the same as MMPs/TIMP-1 imbalanced expression had emerged as a therapeutic target for pulmonary fibrosis (Wang et al., 2011). Notably, the MMP-TIMP regulation is far more convoluted than the basic paradigm that MMPs decrease their expression and TIMPs increase their expression during fibrosis, resulting in ECM deposition. In contrast,

any disruption of their expression should be considered in the context of fibrosis development, as they will contribute to pathological processes through their other functions, including promotion of EMT, influencing cell migration, and differentiation (Chuliá-Peris et al., 2022).

1.3. Fibrosis development in the mare endometrium

One of the initial signs of endometriosis is an abnormal morphological and functional differentiation of endometrial stromal cells. Especially, large polygonal stromal cells that start producing collagen fibers are a hallmark of the onset of fibrosis (Hoffmann et al., 2009a). The cellular activation during endometriosis was demonstrated by the expression analysis of molecular markers of myofibroblasts – ACTA2 in mare endometrium, whose expression was increased in the final stage of endometriosis (Szóstek-Mioduchowska et al., 2019b). Moreover, an increase in the basal secretion of COL1 was observed in explants of mare endometrium categories IIA and IIB (Szóstek-Mioduchowska et al., 2019a). In endometriosis, the pathological accumulation of COL typically emerges in the endometrial lamina propria, surrounding altered endometrial glands (Kenney, 1978; Schöniger & Schoon, 2020). Changes in the expression of MMPs and TIMPs were also observed in the mare endometrium in the course of endometriosis. It has been demonstrated that the concentration of MMP-1, -2, -9, and TIMPs was found to be elevated, while MMP-3 concentration was reduced in the altered endometrium (Szóstek-Mioduchowska et al., 2020). These results indicate that common mechanisms of fibrosis are shared in its development in the course of endometriosis, similar to those occurring in other organs. These findings are further supported by the fact that the most recognized pro-fibrotic factor and regulator of endometrial ECM remodeling, TGF- β 1, directly affects the expression of ACTA2, MMPs, and TIMPs. This cytokine increased the expression of ACTA2 (Szóstek-Mioduchowska et al., 2019b), up-regulated the MMP-1, -9, -13, and TIMP-1 secretion, as well as down-regulated the MMP-3 secretion from fibroblasts derived from category I endometrium (Szóstek-Mioduchowska et al., 2020). In particular, the changes in MMPs expression appear to be important as subsequent studies have indicated that they have the capacity for epigenetic regulation in fibroblasts, as MMP-2 and MMP-9 genes exhibited increased DNA hypermethylation during endometriosis (Alpoim-Moreira et al., 2022a).

In recent years, a vast amount of data concerning the processes involved in the development of mare endometrial fibrosis has been obtained using transcriptomic studies.

In non-cycling mares, an alteration in the expression of 55 transcripts was observed in mares diagnosed with endometriosis. Identified genes were annotated to collagen catabolic process, immune response, and lipid metabolic process (Witkowski et al., 2022). On the other hand, in cycling mares, a change in the expression of 230 genes and 1101 genes was observed in endometria categories IIA and IIB, respectively, when compared to endometria category I. The complexity of the fibrosis development was further indicated by the fact that 2111 genes were differentially expressed between IIB and IIA endometria, suggesting that the progression of fibrosis is an outcome of functional changes and modulation rather than a cumulative, time-dependent effect (Szóstek-Mioduchowska et al., 2023). Dysregulation during the development of mare endometrial fibrosis was also observed through changes in the expression of microRNA (miRNA) – molecules that regulate gene expression through transcriptional and post-transcriptional modulation. Consistent with transcriptome results, most alterations in miRNA were observed in category IIB endometria, revealing their association with different signaling pathways such as Hippo, WNT, and JAK-STAT signaling pathways (Wójtowicz et al., 2023).

In the transcriptomic study, potential upstream regulators of identified genes were presented, among which were different cytokines, growth factors, enzymes, and transcription regulators like IL-6, IL-1 β , IL-4, IL-17, FGF, amphiregulin (AREG), hypoxia inducible factor (HIF)-1 α , or signal transducer and activator of transcription (STAT)-3 (Szóstek-Mioduchowska et al., 2023). According to the findings of previous research, pro-inflammatory cytokines may control the development of fibrosis via their direct impact on fibroblasts or epithelial cells. In endometriosis, elevated expression of IL-1 β and IL-6 has been observed when comparing fibrotic endometrium with a non-fibrotic endometrium (Szóstek et al., 2013). In later years, these cytokines were shown to exert an effect on the production of ECM components like COL1, COL3, and FN, as well as on the MMPs and TIMPs (Szóstek-Mioduchowska et al., 2019a). This indicates that already possessed transcriptomic data can be a valuable reference for further research directions on the topic of endometriosis.

As presented earlier, various signaling pathways serve an important role in the activation of myofibroblasts and fibrosis development. Transcriptomic analysis of mare endometrium with different stages of endometrial fibrosis revealed that altered genes can lead to the enrichment of wound healing, ferroptosis, HIF-1 α , or WNT/ β -catenin signaling pathways (Szóstek-Mioduchowska et al., 2023). Different studies have also

shown the potential role of TGF- β 1 in the development of endometrial fibrosis in *in vitro* condition (Szóstek-Mioduchowska et al., 2020; Szóstek-Mioduchowska et al., 2023; Szóstek-Mioduchowska et al., 2019b). Moreover, in active endometriosis, the NF- κ B signaling pathway, which is an important player in the regulation of cell metabolism, was observed to be activated during the follicular phase of the estrous cycle (Domino et al., 2020). Further analyses revealed that NF- κ B canonical pathway is involved mainly in the destructive form of endometriosis and may play a role in the necrosis of glandular epithelium (Jasiński et al., 2022; Jasiński et al., 2021).

Moreover, functional annotation of genes altered in the course of endometriosis showed their association with inflammation of organs, leukocyte migration, cellular infiltration by M Φ , T helper cell (Th)-1 and Th2 activation pathway, and neutrophil extracellular trap signaling pathway, indicating the importance of the immune system in the development of endometrial fibrosis in mares (Szóstek-Mioduchowska et al., 2023). Previously, neutrophil extracellular trap (NETs) proteins such as elastase (ELA), cathepsin-G (CAT), and myeloperoxidase (MPO) were shown to increase COL1 expression in mare endometrial explants (Rebordão et al., 2018), and recently, the role of Th1 and Th2 lymphocytes was thoroughly studied in the context of the development of fibrosis in the progression of endometriosis (Wójtowicz, 2024). However, there is still a remaining gap in knowledge regarding the direct role of M Φ in the development of mare endometrial fibrosis in the progression of endometriosis, which seems to be relevant, especially in the early stage of the condition.

1.4. Macrophages

Together with monocytes and dendritic cells, M Φ are categorized as a part of the mononuclear phagocyte system. They were believed to belong to the hematopoietic cell lineage that originates from bone marrow progenitor cells, and in the adult organism is replenished only by bone marrow-derived monocytes circulating in the blood (Hume, 2006). In recent years, it has become increasingly evident that tissue-resident M Φ are an integral part of the tissue since the embryogenesis and M Φ subpopulation relies on self-renewal (Dick et al., 2022; Epelman et al., 2014; Hashimoto et al., 2013). However, with developing inflammation, the M Φ population may still be expanded not only by self-renewal but also by differentiation from circulating monocytes. In this instance, monocytes that have been induced by chemokines become precursors for new M Φ – monocyte-derived M Φ that can outnumber those originally residing in the tissue (Fig. 2)

(Davies et al., 2013; Galli et al., 2011; Wynn & Vannella, 2016). Moreover, in virtually every tissue and organ, there are distinct specialized M Φ populations such as Langerhans cells in the skin, Kupffer cells in the liver, alveolar M Φ in the lungs, or lamina propria M Φ within the intestine that play unique roles dependent on the local microenvironment (Nobs & Kopf, 2021). Extensive local and organism specificity does not facilitate characterization of M Φ populations. Macrophages are commonly identified by their cell surface markers, including CD68, CD11b, CD14, CD16, CD64, or F4/80 (which is exclusively expressed in murine M Φ). Notably, some of these markers are also expressed in other cells, such as monocytes and dendritic cells (e.g. CD14). Furthermore, in certain models, they are proposed as population-specific rather than general markers of M Φ , thereby highlighting the absence of uniformity in this field of study (Akinrinmade et al., 2017; Dos Anjos Cassado, 2017; Khazen et al., 2005).

Macrophages are immune guardian cells that are responsible for the regulation of tissue microenvironment and maintenance of local homeostasis. These immune cells are pivotal in defending against infection and tissue damage by their ability to phagocytose and degrade dead cells, cellular debris, and foreign material (Varol et al., 2015). This is possible due to the wide range of sensors and receptors that M Φ possess, e.g. pattern recognition receptors (e.g. TLRs, Nod-like receptors, C-type lectin receptors), scavenger receptors (e.g. scavenger receptor [SR]-A, CD36), or cytokine receptors (e.g. IFN γ receptor, IL-4 receptor, IL-13 receptor, TGF- β receptor) (Ley et al., 2016). Pattern recognition receptors, which specialize in identifying common elements of bacterial and fungal cell walls, such as lipopolysaccharide (LPS), are the primary mediators of pathogen detection. It has been demonstrated that these cells are effective in identifying pathogens that have penetrated the epithelial barrier (Iwasaki & Medzhitov, 2015). Additionally, M Φ are thought of as antigen-presenting cells that expose Th lymphocytes to acquired antigens, trigger the adaptive immune response, and cause naïve T lymphocytes to differentiate into effector T lymphocytes (Ogino & Takeda, 2023). Moreover, stimulation of M Φ receptors results in the release of inflammatory cytokines and growth factors like IL-1 β , IL-6, IL-12, IL-23, TNF- α , and TGF- β , which stimulate and recruit other immune cells like monocytes, neutrophils, eosinophils, and basophils, orchestrating the immunological response (Iwasaki & Medzhitov, 2015). Macrophages are also known as regulators of the local microenvironment. They can sense changes in oxygen levels (HIF-1 α), pH (G protein coupled receptor [GPR]-65), and osmolarity (NOD-like receptor family pyrin domain containing 3 [NLRP3], NOD-like receptor

family CARD domain-containing protein 4 [NLRC4], nuclear factor of activated T-cells 5 [NFAT5]) (Okabe & Medzhitov, 2016). Through integrins and TLRs, these cells may also detect and respond to changes in ECM, especially as some components (e.g. tenascin-C and fibronectin) can orchestrate MΦ recruitment (Okabe & Medzhitov, 2016). Notably, the signals that MΦ obtain are strongly interconnected with the role they play in the tissue, as these cells possess the ability to undergo phenotypic and functional changes into different pro- and anti-inflammatory subpopulations (Wynn & Vannella, 2016).

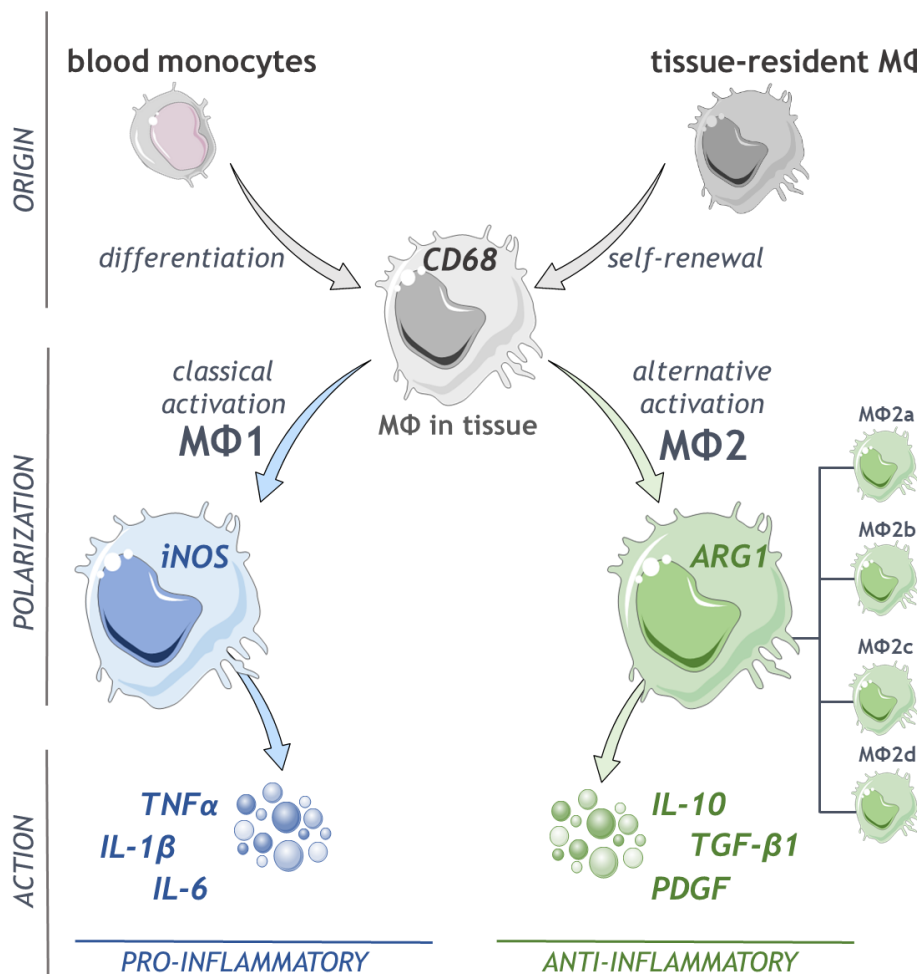


Figure 2. A summary of the origin and distinction of macrophages (MΦ) phenotypes, highlighting their functional and molecular differences.

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1.4.1. Macrophage plasticity

High levels of plasticity and diversity are significant features of the MΦ biology. The recruited and resident MΦ proliferate and undergo marked phenotypic and functional changes that are synchronized with critically timed requirements. Macrophages can be divided into two main phenotypes: pro-inflammatory MΦ1 and anti-inflammatory MΦ2, which are highly interconnected with evolving immune response (Murray & Wynn, 2011). A summary of the distinct MΦ phenotypes, highlighting their functional and molecular differences, is presented in Figure 2. At the early stage of inflammation, the presence of factors like LPS, PAMPs, damage-associated molecular patterns (DAMPs), or Th1-related cytokines, including IFN γ , leads to classical activation of MΦ1 (Sica & Mantovani, 2012). This subpopulation is responsible for higher secretion of inflammatory factors, including cytokines like TNF- α , IL-1, IL-6, IL-12, IL-23. Macrophages type 1 are also responsible for the activation of the oxidase system and induction of antimicrobial ROS (Jiang et al., 2024; Lis-López et al., 2021). Collectively, this enhances their proinflammatory role, which involves antigen presentation for the effector cells, phagocytosis, and immunomodulation via the produced secretome (Fujiwara & Kobayashi, 2005). Mutually, at a later stage of inflammation, the presence of Th2-related cytokines IL-4, IL-13, but also IL-6, IL-10 and PG leads to an alternative activation of MΦ2, which exerts more anti-inflammatory effect through the secretion of molecules such as IL-10, TGF- β 1 (Lis-López et al., 2021; Sica & Mantovani, 2012). The functional polarization of MΦ2 leads to the differentiation into four distinct subtypes: anti-inflammatory MΦ2a, immunomodulatory MΦ2b, immunosuppressive MΦ2c, and tumor-associated MΦ2d (Jiang et al., 2024; Mantovani et al., 2002). The fundamental driver and regulator of this phenotype polarization in MΦ is arginine (ARG) metabolism. The terms “classically-activated” and “alternatively-activated” directly correspond to the ARG dichotomy, which reflects changes in the expression of two genes responsible for the metabolism of ARG that occurs during polarization. Macrophages type 1 expresses an inducible isoform of nitric oxide synthase (iNOS) (Kieler et al., 2021; Rath et al., 2014). This isoform is not present in resting cells, instead, its induction is stimulated in response to the presence of bacterial elements or inflammatory cytokines. Upon activation, iNOS, using NADPH and molecular oxygen, metabolizes ARG into nitric oxide (NO) and citrulline (Aktan, 2004). The rapid production of NO following iNOS activation allows MΦ1 to carry out their pro-inflammatory functions, since NO modulates the inflammatory cascade and mediates viral infections (Laroux et al., 2001; Uehara et al.,

2015). Moreover, NO can be further metabolized into reactive nitrogen species (Rath et al., 2014). On the other hand, MΦ2 are characterized by the expression of arginase 1 (ARG1) (Kieler et al., 2021; Rath et al., 2014), which is a hydrolytic enzyme that metabolizes ARG into ornithine and urea. Not only does ARG1 limit the biological availability of ARG for NO conversion, thus reducing the inflammatory state, but by producing ornithine, it also contributes to the regulation of proline and polyamine synthases and collagen production. This underlines the association of MΦ2 with the orchestration of tissue remodeling (Grasemann et al., 2015; Rath et al., 2014).

1.5. Macrophages' potential for regulation of fibrosis

A growing number of studies have identified MΦ as important regulatory cells in the control of chronic inflammation and the development of fibrosis. Moreover, their high functional plasticity translates into their potential to act as both pro- and anti-fibrotic mediators (Wynn & Barron, 2010; Wynn & Vannella, 2016). The functions attributed to MΦ are primarily directed toward restoring and maintaining tissue homeostasis. Disruption of these regulatory mechanisms can result in the failure to restore physiological balance, thereby contributing to the onset and progression of pathological conditions. This provides a comprehensive explanation for the multifaceted and occasionally contradictory roles that these factors play in diseases such as fibrosis. Given their capacity to both promote and prevent fibrosis, MΦ regulatory mechanism have already been extensively discussed in the context of pulmonary fibrosis (Cheng et al., 2021), renal fibrosis (Meng et al., 2019), intestinal fibrosis (Yao & Tang, 2022), hepatic fibrosis (Sun & Matsukawa, 2024) and cardiac fibrosis (Lafuse et al., 2020) indicating high local specificity of action.

Macrophages type 1 facilitate the removal of pathogens and coordinates the inflammatory response that is essential for the development of fibrosis. They phagocytose the cellular debris and pathogens during an early inflammatory response and promote the inflammation through the production of pro-inflammatory mediators. Persistent MΦ1 activation might result in prolonged chronic inflammation and tissue injury. Through their production of MMPs and by stimulating MMP production in other cells, MΦ1 also promote ECM degradation and contribute to the disruption of tissue architecture (Ge et al., 2024; Murray & Wynn, 2011). Studies have demonstrated predominance of MΦ1 among cells infiltrating the kidneys of patients with diabetic kidney disease and in mouse kidneys with diabetic nephropathy (Ma et al., 2022), as well as elevated levels of MΦ1-

related cytokines in bronchoalveolar lavage fluid collected from patients with idiopathic pulmonary fibrosis (IPF) (Schupp et al., 2015).

As an anti-inflammatory subpopulation, MΦ2 contribute to the development of tissue fibrosis by suppressing the inflammatory response and encouraging tissue repair. Especially MΦ2a have been shown to promote the anti-inflammatory response and orchestrate the repair process, leading to the remodeling of injured tissue. However, their sustained activation has been correlated with fibrosis development through the secretion of pro-fibrotic factors and stimulation of ECM production by activated fibroblasts. In this instance, MΦ2 have been shown to induce EMT of lung alveolar epithelial cells through TGF-β/Smad2 signaling pathway (Zhu et al., 2017). Similarly to MΦ1, by the regulation of MMPs secretion and TIMPs activity regulation, MΦ2 also contributes to the dysregulated process of ECM production and degradation (Ge et al., 2024; Murray & Wynn, 2011).

As MΦ subpopulations may have seemingly contradictory functions, from promoting inflammation and tissue damage to alleviating inflammation and facilitating tissue repair, it is crucial to unravel their dynamic role in the pathogenesis of fibrosis in the course of endometriosis. Thus, it is necessary to accurately identify changes in the distribution and alteration in MΦ subpopulations in the mare endometrium, as well as to recognize the signaling pathways and molecular mechanisms that regulate their intercellular communication with other cell types, such as fibroblasts, but also epithelial, endothelial, and immune cells. Comprehensive knowledge of these changes and interactions can not only contribute to elucidating the complex effects of MΦ subpopulations on the progression of fibrosis but will also provide a basis for developing targeted therapeutic strategies aimed at modulating MΦ function.

2. Research hypotheses

The **primary hypothesis** of the research assumes that variations in the distribution of distinct MΦ subpopulations enhance the development of fibrosis in the progression of equine endometriosis by inducing changes in the expression of ECM-associated components, promoting myofibroblast differentiation, modulating activation of molecular pathways, and altering fibroblasts' functional characteristics (Fig. 3).

Detailed hypotheses were formulated as follows:

Hypothesis I. In the course of endometriosis, the number of MΦ in the equine endometrium is increased with the predominance of proinflammatory MΦ1 (CD68⁺iNOS⁺ARG1⁻ cells) over anti-inflammatory MΦ2 (CD68⁺iNOS⁻ARG1⁺ cells).

Hypothesis II. Equine *in vitro*-generated monocyte-derived (eqMD)-MΦ^{IFN γ /LPS} (akin to MΦ1) and eqMD-MΦ^{IL-4/IL-13} (akin to MΦ2a) show characteristic differences indicating their respective pro-inflammatory and anti-inflammatory roles, which translates into their opposite effect on endometrial fibroblasts and endometrium.

Hypothesis III. As a pro-inflammatory subpopulation, eqMD-MΦ^{IFN γ /LPS} (akin to MΦ1) intensify the inflammation and tissue damage, and promote the development of fibrosis in the equine endometrium.

Hypothesis IV. As an anti-inflammatory subpopulation, eqMD-MΦ^{IL-4/IL-13} (akin to MΦ2a) influence processes and pathways involved in collagen turnover and ECM remodeling.

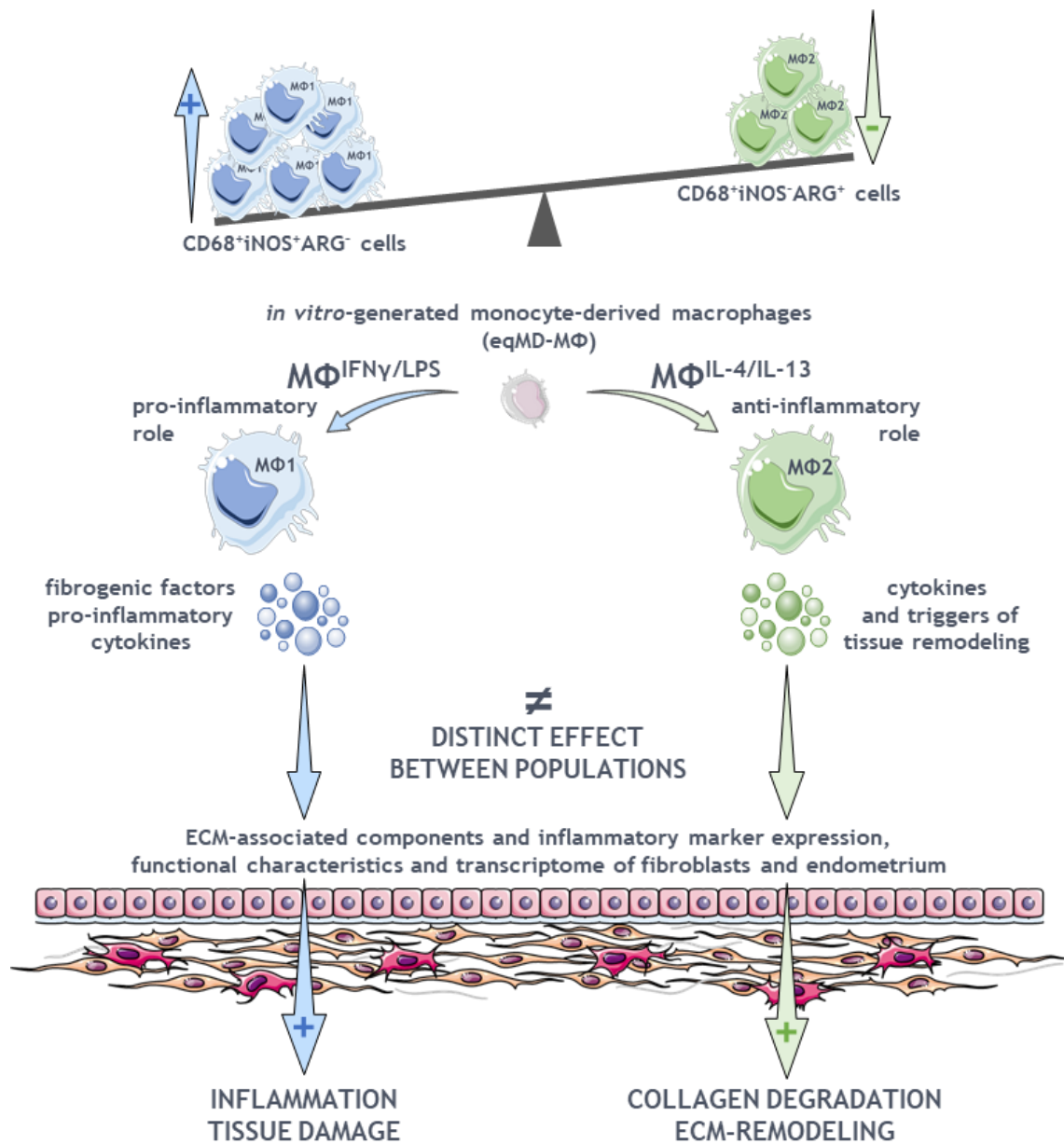


Figure 3. Graphical summary of research hypotheses.

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3. Research objectives

The **main objective** of this dissertation was to determine the role of distinct subpopulations of MΦ in the development of endometrial fibrosis in the course of endometrosis in mares.

The **specific objectives** of the dissertation concern:

1. Analyzing the expression of CD68, the ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells (akin to MΦ1/MΦ2), as well as the immunolocalization of CD68⁺iNOS⁺ and CD68⁺ARG1⁺ cells in endometria classified to category I, IIA, IIB, and III according to Kenney and Doig (1986) in the mid-luteal and follicular phases of the estrous cycle.
2. Acquisition of eqMD-MΦ subpopulations and characterization of their molecular profiles.
3. Identifying the impact of eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} secretomes on the ECM-associated component and inflammatory marker expression, myofibroblasts differentiation, and functional characteristics of *in vitro* cultured fibroblasts derived from category I endometria.
4. Investigating the effect of eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} secretomes on prospective canonical and signaling pathways, identifying their potential upstream regulators, as well as a functional annotation to biological and molecular processes through transcriptomic and bioinformatic analysis in *in vitro* cultured fibroblasts derived from category I endometria.
5. Investigating the effect of intrauterine administration of autologous eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} on ECM-associated component and inflammatory marker expression, and prospective canonical and signaling pathways, identifying their potential upstream regulators, as well as functional annotation to biological and molecular processes through transcriptomic and bioinformatic analysis in category I endometria.

4. Experimental design

In order to validate adopted research hypotheses and achieve the established research objectives, the study presented in the following dissertation was structured into three main Research Tasks, which were further subdivided into individual experiments:

Research Task 1: Determination of the distribution of different types of tissue-resident MΦ in the endometrium of mares with endometrosis

- Experiment 1. The expression of CD68 in the endometrium of mares with endometrosis
- Experiment 2. The ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells in the endometrium of mares with endometrosis
- Experiment 3. The immunolocalization of CD68⁺iNOS⁺ and CD68⁺ARG1⁺ cells in the endometrium of mares with endometrosis

The graphical summary of experiments carried out in the Research Task 1 is presented in Figure 4.

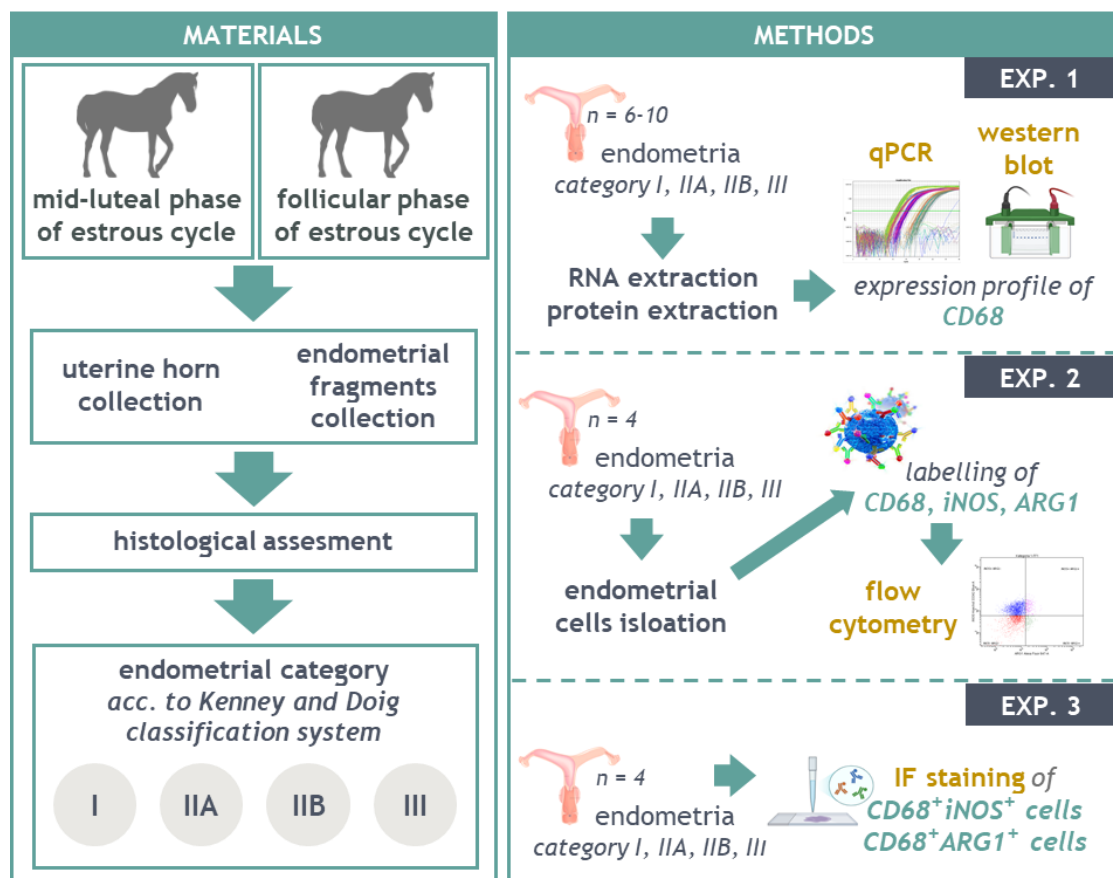


Figure 4. Graphical summary of experiments carried out in the Research Task 1.

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Research Task 2: Investigation of the effect of the secretome of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the mare endometrial fibroblasts

- Experiment 1. The characterization of the molecular profile of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}
- Experiment 2. The effect of the eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome on the ECM-associated components and inflammatory marker expression in endometrial fibroblasts
- Experiment 3. The effect of the eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome on the transcriptome of endometrial fibroblasts
- Experiment 4. The effect of the eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome on the functional characteristics of endometrial fibroblasts

The graphical summary of experiments carried out in the Research Task 2 is presented in Figure 5.

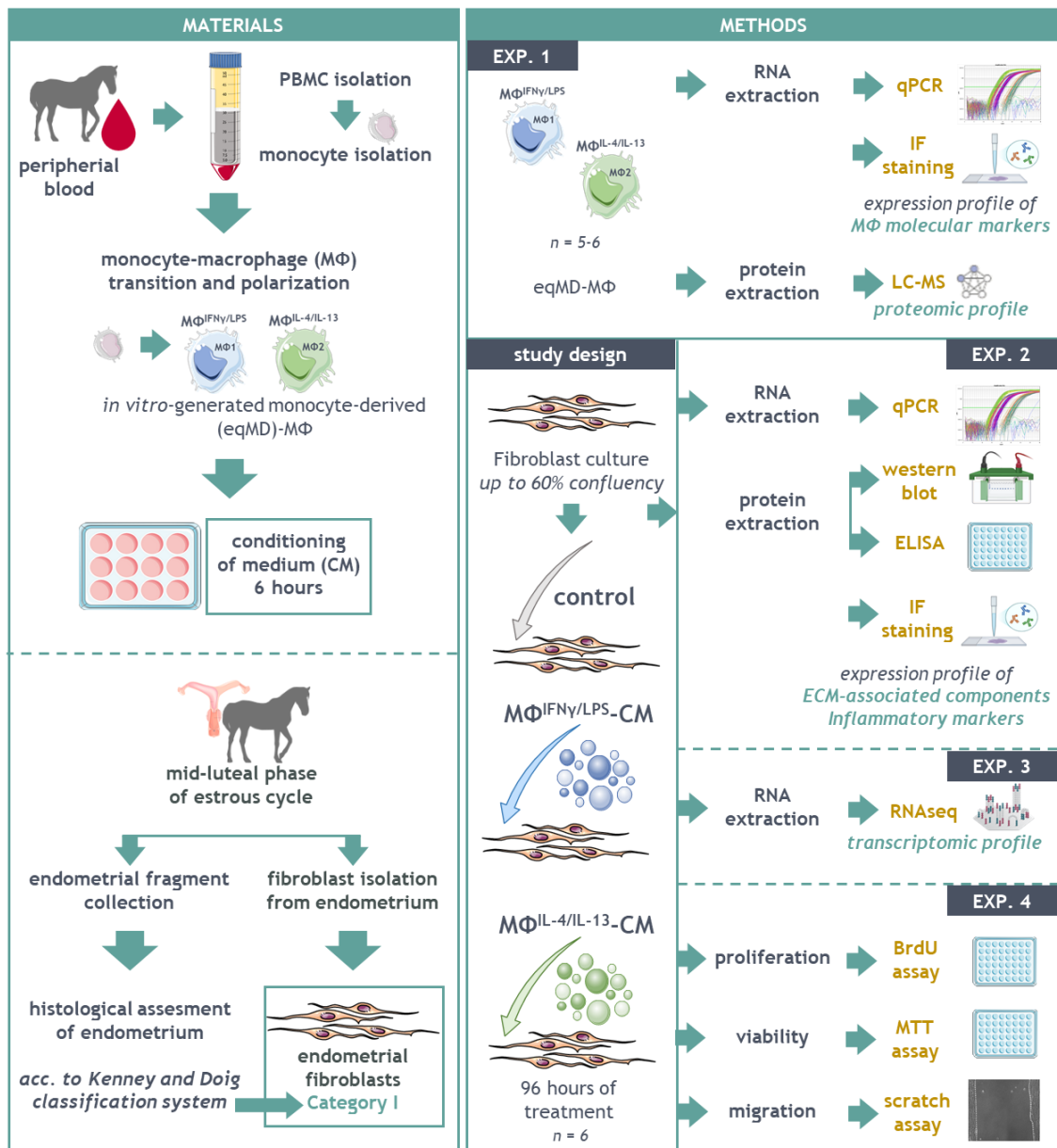


Figure 5. Graphical summary of experiments carried out in the Research Task 2. The figure was prepared with the use of pictures from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License.

Research Task 3: Assessment of the effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the mare endometrium

- Experiment 1. The effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the mRNA transcription of ECM-associated components in mare endometrium
- Experiment 2. The effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the mRNA transcription of inflammatory markers in mare endometrium
- Experiment 3. The effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the transcriptome of mare endometrium

The graphical summary of experiments carried out in the Research Task 3 is presented in Figure 6.

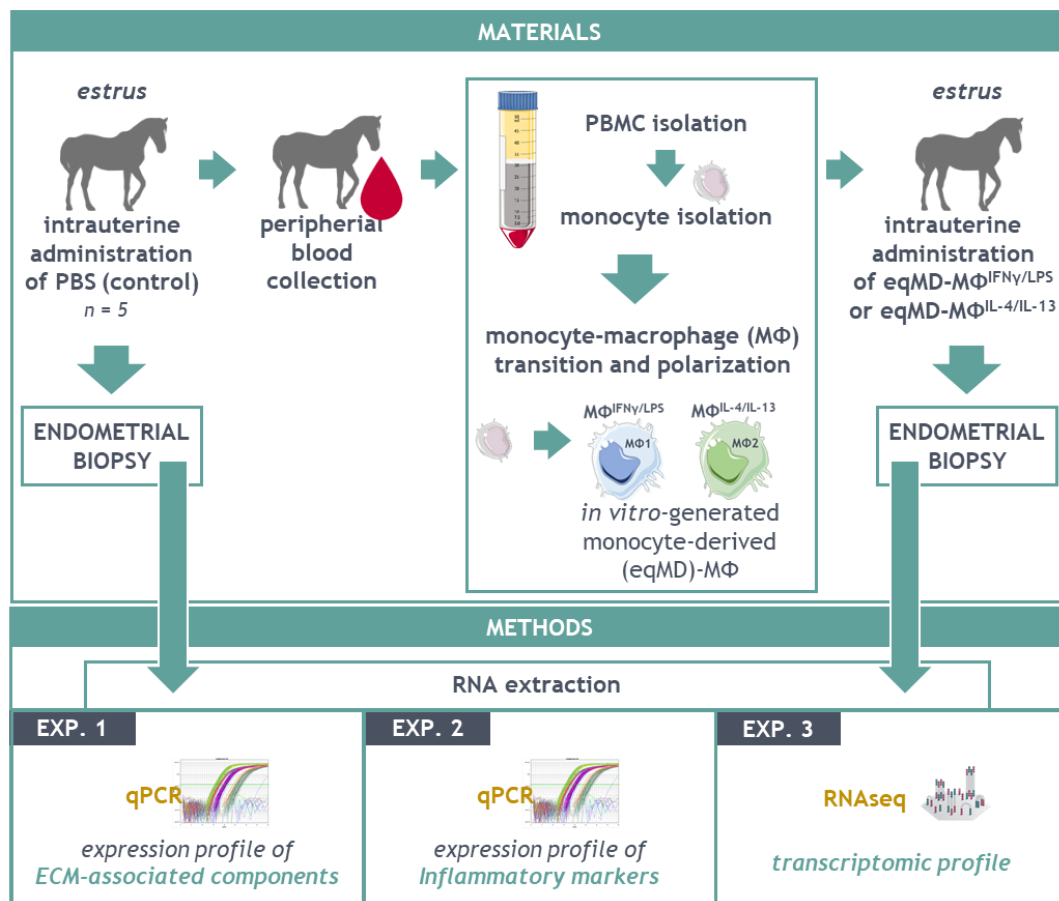


Figure 6. Graphical summary of experiments carried out in the Research Task 3.

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5. Materials and Methods

5.1. Research Material

5.1.1. Ethical statement

All animal procedures were conducted in accordance with the European Union Directive of the European Parliament and of the Council on the protection of animals used for scientific purposes (22 September 2010; no 2010/63/EU), and the Polish Parliament Act on Animal Protection (21 August 1997, Journal of Laws 1997 No 111 item 724) with subsequent updates – the Polish Parliament Act on the protection of animals used for scientific or educational purposes (15 January 2015, Journal of Laws 2015 item 266). All animal procedures were designed to avoid or minimize discomfort, distress, and pain to the animals. Protocols for *in vivo* study and blood collection were reviewed and approved by the Local Ethics Committee for Experiments on Animals in Olsztyn, Poland (Approval No. 27/2022). In addition, animals in a slaughterhouse were handled and humanely euthanized in compliance with European legislation (European Food Safety Authority, AHAW/04-027). Prior to the slaughter, animals were declared clinically healthy by official veterinary inspection. The collection of biological material from slaughtered mares was performed as part of routine meat production practices and, therefore, did not require additional ethical approval.

5.1.2. Animals used for endometrial tissue and blood samples collection

For experiments in Research Tasks 1 and 2, uteri were obtained *post-mortem* from various cyclic cold-blooded mares (weight 600 ± 100 kg, age 2-20 years) at a local abattoir (Rawicz, Poland) during three equine reproductive seasons from April to July 2022-2024. At that time, 109 uteri at the mid-luteal phase and 96 uteri at the follicular phase of the estrous cycle were collected from mares that were slaughtered. The phases of the estrous cycle were identified based on macroscopic observation of ovaries. The mid-luteal phase of the estrous cycle was characterized by the presence of active corpus luteum (CL) and ovarian follicles measuring approximately 15-20 mm in diameter. The follicular phase of the estrous cycle was characterized by the absence of active CL and the presence of follicles of various sizes, but always >35 mm in diameter. During material collection, two sections of endometrium were excised from the tubal horn of uteri, always from the side of an active ovary, and placed in 4% formalin (cat. no. 424321733, Chempur) for further immunofluorescence staining as well as hematoxylin-eosin staining

which is required for retrospective division of endometrium into four categories (category I, IIA, IIB, III) according to Kenney and Doig classification (Kenney & Doig, 1986). Moreover, pieces of endometrium were rapidly frozen in liquid nitrogen for RNA and protein extraction. Horns of uteri collected for isolation of endometrial cells were kept in cold sterile Roswell Park Memorial Institute (RPMI)-1640 medium (cat. no. R8755; Sigma-Aldrich) with gentamicin solution (cat. no. G1272; Sigma-Aldrich) and transported to the laboratory on ice.

For *in vitro* differentiation of MΦ in Research task 2, to avoid the impact of steroid hormone fluctuations, blood (60 ml) was collected in 2023 from healthy non-cycling and non-pregnant mares (n=6), which was confirmed by veterinarian after ultrasonography genital tract examination. Mares were housed in a private facility “Stajnia pod Klonem” located in Szymbark, Poland. Drawn blood was mixed with an anticoagulant consisting of 0.38 M ethylenediaminetetraacetic acid (EDTA; cat. no. 879810112; POCH) and 55 mM acetylsalicylic acid (cat. no. 2091299; Polpharma) and transported to the laboratory in a cool environment.

5.1.3. Experimental animals in *in vivo* study

Ten clinically healthy, non-pregnant, and normally cycling cold-blooded mares (aged 2-5 years, weighing 600 ± 100 kg) were utilized in Research Task 3. The study was conducted during the reproductive season between April and June in 2023 in Poland. Mares were housed in a private facility “Stajnia pod Klonem” located in Szymbark, Poland. Mares were provided with *ad libitum* access to water and were fed a diet comprising hay and cereal grain. Prior to study initiation, each mare underwent a veterinary physical examination to confirm overall health. Moreover, prior to the study, biopsies of the endometrium were taken in order to determine the condition of the uterus. Only mares with endometrium classified to category I of Kenney and Doig classification (Kenney & Doig, 1986) were directed for procedures. A multi-step protocol was implemented to synchronize the estrous cycles of the mares. Three weeks before the start of the experiments, each mare received an intramuscular injection of 40 µg of a synthetic gonadotropin-releasing hormone (GnRH) analog, buserelin (Buserelin aniMedica; AniMedica, Warsaw, Poland). Two weeks later, the mares were administered an intramuscular injection of 5 mg of a synthetic prostaglandin (PG)-F_{2α} analog, dinoprost (Dinolytic; Zoetis, Kalamazoo, Michigan, USA). On the day of the initial experimental session, estrus was confirmed by an experienced veterinarian via ultrasound examination,

which showed estrus endometrium and follicles $\geq 35\text{mm}$. In addition to the ultrasonographic evaluation, visible signs of estrus, such as the presence of vaginal mucus and the exhibition of standing behavior, were also assessed. A detailed description of all experimental procedures is provided in chapter 5.5.

5.2. Material handling

5.2.1. Histological assessment of endometrial sections

Fixed in formalin endometrial sections were embedded in paraffin, cut into 5 μm thick slices, and stained with hematoxylin-eosin according to the standard procedure. Then, Kenney and Doig's classification (Kenney & Doig, 1986) was used to classify endometria retrospectively into four categories. This system of classification is based on the visual evaluation of fibrotic, inflammatory, atrophic, and lymphatic abnormalities. Under this classification system, category I comprises the endometria of mares, devoid of pathological changes or gland alterations. Further, category IIA includes mares with endometria where observed are: the slight amount of stromal fibrosis surrounding the individual gland branches, mild to moderate inflammatory alterations, and the absence of glandular nests in four adjacent fields. In this category, it is generally recognized that 10-35% of glands are pathologically altered. Meanwhile, in mares with endometria classified to category IIB alterations appear in 35-60% of glands, and up to four fibrotic glandular nests with two to four layers are visible in four adjacent fields. Observed fibrotic alterations get more extensive and severe, as well as lymphatic and inflammatory alterations get more widespread and moderately severe. Mares whose endometria exhibit the most advanced pathological changes are classified into category III. It is characterized by alterations in above 60% of endometrial glands which form five or more nests in four adjacent fields while fibrotic and inflammatory alterations are widespread and severe (Schlafer, 2007; Snider et al., 2011).

5.2.2. Endometrial cell isolation

Endometrial cells, including fibroblasts and M Φ , were isolated simultaneously from mare endometrium using a previously described protocol (Szóstek-Mioduchowska et al., 2023) with minor modifications. Specifically, fibroblasts (Research Task 2) were obtained using this protocol, while M Φ (CD68⁺iNOS⁺ARG1⁻ and CD68⁺iNOS⁻ARG1⁺ cells; Research Task 1) were isolated in parallel using a protocol for tissue-resident M Φ

isolation (Dick et al., 2022). The graphical summary of the performed steps is presented in Figure 7.

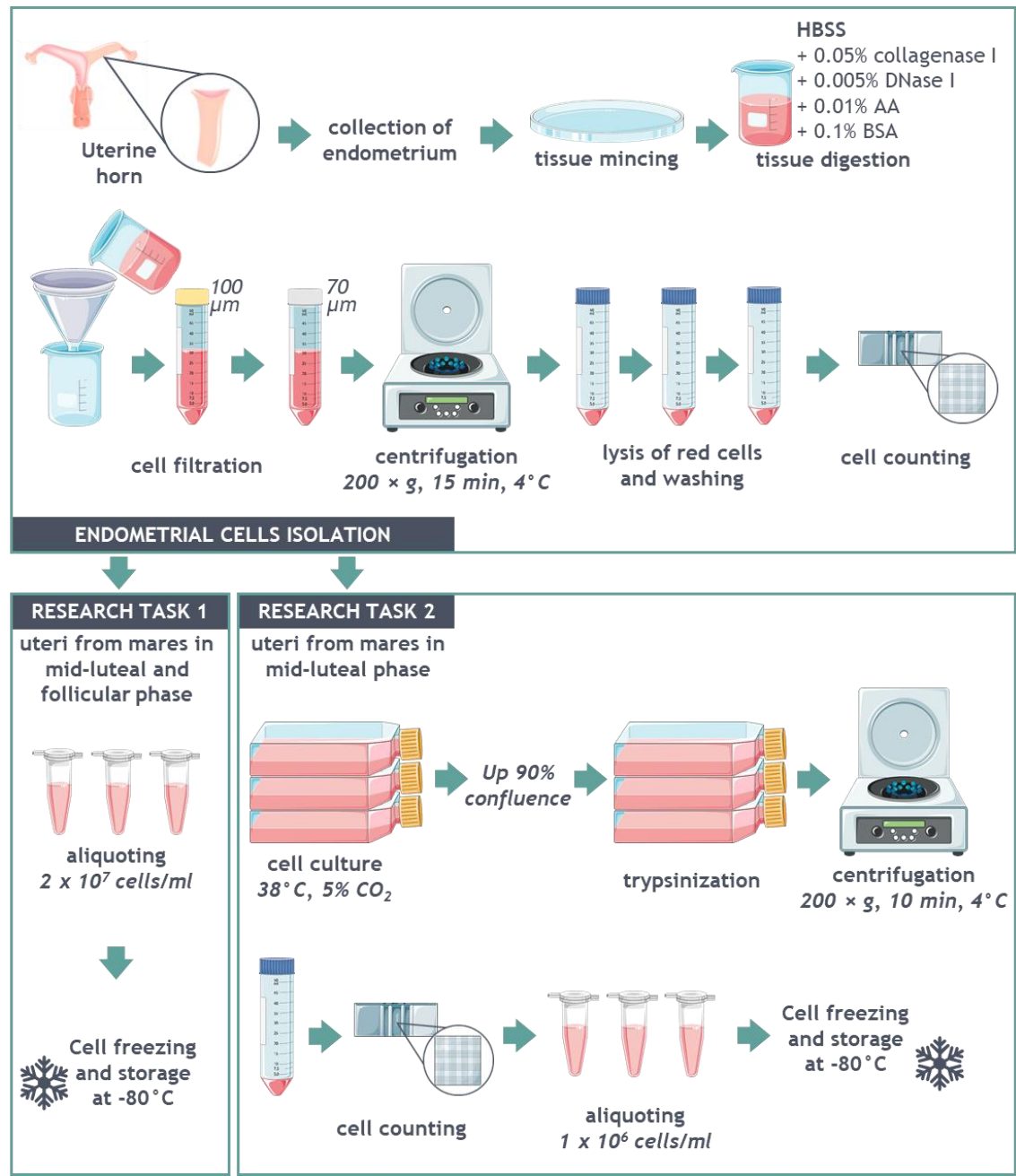


Figure 7. Graphical summary of mare endometrial cell isolation and preparation. The figure was prepared with the use of pictures from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License.

Macrophages were isolated from the endometrial tissue of mares in the mid-luteal and follicular phases of the estrous cycle. Around 10 ml of saline was used to wash the uterine lumen. To expose the endometrial surface, a uterine horn was cut open using scissors and the epithelial cell layer was scrapped off with a scalpel blade. Then, endometrial stripes

were excised from the myometrium either using a scalpel or scissors and minced thoroughly. The minced tissues were digested in 100 ml of sterile Hanks' Balanced Salts Solution (HBSS; cat. no. H1387, Sigma-Aldrich) containing 0.05% (w/v) collagenase I (cat. no. C2674, Sigma-Aldrich), 0.005% (w/v) DNase I (cat. no. 11284932001, Roche, Switzerland), 0.01% antibiotic antimycotic solution (AA) containing penicillin, streptomycin, amphotericin (cat. no. A5955, Sigma-Aldrich) and, 0.1% (w/v) bovine serum albumin (BSA; cat. no. A9418, Sigma-Aldrich) for 45 minutes in 38°C with constant stirring. The cell suspension was then sequentially filtered through nonwoven fabric, 100 µm, and 70 µm strainers to remove any undigested tissue fragments or clumped cells. The filtrate was centrifuged at $200 \times g$ for 15 minutes in 4°C. The obtained pellet was then gently mixed with 1 ml of Red Blood Cell Lysing Buffer Hybri-Max™ (cat. no. R7757, Sigma-Aldrich) and washed three times by centrifugation in HBSS supplemented with AA and 0.1% (w/v) BSA at $200 \times g$ for 15 minutes in 4°C. The obtained endometrial cells pellet was resuspended in fetal bovine serum (FBS; cat. no. 10270106, Gibco™) and counted using a hemocytometer. The viability of endometrial cells was higher than 95% as assessed by the trypan blue exclusion test, using 0.4% Trypan Blue Solution (cat. no. 1520061, Gibco™). For analysis of the ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells in the endometrium of mares using flow cytometry in Research Task 1, the cell suspension was then transferred into cell freezing medium (consisting of 10% DMSO [cat. no. D2650, Sigma-Aldrich] in FBS) at a concentration of 2×10^7 cells per ml, frozen with caution and stored at -80°C to perform analysis after the retrospective evaluation of endometrial categories.

For cell-based analyses in Research Task 2, the fibroblasts were isolated from the endometrium of mares in the mid-luteal phase of the estrous cycle. The mid-luteal phase was selected for experiments based on flow cytometry results obtained in Research Task 1 and presented in chapter 6.1.2. For isolation of mare endometrial fibroblasts, pellet of endometrial cells was suspended in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham medium (DMEM/F-12; cat. no. D8900, Sigma-Aldrich) containing 20% FBS and seeded at a density of 5×10^5 viable cells/ml and cultured at 38.0°C in a humidified atmosphere of 5% CO₂ in the air. To purify the fibroblast population, the medium was changed with fresh DMEM/F-12 containing 10% FBS, 18 hours after plating, by which point fibroblasts had selectively attached. Cells were further cultured until 90% confluence was achieved. Then, the cells were cryopreserved following the protocol previously described by (Szóstek et al., 2012b). Precisely, the cells were first

washed with sterile phosphate-buffered saline (PBS; cat. no. P4417, Sigma-Aldrich) and treated for 3 to 5 minutes with 0.025% trypsin-EDTA solution (cat. no. T4049, Sigma-Aldrich) to detach the cells from the cell culture dish. To inhibit trypsin activity and protect the cells, DMEM/F-12 containing 10% FBS was added after detachment. Subsequently, cells were centrifuged at $200 \times g$ for 10 minutes at 4°C, counted using a hemocytometer, and transferred into a cell freezing medium (consisting of 16% DMSO in FBS) at a concentration of around 1×10^6 cells per ml, frozen with caution and stored at -80°C.

When needed for analyses, cells were thawed at 38°C for 2 minutes and suspended in FBM™ Basal Medium (cat. no. CC-3131, LONZA) supplemented with 100 ng/ml ascorbic acid (cat. no. A4403; Sigma-Aldrich) and FGM™-2 SingleQuots™ supplements (cat. no. CC-4126, LONZA) which includes 2% FBS, insulin, human fibroblastic growth factor (hFGF)-B, gentamycin-amphotericin (GA), and centrifuged at $200 \times g$ for 10 minutes. The obtained pellet was then resuspended in FBM™ Basal Medium with additives and counted using a hemocytometer. The viability of thawed fibroblasts was higher than 90% as assessed by the trypan blue exclusion test. Then, the appropriate amount of FBM™ Basal Medium with additives was added to the cells to obtain a concentration of 5×10^5 viable cells/ml, and fibroblasts were seeded onto T175 cell culture bottles (around 35 ml of cell suspension per bottle). Cells were cultured under normal growth conditions at 38°C in a humidified atmosphere of 5% CO₂ in the air, until reaching 90% of confluence. To ensure sufficient access to nutritional compounds medium was changed every 48 hours. Fibroblasts that had reached 90% confluence were detached using trypsin-EDTA solution as described above and passaged at the appropriate concentrations for the specific experiments. The exact concentration and conditions for subsequent experiments are described in specific studies of Research Task 2.

5.2.3. Acquisition of *in vitro* model of equine monocyte-derived MΦ

In vitro generation of equine monocyte-derived MΦ consisted of three consecutive steps: peripheral blood mononuclear cells (PBMCs) isolation, monocyte isolation, and MΦ differentiation and polarization. The graphical summary of the performed steps is presented in Figure 8.

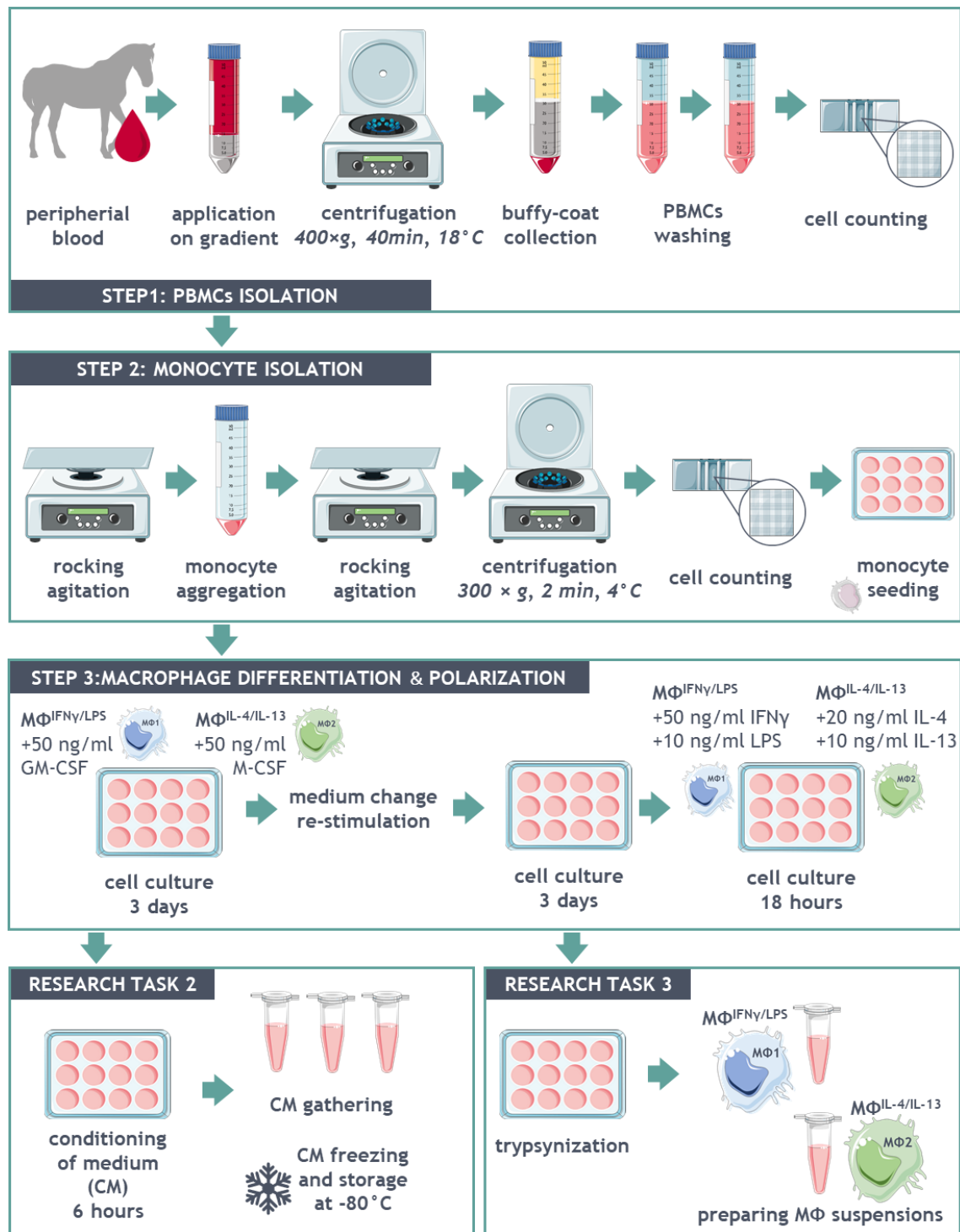


Figure 8. Graphical summary of peripheral blood mononuclear cells (PBMCs) isolation, monocyte isolation, macrophage differentiation and polarization, and preparation for experiments. The figure was prepared with the use of pictures from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License.

Peripheral blood mononuclear cells (n=6) were isolated by density-gradient centrifugation using Ficoll-Paque Plus (cat. no. 17-1440-03, Cytiva) according to the manufacturer's protocol and according to previously described protocol (Mily et al., 2020). Precisely, blood with a coagulant, obtained as described in chapter 5.1.2 was

diluted at a 1:1 ratio with HBSS containing 0.1% BSA. Then, 30 ml of the mixture was gently layered on 20 ml of Ficoll-Paque Plus in sterile 50 ml centrifuge tubes and centrifuged at $400 \times g$ for 40 minutes at 18°C with minimal acceleration and deceleration. Received buffy coats were collected using sterile Pasteur pipettes, transferred to a new sterile 50 ml centrifuge tube, and washed twice with HBSS containing 0.1% BSA at $500 \times g$ for 10 minutes at 18°C . Then, cells were counted using Countess™ 3 FL Automated Cell Counter and suspended at a concentration of 5×10^7 cells/ml in X-VIVO™10 (cat. no. BE04-380Q, LONZA) supplemented with 10% FBS and transferred to new sterile 15 ml centrifuge tubes. The viability of PBMCs was around 99% as assessed by the trypan blue exclusion test using Trypan Blue Solution. For subsequent monocyte isolation, two cycles of cold aggregation were performed according to a method previously published by Santos et al. (2001). Cold aggregation is a method of monocyte purification that takes advantage of the fact that monocytes *in vitro* spontaneously and rapidly aggregate at 4°C (Mentzer et al., 1986). The prepared PBMCs suspension was incubated at 4°C with constant rocking agitation for 30 minutes. Then, cells were placed vertically on ice for around 5 minutes which allowed for spontaneous sedimentation of monocytes. The obtained pellet was resuspended in the same volume of X-VIVO™10 supplemented with 10% FBS and subjected to another cycle of cold aggregation. After the second sedimentation cells were centrifuged at $300 \times g$ for 2 minutes at 4°C and directed for differentiation and polarization of eqMD-M Φ .

Differentiation and polarization of eqMD-M Φ were conducted according to the method previously described by Mily et al. (2020). The pellet of monocytes obtained by the cold aggregation purification method was resuspended in fresh X-VIVO™10 without additives and seeded on 12-well plates at 3.5×10^5 cells/cm². After 2 hours, cells were washed three times, each time with 500 μl PBS and cultured in 1 ml of X-VIVO™10 supplemented with 10% FBS and 50 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF; cat. no. RP0022E-100, Kingfisher Biotech) or 50 ng/ml macrophage colony-stimulating factor (M-CSF; cat. no. 300-25; PeproTech) for polarization toward eqMD-M $\Phi^{\text{IFN}\gamma/\text{LPS}}$ (akin to proinflammatory M Φ 1) and eqMD-M $\Phi^{\text{IL-4/IL-13}}$ (akin to anti-inflammatory M Φ 2a), respectively. After 3 days of cell culture, 500 μl of the medium was removed, the same volume of the fresh medium was added and GM-CSF/M-CSF treatment was repeated. The culture was continued for another 3 days. Then, after a total of 6 days of cell culture, the culture medium was changed to 1 ml of TheraPEAK™ X-VIVO™-10 (cat. no. BP04-743Q, LONZA) supplemented with 10% FBS and 0.01% AA,

and cells were differentiated toward eqMD-M Φ ^{IFN γ /LPS} by treatment with 50 ng/ml IFN γ (cat. no. RP0012E, Kingfisher Biotech), 10 ng/ml LPS (cat. no. LPS25; Sigma-Aldrich) and 10 ng/ml GM-CSF or differentiated toward eqMD-M Φ ^{IL-4/IL-13} by treatment with 20 ng/ml IL-4 (cat. no. RP0003E, Kingfisher Biotech), 10 ng/ml IL-13 (cat. no. RP0102E, Kingfisher Biotech) and 10 ng/ml M-CSF. After 18 hours populations of eqMD-M Φ ^{IFN γ /LPS} or eqMD-M Φ ^{IL-4/IL-13} were obtained.

5.2.4. Utilization of the obtained eqMD-M Φ

For experiments performed in Research Task 2, after 6 days of M Φ differentiation from monocytes and polarization into specific populations, as described in chapter 5.2.3, the cell culture medium was replaced with 1 ml of TheraPEAK™ X-VIVO™-10 supplemented with 0.1% BSA and 0.01% AA. Cells were then incubated for 6 hours to allow conditioning of the media. Conditioning time was determined based on the observation of the cells during the cell culture. The eqMD-M Φ ^{IFN γ /LPS}-conditioned medium (CM) and eqMD-M Φ ^{IL-4/IL-13}-CM were then collected in sterile centrifuge tubes, centrifuged at 300 × g for 5 minutes at room temperature (RT) to remove cellular debris, portioned into volumes suitable for experiments, and stored at -80°C. To obtain the most reliable control group, equal amounts of vehicle (TheraPEAK™ X-VIVO™-10 supplemented with 0.1% BSA and 0.01% AA) were placed at -80°C. Prior to the experiments, eqMD-M Φ ^{IFN γ /LPS}-CM, eqMD-M Φ ^{IL-4/IL-13}-CM, and frozen vehicle were thawed and diluted at a ratio of 1:1, as it was selected in a preliminary study, with a fresh dose of TheraPEAK™ X-VIVO™-10 supplemented with 0.1% BSA and 0.01% AA. Additionally, diluted eqMD-M Φ ^{IFN γ /LPS}-CM, eqMD-M Φ ^{IL-4/IL-13}-CM, and vehicle, before use, were freshly supplemented with 100 ng/ml of ascorbic acid. Given that the treatment time was planned for 96 hours to ensure sufficient access to nutritional compounds, the medium was changed after 48 hours with a fresh portion of eqMD-M Φ ^{IFN γ /LPS}-CM, eqMD-M Φ ^{IL-4/IL-13}-CM, or vehicle.

For procedures performed in Research Task 3, after 6 days of M Φ differentiation from monocytes and polarization into specific populations, as described in chapter 5.2.3, cells were washed with PBS and detached from the cell culture plate using 0.125% trypsin-EDTA solution for 10 minutes. After detachment, TheraPEAK™ X-VIVO™-10 supplemented with 10% FBS was added to inhibit trypsin activity, and the cells were centrifuged at 500 × g for 15 minutes at 4°C and counted using a hemocytometer. For

intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} or eqMD-M Φ ^{IL-4/IL-13}, 2×10^6 cells were suspended in 2 ml of PBS.

5.3. Research Task 1: Determination of the distribution of different types of tissue-resident M Φ in the endometrium of mares with endometrosis

The aim of the Research Task 1 was to analyze the mRNA transcription and protein abundance of CD68, the ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells (akin to proinflammatory M Φ 1/anti-inflammatory M Φ 2a), as well as to characterize the immunolocalization of CD68⁺iNOS⁺ and CD68⁺ARG1⁺ cells in endometria classified to category I, IIA, IIB, and III according to Kenney and Doig (1986) in the mid-luteal and follicular phases of the estrous cycle.

5.3.1. Experiment 1. The expression of CD68 in the endometrium of mares with endometrosis

The research material consisted of endometrial fragments that have been preserved in liquid nitrogen as described in chapter 5.1.2. mRNA transcription of *CD68* in mare endometrium in each category (I, IIA, IIB, and III) in the mid-luteal phase and follicular phase of the estrous cycle (n=10 per category in each phase of the cycle, $\sum n=80$) was analyzed using quantitative Polymerase Chain Reaction (qPCR) as described in chapter 5.6.4. Protein abundance of CD68 in mare endometrium in each category (I, IIA, IIB, and III) in the mid-luteal phase and follicular phase of the estrous cycle (n=6 per category in each phase of the estrous cycle, $\sum n=48$) was analyzed using Western Blot as described in chapter 5.6.8.

5.3.2. Experiment 2. The ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells in the endometrium of mares with endometrosis

The research material consisted of endometrial cells that have been isolated and preserved as described in chapter 5.2.2. The endometrial percentage of CD68⁺iNOS⁺ARG1⁻ and CD68⁺iNOS⁻ARG1⁺ cells in mare endometrium in each category (I, IIA, IIB and III), both in the mid-luteal phase and follicular phase of the estrous cycle (n=4 per category in each phase of the estrous cycle, $\sum n=32$) was analyzed by flow cytometry as described in chapter 5.6.11.

5.3.3. Experiment 3. The immunolocalization of CD68⁺iNOS⁺ and CD68⁺ARG1⁺ cells in the endometrium of mares with endometrosis

The research material consisted of endometrial fragments collected as described in chapter 5.1.2. The endometrial localization of CD68⁺iNOS⁺ and CD68⁺ARG1⁺ cells in mare endometrium in each category (I, IIA, IIB, and III), both in the mid-luteal and follicular phases of the estrous cycle (n=4 per category in each phase of the estrous cycle, Σn=32) was analyzed using immunofluorescence staining as described in chapter 5.6.12.1.

5.4. Research Task 2: Investigation of the effect of the secretome of eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} on the mare endometrial fibroblasts

The aims of the Research Task 2 were to analyze the molecular profile of eqMD-MΦ^{IFN γ /LPS} or eqMD-MΦ^{IL-4/IL-13} as well as to investigate the effect of their secretome on ECM-associated component and proinflammatory cytokine expression. Additionally, the study aimed to assess changes in prospective canonical and signaling pathways, through transcriptomic and bioinformatic analysis, as well as the effect on functional characteristics of mare endometrial fibroblasts in response to treatment with the secretome of eqMD-MΦ^{IFN γ /LPS} or eqMD-MΦ^{IL-4/IL-13}.

5.4.1. Experiment 1. The characterization of the molecular profile of eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13}

The research material consisted of eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} obtained as described in chapter 5.2.3, as well as eqMD-MΦ^{IFN γ /LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM obtained as described in chapter 5.2.4. For mRNA transcription analysis and immunofluorescence staining freshly isolated monocytes were used as a control for MΦ differentiation.

5.4.1.1. Experiment 1.1. The expression of characteristic molecular markers of MΦ in eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13}

For mRNA transcription analysis of markers characteristic for distinct MΦ populations (*CD68*, *NOS2*, *ARG1*, *IL-1 β* , *IL-6*, *IL-12A*, *IL-12B*, *MMP-3*, *MMP-9*, *TGF- β 1*, *TNF- α*) in equine monocytes (n=6), eqMD-MΦ^{IFN γ /LPS} (n=6) and eqMD-MΦ^{IL-4/IL-13} (n=6) using qPCR as described in chapter 5.6.4, cells were disrupted with 1 ml of TRIreagent (cat. no. T9424, Sigma-Aldrich) and stored at -80°C for subsequent RNA extraction as described in chapter 5.6.1.2. To visualize the differences between equine monocytes, eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13}, cells were fixed with 4% paraformaldehyde for immunofluorescence staining as described in chapter 5.6.12.2.

5.4.1.2. Experiment 1.2. The proteomic profile of eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13}

For the analysis of the proteome profiles of eqMD-MΦ^{IFN γ /LPS} (n=5) and eqMD-MΦ^{IL-4/IL-13} (n=5) using LC-MS as described in chapter 5.6.9, eqMD-MΦ were washed three times with sterile-filtered PBS and disrupted with 200 μ l of lysis buffer consisting of 30 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; cat. no. 17-737E, Lonza) pH=8 containing 1% sodium dodecyl sulfate (SDS; cat. no. L3771, Sigma-Aldrich) and SIGMAFAST™ Protease Inhibitor Cocktail Tablets (cat. no. S8830, Sigma-Aldrich) for subsequent protein extraction, as described in chapter 5.6.6.2.

5.4.2. Experiment 2. The effect of the eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} secretome on the ECM-associated components and inflammatory markers expression in endometrial fibroblasts

The research material consisted of endometrial fibroblasts derived from mares (n=6) in the mid-luteal phase of the estrous cycle obtained as described in chapter 5.2.2 as well as MΦ^{IFN γ /LPS}-CM and MΦ^{IL-4/IL-13}-CM obtained and prepared as described in chapter 5.2.4. Upon obtaining 90% confluence, endometrial fibroblasts were passaged and seeded on 12-well culture plates for mRNA transcription analysis, T25 cell culture bottles for protein abundance and enzyme-linked immunosorbent assay (ELISA) analyses, and on Nunc™ Lab-Tek™ Chamber Slide System (cat. no. 177437; Thermo Fisher Scientific) for immunofluorescence staining, at a concentration of 5×10^4 cells/ml in FBM™ Basal Medium supplemented with 100 ng/ml of ascorbic acid and FGM™-2 SingleQuots™ supplements in a volume of 1 ml, 5 ml and 1 ml, respectively. Endometrial fibroblasts

were cultured at 38°C in a humidified atmosphere of 5% CO₂ in the air until they reached the desired 60% confluence. Then, the cells were washed with sterile-filtered PBS, and the culture medium was replaced with eqMD-MΦ^{IFN γ /LPS}-CM, eqMD-MΦ^{IL-4/IL-13}-CM, or vehicle as a control group and cells were cultured further for a total of 96 hours. After treatment, mare endometrial fibroblasts were washed with sterile-filtered PBS.

