SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 35-43

UDC 614.9.616.6-002.618.636.09
DOI: 10.48077/scihor9.2023.35

Inflammatory and anti-inflammatory cytokines in the endometrium of cows during anaphrodisia and estrous cycle

Iryna Bondarenko*
PhD in Veterinary Science, Associate Professor
Sumy National Agrarian University
40021, 160 H. Kondratiev Str., Sumy, Ukraine
https://orcid.org/0000-0002-1019-3446
Andriy Lazorenko
PhD in Veterinary Science, Associate Professor
Sumy National Agrarian University
40021, 160 H. Kondratiev Str., Sumy, Ukraine
https://orcid.org/0000-0002-0916-3901
Yurii Musiienko
PhD in Veterinary Science, Associate Professor
Sumy National Agrarian University
40021, 160 H. Kondratiev Str., Sumy, Ukraine
https://orcid.org/0000-0002-9735-4758
Oleksandr Panasenko
PhD in Veterinary Science, Associate Professor
Sumy National Agrarian University
40021, 160 H. Kondratiev Str., Sumy, Ukraine
https://orcid.org/0000-0003-1257-6341

Article's History:
Received: 9.05.2023
Revised: 24.08.2023
Accepted: 27.09.2023

Abstract. Infertility and sexual cycle disorders in cows are an urgent and widespread problem in cattle breeding, causing significant losses to farms. At the same time, a detailed understanding of the cellular and molecular mechanisms in the bovine uterus is crucial to explain and avoid infertility in dairy cows. The research aims to determine the concentration of tumour necrosis factor (TNFα), interleukin-1 (IL-1) and interlekin-4 (IL-4) in endometrial homogenates during oestrus, proestrus, corpus luteum and anaphrodisia caused by endometritis, as well as lactation. The research material was homogenates of cow endometrium taken from the horns of the uterus in its upper third. The methods used to determine the content of IL-1, IL-4 and TNFα in endometrial samples were enzyme-linked immunosorbent assay with a ratio (tissue homogenate-phosphate-salt buffer). The results of the study revealed changes in the content of IL-4, IL-1 and TNF-α in the functional layer of the endometrium during anaphrodisia and certain stages of the oestrous cycle. A significant increase in the content of TNF-α

Suggested Citation:
Bondarenko, I., Lazorenko, A., Musiienko, Yu., & Panasenko, O. (2023). Inflammatory and anti-inflammatory cytokines in the endometrium of cows during anaphrodisia and estrous cycle. *Scientific Horizons*, 26(9), 35-43. doi: 10.48077/scihor9.2023.35.

*Corresponding author

and a decrease in the levels of IL-1 and IL-4 in the endometrium compared to the same indicator during oestrus were found. No significant difference in the level of IL-1 during proestrus compared to the corpus luteum was found, as a result of the extinction of the transient phlogogenic reaction inherent in the oestrous endometrium at this time, due to the cytokine balance, which prepares the best conditions for zygote implantation. It was found that the level of IL-1 during oestrus significantly increases relative to the corpus luteum and proestrus stages. It was determined that the concentration of IL-4 in the functional layer of the endometrium of cows suffering from afterbirth retention and endometritis is lower than the same indicator during oestrus, which confirms the inhibition of cell proliferation and differentiation. The data obtained can be used to optimise the correction of sexual cycling in cows with anaphrodisia

Keywords: cows; anaphrodisia; sexual cycle; endometrium; TNF-α; IL-1; IL-4

INTRODUCTION

Maintaining and improving the reproductive performance of cows and heifers is one of the key parameters for the economic success of dairy farming. According to M. Bekara & N. Bareille (2019), infertility is a significant problem for dairy farming, as the associated extended calving intervals, reduced milk yields and increased culling rates contribute to huge economic losses. According to P. Lonergan *et al.* (2019), the causes of infertility are diverse and complex and, in addition to controlling the sexual cycle, efficiency of oestrus detection and proper breeding management, monitoring uterine health is a key factor in the proper reproductive performance of a dairy herd.

In the postpartum period in dairy cows, a high risk of clinical or subclinical diseases of the reproductive tract is present, which, following O. Pascottini & S. LeBlanc (2020), is accompanied by glucose intake, hypocalcaemia, lipid mobilisation from body fat, ketosis and the expression of proinflammatory cytokines that affect the immune response and change rapidly but differently in individual cows. At the same time, according to H. Pothmann *et al.* (2021), inflammatory processes and mRNA expression of cytokines at the end of the postpartum period can be considered as multifocal or widespread throughout the endometrium, regardless of the health of the uterus.

I. Sheldon *et al.* (2019) analysed infectious factors and endometrial inflammation in the postpartum period in cows with clinical and subclinical endometritis to determine the main causes of deterioration of the structural and functional state of the uterine mucosa and showed that inflammatory processes that continue in the postpartum period can impair fertility, lead to anaphrodisia, and interfere with embryo development. N. Naim *et al.* (2020) have shown that the reproductive function is realised through the activation of cambial tissues, which include the endometrium, and the physiological restructuring of the latter depends on coordinated intercellular interaction through the cytokine system.

F. Pinaffi (2018) found an important role of gonadal hormones and cytokines in preparing the endometrium for implantation and activating the transformation of epithelial cells and stromal fibroblasts into decidual cells. The cytokines IL1, IL6 and TNFα are considered (Drillich & Wagener, 2018) as mediators of nonspecific inflammatory processes, but their activity is also physiologically enhanced during the early postpartum period with a high degree of mRNA expression of cytokines and acute phase proteins during the third week after delivery, regardless of the health status of the animals.

