ANIMAL SCIENCE AND GENETICS
Published by the Polish Society of Animal Production
vol. 19 (2023), no 4, 51-67
DOI: 10.5604/01.3001.0054.2865

Research Article ***Open Access***

Expression of oestrogen and progesterone receptor and intermediate filament proteins in the oviduct of mares with endometrosis

Magdalena Profaska[1*], Artur Gurgul[2], Małgorzata Kotula-Balak[1], Agnieszka Rak[3], Roberto Sanchez[4], Sylwester Zając[5], Maciej Witkowski[1]

[1]University Centre of Veterinary Medicine JU-UA, Department of Obstetrics, Gynecology and Andrology with Biotechnology of Reproduction, University of Agriculture in Kraków, Kraków, Poland;
[2]Center of Experimental and Innovative Medicine, University of Agriculture in Kraków, Kraków, Poland
[3]Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Kraków, Kraków, Poland
[4]Embryosanchez, Bogota, Colombia
[5]Center of Equine Reproduction - EquiBiotech, Henryków-Urocze, Poland

SUMMARY

Equine endometrosis is one of the major causes of equine infertility, often associated with signs of periglandular fibrosis occurring in the endometrium. As the oviduct is a key location of early embryo development, we hypothesized that disturbances in hormonal action may be associated with a loss of contact between mucosal cells in the uterus and oviducts. This study investigates the expression of intermediate filament proteins (vimentin and desmin), oestrogen receptor alpha (ER*α*), and progesterone receptor A (PRA) in the stroma and epithelia of the endometria and oviducts of healthy mares and mares with endometrosis. Endometrium samples were obtained from 56 mares, culled in a slaughterhouse, and were designated either healthy endometrium (n = 26) or endometrium with signs of moderate to severe endometrosis (n = 20). The phase of the ovarian cycle and the expression of vimentin, desmin, ERα, and PRA were compared between these groups. The expressions of both vimentin and desmin were higher in the endometrial stroma of the endometrosis group than in healthy endometria. The expression of ERα in the healthy endometrial stroma and luminal epithelium during the follicular phase was higher than in the luteal phase, but showed no differences in the endometrium of mares with endometrosis. PRA expression was significantly

Corresponding author e-mail: magdalena.profaska@urk.edu.pl
Received: 9.11.2023 Received in revised form: 8.12.2023
Accepted: 9.12.2023 Published online: 12.12.2023

higher in the stoma oviduct during the follicular phase than in the luteal phase. It seems possible that mucosal cells may support the occurrence of moderate to severe endometrosis through their altered intermediate filament and sex steroid receptor interactions. In addition, the oviduct appears to be less sensitive to hormonal changes than the uterus. Changes in the expression of intermediate filament and steroid hormone proteins may be a leading factor contributing to the functional status of the oviduct in endometrosis.

KEY WORDS: endometrosis; vimentin; desmin; steroid hormone receptor; mare

INTRODUCTION

Endometrosis is a pathological alteration in the endometrium of mares characterized by periglandular fibrosis and the formation of glandular cysts. These changes may affect individual glands and/or several gland branches. No altered glands can exist next to degenerative glands. Depending on the severity of periglandular fibrosis, equine endometrial samples were categorized as normal endometrium (I) or endometrosis, ranging from mild (IIA), to moderate (IIB), to severe (III) (Kenney and Doig 1986). The endometrosis process is classified as non-destructive and destructive, depending on the glandular epithelium status, and also as active or inactive, based on the state of metabolic activity of the stroma (Hoffmann et al., 2009a, Schöniger and Schoon 2020).

Both the immune system and endocrine system play a significant role in the development of endometrial disease (Witkowski et al., 2015; Jasiński et al., 2021, 2022a). Evans et al. (1998) and Hoffmann et al. (2009b) showed that in destructive endometrosis, myofibroblasts destroy the uterine glands, which influence the amount and composition of the extracellular matrix via several secreting mediators. In addition, the impaired contractile activity of the myofibroblasts may result in cyst formation and the retention of secretions in the lumens of the endometrial glands (Evans et al., 1998, Raila et al., 1999, Walter et al., 2001, Hoffmann et al., 2009b). Several studies have shown that connective tissue cells are stimulated to produce and secrete collagen by various endogenous factors, which leads to fibrosis (Alpoim-Moreira et al., 2019, Rebordão et al., 2019, Szóstek-Mioduchowska et al., 2019, Jasiński et al., 2021).