5.4.2.1. Experiment 2.1. The effect of the eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} secretome on the expression of the ECM-associated components in mare endometrial fibroblasts

For mRNA transcription analysis of ECM-associated components (*ACTA2*, *COL1A1*, *COL3A1*, *FN1*, *MMP-2*, *MMP-3*, *MMP-9*, *TIMP-1*, *TIMP-2*) using qPCR as described in chapter 5.6.4, the cells treated with eqMD-MΦ^{IFN γ /LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM for 96 hours were disrupted with 1 ml of TRIreagent and stored at -80°C for subsequent RNA extraction as described in chapter 5.6.1.2. For analysis of protein abundance of ACTA2, COL1, COL3, FN1, MMP-2, MMP-3, MMP-9, using Western Blot as described in chapter 5.6.8, and concentration of TIMP-1 and TIMP-2, using ELISA as described in chapter 5.6.10, the cells treated with eqMD-MΦ^{IFN γ /LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM for 96 hours were disrupted with 200 μ l radioimmunoprecipitation assay (RIPA) (cat. no. 10010263-1; Cayman) supplemented with SIGMAFAST™ Protease Inhibitor Cocktail Tablets and Phosphatase Inhibitor Mix (cat. no. 39050; Serva) and stored at -80°C for subsequent protein extraction as described in chapter 5.6.6.1. To present the localization of ECM-associated components in response to eqMD-MΦ^{IFN γ /LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM treatment for 96 h, mare endometrial fibroblasts were fixed with 4% paraformaldehyde for immunofluorescence staining as described in chapter 5.6.12.2.

5.4.2.2. Experiment 2.2. The effect of the eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} secretome on the expression of inflammatory markers in mare endometrial fibroblasts

For mRNA transcription analysis of proinflammatory cytokines (*TNF- α* , *IL-6*, *IL-1 β* , *TGF- β 1*, *CCN2*, *AREG*) using qPCR as described in chapter 5.6.4, the cells treated with eqMD-MΦ^{IFN γ /LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM for 96 hours were disrupted with 1ml of TRIreagent and stored at -80°C for subsequent RNA extraction as described in chapter 5.6.1.2.

5.4.3. Experiment 3. The effect of the eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome on the transcriptome of endometrial fibroblasts

The research material consisted of endometrial fibroblasts derived from mares (n=6) in the mid-luteal phase of the estrous cycle obtained as described in chapter 5.2.2 as well as eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM obtained and prepared as described in chapter 5.2.4. Upon obtaining 90% confluence, endometrial fibroblasts were passaged and seeded on 12-well culture plates at a concentration of 5×10^4 cells/ml in a volume of 1 ml of FBM™ Basal Medium supplemented with 100 ng/ml of ascorbic acid and FGM™-2 SingleQuots™ supplements. Endometrial fibroblasts were cultured at 38°C in a humidified atmosphere of 5% CO₂ in the air until they reached the desired 60% confluence. Then, the cells were washed with sterile-filtered PBS, and the culture medium was replaced with eqMD-M Φ ^{IFN γ /LPS}-CM, eqMD-M Φ ^{IL-4/IL-13}-CM, or vehicle as a control group and cells were cultured further for a total of 96 hours. After treatment, mare endometrial fibroblasts were washed with sterile-filtered PBS, disrupted with 1 ml of TRIreagent, and stored at -80°C for subsequent RNA extraction as described in chapter 5.6.1.2. The analysis of the transcriptomic profiles of endometrial fibroblasts after treatment with the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM was performed as described in chapter 5.6.5.

5.4.4. Experiment 4. The effect of the eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome on the functional characteristics of endometrial fibroblasts

The research material consisted of endometrial fibroblasts derived from mares (n=6) in the mid-luteal phase of the estrous cycle obtained as described in chapter 5.2.2 as well as eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM obtained and prepared as described in chapter 5.2.4.

For analysis of proliferation and viability of the cells, upon obtaining 90% confluence, endometrial fibroblasts were passaged and seeded on 96-well plates at a concentration of 5×10^4 cells/ml in a volume of 100 μ l of FBM™ Basal Medium supplemented with 100 ng/ml of ascorbic acid and FGM™-2 SingleQuots™ supplements. Endometrial fibroblasts were cultured at 38°C in a humidified atmosphere of 5% CO₂ in the air until they reached the desired 60% confluence. Then, the cells were washed with sterile-filtered PBS, and the culture medium was replaced with eqMD-M Φ ^{IFN γ /LPS}-CM, eqMD-M Φ ^{IL-4/IL-13}-CM, or vehicle as a control group and cells were cultured further for a total of 96 hours. After treatment, the proliferation of mare endometrial fibroblasts was

assessed using bromodeoxyuridine (BrdU) assay as described in chapter 5.6.13.1, whereas the viability of mare endometrial fibroblasts was assessed using MTT assay as described in chapter 5.6.13.2.

For analysis of migration, upon obtaining 90% confluence, endometrial fibroblasts were passaged and seeded in each well of a 2-well silicone insert with a defined cell-free gap (cat. no. 80209, ibidi), which was placed in a 24-well culture plate prior to seeding, at a concentration of 1.4×10^4 cells/ml in a volume of 70 μ l of FBM™ Basal Medium supplemented with 100 ng/ml of ascorbic acid and FGM™-2 SingleQuots™ supplements. Endometrial fibroblasts were cultured at 38°C in a humidified atmosphere of 5% CO₂ in the air up to the point of full confluency. The analysis of cell migration was assessed as described in chapter 5.6.13.3.

5.5. Research Task 3: Assessment of the effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the mare endometrium

The aim of the research task was to analyze the effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the mRNA transcription of ECM-associated components (*ACTA2*, *COL1A1*, *COL3A1*, *FN1*, *MMP-2*, *MMP-3*, *MMP-9*, *TIMP-1*, *TIMP-2*) and inflammatory markers (*TNF- α* , *IL-6*, *IL-1 β* , *TGF- β 1*, *CCN2*, *AREG*) as well as on the prospective canonical and signaling pathways, their potential upstream regulators, and functional annotation to biological and molecular processes through transcriptomic and bioinformatic analysis in the endometrium of mares.

For the *in vivo* study, 10 mares (n=5 in each group) were selected, cared for, and prepared as described in chapter 5.1.3. A schematic overview of the procedures is shown in Figure 9.

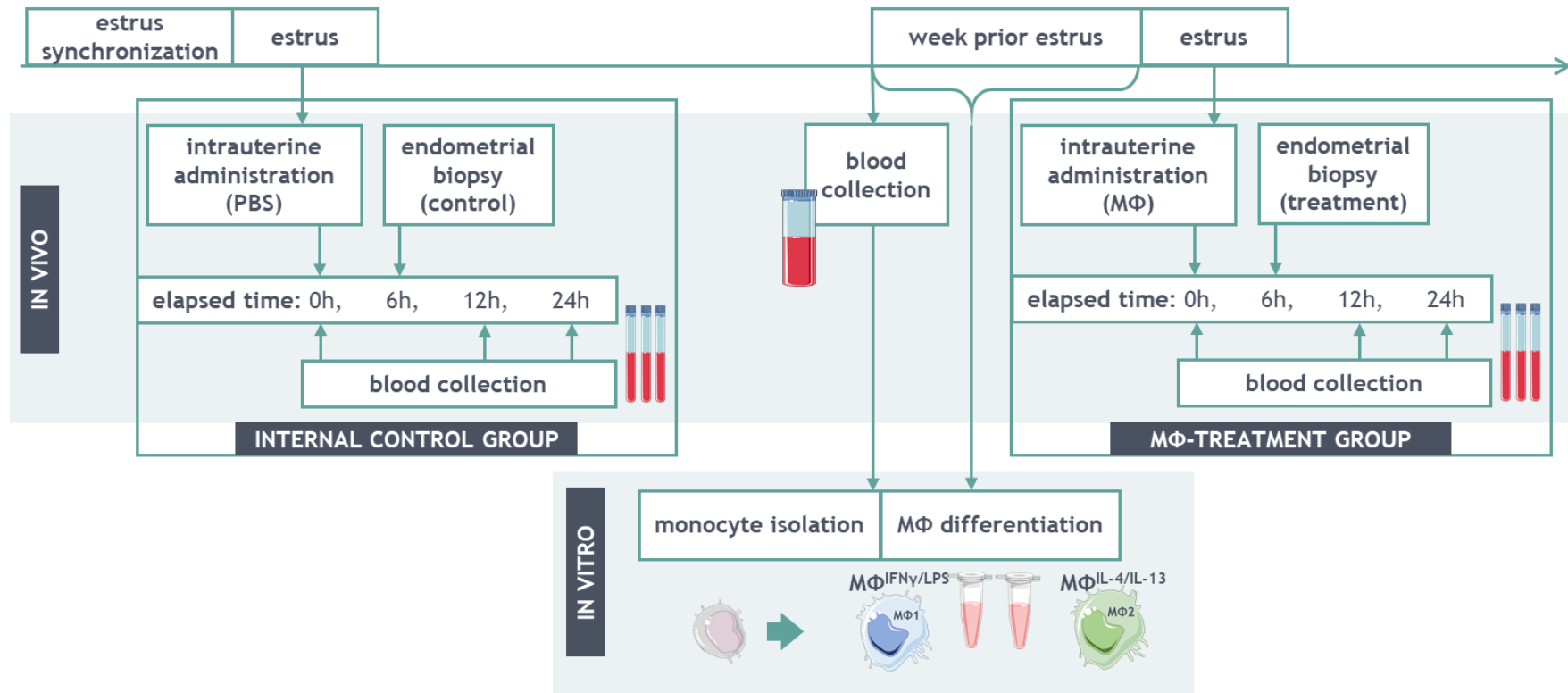


Figure 9. Workflow of *in vivo* experiment in Research Task 3.

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Once the estrus was confirmed and evaluated, each mare was sedated with an intravenous injection of 0.01 mg/kg detomidine hydrochloride (Domosedan; Orion Pharma, Warsaw, Poland). For the analysis of C-reactive protein (CRP) concentration, the sedation of mares was followed by the insertion of a temporary catheter (Intraflon IV cannulae 2.1 × 80 mm 14G, KRUUSE, 121805; KRUSSE Poland) into the jugular vein to facilitate frequent blood sample collection using tubes with clot activator. The catheter was maintained in situ by regular flushing with heparinized saline (10 IU *Heparinum natricum* per 1 ml of saline; Heparinum WZF, Polfa Warszawa S.A.) To establish a reliable control group for the experiment, 4 ml of PBS was administered straight into the uterine lumen of mares using the catheter with the cannula and 5 ml sterile syringe. Prior to administration, the catheter with the cannula was protected by a sanitary sheath providing a sterile environment. The sheath was opened right before the passing through the opening of the cervix. Immediately after administration, 10 ml of blood were drawn for analysis of CRP concentration. Six hours after intrauterine administration of PBS one endometrial biopsy was obtained from the ventral wall at the base of the uterine horn by an experienced veterinarian. Collected biopsies of endometrium were placed in RNAlater (cat. no. R0901; Sigma-Aldrich) and stored at -80°C until RNA extraction was performed as described in chapter 5.6.1.3. Simultaneously, subsequent to the initial procedures, mares were carefully observed, as well as, 12 and 24 hours after intrauterine administration of PBS, blood (10 ml) was draw again for analysis of CRP concentration. Following the initial procedures, a supplementary dose of the synthetic GnRH analog was administered to the mares in order to maintain estrous synchronization.

One week prior to the anticipated subsequent estrus cycle, blood (60 ml) was drawn from synchronized mares and mixed with an anticoagulant consisting of 0.38 M EDTA and 55 mM acetylsalicylic acid and transported to the laboratory in a cool environment for isolation and *in vitro* differentiation of eq-MD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} performed as described in chapter 5.2.3. Following blood collection, the mares received a second dose of the synthetic PGF₂ α analog to further support the synchronization process.

One week later, on the day of the subsequent experimental session, suspensions of autologous eqMD-MΦ^{IFN γ /LPS} or eqMD-MΦ^{IL-4/IL-13} were prepared as described in chapter 5.2.4 and transported to the place of the experiment. Estrus in mares was re-confirmed through both ultrasonographic examination and the assessment of overt estrous behavior. Only mares with confirmed estrus were selected for the continuation of the experimental

procedures. Qualified mares were again sedated with an intravenous injection 0.01 mg/kg of detomidine hydrochloride. Consistent with the initial session, a temporary intravenous catheter was inserted into the jugular vein for the purpose of frequent blood sampling, and the catheter was routinely flushed with heparinized saline to ensure patency. Mares received an intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} or eqMD-M Φ ^{IL-4/IL-13} using the same method as described above. For administration of M Φ , 2ml of autologous eqMD-M Φ ^{IFN γ /LPS} or eqMD-M Φ ^{IL-4/IL-13} suspension was infused into the uterine lumen, followed by 2 ml of PBS, to ensure that all cells were transferred to the uterus and no cellular residue was left in the cannula. Observation of the animal, blood collection, and biopsy collection were performed in the same way as on the day of PBS administration.

5.5.1. Experiment 1. The effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the expression of ECM-associated components in mare endometrium

For mRNA transcription analysis of ECM-associated components (*ACTA2*, *COL1A1*, *COL3A1*, *FN1*, *MMP-2*, *MMP-3*, *MMP-9*, *TIMP-1*, *TIMP-2*) using qPCR as described in chapter 5.6.4, the RNA was extracted from endometrial fragments collected after intrauterine administration of PBS and autologous eqMD-M Φ ^{IFN γ /LPS} or eqMD-M Φ ^{IL-4/IL-13} according to the protocol provided in chapter 5.6.1.3.

5.5.2. Experiment 2. The effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the expression of inflammatory markers in mare endometrium

For mRNA transcription analysis of inflammatory markers (*TNF- α* , *IL-6*, *IL-1 β* , *TGF- β 1*, *CCN2*, *AREG*) using qPCR as described in chapter 5.6.4, in the mare endometrium after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}, the RNA was extracted from endometrial biopsies according to the protocol provided in chapter 5.6.1.3.

5.5.3. Experiment 3. The effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the transcriptome of mare endometrium

For the analysis of the transcriptomic profiles of mare endometrium after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}, performed as described in chapter 5.6.5, the RNA was extracted from endometrial biopsies according to the protocol provided in chapter 5.6.1.3.

5.6. Methods

5.6.1. RNA extraction

5.6.1.1. RNA extraction from endometrial fragments

For mRNA transcription analysis in Research Task 1, total RNA was extracted from endometria classified into categories I, IIA, IIB, and III in the mid-luteal and follicular phases of the estrous cycle with the use of *mirVana*[™] miRNA Isolation Kit (cat. no. AM1560, Invitrogen) according to manufacturer's directions. Precisely, in a mortar that had been refrigerated beforehand, 50 mg of frozen mare's endometrium was ground up with a pestle in the presence of liquid nitrogen to avoid even partial thawing of the tissue. Powdered tissue was transferred into a prechilled 1.5 ml centrifuge tube, 500 µl of Lysis/Binding buffer was added, and such suspension was mixed until all visible clumps were dispersed. Subsequently, 50 µl of miRNA Homogenate Additive was added, mixed thoroughly by vortexing, and kept on ice for 10 minutes. To separate the aqueous and organic phases, 500 µl of Acid-Phenol:Chloroform was added. Samples were then mixed thoroughly by vortexing for a total of 60 seconds and centrifuged at $10.000 \times g$ for 5 minutes at RT. The aqueous (upper) phase was carefully removed without disturbing the lower phase and transferred to a fresh 1.5 ml centrifuge tube. The volume of the collected upper phase was noted, as in the next step 1.25 volume of 100% ethanol (cat. no. 1.08543, Sigma-Aldrich) was added (around 500 µl per sample). Then, the mixture was transferred onto a filter cartridge and centrifuged at $10.000 \times g$ for 15 seconds at RT. The obtained flow-through was discarded and the filter was proceeded for washing. First, 700 µl of Wash Solution 1 was added, samples were centrifuged at $10.000 \times g$ for 10 seconds at RT and flow-through was discarded. Then, the filter was washed twice, each time with the use of 500 µl of Wash Solution 2/3 and centrifugation at $10.000 \times g$ for 10 seconds at RT. In the end, after discarding the final flow-through samples were centrifuged at $10.000 \times g$ for 1 minute at RT to remove any residual fluid from the filter. For the final elution of RNA, filter cartridges were transferred into fresh collection tubes and 100 µl of pre-heated to 95°C nuclease-free water was applied to the center of the filter. Samples were then centrifuged at $10.000 \times g$ for 30 seconds at RT and RNA was recovered.

5.6.1.2. RNA extraction from cells

For mRNA transcription analyses in Research Task 2, total RNA was extracted from eqMD-MΦ^(IFN γ /LPS) and eqMD-MΦ^(IL-4/IL-13) (Research Task 2, Experiment 1) as well as mare endometrial fibroblasts after treatment with eqMD-MΦ^{IFN γ /LPS}-CM,

eqMD-M $\Phi^{IL-4/IL-13}$ -CM, or vehicle for 96 hours (Research Task 2, Experiments 2 and 3) with the use of TRIreagent according to manufacturer's directions. After the cell culture with the appropriate treatments, cells were washed with sterile-filtered PBS, and all wells were drenched with a total of 1 ml of TRIreagent. Precisely, the cell culture was performed in three technical replicates, 333 μ l of TRIreagent was added into each well, and after scraping with 1 ml pipette tip, they were combined in one 1.5 ml centrifuge tube, with a final volume of 1 ml. Before RNA extraction, the collected cells were stored at -80°C. On the day of extraction, samples were removed from -80°C and incubated for 5 minutes at RT. To separate the aqueous and organic phases, 200 μ l of chloroform (cat. no. 234430111; POCH) was added to each tube and mixed thoroughly by pipetting and inverting the centrifuge tubes, and the samples were incubated for 5 minutes at RT. Subsequently, samples were centrifuged at $12.000 \times g$ for 15 minutes at 4°C, and the aqueous (upper) phase was collected and transferred to new 1.5 ml centrifuge tubes. To precipitate RNA, 500 μ l of isopropanol (cat. no. 751500111, POCH) was added to samples, mixed thoroughly by shaking, incubated for 15 minutes on ice, and centrifuged at $12.000 \times g$ for 10 minutes at 4°C. Then, the obtained pellet of RNA was washed twice, each time with the addition of 1 ml of 75% ethanol and centrifugation at $8.000 \times g$ for 6 minutes at 4°C. In the end, the supernatant was discarded, and the obtained pellet of RNA was allowed to dry for 15 to 30 minutes in inverted centrifuge tubes set on paper towels. Thus, recovered RNA was suspended in 30 μ l of nuclease-free water.

5.6.1.3. RNA extraction from endometrial biopsies

For mRNA transcription analyses in Research Task 3, total RNA was extracted from biopsies of mare endometrium after intrauterine administration of autologous eqMD-M $\Phi^{IFN\gamma/LPS}$, eqMD-M $\Phi^{IL-4/IL-13}$ or vehicle with the use of Total RNA Mini (cat. no. 031-100; A&A Biotechnology) according to manufacturer's directions. Precisely, in a mortar that had been refrigerated beforehand, around 50 mg of frozen endometrial biopsy was ground up with a pestle in the presence of liquid nitrogen to avoid even partial thawing of the tissue. Powdered tissue was transferred into a 1.5 ml centrifuge tube with 800 μ l of fenozol, mixed until all visible clumps were dispersed, and incubated for 5 minutes at 50°C. To separate the aqueous and organic phases, 200 μ l of chloroform was added to each tube and mixed gently by inverting the tube, and the samples were incubated for 3 minutes at RT. Subsequently, samples were centrifuged at $13.500 \times g$ for 10 minutes at RT, and the aqueous (upper) phase was collected and transferred to new

1.5 ml centrifuge tubes. Then, 250 μ l of isopropanol was added and thoroughly mixed by shaking. Obtained mixture was applied onto the minicolumn, and centrifuged at $13.500 \times g$ for 1 minute at RT. Minicolumn was transferred to a new 2 ml centrifuge tube and proceeded to washing. Minicolumn was washed twice, each time 700 μ l of Wash Solution A1 was added, samples were centrifuged at $13.500 \times g$ for 1 minute at RT and flow-through was discarded. Then, 200 μ l of Wash Solution A1 was added and samples were centrifuged at $13.500 \times g$ for 2 minutes at RT. After the final wash, minicolumns were transferred to a new 1.5 ml centrifuge tube, and around 30 μ l of ultrapure water was added directly onto the minicolumn resin. Samples were then kept for 3 minutes at RT and centrifuged at $13.500 \times g$ for 1 minute at RT and RNA was recovered.

5.6.2. Quantity and quality analysis of RNA

After each RNA extraction, the quantity and quality of RNA were determined spectrophotometrically with a DeNovix Spectrophotometer. The ratio of absorbance at 260 and 280 nm ($A_{260}/280$) was approximately 1.8 – 2. Additionally, in Research Task 2 and Research Task 3, for samples prepared for Next Generation Sequencing (NGS), RNA integrity number (RIN) was also assessed using Agilent RNA 6000 Nano Kit (cat. no. 5067-1511; Agilent Technologies Inc.) according to manufacturer's direction. After kit arrival, the ladder was heat denatured for 2 minutes at 70°C and immediately cooled down on ice. Then, 1 μ l aliquots of the ladder were prepared and stored at -80°C until the experiments. Prior to experiments, all reagents were equilibrated to RT for 30 minutes. For gel preparation, 550 μ l of Agilent RNA 6000 Nano gel matrix was placed on a spin filter, centrifuged at $1500 \times g$ for 10 minutes at RT, and aliquoted. For Gel-Dye mix preparation, RNA 6000 Nano dye concentrate was vortexed for ten seconds and shortly centrifuged. Then, 1 μ l of RNA 6000 Nano dye concentrate was added to 65 μ l of filtered gel, vortexed thoroughly, and centrifuged at $13.000 \times g$ for 10 minutes at RT. To set up a chip, 9 μ l of prepared gel-day mix was added to a specified well and pressurized using a Chip Priming Station, after which 9 μ l of prepared gel-day mix was added to another two specified wells. Subsequently, 5 μ l of RNA 6000 Nano marker was added into the wells intended for ladder and samples. To minimize the secondary structure, samples were heat denatured for 2 minutes at 70°C, and 1 μ l of the ladder and 1 μ l of each sample was added to the respective wells of a chip. Analysis of a chip was performed using the Agilent 2100 Bioanalyzer and Expert software (Agilent Technologies, Inc.) according to the manufacturer's directions. In Research Task 2, all RNA samples extracted from mare

endometrial fibroblasts after treatment with eqMD-M Φ ^{IFN γ /LPS}-CM, eqMD-M Φ ^{IL-4/IL-13}-CM, or vehicle for 96 hours directed for library preparation had RIN = 10. In Research Task 3, RNA samples extracted from mare endometrium after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS}, eqMD-M Φ ^{IL-4/IL-13}, or vehicle, directed for library preparation had RIN in range from 8.5 to 9.9.

5.6.3. Reverse transcription

Complementary DNA (cDNA) for qPCR analyses was obtained using 1 μ g of total RNA that was extracted as described in chapter 5.6.1. Corresponding to the sample concentration, a volume equivalent to 1 μ g of total RNA was collected and diluted with nuclease-free water to obtain a final volume of 10 μ l. To eliminate DNA from RNA samples, DNase I (cat. no. AMPD-1; Sigma-Aldrich) was used according to the manufacturer's protocol. Precisely, DNase I and Reaction Buffer were mixed in 1:1 proportion and 2 μ l of such mix was added to each RNA sample. Then, samples were incubated for 15 minutes at RT, after which the reaction was inhibited by the addition of 1 μ l of Stop Solution. The process was completed by 10 minutes of incubation at 70°C. After that, samples were put on ice for reverse transcription. To perform the reverse transcription High-Capacity cDNA Reverse Transcription Kit (cat. no. 4368813, Applied Biosystems) was used according to the manufacturer's instructions. First, an adequate volume of 2 $\times$ reverse transcription master mix was prepared, which consisted of 2 μ l of 10 $\times$ RT buffer, 0.8 μ l of 25 $\times$ dNTP mix, 2 μ l of 10 $\times$ RT random primers, 1 μ l of Multiscribe Reverse Transcriptase, 0.2 μ l of nuclease-free water, and 1 μ l of RNase Inhibitor (cat. no. N8080119; Applied Biosystems) for each sample. Then, 7 μ l of the prepared master mix was added to each sample, and reverse transcription was performed in a thermal cycle with predefined steps: step 1 – 10 minutes at 25°C, step 2 – 120 minutes at 37°C and step 3 – 5 minutes at 85°C. Prepared cDNA was stored at -20°C up until qPCR was performed.

5.6.4. qPCR

qPCR analysis was performed with the use of TaqMan Universal PCR Master Mix II (cat. no. 4440049; Applied Biosystems) and Single Tube TaqMan Gene Expression Assays (Life Technologies Thermo Fisher Scientific) according to manufacturer's directions. The qPCR reaction was carried out in a final volume of 10 μ l reaction mix, which consisted of 3 μ l of cDNA (which equaled 10 ng of cDNA), 5 μ l of TaqMan

Universal PCR Master Mix II, 0.5 µl of respective Single Tube TaqMan Gene Expression Assay and 1.5 µl of nuclease-free water. All assays employed for experiments are presented in Table 1. All samples were run in duplicates on 384-well plates. Additionally, instead of template cDNA, nuclease-free water was employed as a negative control. Amplification was performed with 7900HT QPCR System (Agilent Technologies) with settings: 10 minutes at 95°C for initial denaturation and 40 cycles of alternately 15 seconds at 95°C and 60 seconds at 60°C. The data was analyzed using the method described previously (Zhao & Fernald, 2005) (<http://miner.ewindup.cn/miner/>). It is a technique for measuring qPCR results that uses calculations based on the kinetics of individual PCR reactions instead of the standard curve. This method is free from assumptions and subjective evaluations, allowing average efficiency (E) and the cycle number of threshold (Ct) to be directly calculated. The relative concentration of mRNA (R0) was calculated in accordance with the following formula:

$$R0 = \frac{1}{(1 + E)^{Ct}}$$

The relative mRNA transcription was calculated in accordance with the principle:

$$\text{Relative mRNA transcription} = \frac{R0 \text{ of target gene}}{\text{average } R0 \text{ of reference genes}}$$

The choice of the most reliable reference genes for the results' normalization was verified using NormFinder (Andersen et al., 2004). Out of the 7 potential reference genes (*SDHA*, *HPRT1*, *UBE2B*, *B2M*, *RPL32*, *RPS18*, *RPS28*) combination of two with highest combined stability value were chosen – *SDHA:HPRT1* for mRNA transcription analysis in endometrial tissue and mare endometrial fibroblasts (stability value=0.031) and *HPRT1:UBE2B* for mRNA transcription analysis in monocytes and eqMD-MΦ subpopulations (stability value=0.155). Calculated relative mRNA transcription was expressed in arbitrary units.

In eqMD-MΦ subpopulations and mare endometrium (control for method), *ARG1* mRNA transcription was below the level of detection, despite the use of commercially available (Tab.1) or custome-designed probes.

Table 1. The list of TaqMan Gene Expression Assays used for qPCR

gene name	Gene ID	Assay ID
reference genes		
hypoxanthine phosphoribosyl transferase 1	<i>HPRT1</i>	Ec03470217_m1
succinate dehydrogenase complex flavoprotein subunit A	<i>SDHA</i>	Ec03470487_m1
ubiquitin conjugating enzyme E2 B	<i>UBE2B</i>	Ec04660756_m1
ECM-associated components		
actin alpha 2 smooth muscle	<i>ACTA2</i>	Ec07040967_m1
collagen type I alpha 1 chain	<i>COL1A1</i>	Ec03469676_m1
collagen type III alpha 1 chain	<i>COL3A1</i>	Ec03469743_m1
fibronectin 1	<i>FN1</i>	Ec03470760_m1
matrix metalloproteinase 2	<i>MMP2</i>	Ec03469995_m1
matrix metalloproteinase 3	<i>MMP3</i>	Ec03468676_m1
matrix metalloproteinase 9	<i>MMP9</i>	Ec03469193_m1
tissue metalloproteinase inhibitor 1	<i>TIMP1</i>	Ec03468772_m1
tissue metalloproteinase inhibitor 2	<i>TIMP2</i>	Ec03470558_m1
inflammatory markers		
amphiregulin	<i>AREG</i>	Ec06992855_m1
cellular communication network 2	<i>CCN2</i>	Ec06625776_gH
interleukin-1 β	<i>IL-1β</i>	Ec04260296_g1
interleukin-6	<i>IL-6</i>	Ec03468679_m1
transforming growth factor β 1	<i>TGF-β1</i>	Ec03468030_m1
tumor necrosis factor α	<i>TNFα</i>	Ec03467871_m1
MΦ markers		
CD68 molecule	<i>CD68</i>	Ec03468978_m1
nitric oxide synthase 2	<i>NOS2</i>	Ec03467500_m1
arginase 1	<i>ARG1</i>	Ec07052649_m1
interleukin-12A	<i>IL-12A</i>	Ec03468747_m1
interleukin-12B	<i>IL-12B</i>	Ec03468777_m1

5.6.5. Next-generation sequencing

5.6.5.1. Library preparation

The library preparation and NGS were performed by Macrogen Europe, Netherlands. Standard library preparation was performed, which included mRNA selection, fragmentation, cDNA synthesis, end repair, adenylation, indexed adapters ligation, and amplification steps. It was then followed by a qualitative evaluation (Agilent TapeStation 2200) and quantitation (Qubit, Thermo Fisher Scientific). In total, 1000 ng of RNA was used for library construction with the TruSeq mRNA (Illumina, San Diego, CA). The NGS analysis was performed with the use of the NovaSeqX sequencing platform with 150 paired-end sequencing to obtain 40 million total reads per sample.

5.6.5.2. NGS data analysis

Quality assessment of the raw RNA reads was conducted using FastQC to detect adapter contamination and low-quality sequences. Subsequently, Trimmomatic was employed for quality trimming, ensuring that only high-quality reads were retained for downstream analysis. Clean reads were aligned to the *Equus caballus* reference genome (EquCab3.0) using the STAR aligner, with the reference genome and gene annotation files sourced from Ensembl (release 110). STAR produced sorted BAM files, which were then indexed for efficient access during subsequent analysis. Following this, read group information was added to the BAM files to maintain proper sample labeling throughout the pipeline. Gene expression quantification was performed using featureCounts from the Subread package, targeting gene-level annotations. featureCounts was used to map reads to exonic regions, and the resulting gene count matrices provided expression levels for each sample. For differential expression analysis, the gene count data was processed using DESeq2 in the R environment. DESeq2 normalized the data for sequencing depth differences across samples and identified significantly differentially expressed genes (DEGs) between experimental conditions, shedding light on the biological mechanisms underlying the changes in the equine endometrial samples. Differentially expressed genes were identified if adjusted p-value < 0.05 and $|\log_2FC| > 1$, ensuring the inclusion of only the most relevant DEGs.

The characteristics of a given RNA-seq data set among the groups were evaluated using principal component analysis (PCA). To determine the gene network, the obtained data were analyzed using Ingenuity Pathways Analysis (IPA) tools (Ingenuity Systems,

Mountain View, CA), a web-delivered application that enables the identification, visualization, and exploration of molecular interaction networks in gene expression data.

5.6.6. Protein extraction

5.6.6.1. Protein extraction from endometrial fragments and fibroblasts

In Research Task 1, 100 mg of frozen mare's endometrium was ground up with a pestle in a mortar that had been refrigerated beforehand, in the presence of liquid nitrogen to avoid even partial thawing of the tissue. Powdered tissue was transferred into a 1.5 ml centrifuge tube with 400 μ l of supplemented RIPA buffer supplemented with SIGMAFAST™ Protease Inhibitor Cocktail Tablets and Phosphatase Inhibitor Mix. In Research Task 2, after the cell culture with the appropriate treatments, cells were washed with sterile-filtered PBS, 200 μ l of supplemented RIPA buffer was added to T25 cell culture bottles, and cells were scraped on ice using cell scrapers. Cells were collected into a 1.5 ml centrifuge tube, stored at -80°C, and thawed on ice prior to protein extraction. For the extraction procedure, samples were subjected to 5 cycles of sonication using an ultrasonic processor UP50H with settings cycle 0.5 and amplitude 20, while constantly kept on ice followed by 1-hour incubation at 4°C on ice. After that, samples were centrifuged at $12.000 \times g$ for 15 minutes at 4°C and obtained supernatant was collected, aliquoted, and stored at -80°C until analyses.

5.6.6.2. Protein extraction from the eqMD-M Φ

For the analysis of the proteome of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}, proteins were extracted using lysis buffer which consisted of HEPES (pH=8) supplemented with 1% SDS and SIGMAFAST™ Protease Inhibitor Cocktail Tablets. After the appropriate cell culture treatment, cells were washed three times with sterile-filtered PBS and drenched with a total of 200 μ l of lysis buffer. Cells were then scraped using a 1 ml pipette tip and transferred to 1.5 ml centrifuge tubes. Before protein extraction, the collected cells were stored at -80°C. On the day of extraction, samples were thawed on ice. For protein extraction samples were vortexed for 15 minutes, sonicated three times using ultrasonic processor UP50H with settings cycle 0.5 and amplitude 20, while constantly kept on ice, and then boiled at 95°C for 10 minutes. Subsequently, samples were centrifuged at $21.000 \times g$ for 20 minutes at 4°C, and the supernatant was collected, aliquoted, and stored at -80°C until analysis.

5.6.7. Protein concentration analysis

The concentration of extracted proteins was determined using the bicinchoninic acid (BCA) protein assay kit (cat. no. BCA1; Sigma-Aldrich) according to the manufacturer's directions. To perform the assay, 10 µl of protein and 10 µl of the standard curve (range from 125 to 1000 µg/ml of BSA) were placed into wells of 96-well plate. Both, protein samples and standard curve samples were run in duplicates. Then, BCA solution and 4% (w/v) CuSO₄ · 5H₂O Solution were mixed in 49:1 proportion, and 200 µl of such mix was added to each well, followed by 30 minutes of incubation at 37°C. The absorbance was measured using a BioTek Microplate reader at a wavelength of 562 nm, and the concentration of the samples was determined based on the linear trend line of the standard curve.

5.6.8. Western blot

First, 10 µg of extracted proteins (with exception for analysis of protein abundance of MMP-9, where 15 µg of extracted proteins were used) were mixed with 5× SDS gel-loading buffer that contained 0.25 M Tris-HCl (cat. no. T3253, Sigma-Aldrich), 10% SDS, 50% glycerol (cat. no. 44320113, POCH), 0.25% bromophenol blue (cat. no. B-8026, Sigma-Aldrich) and 2% 2-mercaptoethanol (cat. no. M3148, Sigma-Aldrich). Then, for protein denaturation, samples were heated for 5 minutes at 95°C. Before application, samples were shortly centrifuged to remove any residual particles. Prepared samples were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels along with Prestained Plus Protein Ladder (cat. no. 26619, Thermo Fisher Scientific), which was used as a molecular weight indicator. For the first 20 minutes, electrophoresis was carried out at 80V, after which the voltage was increased to 150V and electrophoresis was carried out until the proteins were separated. After electrophoresis, separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes (Immobilon-P Transfer Membrane; cat. no. IPVH00010; Millipore) using a semi-dry transfer method with Trans-Blot Turbo Transfer System (Bio-Rad) with settings: 200 mA and 17 V for 90 minutes. After transfer, to allow specific detection of different molecular weight proteins, the membranes were cut across and the non-specific binding sites were blocked by incubation of the membranes in 5% non-fat dry milk in TBS-T buffer (Tris-buffered saline, containing 0.1% Tween-20 [cat. no. P1379, Sigma-Aldrich]) for 90 minutes at RT. Then, membranes were incubated with primary antibodies overnight at 4°C. For analysis of protein abundance of CD68 in Research Task 1,

membranes were incubated separately with mouse monoclonal anti-CD68 (1:2000; cat. no. 14-0681-82; Invitrogen) and mouse monoclonal anti-GAPDH (internal control; 1:10 000; cat. no. ab8245; Abcam) primary antibodies. For analysis of protein abundance of ECM-associated components in Research Task 2, membranes were incubated separately with rabbit polyclonal anti-collagen type I (1:1000; cat. no. 20121; Novotec), rabbit polyclonal anti-collagen type III (1:750; cat. no. ab7778; Abcam), rabbit polyclonal anti-fibronectin 1 (1:2000; cat. no. ab2413; Abcam), rabbit polyclonal anti-alpha smooth muscle actin (1:2000; cat. no. ab5694; Abcam), rabbit polyclonal anti-metalloproteinase 2 (1:1000; cat. no. ab97779; Abcam), goat polyclonal anti-metalloproteinase 3 (1:1000; cat. no. ab223666; Abcam), rabbit polyclonal anti-metalloproteinase 9 (1:1000; ab73734; Abcam) and mouse monoclonal anti-GAPDH (internal control; 1:10 000; cat. no. ab8245; Abcam) antibodies. Afterward, membranes were washed three times, each time by incubating them in TBS-T for 10 minutes. Taking care to limit access to light, the proteins were detected by incubating the membranes with goat polyclonal anti-rabbit (1:20 000; cat. no. ab205718; Abcam), goat polyclonal anti-mouse (1:7500; cat. no. A90-116P; Bethyl) or goat polyclonal anti-rat (1:5000, cat. no. 112-035-003, Jackson IR) horseradish peroxidase (HRP)-conjugated secondary antibodies for one hour at RT in accordance to the host of primary antibodies. Subsequently, membranes were again washed three times as described above and bands were detected by chemiluminescence using Super Signal West Pico PLUS Chemiluminescent Substrate (cat. no. 34577; ThermoFisher), visualized with ChemiDoc Touch Imaging System (Bio-Rad Laboratories, Inc.) and analyzed by computerized densitometry using Image Lab Software version 6.0 (Bio-Rad Laboratories).

5.6.9. Liquid chromatography-mass spectrometry (LC-MS)

5.6.9.1. Protein digestion

Sample preparation and proteomics analysis were performed at the Mass Spectrometry Laboratory at the Institute of Biochemistry and Biophysics PAS.

Cell lysates of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} obtained as described in chapter 5.6.6.2 were digested with trypsin using a modified SP3 method (Mielecki et al., 2023). Lysates were reduced with 10 mM TCEP for 60 minutes at 60°C. Two types of Sera-MagTM Carboxylate-Modified Magnetic Beads & SpeedBeads (cat. no. 65152105050250 and cat. no. 45152105050250, Cytiva) were combined in equal parts, washed three times with MS-grade water, and resuspended to a working concentration of

10 µg/µl. A volume of 120 µL of the bead mix and 1% formic acid (FA) in acetonitrile (ACN) was added to each sample to reach a final ACN concentration of 85%. After binding, beads were washed with 85% ethanol, reconstituted in 100 mM triethylammonium bicarbonate (TEAB) with 50 mM methyl methanethiosulfonate (MMTS), incubated for 10 minutes at RT, then washed twice with ethanol and once with ACN. Proteins were digested with 3 µg of trypsin in 100 mM TEAB at 37°C for 16 hours. Peptides were collected, and the bead washes – 40 µl of 1% DMSO in water, and 60 µl of water, were pooled. Peptide concentrations were determined using the Pierce Quantitative Colorimetric Peptide Assay. For tandem mass tag (TMT) labeling, 25 µg of peptides were diluted in 100 µL TEAB and labeled with TMT 10plex (Thermo Fisher Scientific) in acetonitrile for 1 hour. Labeling was quenched with hydroxylamine, and peptides were combined and desalted using Oasis HLB columns (Waters). Desalting involved preconditioning columns with methanol, washing with 1.5% ACN/0.1% FA, loading samples, and eluting peptides with 90% ACN/0.1% FA. Eluates were pooled, dried in a SpeedVac, and resuspended in 10 mM ammonium hydroxide for high-pH fractionation.

5.6.9.2. Reversed-phase peptide fractionation at high pH (HpRP) fractionation

The mixed and labeled peptides were fractionated using high-pH reverse-phase chromatography on an XBridge Peptide BEH C18 column (4.6 × 250 mm, 130 Å, 5 µm, Waters). The separation was performed on a Waters Acquity UPLC H-class system at a flow rate of 0.8 ml/min, with fractions collected every minute. The mobile phase system consisted of water (A), acetonitrile (B), and 100 mM ammonium hydroxide (C), with solvent C kept constant at 10% throughout the run. Peptide elution was monitored by measuring absorbance at 214 nm. The gradient was applied over 27 minutes, and fraction collection began at the third minute. The following elution program was used: 3% to 5% solvent B in the first 0.5 minutes, followed by an increase from 5% to 22% over 10.5 minutes, then 22% to 28% over 7 minutes, 28% to 50% over 2.5 minutes, and 50% to 90% in 0.5 minutes. This was followed by an isocratic hold at 90% for 2.5 minutes, a decrease from 90% to 5% over 0.5 minutes, then from 5% to 3% over another 0.5 minutes, with the column re-equilibrated at 3% solvent B for 2.5 minutes. A total of 25 fractions were collected during the process. Every 12th fraction was concatenated, with fractions 1, 13, and 25 pooled together, resulting in 12 final fractions. These fractions were dried

in a SpeedVac and reconstituted in Evosep solvent A (0.1% FA in water). Reconstitution was achieved through 30 minutes of vortexing, followed by sonication.

5.6.9.3. Mass spectrometry

The fractions were analyzed using an LC-MS system consisting of an Evosep One (Evosep Biosystems, Odense, Denmark) coupled to an Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Peptides were loaded onto disposable Evotips Pure C18 trap columns (Evosep Biosystems, Odense, Denmark) following the manufacturer's instructions. Peptide separation was performed on an analytical column (Dr. Maisch C18 AQ, 1.9 μm beads, 150 μm inner diameter, 15 cm length, Bruker) at a flow rate of 220 nl/min over 88 minutes, using the Evosep Extended method (15 samples per day). Mass spectrometry data were acquired in positive mode with a data-dependent acquisition method. For MS1 scans, the resolution was set to 60 000, with a normalized AGC target of 300%, an automatic maximum injection time, and a scan range of 300 to 1700 m/z. MS2 scans were performed with a resolution of 30.000, a standard normalized AGC target, an automatic maximum injection time, and analysis of the top 25 precursors within a 1.2 m/z isolation window. Dynamic exclusion was set to 20 s with a tolerance of ± 10 ppm, a precursor intensity threshold of $5e3$, and a precursor fit threshold of 70%. Precursors were fragmented in HCD mode with a normalized collision energy of 30% and TurboTMT mode set to "TMT Reagents". The spray voltage was maintained at 2.1 kV, the funnel RF level at 40, and the heated capillary temperature at 275°C.

5.6.9.4. LC-MS data analysis

Peptide/protein identification was performed with MaxQuant/Andromeda suite (version 2.5) using reference *Equus caballus* proteome database from Uniprot (version 06_2024, 1 protein sequence per gene, 21429 sequences). The following parameters were used: variable modification – Oxidation (M), static modification – Methylthio (C), enzyme – Trypsin, protein and PSM FDR – 0.01, min. unique peptides – 1. Further analysis based on the ProteinGroups file was performed in Perseus (version 1.6.15). Proteins were identified only by site, proteins from the reversed database, proteins with less than 3 quantitative values in at least one group, and contaminants were removed from the analysis. Values were normalized on loading factors and log2 transformed. Missing

values were imputed from a normal distribution. Two sample t-test with permutative false discovery rate (FDR) was performed to locate statistically significant proteins.

Functional enrichment analysis was conducted to investigate the biological relevance of identified proteins. Data was analyzed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database version 12.0 (Szklarczyk et al., 2023). Default settings were chosen for the analysis: network type: full STRING network; required score: medium confidence (0.400); FDR < 0.05).

5.6.10. Enzyme-linked immunosorbent assay (ELISA)

To analyze the concentration of TIMP1 and TIMP2 in lysates of endometrial fibroblast after treatment with eqMD-M Φ ^{IFN γ /LPS}-CM, eqMD-M Φ ^{IL-4/IL-13}-CM, or vehicle for 96 hours, Horse TIMP1 ELISA Kit (cat. no. orb780284, Biorbyt), with detection range between 0.63-40 ng/ml and sensitivity 0.241 ng/ml and Horse TIMP2 ELISA Kit (cat. no. orb779686; Biorbyt), with detection range between 7.82-500 ng/ml and sensitivity 2.9 ng/ml, were used according to manufacturer's directions. For both, the principle of the test was sandwich enzyme immunoassay. Corresponding to the sample concentration, a volume equivalent to 50 μ g of protein for TIMP1 assay and 30 μ g of protein for TIMP2 assay was collected and diluted with nuclease-free water to obtain a final volume of 100 μ l. After the equilibration of kits at RT, 100 μ l of Standard Working Buffer (gradually diluted according to the instructions) or 100 μ l of samples were added to each well and incubated at 37°C for 80 minutes. After incubation, the liquid was discarded from the plates, and wells were washed three times, each time by adding 200 μ l of 1 $\times$ Wash Buffer. Subsequently, plates were dried by patting into clean absorbent paper and 100 μ l of 1 $\times$ Biotinylated Antibody Working Solution was added to each well and the plates were incubated at 37°C for 50 minutes. Then, the liquid was discarded from the plates, and wells were again washed three times, each time by adding 200 μ l of 1 $\times$ Wash Buffer, which was followed by pat-drying, the addition of 100 μ l of 1 $\times$ Streptavidin-HRP Working Solution to each well and incubation at 37°C for 50 minutes. Lastly, the liquid was again discarded from the plates, wells were washed five times, each time by adding 200 μ l of 1 $\times$ Wash Buffer, pat-dried, and 90 μ l of TMP Substrate Solution was added for 20 minutes of incubation at 37°C in darkness. The reaction was stopped by adding 50 μ l of Stop Solution to each well. Plates were shaken for one minute and absorbance at 450 nm was immediately measured and the concentration of TIMP1 and TIMP2 was determined based on the linear trend line of the standard curve.

5.6.11. Flow cytometry

To perform flow cytometry analysis in Research Task 1, the eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set (cat. no. 00-5523-00, Thermo Fisher Scientific) was used according to manufacturer's directions. Precisely, frozen endometrial cells were thawed at 38°C for 2 minutes, suspended in 5 ml of X-VIVO™10 supplemented with 10% FBS, and centrifuged at $400 \times g$ for 15 minutes at 4°C. The obtained cell pellet was resuspended in 2 ml of flow cytometry buffer (PBS containing 1% BSA) and counted. Then, 1×10^6 cells were transferred to cytometer tubes, 1 ml of flow cytometry buffer was added and tubes were centrifuged at $650 \times g$ for 7 minutes at RT. The supernatant was carefully discarded and the cell pellet was resuspended in 95 µl of flow cytometry buffer. For staining of the surface marker, 5 µl of anti-CD68 antibodies conjugated with PE-Cyanine7 (cat no. 25-0681-82, Invitrogen) was added and cells were incubated for 1 hour at 4°C in darkness. At this point, all procedures were conducted with limited access to light. After incubation, cells were washed with 1 ml of flow cytometry buffer and centrifuged at $650 \times g$ for 7 minutes at RT. Then, the supernatant was carefully discarded and tubes were pulsed vortexed to break down the pellet. Cells were suspended in 1 ml of Foxp3 Fixation/Permeabilization working solution (consisting of Foxp3 Fixation/Permeabilization Concentrate and Foxp3 Fixation/Permeabilization Diluent mixed in 1:3 proportion), pulsed vortexed and incubated for 45 minutes at 4°C in darkness. After incubation, cells were washed twice, each time by adding 2 ml of 1x Permeabilization Buffer and centrifuging at $650 \times g$ for 7 minutes at RT. After the second centrifugation, the supernatant was carefully discarded and cells were resuspended in 91 µl of 1x Permeabilization Buffer. For staining of intracellular markers, 4 µl of anti-arginase 1 antibodies conjugated with Alexa Fluor® 647 (cat. no. NBP1-32731AF647, NovusBio) and 5 µl of anti-iNOS antibody conjugated with Alexa Fluor® 405 (cat. no. NB300-605AF405, NovusBio) were added to each tube and cells were incubated for another 45 minutes at RT in darkness. Then, cells were washed twice, each time by adding 2 ml of 1x Permeabilization Buffer and centrifuging at $650 \times g$ for 7 minutes at RT. The final cell pellet was resuspended in 500 µl of flow cytometry buffer and subjected to flow cytometry analysis using BD FACSaria II Cell Sorter, Beckton Dickinson. As MΦ constitutes an average of 5% of endometrial cells, to increase the reliability of the analysis, one sample was analyzed until 5 000 CD68⁺ events were detected, among which detection of iNOS and ARG1 fluorescence was performed. Gating strategy applied for

selection of CD68⁺ cells, CD68⁺iNOS⁺ cells and CD68⁺ARG1⁺ cells is presented in Figure 10 and Figure 11.

To carry out a reliable multi-color flow cytometry analysis, compensation for fluorochrome-conjugated antibodies was set using UltraComp eBeads™ Compensation Beads (cat. no. 01-2222-41, Invitrogen) according to the manufacturer's directions. Obtained raw data were analyzed using BD FCS Diva Software v6.1.3.

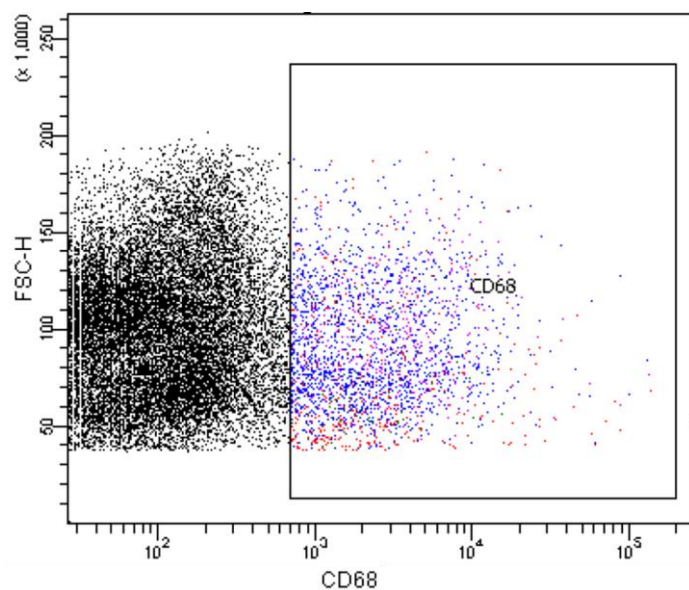


Figure 10. Gating strategy applied for selection of CD68⁺ cells in cells derived from mare endometria.

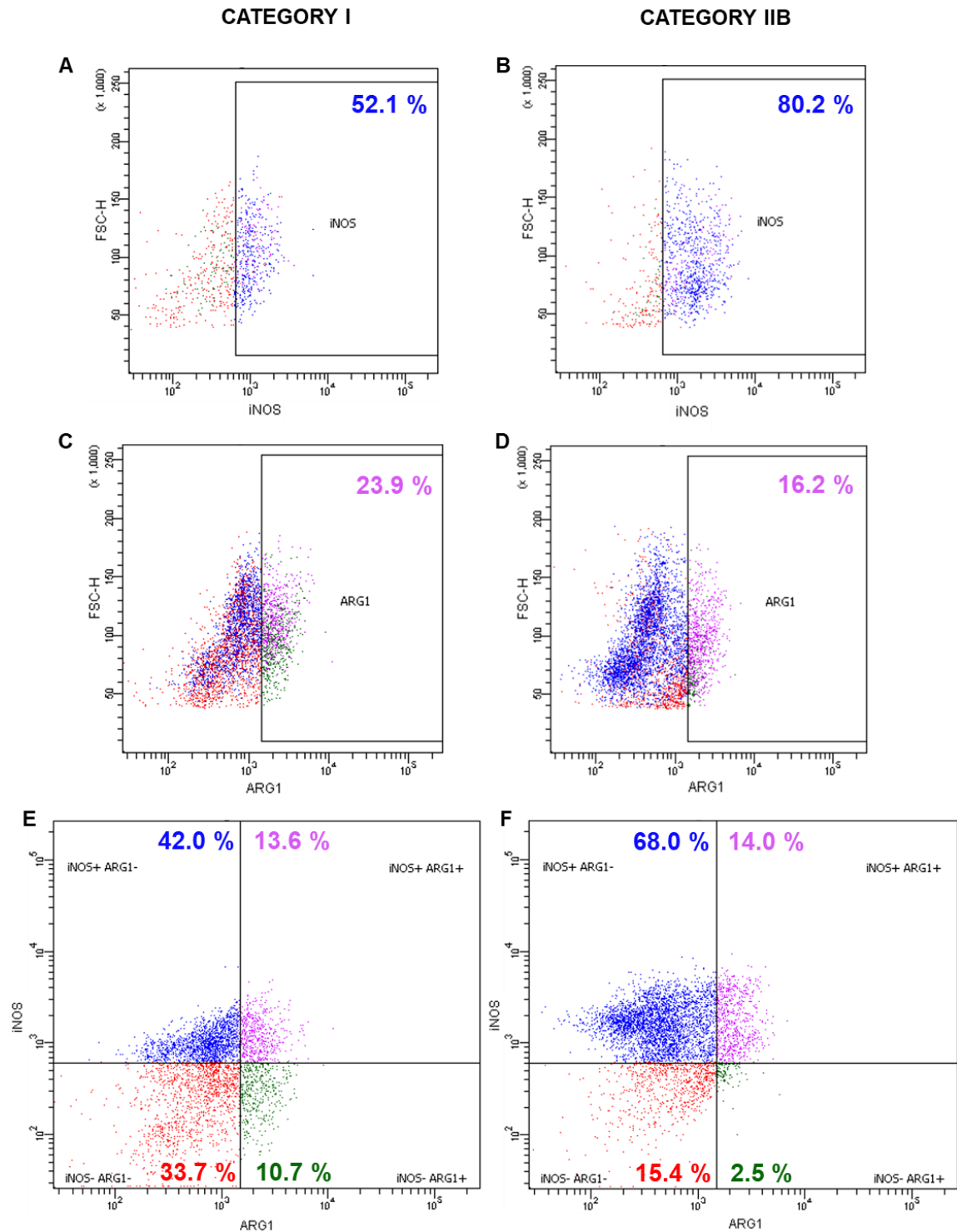


Figure 11. Gating strategy applied for selection of iNOS⁺ cells (A, B) and ARG1⁺ cells (C, D) in population of CD68⁺ cells derived from mare endometria. Gating strategy applied for distinguishing iNOS⁺ARG⁻ cells, iNOS⁻ARG⁺ cells, iNOS⁻ARG1⁻ cells and iNOS⁺ARG1⁺ cells (E, F) in population of CD68⁺ cells derived from mare endometria. Presented are exemplary images for endometria category I (A, C, E) and category IIB (B, D, F) in mid-luteal phase of estrous cycle. The numbers express what percentage the population represents among CD68⁺ cells.

5.6.12. Immunofluorescence staining

5.6.12.1. Immunofluorescence staining in tissue

To deparaffinize the sections of mare endometrium in each category (I, IIA, IIB, and III), both in the mid-luteal phase and follicular phase of the estrous cycle, slides were first kept for one hour at 59°C and incubated in two changes of xylene (cat. no. 1330-20-7, POCH) for 10 minutes at RT. Then, the sections were hydrated in an alcoholic series (incubating twice in 100% ethanol for two minutes, twice in 96% ethanol for two minutes, twice in 70% ethanol for two minutes, and in running tap water for 5 minutes). For antigen retrieval, slides were placed in sodium citrate buffer and boiled for 25 minutes in a water bath set to 95°C, which was followed by 25 minutes of cooling in an RT water bath. For permeabilization, slides were incubated in PBS with 0.3% TritonX-100 for 10 minutes and washed in PBS for 5 minutes. The non-specific binding sites were blocked by 90 minutes of incubation in phosphate buffer (PB) with 1% BSA and 10% normal goat serum. To determine the endometrial localization of CD68⁺iNOS⁺ and CD68⁺ARG1⁺ cells in Research Task 1, a co-staining of CD68 with iNOS or ARG1 was conducted. Immunofluorescence assays were performed with the following primary antibodies: mouse monoclonal anti-CD68 (1:100; cat. no. 14-0681-82; Invitrogen), rabbit superclonal anti-ARG1 (1:50; cat. no. 711765, Thermo Fisher Scientific) and rabbit polyclonal anti-iNOS (1:50; cat. no. PA1-036, Invitrogen) antibodies overnight at 4°C. For staining control, sections were also incubated without the use of primary antibodies. The next day, slides were first warmed for 20 minutes in RT and washed three times with PBS. Taking care to limit access to light, the proteins were detected by incubating the sections with goat polyclonal anti-rat antibodies conjugated with AlexaFluor® 594 (1:400, cat. no. A-11007, Thermo Fisher Scientific) and goat polyclonal anti-rabbit antibodies conjugated with AlexaFluor® 488 (1:400, A-11034, Thermo Fisher) in accordance with a host of primary antibodies. After incubation, slides were washed three times in PBS and mounted in Fluoroshield with 4',6-diamidyno-2-fenyloindol (DAPI) (cat. no. F6057; Sigma-Aldrich), which allowed for counterstaining of nuclei. Imaging was performed using an Inverted epi-fluorescence microscope Axio-Observer (Carl-Zeiss; Germany). All image editions and data analysis were done using Zen Blue Pro software (Carl-Zeiss; Germany).

5.6.12.2. Immunofluorescence staining in cells

After the cell culture with adequate treatments, monocytes, MΦ or fibroblasts were washed three times with warm sterile-filtered PBS for 5 minutes and fixed by incubating with 4% paraformaldehyde for 10 minutes. Then, cells were washed three times with PBS containing 0.1% Tween-20 and permeabilized by incubation with PBS containing 0.5% TritonX-100 for 5 minutes. To block any non-specific binding sites, cells were incubated with 1% BSA in PB for 1 hour at RT. To visualize the differences between monocytes, eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} in Experiment 1 of Research Task 2, cells were incubated overnight, separately with mouse monoclonal anti-CD68 (1:100; cat. no. 14–0681-82; Invitrogen), rabbit superclonal anti-ARG1 (1:50; cat. no. 711765, Thermo Fisher Scientific) and rabbit polyclonal anti-iNOS (1:50; cat. no. PA1-036, Invitrogen) antibodies at 4°C. To visualize the effect of eqMD-MΦ^{IFN γ /LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM on mare endometrial fibroblasts in Experiment 3 of Research Task 2, cells were incubated overnight, separately with rabbit polyclonal anti-alpha smooth muscle actin (1:50; cat. no. ab5694; Abcam), rabbit polyclonal anti-collagen type I (1:50; cat. no. 20121; Novotec), rabbit polyclonal anti-collagen type III (1:200; cat. no. ab7778; Abcam), rabbit polyclonal anti-fibronectin 1 (1:100; cat. no. ab2413; Abcam), rabbit polyclonal anti-metalloproteinase 2 (1:100; cat. no. ab97779; Abcam), goat polyclonal anti-metalloproteinase 3 (1:75; cat. no. ab223666; Abcam) rabbit polyclonal and anti-metalloproteinase 9 (1:200; ab73734; Abcam) antibodies at 4°C. For staining control, cells were also incubated without primary antibodies. Afterward, cells were washed three times with PBS for 5 minutes and incubated for one hour at RT with goat polyclonal anti-rat secondary antibodies conjugated with AlexaFluor® 594 (1:400, cat. no. A-11007, Thermo Fisher Scientific), anti-rabbit secondary antibodies conjugated with goat polyclonal AlexaFluor® 488 (1:400, A-11034, Thermo Fisher) and donkey polyclonal anti-goat secondary antibodies conjugated with AlexaFluor® 488 (1:400; cat. no. A11055, Thermo Fisher Scientific) in accordance to the host of primary antibodies, while taking care to limit access to light. To better visualize the complex structure of monocytes and eqMD-MΦ, cells were simultaneously incubated with Phalloidin-iFluor 594 Reagent (1:1000, cat. no. ab176757, Abcam) which allows to label F-actin in cells. Subsequently, the cells were washed three times with PBS for 5 minutes and mounted in Fluoroshield with DAPI which allowed for counterstaining of nuclei. Analysis was performed using an Inverted epi-fluorescence microscope Axio-Observer supported by Zen Blue Pro software.

5.6.13. Assessment of cell functional characteristic

5.6.13.1. Cell proliferation assay

The proliferation of mare endometrial fibroblasts after treatment with eqMD-M Φ ^{IFN γ /LPS}-CM or eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours, was assessed using an ELISA BrdU incorporation assay (cat. no. 11647229001, Roche) according to the manufacturer's directions. This assay is a colorimetric alternative to quantify cell proliferation based on the measurement of BrdU incorporation during DNA synthesis in proliferating cells. After the cell culture with the appropriate treatments, cells were labeled using 10 μ l of BrdU labeling solution, which equaled the final concentration of 10 μ M of BrdU in a well, and incubated for additional 2 hours (humidified atmosphere, 5% CO₂, 38°C). Wells free of cells, with TheraPEAK™ X-VIVO™-10 supplemented with 0.1% BSA and 0.01% AA itself, were used as a blank sample for a reaction. The analysis was performed in three technical replicates. After incubation, the labeling medium was removed by tapping, and labeled cells were dried for 1 hour at 60°C. Subsequently, 200 μ l of FixDenat solution was added to each well, and cells were incubated at 25°C for 30 minutes. After incubation, FixDenat was removed by flicking and tapping on clean absorbent paper and 100 μ l of Anti-BrdU-POD working solution was added to each well. Cells were incubated at 25°C for 90 minutes, after which antibody conjugate was removed by flicking off and wells were washed three times, each time with 200 μ l of Washing solution. After the last wash, the Washing solution was removed by flicking and tapping on clean absorbent paper and 100 μ l of Substrate Solution was added to each well, and the plate was incubated at 25°C for 10 minutes. The absorbance at 370 nm and 492 nm for reference wavelength was immediately measured. The absorbance of cells treated with the vehicle (control group) was used as a reference value and established as 100%, to which the absorbance of treated cells was related. Results above 100% indicate higher proliferation of the cells, while under 100% indicate lower proliferation in response to treatment.

5.6.13.2. Cell viability assay

The viability of mare endometrial fibroblasts after treatment with eqMD-M Φ ^{IFN γ /LPS}-CM or eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours, was assessed using MTT based *In Vitro* Toxicology Assay Kit (cat. no. TOX1, Sigma-Aldrich) according to manufacturer's directions. It is an assay designed for spectrophotometrically determining cell viability as a function of mitochondrial activity in living cells. After the cell culture

with the appropriate treatments, 10 µl of MTT (which equaled the final concentration of 0.5 mg/ml of MTT) was added to each well of the 96-well culture plate, and incubated for an additional 3 hours (humidified atmosphere, 5% CO₂, 38°C). Wells free of cells, with TheraPEAK™ X-VIVO™-10 supplemented with 0.1% BSA and 0.01% AA itself, were used as a blank sample for a reaction. The analysis was performed in three technical replicates. After the incubation, 100 µl of MTT Solubilization Solution was added to each well and MTT formazan crystals were dissolved thoroughly by trituration. The absorbance at 570 nm and 690 nm (for subtracting the background absorbance) was immediately measured. The absorbance of cells treated with the vehicle (control group) was used as a reference value and established as 100%, to which the absorbance of treated cells was related. Results above 100% indicate higher mitochondrial activity (viability) of the cells, while under 100% indicate lower viability of the cells in response to treatment.

5.6.13.3. Cell migration assay

The cell migration assay was conducted in accordance with the methodology previously described by Bukowska et al. (2018) with modifications. For prevention of cell proliferation, mare endometrial fibroblasts that reached 100% of confluence were incubated for 2 hours with 10 µg/ml mitomycin C (cat. no. M0503, Sigma-Aldrich) in TheraPEAK™ X-VIVO™-10 supplemented with 0.1% BSA and 0.01% AA. After that, inserts were carefully discarded and cells were washed twice, each time with 500 µl of PBS. Subsequently, cells were treated with eqMD-MΦ^{IFN γ /LPS}-CM, eqMD-MΦ^{IL-4/IL-13}-CM or vehicle. The experiment was performed using an inverted epifluorescence microscope Axio-Observer (Carl-Zeiss; Germany) with a cell culture module. All image editions and data analysis were done using Zen Blue Pro software. Two representative regions of interest were chosen for each well and the area between edges was measured. The area at 0h was considered to be 100%. The areas were monitored for 48h and photographed every 12 hours (0h, 12h, 24h, 36h, 48h).

5.6.14. C-reactive protein concentration

Analysis of CRP concentration in serum of mares after intrauterine administration of $M\Phi^{IFN\gamma/LPS}$, $M\Phi^{IL-4/IL-13}$ or vehicle in Research Task 3 was performed by VetLab Group Jędrzychko, Gietrzwałd, Poland. After collection, the serum was aliquoted and frozen at -20°C . 500 μl of frozen serum was sent for analysis with the use of a biochemical analyzer. The detection limit was 1 mg/l, when in the horse the standard level of CRP is < 14 mg/l.

5.6.15. Statistical analyses and preparation of graphs

All statistical analyses were performed using StatSoft, Inc. STATISTICA (data analysis software system; version 13. www.statsoft.com). Several statistical tests were employed, dependent on the nature of the conducted experiments.

In Research Task 1, for analysis of mRNA transcription and protein abundance of CD68, as well as for analysis of $CD68^{+}iNOS^{+}ARG1^{-}$ / $CD68^{+}iNOS^{-}ARG1^{+}$ cells ratio in mare endometrium in each category of endometriosis (I, IIA, IIB, and III) in the mid-luteal phase and follicular phase of the estrous cycle, the Gaussian distribution of the results was tested using the Shaphiro-Wilk normality test. It was then followed by Levene's test to assess the homogeneity of variances. As all assumptions were met, statistical differences were determined using two-way ANOVA followed by a post-hoc Tukey test. In case, where two-way ANOVA showed a significant difference in column factor, the one-way ANOVA was performed to determine the statistical differences between categories of endometria, regardless of the phase of estrous cycle.

In Research Task 2 for analysis of mRNA transcription of $M\Phi$ markers (*CD68*, *NOS2*, *IL-1 β* , *IL-6*, *IL-12A*, *IL-12B*, *MMP-3*, *MMP-9*, *TGF- β 1*, *TNF- α*) in eqMD- $M\Phi^{IFN\gamma/LPS}$ and eqMD- $M\Phi^{IL-4/IL-13}$, mRNA transcription and protein abundance of ECM-associated components (*ACTA2*, *COL1A1*, *COL3A1*, *FN1*, *MMP-2*, *MMP-3*, *MMP-9*, *TIMP-1*, *TIMP-2*), mRNA transcription of inflammatory markers (*AREG*, *CCN2*, *IL-1 β* , *IL-6*, *TGF- β 1*, *TNF- α*) in mare endometrial fibroblasts after treatment with eqMD- $M\Phi^{IFN\gamma/LPS}$ -CM, eqMD- $M\Phi^{IL-4/IL-13}$ -CM, or vehicle for 96 hours, as well as in the proliferation and viability assays, the Gaussian distribution of the results was tested using the Shaphiro-Wilk normality test. It was then followed by Levene's test to assess the homogeneity of variances. In cases when all assumptions were met, statistical differences were determined using the Student's t-test. In the absence of variance homogeneity statistical differences were determined using Student's t-test with independent variance

estimation. In the case of not meeting the assumptions of normal distribution, the nonparametric Mann-Whitney U test was performed. For analysis of the cell migration assay, the Gaussian distribution of the results was tested using the Shaphiro-Wilk normality test and was followed by Mauchly's sphericity test. Based on the results, statistical difference was determined with the use of repeated measures ANOVA with Greenhouse-Geisser correction and followed by post-hoc Tukey test.

In Research Task 3 for analysis of mRNA transcription of ECM-associated components (*ACTA2*, *COL1A1*, *COL3A1*, *FN1*, *MMP-2*, *MMP-9*, *TIMP-1*, *TIMP-2*) and mRNA transcription of inflammatory markers (*AREG*, *CCN2*, *IL-1 β* , *IL-6*, *TGF- β 1*, *TNF- α*) in mare endometrium after intrauterine administration of eqMD-M Φ ^{IFN γ /LPS}, eqMD-M Φ ^{IL-4/IL-13} or vehicle, the Gaussian distribution of the results was tested using the Shaphiro-Wilk normality test. It was then followed by Levene's test to assess the homogeneity of variances. In cases when all assumptions were met, statistical differences were determined using the Student's t-test. In the absence of variance homogeneity statistical differences were determined using Student's t-test with independent variance estimation. In the case of not meeting the assumptions of normal distribution, the nonparametric Mann-Whitney U test was performed. For analysis of CRP level in the serum of mares after intrauterine administration of eqMD-M Φ ^{IFN γ /LPS}, eqMD-M Φ ^{IL-4/IL-13} or vehicle (PBS), the Gaussian distribution of the results was tested using the Shaphiro-Wilk normality test and was followed by Mauchly's sphericity test. Based on the results, statistical difference was determined with the use of repeated measures ANOVA with Greenhouse-Geisser correction and followed by post-hoc Tukey test.

All results were considered as significantly different when the p-value < 0.05. All graphs were prepared using GraphPad Prism version 10.4.1 for Windows (GraphPad Software, San Diego, California USA).

6. Results

6.1. Research Task 1: Determination of the distribution of different types of tissue-resident MΦ in the endometrium of mares with endometriosis

6.1.1. Experiment 1. The expression of CD68 in the endometrium of mares with endometriosis

No significant differences were observed in the mRNA transcription of *CD68* within categories of endometria and phases of the estrous cycle ($p>0.05$, Fig. 12A). However, higher protein abundance of CD68 was observed in category IIA compared to the category I endometria in the follicular phase of the estrous cycle ($p<0.05$, Fig. 12C) as well as in category IIA endometria in the follicular phase of estrous cycle compared to category IIA endometria in the mid-luteal phase of estrous cycle ($p<0.01$, Fig. 12C).

To better understand the differences between studied phases of the estrous cycle additional analysis was performed that considered only the phase of estrous cycle factor with the exclusion of endometrial category. It revealed overall higher expression of both mRNA transcription and protein abundance of CD68 in follicular phase compared to mid-luteal phase of estrous cycle ($p<0.01$, Fig. 12B and $p<0.0001$, Fig. 12D, respectively).