The cytokine system is quite informative about the intercellular interaction of the endometrium, in connection with the prediction of zygote nidulation. According to P. Abeysinghe *et al.* (2023), binding of interleukin-1 to the receptors of maternal placental cells ensures zygote nidulation, proliferation of placental barrier cells, embryogenesis, and an increase in the percentage of zygotes that reach the blastocyst stage. N. Nijkang (2019) points out that the main function of interleukin-4 is to regulate stem cell proliferation by inducing mitogen-activated protein kinases, which transmit intracellular signals that cause cell differentiation, metabolic changes, apoptosis, and stimulate luteal cells to secrete progesterone. Q. Huang (2021) has demonstrated that zygote nidulation occurs when proinflammatory cytokines are expressed, but subsequently, pregnancy proceeds physiologically in the event of a shift in the cytokine balance towards immunosuppressive cytokines (interleukin-4, interleukin-10), as the latter inhibits cellular immunity responses, whereas a significant increase in interleukin-4 indicates a violation of peripheral tolerance and a shift in the local immune response towards Th-1, which causes infertility, and the ratio of interleukin-1 to interleukin-4 is a prognostic indicator in assessing the course of fertilisation and pregnancy.

According to M. Lappas (2017), cytokines affect the regulation of numerous types of intercellular interactions: granulosa cell proliferation, expression of gonadotropin receptors, and, conversely, cytokine imbalance leads to desynchronisation of cell differentiation, implantation disorders and infertility. N. Naim *et al.* (2020) investigated the cytokine-regulated endothelial-haemostatic interaction during maternal placental remodelling, implantation, and pregnancy. Q. Huang *et al.* (2021) note that during endometrial proliferation and differentiation, cytokines stimulate the functional activity of cells and humoral regulation of intercellular and intersystemic connections.

Thus, the analysis of the cytokine profile of tissue extracts of the uterine mucosa of cows at different stages of the sexual cycle will make it possible to determine the fullness of sexual cyclicity, the prerequisites for the development of infertility and will help to optimise prognostic tests for the effectiveness of sexual function correction. The research aims to investigate the content of certain pro- and anti-inflammatory cytokines in cow endometrial tissue extracts at different stages of the sexual cycle and in anaphrodisia following endometritis and after partum retention.

MATERIALS AND METHODS

The research was carried out within the framework of the research work "Study of cellular, biochemical and molecular genetic mechanisms of infectious diseases, metabolic disorders and immunocompensatory processes of counteracting biotic and abiotic factors in obstetric-gynecological, andrological and surgical pathology in animals" (state registration number 0116U005121).

The selection of experimental samples of the uterine mucosa of cows was carried out in farms with different housing technologies in the period from 2016 to 2022: by the tethered method (Shorthorn cow breed, OJSC breeding plant "Mikhailovka", Lebedynsky district, Sumy region with a productivity of <6000 kg and SFH "Vitalia", Burynsky district, Sumy region, Simmental breed of cows with productivity of >6000 kg), as well as the untethered method (LLC AF "Vladana", Ukrainian Black-and-White cows and LLC AF "Lan", Sumy district, Sumy region, Holstein breed, with a productivity of >6000 kg).

The material for the study was fragments of endometrial tissue (2-6 grams) taken from the upper third of the uterine horns of euthanised cows from experimental farms. Endometrial tissue samples were taken from euthanised animals without gynaecological pathology of the reproductive organs, aged 3 to 10 years: in heat (n=5), during the corpus luteum (n=5), proestrus (n=5) and from cows that did not come into heat for more than 60 days after endometritis (n=5) and after delayed afterbirth (n=5).

The selected endometrial samples were washed with 0.9% sodium chloride saline with a theoretical osmolarity of 308 mosmol/l, pH 5.0-7.0. Subsequently, the washed samples were weighed on an electronic analytical balance of the 1st accuracy class AS 82/220 R2, labelled, packed in polystyrene containers, and subjected to cryopreservation at – 20°C. To determine the concentration of cytokines IL-1, IL-4 and TNFα, endometrial samples were homogenised in the cold with the addition of 0.01 M phosphate-salt buffer (pH 7.4) containing 1% Triton X-100 solution in a ratio of 1:40, followed by placement in a +4°C refrigerator for 120 minutes (Lazorenko & Izdepsky, 2012).

The endometrial homogenates were then centrifuged at 3000 rpm for 15 min. In the supernatant, the level of cytokines IL-1, IL-4 and TNFα was determined by enzyme-linked immunosorbent assay using diagnostic test kits manufactured by Peninsula Laboratories Inc, (USA). The actual content of IL-1, IL-4 and TNFα in endometrial samples was determined by considering the ratio (tissue homogenate-phosphate-salt buffer).

Experimental studies were performed by modern methodological approaches, following the requirements of DSTU ISO/IEC 17025:2005 (2006). The animals were kept, and all manipulations were carried out following the Order of the Ministry of Health of Ukraine No. 416/20729 "On Approval of the Procedure for Conducting Animal Tests in Research Institutions" (Law of Ukraine No. 249, 2012) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986). The digital material obtained in the research was processed by variational statistical methods using statistical techniques (STATISTICA 10.0 for Windows) with the determination of the arithmetic mean (M), the statistical error of the arithmetic mean (m), and the probability of difference (p) between two variation series according to the Student's criterion with values of $p \leqslant 0.05$, $p \leqslant 0.01$, and $p \leqslant 0.001$.