Multiple studies have shown changes in the expression patterns of two specific oestrogen receptors (ERs), ERα and ERβ, and two isoforms of progesterone receptors (PRs), PRA and isoform B (PRB), in mare reproductive tissues (Watson et al., 1992, Aupperle et al., 2000, Hartt et al., 2005, Alm et al., 2009, Hoffmann et al., 2009a, Jasiński et al., 2022b). ER and PR expression in the mare's endometrium peaks during oestrus and begins to decrease in early dioestrus, with the lowest levels detected in late dioestrus (Hartt et al., 2005, Alm et al., 2009). There are limited and contradictory reports on sex steroid receptor expression in endometrosis. For example, Watson et al. (1992) did not detect any changes in ER and PR expression in the mare endometrium. Aupperle et al. (2000) demonstrated asynchronous changes in steroid receptors in the reproductive cycle, which were reflected in reduced ERs and PRs in the glandular nests. Hoffmann et al. (2009a) found a decrease in ER and PR expression in the connective tissue cells in fibrotic foci and cells of nests of glandular epithelium. However, in what is referred to as non-destructive

endometrosis, an increase in ER and PR expression in the epithelial cells of nests has been noted. Jasiński et al. (2022b) showed that transcription levels of ER and PR genes decreased with the severity of endometrial fibrosis.

There are several factors affecting the histo-functional status of the endometrium which appear to contribute to abnormal interactions of endometrial cells leading to infertility in the course of endometrosis. These include abnormal abundance of ERs and PRs in the endometrium (Watson et al., 1992, Aupperle et al., 2000, Hartt et al., 2005, Alm et al., 2009, Hoffmann et al. 2009a, Jasiński et al., 2022b), together with disturbances of endometrial immunity, the composition of the extracellular matrix (Evans et al., 1998, Hoffmann et al., 2009b), myofibroblast contractility (Evans et al., 1998, Raila et al., 1999, Walter et al., 2001, Hoffmann et al., 2009b), the cytoskeleton, and the basement membrane of the glandular epithelium (Kenney and Doing 1986, Hoffmann et al., 2009a, Lehmann et al., 2011).

The localization and expression of the two major cytoskeletal proteins, desmin and vimentin, are routinely used to analyse the morphology of myometrial cells in order to follow dynamic changes during the physiological oestrus cycle and to diagnose its pathology (Can et al., 1995). After fertilization, the equine embryo develops in the oviduct for about 5–6 days (Freeman et al., 1991). The oviduct mucosa should provide a suitable environment for gamete transport, fertilization, and embryo development. The underlying question is whether oviduct inflammation (Losinno et al., 1997) or oviduct fibrosis (Pinto-Bravo et al., 2018) is linked to the periglandular fibrosis and glandular cyst formation found in equine endometria.

We hypothesize that the expression and/or amount and/or localization of intermediate filament proteins or steroid hormone receptor proteins are altered in the uterus and oviduct of mares with endometrosis.

The specific aim of the study was to investigate the expression of the intermediate filaments (vimentin and desmin) and steroid hormone receptors (ERα and PRA) in the stroma and epithelium of the endometria of mares with endometrosis and in the oviduct during the follicular and luteal phases.

MATERIALS AND METHODS

Tissue collection

The study was performed on tissue samples from 58 mares (aged from 2 to 22 years) in a slaughterhouse. Because all samples were collected post mortem, the research did not require the consent of the ethics committee. Blood, uteri, oviducts, and ovaries were collected from mares with unknown reproductive histories, from horse slaughterhouses. Before culling, blood samples were collected from the external jugular vein into ethylenediaminetetraacetic acid-coated tubes. Blood samples were centrifuged (2000 x g, 5 min), and then the plasma was separated and frozen. Immediately after culling, the uterus, oviducts, and both ovaries were collected from each mare. Representative samples (approximately 1 cm^3 each) of mucosa from the uterine horn (near the uterine body) and ampulla of both oviducts were collected immediately after culling and fixed in 4% neutral phosphate-buffered formalin. The ovaries were immersed in cold 0.9% sodium chloride (Polfa S.A., Lublin, Poland) and transported to the laboratory at 4°C. The mares were managed and slaughtered following the welfare mandates of

European Council Regulation (EC) No. 1099/2009 and the Polish Regulation of the Minister of Agriculture and Rural Development (Journal of Laws 2004 No. 205 item 2102).

Assignment of mares to specific phases of the ovarian cycle

Each mare was assigned to the anoestrus or cyclic phase of the reproductive cycle, and cyclic mares were further assigned to the follicular or luteal phase, according to the results of blood progesterone (P4) analysis, morphometric examination of the ovaries, and histological evaluation of the uterus.

In the blood samples, the plasma P4 concentration was determined using a commercial radioimmunoassay (RIA) with an intra-assay coefficient of variation < 5.6% and inter-assay coefficient of variation < 8.8% (Fluorometer 1232 with Progesterone Kit, Wallac Oy, Finland). Morphometric evaluation of the ovaries was performed, taking into account the size and number of ovarian follicles, haemorrhagic corpora, and corpora lutea to determine the phase of the oestrus cycle. Mares were considered to be in the anoestrus phase when small, hard ovaries with no active structures were observed (ovarian follicle < 25 mm or corpus luteum), the P4 concentration was < 1 ng/mL, and the luminal epithelium was low, with no sign of endometrial oestrogenization. Of 58 examined mares, nine met these criteria and were excluded from further investigation.