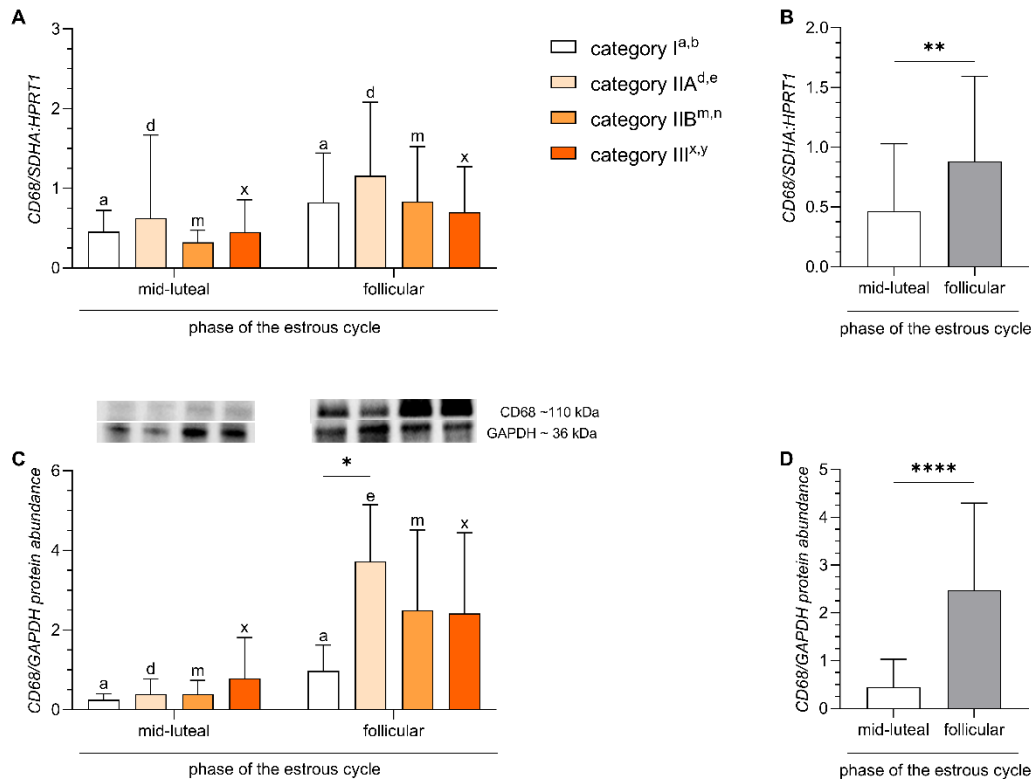


Figure 12. The mRNA transcription (A, B) and protein abundance (C, D) of CD68 in category I, IIA, IIB, and III endometria of mares (A, C) or regardless of the endometrial category (B, D) in the mid-luteal and follicular phases of the estrous cycle (n=10 and n=6; respectively). Results are presented as mean $\pm$ standard deviation (SD). Letters indicate statistical differences between phases of the estrous cycle within the category of endometrium, and asterisks indicate statistical differences between categories of endometrium within the phase of the estrous cycle (*p<0.05) as determined by two-way ANOVA with Tukey's multiple comparisons test (A, C). Asterisk indicate statistical differences between phases of the estrous cycle (**p<0.01; ****p<0.0001) as determined by Student's t test (B, D)

6.1.2. Experiment 2. The ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells in the endometrium of mares with endometrosis

No significant differences were observed in the percentage of CD68⁺iNOS⁺ARG1⁻ cells and CD68⁺iNOS⁻ARG1⁺ cells within categories of endometria and phases of the estrous cycle ($p>0.05$, Fig. 13 A, B).

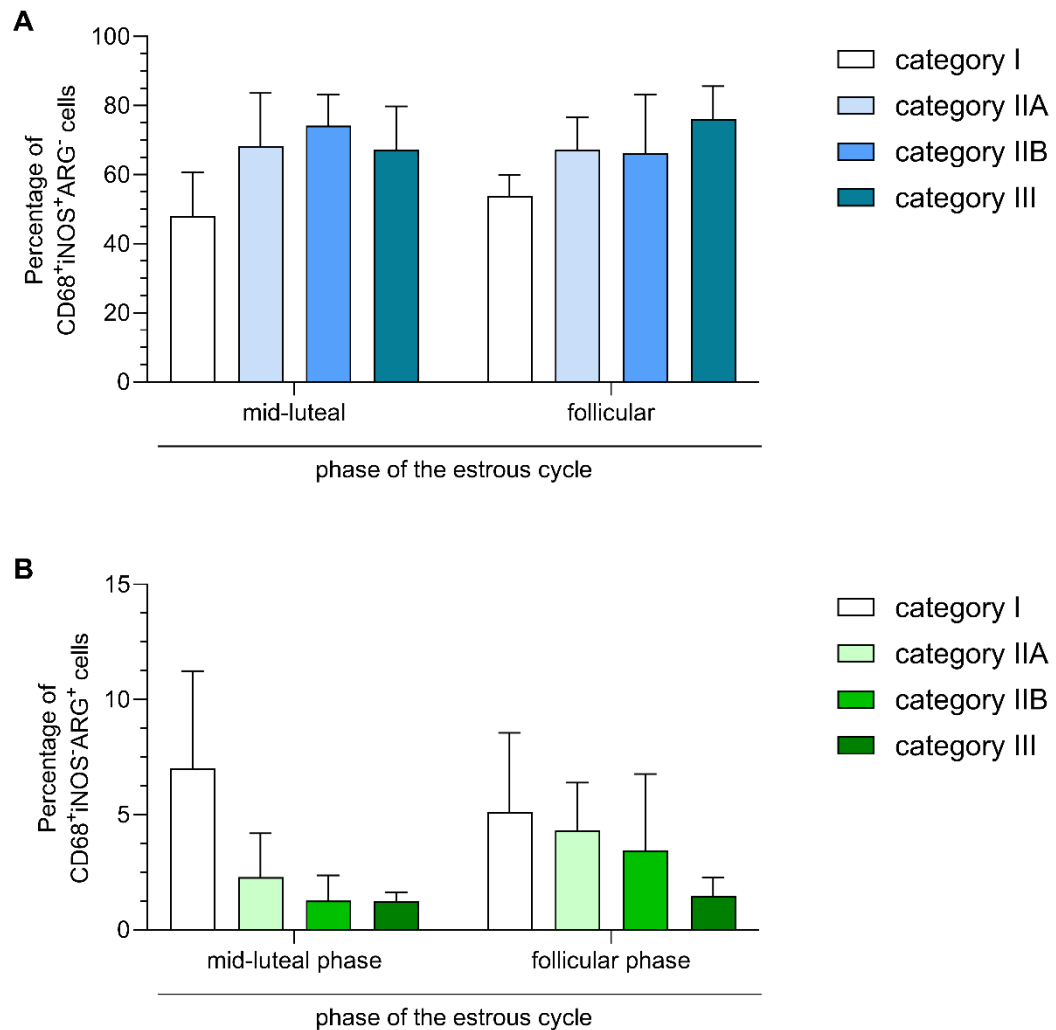


Figure 13. The percentage of CD68⁺iNOS⁺ARG1⁻ cells (A) and CD68⁺iNOS⁻ARG1⁺ cells (B) in categories I, IIA, IIB, and III endometria of mares in the mid-luteal and follicular phases of the estrous cycle (n=4). Results are presented as mean $\pm$ standard deviation (SD). Two-way ANOVA with Tukey's multiple comparisons test was performed for statistical analysis.

The ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells was higher in category IIA (p<0.05; Fig. 14) and category IIB (p<0.0001; Fig. 14) endometria in the mid-luteal phase of the estrous cycle compared to the respective endometria categories in the follicular phase of the estrous cycle. Moreover, the ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells was higher in category IIA (p<0.001; Fig 14), category IIB (p<0.0001; Fig. 14) and category III (p<0.0001; Fig. 14) compared to category I endometria, as well as in category IIB (p<0.0001; Fig. 14) and category III (p<0.001; Fig. 14), when compared to category IIA endometria in the mid-luteal phase of the estrous cycle. The increase of the ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells, was observed only in category III when compared to all endometria categories in the follicular phase of the estrous cycle (p<0.0001; Fig 14).

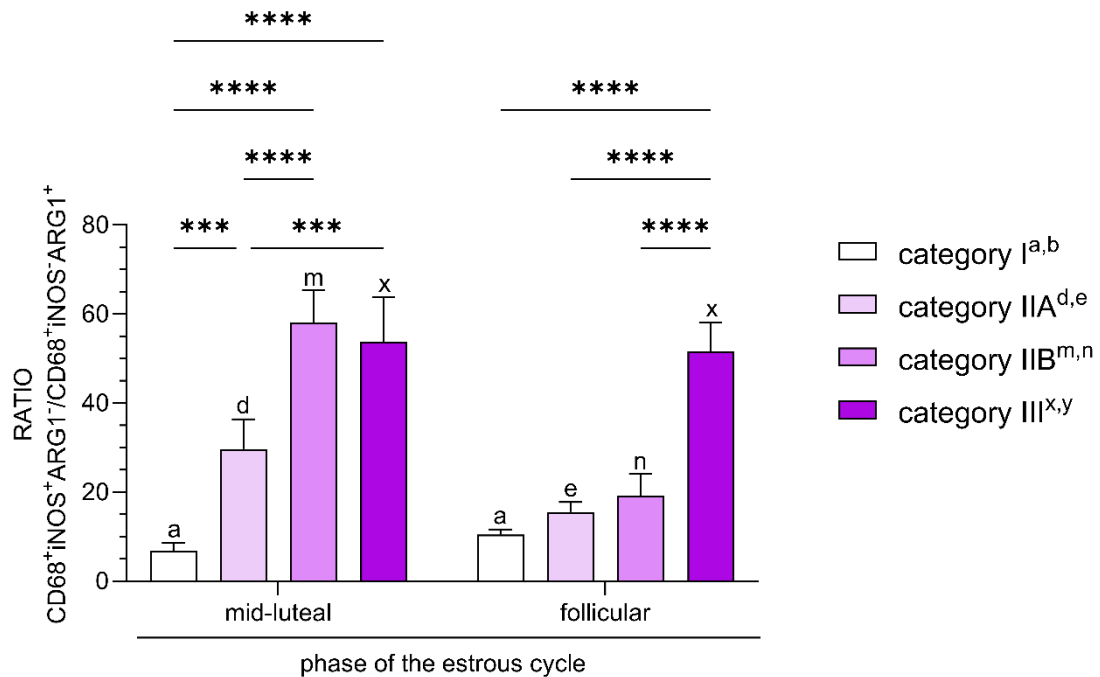


Figure 14. The ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells in category I, IIA, IIB, and III endometria of mares in the mid-luteal and follicular phases of the estrous cycle (n=4). Results are presented as mean $\pm$ standard deviation (SD). Letters indicate statistical differences between phases of the estrous cycle within the category of the endometrium, and asterisks indicate statistical differences between categories within the phase of the estrous cycle (***p<0.001; ****p<0.0001) as determined by two-way ANOVA with Tukey's multiple comparisons test.

To better understand the fluctuations in a number of different MΦ subpopulations, an additional analysis was performed in which the samples were divided only in context of endometrial category, irrespective of the phase of the estrous cycle. It revealed that there is an increase in the percentage of CD68⁺iNOS⁺ARG⁻ cells in category IIA (p<0.05; Fig. 15A), category IIB (p<0.05; Fig. 15A) and category III (p<0.01; Fig. 15A) compared to category I endometria (Fig. 15A). Concomitant decrease in the percentage of CD68⁺iNOS⁻ARG1⁺ cells was observed in category IIA (p<0.05; Fig. 15B), category IIB (p<0.01; Fig. 15B) and category III (p<0.01; Fig. 15B) compared to category I endometria. Concurrently, analysis showed that the ratio of CD68⁺iNOS⁺ARG⁻ / CD68⁺iNOS⁻ARG1⁺ cells is higher in endometria category IIB (p<0.001; Fig. 15C) and category III (p<0.0001; Fig. 15C) when compared to endometria category I, as well as the ratio is higher in endometria category III when compared to endometria category IIA (p<0.001; Fig. 15C).

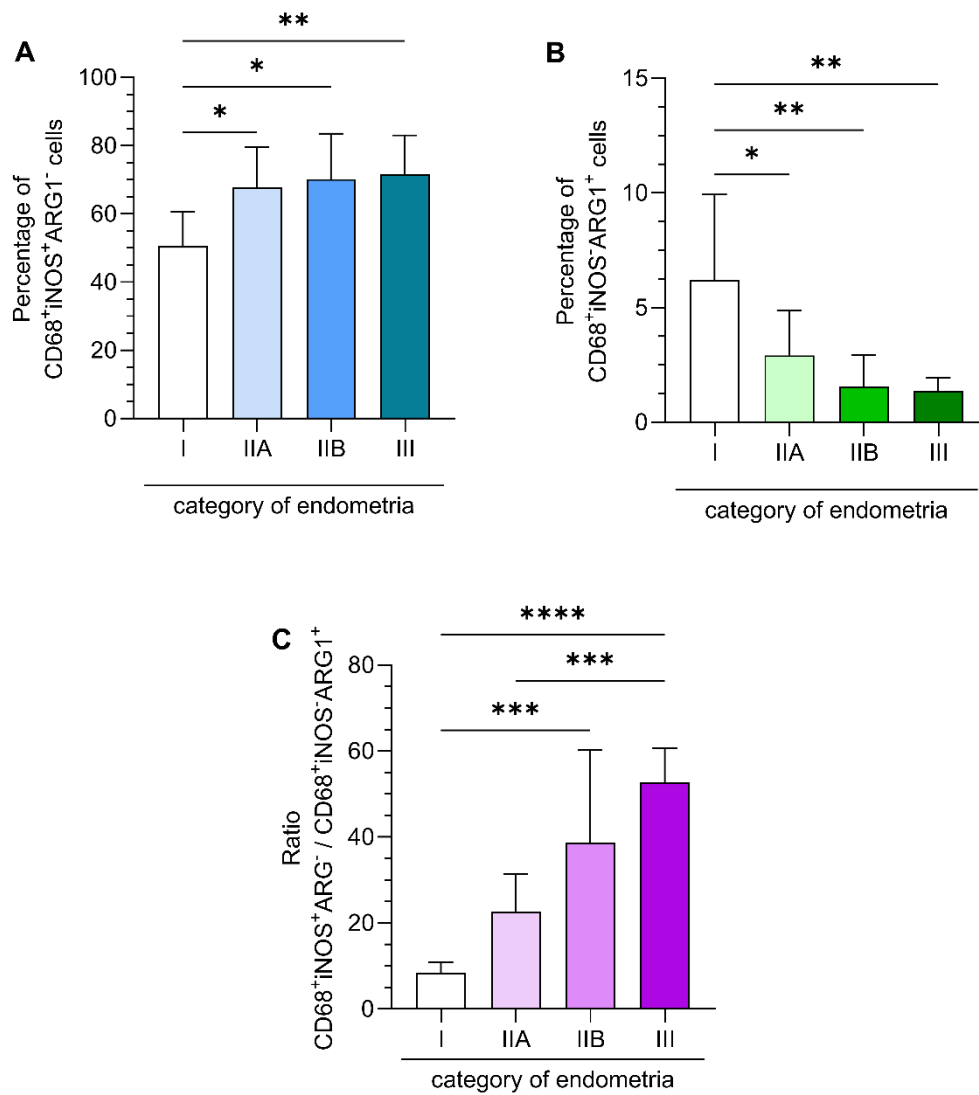


Figure 15. The percentage of CD68⁺iNOS⁺ARG1⁻ cells (A) and CD68⁺iNOS⁻ARG1⁺ cells (B), and the ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells (C) in category I, IIA, IIB and III endometria of mares regardless of the phase of the estrous cycle (n=4). Results are presented as mean $\pm$ standard deviation (SD). Asterisks indicate statistical differences (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001) as determined by one-way ANOVA.

6.1.3. Experiment 3. The immunolocalization of CD68⁺iNOS⁺ and CD68⁺ARG1⁺ cells in the endometrium of mares with endometrosis

The expression of CD68⁺iNOS⁺ cells and CD68⁺ARG1⁺ cells was demonstrated in the endometrium of mares within categories of endometria and phases of the estrous cycle (Fig. 16-19). CD68⁺iNOS⁺ cells and CD68⁺ARG1⁺ cells were observed to be present within the stratum spongiosum and stratum compactum of endometrium, as well as in the region of endometrial glands and in close proximity to the epithelium. No particular differences were observed in the immunolocalization of CD68⁺iNOS⁺ cells and CD68⁺ARG1⁺ cells within categories of endometria and phases of the estrous cycle. Meanwhile, in endometria category III, regardless of the phase of the estrous cycle, CD68⁺iNOS⁺ cells and CD68⁺ARG1⁺ cells scarcely appeared in the vicinity of glandular nests, although such immunolocalization was also observed as presented in Figures 16-19. Analysis of immunolocalization also confirmed the simultaneous presence of CD68⁺ cells with varied expression of studied markers. CD68⁺iNOS⁺ and CD68⁺iNOS⁻ cells as well as CD68⁺ARG1⁺ and CD68⁺ARG1⁻ cells were observed in close proximity to each other (Fig. 20).

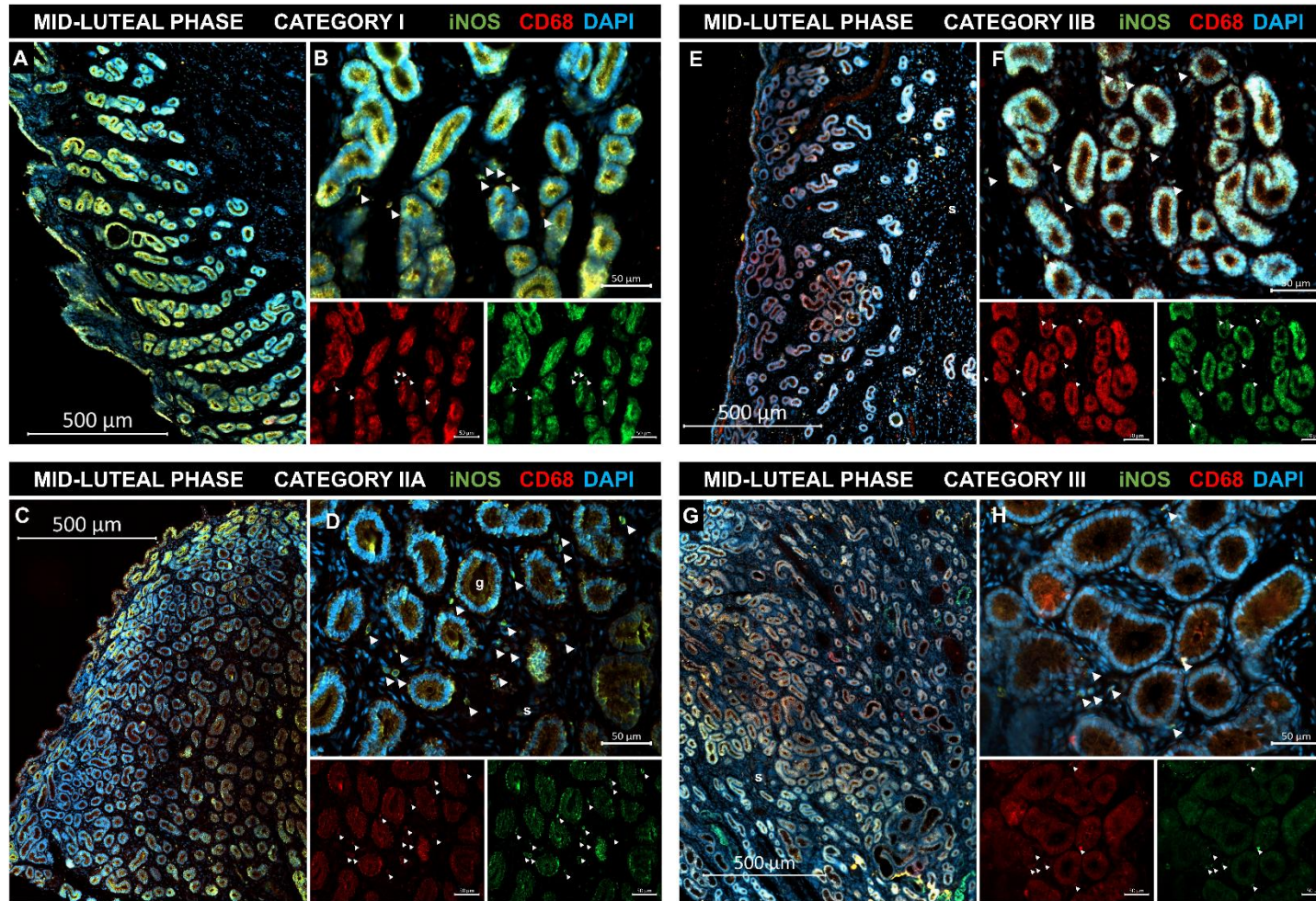


Figure 16. Endometrial infiltration and immunolocalization of CD68⁺iNOS⁺ cells. Representative immunostainings for category I (A, B), IIA (C, D), IIB (E, F), and III (G, H) endometria in the mid-luteal phase of the estrous cycle. A large scan of a tissue section (scale bar 500 μm) and a detailed image (scale bar 50 μm) are shown side by side. The white arrows indicate CD68⁺iNOS⁺ cells.

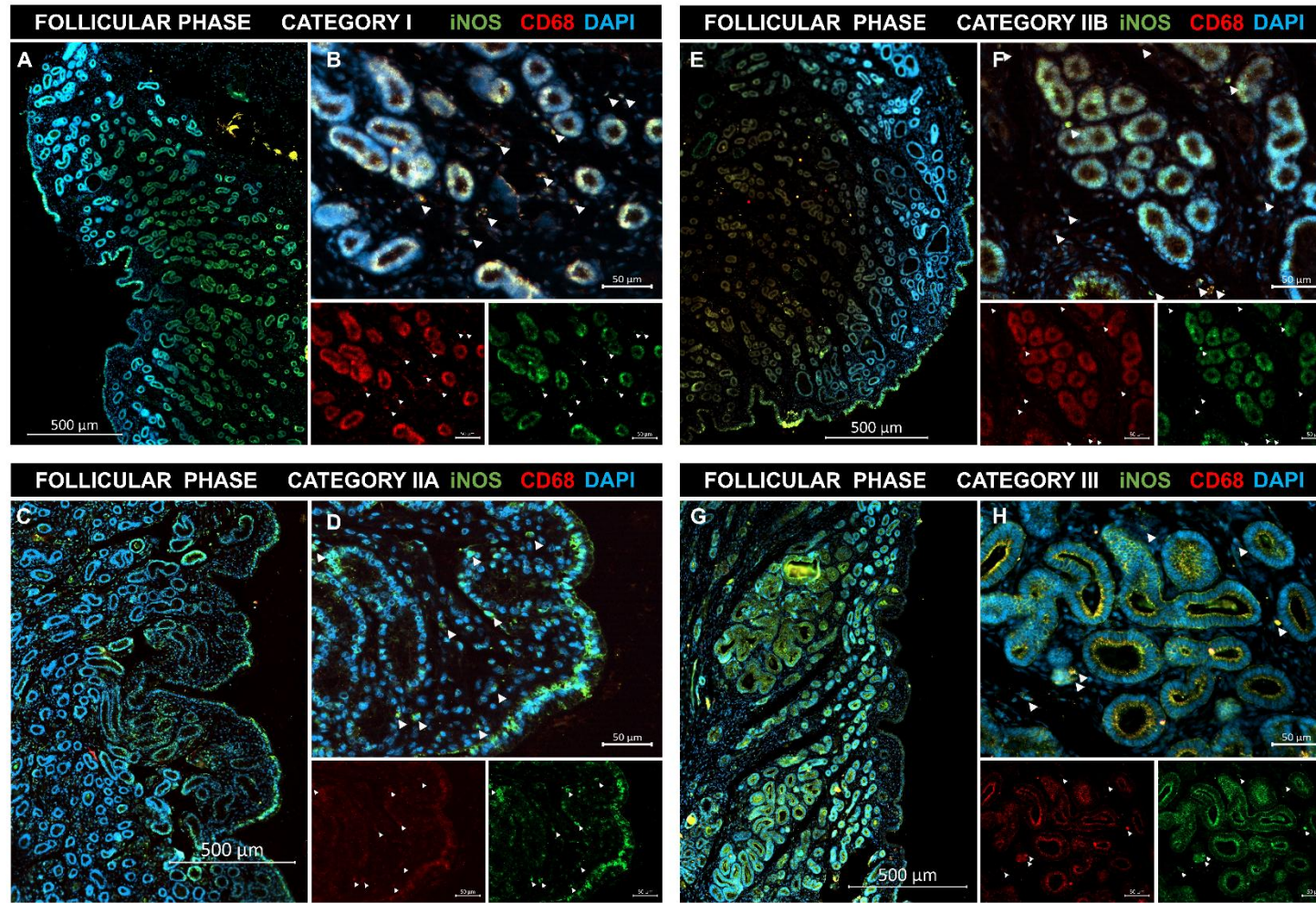


Figure 17. Endometrial infiltration and immunolocalization of CD68⁺iNOS⁺ cells. Representative immunostainings for category I (A, B), IIA (C, D), IIB (E, F), and III (G, H) endometria in the follicular phase of the estrous cycle. A large scan of a tissue section (scale bar 500 μm) and a detailed image (scale bar 50 μm) are shown side by side. The white arrows indicate the CD68⁺iNOS⁺ cells.

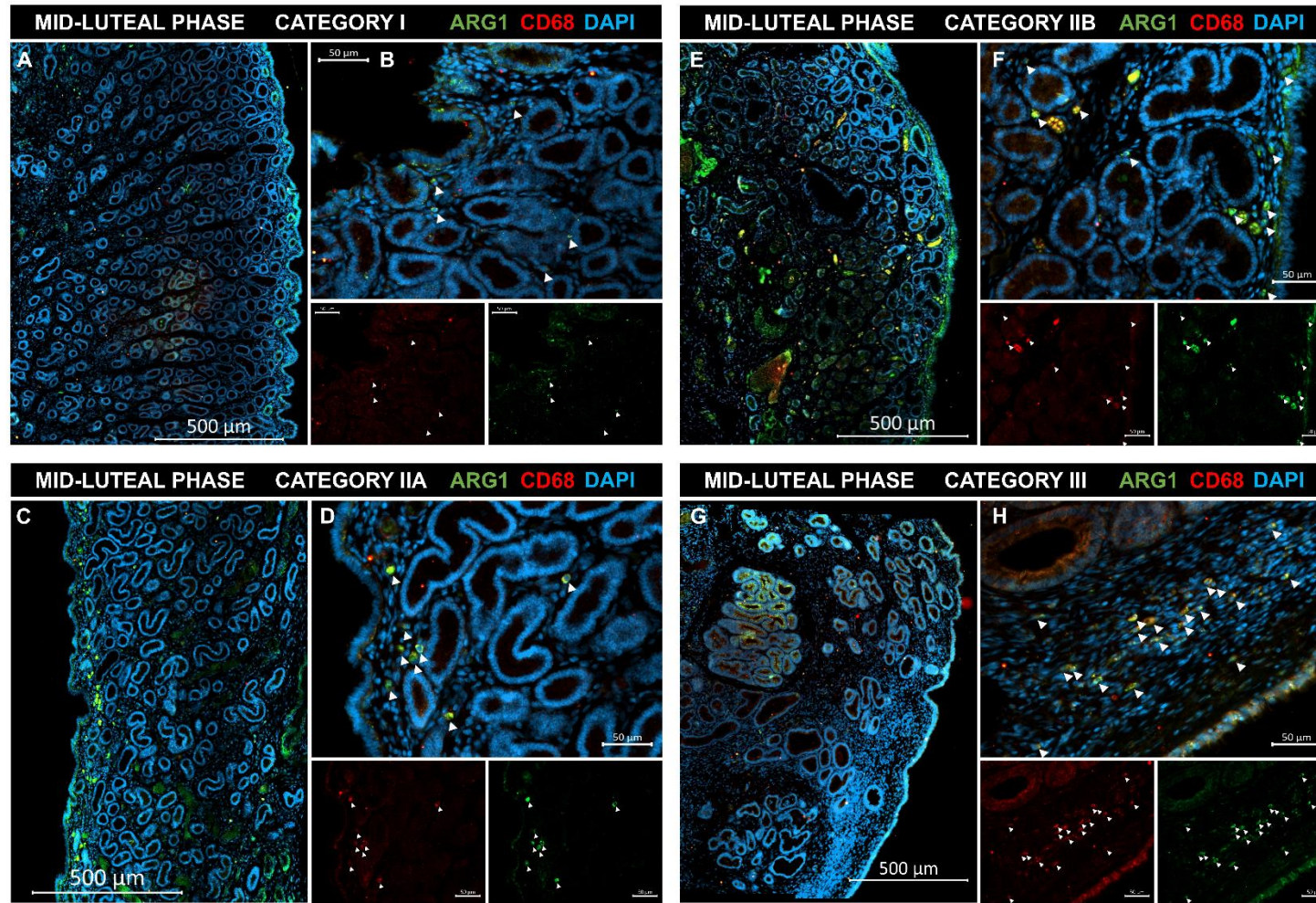


Figure 18. Endometrial infiltration and immunolocalization of CD68⁺ARG1⁺ cells. Representative immunostainings for category I (A, B), IIA (C, D), IIB (E, F), and III (G, H) endometria in the mid-luteal phase of the estrous cycle. A large scan of a tissue section (scale bar 500 μm) and a detailed image (scale bar 50 μm) are shown side by side. The white arrows indicate CD68⁺ARG1⁺ cells.

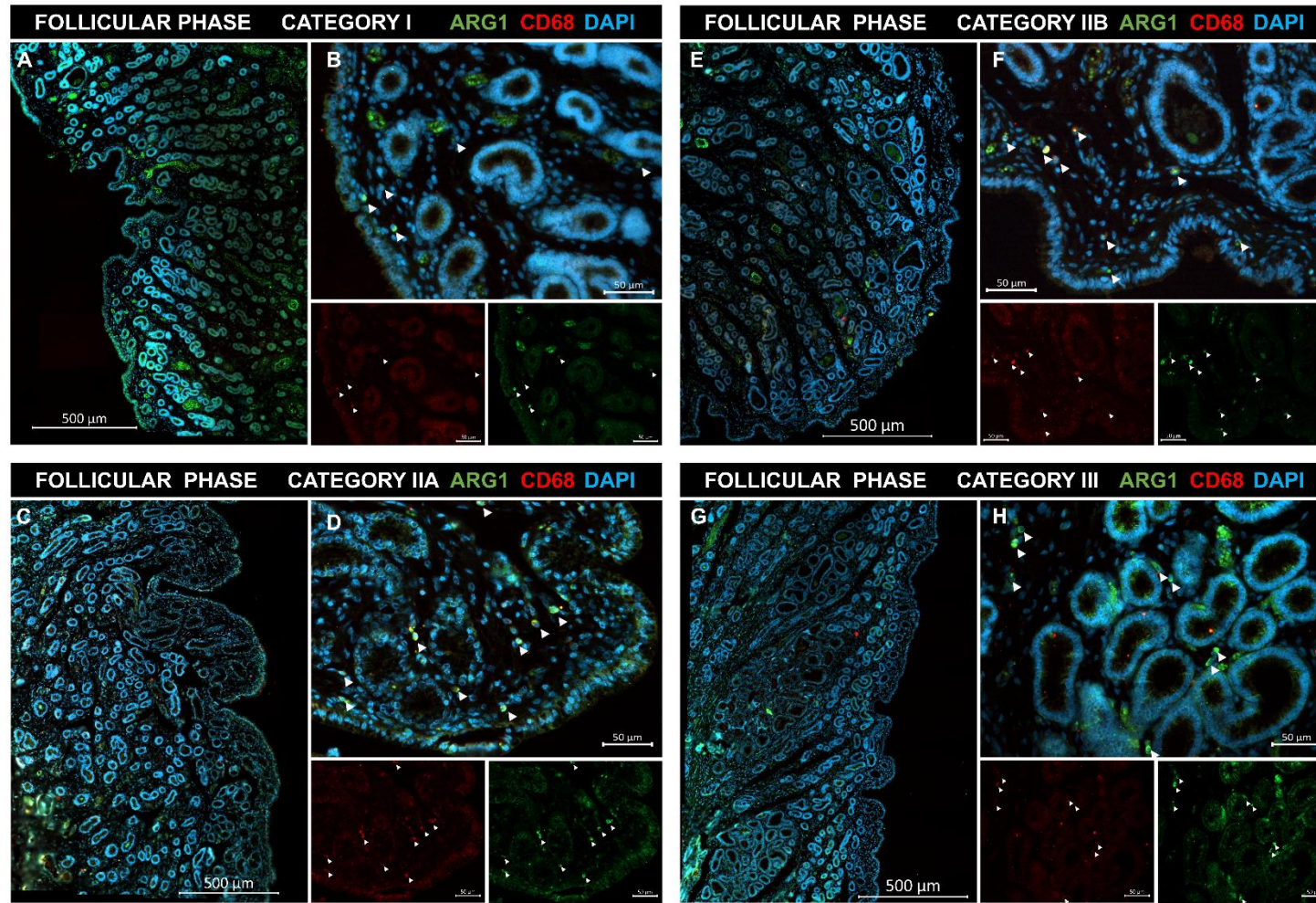


Figure 19. Endometrial infiltration and immunolocalization of CD68⁺ARG1⁺ cells. Representative immunostainings for category I (A, B), IIA (C, D), IIB (E, F), and III (G, H) endometria in the follicular phase of the estrous cycle. A large scan of a tissue section (scale bar 500 µm) and a detailed image (scale bar 50 µm) are shown side by side. The white arrows indicate CD68⁺ARG1⁺ cells.

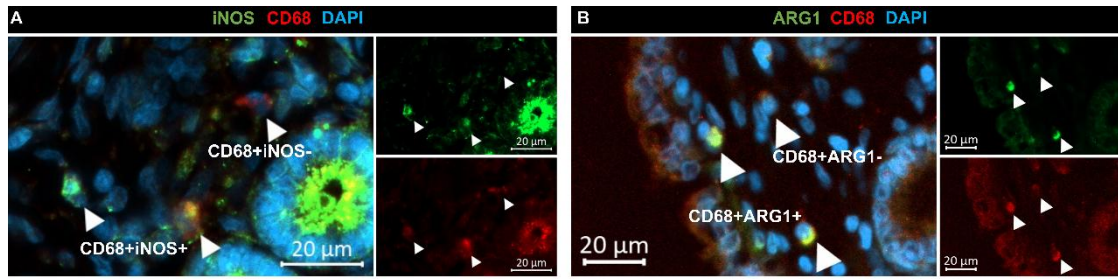


Figure 20. Simultaneous presence of cells with different expression of MΦ markers. Presence of CD68⁺iNOS⁺ cells and CD68⁺iNOS⁻ cells (A) and CD68⁺ARG1⁺ cells and CD68⁺ARG1⁻ cells (B). Scale bar 20 μm. The white arrows indicate identified cells.

6.2. Research Task 2: Investigation of the effect of the secretome of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the mare endometrial fibroblasts

6.2.1. Experiment 1. The characterization of the molecular profile of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}

6.2.1.1. Experiment 1.1. The expression of characteristic molecular markers of M Φ in eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}

The mRNA transcription of *CD68* was higher in eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} when compared to freshly isolated equine peripheral blood monocytes used for M Φ differentiation ($p < 0.05$; Fig. 21A). It was further confirmed by immunofluorescence staining, where the expression of CD68 in monocytes was almost undetectable, in contrary to its expression in both populations of M Φ (Fig. 21B).

The eqMD-M Φ ^{IFN γ /LPS} exhibited higher mRNA transcription of *NOS2* when compared to eqMD-M Φ ^{IL-4/IL-13} ($p < 0.05$; Fig. 22A). Immunofluorescence staining confirmed high expression of iNOS in eqMD-M Φ ^{IFN γ /LPS} (Fig. 22B), and high expression of ARG1 in eqMD-M Φ ^{IL-4/IL-13} (Fig. 22C) indicating successful polarization into two populations of M Φ – akin to M Φ 1 (eqMD-M Φ ^{IFN γ /LPS}) and akin to M Φ 2 (eqMD-M Φ ^{IL-4/IL-13}).

The eqMD-M Φ ^{IFN γ /LPS} exhibited higher mRNA transcription of *IL-1 β* ($p < 0.05$; Fig. 23A), *IL-6* ($p < 0.05$; Fig. 23B), *IL-12A* ($p < 0.05$; Fig. 23C) and *IL-12B* ($p < 0.05$; Fig. 23D) when compared to eqMD-M Φ ^{IL-4/IL-13}. On the other hand, eqMD-M Φ ^{IL-4/IL-13} exhibited higher mRNA transcription of *MMP-3* ($p < 0.05$; Fig. 23E) when compared to eqMD-M Φ ^{IFN γ /LPS}.

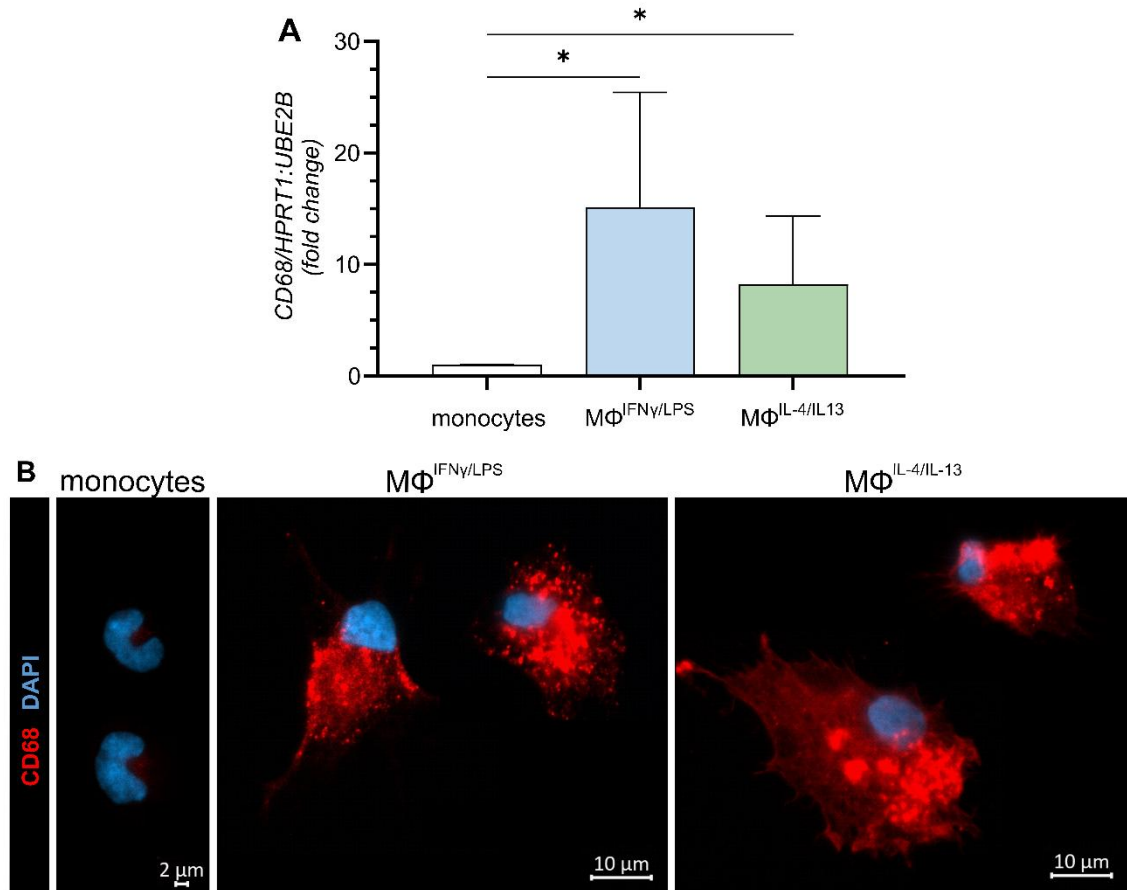


Figure 21. The mRNA transcription of *CD68* in equine monocytes, eqMD-MΦ^{IFNγ/LPS}, and eqMD-MΦ^{IL-4/IL-13} (A; n=6). Results were calculated as the mean ± standard deviation (SD) and expressed as a fold change. Asterisks indicate statistical differences (*p<0.05). Representative immunofluorescence staining of CD68 (B) monocytes, eqMD-MΦ^{IFNγ/LPS} and eqMD-MΦ^{IL-4/IL-13}. The scale bar = 2 μm for monocytes and 10 μm for MΦ (magnification: x63).

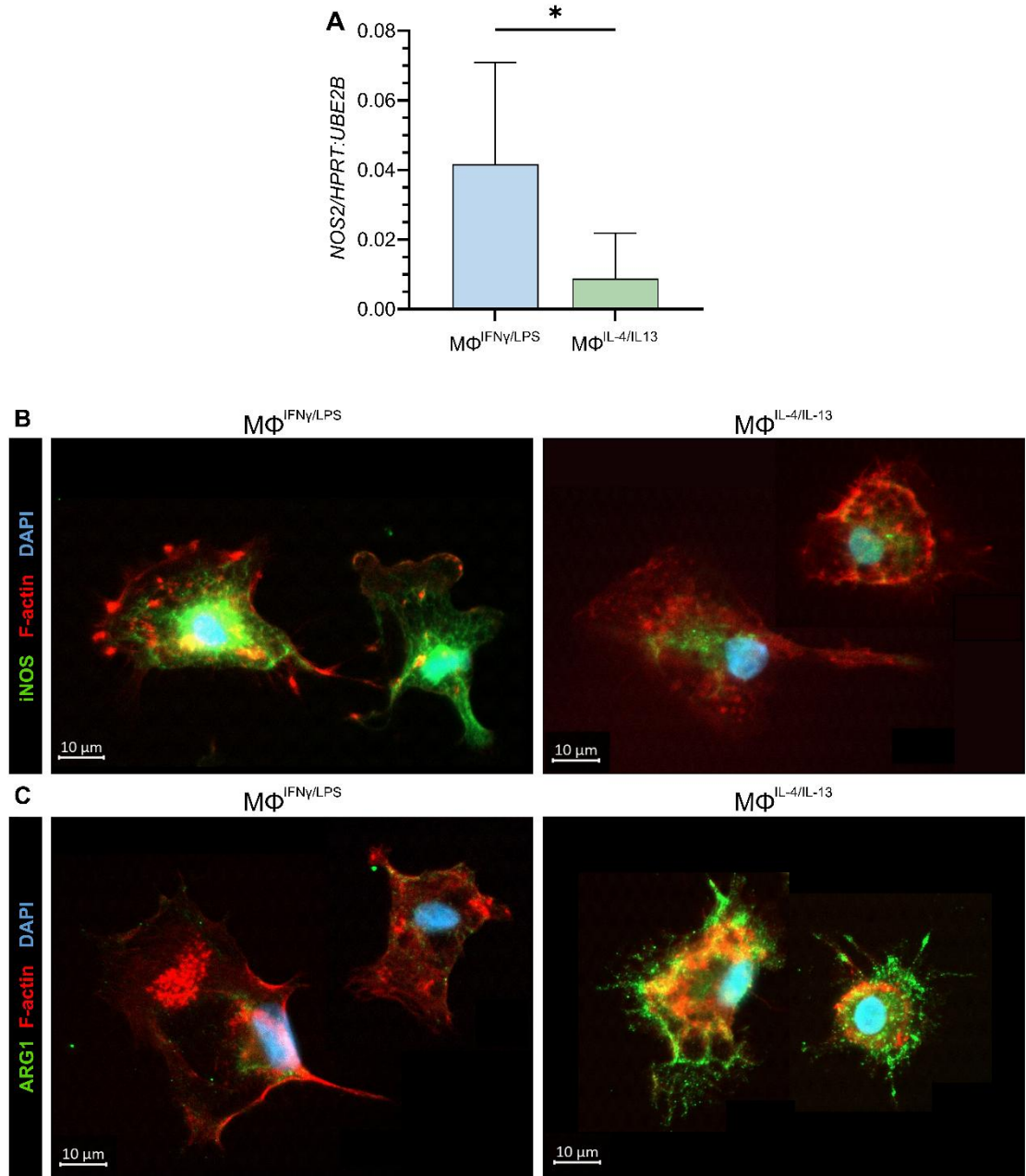


Figure 22. The mRNA transcription of *NOS2* (A) in eqMD- $M\Phi^{IFN\gamma/LPS}$ and eqMD- $M\Phi^{IL-4/IL-13}$ (n=6). Results were calculated as the mean $\pm$ standard deviation (SD) and expressed as a fold change. Asterisks indicate statistical differences (* $p < 0.05$) as determined by Student's t-test. Representative immunofluorescence staining of iNOS (B) and ARG1 (C) in equine eqMD- $M\Phi^{IFN\gamma/LPS}$ and eqMD- $M\Phi^{IL-4/IL-13}$. The scale bar = 10 μm (magnification: x63).

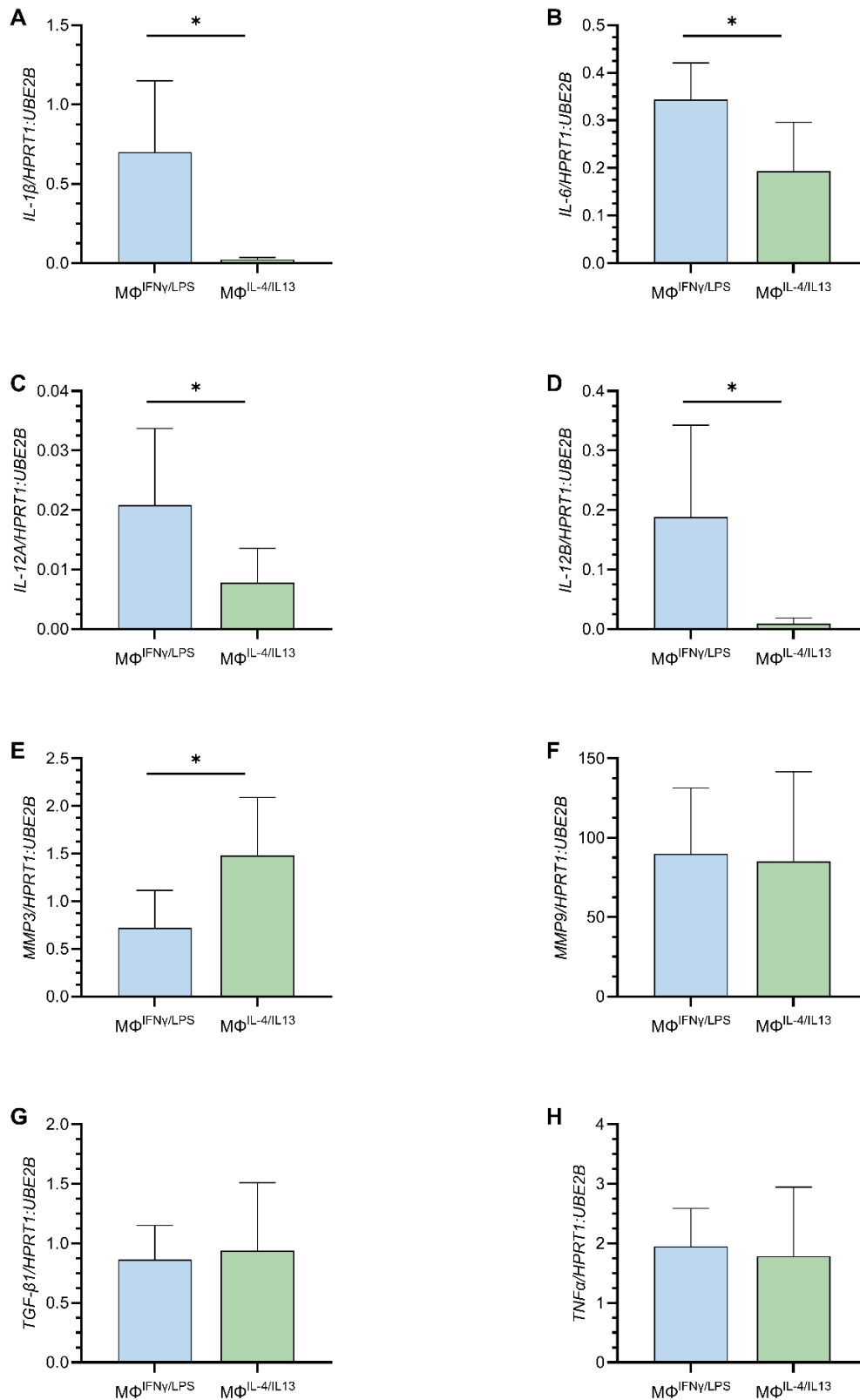


Figure 23. The mRNA transcription of *IL-1 β* (A), *IL-6* (B), *IL-12A* (C), *IL-12B* (D), *MMP-3* (E), *MMP-9* (F), *TGF- β 1* (G) and *TNF- α* (H) in eqMD-M $\Phi^{IFN\gamma/LPS}$ and eqMD-M $\Phi^{IL-4/IL13}$ (n=6). Results were calculated as the mean $\pm$ standard deviation (SD) and presented in arbitrary units. Asterisks indicate statistical differences (* $p < 0.05$) as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution.

6.2.1.2. Experiment 1.2. The proteomic profile of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}

A total of 5500 proteins were identified in eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}, of which 548 were considered as differentially expressed proteins (DEPs). Supplementary Table 1 contains complete lists of all DEPs identified in eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}. The PCA analysis revealed an acceptable clustering of samples within studied eqMD-M Φ subpopulations and indicated the presence of differences in proteomic profiles between eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} (Fig. 24A). Among all DEPs, 97 proteins had significantly higher expression in eqMD-M Φ ^{IFN γ /LPS} than in eqMD-M Φ ^{IL-4/IL-13}. The top 5 DEPs with the highest expression in M Φ ^{IFN γ /LPS} were EPB41L3, LOC100058990, CMKLR1, MX2, and LOC100630879. Simultaneously, 52 proteins had significantly higher expression in eqMD-M Φ ^{IL-4/IL-13} than in eqMD-M Φ ^{IFN γ /LPS}. The top 5 DEPs with the highest expression in eqMD-M Φ ^{IL-4/IL-13} were CLEC10A, SLC29A1, ALDH1A2, SPP1, and TNF. The rest of the identified DEPs showed similar level of expression in both eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} (Fig. 24B).

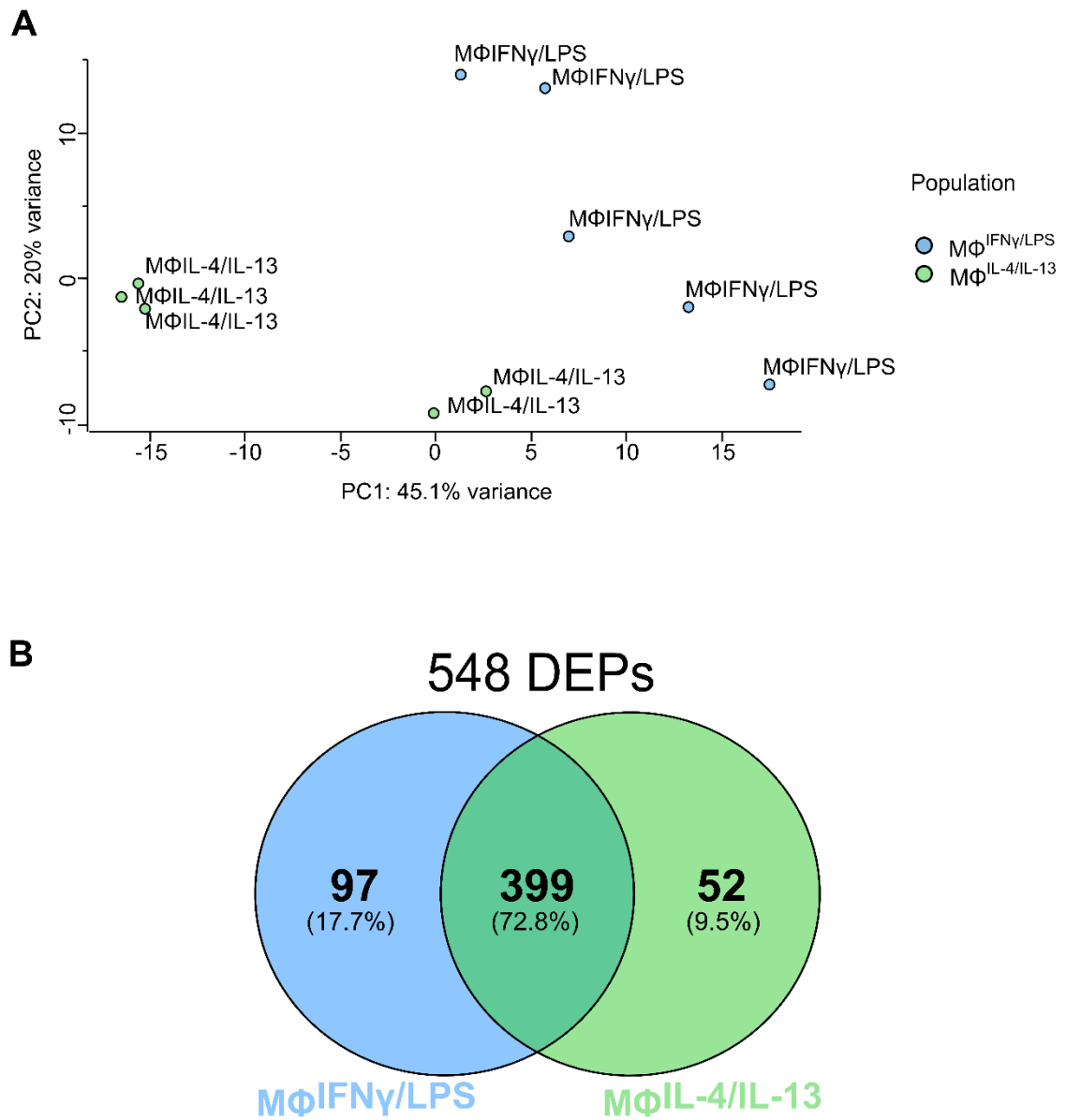


Figure 24. Graphical presentation of principal components analysis (PCA) of the protein abundance pattern in eqMD-MΦ^{IFNγ/LPS} and eqMD-MΦ^{IL-4/IL-13} (A). Venn diagram showing shared and unique differentially expressed proteins (DEPs; $q\text{-value} \leq 0.05$, $FC < -1/FC > 1$) identified in eqMD-MΦ^{IFNγ/LPS} and eqMD-MΦ^{IL-4/IL-13} (B).

Basic functional analysis using the STRING database (with incorporated Reactome database) (Szkarczyk et al., 2023) of DEPs identified in eqMD-M Φ ^{IFN γ /LPS} revealed significant enrichment of: 28 GO: BP, 6 GO: MF, 4 GO: CC, 11 KEGG pathways and 12 Reactome pathways (Fig. 25). Among the enriched were: defense response to virus (GO:0051607), response to virus (GO:0009615), exopeptidase activity (GO:0008238), serine-type carboxypeptidase activity (GO:0004185), lysosome (GO:0005764), cytoplasm (GO:0005737), lysosome (KEGG ID:ecb04142), necroptosis (KEGG ID:ecb04217), immune system (Reactome ID: MAP-168256) and interferon signaling (Reactome ID: MAP-913531). An overview of selected enriched terms is presented in Table 2.

Table 2. An overview of selected terms enriched by differentially expressed proteins (DEPs) identified in eqMD-M Φ ^{IFN γ /LPS}

Term	Term ID	Term description	DEPs count	Strength	Signal	FDR
GO: BP	GO:0051607	Defense response to virus	16	1,31	2,46	2,78E-12
GO: BP	GO:0009615	Response to virus	17	1,21	2,28	3,05E-12
GO: MF	GO:0008238	Exopeptidase activity	7	1,25	0,87	6,10E-04
GO: MF	GO:0004185	Serine-type carboxypeptidase activity	3	2,26	0,76	3,40E-03
GO: CC	GO:0005764	Lysosome	17	1,03	1,66	9,60E-10
GO: CC	GO:0005737	Cytoplasm	62	0,16	0,3	7,50E-03
KEGG	ecb04142	Lysosome	9	1,28	1,51	7,28E-07
KEGG	ecb04217	Necroptosis	9	1,22	1,43	1,12E-06
Reactome	MAP-168256	Immune System	45	0,61	1,15	5,62E-15
Reactome	MAP-913531	Interferon Signaling	14	1,39	2,64	1,59E-12

Analysis of DEPs identified in eqMD-M Φ ^{IFN γ /LPS} allowed to indicate potential protein interactions between proteins and distinguish two main clusters of interconnected proteins. Superimposing the Reactome pathways, data revealed that linked proteins are associated with the immune system, while cluster A contains proteins associated with cytokine signaling in the immune system, interferon signaling, and interferon alpha/beta signaling, while cluster B contains proteins associated with the innate immune system, adaptive immune system and MHC class II antigen presentation (Fig. 26).



Figure 25. Gene ontology, KEGG pathways, and Reactome pathways enriched by differentially expressed proteins (DEPs; $q\text{-value} \leq 0.05$, $\text{FC} < -1/\text{FC} > 1$) identified in eqMD-M $\Phi^{\text{IFN}\gamma/\text{LPS}}$. The vertical axis shows the identified GO terms and KEGG pathways, while the horizontal axis indicates the enrichment ($-\log_{10}\text{FDR}$) of given processes. The size of bubbles refers to the count of DEPs annotated to each term or pathway.

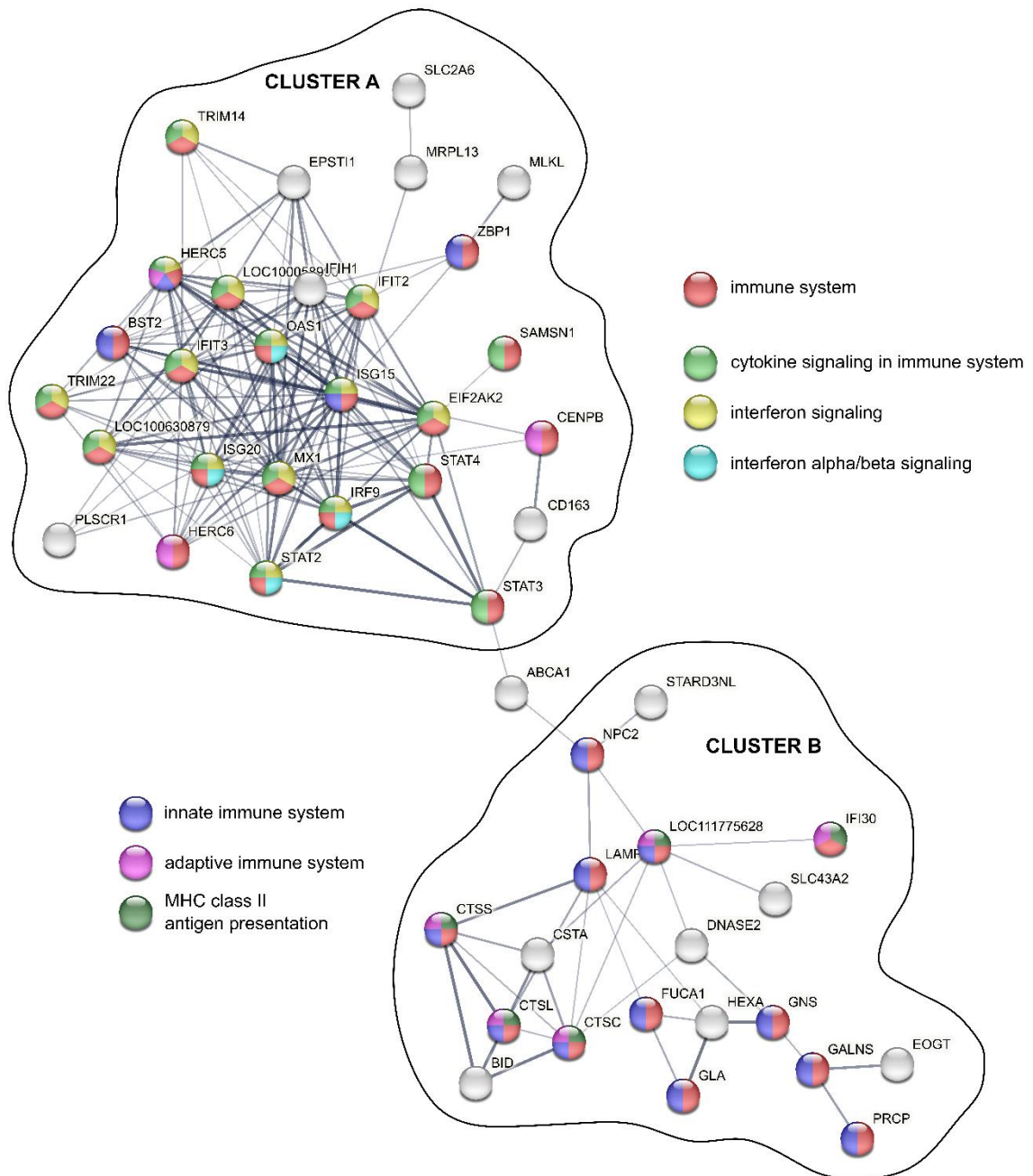


Figure 26. A map of potential protein interactions of differentially expressed proteins (DEPs; $q\text{-value} \leq 0.05$, $FC < -1/FC > 1$) identified in eqMD-M $\Phi^{IFN\gamma/LPS}$ that include direct physical and indirect functional protein linkages. The thickness of the line reflects confidence in the protein-protein interaction. Examples of the enriched Reactome pathways (medium confidence [0.400]; FDR stringency: medium [5 percent]) are marked with colors.

The functional analysis of DEPs identified in eqMD-M Φ ^{IL-4/IL-13} revealed significant enrichment of 20 GO: BP, 4 GO: MF, 17 KEGG pathways, and 11 Reactome pathways (Fig. 27). Among the enriched were cell adhesion (GO:0007155), integrin-mediated signaling pathway (GO:0007229), integrin binding (GO:0005178), fibronectin binding (GO:0001968), ECM-receptor interaction (KEGG ID:ecb04512), focal adhesion (KEGG ID: ecb04510), extracellular matrix organization (Reactome ID: MAP-1474244), and signaling by TGF-beta receptor complex (Reactome ID: MAP-170834). An overview of selected enriched terms is presented in Table 3.

Table 3. An overview of selected terms enriched by differentially expressed proteins (DEPs) identified in eqMD-M Φ ^{IL-4/IL-13}

Term	Term ID	Term description	DEPs count	Strength	Signal	FDR
GO: BP	GO:0007155	Cell adhesion	10	0,78	0,46	1,70E-02
GO: BP	GO:0007229	Integrin-mediated signaling pathway	4	1,36	0,48	3,51E-02
GO: MF	GO:0005178	Integrin binding	7	1,39	1,24	5,99E-05
GO: MF	GO:0001968	Fibronectin binding	3	1,77	0,58	1,99E-02
KEGG	ecb04512	ECM-receptor interaction	5	1,38	0,93	9,20E-04
KEGG	ecb04510	Focal adhesion	5	1,05	0,65	5,20E-03
Reactome	MAP-1474244	Extracellular matrix organization	9	1,02	0,96	1,00E-04
Reactome	MAP-170834	Signaling by TGF-beta Receptor Complex	4	1,25	0,55	1,85E-02

Further analysis of DEPs identified in eqMD-M Φ ^{IL-4/IL-13} indicated that this group of proteins is less interconnected in comparison to DEPs identified in equine eqMD-M Φ ^{IFN γ /LPS}. One main interconnection network was observed with a core created of 4 proteins (FN1, ITGAV, ITGB5, ITGB3) annotated to all Reactome pathways, associated mainly with ECM interactions and TGF- β signaling (Fig. 28).



Figure 27. Gene ontology, KEGG pathways, and Reactome pathways enriched by differentially expressed proteins (DEPs) identified in eqMD-M $\Phi^{\text{IL-4/IL-13}}$. The vertical axis shows the identified GO terms and KEGG pathways, while the horizontal axis indicates the enrichment ($-\log_{10}(\text{FDR})$) of given processes. The size of bubbles refers to the count of DEPs annotated to each term or pathway.

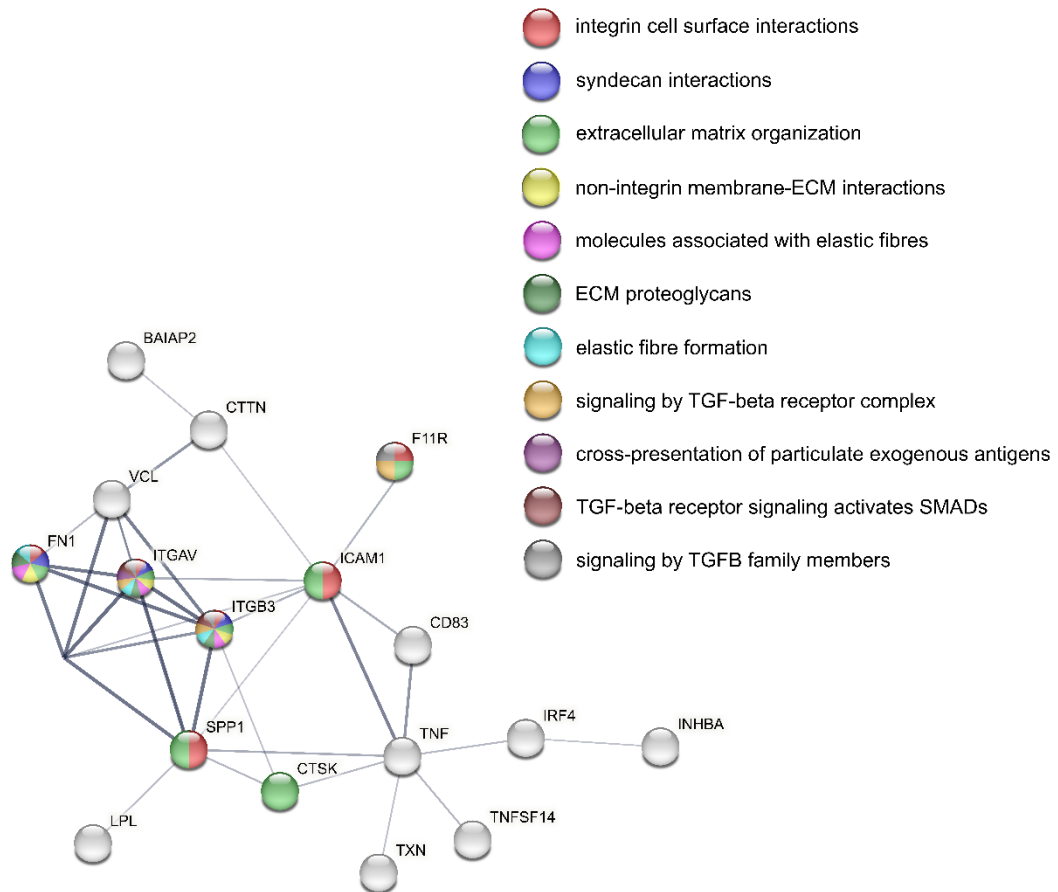


Figure 28. A map of potential protein interactions of differentially expressed proteins (DEPs) identified in eqMD-M Φ ^{IL-4/IL-13} that include direct physical and indirect functional protein linkages. The thickness of the line reflects confidence in the protein-protein interaction. Examples of the enriched Reactome pathways (medium confidence [0.400]; FDR stringency: medium [5 percent]) are marked with colors.

The functional analysis of DEPs considered as common for eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} revealed significant enrichment of 131 GO: BP, 38 GO: MF, 51 GO: CC, 15 KEGG pathways, and 28 Reactome pathways. Among them, several GO were notable for concentrating most of the analyzed proteins: cellular process (GO:0009987), metabolic process (GO:0008152), binding (GO:0005488), catalytic activity (GO:0003824), cellular anatomical entity (GO:0110165), intracellular anatomical structure (GO:0005622). An overview of selected enriched terms is presented in Table 4.

Table 4. An overview of selected terms enriched by differentially expressed proteins (DEPs) identified as common for eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}

Term	Term ID	Term description	DEPs count	Strength	Signal	FDR
GO: BP	GO:0009987	Cellular process	326	0,05	0,31	7,90E-03
GO: BP	GO:0008152	Metabolic process	196	0,12	0,45	2,40E-04
GO: MF	GO:0005488	Binding	285	0,06	0,32	7,20E-03
GO: MF	GO:0003824	Catalytic activity	160	0,16	0,48	1,30E-04
GO: CC	GO:0110165	Cellular, anatomical entity	386	0,02	0,42	4,34E-05
GO:CC	GO:0005622	Intracellular anatomical structure	340	0,11	0,68	4,21E-17

6.2.2. Experiment 2. The effect of the eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome on the ECM-associated components and inflammatory marker expression in endometrial fibroblasts

6.2.2.1. Experiment 2.1. The effect of the eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome on the expression of the ECM-associated components in mare endometrial fibroblasts

The eqMD-M Φ ^{IFN γ /LPS}-CM did not affect, while the M Φ ^{IL-4/IL-13}-CM decreased *COL1A1* mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p>0.05$ and $p<0.01$ respectively; Fig. 29A). On the other hand, the protein abundance of COL1 in mare endometrial fibroblasts was not affected either by the eqMD-M Φ ^{IFN γ /LPS}-CM or eqMD-M Φ ^{IL-4/IL-13}-CM when compared to the control group after 96 hours of treatment ($p>0.05$; Fig. 29B). The immunofluorescence staining confirmed the presence of COL1 in the cytoplasm of mare endometrial fibroblasts after treatment for 96 hours (Fig. 29C).

Both, the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM increased *COL3A1* mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p<0.05$; Fig. 30A). Simultaneously, neither the eqMD-M Φ ^{IFN γ /LPS}-CM or eqMD-M Φ ^{IL-4/IL-13}-CM affected the protein abundance of COL3 in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p>0.05$; Fig 30B). The immunofluorescence staining confirmed the presence of COL3 in the cytoplasm of mare endometrial fibroblasts after treatment for 96 hours (Fig. 30C).

The eqMD-M Φ ^{IFN γ /LPS}-CM decreased, while the eqMD-M Φ ^{IL-4/IL-13}-CM did not affect *FN1* mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p<0.01$ and $p>0.05$ respectively; Fig. 31A). On the contrary, the eqMD-M Φ ^{IFN γ /LPS}-CM did not affect, while eqMD-M Φ ^{IL-4/IL-13}-CM increased the protein abundance of FN1 in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p>0.05$ and $p<0.01$ respectively; Fig 31B). The immunofluorescence staining confirmed the presence of FN1 in the cytoplasm of mare endometrial fibroblasts after treatment for 96 hours (Fig. 31C).