RESULTS AND DISCUSSION

The complex pathogenetic aspects of the interaction of anti-inflammatory and pro-inflammatory cytokines during endometrial remodelling and post-morbid state in cows are not well understood. Therefore, the studies that detail the above processes are new and relevant. The data presented in Figure 1 indicate that during oestrus, the concentration of TNFα in the endometrium is the lowest, amounting to 5.03±2.16 pg/ml, while during proestrus and the corpus luteum, its level increases, compared to the oestrous phase, by almost 4.2-4.3 times (21.15±3.08 pg/ml and 21.63±4.69 pg/ml, $p<0.01$), respectively.

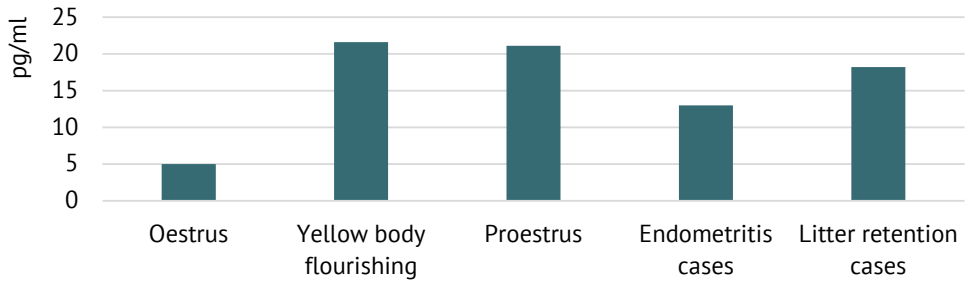

Figure 1. *TNF-α content in cow endometrial homogenates, pg/ml*

Source: *compiled by the authors*

The study of TNF-α content in endometrial homogenates of cows, during anaphrodisia and after endometritis and retained litter, indicates high expression, with the accumulation of this cytokine in the endometrium. In particular, the concentration of TNF-α in endometrial homogenates was significantly higher in patients with endometritis – 2.6 times to 13.07±1.98 pg/ml ($p<0.01$) and 3.6 times after afterbirth to 18.21±3.99 pg/ml ($p<0.01$), compared to the index during oestrus.

At the same time, the level of endometrial TNFα decreased by 1.7 and 1.2 times in cows with endometritis and after delayed afterbirth, respectively, compared to the same indicator in the corpus luteum, respectively. Concerning the proestrus, the content of TNFα in the endometrium decreased by 1.6 times in animals with endometritis and by 1.2 times after delayed birth, respectively. The data shown in Figure 2 demonstrate an increase in the level of IL-1 in cow endometrial homogenates during oestrus (8325.46±69.18 pg/ml), almost 2-fold, compared to the corpus luteum (4153.82±74.23 pg/ml) and proestrus (4092.57±476.05 pg/ml), $p<0.001$, respectively.

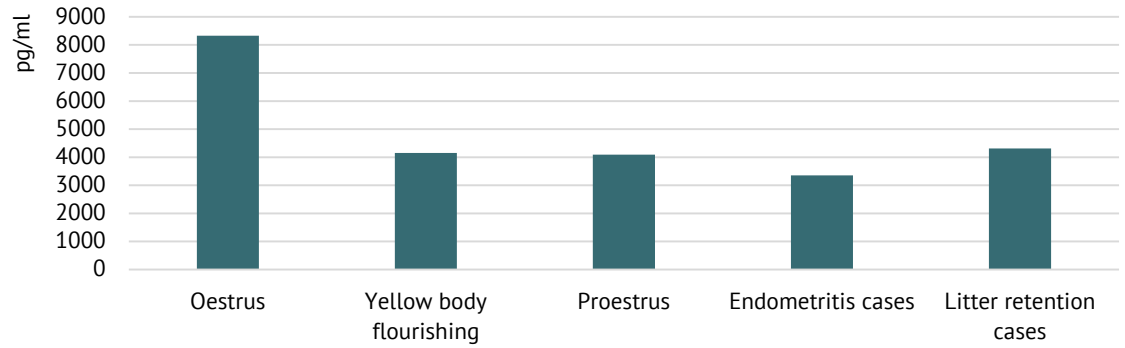

Figure 2. *IL-1 content in cow endometrial homogenates, pg/ml*
Source: *compiled by the authors*

The absence of a significant difference in the content of endometrial IL-1 in proestrus compared to corpus luteum is obviously due to the attenuation of the temporary inflammatory response inherent in the endometrium in oestrus at this period of the sexual cycle and a shift in the cytokine balance in favour of immunosuppressive cytokines, which creates optimal conditions for zygote nidulation. The concentration of IL-1 in the endometrium of anaphroditic cows suffering from endometritis and afterbirth retention was 2.5 times lower than that of cows in heat, 3351.71±1422.42 pg/ml and 3412.43±430.12 pg/ml, respectively, $p<0.01$, respectively, which is associated with the inhibition of the transformation of stromal fibroblasts and epithelial cells into decidual cells, and the impossibility of endometrial remodelling necessary for implantation. Thus, studies have shown that the key proinflammatory cytokine in the endometrium of cows during oestrus is IL-1, while from 7 to 18 days of the sexual cycle its concentration decreases significantly, almost reaching the level of animals that do not show sexual cyclicity, and TNFα takes over the role of the dominant proinflammatory cytokine, the expression of which increases significantly during this period. As can be seen from the data presented in Figure 3, the level of IL-4 in endometrial homogenates during oestrus reaches its maximum value – 407.07±45.78 pg/ml, while during the corpus luteum it begins to show a tendency to decrease by almost 1.4 times (295.31±42.16 pg/ml), and during proestrus it undergoes a significant decrease by 3.4 times (118.56±32.34 pg/ml), $p<0.001$, respectively. At the same time, a significant difference in the concentration of endometrial IL-4 was found between days 7-8 and 17-18 of the sexual cycle, amounting to 2.5 times ($p<0.01$), respectively.

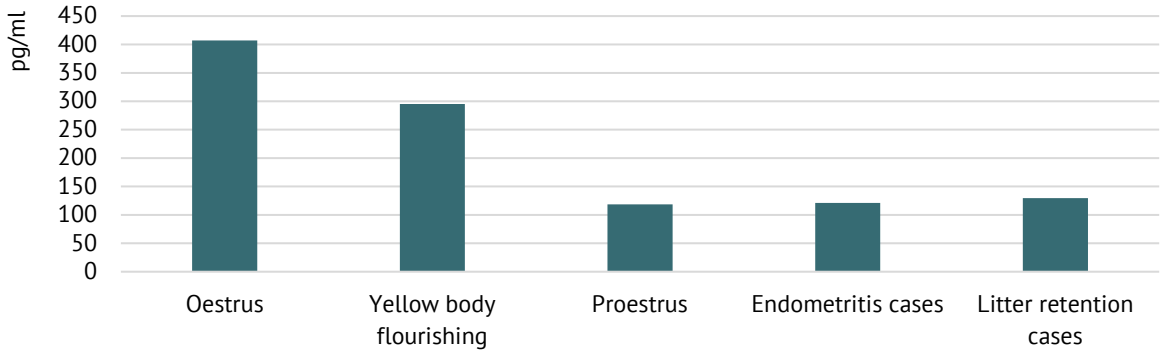

Figure 3. *IL-4 content in cow endometrial homogenates, pg/ml*
Source: *compiled by the authors*

The high concentration of this cytokine during oestrus is probably a consequence of the inhibitory effect of IL-4 on proinflammatory cytokines, the content of which at this time significantly increases, and in particular, the level of IL-1. The level of IL-4 in the endometrial homogenates of cows in the state of anaphrodisia and suffering from endometritis (121.03±52.36 pg/ml) and afterbirth retention (129.58±58.28 pg/ml) underwent a significant decrease compared to the indicator during the oestrous phase of the cycle by 3.4 and 3.2 times, $p<0.001$, respectively.

To assess the cytokine imbalance between proinflammatory IL-1 and anti-inflammatory IL-4, the ratio between IL-1 and IL-4 was calculated as an index reflecting the balance between both interleukins and the balance of cytokine expression. The ratio of IL-1 to IL-4 is an objective criterion for predicting the type of tissue regeneration, since in the event of a local imbalance of these cytokines, the regeneration mechanism undergoes a pathophysiological shift and causes tissue remodelling defects and infertility (Fig. 4)

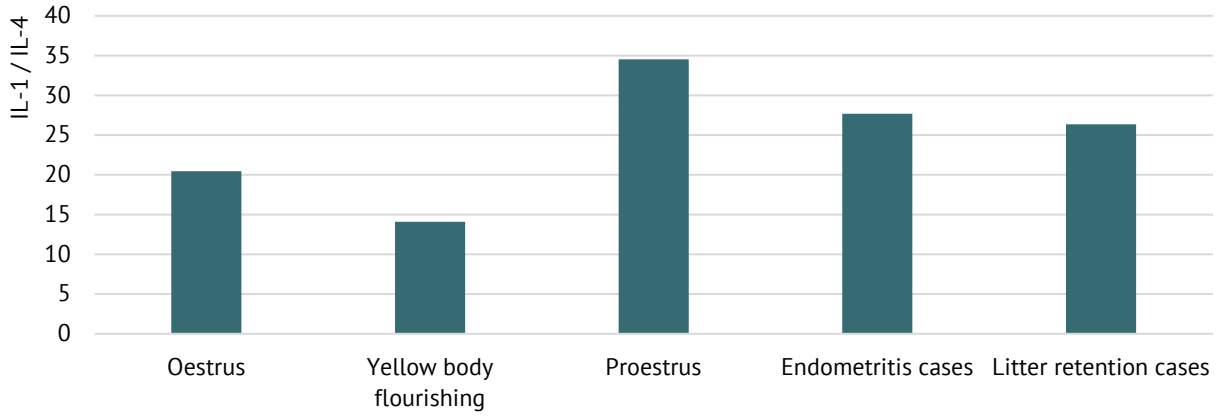

Figure 4. *IL-1 to IL-4 ratio index in cow endometrial homogenates*
Source: *compiled by the authors*

The highest index of the ratio between IL-1 and IL-4 was found to be during proestrus (34.53), while during oestrus it was 20.45, and during the corpus luteum, this ratio had a minimum value of 14.08. The obtained results demonstrate the role of IL-1 and IL-4 in the process of remodelling since changes in concentration confirm the activation of physiological endometrial remodelling during proestrus and inhibition of remodelling during corpus luteum. The index of the ratio between IL-1 and IL-4 in animals with endometritis and retained litter was 27.69 and 26.35, respectively, remaining significantly lower than the same index in the period of proestrus. The abovementioned changes indicate the inhibition of regenerative and restorative reactions due to the extinction of the metabolic and secretory activity of the postmorbid endometrium.