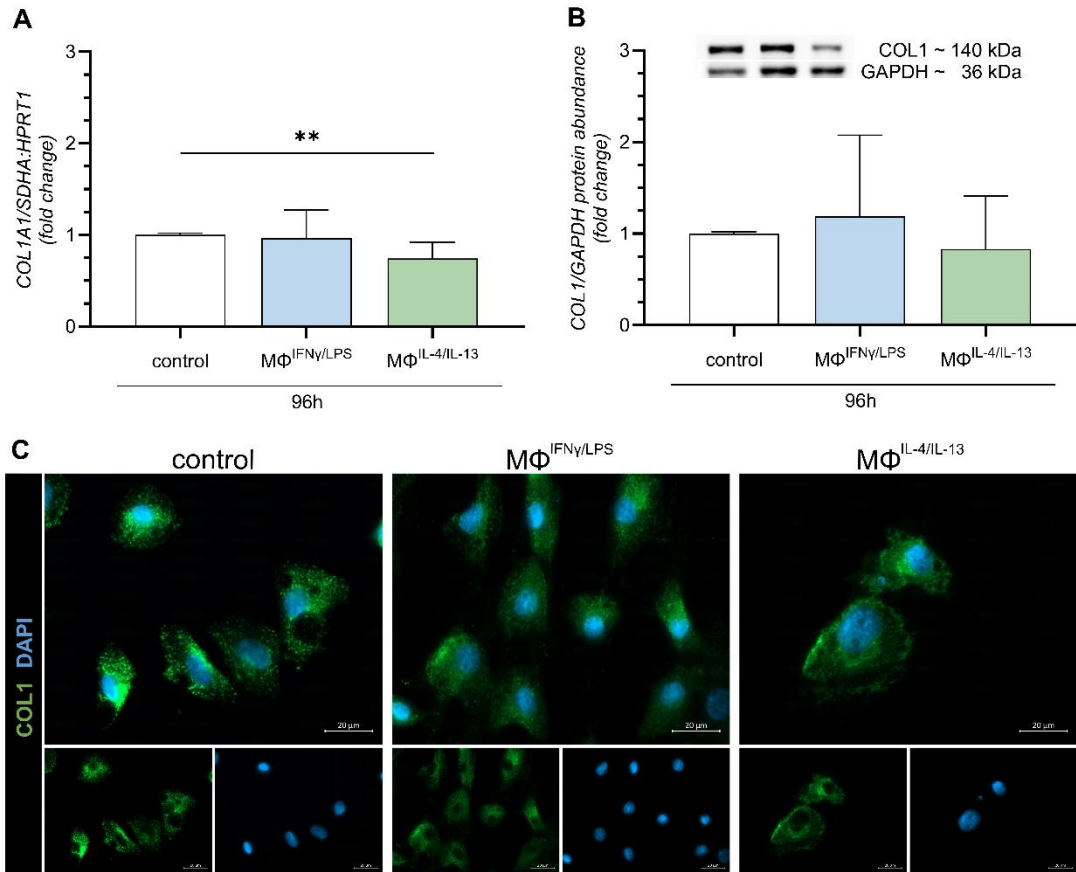


Figure 29. The effect of the eqMD-MΦ^{IFNγ/LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM on *COL1A1* mRNA transcription (A) and COL1 protein abundance (B) in fibroblasts derived from category I endometria (n=6) after 96 hours of treatment. Results were calculated as the mean $\pm$ standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (**p < 0.01) from the control group as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution. Representative immunofluorescence staining of COL1 (C) in mare endometrial fibroblasts after eqMD-MΦ-CM treatment. The scale bar = 20 μ m (magnification: x63).

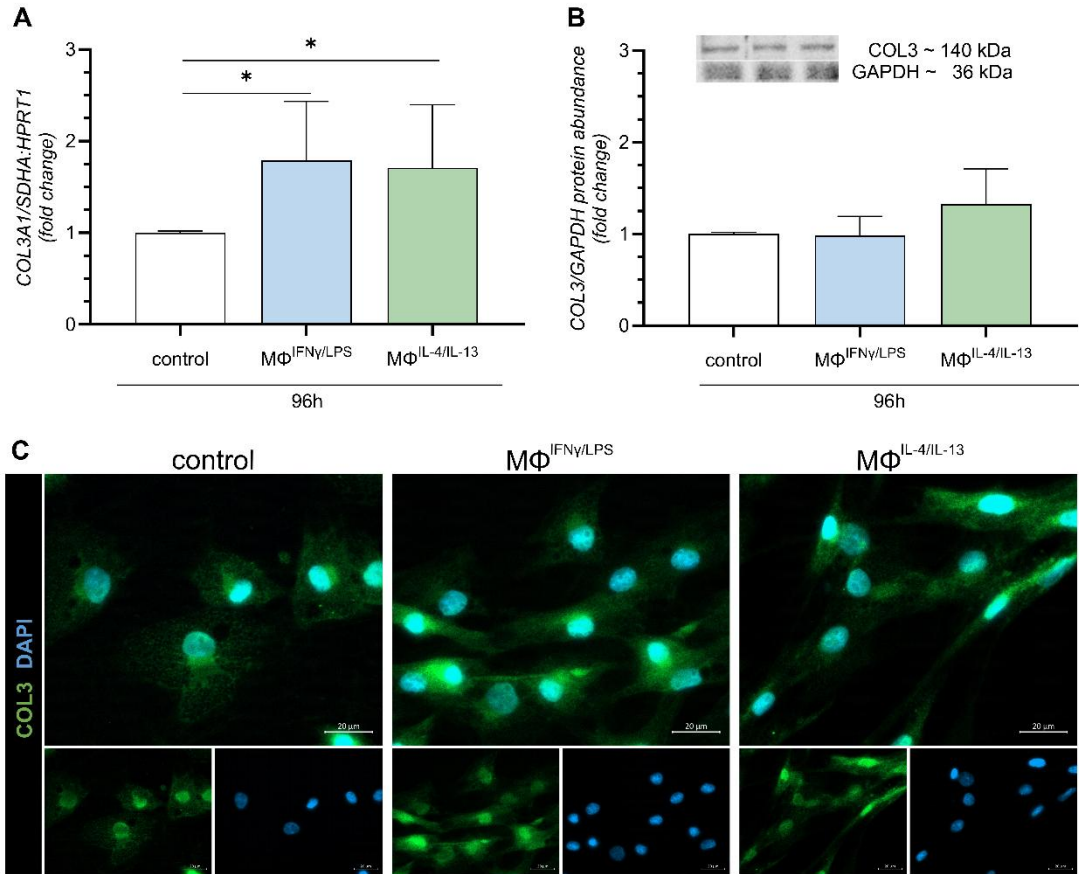


Figure 30. The effect of the eqMD-MΦ^{IFNγ/LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM on *COL3A1* mRNA transcription (A) and COL3 protein abundance (B) in fibroblasts derived from category I endometria (n=6) after 96 hours of treatment. Results were calculated as the mean ± standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (**p < 0.01) from the control group as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution. Representative immunofluorescence staining of COL3 (C) in mare endometrial fibroblasts after eqMD-MΦ-CM treatment. The scale bar = 20 μm (magnification: x63).

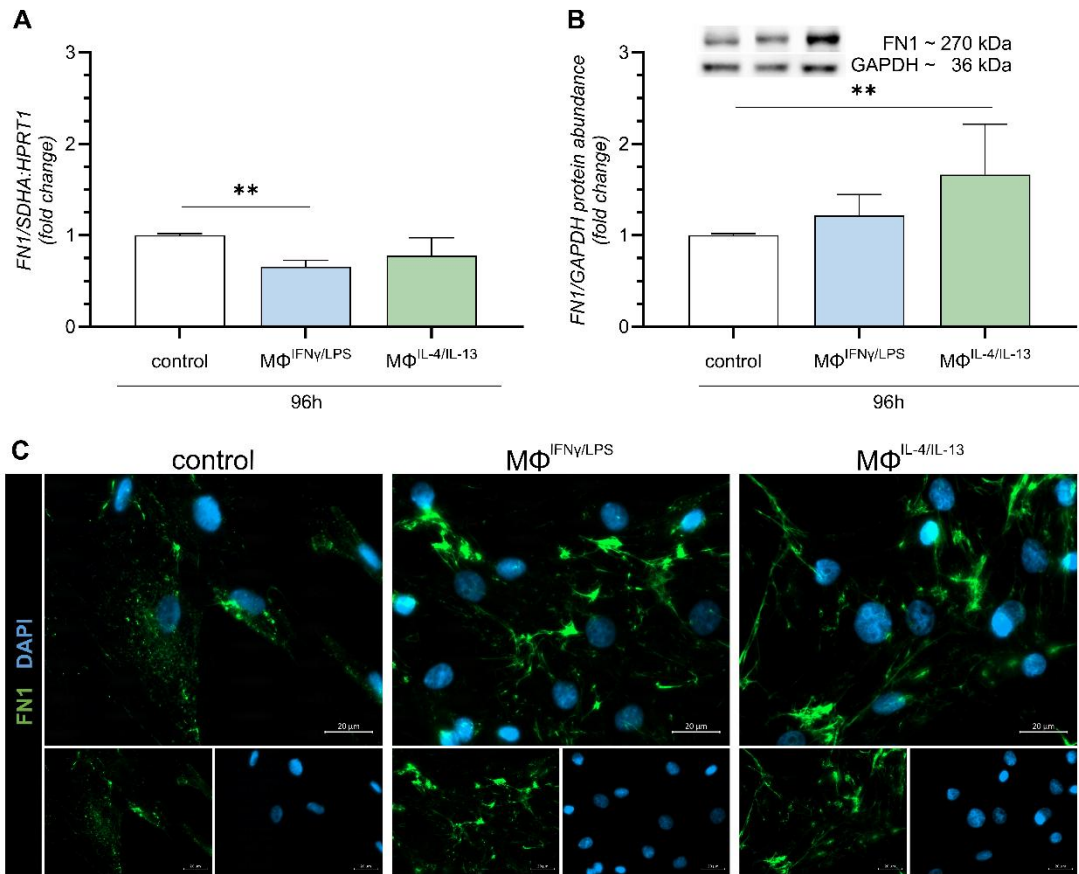


Figure 31. The effect of the eqMD-MΦ^{IFNγ/LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM on *FN1* mRNA transcription (A) and FN1 protein abundance (B) in fibroblasts derived from category I endometria (n=6) after 96 hours of treatment. Results were calculated as the mean ± standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (**p < 0.01) from the control group as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution. Representative immunofluorescence staining of FN1 (C) in mare endometrial fibroblasts after eqMD-MΦ-CM treatment. The scale bar = 20 μm (magnification: x63).

Both the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM decreased *ACTA2* mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p < 0.001$ and $p < 0.01$ respectively; Fig. 32A). Simultaneously, only the eqMD-M Φ ^{IFN γ /LPS}-CM decreased the protein abundance of *ACTA2* when compared to the control group after 96 hours of treatment ($p < 0.01$; Fig. 32B). The immunofluorescence staining confirmed the presence of *ACTA2* in the cytoplasm and nucleus of mare endometrial fibroblasts after treatment for 96 hours (Fig. 32C).

Both the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM had no effect on *MMP-2* mRNA transcription and protein abundance of *MMP-2* in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p > 0.05$; Fig. 33A, B). The immunofluorescence staining confirmed the presence of *MMP-2* in the cytoplasm, especially close to the nucleus, in mare endometrial fibroblasts after treatment for 96 hours (Fig. 33C).

The eqMD-M Φ ^{IFN γ /LPS}-CM did not affect, while the eqMD-M Φ ^{IL-4/IL-13}-CM decreased *MMP-3* mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p > 0.05$ and $p < 0.0001$ respectively; Fig 34A). On the contrary, the eqMD-M Φ ^{IFN γ /LPS}-CM and the eqMD-M Φ ^{IL-4/IL-13}-CM had no effect on protein abundance of *MMP-3* in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p > 0.05$; Fig. 34B). The immunofluorescence staining confirmed the presence of *MMP-3* in the cytoplasm, especially close to the nucleus, in mare endometrial fibroblasts after treatment for 96 hours (Fig. 34C).

The eqMD-M Φ ^{IFN γ /LPS}-CM decreased while the eqMD-M Φ ^{IL-4/IL-13}-CM did not affect the *MMP-9* mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p < 0.05$ and $p > 0.05$ respectively; Fig. 35A). On the contrary, both the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM increased the protein abundance of *MMP-9* in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p < 0.01$ and $p < 0.05$ respectively; Fig. 35B). The immunofluorescence staining and confirmed the presence of *MMP-9* in the nucleus and in the cytoplasm near the nucleus in mare endometrial fibroblasts after treatment for 96 hours (Fig. 35C).

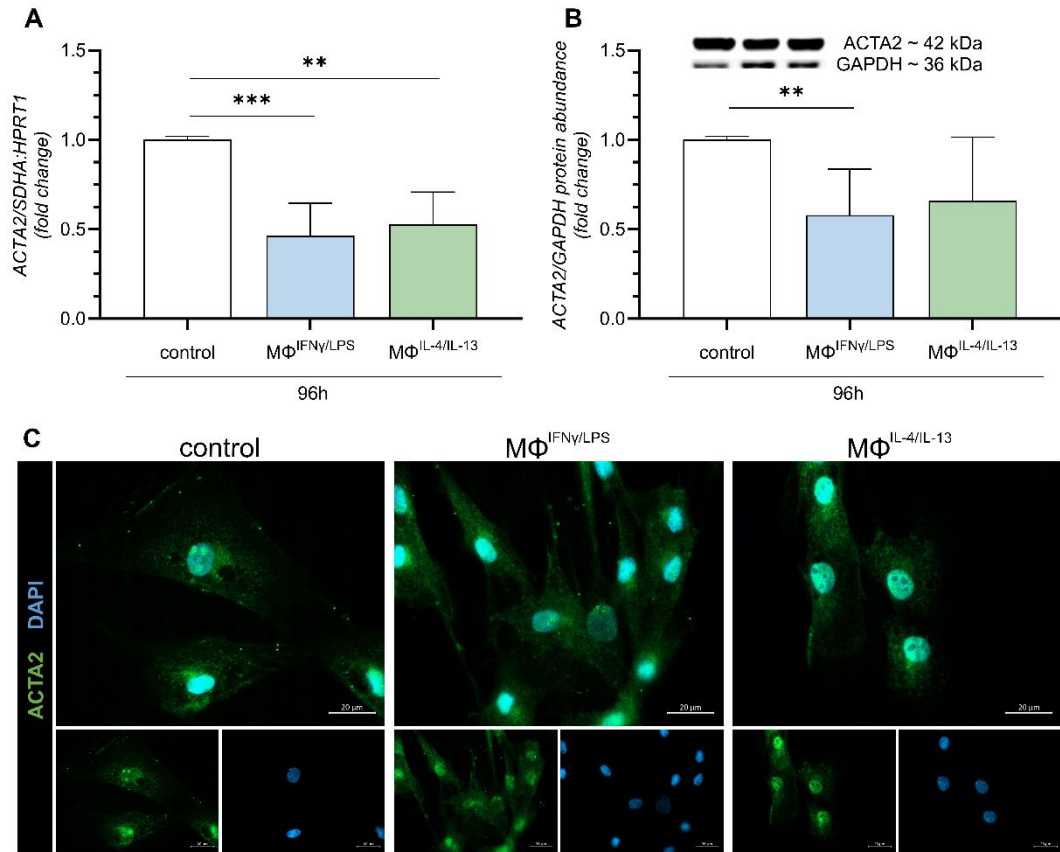


Figure 32. The effect of the eqMD-MΦ^{IFNγ/LPS} or eqMD-MΦ^{IL-4/IL-13} on ACTA2 mRNA transcription (A) and ACTA2 protein abundance (B) in fibroblasts derived from category I endometria (n=6) after 96 hours of treatment. Results were calculated as the mean $\pm$ standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (**p < 0.01) from the control group as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution. Representative immunofluorescence staining of ACTA2 (C) in mare endometrial fibroblasts after eqMD-MΦ-CM treatment. The scale bar = 20 μ m (magnification: x63).

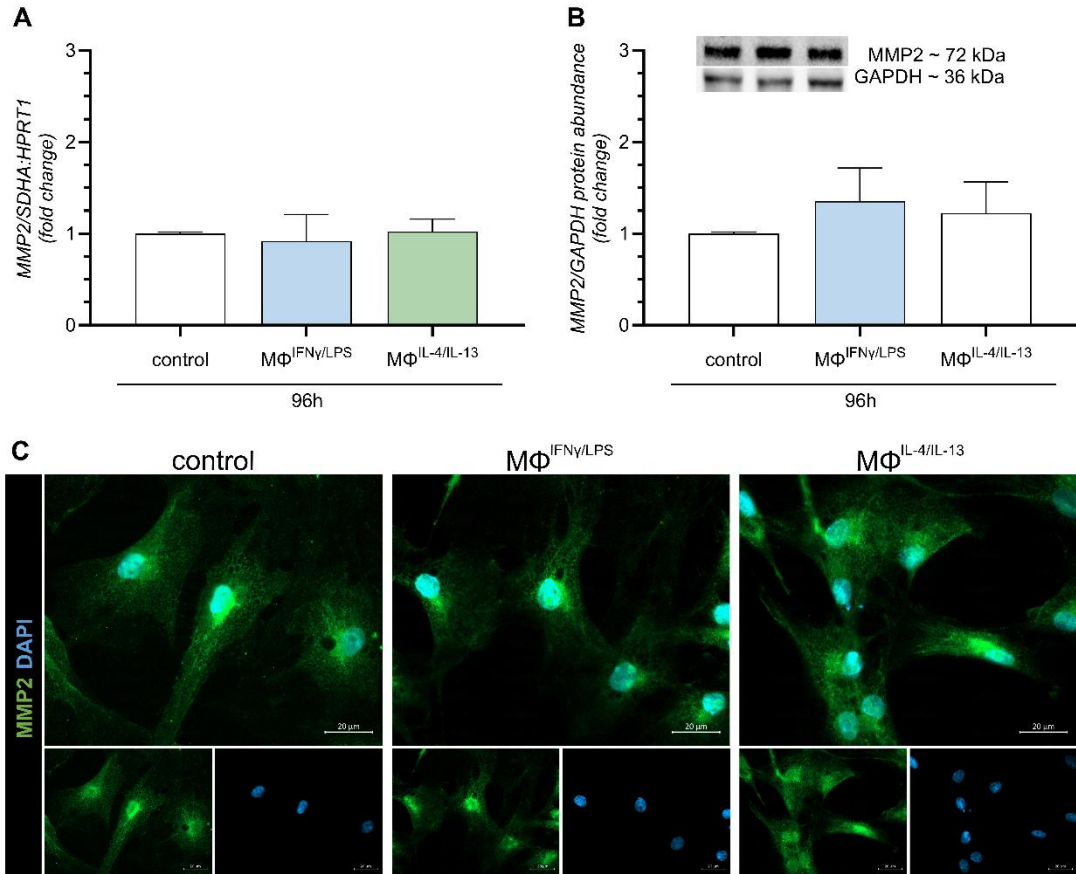


Figure 33. The effect of the eqMD-MΦ^{IFNγ/LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM on *MMP-2* mRNA transcription (A) and *MMP-2* protein abundance (B) in fibroblasts derived from category I endometria (n=6) after 96 hours of treatment. Results were calculated as the mean ± standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (**p < 0.01) from the control group as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution. Representative immunofluorescence staining of *MMP-2* (C) in mare endometrial fibroblasts after eqMD-MΦ-CM treatment. The scale bar = 20 μm (magnification: x63).

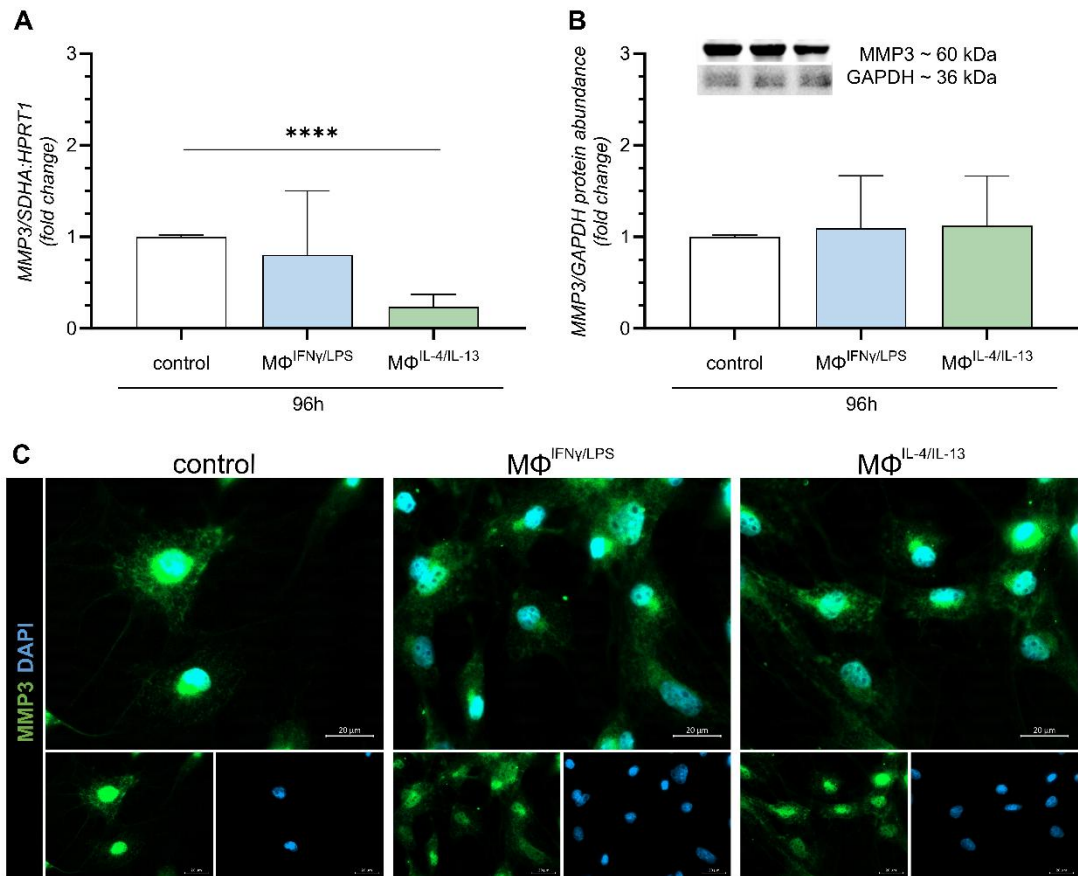


Figure 34. The effect of the eqMD-MΦ^{IFNγ/LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM on *MMP-3* mRNA transcription (A) and *MMP-3* protein abundance (B) in fibroblasts derived from category I endometria (n=6) after 96 hours of treatment. Results were calculated as the mean ± standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (**p < 0.01) from the control group as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution. Representative immunofluorescence staining of *MMP-3* (C) in mare endometrial fibroblasts after eqMD-MΦ-CM treatment. The scale bar = 20 μm (magnification: x63).

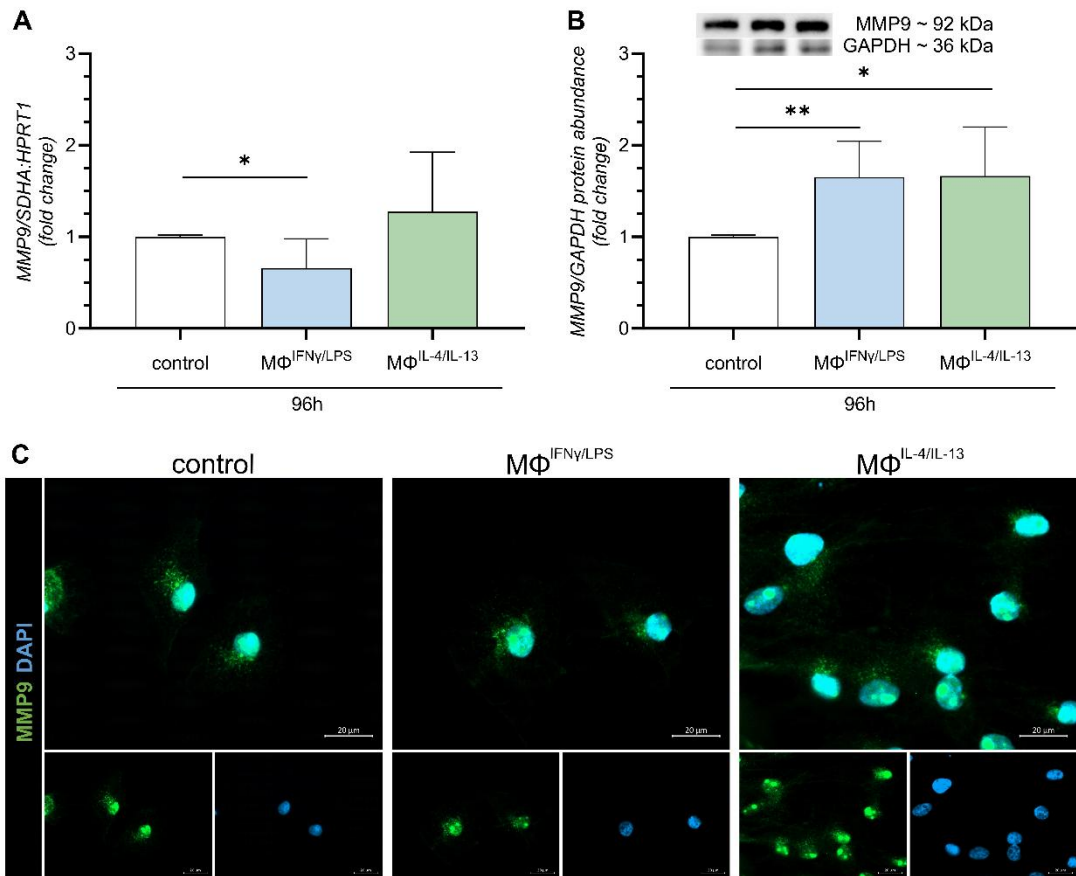


Figure 35. The effect of the eqMD-MΦ^{IFNγ/LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM on *MMP-9* mRNA transcription (A) and *MMP-9* protein abundance (B) in fibroblasts derived from category I endometria (n=6) after 96 hours of treatment. Results were calculated as the mean ± standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (**p < 0.01) from the control group as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution. Representative immunofluorescence staining of *MMP-9* (C) in mare endometrial fibroblasts after eqMD-MΦ-CM treatment. The scale bar = 20 μm (magnification: x63).

The eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM did not affect, while the eqMD-M $\Phi^{IL-4/IL-13}$ -CM increased *TIMP-1* mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p < 0.05$; Fig. 36A). The basal secretion of TIMP-1 was $34,413 \pm 11,709$ ng/ml. The eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM decreased the concentration of TIMP-1 in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p < 0.01$; Fig. 36B).

The eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM increased, while the eqMD-M $\Phi^{IL-4/IL-13}$ -CM did not affect *TIMP-2* mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p < 0.01$; Fig. 36C). The basal secretion of TIMP-2 was $413,219 \pm 234,952$ ng/ml. The eqMD-M $\Phi^{IL-4/IL-13}$ -CM decreased the concentration of TIMP-2 in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p < 0.01$; Fig. 36D).

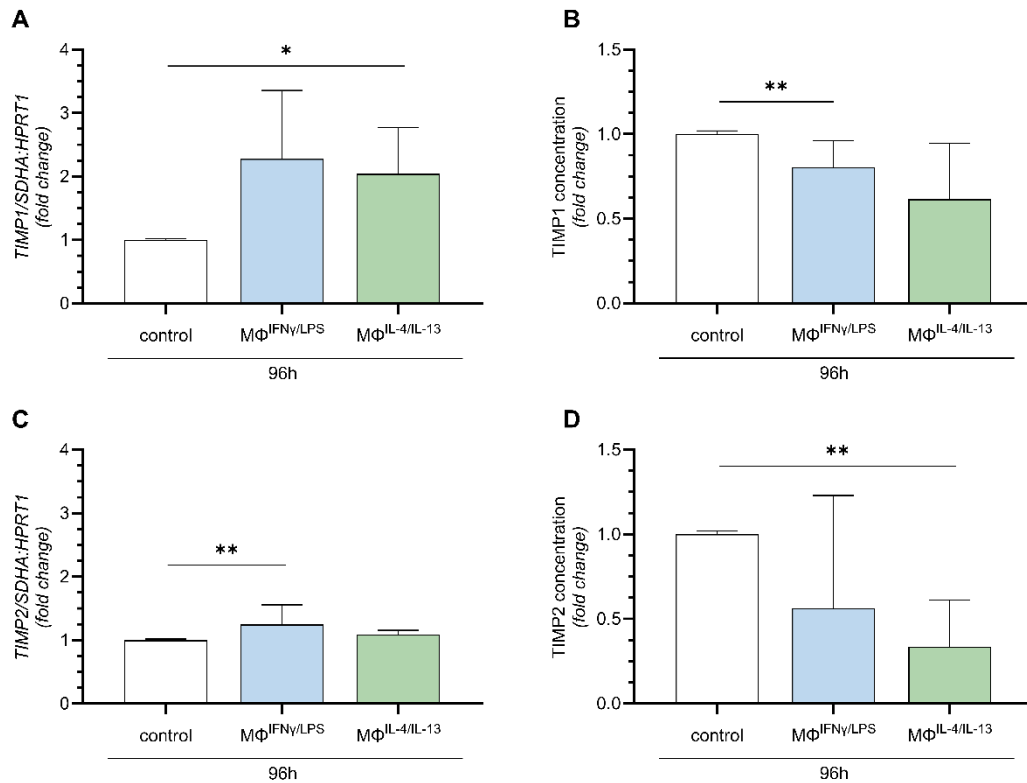


Figure 36. The effect of the eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM or eqMD-M $\Phi^{IL-4/IL-13}$ -CM on *TIMP-1* mRNA transcription (A) and TIMP-1 concentration (B), *TIMP-2* mRNA transcription (C) and TIMP-2 concentration in fibroblasts derived from category I endometria (n=6) after 96 hours of treatment. Results were calculated as the mean $\pm$ standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (* $p < 0.05$; ** $p < 0.01$) from the control group as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution.

6.2.2.2. Experiment 2.2. The effect of the eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome on the expression of inflammatory markers in mare endometrial fibroblasts

The eqMD-M Φ ^{IFN γ /LPS}-CM decreased *CCN2* mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment ($p < 0.05$; Fig. 37B).

The eqMD-M Φ ^{IL-4/IL-13}-CM decreased *AREG* ($p < 0.05$; Fig. 37A), *IL-1 β* ($p < 0.001$; Fig. 37C) mRNA transcription and increased *TNF- α* ($p < 0.05$; Fig. 37F) mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment.

Both the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM increased *IL-6* ($p < 0.05$; Fig. 37D) mRNA transcription and decreased *TGF- β 1* ($p < 0.0001$ and $p < 0.001$ respectively; Fig. 37E) mRNA transcription in mare endometrial fibroblasts as compared to the control group after 96 hours of treatment.

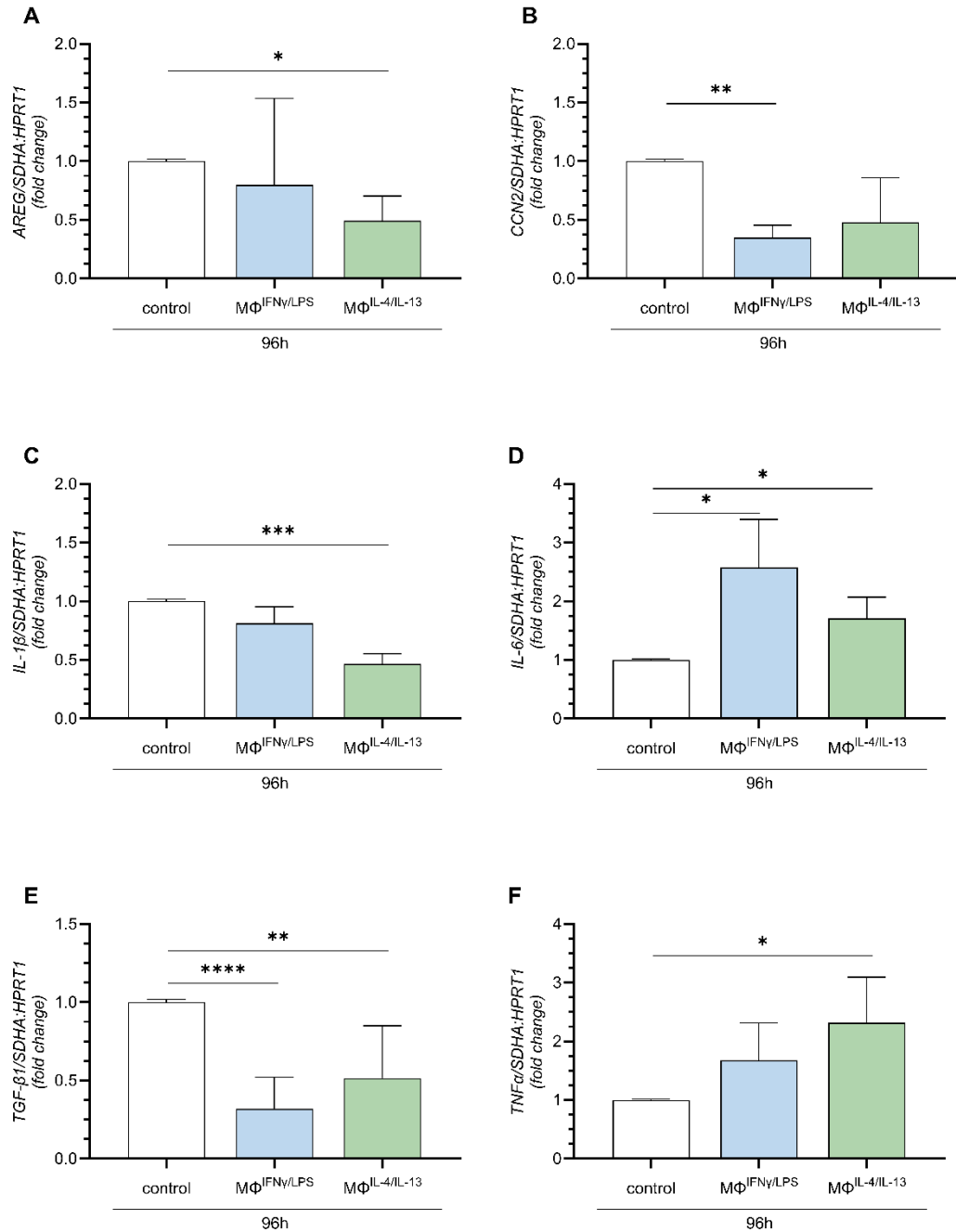


Figure 37. The effect of the eqMD-MΦ^{IFNγ/LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM on *AREG* mRNA transcription (A), *CCN2* mRNA transcription (B), *IL-1β* mRNA transcription (C), *IL-6* mRNA transcription (D), *TNF-α* mRNA transcription (E) and *TGF-β1* mRNA transcription (F) in fibroblasts derived from category I endometria (n=6) after 96 hours of treatment. Results were calculated as the mean ± standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001) from the control group as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution.

6.2.3. Experiment 3. The effect of the eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome on the transcriptome of endometrial fibroblasts

Following filtering and sequence removal, sequencing yielded an average of 18 031 781 reads, of which 17 406 842 were uniquely mapped to the *Equus caballus* reference genome (EquCab3.0). The summary of RNA sequencing reads in endometrial fibroblasts after treatment with eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours is presented in Supplementary Table 2. The PCA analysis tentatively revealed that both the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM affect the transcriptomic profile of mare endometrial fibroblasts (Fig. 38A)

In response to the eqMD-M Φ ^{IFN γ /LPS}-CM treatment, alterations in the expression of 404 genes were demonstrated, of which 211 were up-regulated and 193 were down-regulated (Fig. 38B). The top 5 up-regulated DEGs were *CXCL9*, *CYP2C113*, *SNCB*, *SLC8A2*, *CHI3L1*, while top 5 down-regulated DEGs were *IGF1*, *CCDC3*, *C10H6orf58*, *GREB1*, *PRLR*. Supplementary Table 3 contains complete lists of all DEGs detected in endometrial fibroblasts after treatment with the eqMD-M Φ ^{IFN γ /LPS}-CM for 96 hours, along with their Log2FC and p-adjusted values. Simultaneously, alterations in the expression of 282 genes were shown in response to the eqMD-M Φ ^{IL-4/IL-13}-CM treatment of which 137 were up-regulated and 145 were down-regulated (Fig. 38B). The top 5 up-regulated DEGs were *CXCL9*, *SLC8A2*, *OASL*, *CYBB*, *INSRR*, while the top 5 down-regulated DEGs were *CCDC3*, *C10h6ORF58*, *ODAM*, *PTPRZ1*, *RHOV*. Supplementary Table 4 contains complete lists of all DEGs detected in endometrial fibroblasts after treatment with the eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours, along with their Log2FC and p-adjusted values. Each comparison revealed several novel genes.

To visualize whether changes in the mRNA transcription of mare endometrial fibroblasts were specific to the effect of particular M Φ subpopulation or shared across both, lists of up- and down-regulated DEGs identified after treatment with the eqMD-M Φ ^{IFN γ /LPS}-CM or eqMD-M Φ ^{IL-4/IL-13}-CM were uploaded to the jvenn software (<https://jvenn.toulouse.inrae.fr/app/example.html>). Analysis revealed that 234 DEGs were common for both M Φ populations. Among identified common DEGs, 123 were up-regulated, and 111 were down-regulated after treatment with both the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours (Fig. 38B).

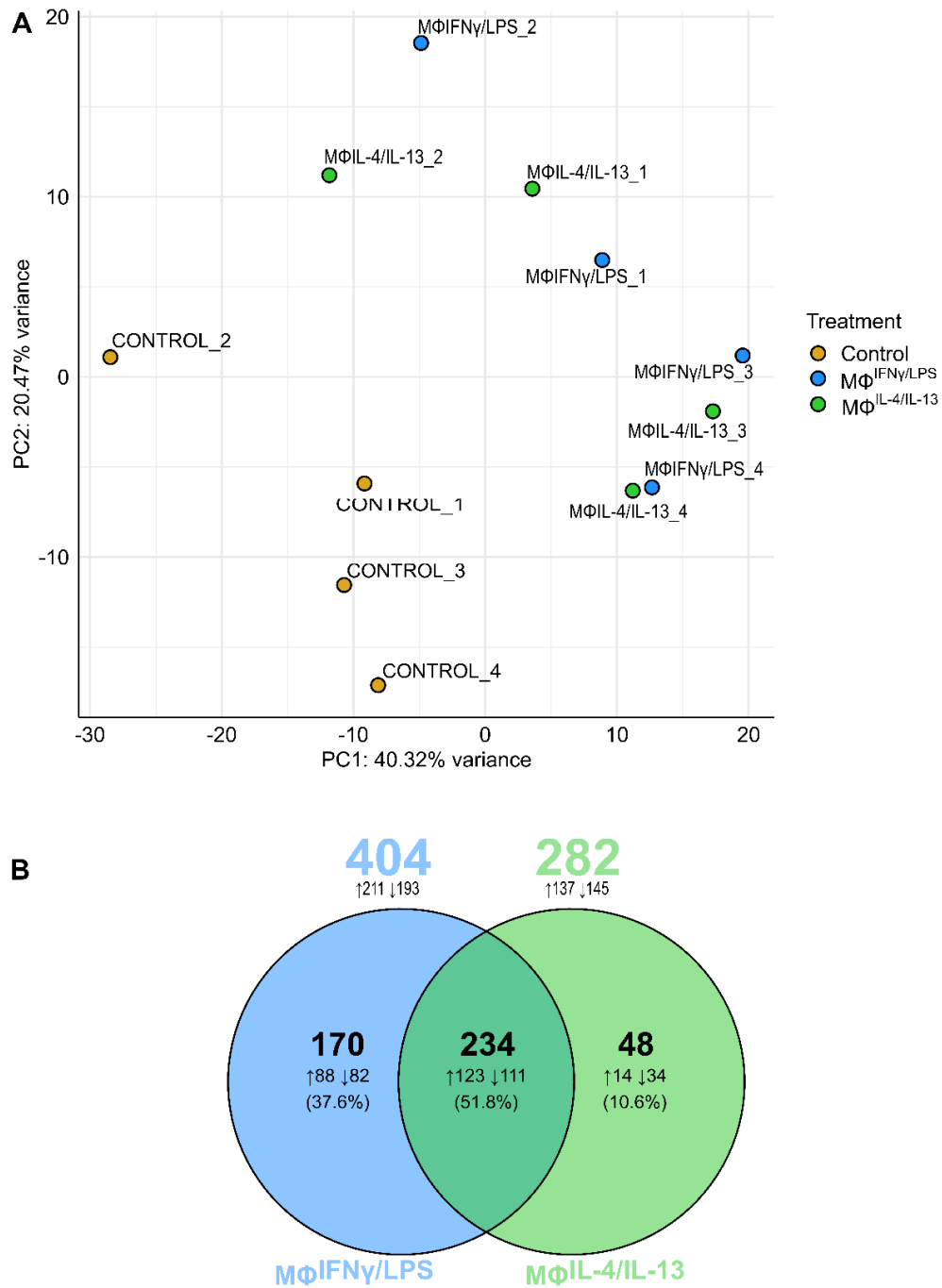


Figure 38. Graphical presentation of principal components analysis (PCA) of the gene expression pattern in mare endometrial fibroblasts treated for 96 hours with the eqMD-MΦ^{IFN γ /LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM (A). Venn diagram showing shared and unique differentially expressed genes (DEGs; p -value ≤ 0.05 and $\log_2FC < -1/\log_2FC > 1$) identified in fibroblasts derived from category I endometria ($n=4$) after the treatment with the eqMD-MΦ^{IFN γ /LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM for 96 hours. Under the total number of DEGs distinguished are quantities of down- and up-regulated DEGs (B).

6.2.3.1. Functional analysis of transcriptomic changes in endometrial fibroblasts after eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome treatment

As a broad range of DEGs were common after treatment with both the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours, primarily, core analysis in the IPA was used to identify the pathways that were significantly enriched specifically by common DEGs. The functional analysis revealed significant enrichment of 75 functional processes (55 with predicted increased activation and 20 with predicted decreased activation) and 25 canonical pathways (21 with predicted increased activation and 4 with predicted decreased activation). The functional annotation of identified common DEGs was associated with increased activation of processes such as migration of cells (*CXCL12*, *FGF19*, *IGF1*, *SOCS3*), cell movement (*BAMBI*, *PDK1*, *STAT1*, *TRIM29*) cell death of connective tissue cells (*ERG*, *LDHA*, *RUNX1*, *TXNIP*), fibrosis of tissue (*CAV1*, *IL13RA2*, *IL1R1*, *VDR*), and soft tissue lesion (*ADAMTS12*, *ASPN*, *MMP15*, *TNXB*). Furthermore, identified common DEGs resulted in the enrichment of canonical pathways such as interferon alpha/beta signaling (*BST2*, *IRF7*, *IRF9*, *STAT1*), pathogen-induced cytokine storm signaling (*CXCL9*, *RIGI*, *TNFSF13B*, *ZBP1*), interferon-gamma signaling (*GBP5*, *IRF8*, *OAS2*, *PML*), and neutrophil degranulation (*ALDOC*, *CYBB*, *PLAC8*, *SNAP25*). At the same time, they led to the hindrance of canonical pathways like gap-junction signaling (*CAV1*, *GJA1*, *PDGFRA*, *TUBB4A*), extra-nuclear estrogen signaling (*BCL2*, *CAV1*, *CAV2*, *ESR1*), and signaling by the TGF- β receptor complex (*BAMBI*, *LTBP1*, *LTBP2*, *LTBP4*). For the common DEGs identified in fibroblasts after treatment with the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours, chosen functional categories are presented in Table 5 and Table 6.

Table 5. Ingenuity pathway analysis (IPA) overview of the common effect of the eqMD-MΦ^{IFNγ/LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM on functional annotations associated with differentially expressed genes (DEGs; p-value ≤ 0.05 and log2FC < -1/log2FC > 1) identified in fibroblasts derived from category I endometria (n=4) after 96 hours of treatment. Presented are selected functional annotations associated with identified common DEGs.

Category	Functional annotation	p-value	Predicted activation state	No. of DEGs
cellular movement	migration of cells	4,11E-25	Increased	107
	cell movement	1,1E-24	Increased	111
	cellular infiltration by phagocytes	1,65E-06	Increased	18
	cell movement of leukocytes	2,37E-11	Increased	41
	migration of mononuclear leukocytes	4,74E-07	Increased	21
cell death and survival	cell death of immune cells	6,52E-07	Increased	26
	cell death of connective tissue cells	1,81E-07	Increased	25
cell morphology	polarization of macrophages	2,98E-06	Increased	9
cellular development and proliferation	differentiation of antigen-presenting cells	7,58E-13	Increased	22
	differentiation of phagocytes	1,26E-12	Increased	24
organismal injury and abnormalities	fibrosis of tissue	1,56E-07	Increased	12
	soft tissue lesion	3,74E-12	Increased	162
	infection of Mammalia	4,31E-10	Decreased	21

Table 6. Ingenuity pathway analysis (IPA) overview of the common effect of the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM on canonical pathways associated with differentially expressed genes (DEGs; p-value ≤ 0.05 and $\log_2FC < -1/\log_2FC > 1$) identified in fibroblasts derived from category I endometria (n=4) after 96 hours of treatment. Presented are selected canonical pathways associated with identified common DEGs.

Canonical pathway	-log (p-value)	Predicted activation state	No. of DEGs
interferon-alpha/beta signaling	14,7	Increased	14
pathogen-induced cytokine storm signaling pathway	6,08	Increased	15
interferon gamma signaling	10,5	Increased	12
neutrophil degranulation	0,689	Increased	6
macrophage classical activation signaling pathway	4,54	Increased	9
gap junction signaling	0,84	Decreased	5
extra-nuclear estrogen signaling	2,46	Decreased	4
signaling by TGF-beta receptor complex	2,98	Decreased	4

Analysis of all 404 DEGs identified in fibroblasts after treatment with the eqMD-M Φ ^{IFN γ /LPS}-CM for 96 hours revealed a significant number of annotated functional categories. A significant enrichment of 147 functional processes (126 with predicted increased activation and 21 with predicted decreased activation) and 36 canonical pathways (27 with predicted increased activation and nine with predicted decreased activation) was observed. Among them were annotations associated with increased activation state of cellular infiltration (*CLC5*, *NFKBIZ*, *TGFB1*, *VDR*), cell movement of neutrophils (*CD74*, *ITGAL*, *MASP1*, *SLIT2*), lymphocyte migration (*ACKR3*, *CAV1*, *LAMA4*, *SMPD2*), activation of cells (*CCN3*, *F2RL2*, *PDGFRA*, *SERPING1*), proliferation of connective tissue cells (*ADAMTSL12*, *APLN*, *PTGES*, *THRB*), and growth of connective tissue (*CCL13*, *FGF19*, *PDK1*, *ZBP1*). Subsequent analysis revealed that DEGs identified in fibroblasts treated with the eqMD-M Φ ^{IFN γ /LPS}-CM could be responsible for the enrichment of canonical pathways like NF- κ B signaling (*EIF2AK2*, *IL1R1*, *PDGFRA*, *TNFSF13B*) and neutrophil extracellular trap signaling (*COL23A1*,

COL4A3, *COL4A5*, *MLKL*) and hindrance of canonical pathways like elastic fiber formation (*FBLN5*, *LOXL3*, *MFAP5*, *TGFB1*) and collagen degradation (*COL23A1*, *COL4A3*, *COL4A5*, *MMP15*). For the common DEGs identified in fibroblasts after treatment with the eqMD-M Φ ^{IFN γ /LPS}-CM for 96 hours, chosen functional categories are presented in Table 7 and Table 8.

Analysis of all 282 DEGs identified in fibroblasts after treatment with the eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours revealed a smaller number of annotated functional categories when compared to DEGs in fibroblasts after M Φ ^{IFN γ /LPS}-CM treatment for 96 hours. A significant enrichment of 96 functional processes (62 with predicted increased activation and 30 with predicted decreased activation) and 25 canonical pathways (19 with predicted increased activation and 6 with predicted decreased activation) was observed. Among the functional alterations annotated specifically to DEGs identified in fibroblasts treated with the eqMD-M Φ ^{IL-4/IL-13}-CM were such as assembly of intercellular junctions (*CLSTN2*, *LRRN1*, *PMP22*, *WNT11*), formation of the plasma membrane (*FLRT2*, *NRG1*, *OCLN*, *THRB*), formation of intercellular junctions (*GJA1*, *IGF1*, *LZTS1*, *UCP2*), development of gap junctions (*CLSTN3*, *NEFL*, *PODXL*, *TOMD1*) with predicted decreased activation, as well as colony formation (*ASPN*, *JAG1*, *OSMR*, *TES*) with predicted increased activation. Subsequent analysis revealed that DEGs from fibroblasts treated with the eqMD-M Φ ^{IL-4/IL-13}-CM showed the enrichment of protein kinase A signaling (*DUSP8*, *PDE4c*, *PTPRO*, *PTPRZ1*) and hindrance of estrogen receptor signaling (*BCL2*, *MMP19*, *PPARGC1A*, *VEGF*) canonical pathways. For the common DEGs identified in fibroblasts after treatment with the eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours, chosen functional categories are presented in Table 7 and Table 8.

Table 7. Ingenuity pathway analysis (IPA) overview of the effect of the eqMD-M Φ ^{IFN γ /LPS}-CM or eqMD-M Φ ^{IL-4/IL-13}-CM on functional annotations associated with differentially expressed genes (DEGs; p-value ≤ 0.05 and log2FC < -1 /log2FC > 1) identified in fibroblasts derived from category I endometria (n=4) after 96 hours of treatment. Presented are selected functional annotations associated with identified DEGs.

	Category	Functional annotation	p-value	Predicted activation state	No. of DEGs
M Φ ^{IFNγ/LPS}	Cellular movement	Cellular infiltration	3,67E-18	Increased	53
		Cell movement of neutrophils	2,86E-09	Increased	28
		Lymphocyte migration	4,22E-10	Increased	32
	Cell-To-Cell Signaling and Interaction	Activation of cells	4,93E-17	Increased	71
	Cellular Growth and Proliferation	Cell proliferation of T lymphocytes	1,35E-06	Increased	33
		Proliferation of connective tissue cells	3,35E-10	Increased	42
	Lipid metabolism	Release of lipid	1,81E-07	Increased	17
		Release of prostaglandin	5,18E-07	Increased	9
		Concentration of triacylglycerol	8,37E-11	Decreased	32
	Connective Tissue Development and Function	Growth of connective tissue	1,64E-12	Increased	48
M Φ ^{IL-4/IL13}	Cellular Assembly and Organization	Development of gap junctions	6,93E-07	Decreased	17
		Formation of plasma membrane	7,82E-07	Decreased	19
		Formation of intercellular junctions	1,05E-07	Decreased	21
		Assembly of intercellular junctions	1,41E-07	Decreased	20
	Cellular Growth and Proliferation	Colony formation	9,62E-12	Increased	44
	Lipid metabolism	Release of prostaglandin	8,04E-06	Increased	7

Table 8. Ingenuity pathways analysis (IPA) overview of the effect of the eqMD-M Φ ^{IFN γ /LPS}-CM or eqMD-M Φ ^{IL-4/IL-13}-CM on canonical pathways associated with differentially expressed genes (DEGs; p-value $\leq$ 0.05 and log2FC < -1 /log2FC > 1) identified in fibroblasts derived from category I endometria (n=4) after 96 hours of treatment. Presented are selected canonical pathways associated with identified DEGs.

	Canonical pathway	-log (p-value)	Predicted activation state	No. of DEGs
M Φ ^{IFNγ/LPS}	NF- κ B Signaling	0	Increased	4
	Neutrophil Extracellular Trap Signaling Pathway	0,495	Increased	7
	Elastic fibre formation	6,94	Decreased	8
	Collagen degradation	1,97	Decreased	4
M Φ ^{IL-4/IL-13}	Protein Kinase A Signaling	0,641	Increased	6
	Sertoli Cell-Sertoli Cell Junction Signaling	2,44	Decreased	8
	Estrogen Receptor Signaling	1,24	Decreased	8

6.2.3.2. Predicted upstream regulators of DEGs identified after eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} secretome treatment

Potential upstream regulators of DEGs identified in mare endometrial fibroblasts after the treatment with eqMD-M Φ ^{IFN γ /LPS}-CM or eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours were predicted using the upstream analysis tool in IPA. Analysis of all 404 identified DEGs in fibroblasts after treatment with eqMD-M Φ ^{IFN γ /LPS}-CM for 96 hours revealed 406 predicted upstream regulators (255 with predicted activated state and 151 with predicted inhibited state), whereas analysis of all 282 identified DEGs in fibroblasts after treatment with the eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours revealed 291 predicted upstream regulators (184 with predicted activated state and 107 with predicted inhibited state). Subsequent analysis showed that 164 predicted activated upstream regulators and 81 predicted inhibited upstream regulators were common for DEGs identified in mare endometrial fibroblasts after the treatment with CM of both eqMD-M Φ subpopulations. Neither upstream regulator has been shown to be simultaneously activated in one group and inhibited in the other (Fig. 39). Identified molecules were mainly convergent among studied eqMD-M Φ subpopulations and exhibited similar activation or inhibition patterns. The activated upstream regulators of DEGs identified in mare endometrial fibroblasts after the treatment with eqMD-M Φ ^{IFN γ /LPS}-CM or eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours included, among others IFN γ , TNF, IL-1 β , NF- κ β , IL-6, HIF-1 α , and mir-183, whereas

among inhibited upstream regulators were mir-145-5p, large tumor suppressor (LATS) family, interleukin 1 receptor antagonist (IL1RN), and triggering receptor expressed on myeloid cells (TREM)-1. Among upstream regulators specific for DEGs identified in mare endometrial fibroblasts after the treatment with eqMD-M Φ ^{IFN γ /LPS}-CM for 96 hours were aryl hydrocarbon receptor (AHR), IL-2, IL-12 with predicted activated state and miR-146, LATS, Yes1 associated transcriptional regulator (YAP1) with predicted inhibited state. Among upstream regulators specific for DEGs identified in mare endometrial fibroblasts after the treatment with the eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours were MMP-2, inhibin subunit beta A (INHBA) with predicted activated state and arginine, TIMP3, ten-eleven translocation methylcytosine dioxygenase (TET)-2 with predicted inhibited state. Chosen predicted activated upstream regulators are presented in Table 9, and chosen predicted inhibited upstream regulators are presented in Table 10.

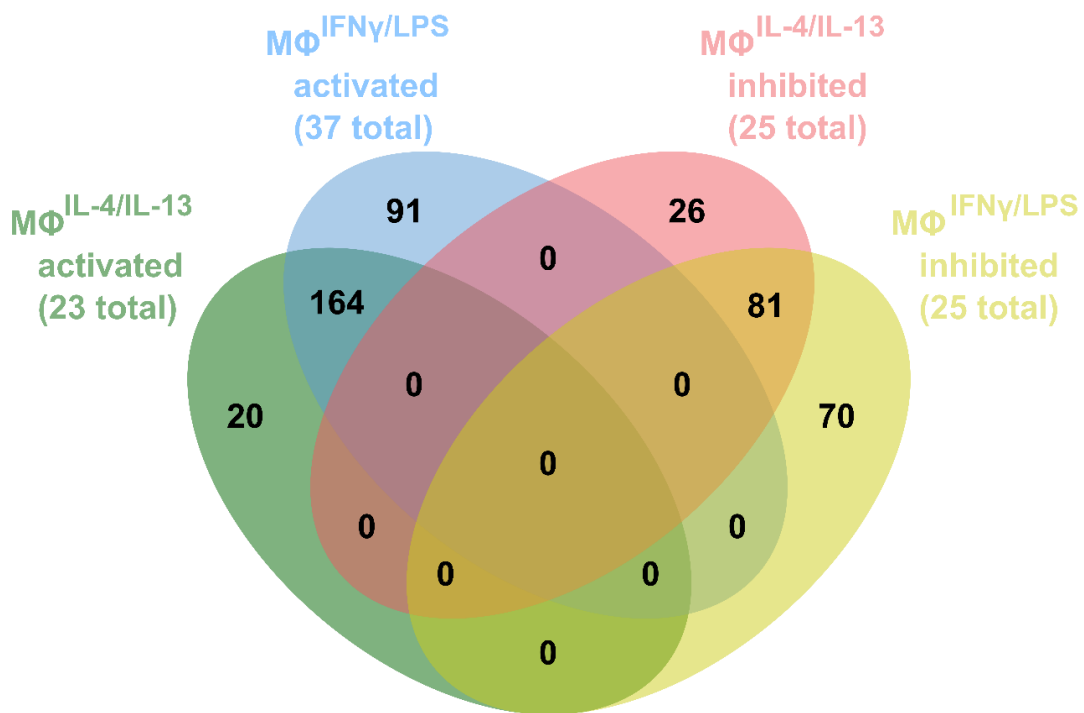


Figure 39. Venn diagram showing shared and unique predicted upstream regulators of differentially expressed genes (DEGs; $p\text{-value} \leq 0.05$ and $\log_2\text{FC} < -1/\log_2\text{FC} > 1$) identified in fibroblasts derived from category I endometria (n=4) after the treatment with the eqMD-MΦ^{IFN γ /LPS}-CM or eqMD-MΦ^{IL-4/IL-13}-CM for 96 hours subdivided into those with predicted activated and inhibited state.

Table 9. Ingenuity pathway analysis (IPA) overview of activated upstream regulators of identified differentially expressed genes (DEGs; p-value ≤ 0.05 and $\log_2FC < -1/\log_2FC > 1$) in fibroblasts derived from category I endometria (n=4) after the treatment with eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM or eqMD-M $\Phi^{IL-4/IL-13}$ -CM for 96 hours. Presented are selected upstream regulators with predicted activated state associated with DEGs identified after the treatment with eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM and DEGs identified after the treatment with the eqMD-M $\Phi^{IL-4/IL-13}$ -CM.

Upstream Regulator	Molecule type	M $\Phi^{IFN\gamma/LPS}$			M $\Phi^{IL-4/IL-13}$		
		z-score	p-value	No. DEGs	z-score	p-value	No. DEGs
IFN γ	cytokine	7,703	6,45E-47	110	5,836	2,79E-34	81
TNF	cytokine	5,998	3,19E-35	111	3,42	5,86E-26	82
IL-1 β	cytokine	5,28	6,38E-29	76	3,567	8,37E-27	63
NF- $\kappa\beta$ (complex)	complex	4,382	1,09E-14	39	3,237	1,64E-10	28
IL-17A	cytokine	3,524	1,98E-08	24	2,52	5,14E-08	20
IL-6	cytokine	3,505	9,72E-09	35	2,65	8,83E-06	24
mir-183	microRNA	3,464	8,23E-12	12	2,813	7,94E-08	8
IL-21	cytokine	3,396	1,06E-06	13	2,335	1,49E-05	10
HIF1A	Transcription regulator	3,255	1,67E-13	36	3,268	1,7E-10	27
TLR4	Transmembrane receptor	2,892	1,05E-12	29	2,043	7,38E-08	19
NOX4	enzyme	2,4	8,79E-05	6	2,204	2,15E-04	5
AHR	Ligand-dependent nuclear receptor	2,506	3,2E-12	34	-	-	-
IL-2	cytokine	2,381	8,47E-07	29	-	-	-
IL-12 (complex)	complex	1,977	1,11E-06	14	-	-	-
MMP-2	peptidase	-	-	-	2	8,02E-04	5
INHBA	growth factor	-	-	-	1,974	7,58E-03	7

Table 10. Ingenuity pathway analysis (IPA) overview of activated upstream regulators of identified differentially expressed genes (DEGs; p-value ≤ 0.05 and $\log_2FC < -1/\log_2FC > 1$) in fibroblasts derived from category I endometria (n=4) after the treatment with eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM or eqMD-M $\Phi^{IL-4/IL-13}$ -CM for 96 hours. Presented are selected upstream regulators with predicted inhibited state associated with DEGs identified after the treatment with eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM and DEGs identified after the treatment with the eqMD-M $\Phi^{IL-4/IL-13}$ -CM.

Upstream Regulator	Molecule type	M $\Phi^{IFN\gamma/LPS}$			M $\Phi^{IL-4/IL-13}$		
		z-score	p-value	No. DEGs	z-score	p-value	No. DEGs
IL1RN	cytokine	-4,909	1,53E-25	7	-3,207	7,51E-11	14
TREM1	transmembrane receptor	-2,691	1,71E-09	17	-2,36	1,7E-09	15
miR-145-5p	mature microRNA	-2,621	0,000297	28	-1,866	4,91E-05	7
miR-146	microRNA	-2,433	8,81E-04	7	-	-	-
LATS (family)	group	-2	0,00612	4	-	-	-
YAP1	transcription regulator	-1,798	1,17E-03	18	-	-	-
arginine	endogenous chemical	-	-	-	-2	7,88E-04	4
TIMP3	other	-	-	-	-2	1,64E-03	4
TET2	enzyme	-	-	-	-1,791	3,09E-04	10

6.2.4. Experiment 4. The effect of the eqMD-M $\Phi^{IFN\gamma/LPS}$ and eqMD-M $\Phi^{IL-4/IL-13}$ secretome on the functional characteristics of endometrial fibroblasts

Both, the eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM and eqMD-M $\Phi^{IL-4/IL-13}$ -CM led to increased proliferation and increased metabolic activity (viability) of endometrial fibroblasts after 96 hours of treatment when compared to the control group ($p < 0.05$ and $p < 0.01$ respectively; Fig. 40A, B). Moreover, treatment with the eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM or eqMD-M $\Phi^{IL-4/IL-13}$ -CM resulted in enhanced endometrial fibroblast migration, particularly at 36 and 48 hours ($p < 0.05$; Fig 41).

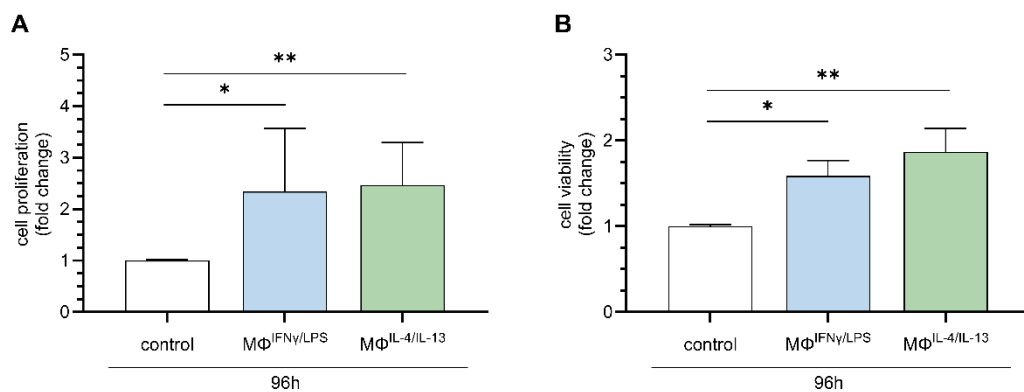


Figure 40. The effect of the eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM and eqMD-M $\Phi^{IL-4/IL-13}$ -CM on the proliferation (A) and metabolic activity (viability) (B) of fibroblasts derived from category I endometria (n=6) after 96 hours of treatment. Results were calculated as the mean $\pm$ standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (* $p < 0.05$; ** $p < 0.01$) from the control group as determined by Student's t test or Mann-Whitney U test, depending on normal distribution.

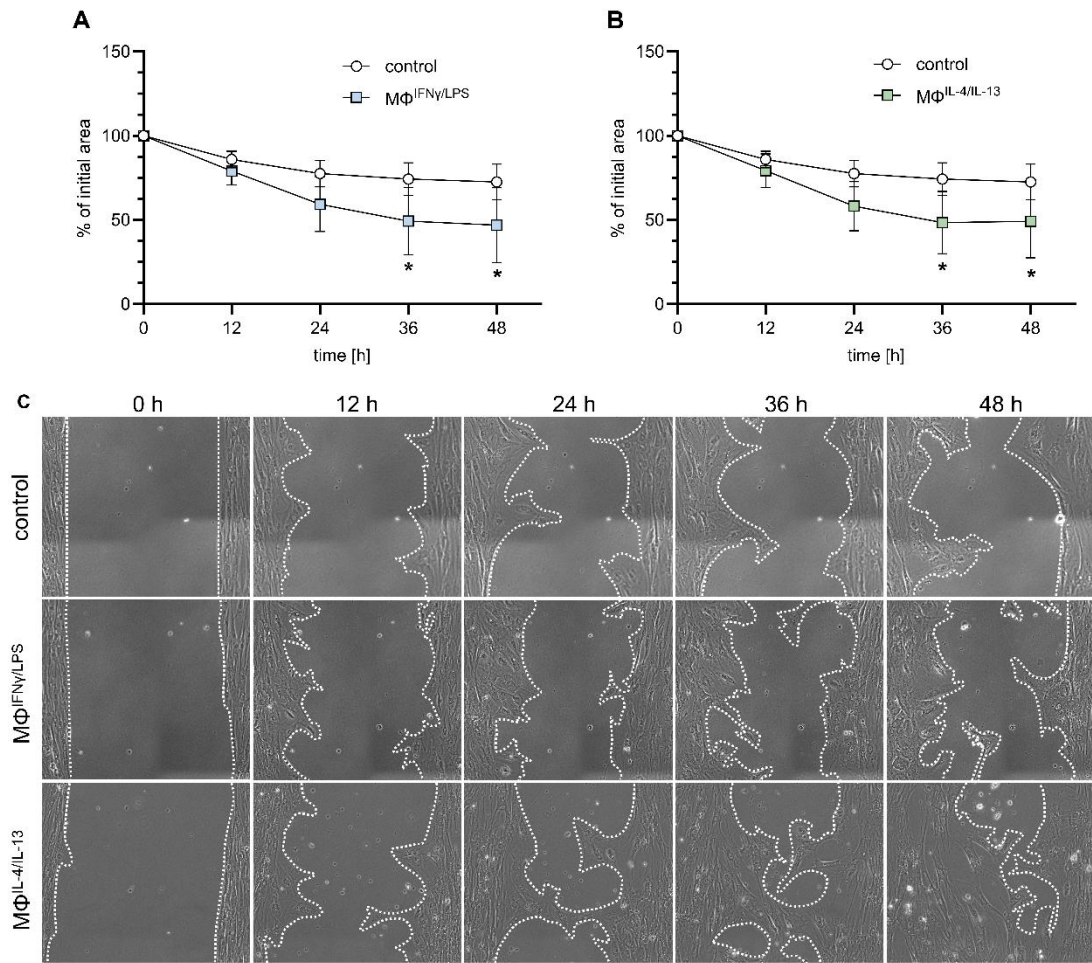


Figure 41. Analysis of migratory abilities of fibroblasts derived from category I endometria (n=6) after treatment with the eqMD-MΦ^{IFNγ/LPS}-CM (A) and eqMD-MΦ^{IL-4/IL-13}-CM (B). Representative pictures of fibroblasts exposed to the CM of eqMD-MΦ taken after 12h, 24h, 36h, and 48h (C). Results were calculated as the mean ± standard deviation (SD) and expressed as a percentage of the initial area. Asterisks indicate statistical differences (*p < 0.05) from the control group as determined by repeated measures ANOVA with Greenhouse-Geisser correction and post-hoc Tukey test.

6.3. Research Task 3: Assessment of the effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the mare endometrium

The CRP levels in serum of mares remained constant 12h and 24h after intrauterine administration of vehicle (PBS) and autologous eqMD-M Φ ^{IFN γ /LPS} or eqMD-M Φ ^{IL-4/IL-13} ($p>0.05$; Fig 42). Moreover, the CRP levels after intrauterine administration of vehicle (PBS) and autologous eqMD-M Φ ^{IFN γ /LPS} or eqMD-M Φ ^{IL-4/IL-13} were at similar levels, markedly below the physiological range ($p>0.05$; Fig. 42).

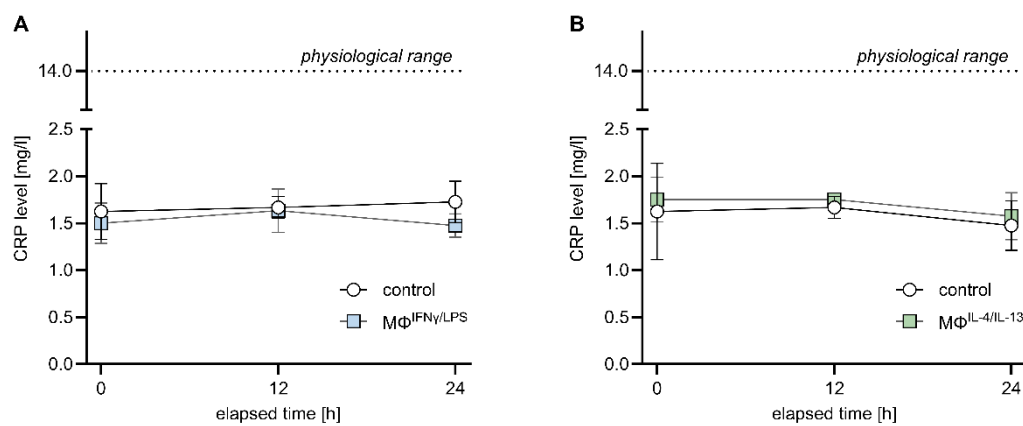


Figure 42. The effect of the intrauterine administration of autologous in eqMD-M Φ ^{IFN γ /LPS} (n=3) (A) or eqMD-M Φ ^{IL-4/IL-13} (n=3) (B) on C-reactive protein (CRP) levels in serum of mares. Results were calculated as the mean $\pm$ standard deviation (SD) as determined by repeated measures ANOVA with Greenhouse-Geisser correction and post-hoc Tukey test.

6.3.1. Experiment 1. The effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the expression of ECM-associated components in mare endometrium

The intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} increased *FN1* (p<0.001; Fig. 43C), *ACTA2* (p<0.01; Fig. 43D), *MMP-2* (p<0.05; Fig. 45E), *TIMP-1* (p<0.01; Fig. 43G) and *TIMP-2* (p<0.01; Fig. 43H) mRNA transcription in mare endometrium 6 hours after the procedure.

The intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13} decreased *COL1A1* (p<0.01; Fig. 43A), *COL3A1* (p<0.001; Fig. 43B), and *FN1* (p<0.05; Fig. 43C) mRNA transcription in mare endometrium 6 hours after the procedure.

Both the intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} or eqMD-M Φ ^{IL-4/IL-13} did not affect the *MMP-9* (p>0.05; Fig. 43F) mRNA transcription in mare endometrium 6 hours after the procedure.

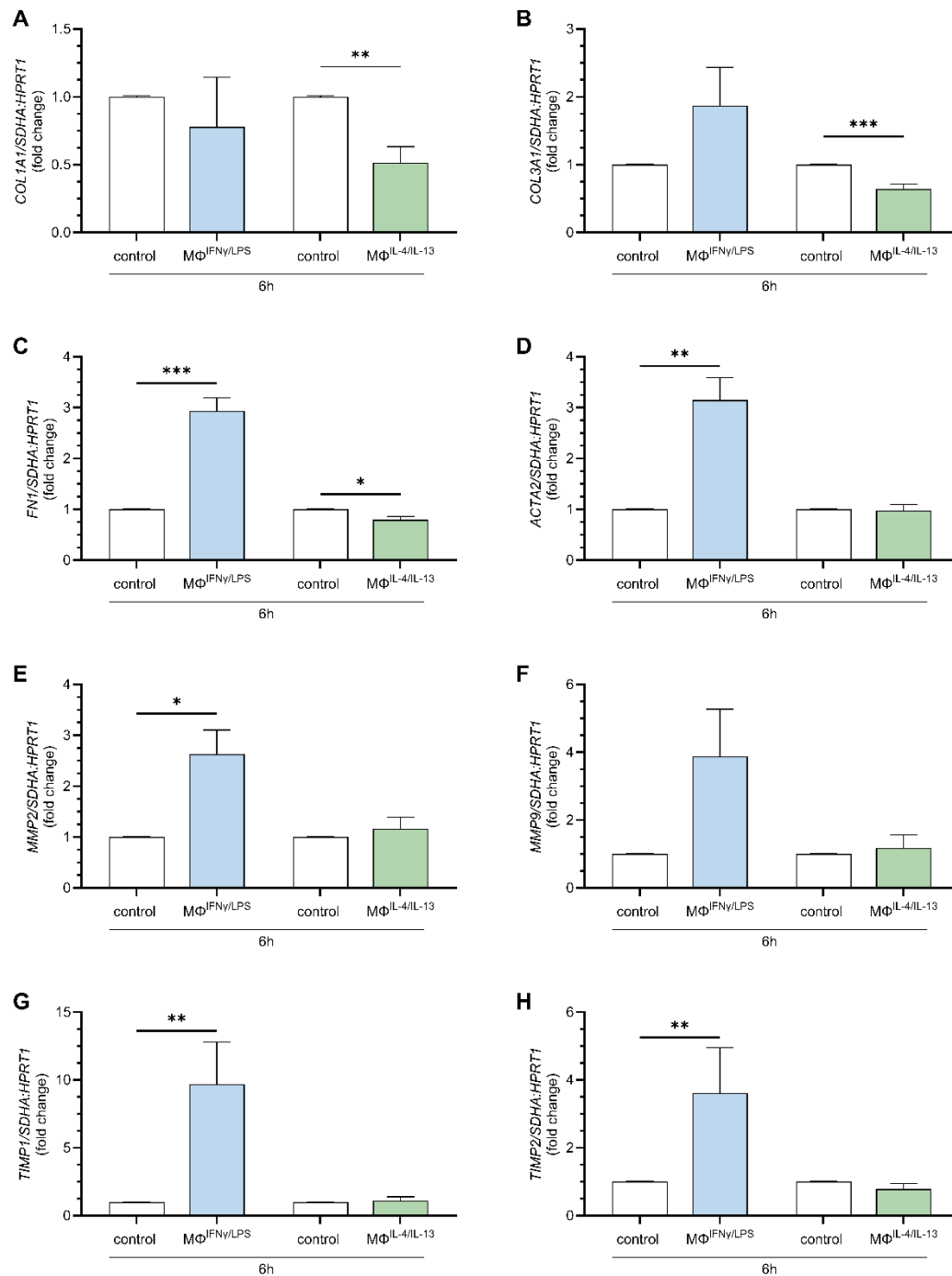


Figure 43. The effect of the intrauterine administration of autologous eqMD-MΦ^{IFNγ/LPS} (n=3) or eqMD-MΦ^{IL-4/IL-13} (n=3) on mRNA transcription of *COL1A1* (A), *COL3A1* (B), *FN1* (C), *ACTA2* (D), *MMP-2* (E), *MMP-9* (F), *TIMP-1* (G), *TIMP-2* (H) in mare endometrium 6 hours after the procedure. Results were calculated as the mean ± standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (*p < 0.05; **p < 0.01; ***p < 0.001) from the control group as determined by Student's t-test or Mann-Whitney U test, depending on normal distribution.

6.3.2. Experiment 2. The effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the expression of inflammatory markers in mare endometrium

The intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} increased *CCN2* ($p < 0.05$; Fig. 44A), *IL-1 β* ($p < 0.05$; Fig. 44C), *IL-6* ($p < 0.05$; Fig. 44D), *TGF- β 1* ($p < 0.05$; Fig. 44E) and *TNF- α* ($p < 0.01$; Fig. 44F) mRNA transcription in mare endometrium 6 hours after the procedure.

The intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13} decreased *IL-1 β* ($p < 0.01$; Fig. 44C) mRNA transcription in mare endometrium 6 hours after the procedure.

Both the intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} or eqMD-M Φ ^{IL-4/IL-13} did not affect the *CD68* ($p > 0.05$; Fig. 44B) mRNA transcription in mare endometrium 6 hours after the procedure.

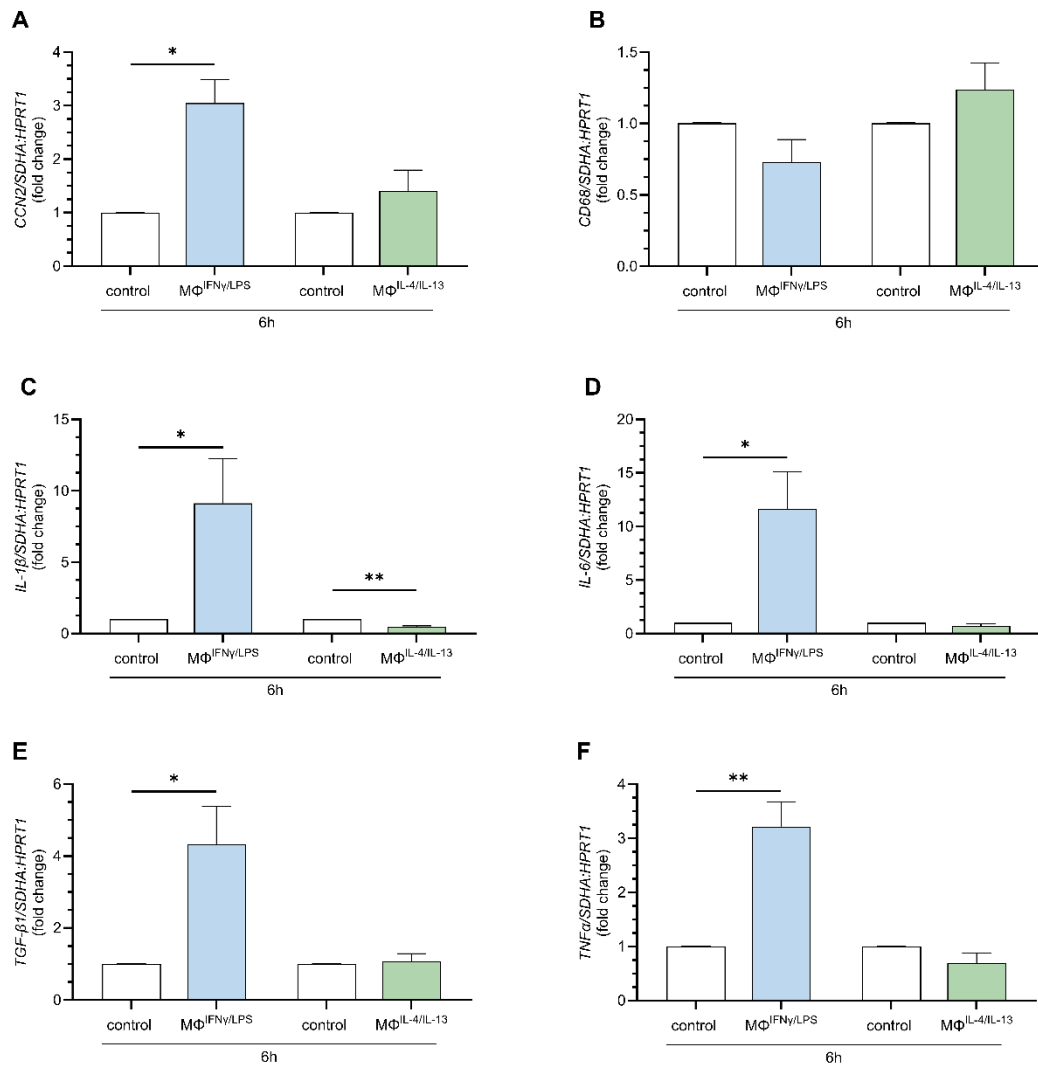


Figure 44. The effect of the intrauterine administration of autologous eqMD-MΦ^{IFNγ}/LPS (n=3) or eqMD-MΦ^{IL-4/IL-13} (n=3) on mRNA transcription of *CCN2* (A), *CD68* (B), *IL-1β* (C), *IL-6* (D), *TGF-β1* (E), *TNF-α* (F) in mare endometrium 6 hours after the procedure. Results were calculated as the mean ± standard deviation (SD) and expressed as a fold change to the control group. Asterisks indicate statistical differences (*p < 0.05; **p < 0.01;) from the control group as determined by Student's t-test Mann-Whitney U test, depending on normal distribution.

6.3.3. Experiment 3. The effect of intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} on the transcriptome of mare endometrium

Following filtering and sequence removal, sequencing yielded an average of 16 606 834 reads, of which 15 847 819 were uniquely mapped to the *Equus caballus* reference genome (EquCab3.0). The summary of RNA sequencing reads in mare endometrium 6 hours after intrauterine administration of M Φ ^{IFN γ /LPS} or M Φ ^{IL-4/IL-13} is presented in Supplementary Table 5. The PCA analysis revealed that intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} or eqMD-M Φ ^{IL-4/IL-13} affect the transcriptomic profile of mare endometrium 6 hours after the procedure (Fig. 45A).

In response to the intrauterine administration of eqMD-M Φ ^{IFN γ /LPS}, alterations in the expression of 127 genes were demonstrated, of which 78 were up-regulated and 49 were down-regulated (Fig. 45B). The top 5 up-regulated DEGs were *SPINK7*, *ENSECAG00000023696*, *CALR4*, *ENSECAG00000031486*, *LRR74A*, while top 5 down-regulated DEGs were *DEPDC1*, *PCDH9*, *ENSECAG00000026891*, *DEPDC1B*, *ENSECAG00000055118*. Supplementary Table 6 contains complete lists of all DEGs detected in mare endometrium 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS}, along with their Log2FC and p-adjusted values. Simultaneously, alterations in the expression of 310 genes were shown in response to the intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13} of which 144 were up-regulated and 166 were down-regulated (Fig. 45B). The top 5 up-regulated DEGs were *HEPACAM*, *CTSL*, *ENSECAG00000057527*, *LRP2*, *ADGRG2*, while the top 5 down-regulated DEGs were *SERPINA6*, *HS6ST3*, *ENSECAG00000043826*, *ENSECAG00000008745*, *CCN5*. Supplementary Table 7 contains complete lists of all DEGs detected in mare endometrium 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13}, along with their Log2FC and p-adjusted values. Each comparison revealed several unique transcripts in addition to known genes.

Analysis using jvenn software revealed that only 12 DEGs were common for both eqMD-M Φ subpopulations. Among identified common DEGs 5 were up-regulated (*OXSM*, *MCOLN2*, *WLS*, *NRCAM*, *INSIG1*) and 6 were down-regulated (*TRIM9*, *CHRM3*, *KIF5C*, *STMN2*, *NEO1*, *CLDN10*). One common gene was characterized by different expression patterns. *ENSECAG00000037336* was up-regulated 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and down-regulated 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13} (Fig. 45B).

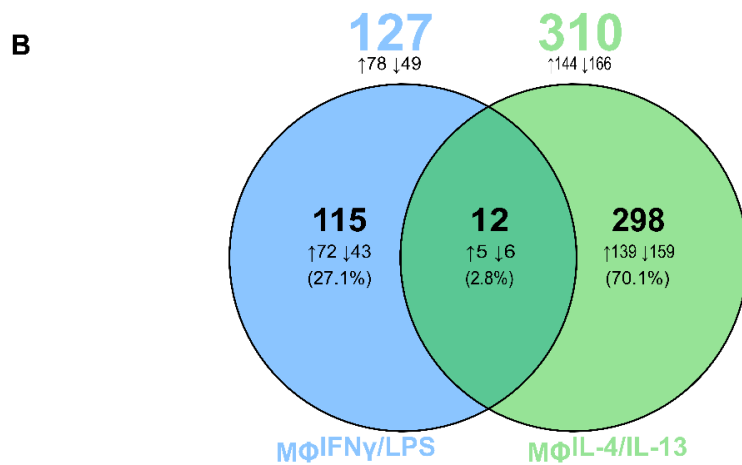
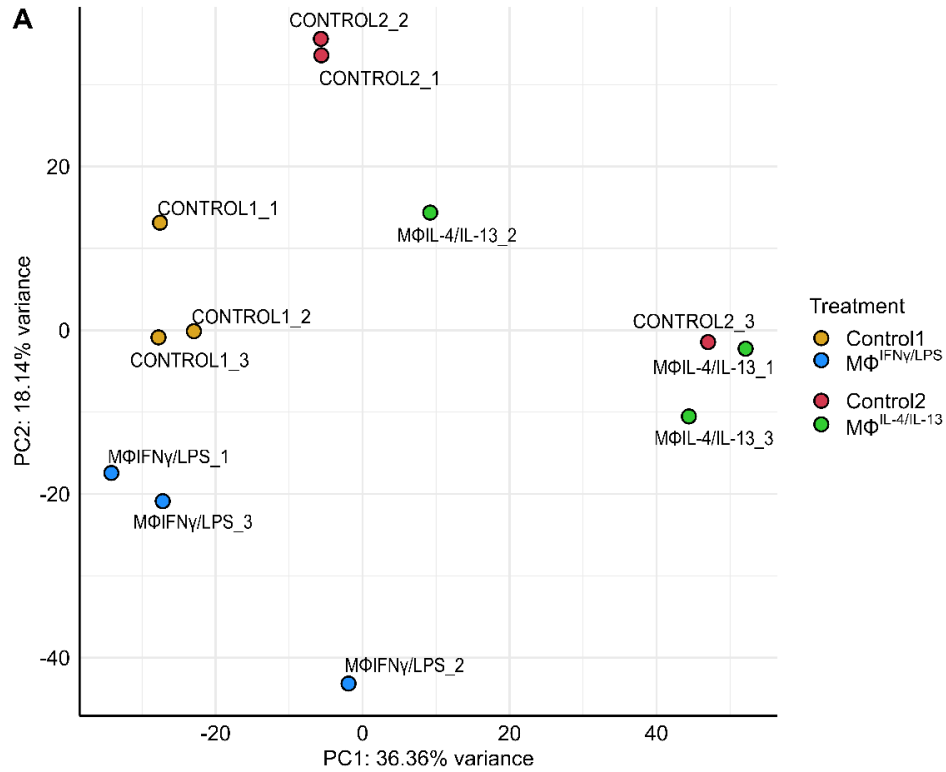


Figure 45. Graphical presentation of principal components analysis (PCA) of the gene expression pattern in mare endometrium 6 hours after the intrauterine administration of eqMD-MΦ^{IFNγ/LPS} (n=3) or eqMD-MΦ^{IL-4/IL-13} (n=3) (A). Venn diagram showing shared and unique differentially expressed genes (DEGs; p-value ≤ 0.05 and log2FC < -1/log2FC > 1) identified in mare endometrium 6 hours after the intrauterine administration of eqMD-MΦ^{IFNγ/LPS} (n=3) or eqMD-MΦ^{IL-4/IL-13} (n=3) (B). Under the total number of DEGs distinguished are quantities of down- and up-regulated DEGs

6.3.3.1. Functional analysis of transcriptomic changes in mare endometrium after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}

The functional analysis in the IPA revealed significant enrichment of 25 functional processes (11 with predicted increased activation and 14 with predicted decreased activation) and 13 canonical pathways (6 with predicted increased activation and 7 with predicted decreased activation) by DEGs identified 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS}. The functional annotation of identified DEGs was associated with increased activation of processes such as abnormal morphology of subcellular (*BUB1*, *CCN2*, *SERPINA3*), upper abdominal cancer (*CLDN10*, *IL1RN*, *TRIM9*), interabdominal organ tumor (*APOA1*, *INSIG1*, *SPINK7*) and decreased activation of processes such as organismal death (*ANLN*, *LIF*, *WLS*), glucose metabolism disorder (*CXCR4*, *HOPX*, *KIF5C*), leukocytosis (*APOA1*, *GRN*, *TRPV6*), release of amino acids (*CHRM3*, *CXCR4*, *PPARGC1A*). Furthermore, identified DEGs resulted in the enrichment of canonical pathways such as cachexia signaling pathway (*CAPN11*, *IL1RN*, *LCN2*), serotonin receptor signaling (*ABHD3*, *MAPK13*, *TRPV6*), multiple sclerosis signaling (*C7*, *LIF*, *MAPK13*), neutrophil degranulation (*GRN*, *LCN2*, *SERPINA3*), and hindrance of canonical pathways such as cell cycle checkpoints (*BUB1B*, *CCNB1*, *MCM2*), MHC class II antigen presentation (*KIF11*, *KIF23*, *KIF5C*). For the DEGs identified 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS}, chosen functional categories are presented in Table 11 and Table 12.

The functional analysis in the IPA revealed significant enrichment of 18 functional processes (7 with predicted increased activation and 11 with predicted decreased activation) and 13 canonical pathways (3 with predicted increased activation and 10 with predicted decreased activation) by DEGs identified 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13}. The functional annotation of identified DEGs was associated with increased activation of processes such as upper abdominal cancer (*AK3*, *DAAM2*, *GPC6*), mean arterial pressure (*ELN*, *INSR*, *SOD3*), and decreased activation of processes such as leukemia (*ADD2*, *PPAT*, *TLL1*), cellular infiltration by granulocytes (*CBFB*, *NQO1*, *VAV3*), cell movement of neutrophils (*CD55*, *PIGR*, *WLS*). Furthermore, identified DEGs resulted in the enrichment of canonical pathways such as, Sheddase signaling pathway (*COL4A6*, *IGFBP3*, *MMP26*), CLEAR signaling pathway (*EIF2AK3*, *INSR*, *VPS26A*), and hindrance of canonical pathways such as insulin secretion signaling pathway (*CACNA1H*, *CHRM3*, *PLCD4*), collagen chain trimerization

(*COL14A1*, *COL21A1*, *COL4A6*), IL-4 signaling (*ANO1*, *COL21A1*, *COL8A1*), post-translational protein phosphorylation (*F5*, *LAMB2*, *RCN1*). For the DEGs identified 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13}, chosen functional categories are presented in Table 11 and Table 13.

Table 11. Ingenuity pathway analysis (IPA) overview of the effect of the intrauterine administration of eqMD-MΦ^{IFNγ/LPS} (n=3) or eqMD-MΦ^{IL-4/IL-13} (n=3) on functional annotation associated with differentially expressed genes (DEGs; p-value ≤ 0.05 and log2FC < -1/log2FC > 1) identified in mare endometrium 6 hours after the procedure. Presented are selected functional annotations.

	Category	Functional annotation	p-value	Predicted activation state	No. of DEGs
MΦ ^{IFNγ/LPS}	Cell Morphology	Abnormal morphology of subcellular	2,98E-03	Increased	13
	Organismal Injury and Abnormalities	Upper abdominal cancer	7,14E-09	Increased	90
		Intraabdominal organ tumor	1,77E-15	Increased	117
		Organismal death	3,30E-09	Decreased	52
		Glucose metabolism disorder	1,10E-03	Decreased	34
		Leukocytosis	2,86E-04	Decreased	6
	Amino Acid Metabolism	Release of amino acids	1,74E-03	Decreased	4
MΦ ^{IL-4/IL-13}	Hematological System Development and Function	Mean arterial pressure	6,90E-03	Increased	6
	Organismal Injury and Abnormalities	Upper abdominal cancer	1,05E-24	Increased	228
		Leukemia	1,06E-09	Decreased	111
	Cellular Movement	Cellular infiltration by granulocytes	1,67E-03	Decreased	12
		Cell movement of neutrophils	3,60E-03	Decreased	14

Table 12. Ingenuity pathway analysis (IPA) overview of the effect of the intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} (n=3) on canonical pathways associated with differentially expressed genes (DEGs; p-value ≤ 0.05 and $\log_2FC < -1/\log_2FC > 1$) identified in mare endometrium 6 hours after the procedure. Presented are selected canonical pathways.

Canonical pathway	-log (p-value)	Predicted activation state	No. of DEGs
Cachexia Signaling Pathway	1,59	Increased	5
Serotonin Receptor Signaling	1,2	Increased	5
Multiple Sclerosis Signaling Pathway	1,76	Increased	4
Neutrophil degranulation	0,771	Increased	4
Cell Cycle Checkpoints	2,1	Decreased	5
MHC class II antigen presentation	2,56	Decreased	4

Table 13. Ingenuity pathway analysis (IPA) overview of the effect of the intrauterine administration of eqMD-M Φ ^{IL-4/IL-13} (n=3) on canonical pathways associated with differentially expressed genes (DEGs; p-value ≤ 0.05 and $\log_2FC < -1/\log_2FC > 1$) identified in mare endometrium 6 hours after the procedure. Presented are selected canonical pathways.

Canonical pathway	-log (p-value)	Predicted activation state	No. of DEGs
Sheddase Signaling Pathway	0,726	Increased	4
CLEAR Signaling Pathway	1,02	Increased	6
Insulin Secretion Signaling Pathway	1,03	Decreased	6
Collagen chain trimerization	2,87	Decreased	4
IL-4 Signaling	0	Decreased	4
Post-translational protein phosphorylation	2,93	Decreased	5

6.3.3.2. Predicted upstream regulators of DEGs identified after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}

Potential upstream regulators of DEGs identified in mare endometrium 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} or M Φ ^{IL-4/IL-13} were predicted using the upstream analysis tool in IPA. Analysis of DEGs identified in mare endometrium 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} revealed 62 predicted upstream regulators (37 with predicted activated state and 25 with predicted inhibited state), whereas analysis of DEGs identified in mare endometrium 6 hours after intrauterine administration of M Φ ^{IL-4/IL-13} revealed 48 predicted upstream regulators (23 with predicted activated state and 25 with predicted inhibited state). Subsequent analysis showed that 2 of the predicted activated upstream regulators (progesterone, PGR) and 3 of the predicted inhibited upstream regulators (NR1H4, CSF2, aflatoxin B1) were common for DEGs identified in mare endometrium 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}. Only one upstream regulator (TP73) was predicted to be simultaneously activated for DEGs identified in mare endometrium 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and inhibited for DEGs identified in mare endometrium 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13} (Fig. 46). Among upstream regulators specific for DEGs identified in mare endometrium 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} were among others, mir-21, IL-13, LPS, miR-139-5p, TP73 with predicted activated state and TBX3, AREG, LATS2 with predicted inhibited state (Table 14). Among upstream regulators specific for DEGs identified in mare endometrium 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13} were among others, IFN- β , G protein α 1, MAPK7, PGE2 with predicted activated state and IGF1, NFKBIA, TP73, TGF- β 1 with predicted inhibited state (Table 15).

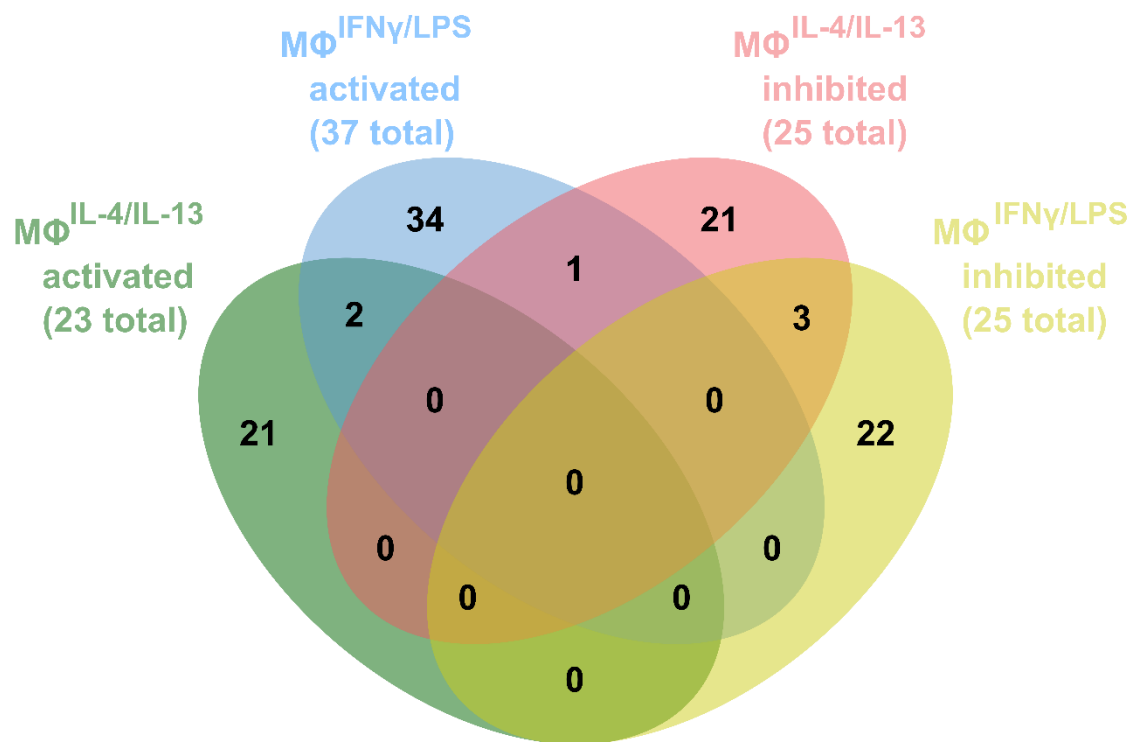


Figure 46. Venn diagram showing shared and unique predicted upstream regulators of differentially expressed genes (DEGs; $p\text{-value} \leq 0.05$ and $\log_2\text{FC} < -1/\log_2\text{FC} > 1$) identified in mare endometrium 6 hours after the intrauterine administration of the eqMD-MΦ^{IFNγ/LPS} (n=3) or eqMD-MΦ^{IL-4/IL-13} (n=3) subdivided into those with predicted activated and inhibited state.

Table 14. Ingenuity pathway analysis (IPA) overview of upstream regulators of differentially expressed genes (DEGs; $p\text{-value} \leq 0.05$ and $\log_2\text{FC} < -1/\log_2\text{FC} > 1$) identified in mare endometrium 6 hours after the intrauterine administration of the eqMD-M $\Phi^{\text{IFN}\gamma/\text{LPS}}$ (n=3). Presented are selected upstream regulators with predicted activated and inhibited state.

Upstream Regulator	Molecule type	z-score	p-value	No. DEGs
mir-21	microRNA	2,449	6,08E-03	6
IL-13	cytokine	2,398	7,29E-05	10
LPS	chemical drug	2,063	6,92E-06	30
miR-139-5p	mature microRNA	1,981	2,98E-05	4
TP73	transcription regulator	1,671	2,24E-03	8
TBX3	transcription regulator	-2,63	6,02E-05	7
AREG	growth factor	-2,449	7,57E-06	6
LATS2	kinase	-1,982	3,00E-04	4

Table 15. Ingenuity pathway analysis (IPA) overview of upstream regulators of differentially expressed genes (DEGs; $p\text{-value} \leq 0.05$ and $\log_2\text{FC} < -1/\log_2\text{FC} > 1$) identified in mare endometrium 6 hours after the intrauterine administration of the eqMD-M $\Phi^{\text{IL-4/IL-13}}$ (n=3). Presented are selected upstream regulators with predicted activated and inhibited state.

Upstream Regulator	Molecule type	z-score	p-value	No. DEGs
IFN- β	group	2,19	1,01E-01	6
G protein $\alpha 1$	group	2	8,93E-03	4
MAPK7	kinase	2	1,78E-02	4
prostaglandin E2	endogenous chemical	1,914	4,21E-02	9
IGF1	growth factor	-3,243	7,34E-02	11
NFKBIA	transcription regulator	-2,53	1,26E-02	11
TP73	transcription regulator	-2,403	4,55E-03	13
TGF- $\beta 1$	growth factor	-1,618	4,36E-08	52

6.4. Comparative analysis of DEGs and upstream regulators identified in endometrial fibroblasts treated with eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM and endometrium after intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}

Analysis using jvenn software revealed that only 4 DEGs (*CXCL6*, *PPARGC1A*, *NRCAM*, *NRBP2*) were simultaneously identified in mare endometrial fibroblasts after treatment with the eqMD-M Φ ^{IFN γ /LPS}-CM for 96 hours and in mare endometrium after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} 6 hours after the procedure (Fig. 47A). Concurrently, 14 DEGs (*CD7*, *ENSECAG00000049355*, *MASP1*, *USP18*, *ENSECAG00000024719*, *PSD3*, *CHML*, *NEBL*, *CDC42EP3*, *GYS2*, *PODN*, *NPNT*, *CDH13*, *GREM1*) were simultaneously identified in mare endometrial fibroblasts after treatment with the eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours and in mare endometrium after intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13} 6 hours after the procedure (Fig. 48A).

An analogous analysis was performed for upstream regulators identified in both Research Tasks and revealed that 7 of predicted activated upstream regulators (LPS, tretinoin, doxorubicin, CHUK, palbociclib, tetradecanoylphorbol acetate, GW3965) and 1 of predicted inhibited upstream regulators (FBXW7) were common for DEGs identified in mare endometrial fibroblasts after treatment with the eqMD-M Φ ^{IFN γ /LPS}-CM for 96 hours and in mare endometrium after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} 6 hours after the procedure. Simultaneously, 2 of predicted inhibited upstream regulators (IRF4, dexamethasone) and 1 of predicted activated upstream regulators (immunoglobulin) for DEGs identified in mare endometrial fibroblasts after treatment with the eqMD-M Φ ^{IFN γ /LPS}-CM for 96 hours and in mare endometrium after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} 6 hours after the procedure (Fig. 47A). Four of predicted activated upstream regulators (IFN α , IFN β , G protein α I, molybdenum disulfide) were common for DEGs identified in mare endometrial fibroblasts after treatment with the eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours and in mare endometrium after intrauterine administration of autologous eqMD-M Φ ^{IL-4/IL-13} 6 hours after the procedure (Fig. 48B).

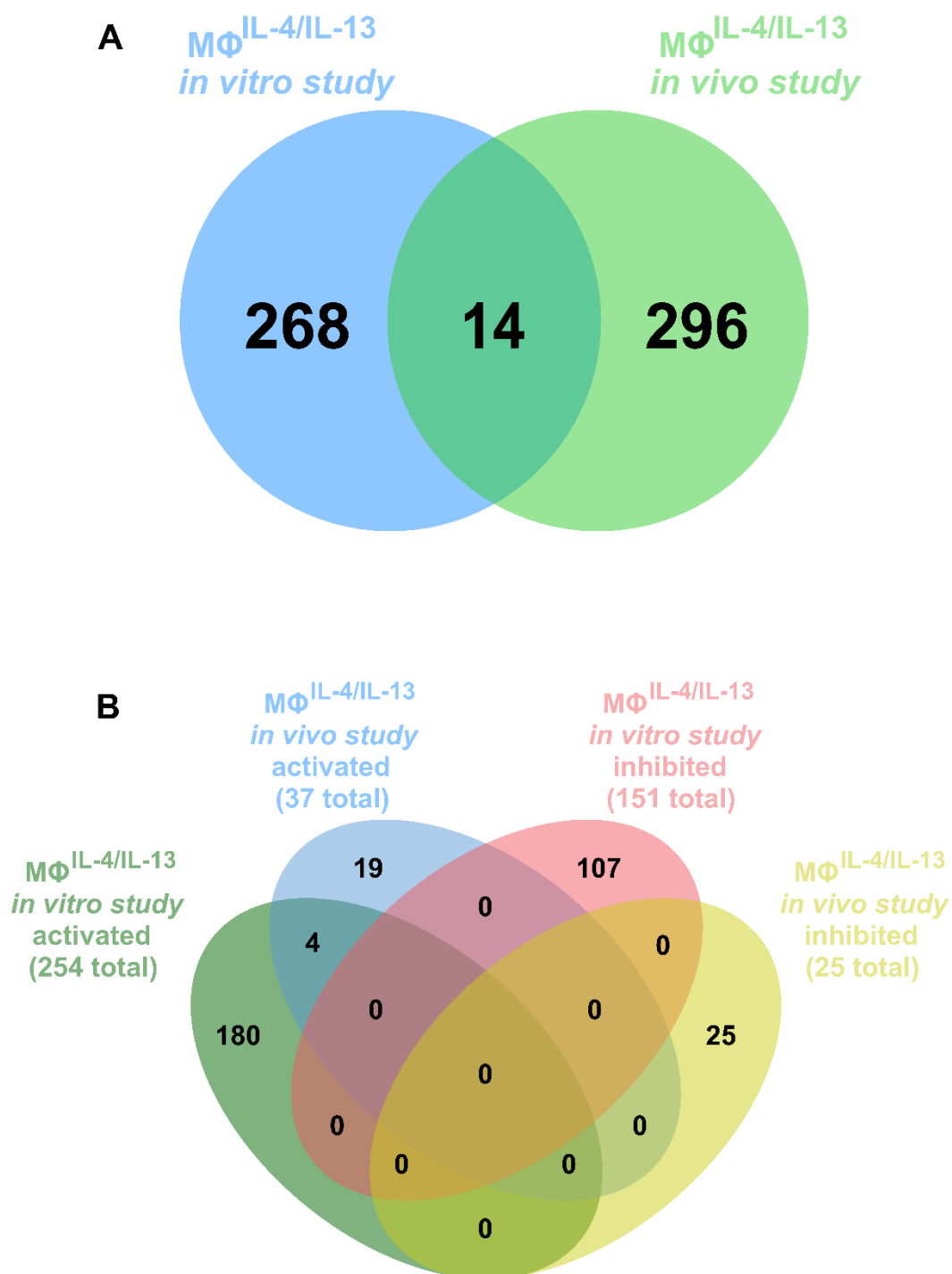


Figure 48. Venn diagram showing shared and unique differentially expressed genes (DEGs; p -value ≤ 0.05 and $\log_2FC < -1/\log_2FC > 1$) identified in mare endometrial fibroblasts after treatment with the eqMD- $M\Phi^{IL-4/IL-13}$ -CM for 96 hours and in mare endometrium after intrauterine administration of autologous eqMD- $M\Phi^{IL-4/IL-13}$ 6 hours after the procedure (A). Venn diagram showing shared and unique predicted upstream regulators of DEGs identified in mare endometrial fibroblasts in *in vitro* study and in mare endometrium in *in vivo* study subdivided into those with predicted activated and inhibited state (B).

7. Discussion

Fibrosis is a multidimensional and complex pathological condition that is widely recognized as a major cause of damaged tissue architecture. Consequently, it is associated with the onset and progression of many chronic diseases (Henderson et al., 2020; Wynn, 2008; Zeisberg & Kalluri, 2013). Endometrial fibrosis is the main hallmark of the development of equine endometrosis. It is therefore vital to research the processes involved in fibrosis development in order to establish a course of action that will identify the underlying cause of endometrosis. Moreover, it can lay a foundation towards finding an effective treatment method. The development of chronic inflammation, the altered expression of ECM-associated components, and the coordinated activation and action of different cell types are intensively studied topics in the context of both fibrosis and endometrosis. In particular, recent studies have demonstrated the critical role that M Φ play in processes associated with the development of fibrosis, with clear distinct differences between pro-inflammatory and anti-inflammatory subpopulations in cases of pulmonary fibrosis (Cheng et al., 2021), renal fibrosis (Meng et al., 2019), intestinal fibrosis (Yao & Tang, 2022), hepatic fibrosis (Sun & Matsukawa, 2024) and cardiac fibrosis (Lafuse et al., 2020). Nevertheless, there exists an insufficiency of knowledge with regard to their function in the development of endometrosis. To date, increased infiltration of M Φ in the mare endometrium has been observed in the early stage of fibrosis development (Szóstek-Mioduchowska et al., 2023). However, there was a lack of information regarding distribution of endometrial M Φ subpopulations both during the estrous cycle and in the course of endometrosis, as well as the manner in which these subpopulations interact with other endometrial cells, particularly fibroblasts, which are the most recognized cells in fibrotic research. The objective of the present dissertation was to address the knowledge gaps surrounding distribution of M Φ subpopulations in mare endometrium using a combination of integrated *in vitro* and *in vivo* models and omics-driven analyses. The results reported herein were intended to demonstrate the specific roles that M Φ subpopulations may play in the development of fibrosis in the course of endometrosis in mares.

7.1 The distribution of different types of tissue-resident MΦ in the endometrium of mares with endometrosis

The obtained results showed that the protein abundance of CD68 was up-regulated in category IIA compared to category I endometria in the follicular phase and to the respective category in the mid-luteal phase of the estrous cycle. These results are consistent with the knowledge regarding the fluctuations of immune cells in the endometrium of cycling mares. Previous studies have demonstrated an increase in the number of MHCII⁺ cells (including MΦ, lymphocytes, monocytes, and dendritic cells) in the mare endometrium in the follicular phase of the estrous cycle (Frayne & Stokes, 1994; Watson & Dixon, 1993). The localization of MHCII⁺ cells was confirmed in the endometrial stratum spongiosum and stratum compactum (Frayne & Stokes, 1994). Physiologically, the follicular phase of the estrous cycle is the period during which the uterus is most exposed to antigens. Consequently, an increase in the number of immune cells provides a state of preparation for mating and subsequent clearance of the uterus. This state of preparation has been shown to persist irrespective of the condition of the endometrium. This finding is supported by the results of a category-independent analysis showing elevated CD68 expression in the follicular phase of the estrous cycle. Recently, the transcriptomic profiling of mare endometria across different categories demonstrated the increased expression of genes annotated to cellular infiltration by MΦ in category IIA endometria in the follicular phase of the estrous cycle when compared to endometria category I. This infiltration was confirmed by immunofluorescence staining of CD68, which revealed a higher number of CD68⁺ cells in category IIA endometria (Szóstek-Mioduchowska et al., 2023). Similarly, the higher expression of CD68 was observed in moderate and advanced stages of oral submucous fibrosis in male patients (Pereira et al., 2015) and also in areas of severe interstitial fibrosis in biopsies from patients with chronic active allograft rejection (Wang et al., 2017). These findings, expanded with results of this dissertation, suggest a potential role for MΦ in the pathogenesis of fibrosis in the mare endometrium. However, no specific changes in CD68 expression was observed in endometria category IIB and III, suggesting that MΦ may play the most significant role in the onset of fibrosis.

In a mouse model of diabetic nephropathy, the concentration of MCP-1, a potent chemoattractant for monocytes and MΦ, in blood serum as well as its mRNA transcription in kidney tissue were significantly increased when compared to the control group (Ma et al., 2022). Moreover, *MCP-1* mRNA transcription was increased in patients with

nonalcoholic steatohepatitis (NASH) and the NASH mouse model, which resulted in increased infiltration of liver MΦ and liver fibrosis (J. Kang et al., 2023). However, no differences in the mRNA transcription of *MCP-1* were demonstrated in mare endometrium both in the course of endometriosis and during the estrous cycle (Domino et al., 2020), which is in accordance with our results. Besides that, an interesting direction to unravel the full picture of MΦ distribution in the tissue would be to adopt more advanced molecular methods of analysis. Endometrial fibrosis is scattered in the endometrium, therefore, changes that are restricted to a specific region may be challenging to detect exclusively by RNA and protein analyses, and it would be beneficial to take into consideration spatial analysis methods, like spatial proteomics and transcriptomics, scRNA-seq, or microdissection. Recently, image-based spatial transcriptomics was used to identify dysregulated niches in the lungs of patients diagnosed with pulmonary fibrosis (Vannan et al., 2025). Similarly, microdissection was proven useful in studying patients with IPF and lung cancer (Iida et al., 2021). Nevertheless, in this dissertation, the higher protein abundance of CD68 was presented in category IIA endometria, thus, the following step was to analyze changes in distribution of distinct MΦ subpopulations in mare endometrium.

The distribution of CD68⁺iNOS⁺ARG1⁻ cells (akin to MΦ1) and CD68⁺iNOS⁻ARG1⁺ cells (akin to MΦ2) in the endometrium category I, IIA, IIB, and III was confirmed in Research Task 1 with the use of flow cytometry. No significant differences in the percentage of CD68⁺iNOS⁺ARG1⁻ cells and CD68⁺iNOS⁻ARG1⁺ cells within categories of endometria and phases of the estrous cycle were observed. However, an increase in the percentage of CD68⁺iNOS⁺ARG1⁻ cells and concomitant decrease in the percentage of CD68⁺iNOS⁻ARG1⁺ cells were observed in all endometrial categories when compared to category I endometria, regardless of the phase of the estrous cycle. The role of MΦ1 and MΦ2 in the development of fibrosis is already well established in the lung, kidney, and liver (Wynn & Barron, 2010; Wynn & Vannella, 2016). However, the conventional concept of anti-fibrotic MΦ1 and pro-fibrotic MΦ2 is outdated as research has repeatedly shown that MΦ subpopulations may exert dual roles depending on the tissue site, physiological context and their general role in a given niche. Moreover, numerous studies have already indicated that there is a large interorgan and interspecies divergence of changes in MΦ polarization during the development of fibrosis. Macrophage infiltration was present in the kidneys of patients with diabetic kidney disease, as well as in a mouse model of diabetic nephropathy. In both cases, MΦ1 predominated among infiltrating cells

(Ma et al., 2022). Likewise, in mouse diabetic myocardial fibrosis, with the progression of the condition, the MΦ1 population increased, while the MΦ2 population decreased in number (Guo et al., 2024). On the other hand, in a mouse model of endometriosis, there was a correlation between infiltration of MΦ2 and progression of lesions and fibrosis development (Duan et al., 2018). Similarly, increased number of MΦ2a was correlated with muscle fibrosis in mouse during aging (Wang et al., 2015). These further underscore that the pro- and anti-fibrotic roles of MΦ subpopulations should be meticulously investigated and specified in particular cases, regardless of the established paradigm. To date, the distribution of MΦ subpopulations in the endometrium has not been demonstrated in mares. Nevertheless, in mares susceptible to breeding-induced endometritis, which can be considered as one of the risk factors for the development of endometriosis, higher levels of iNOS expression were demonstrated in endometrial biopsies (Alghamdi et al., 2005; Woodward et al., 2013). Although iNOS expression also occurs in other cells, it should not be overlooked that it is an important marker of MΦ1, which increase in the mare endometrium with progressive fibrosis, as presented in this dissertation.

The imbalance of MΦ1/MΦ2 in the mare endometrium in the progression of endometriosis represents a significant advance in the investigation of the underlying cause of endometriosis development. Chronic inflammation and impaired reparative processes are hallmarks of the function of MΦ1 and MΦ2. It is evident that these processes are concurrent and substantial in terms of the development of fibrosis. Thus, gaining an insight into MΦ-fibroblasts and MΦ-endometrium interactions may clarify the functions of MΦ subpopulation in mare endometrium.

7.2 The molecular profile of eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13}

From embryogenesis onward, MΦ are an essential component of the tissue and their population mainly relies on self-renewal (Dick et al., 2022; Epelman et al., 2014; Hashimoto et al., 2013). Nevertheless, when inflammation develops, the MΦ population may also be enlarged by differentiation from circulating blood monocytes. In this case, induced monocytes serve as progenitors for monocyte-derived MΦ that may outnumber those that were initially present in the tissue (Davies et al., 2013; Galli et al., 2011; Wynn & Vannella, 2016). For this reason, *in vitro*-generated monocyte-derived MΦ have become a popular tool for studying the role of MΦ in physiological and pathological processes. Beyond them, human THP-1 and mouse RAW264.7 MΦ cell lines (Li et al.,

2021), as well as bone marrow-derived MΦ (Toda et al., 2021), are utilized during research, however, monocyte-derived MΦ seem to better reflect the nature of inflammation. In horses, monocyte-derived MΦ have been utilized in studies of severe equine asthma to check changes in the expression profile of secreted cytokines after exposure to pathogenic stimuli (H. Kang et al., 2023) or to analyze the impact of equine infectious anemia virus on a host response (Du et al., 2015). In such research, controlling the process of differentiation is crucial. The CD68 marker can be used to monitor monocyte-MΦ differentiation *in vitro* (Lis-López et al., 2021). In Research Task 2, in eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} higher expression of CD68 has been demonstrated in comparison to freshly isolated equine peripheral blood monocytes. Along with a significant change in morphology, this confirmed the successful differentiation of monocytes into MΦ. To ensure the proper differentiation and polarization of MΦ subpopulations, it was necessary to perform the characterization of obtained cells, which in this study was accomplished with the use of qPCR, immunofluorescence, and LC-MS.

Macrophages are cells with exceptional plasticity. In response to adequate stimuli, they undergo phenotypic and functional changes that directly translate into their role in microenvironment regulation. Presence of the pathogens-related molecules, like LPS or PAMPs, but also Th1-related cytokines like IFN γ , drives the MΦ polarity in the direction of proinflammatory MΦ1 subpopulation (Sica & Mantovani, 2012). Macrophages type 1 are first responders after infection or injury. Their pro-inflammatory character is further reflected in their secretion of inflammatory cytokines, including IL-1, TNF- α , IL-12, and IL-23, which contribute to activation of different antimicrobial mechanisms and to the polarization of Th1 and Th17, both of which further fuel the inflammatory response (Murray & Wynn, 2011). Such cells are distinguished among MΦ by their high expression of iNOS, which provides MΦ1 with rapid production of high concentrations of NO (MacMicking et al., 1997). Results obtained in Research Task 2 revealed that eqMD-MΦ challenged with IFN γ /LPS exhibited this characteristic high expression of iNOS. It is well established that NO is a modulator of the inflammatory cascade (Laroux et al., 2001) and mediator of viral infections (Uehara et al., 2015) that equips MΦ with the ability to cytotoxically attack viruses, bacteria, and even tumor cells. Results of this dissertation presented that eqMD-MΦ^{IFN γ /LPS} had a higher expression of proteins annotated, among others, to defense response to virus (e.g. ISG15, TRIM22, OAS1), defense response (e.g. SLFN5, CFP, STAT3), and regulation of viral process (e.g. IFIH1,

TRIM14, MX1) when compared to eqMD-M Φ ^{IL-4/IL-13}. Moreover, eqMD-M Φ ^{IFN γ /LPS} exhibited higher mRNA transcription of *IL-1 β* , *IL-6*, *IL-12A*, and *IL-12B* than eqMD-M Φ ^{IL-4/IL-13} and also expressed proteins annotated to cytokine signaling in the immune system (e.g. HERC5, STAT2, SAMS1). A study of the transcriptomic profile of equine alveolar macrophages indicated that LPS-induced alveolar M Φ express genes involved in the initiation and maintenance of inflammation. Among detected were *TNF*, *IL-1 α* , *IL-1 β* , *IL-2R α* , *IL-6*, *IL-27* and chemoattractant chemokines (*CCL2*, *CXCL3*, *CXCL6*) (Karagianni et al., 2017). Moreover, LPS/IFN γ treatment has been proven to increase the quantity of IL-1 β and TNF- α in the secretome of equine peripheral blood CD14⁺ monocyte-derived M Φ (Bowlby et al., 2023). Further functional characterization of the obtained eqMD-M Φ ^{IFN γ /LPS} presented that DEPs characteristic for these cells were also annotated to cellular response to type I interferon (LOC100058990, STAT2, LOC100630879), interferon signaling (e.g. OAS1, HERC5, IFIT2), and interferon alpha/beta signaling (e.g. STAT2, IRF9, ISG20). Interferons are a group of Th1-associated cytokines that have an important role in host defense and response to pathogens. Precisely, IFN γ has been attributed an antiviral and immunomodulatory role by coordinating of both adaptive and immune responses (Mendoza et al., 2019). Thus, the gained results confirm that a population of pro-inflammatory M Φ secreting Th1-stimulating cytokines forms an activity loop with Th1 lymphocytes. In their active state, Th1 lymphocytes secrete IFN γ , further orchestrating the M Φ differentiation in favor of the M Φ 1 subpopulation. Moreover, the obtained data indicate that eqMD-M Φ challenged with IFN γ /LPS can be considered as cells resembling the physiological M Φ 1 subpopulation.

On the other side of the polarization spectrum are alternatively activated M Φ 2a. In response to stimulation with Th2-associated cytokines, such as IL-4 and IL-13, M Φ change their ARG metabolism in favor of the enzyme ARG1 (Rath et al., 2014). Results of this dissertation presented that eqMD-M Φ challenged with IL-4/IL-13 exhibited high expression of ARG1 compared to eqMD-M Φ ^{IFN γ /LPS} as confirmed by immunofluorescence staining. Arginase limits ARG availability for NO synthesis and hydrolyzes it to urea and ornithine. Then ornithine becomes the downstream regulator of proline and polyamine syntheses and a precursor for polyamines and collagen production (Grasemann et al., 2015; Rath et al., 2014). It has been shown that ornithine can accumulate in wounds extracellular space to serve as a proline precursor or to be directly incorporated by wound-derived fibroblasts (Albina et al., 1993). Therefore, M Φ 2a are

demonstrated as those related to processes of tissue repair and ECM remodeling. Results presented in this dissertation revealed that DEPs characteristic for eqMD-MΦ^{IL-4/IL-13} were annotated to ECM organization (e.g. SERPINH1, FN1, SPP1), fibronectin binding (ITGB3, CTSK, ITGAV), ECM proteoglycans (e.g. FN1, ITGB3, ITGB5), and elastic fiber formation (e.g. ITGB3, ITGB5, ITGAV). Moreover, they were also shown to be linked to multicellular organism development (e.g. C1GALT1, VCL, ECE1), anatomical structure development (e.g. SERPINH1, ALDH1A2, SERINC5), and embryonic morphogenesis (e.g. FN1, ALDH1A2, ITGB3). This correlates with the data that MΦ with high expression of ARG1 play their role in ontogeny and organ differentiation during embryogenesis (Dzik, 2014). Macrophages type 2a are also known for their role in tissue remodeling through the secretion of growth factors like PDGF and TGF-β, or direct production of MMPs and TIMPs, which affect local fibroblasts by regulating myofibroblast differentiation and ECM degradation (Murray & Wynn, 2011; Wynn, 2008). Obtained eqMD-MΦ^{IL-4/IL-13} exhibited higher mRNA transcription of *MMP-3* compared to eqMD-MΦ^{IFNγ/LPS}. However, there were no differences in *TGF-β1* mRNA transcription between eqMD-MΦ^{IFNγ/LPS} and eqMD-MΦ^{IL-4/IL-13}. Similarly, Bowlby et al. (2023) presented that TGF-β1 secretion was observed in LPS+IFNγ and IL-4+IL-10 stimulated equine peripheral blood CD14⁺ monocyte-derived MΦ. Nevertheless, analysis of DEPs characteristic for eqMD-MΦ^{IL-4/IL-13} revealed annotation to signaling by TGF-beta receptor complex (ITGB3, F11R, ITGB5, ITGAV), TGF-beta receptor signaling activated SMADs (ITGB3, ITGB5, ITGAV), and signaling by TGFB family members (ITGB3, F11R, ITGB5, ITGAV), confirming the role of this pathway in the MΦ2a functioning. Further involvement of eqMD-MΦ^{IL-4/IL-13} in processes associated with ECM remodeling was indicated by the detection of DEPs associated with integrin-mediated signaling pathways (FN1, ITGB3, ITGB5, ITGAV), integrin binding (e.g. SPP1, F11R, ICAM1), and focal adhesion (e.g. FN1, SPP1, ITGB3). Integrins are vital for sensing physical, dimensional, and biochemical features of ECM (Larsen et al., 2006; Zeltz & Gullberg, 2016). Thus, integrin signaling, which includes focal adhesions, regulates ECM remodeling and also promotes differentiation, proliferation, and cell cycle progression in local cells (Larsen et al., 2006; Mishra & Manavathi, 2021). The obtained data indicate that eqMD-MΦ challenged with IL-4/IL-13 can be considered as cells resembling the physiological MΦ2a subpopulation.

In eqMD-MΦ^{IFNγ/LPS} and eqMD-MΦ^{IL-4/IL-13}, over the 70% of identified proteins had a similar expression level. This finding does not invalidate the outcomes of the functional

analysis, which demonstrate the effective differentiation and polarization of MΦ1 and MΦ2a. The common proteins were those essential for the fundamental cell physiology and processes, like intracellular anatomical structure (e.g. MANF, HEATR1, DOK3, ATP1A1) or metabolic processes (e.g. RPLP2, PRKX, SUGP2). Similarly, the analysis of the proteomic profile of polarized human monocyte-derived MΦ revealed a high number of common proteins between the studied subpopulations, suggesting that these proteins act as a core enabling MΦ polarization (Oates et al., 2023).

Obtained results indicate that eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} resemble the MΦ1 and MΦ2a subpopulations, and correspond to their typical functional paradigm, which has the potential to be correlated with the possible role that MΦ subpopulations may play in the development of fibrosis. Thus, the subsequent step was to analyze the impact of eqMD-MΦ^{IFN γ /LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM on mare endometrial fibroblasts, as well as how intrauterine administration of these cells affect the mare endometrium.

7.3 The effect of the secretome of eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} on the mare endometrial fibroblasts

Fibroblasts are cells that play a central role in the development and progression of fibrosis (Talbot et al., 2022). At the same time, an increasing amount of research indicates that MΦ orchestrating chronic inflammation are master regulators of fibrosis formation (Wynn & Barron, 2010; Wynn & Vannella, 2016). Hence, the importance of studying the interaction between these two groups of cells emerges, especially when it is unclear how exactly distinct MΦ subpopulations contribute to the processes linked to the development of fibrosis in the progression of endometriosis in the mare. As demonstrated in this dissertation, eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} exhibited characteristic proteomic differences, indicating their functional distinction, which gave rise to the premise that these variations would be transposed onto their effect on mare endometrial fibroblasts. The transcriptomic analysis performed in Research Task 2 revealed that the treatment with eqMD-MΦ^{IFN γ /LPS}-CM affected the expression of 404 genes, while the treatment with eqMD-MΦ^{IL-4/IL-13}-CM influenced the expression of 282 genes in mare endometrial fibroblasts. Interestingly, 234 of the identified DEGs were common after treatment with CM of both eqMD-MΦ subpopulations. The identified common DEGs were strongly annotated among others to migration of cells (e.g. *ABCB1*, *LAMA4*, *TUBB4A*) and cell movement (e.g. *BAMBI*, *NEXN*, *SYNM*). This indicates that MΦ,

irrespective of the subpopulation type, exerts a comparable effect on the fundamental cellular processes in mare endometrial fibroblasts. This resemblance was further highlighted by the same effect on the functional characteristics of mare endometrial fibroblasts, as both eqMD-M Φ subpopulations equally increased the viability, proliferation, and migration of mare endometrial fibroblasts. The onset and progression of fibrosis development are significantly influenced by fibroblast proliferation and migration. It has already been established that fibroblast proliferation is induced in cardiac fibrosis (Ebrahimighaei et al., 2024) and is a hallmark of IPF (Roberts et al., 2021). Similarly, fibroblasts derived from the lungs of IPF patients demonstrated enhanced migration rates (Suganuma et al., 1995; Tisler et al., 2020). Thus, the obtained results are the first to indicate that the secretome of eqMD-M Φ subpopulations could exert an effect on mare endometrial fibroblasts related to the development of fibrosis. This possibility was also underscored by the transcriptomic analysis, as it evidenced that common DEGs were annotated to soft tissue lesions (e.g., *ADAMTS12*, *ASPN*, *MMP15*) and fibrosis of tissue (e.g., *IL13RA2*, *IL1R1*, *VDR*).

One of the molecular mechanisms of the development of fibrosis presumes the activation of fibroblasts into ACTA2-expressing myofibroblasts (Schuster et al., 2023). It was reported that endometrial ACTA2 expression is increased in the final stages of endometrosis (Szóstek-Mioduchowska et al., 2019b), as well as the recent transcriptomic study revealed its higher expression in category IIB when compared to category I endometria (Szóstek-Mioduchowska et al., 2023). Results presented in this dissertation revealed that eqMD-M Φ ^{IFN γ /LPS}-CM decreased the protein abundance of ACTA2, while eqMD-M Φ ^{IL-4/IL-13}-CM had no impact on its protein abundance. Studies on different models of fibrosis suggest that the relationship between M Φ subpopulations and myofibroblasts differentiation is organ-specific, and it is inconclusive which subpopulation is solely responsible for the regulation of this process (Chen et al., 2024; Hou et al., 2018; Nakamura et al., 2022; Yuan et al., 2019). In a murine model of IPF, coculture with M Φ 1 showed no effect on myofibroblast differentiation of lung resident mesenchymal stem cells. Moreover, the co-culture of mesenchymal stem cells with M Φ ^{LPS+} caused a significant decrease in myofibroblast number in the context of skeletal muscle regeneration (Venter et al., 2021). On the contrary, it was presented that incubation with M Φ 2 promoted myofibroblasts activation, which was indicated by higher expression of ACTA2 (Hou et al., 2018). Similarly, a shift in M Φ polarization, from M Φ 1 into M Φ 2, was interconnected with the reduction of ACTA2 expression in murine cardiac

fibroblasts (Chen et al., 2024). On the other hand, THP-1 monocyte-derived $M\Phi^{IFN\gamma/LPS}$ and $M\Phi^{IL-4}$ did not affect ACTA2 expression in human vocal fold fibroblasts, and only $M\Phi^{TGF-\beta 1}$ elevated the expression of this marker (Nakamura et al., 2022). Following this lead, it is important to take into consideration factors such as TGF- $\beta 1$, whose role in myofibroblast differentiation is well established (Yuan et al., 2019). Previously, fibroblasts derived from category I endometria have been shown to have an increased expression of ACTA2 after treatment with TGF- $\beta 1$ (Szóstek-Mioduchowska et al., 2019b). Results presented in this dissertation revealed that treatment with eqMD- $M\Phi^{IFN\gamma/LPS}$ -CM and eqMD- $M\Phi^{IL-4/IL-13}$ -CM decreased mRNA transcription of *TGF- $\beta 1$* . Thus, the obtained results indicate that $M\Phi$ subpopulations in mare endometrium do not appear to directly promote the differentiation of fibroblasts into myofibroblasts.

The main hallmark of fibrosis development is the excessive remodeling and deposition of ECM. Thus, to better understand how mare endometrial fibroblasts respond to treatment with CM of eqMD- $M\Phi$ subpopulations, in the context of fibrosis, it is important to comprehend their effect on the expression of ECM-associated components. The results of Research Task 2 showed that solely treatment with eqMD- $M\Phi^{IL-4/IL-13}$ -CM increased the protein abundance of FN1 in mare endometrial fibroblasts. Fibronectin 1 is an ECM component that is highly involved in several biological processes, including cell adhesion, cell migration, and cell differentiation. Overexpression of FN1 has been shown to accelerate the course of several illnesses, including heart failure or neoplastic disease (Patten & Wang, 2021). Moreover, FN1 polymerization has been shown as a vital component for the deposition of COL, thus its high expression plays a role in fibrosis development. Altrock et al. (2015) have shown that experimental liver fibrosis was alleviated by inhibition of FN1 assembly in murine hepatic stellate cells. Fibronectin assembly inhibitor contributed to reduced quantity of COL and FN1 without influencing their synthesis. Similarly, FN1 assembly inhibitor reduced FN1 and COL deposition by human and murine cardiac myofibroblasts. Inhibited mRNA transcription of *FN1* mitigated the pathological cardiac remodeling and fibrosis in a murine model of heart failure *in vivo* and also reduced the pathogenic characteristic of myofibroblasts *in vitro* (Valiente-Alandi et al., 2018). Fibronectin 1 has also been proposed as a prospective marker of diabetic kidney disease, considering its involvement in the immune response and regulation of $M\Phi$ -induced fibrosis (Dou et al., 2023). Importantly, a high correlation was shown between *FN1* and alternative activation of $M\Phi 2$ in diabetic kidney disease (Dou et al., 2023), and downregulation of *FN1* prevented THP-1 monocyte-derived $M\Phi$

polarization into MΦ2 subpopulation *in vitro* (Zhou et al., 2022). The results of this dissertation further support this information, as eqMD-MΦ^{IL-4/IL-13} were presented to have an elevated protein abundance of FN1, as well as among DEPs characteristic for this subpopulation were those annotated to fibronectin binding (ITGB3, CTSK, ITGAV). As FN1 affects MΦ differentiation, and the treatment eqMD-MΦ^{IL-4/IL-13}-CM up-regulated the protein abundance of FN1 in mare endometrial fibroblasts, this data becomes interesting in the context of disturbed endometrial ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells in the course of endometriosis demonstrated in Research Task 1. Differentially expressed genes identified in mare endometrial fibroblasts after treatment with eqMD-MΦ^{IFNγ/LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM were annotated to increased macrophage classical activation signaling pathway (e.g., *CXCL9*, *STAT1*, *TNFSF15*). Simultaneously eqMD-MΦ^{IL-4/IL-13}-CM stimulated surrounding cells to deposit FN1, as it would promote alternative activation of MΦ2, seemingly to restore homeostatic MΦ ratio balance. If this process was to prove unsuccessful, it could contribute to excessive ECM deposition. However, such a relationship requires further in-depth investigation.

The development of fibrosis, as a consequence of an imbalance in the MΦ1/MΦ2 ratio, may be further aggravated by the fact that only DEGs identified in mare endometrial fibroblasts after treatment with the eqMD-MΦ^{IFNγ/LPS}-CM were annotated to decreased activation of elastic fiber formation (e.g., *FBN2*, *LOXL3*, *LTBP1*) and collagen degradation (e.g., *COL23A1*, *COL4A3*, *MMP15*), indicating their potential inhibiting role in ECM remodeling and deposition. The most prominent factors primarily responsible for ECM degradation and remodeling are MMPs. Furthermore, MMPs are also important components for the activity of immune cells, including MΦ. Matrix metalloproteinase 9 is known for the degradation of COL4, which is present in basement membranes. Due to the secretion of MMP-9, MΦ migrate to the site of inflammation and fulfill their protective function (Gong et al., 2008; Opdenakker et al., 2001). Besides the direct secretion, MΦ can also induce the expression of different MMPs in other cells. For example, treatment with CM of MΦ1 resulted in increased mRNA transcription of *MMP-1*, *MMP-2*, *MMP3*, and *MMP-14* in human dermal fibroblasts (Ploeger et al., 2013), as well as co-culture of MΦ subpopulations with tendon fibroblasts revealed elevated *MMP-1a*, *MMP-3*, and *MMP-13* mRNA transcription (Manning et al., 2015). Results presented in this dissertation revealed that treatment with both the eqMD-MΦ^{IFNγ/LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM increased the protein abundance of

MMP-9 in mare endometrial fibroblasts. It has been previously shown that MMP-9, along with MMP-1 and MMP-2, had a higher concentration in mare endometrium in more severe stages of endometriosis (Szóstek-Mioduchowska et al., 2020). Similarly, Robert et al. (2019) demonstrated that co-culture of human hepatic stellate cells with the THP-1 macrophage cell line led to upregulation of *MMP-3* and *MMP-9* mRNA transcription. Moreover, these authors suggested that the LPS-activated inflammasome pathway in MΦ led to MMPs/TIMPs imbalance and enhanced fibrolytic properties in human hepatic stellate cells (Robert et al., 2019). Similarly, upregulation of MMP-9 expression was observed in steatohepatitis fibrosis in mice, which was down-regulated after the depletion of bone marrow-derived MΦ, however, rather as a result of a lack of stellate cells activation (Han et al., 2019). Moreover, MMP-9 is known for its capacity to induce fibroblast migration. Macrophage-secreted MMP-9 has been shown to increase the migration of fibroblasts in a murine model of bleomycin-induced pulmonary fibrosis (Li et al., 2019) and has been associated with direct TGF-β1 activation and gel contraction activity, as demonstrated in human lung fibroblasts and MMP-9-deficient murine pulmonary fibroblasts (Kobayashi et al., 2014). These suggest that elevated *MMP9* mRNA transcription after the treatment with the eqMD-MΦ^{IFNγ/LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM may be implicated not only in ECM remodeling, but also modulate the functional characteristics of mare endometrial fibroblasts. Certainly, MMP-9 is not the only MMP that undergoes alterations in expression resulting from the action of MΦ subpopulations (Manning et al., 2015; Ploeger et al., 2013). The transcriptomic analysis of mare endometrial fibroblasts revealed that treatment with the eqMD-MΦ^{IFNγ/LPS}-CM down-regulated the expression of *MMP-15*, while eqMD-MΦ^{IL-4/IL-13} down-regulated the expression of both *MMP-15* and *MMP-19*. Furthermore, functional antagonists of MMPs – TIMPs, were presented to have altered expression in response to the treatment with eqMD-MΦ^{IFNγ/LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM. Obtained results revealed that treatment with the eqMD-MΦ^{IFNγ/LPS}-CM decreased the concentration of TIMP-1, while treatment with eqMD-MΦ^{IL-4/IL-13}-CM decreased the concentration of TIMP-2 in mare endometrial fibroblasts. It has been established that in severe cases of endometriosis, TIMPs have higher endometrial concentrations (Szóstek-Mioduchowska et al., 2020). Similarly, COL deposition and fibroblasts activation were significantly reduced in murine lungs, indicating that TIMP-1 is essential for the fibrotic response of fibroblasts during pulmonary fibrosis (Dong & Ma, 2017). Moreover, TIMP-1 is a known regulator of MMP-9 activity (Arpino et al., 2015). Its decreased levels indicate the lack of inhibition

of MMP-9 activity. Similar results were obtained in the study presented by Chen et al. (2020) in which the MMP-9/TIMP ratio was significantly higher in LPS-induced acute lung injury in rats. As for TIMP-2, its function is mainly correlated with a profile of MMP-2 activity (Bernardo & Fridman, 2003). These results may suggest that different MΦ subpopulations are responsible for the regulation of ECM degradation through distinct MMPs/TIMPs pathways. Conversely, considering all of the presented data in terms of ECM remodeling, it appears that MΦ2a may be responsible for FN1 synthesis in mare endometrial fibroblasts, thus regulating ECM deposition, while MΦ1 orchestrate the remodeling process by promoting the imbalance of MMP-9/TIMP-1 which may further modulate functional characteristics of mare endometrial fibroblasts and regulate the ECM remodeling.

Macrophages, as a part of the innate immune system, are considered the guardian cells that regulate the tissue microenvironment and orchestrate the process of inflammation (Varol et al., 2015). Macrophages, through their polarization capacity, modulate the production of inflammatory mediators. Upon activation to MΦ1, they release pro-inflammatory cytokines like TNF- α , IL-1 β , or IL-6, which trigger the inflammatory response, while MΦ2a are known for the secretion of anti-inflammatory factors (Murray & Wynn, 2011). This functional subdivision was confirmed in eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} considering their molecular profile obtained in Research Task 2. Part of this immunoregulatory role is the recruitment, polarization, and activation of particular cells of the innate and adaptive immune system (Mack, 2018). The obtained results showed that treatment with both the eqMD-MΦ^{IFN γ /LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM influenced the expression of genes annotated to cell movement of leukocytes (e.g. *CXCL12*, *IL1R1*, *SERPINB7*), migration of mononuclear leukocytes (e.g. *BCL2*, *CD274*, *STAT1*), and cell death of immune cells (e.g. *RIGI*, *TNFSF13B*, *ZBP1*). Especially influenced was expression of genes connected to activation of innate immune system, as identified common DEGs were annotated to cellular infiltration by phagocytes (e.g. *C2*, *EPAS1*, *MASP1*), polarization of macrophages (e.g. *BST2*, *LDHA*, *ZBP1*) differentiation of antigen-presenting cells (e.g. *CELSR3*, *ESR1*, *RUNX1*), differentiation of phagocytes (e.g. *JAG1*, *PLAC8*, *UCP2*), macrophage classical activation signaling pathway (e.g. *CXCL9*, *STAT1*, *TNFSF15*) and neutrophil degranulation (e.g. *ALDOC*, *CYBB*, *SNAP25*). Interestingly, only DEGs identified in endometrial fibroblasts after the treatment with the eqMD-MΦ^{IFN γ /LPS}-CM showed the annotation to cell movement of neutrophils (e.g. *CHI3L1*, *DOCK5*, *NFKBIZ*), neutrophil extracellular trap signaling pathway (e.g. *CYBB*,

MLKL, *SLC25A4*), lymphocyte migration (e.g. *CAV1*, *KCNA3*, *LAT*) and cell proliferation of T lymphocytes (e.g. *CD74*, *IL13RA2*, *IL7*). The detailed influence of neutrophils and lymphocytes on the processes involved in the development of fibrosis in the course of endometriosis has already been described. Neutrophil extracellular trap proteins, including ELA, CAT, and MPO, were shown to increase COL1 expression in mare endometrial explants (Rebordão et al., 2018), and the utilization of specific inhibitors alleviated this effect (Amaral et al., 2023; Amaral et al., 2020a), further supporting the theory that the presence of neutrophil-associated proteins is important for the development of fibrosis in the progression of endometriosis. Concurrently, it was presented that Th1 and Th2 lymphocytes altered the expression of *FN1*, *ACTA2*, *MMP2*, *TIMP1*, and *TIMP2* in mare endometrial fibroblasts and also influenced genes annotated to processes important in the context of endometriosis, like tissue remodeling, collagen binding, or fibroblast proliferation (Wójtowicz, 2024).

Not only immune cells but also fibroblasts actively respond to signals secreted by activated MΦ and subsequently change their expression and secretion of inflammatory cytokines and chemokines, facilitating the local immune response (Bautista-Hernández et al., 2017). In this manner, treatment with the eqMD-MΦ^{IFN γ /LPS}-CM up-regulated the mRNA transcription of *IL-6* in mare endometrial fibroblasts. Interleukin 6 is a pleiotropic cytokine that has a significant impact on the immune system and inflammation, and is known for its important role in fibrosis development (Li et al., 2022). In this instance, Ma et al. (2012) presented that IL-6 knockout in mice reduced angiotensin II-induced cardiac fibrosis and that the production of IL-6 was markedly increased in the co-culture of murine cardiac fibroblasts with MΦ. In the course of the endometriosis, it was already established that there is a higher expression of IL-6 in mare endometrium with fibrosis (Szóstek et al., 2013) and that this cytokine specifically affects the expression of ECM-associated components in mare endometrial explants (Szóstek-Mioduchowska et al., 2019a). Thus, the results indicate that MΦ1 may play a role in promoting the expression of this pro-fibrotic cytokine in mare endometrial fibroblasts. Interestingly, the treatment with the eqMD-MΦ^{IL-4/IL-13}-CM also up-regulated the *IL-6* mRNA transcription. However, taking into consideration the disturbed endometrial ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells, their effect in this matter could be minor. Concurrently, studies have shown that in human and murine MΦ, IL-6 promoted the alternative activation of MΦ2 (Fernando et al., 2014). The same was observed in patients with gastric cancer (Fu et al., 2017). Thus, the results suggest that MΦ2a may

induce IL-6 not to promote an inflammation but rather as a way to restore homeostatic MΦ ratio balance, however, such a hypothesis should be further elucidated.