It is likely that in the present study, the increase in TNFα during active endometrial remodelling during days 7(8) to 17(18) of the sexual cycle is due to increased reproduction of endometrial components. In particular, studies (Abeysinghe *et al.,* 2023) have shown that TNFα initiates the production of other cytokines and prostaglandins, which causes stimulation, proliferation, and differentiation through the pleiotropic effect of different cell types.–Furthermore, according to N. Nijkang (2019), TNFα can induce apoptosis of immunocompetent cells, connective tissue cells, as well as circulating and exudative neutrophils through the activation of cellular caspases that destroy intracellular components. TNF-α, like IL-1 and IL-6, is a powerful stimulator of acute-phase protein synthesis in the event of tissue damage, so even a slight increase in TNF-α levels causes mobilisation of energy reserves and stimulates the body's defence responses.

Thus, the increase in TNFα content in the endometrium from day 7 to day 18 of the sexual cycle indicates the influence of this cytokine on the differentiation and active proliferation of cells of the functional layer of the uterine mucosa, and the activation of humoral regulation of intercellular connections and intersystemic cellular associations. According to M. Segura-Benítez *et al.* (2022), this finding details the peculiarities of intercellular matrix remodelling during the stages of the oestrous cycle and clarifies the optimisation of implantation conditions. There is no characteristic cell proliferation and differentiation in the postmorbid state, as evidenced by a decrease in TNFα. S. Warashina *et al.* (2022) note that this cytokine is a powerful stimulator of acute phase protein synthesis and, in the pathological process, even a slight increase in it causes mobilisation of energy reserves and stimulates the body's defence responses. At the same time, a significant increase in TNFα content in the case of reproductive dysfunction, according to H. Ragsdale *et al.* (2019), indicates its involvement in the regulation of reproductive processes, as high levels of TNFα activate the biosynthesis of oestrogens by cytotoxic and proliferative cell receptor types.

According to F. Almughlliq *et al.* (2019), this illustrates the disorganisation of the connective tissue matrix of the postmorbid endometrium, since anaphrodisia results in a significant decrease in the concentration of connective tissue markers – glycoproteins and glycosaminoglycans, which is a consequence of the decrease in secretory cells due to the induction of their apoptosis under conditions of TNF-α expression. It is known that IL-1 exerts its biological effect during intercellular contact through specific and non-specific receptors on the surface of target cells involved in the process of implantation and pregnancy. Several studies (Kuhla *et al.,* 2019; Koh *et al.,* 2020), that record an increase in IL-1 and TNFα levels during the corpus luteum, i.e., at the beginning of the inhibition stage, are contradictory to other studies (Abeysinghe *et al.,* 2023), who indicate a maximum increase in IL-1 in endometrial cells during the follicular phase compared to the luteal phase. These circumstances give rise to the need to detail these studies, as they do not reflect the overall concentration of cytokines in endometrial tissue.

In the present study, an increase in endometrial IL-1 was detected during hunting. This trend, according to researchers (Huang *et al.,* 2021), is likely due to the ability of this cytokine to stimulate the expression of release factor genes that enhance the production of gonadotropic hormones, followed by the induction of the synthesis of estrogens and other gonadal hormones, activation of the transformation of stromal fibroblasts and epithelial cells into decidual cells, which in general details the process of intercellular matrix remodelling and ensures nidulation.

Studies conducted by M. Lappas (2017), and H. Ragsdale *et al.* (2019) indicate that IL-1 stimulates the transformation of endometrial epithelial cells and stromal fibroblasts into decidual cells, the expression of releasing factor genes, and can affect homeostasis by increasing corticotropin-releasing factor, adrenocorticotropic hormone, and others. At the same time, N. Nijkang *et al.* (2019) point out that the release of hypothalamic monoamines and neuropeptides under the influence of endogenous IL-1 disrupts the synthesis of gonadotropic and sex hormones, thereby causing anaphrodisia.

Opposite to proinflammatory IL-1 and TNFα is IL-4, a pleiotropic cytokine produced by various immune cells during intercellular interaction and involved, on the one hand, in enhancing tissue alteration and destruction, and, on the other hand, in limiting the inflammation focus, increasing tissue barrier functions, activating regeneration, forming the body's defence response, and inhibiting proinflammatory cytokines. According to N. Nijkang *et al.* (2019) and N. Turner *et al.* (2022), it activates the attenuation of the immune response, and catalyses the endometrium's tolerance to the inflammatory process, thereby including the regeneration phase and restoration of the function of the maternal part of the placenta. The results of our research are consistent with the findings of other scientists.

In particular, as shown in studies (Taylor *et al.,* 2021), interleukin-4 is found in endometrial stromal phagocytes and actively regulates stem cell proliferation by inducing mitogen-activated protein kinases. The latter transmits an intracellular signal that causes cell differentiation, metabolic changes, and apoptosis. In addition, IL-4 stimulates luteal cells to increase progesterone. The findings of a significant decrease in IL-4 in endometrial tissue are consistent with the results of studies (Rasheed & Hamid, 2020) and may indicate a lack of cell proliferation and differentiation, and as a result, the inability of postmorbid endometrium to nidulation.

Individual studies (Sheldon *et al.,* 2020) have shown that IL-4 levels in physiological pregnancy decrease compared to pregnancies that are pathological and end in abortion. A significant increase in IL-4 levels indicates a violation of peripheral tolerance and a shift in the local immune response towards IL-1, which causes infertility. Assessment of serum cytokine levels (proinflammatory IL-1 and regulatory IL-4, which is an inducer of activation of the immune system cellular chain) reflects the dynamics of intercellular interaction and the nature of the pathological process (Salas-Huetos, *et al.,* 2020). According to J. Wisler *et al.* (2022), excessive levels of IL-1 destroy intercellular interactions, causing infertility, due to the excessive development of the endometrial inflammatory response during the stage of excitation. The results of studies obtained by M. Segura-Benítez *et al.* (2022) indicate that physiological zygote nidulation is possible under conditions of a balance between the immunomodulatory and immunosuppressive effects of the endometrium, in the implementation of which components of the cytokine system are directly involved.

M. Zargar *et al.* (2020) point to a specific balance between the anti-inflammatory components of the immune system and pro-inflammatory cytokines. It is this balance of inflammatory and anti-inflammatory cytokines that determines the course of inflammation, the development of possible complications, and organ and tissue dysfunctions. F. Almughlliq *et al.* (2019) proved that full endometrial regeneration is possible under conditions of a balanced ratio of IL-1, TNF-α and their antagonist IL-4, which play an important role in the formation of regenerative and repair reactions. In the case of their local imbalance, the regeneration mechanism acquires a pathophysiological shift and causes tissue remodelling defects. The index of the ratio of IL-1 to IL-4 in cows with endometritis and afterbirth retention was significantly lower compared to the index of proestrus.

The cytokine ratio of IL-1, TNF-α and IL-4 is an objective criterion for predicting the type of tissue regeneration. I. Sheldon *et al.* (2020), having studied the ratio of IL-1 and IL-4, proved that cytokine balance is a prognostic indicator in assessing the course of pregnancy. An

excessive increase in the concentration of IL-1 against a sharp decrease in IL-4 leads to abortion. Whereas, according to P. Abeysinghe *et al.* (2023), the correction of the above ratio towards a decrease in IL-1 concentration against the background of an increase in IL-4 ensures a physiological course of pregnancy. Thus, further research on a detailed understanding of the cellular and molecular mechanisms occurring in the bovine endometrium under physiological and pathological conditions is crucial to explain and avoid infertility in dairy cows.

CONCLUSIONS

During oestrus, the concentration of TNFα in the endometrium is as low as possible, amounting to 5.03±2.16 pg/ml, while during proestrus and corpus luteum, its level increases by almost 4.2-4.3 times (21.15±3.08 pg/ml and 21.63±4.69 pg/ml, $p<0.01$), respectively.

The concentration of TNF-α in endometrial homogenates of cows in the state of anaphrodisia is significantly higher in animals that have suffered from endometritis 2.6 times (13.07±1.98 pg/ml, $p<0.01$) and 3.6 times after afterbirth retention (18.21±3.99 pg/ml, $p<0.01$) compared to the indicator during oestrus. The level of IL-1 in cow endometrial homogenates increased during the oestrus period (8325.46±69.18 pg/ml), almost 2 times compared to the corpus luteum (4153.82±74.23 pg/ml) and proestrus (4092.57±476.05 pg/ml), $p<0.001$, respectively.

The concentration of IL-1 in the endometrium of animals in the state of anaphrodisia and suffering from endometritis and retention of afterbirth is 2.5 times lower than that of cows in heat, 2.5 times (3351.71±1422.42 pg/ml and 3412.43±430.12 pg/ml), $p<0.01$, respectively. The content of IL-4 in endometrial homogenates of cows during the heat reaches maximum values – 407.07±45.78 pg/ml, while during the oestrus it begins to show a tendency to decrease by almost 1.4 times (295.31±42.16 pg/ml), and during proestrus it undergoes a significant decrease by 3.4 times (118.56±32.34 pg/ml), $p<0.001$, respectively.

The concentration of IL-4 in endometrial homogenates of anaphroditic cows suffering from endometritis (121.03±52.36 pg/ml) and retention of afterbirth (129.58±58.28 pg/ml) undergoes a significant decrease compared to the indicator during the oestrous phase of the cycle by 3.4 and 3.2 times, $p<0.001$, respectively. The highest index of the ratio between IL-1 and IL-4 was found to be during proestrus – 34.53, while during oestrus it was 20.45, and during the corpus luteum, this ratio was 14.08, while in animals with endometritis and retention of the litter, it was 27.69 and 26.35, respectively, remaining significantly lower than the same index of proestrus. Thus, the prospect of further research is the need to use the data obtained to develop reasonable methods for correcting the sexual cyclicity of cows in the post morbid state.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

REFERENCES

[1] Abeysinghe, P., Turner, N., Mosaad, E., Logan, J., & Mitchell, M. (2023). Dynamics of inflammatory cytokine expression in bovine endometrial cells exposed to cow blood plasma small extracellular vesicles (sEV) may reflect high fertility. *Scientific Reports,* 13(1), article number 5425. doi: 10.1038/s41598-023-32045-1.