The obtained results also revealed that treatment with the eqMD-MΦ^{IFN γ /LPS}-CM down-regulated the *CCN2* mRNA transcription in mare endometrial fibroblasts. Cellular communication network 2, also known as connective tissue growth factor (CTGF), is strongly connected with the development of fibrosis, as it has a significant role in the modulation of ECM deposition (Rebolledo et al., 2021). In human knee arthrofibrosis, MΦ-fibroblasts interaction led to the development of fibrosis induced by *CCN2* expression in fibroblasts, which correlated with the quantity of collagen and elevated fibroblast proliferation and migration (Li et al., 2025). Moreover, the higher expression of *CCN2* was characterized in patients suffering from IPF, where it can regulate the ECM deposition and senescence of cells (Effendi & Nagano, 2022). In case of mare endometrial fibroblasts, the down-regulated *CCN2* mRNA transcription can be considered a defense mechanism during an induced inflammatory response, acting to avoid premature fibrosis during inflammation. However, it does not undermine the role of MΦ1 in orchestrating the pro-inflammatory microenvironment and indirect control of development of fibrosis.

The aforementioned publications concerning IL-6 expression and its effect on mare endometrium in the context of endometriosis also demonstrated the upregulation of IL-1 β expression (Szóstek-Mioduchowska et al., 2019a; Szóstek et al., 2013). The IL-1 family is implicated in nearly all fibrotic diseases and is essential to their onset and progression through modulation of cell proliferation, adhesion or migration, as well as through maintaining of the pro-inflammatory microenvironment (Wang et al., 2023). In this instance, IL-1 β was demonstrated to control the activation of hepatic stellate cells in mice *in vivo*, transitioning hepatic damage to the development of fibrosis (Gieling et al., 2009). Results obtained in Research Task 2 revealed that the eqMD-MΦ^{IL-4/IL-13}-CM down-regulated *IL-1 β* mRNA transcription in mare endometrial fibroblasts, suggesting that MΦ2a can suppress the inflammatory signaling of IL-1 β in mare endometrial fibroblasts. At the same time, the transcriptomic analysis revealed that both CM up-regulated the expression of *IL1R1*, implying that despite the decreased IL-1 β production, fibroblasts may become more sensitive or responsive to IL-1 β signaling due to upregulation of its receptor. Nevertheless, the down-regulation of *IL-1 β* mRNA transcription highlights the anti-inflammatory role of MΦ2a. This is also emphasized by the fact that eqMD-MΦ^{IL-4/IL-13}-CM also down-regulated the mRNA transcription of *AREG*. Amphiregulin is a transmembrane glycoprotein that has been presented to have a role in

the development of tissue fibrosis. In mice, silencing the *AREG* mRNA transcription in the model of renal fibrosis alleviated the upregulated mRNA transcription of *ACTA2*, *COL1*, *COL3*, and *FN1* (Son et al., 2021). Similarly, the knock-out of *AREG* in mice prevented bleomycin-induced skin fibrosis (Zhang et al., 2021). In endometriosis, *AREG* has been recently demonstrated to differentially affect the ECM-associated component mRNA transcription depending on the category of endometrium from which fibroblast originated (Wójtowicz et al., 2025). Thus, the down-regulation of *IL-1 β* and *AREG* may suggest an anti-inflammatory and anti-fibrotic role of M Φ 2a. On the other hand, the eqMD-M $\Phi^{IL-4/IL-13}$ -CM up-regulated *TNF α* mRNA transcription in mare endometrial fibroblasts. The TNF superfamily is associated with the development of fibrosis due to its role in inflammation (Steele et al., 2023). In endometriosis, the up-regulated secretion of *TNF α* has been demonstrated in uterine fluid of mares with ongoing fibrosis (Lecton et al., 2024), as well as its higher mRNA transcription was presented in fibrotic endometrium (Centeno et al., 2024). Notably, *TNF α* pulmonary administration to mice with established pulmonary fibrosis was reported to improve lung function and architecture and to decrease the number of alternatively activated M Φ . This role was also confirmed in a study of bleomycin-induced *TNF α ^{-/-}* mice in which fibrosis and alternative M Φ activation were sustained (Redente et al., 2014). Thus, data presented in this part of the dissertation indicate that treatment with eqMD-M $\Phi^{IL-4/IL-13}$ -CM change the mRNA transcription of inflammatory markers in mare endometrial fibroblasts in an inconclusive way.

The functional analysis of transcriptomic results obtained in Research Task 2 further emphasized the importance of M Φ 1 subpopulation in orchestrating the inflammatory processes in the context of their mediation of fibrosis development in endometriosis, as DEGs identified after the treatment with the eqMD-M $\Phi^{IFN\gamma/LPS}$ -CM were also annotated to NF- κ B signaling pathway (*EIF2AK2*, *IL1R1*, *PDGFRA*, *TNFSF13B*). The NF- κ B signaling pathway is a key regulator of immune responses and inflammation (Mitchell et al., 2016), which has already been thoroughly described in the context of fibrosis development (Tabary et al., 2006; Vellaichamy et al., 2005; Yuan et al., 2019). In the context of endometriosis, NF- κ B signaling pathway activation was observed in mare endometrium with active endometriosis during the follicular phase of the estrous cycle (Domino et al., 2020), as well as its canonical pathway has been proposed to be primarily engaged in a destructive form of endometriosis (Jasiński et al., 2022; Jasiński et al., 2021). Interestingly, Pradere et al. (2013) have demonstrated that liver fibrosis in mice was

promoted by MΦ regulation of NF-κB signaling, which enhanced myofibroblasts' survival rate, and that depletion of MΦ ameliorated the activation of NF-κB in hepatic stellate cells. Moreover, these authors presented that MΦ-dependent NF-κB activation was mediated specifically by IL-1 and TNF-α, which promoted the survival of myofibroblasts, not their activation (Pradere et al., 2013). These results again demonstrate that the pro-inflammatory activity of MΦ1 has the hallmarks of a pro-fibrotic influence on mare endometrial fibroblasts, indicating that this MΦ subpopulation may be an important mediator of the development of fibrosis in the pathogenesis of endometriosis.

Results presented in Research Task 2 further underscore the relevance of the disrupted endometrial ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells in the course of endometriosis, which implies the increase in MΦ1/MΦ2 ratio and imbalance between pro-inflammatory and repair processes. The MΦ1, as a pro-inflammatory population, secrete pro-inflammatory cytokines orchestrating chronic inflammation, while MΦ2a are responsible for promoting tissue healing and remodeling in fibroblasts. In the context of endometrial fibrosis, the decreased number of MΦ2 indicates weakening of their effect. Concurrently, the predominance of MΦ1 may promote the maintenance of inflammation and cytokine-dependent activation of fibroblasts, which induces the excessive ECM deposition in a pathological manner and cause the development of fibrosis.

7.4 The effect of intrauterine administration of autologous eqMD-MΦ^{IFNγ/LPS} and eqMD-MΦ^{IL-4/IL-13} on the mare endometrium

In vitro models offer distinct advantages, including the ability to control the environment, ethical considerations, and reduced costs. These features make them useful for the preliminary screening and acquiring information about direct communication and correlation between isolated cell populations. *In vivo* studies allow the observation of the intricate relationships between different cell populations and provide critical insights into complex biological processes within the dynamic physiological context of a whole organism. Furthermore, these studies enable the determination of the effect of systemic factors, including hormonal regulation, metabolism, or immunological response, on these processes. Therefore, *in vitro* and *in vivo* studies are complementary in biomedical research, however, *in vivo* analyses are essential for ensuring the applicability of obtained knowledge in actual biological systems. As demonstrated in this dissertation, eqMD-MΦ^{IFNγ/LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM affected the endometrial fibroblasts and their response varied depending on the specific MΦ subpopulation. Thus, the

subsequent step of research involved analyzing how the endometrial tissue would respond to the intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}. Intrauterine administration is a common method for studying the effect of action of distinct cell populations and factors on the mare endometrium. For instance, it was utilized to analyze how semen influences regulatory T lymphocytes in mare endometrium in the context of the involvement of the maternal immune system in early pregnancy loss (Hartmann et al., 2018). Moreover, intrauterine administration of mesenchymal stem cells has already been proposed as a possible method for improvement of endometrial condition, raising prospects for a potential treatment method for endometriosis (Mambelli et al., 2014). Thus, a similar approach was employed to study the effect of different M Φ subpopulations on the endometrial cell response. The results of *in vitro* experiments conducted in Research Task 2 revealed a convergent response of mare endometrial fibroblasts to eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM. Meanwhile, the *in vivo* study revealed that intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} induced changes in the transcriptomic profile as well as in mRNA transcription of ECM-associated components and inflammatory markers in the mare endometrium, which often showed an opposite pattern. This indicated that not only fibroblasts but also other endometrial cells (e.g. epithelial, endothelial, and immunological cells) are responsible for mediating the response of the endometrium to the different M Φ subpopulations. It should be recalled that cells other than fibroblasts can also be activated into myofibroblasts. Epithelial cells and endothelial cells in fibrotic foci have been demonstrated to transition into mesenchymal cells during EMT and EndMT, respectively (Wynn, 2008), and these processes can be orchestrated through M Φ modulation. It has been presented that human peritoneal mesothelial cells lost their epithelial morphology and exhibited myofibroblast-characteristic expression of ACTA2 in response to the co-culture with M Φ 1 in the context of the development of peritoneal fibrosis (Shi et al., 2016). Similarly, cardiac M Φ has been presented to promote EndMT in murine endothelial cells through *MMP-14*-mediated activation of latent TGF- β 1 (Alonso-Herranz et al., 2020). Furthermore, the general role of these cells was broadly studied in the development of pulmonary fibrosis (Confalonieri et al., 2022; Yanagihara et al., 2023) and renal fibrosis (Yang et al., 2019), as well as epithelial cells from mare endometrium have already been presented to change their MMPs and TIMPs secretion in response to the pro-fibrotic stimuli in the context of endometriosis progression (Szóstek-Mioduchowska et al., 2020).

As with the results obtained in the *in vitro* experiments conducted in Research Task 2, the results of the *in vivo* study performed in Research Task 3 revealed that in response to the distinct MΦ subpopulations, the expression of ECM-associated components has been altered. In this instance, the elevated *FN1* mRNA transcription in mare endometrium after the intrauterine administration of eqMD-MΦ^{IFN γ /LPS} was observed. Concurrently, the intrauterine administration of eqMD-MΦ^{IL-4/IL-13} resulted in decreased mRNA transcription of *COL1A1*, *COL3A1*, and *FN1*. These findings are in accordance with the observation that the increase in fibroblast activation, as indicated by elevated *ACTA2* mRNA transcription, was only present in response to the intrauterine administration of eqMD-MΦ^{IFN γ /LPS}. Moreover, the intrauterine administration of eqMD-MΦ^{IFN γ /LPS} resulted in elevated *MMP-2*, *TIMP-1*, and *TIMP-2* mRNA transcription in the mare endometrium. These results suggest that eqMD-MΦ^{IFN γ /LPS} and eqMD-MΦ^{IL-4/IL-13} exert disparate effects on the mare endometrium in the context of ECM deposition and remodeling. Moreover, they are pointing out the potential division into pro- and anti-fibrotic roles that MΦ subpopulations may play during the development of endometriosis. Key findings concerning endometriosis include upregulated COL1 secretion in explants of endometria with developing fibrosis (category IIA and IIB) and increased *ACTA2* expression in endometria with advanced endometriosis, which indicates myofibroblast activation (Szóstek-Mioduchowska et al., 2019a, b). Moreover, elevated MMP-1, -2, -9, and TIMPs levels reported in the course of endometriosis suggest dysregulation of ECM turnover in pathological endometrium (Szóstek-Mioduchowska et al., 2020). Taking this into consideration, the results obtained in Research Task 3 imply that MΦ1 might be involved in the elevated expression pattern of ECM-associated components in the mare endometrium, possibly serving as one of the mechanisms of regulation during ECM remodeling. Even if the intrauterine administration of eqMD-MΦ^{IFN γ /LPS} did not exert a direct impact on endometrial *COL1A1* and *COL3A1* mRNA transcription, it increased the mRNA transcription of *FN1*, which has been demonstrated to be an essential element for COL deposition, as well as its overexpression has been associated with liver and cardiac fibrosis (Altrock et al., 2015; Valiente-Alandi et al., 2018). Furthermore, the transcriptomic analysis revealed that intrauterine administration of eqMD-MΦ^{IFN γ /LPS} up-regulated the expression of *COL22A1*, which has already been proposed as an early response gene in the development of fibrosis in systemic sclerosis (Watanabe et al., 2019). Considering also that the intrauterine administration of eqMD-MΦ^{IFN γ /LPS} increased the endometrial mRNA transcription of *TIMP-1* and *TIMP-2*, which are known for inhibiting

MMPs-mediated ECM degradation (Ries, 2014) and that TIMP-1 has been associated with promoting the multi-walled carbon nanotube-induced lung fibrosis in mice (Dong & Ma, 2017), one may be inclined to conclude that MΦ1 induces the gene expression pattern in the endometrium that promotes the fibrotic process. In contrast, the intrauterine administration of eqMD-MΦ^{IL-4/IL-13} down-regulated the endometrial *COL1A1*, *COL3A3*, and *FN1* mRNA transcription as well as affected expression of genes annotated to decreased collagen chain trimerization (*COL14A1*, *COL21A1*, *COL4A6*, *COL8A1*), inducing their down-regulation. This indicated opposing effects between studied MΦ subpopulations, and due to the inhibition of ECM component mRNA transcription, implies the potential anti-fibrotic role of MΦ2a in the process of ECM deposition in equine endometrium.

The results obtained in Research Task 3 demonstrated a significant divergence of the MΦ subpopulations in the context of modulating the mRNA transcription of inflammatory markers in the mare endometrium. The intrauterine administration of eqMD-MΦ^{IFN γ /LPS} increased *CCN2*, *IL-1 β* , *IL-6*, *TGF- β 1*, and *TNF- α* mRNA transcription in mare endometrium, again highlighting the pro-inflammatory nature of eqMD-MΦ^{IFN γ /LPS}. Notably, elevated expression of both IL-6 and IL-1 β has been demonstrated in the fibrotic endometrium of mares (Szóstek et al., 2013) as well as their pro-fibrotic action on the expression of ECM-associated components (Szóstek-Mioduchowska et al., 2019a). Furthermore, TGF- β 1 has been excessively studied in the context of endometriosis, and its role in the regulation of the expression of ECM-associated components and promotion of fibroblasts activation, proliferation, and collagen gel contraction has already been well established (Szóstek-Mioduchowska et al., 2020; Szóstek-Mioduchowska et al., 2019b; Wójtowicz et al., 2025).

It is interesting that, eqMD-MΦ^{IL-4/IL-13}-CM and intrauterine administration eqMD-MΦ^{IL-4/IL-13} reduced *IL-1 β* mRNA transcription in mare endometrial fibroblasts *in vitro* as well as in endometrium, respectively. Nearly all fibrotic disorders are linked to the IL-1 family, which plays a crucial role in their development and progression (Wang et al., 2023). Moreover, the transcriptomic analysis revealed that intrauterine administration of eqMD-MΦ^{IFN γ /LPS} up-regulated the endometrial expression of *IL1RN*, which is a gene encoding IL-1 receptor antagonist (IL-1Ra) that inhibits the physiological activities of IL-1 β . Dysregulation of IL-1Ra/IL-1 β balance has been reported in the bronchoalveolar lavage fluid and serum of patients with IPF fibrosis. Moreover, single-nucleotide polymorphism in *IL1RN* has been associated with the susceptibility to this condition

(Barlo et al., 2011). Considering these and the aforementioned results of pro-fibrotic IL-1 β action on the expression of ECM-associated components in mare endometrium (Szóstek-Mioduchowska et al., 2019a) further investigation should be directed towards modulation of IL-1 β expression. In conclusion, all studied inflammatory markers affected by eqMD-M Φ ^{IFN γ /LPS} further emphasize the role of inflammation in the fibrosis development, and again imply that M Φ 1 may be primarily responsible for their expression upregulation in mare endometrium.

The transcriptomic analysis of mare endometrium after intrauterine administration of M Φ subpopulations allowed to further underscore the differences between their respective exerted effects. Intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13} affected the expression of 127 and 310 genes, respectively, and among them, less than 3% were common for both eqMD-M Φ subpopulations. These results are the opposite of those presented earlier in this dissertation, as in response to the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM over the 50% of the identified DEGs were common for both subpopulations. Functional analysis of DEGs identified after intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} revealed, among others, their annotation to increased abnormal morphology of subcellular (e.g. *CCN2*, *GSTK1*, *PPARGC1A*), cachexia signaling pathway (e.g. *CAPN11*, *IL1RN*, *LCN2*), multiple sclerosis signaling pathway (e.g. *C7*, *LIF*, *MAPK13*), as well as decreased cell cycle checkpoints (e.g. *BUB1B*, *CCNB1*, *CENPF*). A condition known as cachexia can develop at all stages of cancer and is correlated with an unfavorable prognosis. It is marked as a significant decrease in body weight, as a result of depletion of adipose tissue and skeletal muscle. This dysregulation can be driven by a variety of molecular mechanisms, including the action of pro-inflammatory cytokines like IL-1 and IL-6, as well as by activation of NF- κ B or IGF1-AKT-mTOR pathways (Miyamoto et al., 2016; Neshan et al., 2024). The findings indicate the existence of molecular mechanisms that are shared by both fibrosis and cachexia. In this instance, in tumor biopsies collected from colorectal cancer patients with cachexia, elevated protein expression of inflammatory cytokines has been demonstrated, as well as increased presence of ACTA2 and COL deposition (Lima et al., 2019). Similarly, excessive deposition of COL and elastic fibers, as well as elevated mRNA transcription of *COLI*, *COLIII*, *COLVI* and *FN*, has been reported in the adipose tissue of gastrointestinal cancer patients with cachexia. It was correlated with up-regulated TGF- β 1 and distinct ACTA2 expression in the close surrounding of the adipocytes (Alves et al., 2017). The results suggest that intrauterine administration of

eqMD-M Φ ^{IFN γ /LPS} modulate genes annotated to activated cachexia signaling pathway in the endometrium. To the best of my knowledge, no other studies have been conducted about the cachexia signaling pathway in mare endometrosis. However, the extant literature suggests a correlation between cachexia and fibrosis, which represents a promising research direction.

As previously demonstrated in this dissertation, mainly the eqMD-M Φ ^{IFN γ /LPS}-CM affected expression of genes related to the activation and action of neutrophils in mare endometrial fibroblasts. Consistent with these results, the transcriptomic analysis revealed that DEGs identified in mare endometrium after the intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} were annotated to the increased neutrophil degranulation (e.g. *GRN*, *PADI2*, *SERPINA3*). The M Φ -neutrophil cross-talk has been proposed as an important part of orchestrating the onset and resolution of inflammation, as well as its role in proliferative and remodeling phases of tissue repair has already been recognized (Bouchery & Harris, 2019). In neutrophils, there are found at least 4 distinct kinds of granules: primary granules, secondary granules, tertiary granules, and secretory vesicles. The first type of granules is responsible for the storage of toxic mediators like ELA, MPO, CAT, and defensins, while in secondary and tertiary granules, MMP-9 and lactoferrin are stored. The secretory vesicles, on the other hand, are speculated to contain extracellular fluid. The induced release of these granules, referred to as degranulation, has been identified as one of the main causes of pulmonary disorders, including fibrosis (Lacy, 2006). Moreover, in patients with cystic fibrosis, neutrophils have been shown to have an increased tendency to release the granules (Koller et al., 1995). Equally, an important role of neutrophil-associated proteins has already been demonstrated in the context of fibrosis development in the course of endometrosis. Elastase, MPO, and CAT have been demonstrated to mostly increase the COL1 protein abundance and *COL3* mRNA transcription in explants of mare endometrium, however, the response depended on dosage, phase of the estrous cycle, and category of endometrium (Rebordão et al., 2018). Moreover, the selective inhibition of their activity presented promising evidence in the context of endometrosis alleviation. Inhibition of ELA has been proposed as a potential way of reduction of the development of endometrial fibrosis through regulation of COL1 deposition, regulation of MMP2 and MMP9 expression and gelatinolytic activity (Amaral et al., 2020b; Amaral et al., 2021b) as well as through inhibition of PGF2 α secretion and stimulation of PGE2 production (Amaral et al., 2018). Similarly, inhibition of CAT reduced *COL1A2* mRNA transcription (Amaral et al., 2020a), as well as inhibition of

MPO decreased the COL1 protein abundance and influenced MMP2 and MMP9 gelatinolytic activity in endometrial explants (Amaral et al., 2021a). In this context, presented in this dissertation modulation of genes associated with activated neutrophil degranulation in response to the intrauterine administration of eqMD-M Φ ^{IFN γ /LPS} highly suggests the pro-fibrotic role of M Φ 1, and their modulation of neutrophil action via effect on fibroblasts in the course of endometriosis. The results further underscore the opposing role of M Φ subpopulation in mare endometrium, as DEGs identified after the intrauterine administration of eqMD-M Φ ^{IL-4/IL-13} were annotated to the decreased cellular infiltration by granulocytes (e.g. *CBFB*, *PRDM1*, *WLS*) and cell movement of neutrophils (e.g. *DPP4*, *MASP1*, *ST3GAL4*).

The opposing role of M Φ subpopulation is further accentuated by the results of functional analysis which revealed that DEGs identified after the intrauterine administration of eqMD-M Φ ^{IL-4/IL-13} were associated with increased Sheddase signaling (e.g. *IGFBP3*, *MMP26*, *PLG*) and CLEAR signaling (e.g. *EIF2AK3*, *INSR*, *KCNIP3*) pathways. Sheddases are a group of enzymes responsible for the release of extracellular domains of integral membrane proteins from the cell surface, a process referred to as shedding. A disintegrin and metalloproteases (ADAM) are enzymes belonging to the sheddase family that control intracellular signaling through shedding of growth factors and modulating cell-cell adhesion. Therefore, sheddases regulate the intricate interactions between the extracellular space and cell surface molecules and control factors involved in the proliferation and survival of cells (Kheradmand & Werb, 2002). Notably, with their proteolytic activity, sheddases may contribute to the regulation of the action of pro-inflammatory and pro-fibrotic factors. For instance, IL-6 activity may be altered in consequence of shedding of its receptor and promotion of the soluble IL-6 receptor form (Müllberg et al., 1993; Rose-John, 2012), as well as TNF- α activity, which is a well-characterized substrate of sheddases, is released from its membrane-bound precursor due to shedding (Black et al., 1997). Thus, different sheddases have been presented to be connected with the development of fibrosis, like soluble tumor necrosis factor receptor p55 (sTNFRp55) in systemic sclerosis (Terao et al., 2010), ADAM17 in chronic kidney diseases and kidney fibrosis (Gooz et al., 2012) or ADAM10 in murine model of pulmonary fibrosis (Lagares et al., 2017). Furthermore, the transcriptomic profiling of mare endometrium revealed *ADAM28* upregulation in category IIA endometria, as well as *ADAM33* upregulation and *ADAM12* downregulation in category IIB endometria, suggesting that there are potential disturbances in Sheddase signaling, which could be

related to the development of fibrosis in the course of endometriosis. Taking into consideration the decreased number of CD68⁺iNOS⁻ARG1⁺ cells in mare endometrium in the course of endometriosis, to which correspond eqMD-MΦ^{IL-4/IL-13}, the obtained results indicate that due to the reduced action of MΦ2a, the proteolytic action of sheddases may be impaired, thus affecting the development of endometrial fibrosis. However, such a connection should be directed to further studies.

Equally intriguing seems to be the role of the CLEAR signaling pathway. The CLEAR signaling pathway, which stands for Coordinated Lysosomal Expression and Regulation signaling pathway, is responsible for the regulation of lysosomal biogenesis and function, including cellular processes like exocytosis, membrane repair, or autophagy (Palmieri et al., 2011). Lysosomes have been recognized as multipurpose subcellular compartments that play a role in the maintenance of homeostasis. They have been indicated to be involved in the regulation of cell adhesion and migration, as well as in the regulation of cell metabolism through mediating the intracellular signals. Moreover, lysosomes have been recognized as important regulators of the immune response. Thus, dysregulation of their function has already been recognized as a significant contributing factor in the development and progression of different pathologies and diseases such as fibrosis (Itoh et al., 2023; Martinez-Fabregas et al., 2022). For example, lysosomes have been shown to accumulate cholesterol to maintain the senescence-associated secretory phenotype connected with inflammation alterations in mice during osteoarthritis (Roh et al., 2023). Notably, lysosomes fulfill their roles through the regulation of the mTOR/TFEB axis in cells (Martinez-Fabregas et al., 2022). Transcription factor EB (TFEB), which can be considered a master regulator of CLEAR signaling, regulates cellular functions like autophagy and lysosomal biogenesis and has been shown to induce the intracellular clearance of pathogenic factors (Napolitano & Ballabio, 2016; Settembre et al., 2011). As the modulation of TFEB raises hope for the development of novel treatment approaches, it has already been targeted in studies concerning the development of fibrosis. It has been demonstrated that TFEB antagonist desloratadine promoted nuclear localization of TFEB and thus induced autophagy and lysosome biogenesis in hepatic cell lines as well as in the liver of desloratadine-treated mice *in vivo* (Lin et al., 2024). In silica-induced pulmonary fibrosis in mice, TFEB overexpression enhanced autophagic influx and alleviated inflammatory cytokine levels, lysosomal dysfunction, and apoptosis, thus reducing fibrosis (He et al., 2020). Moreover, TFEB was presented to be down-regulated in human and mouse fibrotic kidneys, and the induction of its kidney-specific

overexpression alleviated the pathogenesis of renal fibrosis in mice. Notably, the observed prevention of ECM components deposition by TFEB was a result of autophagic flux integrity and blockage of G2/M arrest in cells (Ren et al., 2024). Taking the above into consideration, it can be concluded that the insufficiency of the CLEAR pathway hinders the removal of pro-inflammatory and pro-fibrotic factors and ECM remodeling, thus intensifying the fibrosis development. This point emphasizes the importance of changes in the MΦ subpopulation distribution presented in this dissertation, as the MΦ subpopulation, which was observed to decrease in number in the course of endometriosis (akin to MΦ2), was the subpopulation demonstrated to be responsible for activation of the CLEAR signaling pathway.

The results obtained in Research Task 3 underscore the different role of the MΦ subpopulation in the mare's endometrium. The obtained data are all the more interesting given the imbalanced endometrial ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells in the course of endometriosis demonstrated in this dissertation in Research Task 1. The eqMD-MΦ^{IFN γ /LPS} showed a rather consistent pro-fibrotic role after their intrauterine administration, while eqMD-MΦ^{IL-4/IL-13} exhibited anti-fibrotic mode of action. Given the significant predominance of CD68⁺iNOS⁺ARG1⁻ cells in pathological endometrium the overall results indicate the importance of disrupted dynamics of MΦ subpopulations in the fibrosis development during the pathogenesis of endometriosis.

7.5 Prospects of the obtained results

Despite the numerous studies and years of research, the pathogenesis of endometriosis remains unclear and not well understood. This lack of knowledge prevents the development of an effective treatment method and the improvement of the reproductive success of the mares. In recent years, great care has been taken to understand the importance of the immune system in the development of endometrial fibrosis, as well as a vast amount of new data has been acquired with the use of transcriptomic methods. The results demonstrated in this dissertation have expanded the range of knowledge with the information about changes in the distribution and alteration in MΦ subpopulations in the mare endometrium and have allowed recognition of potential molecular mechanism that may underlie the development of endometrial fibrosis. The presented results strongly emphasize the importance of a disturbed balance between MΦ subpopulations in the pathogenesis of mare endometrial fibrosis, which raises a potential direction for the development of novel treatment. It is worthwhile to determine the underlying cause of the

observed MΦ1/MΦ2 imbalance, as its alleviation may improve the endometrial score. The potential way of compensation constitutes the intrauterine administration of autologous eqMD-MΦ^{IL-4/IL-13}. Realizing, however, that such a complex method can hardly be considered as on-site treatment, it is also worth considering modulating the processes that can be impaired due to the decreased number of MΦ2. Among molecular pathways altered in response to the intrauterine administration of eqMD-MΦ^{IL-4/IL-13} was the CLEAR signaling pathway. The master regulator of this pathway, TFEB, is responsible for the regulation of lysosome biogenesis and autophagy (Lin et al., 2024). It has already been hypothesized that TFEB suppression may be related to the DNMT3a-associated TFEB promoter hypermethylation, which may be alleviated by 5-aza-2-deoxycytidine (decitabine) (Lin et al., 2024). Notably, the increase in DNMT3a in mare endometrial fibroblasts challenged with TGF-β1 has already been presented, and this state was eased by the decitabine treatment (Alpoim-Moreira et al., 2023). A combination of this insight may constitute a perspective of the obtained results in the context of the endometriosis treatment development.

8. Summary of results

1. An increase in the percentage of CD68⁺iNOS⁺ARG1⁻ cells and a decrease in the percentage of CD68⁺iNOS⁻ARG1⁺ cells were observed in endometria categories IIA, IIB, and III when compared to endometria category I, regardless of the phase of the estrous cycle.
2. The ratio of CD68⁺iNOS⁺ARG1⁻ / CD68⁺iNOS⁻ARG1⁺ cells was higher in endometria categories IIA, IIB, and III when compared to endometria category I, regardless of the phase of the estrous cycle.
3. The eqMD-MΦ^{IFN γ /LPS} (akin to MΦ1) and eqMD-MΦ^{IL-4/IL-13} (akin to MΦ2a) demonstrate distinct molecular profiles reflecting the potential pro-inflammatory role of eqMD-MΦ^{IFN γ /LPS} and involvement of eqMD-MΦ^{IL-4/IL-13} in tissue remodeling.
4. The eqMD-MΦ^{IFN γ /LPS}-CM decreased the expression of ACTA2 and TIMP-1, and increased the expression of MMP-9, while eqMD-MΦ^{IL-4/IL-13}-CM decreased the expression of TIMP-2, and increased the expression of FN1 and MMP-9 in endometrial fibroblasts *in vitro*. Moreover, the CM of both eqMD-MΦ subpopulations altered the mRNA transcription of inflammatory markers in endometrial fibroblasts *in vitro*, with the fibroblast response varying in dependence on the specific MΦ subpopulation.
5. The eqMD-MΦ^{IFN γ /LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM altered the expression of 406 and 282 genes in endometrial fibroblasts, respectively. Functional analysis revealed great resemblance in fibroblast response to the CM of both eqMD-MΦ subpopulations, highlighting their annotation to e.g., fibrosis, migration of cells, and cellular infiltration by phagocytes. Specifically, DEGs identified in endometrial fibroblasts *in vitro* after the treatment with the eqMD-MΦ^{IFN γ /LPS}-CM showed the annotation to cell movement of neutrophils, neutrophil extracellular trap signaling pathway, lymphocyte migration, cell proliferation of T lymphocytes, and NF- κ B signaling pathway.
6. The eqMD-MΦ^{IFN γ /LPS}-CM and eqMD-MΦ^{IL-4/IL-13}-CM increased viability, proliferation, and migration of endometrial fibroblasts *in vitro*.
7. The intrauterine administration of eqMD-MΦ^{IFN γ /LPS} increased the mRNA transcription of ECM-associated components (*FN1*, *ACTA2*, *MMP-2*, *TIMP-1*, *TIMP-2*) and inflammatory markers (*CCN2*, *IL-1 β* , *IL-6*, *TGF- β 1*, *TNF- α*) in mare

endometrium. Moreover, the intrauterine administration of eqMD-M $\Phi^{IFN\gamma/LPS}$ affected 127 genes associated with, e.g., cachexia signaling and neutrophil degranulation in mare endometrium.

8. The intrauterine administration of eqMD-M $\Phi^{IL-4/IL-13}$ decreased the mRNA transcription of ECM-associated components (*COL1A1*, *COL3A1*, *FN1*) and inflammatory marker (*IL-1 β*) in mare endometrium. Moreover, the intrauterine administration of eqMD-M $\Phi^{IL-4/IL-13}$ influenced 310 genes involved, e.g. in Sheddase and CLEAR signaling pathways in mare endometrium.

9. Conclusions

The achievement of established research objectives and validation of adopted research hypotheses allowed to formulate the following **main conclusion** of presented dissertation that:

Changes in the dynamics of endometrial M Φ 1/M Φ 2 subpopulations may induce disturbance in expression of genes annotated to inflammatory process and ECM remodeling in mare endometrium leading to dysregulation of molecular pathways in a manner that promotes the development of endometrial fibrosis in the course of endometrosis.

Detailed conclusions were formulated as follows:

1. The disruption of M Φ 1/M Φ 2 ratio with the predominance of M Φ 1 over the M Φ 2 subpopulation may affect processes associated with the development of fibrosis in the mare endometrium.
2. Macrophages, irrespective of the subpopulation, exert a comparable effect on the fundamental cellular processes in mare endometrial fibroblasts, including viability, proliferation, and migration.
3. Macrophages type 1 do not directly promote ECM deposition in endometrial fibroblasts, however, they contribute to maintaining the pro-inflammatory microenvironment by releasing pro-inflammatory cytokines and modulating the expression of genes involved in activation of other immune cells, which may initiate the development of fibrosis.
4. Macrophages type 1 modulate genes involved in inflammatory response and neutrophil degranulation, and due to their increased distribution orchestrate the pro-fibrotic processes in mare endometrium in the course of endometrosis.
5. Macrophages type 2 modulate genes annotated to tissue remodeling and inflammatory processes in endometrial fibroblasts, which suggests their importance in ECM turnover.
6. Macrophages type 2 affect genes involved with activated Sheddase signaling and CLEAR signaling pathways, however due to their decreased distribution in the course of endometrosis, these processes are dysregulated, which may contribute to the development of fibrosis in the mare endometrium.

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14. Supplementary data

Supplementary Table 1. The full list of differentially expressed proteins (DEPs) identified in eqMD-MΦ. LogFC < -1.5 indicates DEPs with higher expression in eqMD-MΦ^{IFN γ /LPS} while LogFC > 1.5 indicates DEPs with higher expression in eqMD-MΦ^{IL-4/IL-13}. All other DEPs are considered common for both MΦ populations.

No.	Protein ID	Protein Symbol	Protein name	q-value	LogFC
1	A0A9L0TSA4	CLEC10A	C-type lectin domain containing 10A	0,0409	2,6815
2	A0A3Q2HRQ8	SLC29A1	Solute carrier family 29 member 1	0,0192	2,6557
3	F7CVL4	ALDH1A2	Retinal dehydrogenase 2	0,0142	2,4293
4	F7AYC1	SPP1	Secreted phosphoprotein 1	0,0280	2,4127
5	P29553 A0A0U5J516	TNF	Tumor necrosis factor	0,0176	2,3142
6	F6Z1I4	NTRK1	Tyrosine-protein kinase receptor	0,0038	2,2203
7	A0A5F5PGU3	FAM171B	Family with sequence similarity 171 member B	0,0192	2,1532
8	F6YS25	CA2	Carbonic anhydrase	0,0447	2,0641
9	A0A9L0SV04	CRIP2	Cysteine-rich protein 2	0,0445	2,0434
10	A0A9L0TAD2	PTPN21	Tyrosine-protein phosphatase non-receptor type	0,0232	2,0280
11	A0A9L0TK01	DNMT3A	DNA (cytosine-5-)-methyltransferase	0,0040	1,9949
12	F6ZHY6	GFOD1	Gfo/Idh/MocA-like oxidoreductase domain containing 1	0,0117	1,9930
13	Q28377	FN1	Fibronectin	0,0186	1,9117
14	A0A3Q2HM94	SLC2A3	Solute carrier family 2, facilitated glucose transporter member 3	0,0031	1,8991
15	A0A9L0SC90	ITGB3	Integrin beta	0,0232	1,8310
16	A0A3Q2L6U1	TSPAN33	Tetraspanin	0,0229	1,8292
17	A0A3Q2HYQ3	DIPK1A	Divergent protein kinase domain 1A	0,0361	1,8221
18	A0A9L0QYF4	CTSK	Cathepsin K	0,0468	1,8217
19	F6R6W0		C-C motif chemokine	0,0228	1,8202
20	F6PWV8	SERPINH1	Serpin H1	0,0389	1,8169
21	A2PYL6	HK2	Hexokinase-2	0,0139	1,8168
22	F6X4S0	CTTN	Cortactin	0,0280	1,8081
23	A0A9L0S9S1	CKB	Creatine kinase B-type	0,0092	1,7752
24	A0A9L0S576	FHL3	Four and a half LIM domains 3	0,0105	1,7596
25	A0A3Q2IGU2	BAIAP2	BAR/IMD domain-containing adapter protein 2	0,0259	1,7368
26	P55102 A0A3Q2LFL1	INHBA	Inhibin beta A chain	0,0230	1,7312
27	A0A3Q2LU93	MAP4K3	Mitogen-activated protein kinase	0,0480	1,7236
28	F6SZH4	TNFSF14	TNF superfamily member 14	0,0275	1,6946
29	F7B6C3	F11R	Junctional adhesion molecule A	0,0167	1,6802
30	F7DBA2 F7D1L7	CHSY1	Hexosyltransferase	0,0103	1,6739
31	F7DBA2 F7D1L7	CHSY3	Hexosyltransferase	0,0103	1,6739
32	F6YJP4	TRIM47	Tripartite motif containing 47	0,0311	1,6715
33	A0A9L0TK47	ICAM1	Intercellular adhesion molecule 1	0,0458	1,6591
34	F6YLI8	GOLIM4	Golgi integral membrane protein 4	0,0375	1,6543
35	A0A3Q2HYC4	LPL	Lipoprotein lipase	0,0324	1,6506
36	A0A3Q2HBK3	WFS1	Wolframin ER transmembrane glycoprotein	0,0199	1,6497

37	F7BHY8	MDFIC	MyoD family inhibitor domain containing	0,0138	1,6263
38	A0A3Q2HXX9	ESYT3	Extended synaptotagmin 3	0,0141	1,5976
39	A0A9L0SEG4	CD83	CD83 molecule	0,0457	1,5881
40	A0A9L0T7T3	C1GALT1	Glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1	0,0190	1,5850
41	F7B0S3	MFGE8	Milk fat globule EGF and factor V/VIII domain containing	0,0192	1,5786
42	F6XPN8	SERINC5	Serine incorporator 5	0,0376	1,5771
43	A0A9L0RRQ9	UQCC1	Ubiquinol-cytochrome c reductase complex assembly factor 1	0,0322	1,5674
44	A0A3Q2IDN7	ITGB5	Integrin beta	0,0051	1,5514
45	F6XU44	LPCAT1	Lysophosphatidylcholine acyltransferase 1	0,0274	1,5429
46	F7AMZ3	CERS2	Ceramide synthase 2	0,0138	1,5333
47	A0A9L0TPB8	ECE1	Endothelin-converting enzyme 1	0,0379	1,5258
48	A0A9L0RX02	VCL	Vinculin	0,0298	1,5194
49	A0A3Q2H263	IRF4	Interferon regulatory factor 4	0,0273	1,5160
50	F6UJP0	AGAP3	ArfGAP with GTPase domain, ankyrin repeat and PH domain 3	0,0199	1,5096
51	F7AIP7	SLC1A5	Amino acid transporter	0,0289	1,5059
52	O97508	TXN	Thioredoxin	0,0199	1,5016
53	A0A3Q2HFV7	PLXNB2	Plexin B2	0,0261	1,4996
54	A0A9L0RGX7	MYO1E	Myosin IE	0,0158	1,4974
55	A0A3Q2HG61		Uncharacterized protein	0,0184	1,4948
56	A0A3Q2ICF1	NABP1	SOSS complex subunit B2	0,0043	1,4905
57	A0A9L0T4B9	EPHA2	receptor protein-tyrosine kinase	0,0168	1,4891
58	F6YF95	CNBP	CCHC-type zinc finger nucleic acid binding protein	0,0173	1,4880
59	F7DKV6	PRKX	Protein kinase X-linked	0,0188	1,4875
60	A0A3Q2HAZ6	MTUS1	Microtubule associated scaffold protein 1	0,0445	1,4752
61	A0A9L0SM87	CSRP1	Cysteine and glycine-rich protein 1	0,0115	1,4613
62	F7CLQ3	FAM20C	FAM20C golgi associated secretory pathway kinase	0,0275	1,4604
63	F6SXC4	CSF1	Colony stimulating factor 1	0,0130	1,4541
64	F7DPK4	SLC20A1	Phosphate transporter	0,0326	1,4511
65	A0A3Q2KTJ7	SLC4A7	Anion exchange protein	0,0185	1,4284
66	F6XV19	PARVB	Parvin beta	0,0437	1,4250
67	A0A9L0SLG9	PKIB	cAMP-dependent protein kinase inhibitor	0,0057	1,4157
68	A0A9L0R5C7	TPRG1L	Tumor protein p63 regulated 1 like	0,0409	1,4064
69	A0A5F5PVH8	PC	Pyruvate carboxylase	0,0467	1,4048
70	A0A5F5PGJ5	CADM1	Cell adhesion molecule 1	0,0180	1,3996
71	F6ZNQ1	RGS14	Regulator of G protein signaling 14	0,0177	1,3927
72	F6QJZ7	UTP3	UTP3 small subunit processome component	0,0259	1,3879
73	A0A9L0S9Q4	DDX21	RNA helicase	0,0242	1,3859
74	A0A5F5PZA2	PFKP	ATP-dependent 6-phosphofructokinase	0,0072	1,3852
75	A0A9L0R8N5	C13H7orf50	Chromosome 13 C7orf50 homolog	0,0325	1,3779
76	F6YQT2	GRSF1	G-rich RNA sequence binding factor 1	0,0382	1,3767
77	A0A3Q2H4F8	NAT10	RNA cytidine acetyltransferase	0,0314	1,3742
78	F6QLG3	BCL2L13	BCL2 like 13	0,0274	1,3731
79	F6ZLV9	ATF3	Activating transcription factor 3	0,0234	1,3698

80	A0A3Q2HJE3	CAMSAP2	Calmodulin regulated spectrin associated protein family member 2	0,0375	1,3698
81	F6PSS0	RRS1	Ribosome biogenesis regulatory protein	0,0436	1,3648
82	F6Q9S8	CD81	CD81 molecule	0,0492	1,3639
83	F7DJD8	RASA1	RAS p21 protein activator 1	0,0233	1,3549
84	F6WZ26	PIKFYVE	1-phosphatidylinositol-3-phosphate 5-kinase	0,0168	1,3548
85	A0A9L0S7N4	FHOD1	Formin homology 2 domain containing 1	0,0310	1,3516
86	A0A9L0TN00	MGLL	Monoglyceride lipase	0,0278	1,3515
87	F6URL2	TIAM1	TIAM Rac1 associated GEF 1	0,0229	1,3486
88	A0A3Q2I700	DLC1	Rho GTPase-activating protein 7	0,0280	1,3477
89	A0A9L0RNA2	NOCT	Nocturnin	0,0435	1,3466
90	F6VF01	SLC25A22	Mitochondrial glutamate carrier 1	0,0340	1,3424
91	F7CFY5	MAT2A	S-adenosylmethionine synthase	0,0210	1,3409
92	F6WM60	UBTD1	Ubiquitin domain-containing protein 1	0,0145	1,3394
93	F7D137	UBTD2	Ubiquitin domain containing 2	0,0145	1,3394
94	F7CNS0	SUGP2	SURP and G-patch domain containing 2	0,0143	1,3340
95	A0A3Q2HJF2	AHNAK	AHNAK nucleoprotein	0,0433	1,3119
96	A0A9L0SIH8	GLS	glutaminase	0,0121	1,3103
97	F6U429	PDCD11	Programmed cell death 11	0,0091	1,3102
98	F6QDS7	RASGRP3	RAS guanyl releasing protein 3	0,0360	1,3091
99	A0A5F5PV09	ACSL4	long-chain-fatty-acid--CoA ligase	0,0233	1,3045
100	F6UZL9	SMARCC1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin subfamily c member 1	0,0465	1,3034
101	A0A5F5Q1W1	PPP1CB	Protein phosphatase 1 catalytic subunit beta	0,0416	1,3019
102	F6TEU9 F6Q557	LOC100071373	Pre-mRNA-splicing factor SYF2	0,0187	1,2983
103	A0A9L0SR55	PAM16	Presequence translocase associated motor 16	0,0324	1,2939
104	A0A3Q2GZY0	R3HCC1	R3H domain and coiled-coil containing 1	0,0235	1,2922
105	F7D618	SLC38A2	Sodium-coupled neutral amino acid symporter 2	0,0268	1,2913
106	A0A9L0RG58	BMS1	BMS1 ribosome biosis factor	0,0235	1,2869
107	A0A3Q2IEC5	ITPKB	Kinase	0,0350	1,2826
108	A0A9L0S1Y4	POLB	DNA polymerase	0,0184	1,2787
109	F7AM44	HERC2	HECT-type E3 ubiquitin transferase	0,0233	1,2772
110	F6T9G2	FYN	Tyrosine-protein kinase	0,0236	1,2770
111	A0A3Q2LKZ8	MICAL2	F-actin monooxygenase	0,0095	1,2763
112	A0A3Q2HLY4	PLPBP	Pyridoxal phosphate homeostasis protein	0,0167	1,2757
113	F6YTK4	LARS1	leucine--tRNA ligase	0,0466	1,2623
114	F6RX11	RPS20	Small ribosomal subunit protein uS10	0,0233	1,2607
115	A0A9L0S339	ARL2BP	ADP-ribosylation factor-like protein 2-binding protein	0,0288	1,2594
116	A0A9L0T1M7	MLLT11	Protein AF1q	0,0155	1,2544
117	A0A9L0R9C6	CPD	Carboxypeptidase D	0,0360	1,2540
118	A0A9L0SFB3	PAK1IP1	PAK1 interacting protein 1	0,0218	1,2513
119	A0A9R1SR95	TUBB	Tubulin beta chain	0,0269	1,2483
120	A0A5F5PM22	TUBB2B	Tubulin beta chain	0,0269	1,2483
121	F7AXR6	EIF1B	Eukaryotic translation initiation factor 1B	0,0323	1,2478
122	A0A9L0S1Q6	EMD	Emerin	0,0337	1,2472

123	A0A3Q2HGI9	PLXND1	Plexin D1	0,0361	1,2429
124	A0A9L0TTA4	RPS24	40S ribosomal protein S24	0,0361	1,2420
125	F6QGG8	AFF1	ALF transcription elongation factor 1	0,0491	1,2392
126	F7ALM2	HGS	Hepatocyte growth factor-regulated tyrosine kinase substrate	0,0314	1,2351
127	F6YAK1	HEATR1	HEAT repeat-containing protein 1	0,0182	1,2303
128	A0A3Q2GTG1	NAP1L1	Nucleosome assembly protein 1 like 1	0,0310	1,2277
129	A0A3Q2H8U1	WWP1	E3 ubiquitin-protein ligase	0,0362	1,2262
130	F6VA59	NAA50	N-alpha-acetyltransferase 50	0,0382	1,2250
131	A0A9L0RL06	ANKRD28	Ankyrin repeat domain 28	0,0233	1,2249
132	F6YC63	ARHGAP25	Rho GTPase activating protein 25	0,0269	1,2168
133	A0A5F5PXN6	CYLD	Ubiquitin carboxyl-terminal hydrolase CYLD	0,0318	1,2168
134	A0A3Q2H6Q8	SRC	Tyrosine-protein kinase	0,0459	1,2146
135	A0A5F5PKJ8	STAM	Signal transducing adaptor molecule	0,0360	1,2131
136	A0A5F5PLG3	RPS19	Ribosomal protein S19	0,0456	1,2123
137	A0A9L0RJ0	RPS6KA3	Ribosomal protein S6 kinase	0,0380	1,2068
138	A0A9L0SAF1	ATP7A	P-type Cu(+) transporter	0,0315	1,2036
139	A0A9L0TPI1	TMEM26	Transmembrane protein 26	0,0229	1,2034
140	A0A9L0S2J4	PPM1H	Protein phosphatase 1H	0,0456	1,2028
141	F6SJM8	ATP5F1C	ATP synthase subunit gamma	0,0498	1,2027
142	A0A3Q2IBX0	DNAJB2	DnaJ heat shock protein family (Hsp40) member B2	0,0387	1,2026
143	A0A9L0RZU7	MTHFD2	methenyltetrahydrofolate cyclohydrolase	0,0189	1,2016
144	A0A3Q2HJH0	PCM1	Pericentriolar material 1	0,0231	1,1993
145	F7DRP9	NACA	NAC-A/B domain-containing protein	0,0381	1,1991
146	F7BLB3	ESF1	ESF1 nucleolar pre-rRNA processing protein homolog	0,0167	1,1965
147	A0A3Q2H4J2	DNAJC11	DnaJ heat shock protein family (Hsp40) member C11	0,0241	1,1964
148	A0A5F5Q172	RPS2	Small ribosomal subunit protein uS5	0,0282	1,1960
149	A0A3Q2HW53		Small ribosomal subunit protein uS5 C-terminal domain-containing protein	0,0282	1,1960
150	F6S2Y2	NOP2	NOP2 nucleolar protein	0,0439	1,1953
151	A0A3Q2HJD7	PDRG1	p53 and DNA damage-regulated protein 1	0,0326	1,1922
152	F6PH26	POLR1B	DNA-directed RNA polymerase subunit beta	0,0417	1,1907
153	P18907 A0A3Q2LSQ9	ATP1A1	Sodium/potassium-transporting ATPase subunit alpha-1	0,0360	1,1906
154	A0A9L0RFE7	PI4K2B	Phosphatidylinositol 4-kinase type 2	0,0251	1,1902
155	A0A9L0SNW7	UTP20	UTP20 small subunit processome component	0,0417	1,1886
156	A0A3Q2H0T6	MMGT1	Membrane magnesium transporter	0,0359	1,1883
157	F6VSD4	XPNPEP1	X-prolyl aminopeptidase 1	0,0364	1,1877
158	A0A3Q2H191	ADD1	Adducin 1	0,0230	1,1865
159	A0A9L0SJ19	RPS10	Small ribosomal subunit protein eS10	0,0185	1,1827
160	A0A9L0RVC3	DNAJC19	Mitochondrial import inner membrane translocase subunit TIM14	0,0376	1,1820
161	F7DW15	RPS8	40S ribosomal protein S8	0,0415	1,1790
162	F6PI74	METTL9	Methyltransferase 9, His-X-His N1-histidine	0,0326	1,1770
163	A0A3Q2HUR7	DOCK4	Dedicator of cytokinesis 4	0,0190	1,1763
164	A0A5F5PKB4	RPS15	Small ribosomal subunit protein uS19	0,0374	1,1748

165	A0A9L0R2X5	SYAP1	Synapse associated protein 1	0,0334	1,1738
166	A0A5F5PZI1	GOLM2	Protein GOLM2	0,0098	1,1718
167	A0A3Q2I6H8	IQSEC1	IQ motif and Sec7 domain ArfGEF 1	0,0323	1,1661
168	F6Z7H2	ABCF3	ATP binding cassette subfamily F member 3	0,0211	1,1660
169	F6Y6S9	NOC2L	NOC2 like nucleolar associated transcriptional repressor	0,0273	1,1628
170	F7DZB2	ZYX	Zyxin	0,0192	1,1624
171	A0A9L0RPN6	RPL28	Large ribosomal subunit protein eL28	0,0445	1,1604
172	F6QMC8	WDR36	WD repeat domain 36	0,0437	1,1603
173	A0A9L0TL27	DDX27	RNA helicase	0,0292	1,1597
174	F6W8S4	TIMM50	Mitochondrial import inner membrane translocase subunit TIM50	0,0236	1,1559
175	A0A9L0S0B3	PES1	Pescadillo homolog	0,0230	1,1546
176	F7BZ33	PUS3	Pseudouridine synthase 3	0,0230	1,1519
177	A0A3Q2L1Q1	METTL16	U6 small nuclear RNA (adenine-(43)-N(6))-methyltransferase	0,0298	1,1466
178	F6YAX8	TSR1	Pre-rRNA-processing protein TSR1 homolog	0,0461	1,1460
179	F6QI34	HERC4	HECT and RLD domain containing E3 ubiquitin protein ligase 4	0,0428	1,1447
180	F7AXG5	UTP6	UTP6 small subunit processome component	0,0411	1,1436
181	A0A5F5Q456	NF2	NF2, moesin-ezrin-radixin like (MERLIN) tumor suppressor	0,0414	1,1434
182	F6TIM4	FBL	Fibrillarin	0,0475	1,1413
183	A0A9L0RAB4	ARMC10	Armadillo repeat containing 10	0,0142	1,1391
184	A0A9L0R2N1	CEBPZ	CCAAT enhancer binding protein zeta	0,0224	1,1376
185	F7CG77	CCT6A	T-complex protein 1 subunit zeta	0,0325	1,1291
186	A0A3Q2KMX9	NOP9	NOP9 nucleolar protein	0,0365	1,1286
187	F7BZX0	AAMP	Angio associated migratory cell protein	0,0438	1,1284
188	F7AQ99	ZPR1	ZPR1 zinc finger	0,0433	1,1261
189	F7A4T7	SPATS2	Spermatosis associated serine rich 2	0,0450	1,1259
190	F6S4B7	UBXN1	UBX domain protein 1	0,0408	1,1250
191	A0A5F5PYL8	UCHL5	Ubiquitin carboxyl-terminal hydrolase	0,0373	1,1237
192	H9GZL8	RPLP2	Large ribosomal subunit protein P2	0,0387	1,1232
193	A0A9L0RFI0	FMC1	Protein FMC1 homolog	0,0377	1,1230
194	F6XJW2	MDN1	Midasin	0,0465	1,1230
195	A0A9L0S3F5	SUGT1	SGT1 homolog, MIS12 kinetochore complex assembly cochaperone	0,0445	1,1217
196	A0A3Q2HTL6	MYO18A	Myosin XVIIIa	0,0364	1,1192
197	A0A9L0SRW6	UFSP2	Ufm1-specific protease 2	0,0415	1,1176
198	A0A9L0SDR4	MITD1	Microtubule interacting and trafficking domain containing 1	0,0311	1,1111
199	A0A9L0RUD6	BICD2	BICD cargo adaptor 2	0,0414	1,1098
200	A0A5F5PNJ9	APOO	MICOS complex subunit	0,0466	1,1052
201	A0A9L0TE36	RANGAP1	Ran GTPase activating protein 1	0,0316	1,1036
202	F6USV6	NOL11	Nucleolar protein 11	0,0434	1,1032
203	A0A3Q2HWP9	VCP	Transitional endoplasmic reticulum ATPase	0,0258	1,0970
204	A0A9L0RQZ4	CSTF3	Cleavage stimulation factor subunit 3	0,0243	1,0957
205	A0A9L0SHT3	COG6	Conserved oligomeric Golgi complex subunit 6	0,0457	1,0954
206	A0A9L0S2X2	ZFR	Zinc finger RNA-binding protein	0,0310	1,0949

207	F6TK31	IKBK	NF-kappa-B essential modulator	0,0230	1,0893
208	F6XTN1	EIF4A1	Eukaryotic initiation factor 4A-I	0,0230	1,0852
209	A0A9L0R6B9	UPF1	UPF1 RNA helicase and ATPase	0,0334	1,0839
210	A0A3Q2GXB8	YTHDF3	YTH domain-containing family protein	0,0181	1,0795
211	A0A9L0TP98	NUP205	Nucleoporin 205	0,0453	1,0761
212	A0A9L0SGI0	NUP93	Nuclear pore complex protein Nup93	0,0479	1,0698
213	F7D5H8	ATG2A	Autophagy related 2A	0,0235	1,0694
214	A0A9L0TC24	EIF3E	Eukaryotic translation initiation factor 3 subunit E	0,0236	1,0580
215	F6TQ05	EPN1	Epsin 1	0,0315	-1,0704
216	A0A9L0S9Z4	CCDC6	Coiled-coil domain containing 6	0,0298	-1,0807
217	A0A9L0R567	HYOU1	Hypoxia up-regulated protein 1	0,0121	-1,0933
218	A0A3Q2I2Y1	TBC1D5	TBC1 domain family member 5	0,0498	-1,0958
219	A0A9L0SEP4	DDB1	DNA damage-binding protein 1	0,0388	-1,0961
220	A0A3Q2GU70	ADCY7	Adenylate cyclase type 7	0,0440	-1,0971
221	A0A3Q2LQH7	GNB1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	0,0228	-1,1001
222	A0A9L0S111	CDK19	Cyclin dependent kinase 19	0,0231	-1,1058
223	A0A9L0SED4	DIP2B	Disco interacting B	0,0269	-1,1060
224	A0A9L0TDV8	DPP3	Dipeptidyl peptidase 3	0,0472	-1,1094
225	A0A3Q2GVI2	UGGT1	UDP-glucose glycoprotein glucosyltransferase 1	0,0192	-1,1115
226	A0A3Q2L9D5	CUL2	Cullin 2	0,0437	-1,1165
227	F7B821	P4HB	Protein disulfide-isomerase	0,0388	-1,1175
228	A0A9L0RIP8	EHBP1L1	EH domain binding protein 1 like 1	0,0466	-1,1187
229	A0A3Q2I8R0	STK25	non-specific serine/threonine protein kinase	0,0233	-1,1231
230	A0A9L0RJF3	VRK3	VRK serine/threonine kinase 3	0,0214	-1,1233
231	A0A3Q2IDB9	DOCK11	Dedicator of cytokinesis 11	0,0309	-1,1242
232	F6QJ19	OVCA2	Esterase OVCA2	0,0456	-1,1260
233	A0A9L0R0W1	OTUD4	ubiquitinyl hydrolase 1	0,0233	-1,1276
234	A0A5F5PST9	C11orf98	Chromosome 11 open reading frame 98	0,0310	-1,1278
235	A0A9L0SI99	TMEM268	Transmembrane protein 268	0,0465	-1,1314
236	Q257U4	ATP2A2	Calcium-transporting ATPase	0,0407	-1,1314
237	A0A9L0TGF3	SYNE3	Spectrin repeat containing nuclear envelope family member 3	0,0459	-1,1345
238	F7AGZ6	PEX5	Peroxisomal targeting signal 1 receptor	0,0459	-1,1354
239	A0A9L0R4V2	CASP9	Caspase 9	0,0324	-1,1364
240	A0A9L0TPR2	TEC	Tyrosine-protein kinase	0,0184	-1,1385
241	F6SFH9	CHMP6	Charged multivesicular body protein 6	0,0416	-1,1387
242	F6W039	ARHGDIA	Rho GDP-dissociation inhibitor 1	0,0427	-1,1404
243	A0A9L0TCD0	TIGD5	Tigger transposable element derived 5	0,0193	-1,1420
244	A0A9L0TA91	SNX5	Sorting nexin	0,0180	-1,1425
245	F7BUY8	INPP5D	phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase	0,0265	-1,1464
246	F6YR18	RUFY3	RUN and FYVE domain containing 3	0,0457	-1,1480
247	A0A9L0SGL1	PPP2R2A	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B	0,0484	-1,1490
248	F7BV54	USP8	ubiquitinyl hydrolase 1	0,0311	-1,1498
249	F7A5V9	RABGAP1	RAB GTPase activating protein 1	0,0425	-1,1515
250	A0A3Q2H5U7	IGSF8	Immunoglobulin superfamily member 8	0,0105	-1,1529

251	A0A3Q2HPF5	EML4	EMAP like 4	0,0232	-1,1531
252	F7DC79	ANKS1A	Ankyrin repeat and sterile alpha motif domain containing 1A	0,0280	-1,1552
253	A0A3Q2I1F5	GMIP	GEM interacting protein	0,0297	-1,1592
254	A0A3Q2L6D8	PDCD6IP	Programmed cell death 6 interacting protein	0,0413	-1,1593
255	F6TQU9	ZNFX1	Zinc finger NFX1-type containing 1	0,0382	-1,1597
256	A0A3Q2HWR6	OSBPL3	Oxysterol-binding protein	0,0258	-1,1608
257	A0A9L0SYX6	ATAD1	Outer mitochondrial transmembrane helix translocase	0,0106	-1,1621
258	A0A9L0RDW4	CCM2	CCM2 scaffold protein	0,0302	-1,1634
259	F6PHS4	FKBP15	peptidylprolyl isomerase	0,0379	-1,1658
260	A0A9L0R5S8	AP2A2	AP-2 complex subunit alpha	0,0250	-1,1666
261	A0A3Q2KN14	CERK	Ceramide kinase	0,0302	-1,1680
262	F6YJI4	MINK1	non-specific serine/threonine protein kinase	0,0064	-1,1684
263	A0A3Q2HUG6	WDR81	WD repeat domain 81	0,0413	-1,1687
264	A0A9L0SYU9	LAMTOR5	Ragulator complex protein LAMTOR5	0,0375	-1,1727
265	F6RMP1	AP5Z1	Adaptor related protein complex 5 subunit zeta 1	0,0227	-1,1731
266	A0A9L0S5Q0	ACOT9	Acyl-CoA thioesterase 9	0,0289	-1,1734
267	A0A9L0RQ39	VCPIP1	ubiquitinyl hydrolase 1	0,0302	-1,1738
268	F6WTW0	STAT6	Signal transducer and activator of transcription	0,0274	-1,1740
269	A0A5F5PS97	ITPR2	Inositol 1,4,5-trisphosphate receptor	0,0388	-1,1744
270	F6U999	MANF	Mesencephalic astrocyte derived neurotrophic factor	0,0288	-1,1782
271	A0A3Q2I6V8	PARK7	protein deglycase	0,0408	-1,1797
272	F6SSB5	PARP9	Poly(ADP-ribose) polymerase family member 9	0,0262	-1,1808
273	A0A5F5PNE8	SNTB1	Syntrophin beta 1	0,0142	-1,1835
274	A0A5F5Q3F6	WASHC5	WASH complex subunit 5	0,0325	-1,1849
275	F6QDX4	TICAM2	Toll-like receptor adaptor molecule 2	0,0373	-1,1857
276	A0A9L0RLV2	OXSRI	Serine/threonine-protein kinase OSR1	0,0434	-1,1869
277	F6SMA2	FKBP2	peptidylprolyl isomerase	0,0455	-1,1870
278	F7A575	ERBIN	ErbB2 interacting protein	0,0326	-1,1896
279	A0A3Q2H905 A0A3Q2H2F5	PTPRJ	protein-tyrosine-phosphatase	0,0142	-1,1902
280	A0A3Q2HT77	LOC111771846	protein-tyrosine-phosphatase	0,0142	-1,1902
281	A0A3Q2IDV7	LOC111775907	Fibronectin type-III domain-containing protein	0,0142	-1,1902
282	F7C9H8	LRPAP1	LDL receptor related protein associated protein 1	0,0129	-1,1924
283	A0A9L0T9L8	PRKAA1	Acetyl-CoA carboxylase kinase	0,0259	-1,1924
284	A0A9L0SU58	FGD3	FYVE, RhoGEF and PH domain containing 3	0,0365	-1,1945
285	A0A3Q2LDC2	PPA1	Inorganic pyrophosphatase	0,0232	-1,1951
286	F6UYU3	DMXL2	Dmx like 2	0,0231	-1,1970
287	A0A9L0TEK7	SNX2	Sorting nexin-2	0,0278	-1,2023
288	Q867C9	PFKM	ATP-dependent 6-phosphofructokinase, muscle type	0,0480	-1,2038
289	A0A9L0RR79	PFKM	ATP-dependent 6-phosphofructokinase	0,0480	-1,2038
290	F7AFE1	FRMD4A	FERM domain containing 4A	0,0485	-1,2040
291	A0A3Q2HPN1	LOC111771754	Cytohesin Ubiquitin Protein Inducing domain-containing protein	0,0485	-1,2040

292	A0A3Q2IAU4	ELMO1	Engulfment and cell motility 1	0,0119	-1,2073
293	A0A3Q2KIZ6	RAB18	Ras-related protein Rab-18	0,0211	-1,2080
294	A0A9L0RP89	CGAS	Cyclic GMP-AMP synthase	0,0326	-1,2129
295	A0A9L0T2A9	DNAJC1	DnaJ heat shock protein family (Hsp40) member C1	0,0312	-1,2132
296	F6ZLS9	ABCA3	ATP binding cassette subfamily A member 3	0,0233	-1,2154
297	A0A5F5PQR8	GAK	Cyclin G associated kinase	0,0190	-1,2160
298	K9K202 F6SXQ9	CDC42	Cell division control protein 42 homolog	0,0446	-1,2195
299	A0A5F5PW77	SP100	SP100 nuclear antigen	0,0467	-1,2226
300	A0A9L0T5R3	CYRIB	CYFIP related Rac1 interactor B	0,0129	-1,2226
301	A0A9L0RWK4		NADP-dependent oxidoreductase domain-containing protein	0,0239	-1,2228
302	A0A2L0PQU5	LOC100065387	Carcinoembryonic antigen-related cell adhesion molecule 1 isoform 4L	0,0365	-1,2229
303	A0A9L0R2E0	PRKCD	Protein kinase C delta type	0,0186	-1,2255
304	A0A3Q2KRZ0	DIAPH2	Diaphanous related formin 2	0,0230	-1,2274
305	A0A3Q2H7Z7	SIRT2	NAD-dependent protein deacetylase	0,0453	-1,2328
306	F7A2L7	NUDT4	diphosphoinositol-polyphosphate diphosphatase	0,0171	-1,2332
307	A0A3Q2GVQ6	NUDT11	diphosphoinositol-polyphosphate diphosphatase	0,0171	-1,2332
308	A0A3Q2ICJ7	GPCPD1	Glycerophosphocholine phosphodiesterase 1	0,0261	-1,2348
309	A0A9L0S1B9	GRAP2	GRB2 related adaptor protein 2	0,0454	-1,2357
310	A0A9L0T7L4	CTNNA1	Catenin alpha-1	0,0238	-1,2371
311	A0A3Q2IAK4	DENND1A	DENN domain containing 1A	0,0416	-1,2377
312	F7D917	PGD	6-phosphogluconate dehydrogenase, decarboxylating	0,0490	-1,2398
313	A0A9L0S6H6	POR	NADPH--cytochrome P450 reductase	0,0231	-1,2402
314	F7DH20	AP2A1	AP-2 complex subunit alpha	0,0192	-1,2411
315	F6YP32	DPP7	Dipeptidyl peptidase 7	0,0210	-1,2432
316	F7BQ20	PML	PML nuclear body scaffold	0,0240	-1,2437
317	A0A9L0RKD4	MAPK14	Mitogen-activated protein kinase 14	0,0058	-1,2481
318	F6RLH2	PYCARD	PYD and CARD domain containing	0,0428	-1,2495
319	F7BNN7	STK26	non-specific serine/threonine protein kinase	0,0418	-1,2496
320	F6SPB2	GBP5	Guanylate binding protein 5	0,0325	-1,2517
321	A0A9L0SFL2	RPIA	ribose-5-phosphate isomerase	0,0324	-1,2526
322	F7DK60	AP2M1	AP-2 complex subunit mu	0,0066	-1,2528
323	A0A3Q2I9H9	WDR91	WD repeat-containing protein 91	0,0312	-1,2544
324	F6RUP0	PRDX4	thioredoxin-dependent peroxiredoxin	0,0411	-1,2563
325	F6V2X7	PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	0,0239	-1,2566
326	A0A9L0TQP3	AP1B1	AP complex subunit beta	0,0070	-1,2572
327	F6YY53	MAP3K2	Mitogen-activated protein kinase kinase kinase 2	0,0458	-1,2582
328	A0A9L0TJ76	GNAI2	Guanine nucleotide-binding protein G(i) subunit alpha-2	0,0265	-1,2583
329	F6YTU4	DOK3	Docking protein 3	0,0448	-1,2595
330	A0A5K1UAF9	PNPLA6	Patatin like phospholipase domain containing 6	0,0408	-1,2600
331	H9H012	CRELD2	protein disulfide-isomerase	0,0067	-1,2619
332	F6SZJ2	EHD4	EH domain containing 4	0,0231	-1,2629

333	F7E0S8	RGS19	Regulator of G protein signaling 19	0,0289	-1,2685
334	A0A9L0S5M9	RGS20	Regulator of G protein signaling 20	0,0289	-1,2685
335	F6RVT9	TM7SF3	Transmembrane 7 superfamily member 3	0,0325	-1,2703
336	F6UJ24	TRAFD1	TRAF-type zinc finger domain-containing protein 1	0,0281	-1,2712
337	F7BPU9	PARP3	Poly [ADP-ribose] polymerase	0,0140	-1,2712
338	A0A3Q2H5A0	AMPD2	AMP deaminase	0,0325	-1,2719
339	A0A9L0SWH3	HELZ2	RNA helicase	0,0233	-1,2763
340	A0A3Q2HRQ0	LOC100072612	Purine nucleoside phosphorylase	0,0386	-1,2765
341	A0A9L0TKM4	TNFAIP2	TNF alpha induced protein 2	0,0440	-1,2785
342	F7BHT1	AIDA	Axin interactor, dorsalization associated	0,0231	-1,2803
343	F6R9H6	DOCK2	Dedicator of cytokinesis 2	0,0299	-1,2807
344	A0A3Q2IJY8	CMTR1	Cap-specific mRNA (nucleoside-2-O-)-methyltransferase 1	0,0326	-1,2816
345	F7B2Y1	MYO5A	Myosin VA	0,0375	-1,2822
346	A0A9L0RP63		Caspase-13-like	0,0326	-1,2822
347	A0A9L0TIA5	NUCB2	Nucleobindin 2	0,0455	-1,2865
348	A0A3Q2HQZ4	USP25	ubiquitinyl hydrolase 1	0,0228	-1,2876
349	F7DSP6	OAS2	2-5 oligoadenylate synthase	0,0137	-1,2898
350	A0A9L0RFY2	SLC25A21	Mitochondrial 2-oxodicarboxylate carrier	0,0446	-1,2915
351	A0A3Q2H584	FCHO2	F-BAR domain only protein 2	0,0380	-1,2940
352	A0A3Q2L886	SLC15A3	Solute carrier family 15 member 3	0,0234	-1,2957
353	F6WKI2	GCLM	GCS light chain	0,0491	-1,2980
354	A0A9L0TB67	UBE2Q1	Ubiquitin conjugating enzyme E2 Q1	0,0145	-1,2985
355	A0A3Q2GTP1	SH3GLB1	Endophilin-B1	0,0229	-1,2994
356	F6Y6L2	SIDT2	SID1 transmembrane family member 2	0,0445	-1,3021
357	A0A9L0R2K2	OGFR	Opioid growth factor receptor	0,0231	-1,3038
358	A0A5F5PF68	LOC100069287	Ig-like domain-containing protein	0,0296	-1,3039
359	F7C6Y7	BACH1	BTB domain and CNC homolog 1	0,0184	-1,3042
360	A0A9L0R9N8	PARP12	Poly(ADP-ribose) polymerase family member 12	0,0144	-1,3098
361	A0A9L0SNS8	IL1RAP	Interleukin 1 receptor accessory protein	0,0273	-1,3199
362	A0A5F5PXT6	STAT1	Signal transducer and activator of transcription	0,0064	-1,3208
363	A0A5F5PUT5	FYB1	FYN binding protein 1	0,0317	-1,3215
364	F7BZV9	PREX1	Phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor 1	0,0172	-1,3221
365	A0A9L0R497	DCTPP1	selenide, water dikinase	0,0092	-1,3255
366	A0A3Q2HQA0	CYTH1	Cytohesin 1	0,0180	-1,3257
367	A0A9L0RYK8	TTC39C	Tetratricopeptide repeat domain 39C	0,0323	-1,3260
368	A0A3Q2HH31	SOWAHD	Sosondowah ankyrin repeat domain family member D	0,0274	-1,3266
369	A0A9L0S078	FCER1G	High affinity immunoglobulin epsilon receptor subunit gamma	0,0117	-1,3269
370	A0A3Q2HVA3 A0A3Q2KYR8	LOC111771768	BTB domain-containing protein	0,0211	-1,3282
371	A0A5F5PKL3	LOC100062210	FAM21/CAPZIP domain-containing protein	0,0363	-1,3283
372	A0A9L0SQ13	MPP1	55 kDa erythrocyte membrane protein	0,0230	-1,3287
373	F6T5E1	TFE3	Transcription factor binding to IGHM enhancer 3	0,0303	-1,3287
374	A0A9L0R6L1	GGA2	Golgi associated, gamma adaptin ear containing, ARF binding protein 2	0,0322	-1,3313