[2] Almughlliq, F.B., Koh, Y.Q., Peiris, H.N., Vaswani, K., Holland, O., Meier, S., Roche, J.R., Burke, C.R., Crookenden, M.A., Arachchige B.J., Reed, S., & Mitchell, M.D. (2019). Circulating exosomes may identify biomarkers for cows at risk for metabolic dysfunction. *Scientific Reports,* 9(1), article number 13879. doi: 10.1038/s41598-019-50244-7.

[3] Bekara, M.A., & Bareille, N. (2019). Quantification by simulation of the effect of herd management practices and cow fertility on the reproductive and economic performance of Holstein dairy herds. *Journal of Dairy Science,* 102(10), 9435-9457. doi: 10.3168/jds.2018-15484.

[4] Drillich, M., & Wagener, K. (2018). Pathogenesis of uterine diseases in dairy cattle and implications for fertility. Animal *Reproduction*, 15(1), 879-885. doi: 10.21451/1984-3143-AR2018-0023.

[5] European convention for the protection of vertebrate animals used for experimental and other scientific purposes. (1986). Retrieved from https://rm.coe.int/168007a67b.

[6] Huang, Q., Jin, X., Li, P., Zheng, Z., Jiang, Y., & Liu, H. (2021). Elevated inflammatory mediators from the maternal-fetal interface to fetal circulation during labor. *Cytokine,* 148, article number 155707. doi: 10.1016/j.cyto.2021.155707.

[7] Koh, Y.Q., Peiris, H.N., Vaswani, K., Almughlliq, F.B., Meier, S., Burke, C.R., Roche, J.R., Reed, C.B., & Mitchel, M.D. (2020). Exosomes from dairy cows of divergent fertility; Action on endometrial cells. *Journal of Reproductive Immunology,* 137, article number 102624. doi: 10.1016/j.jri.2019.102624.

[8] Kuhla, B., Kaever, V., Tuchscherer, A., & Kuhla, A. (2019). Involvement of plasma endocannabinoids and the hypothalamic endocannabinoid system in increasing feed intake after parturition of dairy cows. *Neuroendocrinology*, 110(3-4), 246-257. doi: 10.1159/000501208.

[9] Lappas, M. (2017). The IL-1β signalling pathway and its role in regulating pro-inflammatory and pro-labour mediators in human primary myometrial cells. *Reproductive Biology,* 17, 333-340. doi: 10.1016/j.repbio.2017.09.006.

[10] Lazorenko, A., & Izdepsky, V. (2012). Tumor necrosis factor and the modified citrullinated vimentin in developing immunodependent inflammation of connective tissue formations of the horses' hoofs. *Veterinary Medicine of Ukraine*, 1, 27-29.
[11] Lonergan, P., Sánchez, J.M., Mathew, D.J., Passaro, C., & Fair, T. (2019). Embryo development in cattle and interactions with the reproductive tract. *Reproduction, Fertility and Development,* 31(1), 118-125. doi: 10.1071/RD18409.
[12] Naim, N., Amrit, F.R., McClendon, T.B., Yanowitz, J.L., & Ghazi, A. (2020). The molecular tug of war between immunity and fertility: Emergence of conserved signaling pathways and regulatory mechanisms. *BioEssays,* 42(12), article number e2000103. doi: 10.1002/bies.202000103.
[13] Nijkang, N., Anderson, L., Markham, R., & Manconi, F. (2019). Endometrial polyps: Pathogenesis, sequelae and treatment. *SAGE Open Medicine,* 7, article number 2050312119848247. doi: 10.1177/2050312119848247.
[14] Pascottini, O.B., & LeBlanc, S.J. (2020). Modulation of immune function in the bovine uterus peripartum. *Theriogenology,* 150, 193-200. doi: 10.1016/j.theriogenology.2020.01.042.
[15] Pinaffi, F.L.V., Araujo, E.R., & Ginther, O.J. (2018). Role of luteal biosynthesis of prostaglandin F2α on function and structure of the corpus luteum during luteolysis in heifers. *Domestic Animal Endocrinology*, 63, 10-14. doi: 10.1016/j.domaniend.2017.10.007.
[16] Pothmann, H., Flick, P., Tichy A., Gabler, C., & Drillich, M. (2021). Messenger RNA expression of selected factors at different sites of the bovine endometrium associated with uterine health. *Frontiers in Veterinary Science*, 8, article number 649758. doi: 10.3389/fvets.2021.649758.
[17] Ragsdale, H.B., Kuzawa, C.W., Borja, J.B., Avila, J.L., & McDade, T.W. (2019). Regulation of inflammation during gestation and birth outcomes: Inflammatory cytokine balance predicts birth weight and length. *American Journal of Human Biology,* 31(3), article number e23245. doi: 10.1002/ajhb.23245.
[18] Rasheed, H.A.M, & Hamid, P. (2020). Inflammation to infertility: Panoramic view on endometriosis. *Cureus,* 12(11), article number e11516. doi: 10.7759/cureus.11516.
[19] Salas-Huetos, A., James, E.R., Aston, K.I., Carrell, D.T., Jenkins, T.G., & Yeste, M. (2020). The role of miRNAs in male human reproduction: A systematic review. *Andrology,* 8(1), 7-26. doi: 10.1111/andr.12714.
[20] Segura-Benítez, M., Carbajo-García, M.C., Corachán, A., Faus, A., Pellicer, A., & Ferrero, H. (2022). Proteomic analysis of extracellular vesicles secreted by primary human epithelial endometrial cells reveals key proteins related to embryo implantation. *Reproductive Biology and Endocrinology,* 20, article number 3. doi: 10.1186/s12958-021-00879-x.
[21] Sheldon, I.M., Cronin, J.G., & Bromfield, J.J. (2019). Tolerance and innate immunity shape the development of postpartum uterine disease and the impact of endometritis in dairy cattle. *Annual Review of Animal Biosciences*, 7, 361-384. doi: 10.1146/annurev-animal-020518-115227.
[22] Sheldon, I.M., Molinari, P.C., Ormsby, T.R., & Bromfield, J.J. (2020). Preventing postpartum uterine disease in dairy cattle depends on avoiding, tolerating and resisting pathogenic bacteria. *Theriogenology*, 150, 158-165. doi: 10.1016/j.theriogenology.2020.01.017.
[23] Taylor, H.S., Kotlyar, A.M., & Flores, V.A. (2021). Endometriosis is a chronic systemic disease: Clinical challenges and novel innovations. *Lancet,* 397(10276), 839-852. doi: 10.1016/s0140-6736(21)00389-5.
[24] Turner, N.P., Abeysinghe, P., Kwan Cheung, K.A., Vaswani, K., Logan, J., Sadowski, P., & Mitchell, M.D. (2022). A comparison of blood plasma small extracellular vesicle enrichment strategies for proteomic analysis. *Proteomes,* 10(2), article number 19. doi: 10.3390/proteomes10020019.
[25] Warashina S., Zouda, M., Mohri, K., Wada, Y., Maeda, K., Watanabe, Y., & Mukai, H. (2022). ^{64}Cu-labeling of small extracellular vesicle surfaces via a cross-bridged macrocyclic chelator for pharmacokinetic study by positron emission tomography imaging. *International Journal of Pharmaceutics,* 25(624), article number 121968. doi: 10.1016/j.ijpharm.2022.121968.
[26] Wisler, J.R., Singh, K., McCarty, A., Harkless, R., Karpurapu, M., Hernandez, E., Mukherjee, D., Abouhashem A.S., Christman, J.W., & Sen, C.K. (2022). Exosomal transfer of DNA methyl-transferase mRNA induces an immunosuppressive phenotype in human monocytes. *Shock,* 57(6), 218-227. doi: 10.1097/shk.0000000000001928.
[27] Zargar, M., Ghafourian, M., Nikbakht, R., Mir Hosseini, V., & Moradi Choghakabodi, P. (2020). Evaluating chronic endometritis in women with recurrent implantation failure and recurrent pregnancy loss by hysteroscopy and immunohistochemistry. *Journal of Minimally Invasive Gynecology,* 27(1), 116-121. doi: 10.1016/j.jmig.2019.02.016.