375	A0A5F5PXE6	LOC100072592	Purine nucleoside phosphorylase	0,0442	-1,3323
376	F7BTU9	HELB	DNA helicase B	0,0059	-1,3324
377	A0A3Q2H7S2 O77793	PLA2G4A	Phospholipase A2	0,0176	-1,3349
378	F6Y2D5	RNPEP	Arginyl aminopeptidase	0,0311	-1,3360
379	A0A3Q2I4U5	ZNF579	Zinc finger protein 579	0,0417	-1,3363
380	A0A9L0S512	ACP2	Lysosomal acid phosphatase	0,0444	-1,3368
381	A0A9L0QY51	P2RX7	P2X purinoceptor	0,0360	-1,3370
382	F7CNA2	NKAPD1	NKAP domain containing 1	0,0418	-1,3374
383	A0A9L0RJM2	IFI44L	Interferon induced protein 44 like	0,0402	-1,3376
384	A0A3Q2HBR1	SRGAP1	SLIT-ROBO Rho GTPase activating protein 1	0,0071	-1,3381
385	A0A3Q2H729	ANKRD22	Ankyrin repeat domain 22	0,0424	-1,3383
386	A0A9L0RS68	CROCC	Ciliary rootlet coiled-coil, rootletin	0,0313	-1,3395
387	A0A3Q2LMX0	EPB41L2	Erythrocyte membrane protein band 4.1 like 2	0,0233	-1,3407
388	A0A9L0T0J1	LPCAT2	Lysophosphatidylcholine acyltransferase 2	0,0310	-1,3422
389	F6UWD8	OPTN	Optineurin	0,0388	-1,3446
390	A0A5F5PG64	GAS7	Growth arrest specific 7	0,0418	-1,3454
391	F6UCN5	UPP1	Uridine phosphorylase	0,0244	-1,3464
392	F7CFS8	UBA7	Ubiquitin like modifier activating enzyme 7	0,0172	-1,3470
393	A0A5F5PT09	LGMN	Legumain	0,0193	-1,3482
394	F6RYF9	ENPP4	bis(5-adenosyl)-triphosphatase	0,0381	-1,3490
395	F7CAM7	ZCCHC2	Zinc finger CCHC-type containing 2	0,0313	-1,3495
396	F7AQA4	DPYD	Dihydropyrimidine dehydrogenase [NADP(+)]	0,0385	-1,3506
397	F6Y5H9	MERTK	receptor protein-tyrosine kinase	0,0452	-1,3525
398	F6PQ62	CMPK2	Cytidine/uridine monophosphate kinase 2	0,0069	-1,3554
399	F6PPI2	TDRD7	Tudor domain-containing protein 7	0,0193	-1,3564
400	A0A9L0T020	SRGAP2	SLIT-ROBO Rho GTPase activating protein 2	0,0106	-1,3579
401	A0A9L0RKX0	TRIM21	RING-type E3 ubiquitin transferase	0,0220	-1,3617
402	A0A9L0RSF5	ERP29	Endoplasmic reticulum resident protein 29	0,0030	-1,3649
403	A0A3Q2H748	TSPEAR	Thrombospondin type laminin G domain and EAR repeats	0,0230	-1,3759
404	A0A9L0RA22	SKAP2	Src kinase-associated phosphoprotein 2	0,0231	-1,3786
405	F6T9Z6 A0A3Q2HR99	LOC100067491	Solute carrier family 23 member 1	0,0195	-1,3837
406	A0A3Q2GVE2	RNF213	Ring finger protein 213	0,0297	-1,3884
407	F6S1K6	PARP14	Poly [ADP-ribose] polymerase	0,0265	-1,3887
408	A0A3Q2I8C9	ACO1	Cytoplasmic aconitate hydratase	0,0326	-1,3913
409	F6PNF5	TTYH3	Protein tweety homolog	0,0485	-1,3920
410	F6W5X0	RIT1	Ras like without CAAX 1	0,0430	-1,3966
411	A0A9L0RVN1	HSPBAP1	HSPB1 associated protein 1	0,0178	-1,3974
412	F6VK27	MECR	Enoyl-[acyl-carrier-protein] reductase, mitochondrial	0,0025	-1,3998
413	F6Q015	GBP1	Guanylate binding protein 1	0,0449	-1,4041
414	A0A5F5PFY0	SLC25A29	Solute carrier family 25 member 29	0,0143	-1,4044
415	A0A3Q2HQP5	XAF1	XIAP associated factor 1	0,0074	-1,4058
416	F7CGD4	PTK2B	non-specific protein-tyrosine kinase	0,0484	-1,4110
417	A0A5F5PUU9	TRPM2	Transient receptor potential cation channel subfamily M member 2	0,0312	-1,4132

418	A0A9L0TNS7	SP110	SP110 nuclear body protein	0,0036	-1,4132
419	A0A9L0T053	RNF34	E3 ubiquitin-protein ligase RNF34	0,0447	-1,4157
420	A0A3Q2LH48	STX2	Syntaxin 2	0,0230	-1,4186
421	F6WCN4	DOK2	Docking protein 2	0,0410	-1,4256
422	F6Z1C6	DTX3L	E3 ubiquitin-protein ligase	0,0360	-1,4265
423	A0A9L0S7G5	NMI	N-myc and STAT interactor	0,0155	-1,4348
424	F6TDD3	ECHDC1	Ethylmalonyl-CoA decarboxylase	0,0143	-1,4352
425	H9H007	ALDH1L1	10-formyltetrahydrofolate dehydrogenase	0,0416	-1,4354
426	A0A3Q2H6H0	PARP15	Poly [ADP-ribose] polymerase	0,0064	-1,4376
427	F6UD42	GNGT2	Guanine nucleotide-binding protein subunit gamma	0,0444	-1,4417
428	A0A9L0RZS6	DDX60	DExH/H-box helicase 60	0,0171	-1,4425
429	F6Y6X8	CD4	T-cell surface glycoprotein CD4	0,0231	-1,4438
430	A0A9L0T6W8	PLPP3	Phospholipid phosphatase 3	0,0312	-1,4454
431	A0A3Q2I375	UGP2	UTP--glucose-1-phosphate uridylyltransferase	0,0294	-1,4459
432	A0A3Q2HTX9	OAS3	2-5 oligoadenylate synthase	0,0012	-1,4483
433	F6W6W9 F7DBR0	LOC100146833	aldehyde oxidase	0,0313	-1,4536
434	A0A9L0RWJ3	LOC100053055	Ig-like domain-containing protein	0,0325	-1,4537
435	A0A3Q2H0S0		Ig-like domain-containing protein	0,0457	-1,4587
436	F7AB03	GRN	Granulin	0,0068	-1,4589
437	A0A3Q2HHW6	PLXNC1	Plexin C1	0,0446	-1,4615
438	A0A5F5PM43	DNTTIP1	Deoxynucleotidyltransferase terminal-interacting protein 1	0,0456	-1,4623
439	A0A9L0TU63	CDS1	Phosphatidate cytidylyltransferase	0,0171	-1,4639
440	A0A9L0T5D8	CTSO	Cathepsin O	0,0231	-1,4657
441	P12762 A0A9L0RQC6	ALDH2	Aldehyde dehydrogenase, mitochondrial	0,0327	-1,4764
442	A0A9L0S3Z2	MNDA	Myeloid cell nuclear differentiation antigen	0,0445	-1,4798
443	A0A3Q2KXH2	LOC111767504	Aminopeptidase	0,0166	-1,4805
444	A0A3Q2HF85	EZR	Ezrin	0,0247	-1,4821
445	A0A3Q2LHE7	SLC15A4	Solute carrier family 15 member 4	0,0180	-1,4853
446	F7AQZ6	LYN	Tyrosine-protein kinase	0,0157	-1,4872
447	A0A9L0S005	HMOX1	Heme oxygenase 1	0,0093	-1,4901
448	F6YQ33	CARMIL1	Capping protein regulator and myosin 1 linker 1	0,0195	-1,4928
449	F7B1V5	GIMAP8	GTPase, IMA family member 8	0,0447	-1,4960
450	F6RC31	SGSH	N-sulfoglucosamine sulfohydrolase	0,0365	-1,4975
451	A0A3Q2I8H8	LGALS3BP	Galectin-3-binding protein	0,0141	-1,4986
452	F7BNI3	DNASE2	Deoxyribonuclease-2-alpha	0,0479	-1,5057
453	A0A9L0SKI6	ADA	Adenosine deaminase	0,0217	-1,5060
454	A0A9L0SQC1	STAT3	Signal transducer and activator of transcription	0,0066	-1,5067
455	A0A9L0S7H7	SMPDL3A	Sphingomyelin phosphodiesterase acid like 3A	0,0266	-1,5125
456	A0A5F5Q248	MYDGF	Myeloid derived growth factor OS=Equus caballus OX=9796 PE=1 SV=2	0,0366	-1,5185
457	F6U1D9	STAT2	Signal transducer and activator of transcription	0,0091	-1,5258
458	A0A9L0S6I5	SLFN5	Schlafen family member 5	0,0359	-1,5266
459	F7DK01	SP140	SP140 nuclear body protein	0,0191	-1,5286

460	A0A3Q2HBZ4	STARD3NL	MLN64 N-terminal domain-like protein-like protein	0,0091	-1,5302
461	A0A9L0RXA3	LOC100060591	Immunoglobulin subtype domain-containing protein	0,0313	-1,5304
462	A0A3Q2HMK7	HERC6	HECT and RLD domain containing E3 ubiquitin protein ligase family member 6	0,0076	-1,5317
463	Q28379	MX1	Interferon-induced GTP-binding protein Mx1	0,0265	-1,5327
464	F7BTX8	LOC100063866	AIG1-type G domain-containing protein	0,0281	-1,5348
465	A0A9L0RKU6		FAD-binding PCMH-type domain-containing protein OS=Equus caballus OX=9796 PE=1 SV=1	0,0365	-1,5365
466	A0A3Q2I189	GNS	N-acetylglucosamine-6-sulfatase	0,0334	-1,5369
467	A0A3Q2HCB4	SLC2A6	Solute carrier family 2 member 6	0,0411	-1,5384
468	A0A9L0SEM9	GALNS	N-acetylgalactosamine-6-sulfatase	0,0311	-1,5389
469	F7B5P1	CNDP2	Cytosolic non-specific dipeptidase	0,0094	-1,5403
470	A0A9L0SN53	IRF9	Interferon regulatory factor 9	0,0092	-1,5510
471	A0A9L0T6H9	STAT4	Signal transducer and activator of transcription	0,0273	-1,5591
472	A0A9L0REW1	TMCC3	Transmembrane and coiled-coil domain family 3	0,0490	-1,5728
473	F6YA86	ITPR1	Inositol 1,4,5-trisphosphate receptor	0,0457	-1,5819
474	F6VUW2	CTSS	Cathepsin S	0,0234	-1,5863
475	F6UTR4	TBC1D9	TBC1 domain family member 9	0,0091	-1,5958
476	F6SGU1	EOGT	EGF domain-specific O-linked N-acetylglucosamine transferase	0,0185	-1,6060
477	F6U4D0	MRPL13	Mitochondrial ribosomal protein L13	0,0373	-1,6086
478	A0A9L0RQB5	BID	BH3-interacting domain death agonist	0,0468	-1,6196
479	A0A9L0SI36	OAS1	2-5 oligoadenylate synthase	0,0141	-1,6246
480	A0A5F5PZT3 A0A5F5PPS9	LOC111775633	Pro-cathepsin H	0,0184	-1,6251
481	A0A9L0TFY0	SETX	Senataxin	0,0070	-1,6291
482	A0A5F5PJ15	IL4I1	Interleukin 4 induced 1	0,0068	-1,6421
483	A0A9L0RRQ4	DAB2	DAB adaptor protein 2	0,0057	-1,6458
484	F6SH38	LAMP1	Lysosomal associated membrane protein 1	0,0279	-1,6556
485	F7ACF3	SCPEP1	Carboxypeptidase	0,0234	-1,6596
486	A0A9L0TQS8	RIGI	RNA helicase	0,0000	-1,6627
487	A0A9L0TLG5	EIF2AK2	Eukaryotic translation initiation factor 2 alpha kinase 2	0,0000	-1,6648
488	F6SS69	FUCA1	Alpha-L-fucosidase	0,0187	-1,6785
489	F6S337	MLKL	Mixed lineage kinase domain like pseudokinase	0,0187	-1,6919
490	F6T4L9	CD38	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1	0,0326	-1,6947
491	A0A3Q2LHV6	CTSC	Dipeptidyl peptidase 1	0,0497	-1,7124
492	A0A3Q2HL98	RNF13	RING-type E3 ubiquitin transferase	0,0186	-1,7240
493	F6TA46		Interferon gamma inducible protein 16	0,0156	-1,7256
494	A0A5F5PPC4	HEXA	Beta-hexosaminidase	0,0186	-1,7316
495	F6R5V4	CPVL	Carboxypeptidase vitellogenic like	0,0230	-1,7378
496	F6TDS2	HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5	0,0076	-1,7448
497	F6RQE8 A0A5F5PP62	LOC100050797	Interferon-induced transmembrane protein 1-like	0,0230	-1,7458
498	A0A9L0RUE6	IGSF6	Immunoglobulin superfamily member 6	0,0447	-1,7748
499	F7CN95	SIGLEC1	Sialic acid binding Ig like lectin 1	0,0087	-1,7771

500	A0A3Q2HFH0	ENPP5	Ectonucleotide pyrophosphatase/phosphodiesterase family member 5	0,0164	-1,8091
501	F6UJZ8	PRCP	Prolylcarboxypeptidase	0,0492	-1,8227
502	A0A9L0RC22	LOC100146699	AIG1-type G domain-containing protein	0,0445	-1,8316
503	F6QSK3	ABCA1	ATP binding cassette subfamily A member 1	0,0387	-1,8653
504	F7BTI3 A0A3Q2L588		AIG1-type G domain-containing protein	0,0437	-1,8764
505	F7CSX2	CXCL9	C-X-C motif chemokine	0,0365	-1,8837
506	F7CY40		Interferon gamma inducible protein 16	0,0228	-1,8851
507	F6QKQ9	GAS2L3	Growth arrest specific 2 like 3	0,0409	-1,8937
508	A0A3Q2LNF2	ZBP1	Z-DNA binding protein 1	0,0141	-1,9030
509	F6S4G6	PLSCR1	Phospholipid scramblase	0,0374	-1,9058
510	F6SP74	CFP	Complement factor properdin	0,0139	-1,9110
511	F7B6D0	GLA	Alpha-galactosidase	0,0324	-1,9264
512	F6W0G6	SARAF	Store-operated calcium entry-associated regulatory factor OS=Equus caballus OX=9796 GN=SARAF PE=3 SV=2	0,0017	-1,9395
513	Q7YS54 F6WG65	GSDME	Gasdermin-E	0,0129	-1,9505
514	A0A9L0TA95	LAIR1	Ig-like domain-containing protein	0,0140	-1,9528
515	F7B6K6	TRIM22	Tripartite motif containing 22	0,0038	-1,9537
516	A0A9L0S681	SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1	0,0000	-1,9691
517	A0A9L0SHZ3	NPC2	NPC intracellular cholesterol transporter 2	0,0458	-1,9837
518	F7BN14	IFI30	Gamma-interferon-inducible lysosomal thiol reductase	0,0096	-1,9995
519	F6SEZ0	SLC7A11	Solute carrier family 7 member 11	0,0368	-2,0256
520	A0A9L0RES1	TRIM14	Tripartite motif containing 14	0,0040	-2,0638
521	A0A3Q2LPJ8	ADGRE1	Adhesion G protein-coupled receptor E1	0,0312	-2,0926
522	F6RN56	IFIT3	Interferon induced protein with tetratricopeptide repeats 3	0,0068	-2,1134
523	F6V5D4	PHACTR2	Phosphatase and actin regulator	0,0163	-2,1179
524	A0A3Q2LJV0	SLC27A3	long-chain-fatty-acid--CoA ligase	0,0093	-2,1308
525	F7BZ41	CTSL	Cathepsin L	0,0230	-2,1374
526	A0A5F5PQ31	ISG20	Interferon stimulated exonuclease 20	0,0210	-2,1385
527	A0A9L0R6G9	TRANK1	Tetratricopeptide repeat and ankyrin repeat containing 1	0,0013	-2,1461
528	F6TAZ0	GLUL	Glutamine synthetase	0,0159	-2,1649
529	A0A3Q2H807	SLC39A14	Metal cation symporter ZIP14	0,0144	-2,1692
530	A0A3Q2I8S7	LOC100630879	Interferon induced protein with tetratricopeptide repeats 1B	0,0272	-2,1860
531	A0A5F5PN31	BST2	Bone marrow stromal cell antigen 2	0,0045	-2,2139
532	F7BPQ4	SLC43A2	Large neutral amino acids transporter small subunit 4	0,0242	-2,2450
533	F6YCC0	CSTA	Cystatin A	0,0067	-2,2749
534	F6SD45		Uncharacterized protein	0,0107	-2,3327
535	F7DMW5	LOC100054321	Ig-like domain-containing protein	0,0243	-2,3867
536	A0A3Q2GTQ4	MX1	Interferon-induced GTP-binding protein Mx1	0,0020	-2,4055
537	A0A3Q2HVG2	EPSTI1	Epithelial stromal interaction 1	0,0034	-2,4056
538	F6ZJN9	LOC100065733	Ig-like domain-containing protein	0,0061	-2,4115
539	A0A9L0SDX4	CD163	CD163 molecule	0,0141	-2,4391

540	F6W4E1	IFIH1	RNA helicase	0,0000	-2,6160
541	A0A9L0QZV7	ISG15	ISG15 ubiquitin like modifier	0,0297	-2,8630
542	A0A3Q2H6Z3	IFIT2	Interferon induced protein with tetratricopeptide repeats 2	0,0164	-2,8653
543	F6WJ28	AOAH	Acyloxyacyl hydrolase	0,0066	-3,1049
544	A0A5F5PQI8	LOC100630879	Interferon induced protein with tetratricopeptide repeats 1	0,0015	-3,4880
545	A0A3Q2IER4	MX2	Interferon-induced GTP-binding protein Mx2	0,0000	-3,6700
546	A0A9L0TNB6	CMKLR1	Chemerin-like receptor 1	0,0141	-3,9850
547	A0A3Q2LU07	LOC100058990	Interferon induced protein with tetratricopeptide repeats 1	0,0116	-3,9961
548	A0A5F5PL53	EPB41L3	Erythrocyte membrane protein band 4.1 like 3	0,0164	-4,7893

Supplementary Table 2. Statistical analysis of RNA sequencing reads in endometrial fibroblasts after treatment with the eqMD-M Φ ^{IFN γ /LPS}-CM and eqMD-M Φ ^{IL-4/IL-13}-CM for 96 hours

Index	Sample name	Raw reads	Filtered reads	Uniquely mapped reads	% uniquely mapped reads
1.	mare_1_control	19136245	686228	18450017	96,41
2.	mare_2_control	18407023	697032	17816562	96,79
3.	mare_3_control	21436447	591224	20626489	96,22
4.	mare_4_control	16522082	631018	16017044	96,94
5.	mare_1_M Φ ^{IFNγ/LPS}	20241399	590118	19461571	96,15
6.	mare_2_M Φ ^{IFNγ/LPS}	18361926	552800	17809126	96,99
7.	mare_3_M Φ ^{IFNγ/LPS}	16814713	560337	16244195	96,61
8.	mare_4_M Φ ^{IFNγ/LPS}	13915076	809958	13247850	95,21
9.	mare_1_M Φ ^{IL-4/IL-13}	19922599	570518	19225567	96,50
10.	mare_2_M Φ ^{IL-4/IL-13}	16920221	505038	16359884	96,69
11.	mare_3_M Φ ^{IL-4/IL-13}	16442252	667226	15927306	96,87
12.	mare_4_M Φ ^{IL-4/IL-13}	18261393	564896	17696497	96,91
mean		18031781	618866	17406842	96,52

Supplementary Table 3. The full list of differentially expressed genes (DEGs) identified in endometrial fibroblasts after treatment with eqMD-M Φ ^{IFN γ /LPS}-CM for 96 hours.

No.	Ensemble gene ID	Gene symbol	Gene name	log2FC	Padj
1	ENSECAG00000046132	CXCL9	C-X-C motif chemokine ligand 9	6,8830	2,594E-05
2	ENSECAG00000039250	CYP2C113	cytochrome P450 family 2 subfamily C member 113	6,6652	2,584E-04
3	ENSECAG00000039617	SNCB	synuclein beta	5,9824	5,415E-03
4	ENSECAG00000008392	SLC8A2	solute carrier family 8 member A2	5,6190	4,086E-07
5	ENSECAG00000029465	CHI3L1	chitinase 3 like 1	5,2671	5,944E-23
6	ENSECAG00000017917	CYBB	cytochrome b-245 beta chain	5,2558	6,814E-06
7	ENSECAG00000041545	GJD3	gap junction protein delta 3	5,0493	3,807E-05
8	ENSECAG00000005126	TRIM22	tripartite motif containing 22	4,8860	5,233E-03
9	ENSECAG00000014645	OASL	2'-5'-oligoadenylate synthetase-like	4,8348	2,165E-06
10	ENSECAG00000004709	C2	complement C2	4,6884	1,094E-08
11	ENSECAG00000015449	IRF8	interferon regulatory factor 8	4,3180	3,574E-22
12	ENSECAG00000016858	PXT1	peroxisomal testis enriched protein 1	4,1644	8,475E-03
13	ENSECAG00000019551	CXCL12	C-X-C motif chemokine ligand 12	3,9466	8,882E-05
14	ENSECAG00000017185	ZBP1	Z-DNA binding protein 1	3,8214	4,503E-10
15	ENSECAG00000015034	PLAC8B	placenta associated 8 B	3,7182	2,198E-04
16	ENSECAG00000024259	Eqca-DQA1		3,5285	2,079E-03
17	ENSECAG00000008059	PDE4C	phosphodiesterase 4	3,5233	5,237E-04
18	ENSECAG00000046493	GBP5	guanylate binding protein 5	3,4819	6,622E-09
19	ENSECAG00000010055	RSAD2	radical S-adenosyl methionine domain containing 2	3,4726	4,782E-04
20	ENSECAG00000024959	FBXO39	F-box protein 39	3,4722	1,317E-05
21	ENSECAG00000032492	ENSECAG00000032492		3,4130	1,634E-03
22	ENSECAG00000008615	ENSECAG00000008615		3,2589	3,069E-04
23	ENSECAG00000006205	CD7	CD7 molecule	3,2425	3,415E-05
24	ENSECAG00000014867	PLBD1	phospholipase B domain containing 1	3,1870	5,865E-04
25	ENSECAG00000010153	IFIT3	interferon induced protein with tetratricopeptide repeats 3	3,1639	3,184E-05
26	ENSECAG00000023264	RTP4	receptor transporter protein 4	3,1540	1,934E-03
27	ENSECAG00000051569	ENSECAG00000051569		3,1437	1,192E-04
28	ENSECAG00000019758	TNFSF13B	TNF superfamily member 13b	3,1211	4,176E-09
29	ENSECAG00000033385	LGALS3BP	galectin 3 binding protein	3,0613	1,521E-07
30	ENSECAG00000055535	ENSECAG00000055535		3,0384	1,846E-09
31	ENSECAG00000048862	ENSECAG00000048862		2,9994	9,764E-04
32	ENSECAG00000022188	WNT11	Wnt family member 11	2,9979	1,482E-05
33	ENSECAG00000009744	IRF7	interferon regulatory factor 7	2,9094	2,416E-04
34	ENSECAG00000008503	CD74	CD74 molecule	2,9005	3,603E-02
35	ENSECAG00000024961	XAF1	XIAP associated factor 1	2,8278	1,236E-05
36	ENSECAG00000010185	IFIT2	interferon induced protein with tetratricopeptide repeats 2	2,7865	5,323E-09
37	ENSECAG00000010963	SERPING1	serpin family G member 1	2,7234	7,675E-06

38	ENSECAG00000052431	ENSECAG00000052431		2,6795	1,044E-02
39	ENSECAG00000008763	LYPD3	LY6/PLAUR domain containing 3	2,6771	5,287E-04
40	ENSECAG00000001466	PLEKHB1	pleckstrin homology domain containing B1	2,5323	7,534E-05
41	ENSECAG00000011404	ENSECAG00000011404		2,5219	4,798E-05
42	ENSECAG00000011854	CFB	complement factor B	2,5056	8,950E-05
43	ENSECAG00000001633	FZD10	frizzled class receptor 10	2,5024	1,825E-05
44	ENSECAG00000019329	MPP3	MAGUK p55 scaffold protein 3	2,4937	1,197E-03
45	ENSECAG00000019411	HERC6	HECT and RLD domain containing E3 ubiquitin protein ligase family member 6	2,4773	3,631E-06
46	ENSECAG00000012773	CMPK2	cytidine/uridine monophosphate kinase 2	2,4603	3,415E-05
47	ENSECAG00000003563	BST2	bone marrow stromal cell antigen 2	2,4380	1,142E-05
48	ENSECAG00000012742	CXCL6	C-X-C motif chemokine ligand 6	2,3192	3,808E-03
49	ENSECAG00000013037	ALDOC	aldolase, fructose-bisphosphate C	2,3168	1,122E-12
50	ENSECAG00000033890	CIDEC	cell death inducing DFFA like effector c	2,3007	1,635E-02
51	ENSECAG00000009020	IFI44	interferon induced protein 44	2,2764	2,200E-02
52	ENSECAG00000027808	PLCXD1	phosphatidylinositol specific phospholipase C X domain containing 1	2,2531	2,936E-03
53	ENSECAG00000012514	ENSECAG00000012514		2,2505	6,955E-09
54	ENSECAG00000020294	DCLK3	doublecortin like kinase 3	2,2492	6,412E-06
55	ENSECAG00000020326	CLSTN3	calsyntenin 3	2,2289	4,190E-11
56	ENSECAG00000014218	SIGLEC1	sialic acid binding Ig like lectin 1	2,2102	1,598E-08
57	ENSECAG00000007468	GAS2	growth arrest specific 2	2,2043	7,094E-04
58	ENSECAG00000036591	APLN	apelin	2,2028	4,244E-02
59	ENSECAG00000035279	IGFBP2	insulin like growth factor binding protein 2	2,1976	9,542E-26
60	ENSECAG00000008809	OAS3	2'-5'-oligoadenylate synthetase 3	2,1896	2,069E-06
61	ENSECAG00000030750	ENSECAG00000030750		2,1784	7,786E-04
62	ENSECAG00000017172	IFI27	interferon, alpha-inducible protein 27	2,1652	1,050E-03
63	ENSECAG00000055770	KCNE4	potassium voltage-gated channel subfamily E regulatory subunit 4	2,1413	5,728E-04
64	ENSECAG00000023657	FAM20A	FAM20A golgi associated secretory pathway pseudokinase	2,1398	4,232E-02
65	ENSECAG00000045402	ENSECAG00000045402		2,0921	2,271E-06
66	ENSECAG00000015395	HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5	2,0918	4,503E-10
67	ENSECAG00000049617	ENSECAG00000049617		2,0883	1,794E-02
68	ENSECAG00000059003	ISG15	ISG15 ubiquitin like modifier	2,0733	1,227E-02
69	ENSECAG00000018752	APOL6	apolipoprotein L6	2,0587	1,581E-06
70	ENSECAG00000033029	ENSECAG00000033029		2,0519	1,508E-03
71	ENSECAG00000057145	C3	complement C3	2,0327	3,631E-06

72	ENSECAG00000014519	ETV7	ETS variant transcription factor 7	2,0295	2,441E-06
73	ENSECAG00000018398	TRIM69	tripartite motif containing 69	2,0140	4,459E-02
74	ENSECAG00000023693	ECI1	enoyl-CoA delta isomerase 1	2,0103	1,873E-02
75	ENSECAG00000009211	ANGPT4	angiopoietin 4	2,0008	6,351E-07
76	ENSECAG00000030455	ENSECAG00000030455		1,9996	2,603E-03
77	ENSECAG00000021429	C1R	complement C1r	1,9982	1,501E-07
78	ENSECAG00000017443	TRIM14	tripartite motif containing 14	1,9881	3,850E-02
79	ENSECAG00000001064	RET	ret proto-onco	1,9848	1,546E-02
80	ENSECAG00000011776	MX1	MX dynamin like GTPase 1	1,9821	2,881E-02
81	ENSECAG00000047393	ENSECAG00000047393		1,9776	4,754E-03
82	ENSECAG00000001481	SAMD9L	sterile alpha motif domain containing 9 like	1,9531	4,754E-05
83	ENSECAG00000019438	OSMR	oncostatin M receptor	1,9176	3,574E-22
84	ENSECAG00000009618	IFI44L	interferon induced protein 44 like	1,9127	2,221E-02
85	ENSECAG00000053648	DDX60	DExH/H-box helicase 60	1,9082	1,604E-02
86	ENSECAG00000016312	CD274	CD274 molecule	1,8964	3,937E-03
87	ENSECAG00000009519	C1S	complement C1s	1,8944	2,333E-05
88	ENSECAG00000000284	USP18	ubiquitin specific peptidase 18	1,8897	2,288E-03
89	ENSECAG00000003260	ITIH5	inter-alpha-trypsin inhibitor heavy chain 5	1,8835	6,866E-05
90	ENSECAG00000012791	GBP6	guanylate binding protein family member 6	1,8693	3,150E-08
91	ENSECAG00000002623	NHLRC1	NHL repeat containing E3 ubiquitin protein ligase 1	1,8541	4,703E-02
92	ENSECAG00000051521	ENSECAG00000051521		1,8506	3,292E-03
93	ENSECAG00000018649	UCP2	uncoupling protein 2	1,8345	9,108E-05
94	ENSECAG00000019932	ENSECAG00000019932		1,8296	2,578E-04
95	ENSECAG00000007881	IFIH1	interferon induced with helicase C domain 1	1,8271	8,797E-04
96	ENSECAG00000014594	EPAS1	endothelial PAS domain protein 1	1,8171	1,718E-12
97	ENSECAG00000006853	UBD	ubiquitin D	1,8144	3,450E-02
98	ENSECAG00000049355	ENSECAG00000049355		1,8060	7,339E-04
99	ENSECAG00000014422	OAS2	2'-5'-oligoadenylate synthetase 2	1,7876	6,804E-09
100	ENSECAG00000016578	ENSECAG00000016578		1,7807	4,117E-02
101	ENSECAG00000007991	WDR86	WD repeat domain 86	1,7663	4,602E-02
102	ENSECAG00000024148	RAB39A	RAB39A, member RAS onco family	1,7576	2,713E-03
103	ENSECAG00000011704	TNFAIP2	TNF alpha induced protein 2	1,7503	6,023E-16
104	ENSECAG00000024062	DDX60L	DExH/H-box 60 like	1,7380	5,828E-06
105	ENSECAG00000020767	IL7	interleukin 7	1,7322	1,257E-02
106	ENSECAG00000009039	STAT1	signal transducer and activator of transcription 1	1,7137	3,627E-13
107	ENSECAG00000004572	CH25H	cholesterol 25-hydroxylase	1,7111	7,959E-03
108	ENSECAG00000000373	LOXL3	lysyl oxidase like 3	1,6763	3,427E-03
109	ENSECAG00000000910	ENSECAG00000000910		1,6732	9,102E-07
110	ENSECAG00000034282	CCL8	chemokine (C-C motif) ligand 8	1,6687	5,350E-10

111	ENSECAG00000000943	PTGES	prostaglandin E synthase	1,6620	5,126E-04
112	ENSECAG00000012132	ENSECAG00000012132		1,6238	1,637E-04
113	ENSECAG00000017217	CA12	carbonic anhydrase 12	1,6144	2,739E-02
114	ENSECAG00000018332	AK4	adenylate kinase 4	1,5812	2,388E-04
115	ENSECAG00000035459	BATF2	basic leucine zipper ATF-like transcription factor 2	1,5740	3,766E-14
116	ENSECAG00000000942	ACKR3	atypical chemokine receptor 3	1,5686	1,812E-03
117	ENSECAG00000008259	MASP1	MBL associated serine protease 1	1,5614	1,489E-02
118	ENSECAG00000020937	KCNA3	potassium voltage-gated channel subfamily A member 3	1,5486	3,184E-02
119	ENSECAG00000017839	ENSECAG00000017839		1,5441	1,102E-07
120	ENSECAG00000029514	SLC2A6	solute carrier family 2 member 6	1,5176	2,093E-07
121	ENSECAG00000016006	CELSR3	cadherin EGF LAG seven-pass G-type receptor 3	1,5029	6,068E-03
122	ENSECAG00000021989	RIGI	RNA sensor RIG-I	1,4998	2,015E-05
123	ENSECAG00000006213	PDK1	pyruvate dehydrogenase kinase 1	1,4851	5,512E-03
124	ENSECAG00000018324	SLC43A2	solute carrier family 43 member 2	1,4590	1,481E-04
125	ENSECAG00000013138	RAB27B	RAB27B, member RAS onco family	1,4380	3,426E-02
126	ENSECAG00000016394	IL1R1	interleukin 1 receptor type 1	1,4349	2,278E-07
127	ENSECAG00000008485	ITGAL	integrin subunit alpha L	1,4296	2,589E-02
128	ENSECAG00000016121	ADAMTS12	ADAM metalloproteinase with thrombospondin type 1 motif 12	1,4259	9,960E-08
129	ENSECAG00000014658	SCD	stearoyl-CoA desaturase	1,4253	7,769E-06
130	ENSECAG00000014969	TP53I3	tumor protein p53 inducible protein 3	1,4181	1,373E-03
131	ENSECAG00000005086	EGLN3	egl-9 family hypoxia inducible factor 3	1,4172	1,190E-07
132	ENSECAG00000001249	SOCS3	suppressor of cytokine signaling 3	1,4128	9,749E-10
133	ENSECAG00000021670	GGT5	gamma-glutamyltransferase 5	1,4059	3,597E-04
134	ENSECAG00000010128	SEMA4D	semaphorin 4D	1,3921	2,726E-02
135	ENSECAG00000024719	ENSECAG00000024719		1,3839	2,058E-03
136	ENSECAG00000035238	IFITM5	interferon induced transmembrane protein 5	1,3811	1,814E-03
137	ENSECAG00000019061	ADAMTS3	ADAM metalloproteinase with thrombospondin type 1 motif 3	1,3757	1,540E-06
138	ENSECAG00000017426	FGF11	fibroblast growth factor 11	1,3735	4,473E-04
139	ENSECAG00000053139	ENSECAG00000053139		1,3595	6,935E-03
140	ENSECAG00000018133	VSIR	V-set immunoregulatory receptor	1,3583	9,321E-04
141	ENSECAG00000017324	ENSECAG00000017324		1,3540	2,715E-04
142	ENSECAG00000004433	ENSECAG00000004433		1,3491	1,278E-02
143	ENSECAG00000016217	RNF213	ring finger protein 213	1,3400	1,199E-05
144	ENSECAG00000012331	PARP9	poly(ADP-ribose) polymerase family member 9	1,3385	5,354E-06
145	ENSECAG00000008852	ABCA1	ATP binding cassette subfamily A member 1	1,3350	5,657E-06
146	ENSECAG00000021964	ASB9	ankyrin repeat and SOCS box containing 9	1,3298	3,937E-03

147	ENSECAG00000024429	IRF9	interferon regulatory factor 9	1,3029	2,873E-04
148	ENSECAG00000009402	VEGFA	vascular endothelial growth factor A	1,2972	1,602E-07
149	ENSECAG00000028144	TXNIP	thioredoxin interacting protein	1,2961	1,916E-04
150	ENSECAG00000006554	PRRG4	proline rich and Gla domain 4	1,2880	5,797E-10
151	ENSECAG00000015128	CAPG	capping actin protein, gelsolin like	1,2873	1,053E-06
152	ENSECAG00000003345	ENO1	enolase 1	1,2836	7,205E-08
153	ENSECAG00000008906	PARP15	poly(ADP-ribose) polymerase family member 15	1,2829	2,570E-02
154	ENSECAG00000008287	SLC39A14	solute carrier family 39 member 14	1,2814	1,603E-04
155	ENSECAG00000013435	OAS1	2'-5'-oligoadenylate synthetase 1	1,2753	4,829E-04
156	ENSECAG00000000968	ENSECAG00000000968		1,2664	3,087E-02
157	ENSECAG00000010558	PTGFR	prostaglandin F receptor	1,2583	2,060E-05
158	ENSECAG00000004617	PML	PML nuclear body scaffold	1,2580	3,466E-08
159	ENSECAG00000019809	CGAS	cyclic GMP-AMP synthase	1,2495	8,063E-06
160	ENSECAG00000010206	SMTNL1	smoothelin like 1	1,2397	9,512E-03
161	ENSECAG00000016615	NID1	nidogen 1	1,2332	4,334E-10
162	ENSECAG000000032131	UBE2L6	ubiquitin conjugating enzyme E2 L6	1,2293	2,190E-08
163	ENSECAG00000021220	GBP1	guanylate binding protein 1	1,2076	8,214E-05
164	ENSECAG00000013124	BCL3	BCL3 transcription coactivator	1,2012	2,872E-03
165	ENSECAG00000024006	NSMCE1	NSE1 homolog, SMC5-SMC6 complex component	1,1977	2,562E-02
166	ENSECAG00000017104	ERRFI1	ERBB receptor feedback inhibitor 1	1,1890	2,483E-03
167	ENSECAG00000019115	IL13RA1	interleukin 13 receptor subunit alpha 1	1,1861	1,296E-07
168	ENSECAG00000011496	NFKBIZ	NFKB inhibitor zeta	1,1749	1,217E-03
169	ENSECAG00000017483	PSD3	pleckstrin and Sec7 domain containing 3	1,1688	8,950E-05
170	ENSECAG00000041760	ENSECAG00000041760		1,1677	2,457E-02
171	ENSECAG00000037655	GDNF	glial cell derived neurotrophic factor	1,1640	2,221E-02
172	ENSECAG00000009686	DTX3L	deltex E3 ubiquitin ligase 3L	1,1638	4,335E-06
173	ENSECAG00000011037	WARS1	tryptophanyl-tRNA synthetase 1	1,1606	1,142E-05
174	ENSECAG00000023077	SNAP25	synaptosome associated protein 25	1,1603	2,178E-02
175	ENSECAG00000015822	VDR	vitamin D receptor	1,1521	5,251E-05
176	ENSECAG00000011641	TVP23A	trans-golgi network vesicle A	1,1372	2,702E-03
177	ENSECAG00000039673	ENSECAG00000039673		1,1366	3,624E-03
178	ENSECAG00000008186	SP100	SP100 nuclear antigen	1,1346	1,725E-03
179	ENSECAG00000011726	EIF2AK2	eukaryotic translation initiation factor 2 alpha kinase 2	1,1346	1,168E-04
180	ENSECAG00000017842	ABCB1	ATP binding cassette subfamily B member 1	1,1286	6,702E-03
181	ENSECAG00000020669	LDHA	lactate dehydrogenase A	1,1225	2,340E-03
182	ENSECAG00000004523	TIFA	TRAF interacting protein with forkhead associated domain	1,1172	4,181E-02
183	ENSECAG00000022842	PLOD1	procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	1,1030	3,631E-06
184	ENSECAG00000039033	MTARC1	mitochondrial amidoxime reducing component 1	1,1020	2,221E-02

185	ENSECAG00000017458	BNIP3	BCL2 interacting protein 3	1,1001	2,035E-02
186	ENSECAG00000021836	OGFR	opioid growth factor receptor	1,0967	4,545E-04
187	ENSECAG00000001399	SAMD9	sterile alpha motif domain containing 9	1,0957	2,040E-03
188	ENSECAG00000009949	P2RX7	purinergic receptor P2X 7	1,0939	1,630E-03
189	ENSECAG00000018584	LAMA4	laminin subunit alpha 4	1,0937	2,063E-06
190	ENSECAG00000019391	TNFSF10	TNF superfamily member 10	1,0934	2,643E-02
191	ENSECAG00000017558	MLKL	mixed lineage kinase domain like pseudokinase	1,0846	2,702E-03
192	ENSECAG00000018176	PDGFRA	platelet derived growth factor receptor alpha	1,0831	8,710E-04
193	ENSECAG00000009787	SNAI1	snail family transcriptional repressor 1	1,0773	1,028E-03
194	ENSECAG00000010233	WDR25	WD repeat domain 25	1,0756	2,554E-03
195	ENSECAG00000021206	HAS2	hyaluronan synthase 2	1,0717	4,924E-02
196	ENSECAG00000010531	F2RL2	coagulation factor II thrombin receptor like 2	1,0705	1,137E-04
197	ENSECAG00000033034	RAB20	RAB20, member RAS oncogene family	1,0606	1,738E-02
198	ENSECAG00000003462	RUNX1	RUNX family transcription factor 1	1,0586	2,131E-04
199	ENSECAG00000016972	SMPD2	sphingomyelin phosphodiesterase 2	1,0559	2,869E-02
200	ENSECAG00000022326	STAT2	signal transducer and activator of transcription 2	1,0450	2,190E-08
201	ENSECAG00000012484	NAMPT	nicotinamide phosphoribosyltransferase	1,0373	3,170E-05
202	ENSECAG00000036510	ENSECAG00000036510		1,0358	3,455E-02
203	ENSECAG00000023118	MCC	MCC regulator of WNT signaling pathway	1,0344	2,702E-03
204	ENSECAG00000022050	HVCN1	hydrogen voltage gated channel 1	1,0300	1,420E-02
205	ENSECAG00000050186	ENSECAG00000050186		1,0291	2,268E-02
206	ENSECAG00000009543	EPSTI1	epithelial stromal interaction 1	1,0215	1,056E-02
207	ENSECAG00000022051	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	1,0160	2,660E-07
208	ENSECAG00000003310	LY6E	lymphocyte antigen 6 family member E	1,0069	9,200E-05
209	ENSECAG00000019371	SLC1A3	solute carrier family 1 member 3	1,0041	4,834E-02
210	ENSECAG00000013771	PDE4D	phosphodiesterase 4D	1,0032	4,128E-03
211	ENSECAG00000013982	TUBA8	tubulin alpha 8	1,0023	2,689E-03
212	ENSECAG00000049917	CHML	CHM like Rab escort protein	-1,0052	5,119E-03
213	ENSECAG00000024742	PFN2	profilin 2	-1,0143	1,217E-03
214	ENSECAG00000012520	SRD5A1	steroid 5 alpha-reductase 1	-1,0164	3,257E-02
215	ENSECAG00000046549	ENSECAG00000046549		-1,0209	3,715E-02
216	ENSECAG00000014513	TNFSF15	TNF superfamily member 15	-1,0220	4,952E-04
217	ENSECAG00000054559	LRRC8C	leucine rich repeat containing 8 VRAC subunit C	-1,0233	1,186E-02
218	ENSECAG00000030803	ARMCX4	armadillo repeat containing X-linked 4	-1,0249	3,680E-02
219	ENSECAG00000032483	GPR85	G protein-coupled receptor 85	-1,0399	1,209E-02
220	ENSECAG00000020093	NPR3	natriuretic peptide receptor 3	-1,0508	4,382E-02
221	ENSECAG00000013233	FAT4	FAT atypical cadherin 4	-1,0511	4,798E-05
222	ENSECAG00000015915	ABCC13	ATP binding cassette subfamily C member 13	-1,0555	4,075E-03

223	ENSECAG00000023825	EXOC6	exocyst complex component 6	-1,0560	3,048E-02
224	ENSECAG00000019007	THRB	thyroid hormone receptor beta	-1,0577	1,286E-03
225	ENSECAG00000013108	C1H1orf198	chromosome 1 C1orf198 homolog	-1,0637	1,604E-02
226	ENSECAG00000029421	HSPA2	heat shock protein family A (Hsp70) member 2	-1,0653	3,920E-02
227	ENSECAG00000024113	ABLIM1	actin binding LIM protein 1	-1,0662	3,260E-02
228	ENSECAG00000015468	MGAT4A	alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase A	-1,0731	2,387E-02
229	ENSECAG00000000593	PTN	pleiotrophin	-1,0815	4,022E-02
230	ENSECAG00000015608	CRIM1	cysteine rich transmembrane BMP regulator 1	-1,0821	5,193E-03
231	ENSECAG00000005884	CRYL1	crystallin lambda 1	-1,0846	4,564E-02
232	ENSECAG00000025107	COBLL1	cordon-bleu WH2 repeat protein like 1	-1,0858	4,034E-25
233	ENSECAG00000014174	TOM1L1	target of myb1 like 1 membrane trafficking protein	-1,0869	6,366E-03
234	ENSECAG00000020705	DUSP8	dual specificity phosphatase 8	-1,0915	3,056E-04
235	ENSECAG00000017048	CD101	CD101 molecule	-1,1032	1,913E-02
236	ENSECAG00000013677	FRY	FRY microtubule binding protein	-1,1033	5,867E-05
237	ENSECAG00000010297	FYB2	FYN binding protein 2	-1,1154	3,257E-02
238	ENSECAG00000000259	RTKN2	rhotekin 2	-1,1236	1,215E-02
239	ENSECAG00000037857	CAV2	caveolin 2	-1,1245	4,782E-04
240	ENSECAG00000015772	GALNT17	polypeptide N-acetylgalactosaminyltransferase 17	-1,1314	3,820E-02
241	ENSECAG00000015180	NEXN	nexilin F-actin binding protein	-1,1353	4,919E-03
242	ENSECAG00000017905	LTBP4	latent transforming growth factor beta binding protein 4	-1,1441	2,284E-04
243	ENSECAG00000020944	SLC25A4	solute carrier family 25 member 4	-1,1678	4,880E-04
244	ENSECAG00000020200	DPYSL3	dihydropyrimidinase like 3	-1,1859	5,891E-05
245	ENSECAG00000032702	ENSECAG00000032702		-1,1861	8,162E-03
246	ENSECAG00000030788	DIO2	iodothyronine deiodinase 2	-1,1863	1,784E-04
247	ENSECAG00000010747	SH3BGR	SH3 domain binding glutamate rich protein	-1,1870	8,283E-03
248	ENSECAG00000020201	GCNT1	glucosaminyl (N-acetyl) transferase 1	-1,1887	1,716E-03
249	ENSECAG00000004587	ANKRD34B	ankyrin repeat domain 34B	-1,2027	1,604E-02
250	ENSECAG00000008793	PGBD1	piggyBac transposable element derived 1	-1,2034	2,090E-02
251	ENSECAG00000009288	ICA1L	islet cell autoantigen 1 like	-1,2158	1,778E-08
252	ENSECAG00000018631	LTBP2	latent transforming growth factor beta binding protein 2	-1,2166	8,668E-03
253	ENSECAG00000016917	TES	testin LIM domain protein	-1,2201	7,096E-03
254	ENSECAG00000014287	OMD	osteomodulin	-1,2225	1,532E-02
255	ENSECAG00000020596	HMGB3	high mobility group box 3	-1,2344	8,274E-05
256	ENSECAG00000009134	CAV1	caveolin 1	-1,2413	1,007E-06
257	ENSECAG00000014757	MFS13A	major facilitator superfamily domain containing 13A	-1,2416	3,401E-06
258	ENSECAG00000021497	EMP1	epithelial membrane protein 1	-1,2416	1,588E-02
259	ENSECAG00000003230	AKAP5	A-kinase anchoring protein 5	-1,2425	1,629E-02

260	ENSECAG00000006524	FANK1	fibronectin type III and ankyrin repeat domains 1	-1,2628	1,843E-03
261	ENSECAG00000011671	TGFB1	transforming growth factor beta 1	-1,2637	2,283E-04
262	ENSECAG00000012116	CARNS1	carnosine synthase 1	-1,2698	4,862E-02
263	ENSECAG00000021665	SGMS2	sphingomyelin synthase 2	-1,2782	1,230E-03
264	ENSECAG00000016126	RELL1	RELT like 1	-1,2807	1,209E-02
265	ENSECAG00000007385	NIBAN1	niban apoptosis regulator 1	-1,2860	2,228E-02
266	ENSECAG00000021184	LAT	linker for activation of T cells	-1,2862	3,056E-04
267	ENSECAG00000000196	MMP15	matrix metalloproteinase 15	-1,3075	5,721E-04
268	ENSECAG00000020437	BCL2	BCL2 apoptosis regulator	-1,3132	4,780E-04
269	ENSECAG00000013814	HECTD2	HECT domain E3 ubiquitin protein ligase 2	-1,3179	5,146E-03
270	ENSECAG00000023607	DOCK5	dedicator of cytokinesis 5	-1,3399	4,952E-04
271	ENSECAG00000025178	PMP22	peripheral myelin protein 22	-1,3429	9,551E-12
272	ENSECAG00000002874	LRRN1	leucine rich repeat neuronal 1	-1,3522	2,303E-02
273	ENSECAG00000036646	FGF19	fibroblast growth factor 19	-1,3559	2,891E-02
274	ENSECAG00000005525	GJA1	gap junction protein alpha 1	-1,3650	1,156E-08
275	ENSECAG00000006848	SLC27A6	solute carrier family 27 member 6	-1,3663	1,400E-04
276	ENSECAG00000017059	OTULINL	OTU deubiquitinase with linear linkage specificity like	-1,3813	1,075E-03
277	ENSECAG00000016360	PDE3A	phosphodiesterase 3A	-1,3830	4,499E-04
278	ENSECAG00000022054	CLSTN2	calsynenin 2	-1,3946	1,018E-03
279	ENSECAG00000028067	NPTXR	neuronal pentraxin receptor	-1,3987	1,887E-02
280	ENSECAG00000039591	ZNF469	zinc finger protein 469	-1,4059	4,758E-07
281	ENSECAG00000038375	NEBL	nebulin	-1,4121	4,435E-03
282	ENSECAG00000004050	SLC8A1	solute carrier family 8 member A1	-1,4298	1,294E-04
283	ENSECAG00000000487	SYNM	synemin	-1,4410	7,947E-09
284	ENSECAG00000025017	KHK	ketohexokinase	-1,4422	3,498E-02
285	ENSECAG00000016661	NR4A1	nuclear receptor subfamily 4 group A member 1	-1,4494	1,320E-02
286	ENSECAG00000016339	ADAMTS1	ADAM metalloproteinase with thrombospondin type 1 motif 1	-1,4509	4,287E-03
287	ENSECAG00000009164	PPARGC1A	PPARG coactivator 1 alpha	-1,4533	6,297E-04
288	ENSECAG00000018069	ARAP2	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2	-1,4683	2,209E-02
289	ENSECAG00000014891	NRCAM	neuronal cell adhesion molecule	-1,4769	1,991E-03
290	ENSECAG00000023089	SLAIN1	SLAIN motif family member 1	-1,4790	8,528E-03
291	ENSECAG00000022874	SCG5	secretogranin V	-1,4817	3,233E-02
292	ENSECAG00000020112	LZTS1	leucine zipper tumor suppressor 1	-1,4919	1,407E-03
293	ENSECAG00000018803	PGM2L1	phosphoglucomutase 2 like 1	-1,5067	2,584E-04
294	ENSECAG00000029885	CHST7	carbohydrate sulfotransferase 7	-1,5320	6,246E-03
295	ENSECAG00000002579	MATN2	matrilin 2	-1,5394	5,850E-03
296	ENSECAG00000009940	NRBP2	nuclear receptor binding protein 2	-1,5467	2,278E-07
297	ENSECAG00000036059	NALF1	NALCN channel auxiliary factor 1	-1,5519	4,971E-02
298	ENSECAG00000012011	FBN2	fibrillin 2	-1,5556	2,072E-03
299	ENSECAG00000031983	MTUS1	microtubule associated scaffold protein 1	-1,5729	6,119E-06
300	ENSECAG00000013817	CYP1A1	cytochrome P450 family 1 subfamily A member 1	-1,5823	2,200E-02

301	ENSECAG00000010939	ANGPTL1	angiopoietin like 1	-1,5838	1,179E-04
302	ENSECAG00000012066	FBLN5	fibulin 5	-1,5886	1,860E-07
303	ENSECAG00000004384	RTN4RL1	reticulon 4 receptor like 1	-1,6058	5,499E-03
304	ENSECAG00000018280	TACC2	transforming acidic coiled-coil containing protein 2	-1,6083	1,669E-16
305	ENSECAG00000017407	MOXD1	monooxygenase DBH like 1	-1,6094	4,670E-04
306	ENSECAG00000028572	ENSECAG00000028572		-1,6364	2,025E-02
307	ENSECAG00000054414	PLA1A	phospholipase A1 member A	-1,6429	8,625E-03
308	ENSECAG00000013651	TRIM29	tripartite motif containing 29	-1,6462	3,276E-02
309	ENSECAG00000008652	KREMEN1	kringle containing transmembrane protein 1	-1,6572	2,562E-02
310	ENSECAG00000041924	COL23A1	collagen type XXIII alpha 1 chain	-1,6728	1,825E-02
311	ENSECAG00000017802	OGN	osteoglycin	-1,6835	1,726E-02
312	ENSECAG00000000324	RASEF	RAS and EF-hand domain containing	-1,7120	3,548E-02
313	ENSECAG00000017701	CELSR2	cadherin EGF LAG seven-pass G-type receptor 2	-1,7220	2,228E-02
314	ENSECAG00000022407	SLIT2	slit guidance ligand 2	-1,7257	4,659E-02
315	ENSECAG00000015566	NSG1	neuronal vesicle trafficking associated 1	-1,7263	1,847E-02
316	ENSECAG00000009244	PAX8	paired box 8	-1,7272	4,834E-02
317	ENSECAG00000020494	B3GALT2	beta-1,3-galactosyltransferase 2	-1,7419	3,614E-02
318	ENSECAG00000023163	CXADR	CXADR Ig-like cell adhesion molecule	-1,7636	9,325E-04
319	ENSECAG00000007718	TMOD1	tropomodulin 1	-1,7710	8,452E-07
320	ENSECAG00000006674	RRAD	RRAD, Ras related glycolysis inhibitor and calcium channel regulator	-1,7788	9,844E-03
321	ENSECAG00000009623	OLFML2B	olfactomedin like 2B	-1,7851	1,808E-03
322	ENSECAG00000032860	ENSECAG00000032860		-1,7867	3,629E-04
323	ENSECAG00000002935	KCNF1	potassium voltage-gated channel modifier subfamily F member 1	-1,8090	2,964E-03
324	ENSECAG00000020618	C2H8orf58	chromosome 2 C8orf58 homolog	-1,8133	3,354E-02
325	ENSECAG00000008946	ENSECAG00000008946		-1,8278	1,412E-02
326	ENSECAG00000015134	PELI2	pellino E3 ubiquitin protein ligase family member 2	-1,8515	2,051E-04
327	ENSECAG00000033567	ENSECAG00000033567		-1,8844	2,258E-04
328	ENSECAG00000019691	LTBP1	latent transforming growth factor beta binding protein 1	-1,8872	6,430E-07
329	ENSECAG00000012993	JAG1	jagged canonical Notch ligand 1	-1,8986	2,872E-04
330	ENSECAG00000009311	SH3TC2	SH3 domain and tetratricopeptide repeats 2	-1,9107	9,296E-03
331	ENSECAG00000007658	MGLL	monoglyceride lipase	-1,9257	8,157E-04
332	ENSECAG00000009779	SORCS2	sortilin related VPS10 domain containing receptor 2	-1,9328	7,393E-03
333	ENSECAG00000022741	CDC42EP3	CDC42 effector protein 3	-1,9539	1,501E-06
334	ENSECAG00000003067	PWWP3B	PWWP domain containing 3B	-1,9655	1,183E-02
335	ENSECAG00000022441	ESR1	estrogen receptor 1	-1,9881	2,173E-05
336	ENSECAG00000057017	ABCA8	ATP binding cassette subfamily A member 8	-1,9976	1,798E-02
337	ENSECAG00000047700	ENSECAG00000047700		-1,9984	4,633E-02

338	ENSECAG00000003193	BAMBI	BMP and activin membrane bound inhibitor	-2,0178	3,870E-08
339	ENSECAG000000036081	SOBP	sine oculis binding protein homolog	-2,0696	1,092E-03
340	ENSECAG000000010262	PERP	p53 apoptosis effector related to PMP22	-2,0861	4,499E-02
341	ENSECAG000000013781	CRACD	capping protein inhibiting regulator of actin dynamics	-2,0988	9,600E-19
342	ENSECAG000000020932	SVIL	supervillin	-2,1215	2,579E-07
343	ENSECAG000000013428	ID4	inhibitor of DNA binding 4	-2,1331	3,525E-02
344	ENSECAG000000016015	PCDH19	protocadherin 19	-2,1761	1,121E-06
345	ENSECAG000000013278	HES7	hes family bHLH transcription factor 7	-2,1809	3,292E-03
346	ENSECAG000000015840	PODN	podocan	-2,2374	1,080E-05
347	ENSECAG000000012115	SEMA3D	semaphorin 3D	-2,2446	9,600E-19
348	ENSECAG000000023960	KLHL30	kelch like family member 30	-2,2622	1,407E-07
349	ENSECAG000000044796	GREM1	gremlin 1, DAN family BMP antagonist	-2,2625	9,883E-03
350	ENSECAG000000024016	IL13RA2	interleukin 13 receptor subunit alpha 2	-2,2855	3,262E-04
351	ENSECAG000000014947	OPN3	opsin 3	-2,2958	3,781E-02
352	ENSECAG000000001834	DGKI	diacylglycerol kinase iota	-2,2995	7,451E-03
353	ENSECAG000000028496	ENSECAG00000028496		-2,3048	1,328E-02
354	ENSECAG000000013723	SLC7A8	solute carrier family 7 member 8	-2,3185	1,513E-02
355	ENSECAG000000018340	USP43	ubiquitin specific peptidase 43	-2,3281	1,235E-04
356	ENSECAG000000008518	NPNT	nephronectin	-2,3483	4,933E-02
357	ENSECAG000000018771	PKNOX2	PBX/knotted 1 homeobox 2	-2,3571	1,286E-03
358	ENSECAG000000006980	FEZ1	fasciculation and elongation protein zeta 1	-2,3669	1,004E-03
359	ENSECAG000000013088	CDKL2	cyclin dependent kinase like 2	-2,3930	4,548E-02
360	ENSECAG000000038712	TNXB	tenascin XB	-2,3967	8,758E-07
361	ENSECAG000000004725	XIRP1	xin actin binding repeat containing 1	-2,4571	9,098E-03
362	ENSECAG000000004502	WFDC1	WAP four-disulfide core domain 1	-2,4706	9,374E-12
363	ENSECAG000000034852	COL4A5	collagen type IV alpha 5 chain	-2,4966	2,186E-02
364	ENSECAG000000026951	ARVCF	ARVCF delta catenin family member	-2,5279	2,932E-03
365	ENSECAG000000015834	DMD	dystrophin	-2,5285	5,543E-03
366	ENSECAG000000011887	ADAMTSL2	ADAMTS like 2	-2,5345	2,715E-04
367	ENSECAG000000010725	STXBP5L	syntaxin binding protein 5L	-2,5348	3,631E-06
368	ENSECAG000000016251	ENSECAG000000016251		-2,5493	3,429E-02
369	ENSECAG000000007878	FRMD3	FERM domain containing 3	-2,5879	1,564E-03
370	ENSECAG000000000239	FNDC1	fibronectin type III domain containing 1	-2,5994	4,966E-02
371	ENSECAG000000019060	ESYT3	extended synaptotagmin 3	-2,6146	1,458E-02
372	ENSECAG000000015341	KMO	kynurenine 3-monooxygenase	-2,6472	1,141E-02
373	ENSECAG000000018511	SORBS1	sorbin and SH3 domain containing 1	-2,7045	1,320E-04
374	ENSECAG000000021360	KCNMB1	potassium calcium-activated channel subfamily M regulatory beta subunit 1	-2,7136	6,874E-04
375	ENSECAG000000012750	SLC6A6	solute carrier family 6 member 6	-2,7540	1,271E-04

376	ENSECAG00000006846	GFRA1	GDNF family receptor alpha 1	-2,7823	9,483E-03
377	ENSECAG00000037214	TUBB4A	tubulin beta 4A class IVa	-2,7826	9,200E-05
378	ENSECAG00000014164	ADAMTS8	ADAM metalloproteinase with thrombospondin type 1 motif 8	-2,9008	2,317E-11
379	ENSECAG00000021691	RHOV	ras homolog family member V	-2,9061	3,108E-02
380	ENSECAG00000023161	IGSF10	immunoglobulin superfamily member 10	-3,0847	6,612E-05
381	ENSECAG00000022234	CSDC2	cold shock domain containing C2	-3,1000	2,035E-02
382	ENSECAG00000010667	ERG	ETS transcription factor ERG	-3,1802	2,213E-02
383	ENSECAG00000015669	RAB3B	RAB3B, member RAS onco family	-3,1863	8,950E-05
384	ENSECAG00000041748	COL4A3	collagen type IV alpha 3 chain	-3,2141	3,361E-02
385	ENSECAG00000034421	GJB2	gap junction protein beta 2	-3,2441	1,749E-02
386	ENSECAG00000024570	BRINP2	BMP/retinoic acid inducible neural specific 2	-3,3467	9,844E-03
387	ENSECAG00000023104	ITGA11	integrin subunit alpha 11	-3,3888	4,955E-03
388	ENSECAG00000006269	SHISAL1	shisa like 1	-3,5008	2,990E-02
389	ENSECAG00000023039	CCN3	cellular communication network factor 3	-3,6300	1,298E-03
390	ENSECAG00000009607	PTCHD1	patched domain containing 1	-3,6520	8,324E-03
391	ENSECAG00000011264	SYTL5	synaptotagmin like 5	-3,7151	3,800E-03
392	ENSECAG00000024951	SERPINB7	serpin family B member 7	-3,8183	2,096E-03
393	ENSECAG00000006057	ODAM	odontogenic, ameloblast associated	-3,9013	2,609E-03
394	ENSECAG00000015583	TCN1	transcobalamin 1	-4,1073	4,834E-02
395	ENSECAG00000016407	KCNB2	potassium voltage-gated channel subfamily B member 2	-4,2236	1,227E-02
396	ENSECAG00000032656	OR9I18	olfactory receptor family 9 subfamily I member 18	-4,2485	3,589E-02
397	ENSECAG00000011293	PTPRZ1	protein tyrosine phosphatase receptor type Z1	-4,2956	5,402E-04
398	ENSECAG00000041218	LPAR4	lysophosphatidic acid receptor 4	-4,3054	1,448E-02
399	ENSECAG00000007047	ASPN	asporin	-4,3964	1,503E-04
400	ENSECAG00000009483	PRLR	prolactin receptor	-4,4339	1,830E-02
401	ENSECAG00000018308	GREB1	growth regulating estrogen receptor binding 1	-4,8052	1,075E-03
402	ENSECAG00000000740	C10H6orf58	chromosome 10 C6orf58 homolog	-5,7053	7,205E-08
403	ENSECAG00000036634	CCDC3	coiled-coil domain containing 3	-6,3417	3,292E-03
404	ENSECAG00000010109	IGF1	insulin like growth factor 1	-6,7785	1,612E-06

Supplementary Table 4. The full list of differentially expressed genes (DEGs) identified in endometrial fibroblasts after treatment with eqMD-MΦ^{IL-4/IL-13}-CM for 96 hours.