Уміст прозапальних та протизапальних цитокінів у ендометрії корів за анафродизії та естрального циклу

Ірина Вікторівна Бондаренко
Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0002-1019-3446

Андрій Борисович Лазоренко
Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0002-0916-3901

Юрій Володимирович Мусієнко
Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0002-9735-4758

Олександр Сергійович Панасенко
Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0003-1257-6341

Анотація. Неплідність та порушення статевої циклічності в корів, є актуальною і поширеною проблемою в скотарстві, що завдає істотних збитків господарствам. Водночас, детальне розуміння клітинних і молекулярних механізмів у матці великої рогатої худоби має вирішальне значення для пояснення та уникнення неплідності у молочних корів. Метою досліджень було визначити концентрацію фактору некрозу пухлин (TNFα), интерлейкіну – 1 (IL-1) та интерлейкіну – 4 (IL-4) у ендометріальних гомогенатах за еструсу, проеструсу, розквіту жовтого тіла й анафродизії спричиненої прехворюванням на ендометрит, а також затримку посліду. Матеріалом для дослідження були гомогенати ендометрію корів, відібраного з рогів матки у верхній її третині. Використані методи визначення вмісту IL-1, IL-4 та TNFα у зразках ендометрію – імуноферментний твердофазний ELISA аналіз із урахуванням співвідношення (тканинний гомогенат-фосфатно-сольовий буфер). За результатами досліджень з'ясовано зміни умісту IL-4, IL-1 та TNF-α, у функціональному шарі ендометрія за анафродизії та окремих стадій естрального циклу. Встановлено істотне зростання вмісту TNF-α та зниження рівнів IL-1 і IL-4 у ендометрії відносно аналогічного показника в період еструсу. З'ясовано відсутність вірогідної різниці рівня IL-1 за проеструсу порівняно з розквітом жовтого тіла, як наслідок згасання в цей час, притаманної естральному ендометрію транзиторної флогогенної реакції, через цитокіновий баланс, що готує найкращі умови для імплантації зиготи. Встановлено, що рівень IL-1 за еструсу вірогідно збільшується, відносно стадії розквіту жовтого тіла та стадії проеструсу. Визначено, що концентрація IL-4 у функційному шарі ендометрія корів, які перехворіли на затримання посліду та ендометрит, є нижчою за аналогічний показник за еструсу, що підтверджує пригнічення проліферації й диференціації клітин. Отримані дані, можуть бути використані для оптимізації корекції статевої циклічності корів із анафродизією

Ключові слова: корови; анафродизія; статевий цикл; ендометрій; TNF-α; IL-1; IL-4