No.	Ensemble gene ID	Gene symbol	Gene name	log2FC	Padj
1	ENSECAG00000046132	CXCL9	C-X-C motif chemokine ligand 9	5,88124	7,240E-04
2	ENSECAG00000008392	SLC8A2	solute carrier family 8 member A2	5,44297	1,579E-06
3	ENSECAG00000014645	OASL	2'-5'-oligoadenylate synthetase-like	4,43477	2,686E-05
4	ENSECAG00000017917	CYBB	cytochrome b-245 beta chain	4,34464	5,781E-04
5	ENSECAG00000014650	INSRR	insulin receptor related receptor	3,98385	2,025E-03
6	ENSECAG00000029465	CHI3L1	chitinase 3 like 1	3,87743	7,613E-12
7	ENSECAG00000019551	CXCL12	C-X-C motif chemokine ligand 12	3,70819	4,109E-04
8	ENSECAG00000008059	PDE4C	phosphodiesterase 4C	3,56716	6,009E-04
9	ENSECAG00000015449	IRF8	interferon regulatory factor 8	3,43586	4,158E-13
10	ENSECAG00000004709	C2	complement C2	3,31009	3,311E-04
11	ENSECAG00000052431	ENSECAG00000052431		3,18686	1,535E-03
12	ENSECAG00000015034	PLAC8B	placenta associated 8 B	3,14835	4,044E-03
13	ENSECAG00000046493	GBP5	guanylate binding protein 5	3,01522	1,388E-06
14	ENSECAG00000017185	ZBP1	Z-DNA binding protein 1	2,94570	6,395E-06
15	ENSECAG00000024959	FBXO39	F-box protein 39	2,92192	6,374E-04
16	ENSECAG00000006205	CD7	CD7 molecule	2,84490	6,433E-04
17	ENSECAG00000022188	WNT11	Wnt family member 11	2,77624	1,232E-04
18	ENSECAG00000033385	LGALS3BP	galectin 3 binding protein	2,65603	1,289E-05
19	ENSECAG00000008615	ENSECAG00000008615		2,64306	8,066E-03
20	ENSECAG00000014218	SIGLEC1	sialic acid binding Ig like lectin 1	2,54939	1,416E-11
21	ENSECAG00000019758	TNFSF13B	TNF superfamily member 13b	2,53625	6,212E-06
22	ENSECAG00000010963	SERPING1	serpin family G member 1	2,52959	6,137E-05
23	ENSECAG00000051569	ENSECAG00000051569		2,47822	6,853E-03
24	ENSECAG00000055535	ENSECAG00000055535		2,44766	4,801E-06
25	ENSECAG00000010153	IFIT3	interferon induced protein with tetratricopeptide repeats 3	2,33786	6,135E-03
26	ENSECAG00000027808	PLCXD1	phosphatidylinositol specific phospholipase C X domain containing 1	2,31565	2,803E-03
27	ENSECAG00000009744	IRF7	interferon regulatory factor 7	2,29878	9,245E-03
28	ENSECAG00000001466	PLEKHB1	pleckstrin homology domain containing B1	2,29720	6,942E-04
29	ENSECAG00000048862	ENSECAG00000048862		2,29497	3,022E-02
30	ENSECAG00000024961	XAF1	XIAP associated factor 1	2,28274	1,143E-03
31	ENSECAG00000015333	ADAP2	ArfGAP with dual PH domains 2	2,26846	3,492E-02
32	ENSECAG00000035279	IGFBP2	insulin like growth factor binding protein 2	2,24628	5,093E-27
33	ENSECAG00000013138	RAB27B	RAB27B, member RAS onco family	2,18310	2,920E-04
34	ENSECAG00000033890	CIDEA	cell death inducing DFFA like effector c	2,17016	3,508E-02
35	ENSECAG00000019329	MPP3	MAGUK p55 scaffold protein 3	2,13515	1,191E-02

36	ENSECAG00000013037	ALDOC	aldolase, fructose-bisphosphate C	2,08793	4,661E-10
37	ENSECAG00000008763	LYPD3	LY6/PLAUR domain containing 3	2,05344	2,072E-02
38	ENSECAG00000003563	BST2	bone marrow stromal cell antigen 2	2,03792	6,374E-04
39	ENSECAG00000020326	CLSTN3	calsyntenin 3	2,00855	7,658E-09
40	ENSECAG00000014802	KCND1	potassium voltage-gated channel subfamily D member 1	1,99360	1,698E-04
41	ENSECAG00000009211	ANGPT4	angiopoietin 4	1,99274	1,049E-06
42	ENSECAG00000010185	IFIT2	interferon induced protein with tetratricopeptide repeats 2	1,98262	1,495E-04
43	ENSECAG00000019411	HERC6	HECT and RLD domain containing E3 ubiquitin protein ligase family member 6	1,97733	6,421E-04
44	ENSECAG00000016312	CD274	CD274 molecule	1,96602	3,345E-03
45	ENSECAG00000001633	FZD10	frizzled class receptor 10	1,93564	2,785E-03
46	ENSECAG000000055770	KCNE4	potassium voltage-gated channel subfamily E regulatory subunit 4	1,92890	3,607E-03
47	ENSECAG00000001064	RET	ret proto-onco	1,90244	2,907E-02
48	ENSECAG00000012773	CMPK2	cytidine/uridine monophosphate kinase 2	1,89375	3,851E-03
49	ENSECAG00000007991	WDR86	WD repeat domain 86	1,87683	3,662E-02
50	ENSECAG00000016578	ENSECAG00000016578		1,87341	3,383E-02
51	ENSECAG00000020294	DCLK3	doublecortin like kinase 3	1,86201	5,328E-04
52	ENSECAG00000019438	OSMR	oncostatin M receptor	1,84973	3,809E-20
53	ENSECAG00000017172	IFI27	interferon, alpha-inducible protein 27	1,84839	1,050E-02
54	ENSECAG00000012514	ENSECAG00000012514		1,82893	1,145E-05
55	ENSECAG00000015395	HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5	1,78465	3,899E-07
56	ENSECAG00000008809	OAS3	2'-5'-oligoadenylate synthetase 3	1,77659	3,516E-04
57	ENSECAG00000045402	ENSECAG00000045402		1,70951	3,311E-04
58	ENSECAG00000021343	NRG1	neuregulin 1	1,69908	4,740E-04
59	ENSECAG00000012791	GBP6	guanylate binding protein family member 6	1,66050	2,066E-06
60	ENSECAG00000018752	APOL6	apolipoprotein L6	1,63902	4,297E-04
61	ENSECAG00000049355	ENSECAG00000049355		1,62249	4,400E-03
62	ENSECAG00000000949	GPR84	G protein-coupled receptor 84	1,60714	4,259E-02
63	ENSECAG00000021429	C1R	complement C1r	1,60351	7,949E-05
64	ENSECAG00000033029	ENSECAG00000033029		1,59872	3,060E-02
65	ENSECAG00000018649	UCP2	uncoupling protein 2	1,58818	1,488E-03
66	ENSECAG00000016394	IL1R1	interleukin 1 receptor type 1	1,58188	9,411E-09
67	ENSECAG00000009519	C1S	complement C1s	1,56721	1,119E-03
68	ENSECAG00000002867	F2RL1	F2R like trypsin receptor 1	1,55709	1,118E-02
69	ENSECAG00000030455	ENSECAG00000030455		1,55555	4,503E-02
70	ENSECAG00000008259	MASP1	MBL associated serine protease 1	1,55359	2,007E-02
71	ENSECAG00000000284	USP18	ubiquitin specific peptidase 18	1,55227	2,584E-02

72	ENSECAG00000030750	ENSECAG00000030750		1,54451	4,704E-02
73	ENSECAG00000000942	ACKR3	atypical chemokine receptor 3	1,53849	3,227E-03
74	ENSECAG00000035238	IFITM5	interferon induced transmembrane protein 5	1,53281	4,872E-04
75	ENSECAG00000001481	SAMD9L	sterile alpha motif domain containing 9 like	1,52913	3,689E-03
76	ENSECAG00000011704	TNFAIP2	TNF alpha induced protein 2	1,52198	1,021E-11
77	ENSECAG00000014422	OAS2	2'-5'-oligoadenylate synthetase 2	1,51824	2,385E-06
78	ENSECAG00000000373	LOXL3	lysyl oxidase like 3	1,49825	1,570E-02
79	ENSECAG00000017839	ENSECAG00000017839		1,47592	7,240E-07
80	ENSECAG00000018332	AK4	adenylate kinase 4	1,46538	1,143E-03
81	ENSECAG00000024719	ENSECAG00000024719		1,45936	1,348E-03
82	ENSECAG00000004572	CH25H	cholesterol 25-hydroxylase	1,45744	4,382E-02
83	ENSECAG00000028144	TXNIP	thioredoxin interacting protein	1,45183	2,227E-05
84	ENSECAG00000000296	LAMC3	laminin subunit gamma 3	1,44489	8,875E-03
85	ENSECAG00000003260	ITIH5	inter-alpha-trypsin inhibitor heavy chain 5	1,41976	7,385E-03
86	ENSECAG00000024062	DDX60L	DEXD/H-box 60 like	1,41252	6,762E-04
87	ENSECAG00000014519	ETV7	ETS variant transcription factor 7	1,38844	5,160E-03
88	ENSECAG00000006213	PDK1	pyruvate dehydrogenase kinase 1	1,38342	1,521E-02
89	ENSECAG00000009402	VEGFA	vascular endothelial growth factor A	1,38134	2,419E-08
90	ENSECAG00000000910	ENSECAG00000000910		1,38030	1,494E-04
91	ENSECAG00000053139	ENSECAG00000053139		1,37894	7,631E-03
92	ENSECAG00000015822	VDR	vitamin D receptor	1,37747	6,650E-07
93	ENSECAG00000009039	STAT1	signal transducer and activator of transcription 1	1,37543	2,458E-08
94	ENSECAG00000007881	IFIH1	interferon induced with helicase C domain 1	1,37227	3,044E-02
95	ENSECAG00000000943	PTGES	prostaglandin E synthase	1,36479	9,821E-03
96	ENSECAG00000018324	SLC43A2	solute carrier family 43 member 2	1,35075	7,634E-04
97	ENSECAG00000016006	CELSR3	cadherin EGF LAG seven-pass G-type receptor 3	1,33352	2,659E-02
98	ENSECAG00000014594	EPAS1	endothelial PAS domain protein 1	1,31651	1,958E-06
99	ENSECAG00000001249	SOCS3	suppressor of cytokine signaling 3	1,29039	5,834E-08
100	ENSECAG00000013124	BCL3	BCL3 transcription coactivator	1,28930	1,493E-03
101	ENSECAG00000014969	TP53I3	tumor protein p53 inducible protein 3	1,27595	7,333E-03
102	ENSECAG00000011669	TNN	tenascin N	1,25802	1,434E-02
103	ENSECAG00000012132	ENSECAG00000012132		1,24611	1,008E-02
104	ENSECAG00000016121	ADAMTS12	ADAM metalloproteinase with thrombospondin type 1 motif 12	1,24611	7,699E-06
105	ENSECAG00000017483	PSD3	pleckstrin and Sec7 domain containing 3	1,24412	2,694E-05
106	ENSECAG00000019932	ENSECAG00000019932		1,22592	4,259E-02
107	ENSECAG00000021670	GGT5	gamma-glutamyltransferase 5	1,22290	4,227E-03
108	ENSECAG00000017426	FGF11	fibroblast growth factor 11	1,19602	4,320E-03

109	ENSECAG00000020769	FGF1	fibroblast growth factor 1	1,19076	3,348E-03
110	ENSECAG00000018176	PDGFRA	platelet derived growth factor receptor alpha	1,18872	2,626E-04
111	ENSECAG00000003599	ENSECAG00000003599		1,17380	5,296E-03
112	ENSECAG00000003345	ENO1	enolase 1	1,15453	2,797E-06
113	ENSECAG00000017842	ABCB1	ATP binding cassette subfamily B member 1	1,15068	6,935E-03
114	ENSECAG00000023077	SNAP25	synaptosome associated protein 25	1,14578	3,068E-02
115	ENSECAG00000007808	DCBLD1	discoidin, CUB and LCCL domain containing 1	1,13462	5,749E-05
116	ENSECAG00000016615	NID1	nidogen 1	1,13316	1,926E-08
117	ENSECAG00000016217	RNF213	ring finger protein 213	1,12921	5,677E-04
118	ENSECAG00000020669	LDHA	lactate dehydrogenase A	1,12676	3,036E-03
119	ENSECAG00000011641	TVP23A	trans-golgi network vesicle A	1,12461	4,158E-03
120	ENSECAG00000012331	PARP9	poly(ADP-ribose) polymerase family member 9	1,12122	3,770E-04
121	ENSECAG00000035459	BATF2	basic leucine zipper ATF-like transcription factor 2	1,11975	7,327E-07
122	ENSECAG00000018133	VSIR	V-set immunoregulatory receptor	1,11464	1,438E-02
123	ENSECAG00000003462	RUNX1	RUNX family transcription factor 1	1,09401	1,584E-04
124	ENSECAG00000021964	ASB9	ankyrin repeat and SOCS box containing 9	1,09317	3,783E-02
125	ENSECAG00000007788	VAMP5	vesicle associated membrane protein 5	1,08819	1,920E-09
126	ENSECAG00000034282	CCL8	chemokine (C-C motif) ligand 8	1,07460	5,358E-04
127	ENSECAG00000004617	PML	PML nuclear body scaffold	1,05308	1,061E-05
128	ENSECAG00000021989	RIGI	RNA sensor RIG-I	1,04664	9,539E-03
129	ENSECAG00000018874	PRICKLE1	prickle planar cell polarity protein 1	1,04078	4,128E-06
130	ENSECAG00000039033	MTARC1	mitochondrial amidoxime reducing component 1	1,04020	4,568E-02
131	ENSECAG00000024429	IRF9	interferon regulatory factor 9	1,03097	1,011E-02
132	ENSECAG00000009400	ARRDC4	arrestin domain containing 4	1,02579	5,535E-07
133	ENSECAG00000017458	BNIP3	BCL2 interacting protein 3	1,01988	4,805E-02
134	ENSECAG00000018584	LAMA4	laminin subunit alpha 4	1,01040	2,108E-05
135	ENSECAG00000017104	ERRF1	ERBB receptor feedback inhibitor 1	1,00740	2,027E-02
136	ENSECAG00000019061	ADAMTS3	ADAM metalloproteinase with thrombospondin type 1 motif 3	1,00045	1,750E-03
137	ENSECAG00000032131	UBE2L6	ubiquitin conjugating enzyme E2 L6	1,00011	1,642E-05
138	ENSECAG00000013108	C1H1orf198	chromosome 1 C1orf198 homolog	- 1,00009	3,463E-02
139	ENSECAG00000037857	CAV2	caveolin 2	- 1,00862	3,144E-03
140	ENSECAG00000020944	SLC25A4	solute carrier family 25 member 4	- 1,01006	4,774E-03
141	ENSECAG00000014174	TOM1L1	target of myb1 like 1 membrane trafficking protein	- 1,01401	1,505E-02
142	ENSECAG00000020705	DUSP8	dual specificity phosphatase 8	- 1,02312	1,013E-03
143	ENSECAG00000015608	CRIM1	cysteine rich transmembrane BMP regulator 1	- 1,02761	1,208E-02
144	ENSECAG00000049917	CHML	CHM like Rab escort protein	- 1,02939	4,981E-03

145	ENSECAG00000003193	BAMBI	BMP and activin membrane bound inhibitor	- 1,03571	9,798E-03
146	ENSECAG00000009011	FLRT2	fibronectin leucine rich transmembrane protein 2	- 1,04212	5,964E-07
147	ENSECAG00000019648	MID1	midline 1	- 1,04447	1,016E-05
148	ENSECAG00000017921	SUSD1	sushi domain containing 1	- 1,04563	5,549E-03
149	ENSECAG00000017905	LTBP4	latent transforming growth factor beta binding protein 4	- 1,05069	1,213E-03
150	ENSECAG00000030788	DIO2	iodothyronine deiodinase 2	- 1,05658	1,564E-03
151	ENSECAG00000006315	XYLT1	xylosyltransferase 1	- 1,05685	1,372E-05
152	ENSECAG00000025178	PMP22	peripheral myelin protein 22	- 1,05881	3,614E-07
153	ENSECAG00000019691	LTBP1	latent transforming growth factor beta binding protein 1	- 1,06367	1,937E-02
154	ENSECAG00000009940	NRBP2	nuclear receptor binding protein 2	- 1,07271	1,023E-03
155	ENSECAG00000046549	ENSECAG00000046549		- 1,07811	3,122E-02
156	ENSECAG00000021665	SGMS2	sphingomyelin synthase 2	- 1,08172	1,173E-02
157	ENSECAG00000010297	FYB2	FYN binding protein 2	- 1,10016	4,549E-02
158	ENSECAG00000015180	NEXN	nexilin F-actin binding protein	- 1,11965	7,569E-03
159	ENSECAG00000016917	TES	testin LIM domain protein	- 1,13009	1,973E-02
160	ENSECAG00000020596	HMGB3	high mobility group box 3	- 1,14831	4,438E-04
161	ENSECAG00000007861	PAPLN	papilin, proteoglycan like sulfated glycoprotein	- 1,14855	1,277E-02
162	ENSECAG00000016126	RELL1	RELT like 1	- 1,16513	3,529E-02
163	ENSECAG00000000487	SYNM	synemin	- 1,16554	8,074E-06
164	ENSECAG00000039591	ZNF469	zinc finger protein 469	- 1,16740	7,818E-05
165	ENSECAG00000019007	THRB	thyroid hormone receptor beta	- 1,17408	4,213E-04
166	ENSECAG00000009637	OCA2	OCA2 melanosomal transmembrane protein	- 1,17602	5,026E-03
167	ENSECAG00000031983	MTUS1	microtubule associated scaffold protein 1	- 1,17888	1,767E-03
168	ENSECAG00000051604	ENSECAG00000051604		- 1,18029	4,804E-04
169	ENSECAG00000004258	IL17RB	interleukin 17 receptor B	- 1,18196	2,954E-02
170	ENSECAG00000038375	NEBL	nebullette	- 1,18702	2,899E-02
171	ENSECAG00000022741	CDC42EP3	CDC42 effector protein 3	- 1,19726	1,201E-02
172	ENSECAG00000020201	GCNT1	glucosaminyl (N-acetyl) transferase 1	- 1,21398	1,719E-03
173	ENSECAG00000005525	GJA1	gap junction protein alpha 1	- 1,21714	8,752E-07
174	ENSECAG00000004587	ANKRD34B	ankyrin repeat domain 34B	- 1,22375	1,748E-02
175	ENSECAG00000024742	PFN2	profilin 2	- 1,22825	5,586E-05
176	ENSECAG00000006848	SLC27A6	solute carrier family 27 member 6	- 1,23783	1,013E-03
177	ENSECAG00000022441	ESR1	estrogen receptor 1	- 1,25919	2,359E-02

178	ENSECAG00000014649	GYS2	glycogen synthase 2	- 1,27792	4,549E-02
179	ENSECAG00000020112	LZTS1	leucine zipper tumor suppressor 1	- 1,29543	9,991E-03
180	ENSECAG00000002579	MATN2	matrilin 2	- 1,31788	2,858E-02
181	ENSECAG00000036646	FGF19	fibroblast growth factor 19	- 1,33002	4,259E-02
182	ENSECAG00000001845	P2RY1	purinergic receptor P2Y1	- 1,36193	2,889E-02
183	ENSECAG00000033567	ENSECAG00000033567		- 1,36494	2,014E-02
184	ENSECAG00000017059	OTULINL	OTU deubiquitinase with linear linkage specificity like	- 1,39083	1,302E-03
185	ENSECAG00000020718	SEPTIN5	septin 5	- 1,39495	1,486E-02
186	ENSECAG00000018631	LTBP2	latent transforming growth factor beta binding protein 2	- 1,39698	2,167E-03
187	ENSECAG00000028562	ENSECAG00000028562		- 1,40531	1,466E-02
188	ENSECAG00000009864	PTPRD	protein tyrosine phosphatase receptor type D	- 1,40641	1,514E-02
189	ENSECAG00000023963	NMNAT2	nicotinamide nucleotide adenylyltransferase 2	- 1,41466	4,842E-02
190	ENSECAG00000010747	SH3BGR	SH3 domain binding glutamate rich protein	- 1,41661	1,251E-03
191	ENSECAG00000036081	SOBP	sine oculis binding protein homolog	- 1,41781	3,158E-02
192	ENSECAG00000006717	ASS1	argininosuccinate synthase 1	- 1,42593	4,210E-02
193	ENSECAG00000012066	FBLN5	fibulin 5	- 1,44279	4,801E-06
194	ENSECAG00000007718	TMOD1	tropomodulin 1	- 1,44699	1,040E-04
195	ENSECAG00000021602	PARM1	prostate androgen-regulated mucin-like protein 1	- 1,45598	2,404E-03
196	ENSECAG00000010967	MMP19	matrix metalloproteinase 19	- 1,46042	9,079E-06
197	ENSECAG00000007658	MGLL	monoglyceride lipase	- 1,46046	2,157E-02
198	ENSECAG00000023089	SLAIN1	SLAIN motif family member 1	- 1,50176	9,523E-03
199	ENSECAG00000021979	CARD10	caspase recruitment domain family member 10	- 1,50664	1,421E-02
200	ENSECAG00000017771	ATP1B1	ATPase Na ⁺ /K ⁺ transporting subunit beta 1	- 1,54273	1,681E-02
201	ENSECAG00000010939	ANGPTL1	angiopoietin like 1	- 1,54854	2,369E-04
202	ENSECAG00000032860	ENSECAG00000032860		- 1,55815	3,254E-03
203	ENSECAG00000008793	PGBD1	piggyBac transposable element derived 1	- 1,58155	2,088E-03
204	ENSECAG00000014513	TNFSF15	TNF superfamily member 15	- 1,58284	5,489E-09
205	ENSECAG00000000460	FAM185A	family with sequence similarity 185 member A	- 1,58725	6,984E-04
206	ENSECAG00000013651	TRIM29	tripartite motif containing 29	- 1,60404	4,704E-02
207	ENSECAG00000007385	NIBAN1	niban apoptosis regulator 1	- 1,61602	2,551E-03
208	ENSECAG00000015840	PODN	podocan	- 1,63144	3,745E-03
209	ENSECAG00000015134	PELI2	pellino E3 ubiquitin protein ligase family member 2	- 1,63423	1,711E-03
210	ENSECAG00000018280	TACC2	transforming acidic coiled-coil containing protein 2	- 1,64392	4,807E-17

211	ENSECAG00000012507	CRYAB	crystallin alpha B	- 1,66552	1,563E-08
212	ENSECAG00000009134	CAV1	caveolin 1	- 1,66681	7,613E-12
213	ENSECAG00000001775	RAB38	RAB38, member RAS onco family	- 1,70969	9,671E-03
214	ENSECAG00000004384	RTN4RL1	reticulon 4 receptor like 1	- 1,71059	3,645E-03
215	ENSECAG00000000196	MMP15	matrix metalloproteinase 15	- 1,73898	2,460E-06
216	ENSECAG00000029885	CHST7	carbohydrate sulfotransferase 7	- 1,75959	1,481E-03
217	ENSECAG00000020932	SVIL	supervillin	- 1,79319	3,048E-05
218	ENSECAG00000028067	NPTXR	neuronal pentraxin receptor	- 1,79657	2,874E-03
219	ENSECAG00000000324	RASEF	RAS and EF-hand domain containing	- 1,80043	3,166E-02
220	ENSECAG00000011467	ALPL	alkaline phosphatase, biomineralization associated	- 1,80102	3,813E-05
221	ENSECAG00000006980	FEZ1	fasciculation and elongation protein zeta 1	- 1,80424	1,681E-02
222	ENSECAG00000006674	RRAD	RRAD, Ras related glycolysis inhibitor and calcium channel regulator	- 1,80491	1,118E-02
223	ENSECAG00000016015	PCDH19	protocadherin 19	- 1,81252	9,680E-05
224	ENSECAG00000004050	SLC8A1	solute carrier family 8 member A1	- 1,82102	7,644E-07
225	ENSECAG00000022874	SCG5	secretogranin V	- 1,84364	5,851E-03
226	ENSECAG00000018511	SORBS1	sorbin and SH3 domain containing 1	- 1,84853	2,389E-02
227	ENSECAG00000012993	JAG1	jagged canonical Notch ligand 1	- 1,85265	6,009E-04
228	ENSECAG00000020437	BCL2	BCL2 apoptosis regulator	- 1,85516	3,262E-07
229	ENSECAG00000015484	ATP6V0D2	ATPase H ⁺ transporting V0 subunit d2	- 1,86868	7,114E-04
230	ENSECAG00000002874	LRRN1	leucine rich repeat neuronal 1	- 1,91463	5,275E-04
231	ENSECAG00000012115	SEMA3D	semaphorin 3D	- 1,94256	7,805E-15
232	ENSECAG00000028572	ENSECAG00000028572		- 1,96810	5,087E-03
233	ENSECAG00000010725	STXBP5L	syntaxin binding protein 5L	- 2,02930	1,510E-04
234	ENSECAG00000009164	PPARGC1A	PPARG coactivator 1 alpha	- 2,03678	1,513E-06
235	ENSECAG00000001834	DGKI	diacylglycerol kinase iota	- 2,06652	2,155E-02
236	ENSECAG00000021360	KCNMB1	potassium calcium-activated channel subfamily M regulatory beta subunit 1	- 2,09406	1,427E-02
237	ENSECAG00000012750	SLC6A6	solute carrier family 6 member 6	- 2,10571	4,278E-03
238	ENSECAG00000002935	KCNF1	potassium voltage-gated channel modifier subfamily F member 1	- 2,11907	5,892E-04
239	ENSECAG00000013781	CRACD	capping protein inhibiting regulator of actin dynamics	- 2,14529	2,640E-19
240	ENSECAG00000003776	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	- 2,17753	4,075E-02
241	ENSECAG00000018340	USP43	ubiquitin specific peptidase 43	- 2,18138	4,253E-04

242	ENSECAG00000026951	ARVCF	ARVCF delta catenin family member	- 2,19011	1,118E-02
243	ENSECAG00000022407	SLIT2	slit guidance ligand 2	- 2,20146	7,248E-03
244	ENSECAG00000054414	PLA1A	phospholipase A1 member A	- 2,21446	2,733E-04
245	ENSECAG00000000278	FCAMR	Fc alpha and mu receptor	- 2,21555	1,331E-02
246	ENSECAG00000028496	ENSECAG00000028496		- 2,22993	2,214E-02
247	ENSECAG00000022054	CLSTN2	calsyntenin 2	- 2,23126	1,092E-08
248	ENSECAG00000023960	KLHL30	kelch like family member 30	- 2,27869	1,559E-07
249	ENSECAG00000011264	SYTL5	synaptotagmin like 5	- 2,31616	2,541E-02
250	ENSECAG00000004502	WFDC1	WAP four-disulfide core domain 1	- 2,37250	8,858E-11
251	ENSECAG00000024870	CWH43	cell wall biosis 43 C-terminal homolog	- 2,37881	9,798E-03
252	ENSECAG00000038712	TNXB	tenascin XB	- 2,40778	1,056E-06
253	ENSECAG00000004778	RTN4R	reticulon 4 receptor	- 2,48702	6,619E-03
254	ENSECAG00000018771	PKNOX2	PBX/knotted 1 homeobox 2	- 2,48715	8,204E-04
255	ENSECAG00000037214	TUBB4A	tubulin beta 4A class IVa	- 2,51024	4,636E-04
256	ENSECAG00000022890	NEFL	neurofilament light chain	- 2,58538	3,511E-02
257	ENSECAG00000008518	NPNT	nephronectin	- 2,60055	3,133E-02
258	ENSECAG00000009118	OCLN	occludin	- 2,61133	4,877E-02
259	ENSECAG00000007878	FRMD3	FERM domain containing 3	- 2,63220	1,704E-03
260	ENSECAG00000015669	RAB3B	RAB3B, member RAS onco family	- 2,66901	1,057E-03
261	ENSECAG00000013817	CYP1A1	cytochrome P450 family 1 subfamily A member 1	- 2,78203	7,312E-06
262	ENSECAG00000018178	EPDR1	ependymin related 1	- 2,82561	1,670E-02
263	ENSECAG00000021162	CDH13	cadherin 13	- 2,90961	4,805E-02
264	ENSECAG00000023161	IGSF10	immunoglobulin superfamily member 10	- 2,96218	1,380E-04
265	ENSECAG00000044796	GREM1	gremlin 1, DAN family BMP antagonist	- 3,00098	3,386E-04
266	ENSECAG00000024951	SERPINF7	serpin family B member 7	- 3,02076	1,803E-02
267	ENSECAG00000024016	IL13RA2	interleukin 13 receptor subunit alpha 2	- 3,15020	8,137E-07
268	ENSECAG00000007047	ASPN	asporin	- 3,15621	1,709E-02
269	ENSECAG00000024570	BRINP2	BMP/retinoic acid inducible neural specific 2	- 3,26007	1,640E-02
270	ENSECAG00000053848	KRT16	keratin 16	- 3,28622	4,074E-02
271	ENSECAG00000016251	ENSECAG00000016251		- 3,49205	5,041E-03
272	ENSECAG00000014164	ADAMTS8	ADAM metalloproteinase with thrombospondin type 1 motif 8	- 3,52694	4,905E-15
273	ENSECAG00000015695	PTPRO	protein tyrosine phosphatase receptor type O	- 3,55423	8,537E-03
274	ENSECAG00000018308	GREB1	growth regulating estrogen receptor binding 1	- 3,64657	2,917E-02

275	ENSECAG00000010109	IGF1	insulin like growth factor 1	- 3,77363	6,207E-05
276	ENSECAG00000010667	ERG	ETS transcription factor ERG	- 4,05812	1,118E-02
277	ENSECAG00000015341	KMO	kynurenine 3-monooxygenase	- 4,05851	2,487E-03
278	ENSECAG00000021691	RHOV	ras homolog family member V	- 4,27924	1,312E-02
279	ENSECAG00000011293	PTPRZ1	protein tyrosine phosphatase receptor type Z1	- 4,29523	7,079E-04
280	ENSECAG00000006057	ODAM	odontogenic, ameloblast associated	- 4,43361	1,056E-03
281	ENSECAG00000000740	C10H6orf58	chromosome 10 C6orf58 homolog	- 4,93449	2,175E-07
282	ENSECAG00000036634	CCDC3	coiled-coil domain containing 3	- 5,66242	1,542E-02

Supplementary Table 5. Statistical analysis of RNA sequencing reads in mare endometrium 6 hours after intrauterine administration of autologous eqMD-M Φ ^{IFN γ /LPS} and eqMD-M Φ ^{IL-4/IL-13}

Index	Sample name	Raw reads	Filtered reads	Uniquely mapped reads	% uniquely mapped reads
1.	mare_1_control	14 853 628	582 785	14 270 843	96,08
2.	mare_2_control	16 045 100	683 969	15 361 131	95,74
3.	mare_3_control	17 710 088	710 939	16 999 149	95,99
4.	mare_1_ M Φ (IFN γ /LPS)	16 761 164	694 523	16 066 641	95,86
5.	mare_2_ M Φ (IFN γ /LPS)	14 913 183	659 437	14 253 746	95,58
6.	mare_3_ M Φ (IFN γ /LPS)	14 660 037	600 810	14 059 227	95,9
7.	mare_4_control	18 618 915	938 312	17 680 603	94,96
8.	mare_5_control	19 227 903	975 199	18 252 704	94,93
9.	mare_6_control	15 401 384	747 304	14 654 080	95,15
10.	mare_4_ M Φ (IL-4/IL-13)	18 453 580	910 445	17 543 135	95,07
11.	mare_5_ M Φ (IL-4/IL-13)	14 679 991	744 920	13 935 071	94,93
12.	mare_6_ M Φ (IL-4/IL-13)	17 957 037	859 544	17 097 493	95,21
mean		16 606 834	759 016	15 847 819	95,45

Supplementary Table 6. The full list of differentially expressed genes (DEGs) identified in mare endometrium 6 hours after intrauterine administration of autologous eqMD-MΦ^{IFN γ /LPS}

No.	Ensemble gene ID	Gene symbol	Gene name	log2FC	Padj
1	ENSECAG00000034028	SPINK7	serine peptidase inhibitor Kazal type 7	8,3910	1,895E-04
2	ENSECAG00000023696	ENSECAG00000023696		8,3525	1,147E-06
3	ENSECAG00000015205	CALR4	calreticulin 4	6,4919	4,240E-02
4	ENSECAG00000031486	ENSECAG00000031486		6,0257	9,709E-03
5	ENSECAG00000007514	LRRC74A	leucine rich repeat containing 74A	5,4134	3,071E-02
6	ENSECAG00000031280	ENSECAG00000031280		4,9576	6,520E-08
7	ENSECAG00000021128	SLC6A20	solute carrier family 6 member 20	4,6296	5,983E-07
8	ENSECAG00000024782	HYAL4	hyaluronidase 4	4,5231	1,875E-02
9	ENSECAG00000008347	ENSECAG00000008347		4,4937	1,886E-02
10	ENSECAG00000027864	IL1RN	interleukin 1 receptor antagonist	4,4917	4,240E-02
11	ENSECAG00000020957	LCN2	lipocalin 2	4,3236	2,872E-02
12	ENSECAG00000030554	LIF	LIF interleukin 6 family cytokine	4,2298	4,509E-02
13	ENSECAG00000009752	APOA1	apolipoprotein A1	4,1317	3,305E-04
14	ENSECAG00000018390	FFAR4	free fatty acid receptor 4	3,8798	4,802E-02
15	ENSECAG00000017615	CDH17	cadherin 17	3,7507	1,094E-02
16	ENSECAG00000060192	ENSECAG00000060192		3,6927	1,841E-03
17	ENSECAG00000008154	PAQR5	progesterin and adipoQ receptor family member 5	3,5855	1,087E-02
18	ENSECAG00000021856	PADI2	peptidyl arginine deiminase 2	3,5047	1,177E-02
19	ENSECAG00000016610	CCN2	cellular communication network factor 2	3,4100	2,716E-02
20	ENSECAG00000022404	TRPV6	transient receptor potential cation channel subfamily V member 6	3,3838	3,921E-06
21	ENSECAG00000022912	ENSECAG00000022912		3,3721	1,532E-04
22	ENSECAG00000024110	PENK	proenkephalin	3,3547	4,351E-03
23	ENSECAG00000018939	SDCBP2	syndecan binding protein 2	3,2997	2,608E-02
24	ENSECAG00000009125	UPK3A	uroplakin 3A	3,2842	1,801E-02
25	ENSECAG00000041368	COL22A1	collagen type XXII alpha 1 chain	3,1433	3,034E-02
26	ENSECAG00000031539	ZNF697	zinc finger protein 697	3,1336	1,841E-03
27	ENSECAG00000016773	NABP1	nucleic acid binding protein 1	3,1170	2,432E-02
28	ENSECAG00000038352	ENSECAG00000038352		3,1101	1,421E-03
29	ENSECAG00000000132	MAOB	monoamine oxidase B	2,9663	1,875E-02
30	ENSECAG00000013486	C4BPA	complement component 4 binding protein alpha	2,8478	1,895E-04
31	ENSECAG00000012113	ACP5	acid phosphatase 5, tartrate resistant	2,8121	1,230E-04
32	ENSECAG00000006002	CATSPERB	cation channel sperm associated auxiliary subunit beta	2,6683	3,920E-02
33	ENSECAG00000008834	SULT1C4	sulfotransferase family 1C member 4	2,6337	1,900E-03
34	ENSECAG00000000758	CAPN11	calpain 11	2,5231	4,351E-03

35	ENSECAG00000043283	AGR2	anterior gradient 2, protein disulphide isomerase family member	2,2723	1,841E-03
36	ENSECAG00000020411	ENSECAG00000020411		2,2580	3,896E-02
37	ENSECAG00000001076	ENSECAG00000001076		2,1106	1,844E-02
38	ENSECAG00000007305	TC2N	tandem C2 domains, nuclear	2,0678	4,115E-02
39	ENSECAG00000010178	ITGB4	integrin subunit beta 4	2,0402	4,351E-03
40	ENSECAG00000053054	ENSECAG00000053054		1,9655	1,875E-02
41	ENSECAG00000030024	MAPK13	mitogen-activated protein kinase 13	1,9621	2,214E-02
42	ENSECAG00000042952	GPX3	glutathione peroxidase 3	1,8936	2,395E-02
43	ENSECAG00000029287	HOPX	HOP homeobox	1,8512	4,351E-03
44	ENSECAG00000007629	DNAJC1	DnaJ heat shock protein family (Hsp40) member C1	1,8511	4,825E-05
45	ENSECAG00000023764	GRK5	G protein-coupled receptor kinase 5	1,8282	3,165E-03
46	ENSECAG00000010188	ABHD3	abhydrolase domain containing 3, phospholipase	1,7823	8,810E-03
47	ENSECAG00000037336	ENSECAG00000037336		1,7630	3,086E-02
48	ENSECAG00000013482	SERPINA3	serpin family A member 3	1,7378	4,115E-02
49	ENSECAG00000058962	VNN1		1,7228	1,554E-02
50	ENSECAG00000051919	ENSECAG00000051919		1,6755	3,075E-02
51	ENSECAG00000007244	LRP8	LDL receptor related protein 8	1,6220	1,844E-02
52	ENSECAG00000022803	GSTK1	glutathione S-transferase kappa 1	1,6160	8,805E-03
53	ENSECAG00000011008	MCOLN2	mucolipin TRP cation channel 2	1,6129	1,065E-02
54	ENSECAG00000054716	ENSECAG00000054716		1,6074	2,414E-02
55	ENSECAG00000012362	C7	complement C7	1,5831	1,209E-04
56	ENSECAG00000018479	PREX2	phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor 2	1,5816	2,594E-02
57	ENSECAG00000019231	XKRX	XK related X-linked	1,5683	4,351E-03
58	ENSECAG00000008891	NCDN	neurochondrin	1,5614	1,602E-08
59	ENSECAG00000003704	TMEM139	transmembrane protein 139	1,4974	4,115E-02
60	ENSECAG00000020022	INSIG1	insulin induced 1	1,4611	1,844E-02
61	ENSECAG00000009400	ARRDC4	arrestin domain containing 4	1,4458	1,209E-04
62	ENSECAG00000013869	CFI	complement factor I	1,4314	2,432E-02
63	ENSECAG00000015854	HNF1B	HNF1 homeobox B	1,4083	2,188E-02
64	ENSECAG00000018670	ALCAM	activated leukocyte cell adhesion molecule	1,3921	1,065E-02
65	ENSECAG00000006989	SELENOW	selenoprotein W	1,3722	4,363E-02
66	ENSECAG00000026969	RIMKLB	ribosomal modification protein rimK like family member B	1,3559	4,536E-03
67	ENSECAG00000010909	TNS2	tensin 2	1,3149	4,563E-03
68	ENSECAG00000019217	HM13	histocompatibility minor 13	1,2405	4,240E-02
69	ENSECAG00000010601	FKBP2	FKBP prolyl isomerase 2	1,2172	2,864E-02
70	ENSECAG00000009164	PPARGC1A	PPARG coactivator 1 alpha	1,1996	4,240E-02
71	ENSECAG00000013765	MTMR10	myotubularin related protein 10	1,1494	7,623E-03
72	ENSECAG00000014891	NRCAM	neuronal cell adhesion molecule	1,1453	2,432E-02
73	ENSECAG00000012830	CHN2	chimerin 2	1,1228	2,214E-02

74	ENSECAG00000012045	WLS	Wnt ligand secretion mediator	1,1025	4,651E-02
75	ENSECAG00000018046	GRN	granulin	1,0978	3,214E-03
76	ENSECAG00000009940	NRBP2	nuclear receptor binding protein 2	1,0622	4,904E-02
77	ENSECAG00000013630	SULF2	sulfatase 2	1,0447	3,998E-02
78	ENSECAG00000000703	OXSM	3-oxoacyl-ACP synthase, mitochondrial	1,0405	1,844E-02
79	ENSECAG00000019631	MCM2	minichromosome maintenance complex component 2	-1,0693	2,401E-02
80	ENSECAG00000010768	LRBA	LPS responsive beige-like anchor protein	-1,1605	2,432E-02
81	ENSECAG00000024743	NEO1	neogenin 1	-1,1984	3,071E-02
82	ENSECAG00000013822	KIF5C	kinesin family member 5C	-1,3793	3,021E-02
83	ENSECAG00000003278	TRIM9	tripartite motif containing 9	-1,4103	1,639E-02
84	ENSECAG00000043845	ENSECAG0000 0043845		-1,4262	2,432E-02
85	ENSECAG00000013363	DACT2	dishevelled binding antagonist of beta catenin 2	-1,4652	9,730E-04
86	ENSECAG00000025133	SYDE2	synapse defective Rho GTPase homolog 2	-1,5054	3,071E-02
87	ENSECAG00000007176	ELOVL7	ELOVL fatty acid elongase 7	-1,5243	5,983E-07
88	ENSECAG00000016284	SLCO5A1	solute carrier organic anion transporter family member 5A1	-1,8029	1,827E-02
89	ENSECAG00000017647	RAD51	RAD51 recombinase	-1,8412	2,214E-02
90	ENSECAG00000001472	ENSECAG0000 0001472		-1,9542	3,967E-04
91	ENSECAG00000024196	KIFC1	kinesin family member C1	-2,0438	3,149E-02
92	ENSECAG00000003837	CXCR4	C-X-C motif chemokine receptor 4	-2,0666	4,578E-03
93	ENSECAG00000011226	CENPQ	centromere protein Q	-2,1847	2,432E-02
94	ENSECAG00000020983	CCDC196	coiled-coil domain containing 196	-2,1903	1,217E-02
95	ENSECAG00000006581	STPG1	sperm tail PG-rich repeat containing 1	-2,3749	2,401E-02
96	ENSECAG00000015475	RBBP8NL	RBBP8 N-terminal like	-2,4816	1,844E-02
97	ENSECAG00000055431	ENSECAG0000 0055431		-2,5298	1,421E-03
98	ENSECAG00000006607	KIF11	kinesin family member 11	-2,5779	4,131E-02
99	ENSECAG00000013053	TOP2A	DNA topoisomerase II alpha	-2,5942	1,360E-02
100	ENSECAG00000010286	CHRM3	cholinergic receptor muscarinic 3	-2,5968	4,550E-03
101	ENSECAG00000017894	CKAP2	cytoskeleton associated protein 2	-2,6001	2,188E-02
102	ENSECAG00000013238	FAM83D	family with sequence similarity 83 member D	-2,6921	4,363E-02
103	ENSECAG00000015208	SYP	synaptophysin	-2,6985	3,750E-02
104	ENSECAG00000049939	ENSECAG0000 0049939		-2,7700	4,536E-03
105	ENSECAG00000013100	PI15	peptidase inhibitor 15	-2,8514	1,875E-02
106	ENSECAG00000026997	CLDN10	claudin 10	-2,9316	2,618E-03
107	ENSECAG00000030145	ENSECAG0000 0030145		-2,9412	3,896E-02
108	ENSECAG00000031827	ENSECAG0000 0031827		-2,9548	2,216E-02
109	ENSECAG00000010821	RACGAP1	Rac GTPase activating protein 1	-2,9916	4,052E-02
110	ENSECAG00000020698	MELK	maternal embryonic leucine zipper kinase	-3,1092	1,875E-02
111	ENSECAG00000014536	KIF23	kinesin family member 23	-3,1367	3,638E-02

112	ENSECAG00000000278	FCAMR	Fc alpha and mu receptor	-3,1526	2,401E-02
113	ENSECAG00000012742	CXCL6	C-X-C motif chemokine ligand 6	-3,2100	1,442E-02
114	ENSECAG00000020111	IQGAP3	IQ motif containing GTPase activating protein 3	-3,3841	3,411E-02
115	ENSECAG00000058067	KCNJ10	potassium inwardly rectifying channel subfamily J member 10	-3,4193	3,479E-02
116	ENSECAG00000018134	STMN2	stathmin 2	-3,4896	2,200E-02
117	ENSECAG00000016382	ANLN	anillin, actin binding protein	-3,5113	3,302E-02
118	ENSECAG00000024127	CENPF	centromere protein F	-3,6401	1,900E-03
119	ENSECAG00000031808	SAPCD2	suppressor APC domain containing 2	-3,7119	3,638E-02
120	ENSECAG00000015806	CCNB1	cyclin B1	-3,7162	2,432E-02
121	ENSECAG00000021452	BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B	-3,7702	1,844E-02
122	ENSECAG00000010053	HJURP	Holliday junction recognition protein	-3,9895	5,250E-03
123	ENSECAG00000055118	ENSECAG00000055118		-4,1817	2,919E-03
124	ENSECAG00000007620	DEPDC1B	DEP domain containing 1B	-4,4186	2,709E-02
125	ENSECAG00000026891	ENSECAG00000026891		-4,5553	3,479E-02
126	ENSECAG00000017502	PCDH9	protocadherin 9	-5,0838	4,131E-02
127	ENSECAG00000008461	DEPDC1	DEP domain containing 1	-5,3416	2,872E-02

Supplementary Table 7. The full list of differentially expressed genes (DEGs) identified in mare endometrium 6 hours after intrauterine administration of autologous eqMD-MΦ^{IL-4/IL-13}

No.	Ensemble gene ID	Gene symbol	Gene name	log2FC	Padj
1	ENSECAG00000007839	HEPACAM	hepatic and glial cell adhesion molecule	7,061	2,967E-10
2	ENSECAG00000007210	CTSL	cathepsin L	5,152	3,811E-02
3	ENSECAG00000057527	ENSECAG00000057527		4,847	4,249E-03
4	ENSECAG00000000435	LRP2	LDL receptor related protein 2	4,005	8,143E-05
5	ENSECAG00000023252	ADGRG2	adhesion G protein-coupled receptor G2	3,982	5,055E-05
6	ENSECAG00000020912	DTNA	dystrobrevin alpha	3,690	2,126E-02
7	ENSECAG00000016537	IL12RB2	interleukin 12 receptor subunit beta 2	3,510	7,163E-05
8	ENSECAG00000012212	LRP1B	LDL receptor related protein 1B	3,351	6,889E-05
9	ENSECAG00000014649	GYS2	glycogen synthase 2	3,255	1,818E-02
10	ENSECAG00000017358	COLGALT2	collagen beta(1-O)galactosyltransferase 2	3,177	2,181E-03
11	ENSECAG00000017357	DPP4	dipeptidyl peptidase 4	3,121	1,169E-03
12	ENSECAG00000013884	CACNB2	calcium voltage-gated channel auxiliary subunit beta 2	3,089	2,545E-03
13	ENSECAG00000009463	CCDC15	coiled-coil domain containing 15	3,075	5,055E-05
14	ENSECAG00000014708	PTH1H	parathyroid hormone like hormone	3,071	2,494E-02
15	ENSECAG00000024927	ANO5	anoctamin 5	2,997	1,142E-03
16	ENSECAG00000038375	NEBL	nebulin	2,957	5,811E-06
17	ENSECAG00000006671	TMPRSS11F	transmembrane serine protease 11F	2,949	2,372E-02
18	ENSECAG00000006860	F5	coagulation factor V	2,874	3,477E-02
19	ENSECAG00000000896	ALDH3B2	aldehyde dehydrogenase 3 family member B2	2,864	3,400E-02
20	ENSECAG00000024701	AOX4	aldehyde oxidase 4	2,860	1,753E-02
21	ENSECAG00000019335	TLCD4	TLC domain containing 4	2,814	2,020E-02
22	ENSECAG00000017238	ENSECAG00000017238		2,777	2,181E-03
23	ENSECAG00000011304	FKBP5	FKBP prolyl isomerase 5	2,728	3,581E-03
24	ENSECAG00000015915	ABCC13	ATP binding cassette subfamily C member 13	2,710	2,584E-03
25	ENSECAG00000006613	FBXO45	F-box protein 45	2,441	1,393E-03
26	ENSECAG00000024183	SERPINB11	serpin family B member 11	2,437	3,205E-02
27	ENSECAG00000017897	FAM167A	family with sequence similarity 167 member A	2,398	1,271E-02
28	ENSECAG00000008185	KL	klotho	2,398	3,446E-03
29	ENSECAG00000001545	TSPAN12	tetraspanin 12	2,364	1,253E-02
30	ENSECAG00000033324	ENSECAG00000033324		2,344	3,125E-02
31	ENSECAG00000011663	NPTX1	neuronal pentraxin 1	2,340	4,981E-02
32	ENSECAG00000018188	KYNU	kynureninase	2,311	1,078E-02
33	ENSECAG00000008862	ABHD15	abhydrolase domain containing 15	2,293	1,523E-03
34	ENSECAG00000004372	SLC22A3	solute carrier family 22 member 3	2,256	1,253E-02
35	ENSECAG00000050552	ENSECAG00000050552		2,241	3,504E-02
36	ENSECAG00000013456	PRPSAP1	phosphoribosyl pyrophosphate synthetase associated protein 1	2,194	3,827E-03

37	ENSECAG00000000152	KITLG	KIT ligand	2,192	4,176E-02
38	ENSECAG00000039385	ENSECAG00000039385		2,179	3,557E-02
39	ENSECAG00000024677	SIDT1	SID1 transmembrane family member 1	2,159	4,438E-03
40	ENSECAG00000038024	MMP26	matrix metalloproteinase 26	2,099	4,250E-02
41	ENSECAG00000024797	PDE3B	phosphodiesterase 3B	2,081	3,011E-02
42	ENSECAG00000015137	ENSECAG00000015137		2,045	3,423E-02
43	ENSECAG00000013422	SLC12A2	solute carrier family 12 member 2	2,037	4,103E-02
44	ENSECAG00000013029	CADM2	cell adhesion molecule 2	2,033	2,430E-03
45	ENSECAG00000011986	NAPEPLD	N-acyl phosphatidylethanolamine phospholipase D	1,974	1,753E-02
46	ENSECAG00000021329	CHRNA5	cholinergic receptor nicotinic alpha 5 subunit	1,972	5,273E-05
47	ENSECAG00000023870	ACSS1	acyl-CoA synthetase short chain family member 1	1,959	2,151E-02
48	ENSECAG00000018059	SC5D	sterol-C5-desaturase	1,946	3,799E-02
49	ENSECAG00000000284	USP18	ubiquitin specific peptidase 18	1,923	1,276E-02
50	ENSECAG00000028964	ENSECAG00000028964		1,920	9,865E-08
51	ENSECAG00000002251	TLL1	tolloid like 1	1,911	7,298E-03
52	ENSECAG00000039846	ENSECAG00000039846		1,821	1,492E-02
53	ENSECAG00000010445	ST3GAL6	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	1,812	2,324E-02
54	ENSECAG00000036783	SVIP	small VCP interacting protein	1,799	3,485E-02
55	ENSECAG00000017481	SLCO1A2	solute carrier organic anion transporter family member 1A2	1,781	1,654E-02
56	ENSECAG00000020378	NQO1	NAD(P)H quinone dehydrogenase 1	1,774	3,100E-02
57	ENSECAG00000007979	PLG	plasminogen	1,760	1,191E-02
58	ENSECAG00000011657	HEXB	hexosaminidase subunit beta	1,749	1,161E-02
59	ENSECAG00000011008	MCOLN2	mucolipin TRP cation channel 2	1,741	2,181E-03
60	ENSECAG00000004928	EXO5	exonuclease 5	1,701	3,581E-03
61	ENSECAG00000019398	TFPI2	tissue factor pathway inhibitor 2	1,681	3,952E-02
62	ENSECAG00000023642	SPHK1	sphingosine kinase 1	1,654	3,504E-02
63	ENSECAG00000021092	KLF15	KLF transcription factor 15	1,629	1,314E-02
64	ENSECAG00000015394	SLC25A35	solute carrier family 25 member 35	1,619	1,732E-02
65	ENSECAG00000053825	ENSECAG00000053825		1,617	4,196E-02
66	ENSECAG00000012693	LONRF1	LON peptidase N-terminal domain and ring finger 1	1,609	1,142E-03
67	ENSECAG00000016183	STARD4	StAR related lipid transfer domain containing 4	1,599	4,304E-02
68	ENSECAG00000019233	ELMO1	engulfment and cell motility 1	1,584	9,331E-03
69	ENSECAG00000016667	ENSECAG00000016667		1,563	2,387E-03
70	ENSECAG00000030092	ENSECAG00000030092		1,545	1,753E-02
71	ENSECAG00000011249	AP1S2	adaptor related protein complex 1 subunit sigma 2	1,533	3,504E-02
72	ENSECAG00000018846	NUDCD2	NudC domain containing 2	1,502	3,841E-02
73	ENSECAG00000010558	PTGFR	prostaglandin F receptor	1,482	3,557E-02

74	ENSECAG00000016117	ASB13	ankyrin repeat and SOCS box containing 13	1,477	4,309E-02
75	ENSECAG00000009507	ENSECAG00000009507		1,473	4,649E-02
76	ENSECAG00000021803	MFS4B	major facilitator superfamily domain containing 4B	1,468	3,127E-02
77	ENSECAG00000014879	CBFB	core-binding factor subunit beta	1,439	2,879E-02
78	ENSECAG00000005590	FAM118B	family with sequence similarity 118 member B	1,432	4,747E-02
79	ENSECAG00000049917	CHML	CHM like Rab escort protein	1,419	2,991E-02
80	ENSECAG00000026844	UTP23	UTP23 small subunit processome component	1,398	3,781E-05
81	ENSECAG00000022445	SNX16	sorting nexin 16	1,391	2,376E-03
82	ENSECAG00000008214	MCCC1	methylcrotonyl-CoA carboxylase subunit 1	1,386	4,785E-02
83	ENSECAG00000033399	TSC22D3	TSC22 domain family member 3	1,382	2,181E-03
84	ENSECAG00000014107	C18H2orf69	chromosome 18 C2orf69 homolog	1,370	1,881E-03
85	ENSECAG00000014013	GOLT1B	golgi transport 1B	1,367	2,539E-02
86	ENSECAG00000055802	ABRAXAS1	abraxas 1, BRCA1 A complex subunit	1,344	4,882E-02
87	ENSECAG00000017783	FST	folliculin	1,333	3,917E-02
88	ENSECAG00000033381	ZBTB18	zinc finger and BTB domain containing 18	1,304	4,170E-03
89	ENSECAG00000011111	FMNL2	formin like 2	1,298	4,785E-02
90	ENSECAG00000012033	THAP5	THAP domain containing 5	1,284	5,811E-06
91	ENSECAG00000004353	FAHD1	fumarylacetoacetate hydrolase domain containing 1	1,280	1,755E-03
92	ENSECAG00000013680	TLE1	TLE family member 1, transcriptional corepressor	1,280	2,150E-02
93	ENSECAG00000004874	EPM2AIP1	EPM2A interacting protein 1	1,265	2,102E-02
94	ENSECAG00000024188	ANKRD49	ankyrin repeat domain 49	1,257	2,830E-03
95	ENSECAG00000059644	ENSECAG00000059644		1,256	4,347E-02
96	ENSECAG00000010269	NIPAL2	NIPA like domain containing 2	1,249	6,678E-03
97	ENSECAG00000016315	SERINC5	serine incorporator 5	1,247	3,078E-02
98	ENSECAG00000020022	INSIG1	insulin induced 1	1,235	3,234E-02
99	ENSECAG00000017105	SEC62	SEC62 homolog, preprotein translocation factor	1,228	6,823E-03
100	ENSECAG00000011527	CASD1	CAS1 domain containing 1	1,218	1,406E-02
101	ENSECAG00000017800	GRAMD2B	GRAM domain containing 2B	1,215	4,170E-03
102	ENSECAG00000000971	SUCO	SUN domain containing ossification factor	1,214	1,755E-03
103	ENSECAG00000014891	NRCAM	neuronal cell adhesion molecule	1,213	7,507E-03
104	ENSECAG00000021247	DET1	DET1 partner of COP1 E3 ubiquitin ligase	1,204	2,542E-02
105	ENSECAG00000015720	HPS5	HPS5 biosynthesis of lysosomal organelles complex 2 subunit 2	1,203	2,184E-02
106	ENSECAG00000021484	CEMIP2	cell migration inducing hyaluronidase 2	1,196	4,309E-02
107	ENSECAG00000017205	CCPG1	cell cycle progression 1	1,196	2,173E-02
108	ENSECAG00000005959	GPR160	G protein-coupled receptor 160	1,184	2,425E-03
109	ENSECAG00000010584	DYNC1L1	dynein cytoplasmic 1 light intermediate chain 1	1,181	1,752E-03
110	ENSECAG00000010554	MSMO1	methylsterol monooxygenase 1	1,177	3,423E-02
111	ENSECAG00000013632	RSPRY1	ring finger and SPRY domain containing 1	1,168	3,245E-02

112	ENSECAG00000010414	PON2	paraoxonase 2	1,163	1,732E-02
113	ENSECAG00000012045	WLS	Wnt ligand secretion mediator	1,162	1,458E-02
114	ENSECAG00000016813	ENSECAG00000016813		1,162	4,404E-02
115	ENSECAG00000019968	SLC16A2	solute carrier family 16 member 2	1,161	2,879E-02
116	ENSECAG00000015574	NFIA	nuclear factor I A	1,154	1,753E-02
117	ENSECAG00000005770	BNIP3L	BCL2 interacting protein 3 like	1,154	3,477E-02
118	ENSECAG00000019332	AVL9	AVL9 cell migration associated	1,149	1,454E-02
119	ENSECAG00000017379	CYP27C1	cytochrome P450 family 27 subfamily C member 1	1,140	1,604E-02
120	ENSECAG00000020856	EIF2AK3	eukaryotic translation initiation factor 2 alpha kinase 3	1,136	2,233E-02
121	ENSECAG00000003495	MFHAS1	multifunctional ROCO family signaling regulator 1	1,134	1,383E-02
122	ENSECAG00000006243	RANBP6	RAN binding protein 6	1,116	1,955E-03
123	ENSECAG00000011235	AK3	adenylate kinase 3	1,116	4,836E-02
124	ENSECAG00000000703	OXSM	3-oxoacyl-ACP synthase, mitochondrial	1,109	5,828E-03
125	ENSECAG00000009214	SACM1L	SAC1 like phosphatidylinositol phosphatase	1,104	3,072E-02
126	ENSECAG00000026810	FAM133B	family with sequence similarity 133 member B	1,103	1,605E-02
127	ENSECAG00000012925	NAA30	N-alpha-acetyltransferase 30, NatC catalytic subunit	1,094	2,433E-02
128	ENSECAG00000014420	TRNT1	tRNA nucleotidyl transferase 1	1,093	3,586E-02
129	ENSECAG00000016417	TBC1D4	TBC1 domain family member 4	1,092	1,732E-02
130	ENSECAG00000008822	FASTKD2	FAST kinase domains 2	1,090	4,909E-02
131	ENSECAG00000006458	TAF13	TATA-box binding protein associated factor 13	1,080	1,439E-02
132	ENSECAG00000009339	THNSL1	threonine synthase like 1	1,075	3,345E-02
133	ENSECAG00000008173	HSD17B11	hydroxysteroid 17-beta dehydrogenase 11	1,053	2,484E-02
134	ENSECAG00000003237	GCC2	GRIP and coiled-coil domain containing 2	1,044	2,324E-02
135	ENSECAG00000018199	KCNN3	potassium calcium-activated channel subfamily N member 3	1,043	1,271E-02
136	ENSECAG00000007686	RP1	RP1 axonemal microtubule associated	1,041	7,444E-03
137	ENSECAG00000022265	SMC6	structural maintenance of chromosomes 6	1,040	2,533E-02
138	ENSECAG00000020948	PANK3	pantothenate kinase 3	1,036	2,567E-02
139	ENSECAG00000024125	DPY19L4	dpy-19 like 4	1,034	1,649E-02
140	ENSECAG00000022647	CHMP4C	charged multivesicular body protein 4C	1,032	2,181E-03
141	ENSECAG00000006925	WDR53	WD repeat domain 53	1,013	3,373E-02
142	ENSECAG00000000194	ZMYM5	zinc finger MYM-type containing 5	1,009	2,425E-03
143	ENSECAG00000023644	PPAT	phosphoribosyl pyrophosphate amidotransferase	1,007	2,209E-02
144	ENSECAG00000009719	EBAG9	estrogen receptor binding site associated antigen 9	1,002	3,715E-02
145	ENSECAG00000017546	EXD3	exonuclease 3'-5' domain containing 3	-1,019	4,347E-02
146	ENSECAG00000040695	ENSECAG00000040695		-1,044	3,245E-02
147	ENSECAG00000012582	PLCD4	phospholipase C delta 4	-1,047	4,404E-02

148	ENSECAG00000002855	FCGRT	Fc gamma receptor and transporter	-1,049	1,604E-02
149	ENSECAG00000003278	TRIM9	tripartite motif containing 9	-1,059	1,032E-02
150	ENSECAG00000009044	PRDM1	PR/SET domain 1	-1,082	2,996E-02
151	ENSECAG00000011019	KCNAB3	potassium voltage-gated channel subfamily A regulatory beta subunit 3	-1,118	3,237E-02
152	ENSECAG00000016215	PARP6	poly(ADP-ribose) polymerase family member 6	-1,129	6,823E-03
153	ENSECAG00000024743	NEO1	neogenin 1	-1,141	1,959E-02
154	ENSECAG00000019766	EML1	EMAP like 1	-1,144	4,050E-02
155	ENSECAG00000021331	NOTCH3	notch receptor 3	-1,171	3,373E-02
156	ENSECAG00000029352	FNDC10	fibronectin type III domain containing 10	-1,172	4,786E-02
157	ENSECAG00000036248	ENSECAG00000036248		-1,182	8,841E-03
158	ENSECAG00000000864	DNAAF8	dynein axonemal assembly factor 8	-1,189	3,504E-02
159	ENSECAG00000010090	TMC8	transmembrane channel like 8	-1,200	4,849E-02
160	ENSECAG00000038328	ENSECAG00000038328		-1,201	2,098E-02
161	ENSECAG00000021605	LURAP1	leucine rich adaptor protein 1	-1,201	3,623E-02
162	ENSECAG00000001634	INSYN1	inhibitory synaptic factor 1	-1,204	4,225E-02
163	ENSECAG00000024719	ENSECAG00000024719		-1,221	4,077E-02
164	ENSECAG00000045036	HAPLN3	hyaluronan and proteoglycan link protein 3	-1,224	3,384E-02
165	ENSECAG00000015840	PODN	podocan	-1,227	1,057E-02
166	ENSECAG00000020868	SYNGAP1	synaptic Ras GTPase activating protein 1	-1,230	2,625E-02
167	ENSECAG00000008118	DTX4	deltex E3 ubiquitin ligase 4	-1,232	4,304E-02
168	ENSECAG00000013066	ZCCHC14	zinc finger CCHC-type containing 14	-1,238	3,026E-02
169	ENSECAG00000051883	ENSECAG00000051883		-1,241	4,424E-02
170	ENSECAG00000012427	BACE1	beta-secretase 1	-1,241	4,304E-02
171	ENSECAG00000033093	VWA1	von Willebrand factor A domain containing 1	-1,272	4,652E-02
172	ENSECAG00000017156	ST3GAL4	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	-1,277	4,904E-02
173	ENSECAG00000020735	RBPM5	RNA binding protein, mRNA processing factor	-1,283	6,691E-04
174	ENSECAG00000039969	KCTD17	potassium channel tetramerization domain containing 17	-1,283	4,260E-02
175	ENSECAG00000000475	DNAH1	dynein axonemal heavy chain 1	-1,289	3,255E-02
176	ENSECAG00000021462	SFMBT2	Scm like with four mbt domains 2	-1,298	1,523E-03
177	ENSECAG00000049355	ENSECAG00000049355		-1,302	2,867E-02
178	ENSECAG00000017555	TF	transferrin	-1,311	2,637E-02
179	ENSECAG00000009443	MAP3K21	mitogen-activated protein kinase kinase kinase 21	-1,325	1,383E-02
180	ENSECAG00000014443	HSD11B1L	hydroxysteroid 11-beta dehydrogenase 1 like	-1,341	3,044E-02
181	ENSECAG00000012344	PTPRU	protein tyrosine phosphatase receptor type U	-1,363	3,623E-02
182	ENSECAG00000046205	CFAP52	cilia and flagella associated protein 52	-1,366	4,503E-02

183	ENSECAG00000009501	DAAM2	dishevelled associated activator of morphosis 2	-1,377	6,059E-04
184	ENSECAG00000014593	ENSECAG00000014593		-1,389	2,928E-02
185	ENSECAG00000054507	ENSECAG00000054507		-1,392	4,891E-02
186	ENSECAG00000022741	CDC42EP3	CDC42 effector protein 3	-1,408	3,988E-02
187	ENSECAG00000055761	ENSECAG00000055761		-1,438	1,175E-02
188	ENSECAG00000008333	MSC	musculin	-1,456	4,048E-02
189	ENSECAG00000022734	SGPP2	sphingosine-1-phosphate phosphatase 2	-1,496	3,044E-02
190	ENSECAG00000020314	ANO1	anoctamin 1	-1,498	3,485E-02
191	ENSECAG00000005796	ATP2C2	ATPase secretory pathway Ca2+ transporting 2	-1,501	3,586E-02
192	ENSECAG00000013822	KIF5C	kinesin family member 5C	-1,502	4,430E-03
193	ENSECAG00000011939	CLIP2	CAP-Gly domain containing linker protein 2	-1,502	1,753E-02
194	ENSECAG00000022152	CACHD1	cache domain containing 1	-1,513	4,077E-02
195	ENSECAG00000000791	DMTN	dematin actin binding protein	-1,534	2,584E-03
196	ENSECAG00000009746	UBXN11	UBX domain protein 11	-1,538	1,175E-02
197	ENSECAG00000012889	TMEM108	transmembrane protein 108	-1,538	2,906E-02
198	ENSECAG00000019690	NFIX	nuclear factor I X	-1,546	2,512E-02
199	ENSECAG00000009382	SH3BP4	SH3 domain binding protein 4	-1,562	2,614E-05
200	ENSECAG00000022638	PLD1	phospholipase D1	-1,576	2,150E-02
201	ENSECAG00000006205	CD7	CD7 molecule	-1,580	1,253E-02
202	ENSECAG00000024612	SNED1	sushi, nidogen and EGF like domains 1	-1,581	2,637E-02
203	ENSECAG00000016283	ZNF592	zinc finger protein 592	-1,586	6,823E-03
204	ENSECAG00000004904	SMO	smoothened, frizzled class receptor	-1,586	1,984E-02
205	ENSECAG00000021805	LAMB2	laminin subunit beta 2	-1,595	1,271E-02
206	ENSECAG00000042229	SAXO3	stabilizer of axonemal microtubules 3	-1,622	1,454E-02
207	ENSECAG00000017483	PSD3	pleckstrin and Sec7 domain containing 3	-1,631	4,176E-02
208	ENSECAG00000003966	GPC4	glypican 4	-1,643	1,232E-02
209	ENSECAG00000022754	ASPHD2	aspartate beta-hydroxylase domain containing 2	-1,644	4,304E-02
210	ENSECAG00000022068	RCN1	reticulocalbin 1	-1,664	5,762E-04
211	ENSECAG00000024568	ADD2	adducin 2	-1,665	4,304E-02
212	ENSECAG00000022973	HTR7	5-hydroxytryptamine receptor 7	-1,674	2,339E-02
213	ENSECAG00000038056	ENSECAG00000038056		-1,676	3,919E-03
214	ENSECAG00000030951	PTP4A3	protein tyrosine phosphatase 4A3	-1,693	1,757E-02
215	ENSECAG00000023397	C1QTNF1	C1q and TNF related 1	-1,694	4,225E-02
216	ENSECAG00000010544	SKAP1	src kinase associated phosphoprotein 1	-1,705	4,309E-02
217	ENSECAG00000022980	ENTPD8	ectonucleoside triphosphate diphosphohydrolase 8	-1,706	8,793E-03
218	ENSECAG00000003626	ZCCHC12	zinc finger CCHC-type containing 12	-1,720	4,498E-02
219	ENSECAG00000037336	ENSECAG00000037336		-1,730	2,986E-02
220	ENSECAG00000051098	ENSECAG00000051098		-1,777	6,678E-03

221	ENSECAG00000014219	SRCIN1	SRC kinase signaling inhibitor 1	-1,781	2,376E-03
222	ENSECAG00000006457	DNHD1	dynein heavy chain domain 1	-1,796	4,430E-03
223	ENSECAG00000000773	GGT7	gamma-glutamyltransferase 7	-1,805	2,226E-02
224	ENSECAG00000016231	HSPA12A	heat shock protein family A (Hsp70) member 12A	-1,811	3,841E-02
225	ENSECAG00000003445	C22H2orf96	chromosome 22 C20orf96 homolog	-1,827	3,564E-02
226	ENSECAG00000016576	C1QTNF12	C1q and TNF related 12	-1,832	4,516E-02
227	ENSECAG00000057433	ENSECAG00000057433		-1,834	3,159E-02
228	ENSECAG00000010917	HR	HR lysine demethylase and nuclear receptor corepressor	-1,843	9,091E-03
229	ENSECAG00000029387	NME9	NME/NM23 family member 9	-1,844	4,098E-03
230	ENSECAG00000014573	TMEM44	transmembrane protein 44	-1,844	3,400E-02
231	ENSECAG00000010127	INSR	insulin receptor	-1,849	2,845E-02
232	ENSECAG00000017304	MAPK8IP2	mitogen-activated protein kinase 8 interacting protein 2	-1,856	2,425E-03
233	ENSECAG00000021134	AQP3	aquaporin 3	-1,877	6,302E-03
234	ENSECAG00000031152	EMX2	empty spiracles homeobox 2	-1,897	2,545E-03
235	ENSECAG00000000544	HECW2	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2	-1,918	4,077E-02
236	ENSECAG00000011607	C8H2orf31	chromosome 8 C22orf31 homolog	-1,923	1,492E-02
237	ENSECAG00000018229	ZDHC14	zinc finger DHHC-type palmitoyltransferase 14	-1,929	2,186E-02
238	ENSECAG00000007044	FRAS1	Fraser extracellular matrix complex subunit 1	-2,006	7,584E-03
239	ENSECAG00000022804	DKK3	dickkopf WNT signaling pathway inhibitor 3	-2,042	2,484E-02
240	ENSECAG00000021162	CDH13	cadherin 13	-2,043	4,904E-02
241	ENSECAG00000007161	CYP2F1	cytochrome P450 family 2 subfamily F member 1	-2,044	3,536E-02
242	ENSECAG00000011631	EPHB2	EPH receptor B2	-2,047	2,804E-02
243	ENSECAG00000009934	KCNH3	potassium voltage-gated channel subfamily H member 3	-2,061	4,225E-02
244	ENSECAG00000017268	ATP8A2	ATPase phospholipid transporting 8A2	-2,069	4,924E-02
245	ENSECAG00000012871	UPK1A	uroplakin 1A	-2,084	1,035E-02
246	ENSECAG00000047513	CLDN5	claudin 5	-2,105	3,574E-02
247	ENSECAG00000008360	EFNB3	ephrin B3	-2,135	4,924E-02
248	ENSECAG00000018739	GRIK5	glutamate ionotropic receptor kainate type subunit 5	-2,142	1,142E-03
249	ENSECAG00000019508	COL8A1	collagen type VIII alpha 1 chain	-2,152	4,293E-02
250	ENSECAG00000022241	CACNA1H	calcium voltage-gated channel subunit alpha1 H	-2,160	1,605E-02
251	ENSECAG00000008518	NPNT	nephronectin	-2,172	3,044E-02
252	ENSECAG00000006846	GFRA1	GDNF family receptor alpha 1	-2,192	3,044E-02
253	ENSECAG00000021634	SLC29A4	solute carrier family 29 member 4	-2,276	3,485E-02
254	ENSECAG00000016129	ABLIM3	actin binding LIM protein family member 3	-2,336	3,477E-02
255	ENSECAG00000000234	GPC6	glypican 6	-2,363	2,102E-02
256	ENSECAG00000020485	PCOLCE2	procollagen C-endopeptidase enhancer 2	-2,406	3,811E-02
257	ENSECAG00000019060	ESYT3	extended synaptotagmin 3	-2,418	2,756E-02

258	ENSECAG00000057869	ENSECAG00000057869		-2,420	5,723E-03
259	ENSECAG00000016691	NTNG1	netrin G1	-2,457	1,343E-02
260	ENSECAG00000035543	SOD3	superoxide dismutase 3	-2,506	2,543E-02
261	ENSECAG00000022200	ENSECAG00000022200		-2,535	4,535E-02
262	ENSECAG00000009462	COL14A1	collagen type XIV alpha 1 chain	-2,554	1,249E-02
263	ENSECAG00000010286	CHRM3	cholinergic receptor muscarinic 3	-2,561	4,066E-04
264	ENSECAG00000000161	DPP10	dipeptidyl peptidase like 10	-2,607	4,858E-02
265	ENSECAG00000015516	APBA2	amyloid beta protein binding family A member 2	-2,656	4,077E-02
266	ENSECAG00000018104	IGFBP3	insulin like growth factor binding protein 3	-2,673	2,542E-02
267	ENSECAG00000010751	SCN8A	sodium voltage-gated channel alpha subunit 8	-2,700	6,823E-03
268	ENSECAG00000011099	VAV3	vav guanine nucleotide exchange factor 3	-2,724	7,460E-03
269	ENSECAG00000057027	ENSECAG00000057027		-2,724	1,288E-02
270	ENSECAG00000016196	PIGR	polymeric immunoglobulin receptor	-2,746	1,492E-02
271	ENSECAG00000018061	LAMA1	laminin subunit alpha 1	-2,763	4,098E-03
272	ENSECAG00000017742	ERN2	endoplasmic reticulum to nucleus signaling 2	-2,773	3,485E-02
273	ENSECAG00000021274	COL4A6	collagen type IV alpha 6 chain	-2,798	2,637E-02
274	ENSECAG00000040154	ELN	elastin	-2,817	3,446E-03
275	ENSECAG00000021358	SFRP1	secreted frizzled related protein 1	-2,936	7,544E-03
276	ENSECAG00000018134	STMN2	stathmin 2	-2,953	2,986E-02
277	ENSECAG00000026997	CLDN10	claudin 10	-2,964	1,070E-03
278	ENSECAG00000043912	PRRT1B	proline rich transmembrane protein 1B	-3,048	3,446E-03
279	ENSECAG00000044796	GREM1	gremlin 1, DAN family BMP antagonist	-3,072	3,223E-02
280	ENSECAG00000017487	MXRA5	matrix remodeling associated 5	-3,094	3,577E-03
281	ENSECAG00000008520	SEMA5B	semaphorin 5B	-3,120	4,293E-02
282	ENSECAG00000020165	PPP1R1B	protein phosphatase 1 regulatory inhibitor subunit 1B	-3,145	1,439E-02
283	ENSECAG00000021322	AMZ1	archaelysin family metallopeptidase 1	-3,218	2,020E-02
284	ENSECAG00000008591	CHST8	carbohydrate sulfotransferase 8	-3,221	1,253E-02
285	ENSECAG00000036791	SP5	Sp5 transcription factor	-3,242	7,632E-04
286	ENSECAG00000008843	PODXL2	podocalyxin like 2	-3,326	2,062E-02
287	ENSECAG00000010737	LKAAEAR1	LKAAEAR motif containing 1	-3,342	1,558E-02
288	ENSECAG00000009887	COL21A1	collagen type XXI alpha 1 chain	-3,407	1,174E-02
289	ENSECAG00000012686	SNCAIP	synuclein alpha interacting protein	-3,470	3,073E-03
290	ENSECAG00000008259	MASP1	MBL associated serine protease 1	-3,473	1,035E-02
291	ENSECAG00000007774	LY6H	lymphocyte antigen 6 family member H	-3,478	9,076E-03
292	ENSECAG00000007511	SPOCK1	SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1	-3,516	2,376E-03
293	ENSECAG00000031446	KCNIP3	potassium voltage-gated channel interacting protein 3	-3,543	3,581E-03
294	ENSECAG00000002236	KCNC1	potassium voltage-gated channel subfamily C member 1	-3,579	3,234E-02

295	ENSECAG00000018261	RGS4	regulator of G protein signaling 4	-3,630	5,811E-06
296	ENSECAG00000013207	SHISA2	shisa family member 2	-3,630	6,841E-03
297	ENSECAG00000000813	SCN5A	sodium voltage-gated channel alpha subunit 5	-3,650	2,685E-02
298	ENSECAG00000024631	CPXM2	carboxypeptidase X, M14 family member 2	-3,731	1,000E-02
299	ENSECAG00000006176	CNTFR	ciliary neurotrophic factor receptor	-3,761	7,260E-03
300	ENSECAG00000014683	BRINP1	BMP/retinoic acid inducible neural specific 1	-4,021	2,542E-02
301	ENSECAG00000011240	CNTN1	contactin 1	-4,240	3,687E-02
302	ENSECAG00000011581	ENSECAG00000011581		-4,607	3,423E-02
303	ENSECAG00000011934	LGR6	leucine rich repeat containing G protein-coupled receptor 6	-4,729	3,073E-03
304	ENSECAG00000019566	PHYHIPL	phytanoyl-CoA 2-hydroxylase interacting protein like	-4,875	4,176E-02
305	ENSECAG00000000538	ZNF385B	zinc finger protein 385B	-4,941	6,841E-03
306	ENSECAG00000014566	CCN5	cellular communication network factor 5	-5,287	3,221E-02
307	ENSECAG00000008745	ENSECAG00000008745		-5,524	2,209E-02
308	ENSECAG00000043826	ENSECAG00000043826		-5,528	4,891E-02
309	ENSECAG00000003050	HS6ST3	heparan sulfate 6-O-sulfotransferase 3	-5,922	8,494E-04
310	ENSECAG00000015152	SERPINA6	serpin family A member 6	-6,445	1,616E-02