Mares were considered to be in the follicular phase when a follicle of diameter > 30 mm was present in the ovary, the P4 concentration was < 2 ng/mL, and histological signs of severe to moderate endometrial oestrogenization were observed. Of 58 examined mares, 14 mares met these criteria and were assigned to the follicular phase group. In these mares, luminal cylindrical epithelia (up to 50 μm) containing cytoplasmic vacuoles and endometrial glands arranged in characteristic longitudinal packets were revealed (Fig. 1A, C, E).

Mares were considered to be in the luteal phase when the corpus luteum was present on the ovary, the P4 concentration was > 2 ng/mL, and no signs of endometrial oestrogenization were observed. Of 58 examined mares, 35 met these criteria and were assigned to the luteal phase group. In these mares, a low luminal cylindrical epithelium approximately 20 μm high and well-developed, twisted endometrial glands were observed (Fig. 1B, D, F).

Histopathological staining and analysis of mucosa samples

Tissue samples for histopathological examination were fixed in formalin at 4°C for 24 h and then transferred to 70% ethanol (Sigma-Aldrich, Poland) for one week. Next, the tissue samples were embedded in a paraffin equivalent (Sigma-Aldrich, Poland) for standard histological staining procedures (Witkowski et al., 2021).

Haematoxylin and eosin (H&E)-stained tissue slides of the mucosa were examined under a light microscope (Nikon 104, Nikon, Germany) and classified according to criteria presented by Kenney and Doig (1986) as modifed by Schoon et al. (1997).

Healthy endometria were observed in 26 mares and classified as category I (n = 26), while the microscopic hallmarks of endometrosis in 23 mares were classified as categories IIA (n = 3), IIB (n = 14), and III (n = 6), corresponding to mild, moderate and severe fibrosis, respectively.

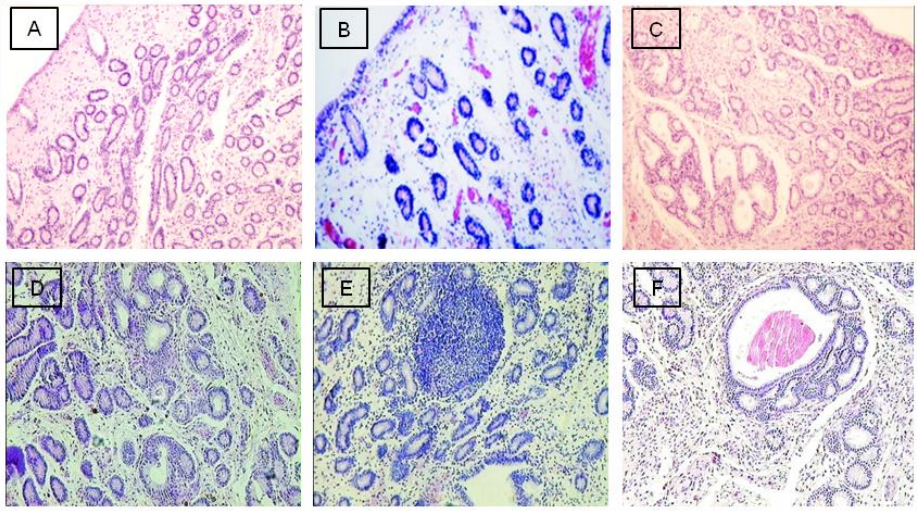


Figure 1. Various categories of endometrosis in two phases of the ovarian cycle. Category I – healthy endometrium in the follicular phase (A) and luteal phase (B); category IIB – moderate endometrosis in the follicular phase (C, E) and luteal phase (D, F); category III – severe endometrosis in the follicular phase and luteal phase (not shown). Randomly chosen representative microphotographs: H&E staining, magnification 40×.

In samples classified as category I, no periglandular fibrous capsule and no glandular nests were observed. In this category, there were signs of endometrial oestrogenization in six samples (Fig. 1A), while no signs were found in 20 samples (Fig.1B). In samples classified as category IIA, two-layer periglandular fibrous capsules formed around individual branches of the uterine glands, and no glandular nests were observed. In this category, signs of endometrial oestrogenization were observed in only three samples. Due to the small size of this group and the lack of representation for both phases of the cycle, the samples classified as group IIA were excluded from further analysis. In samples classified as category IIB, multilayer layers (> 2–3 layers) of the periglandular fibrous capsule formed around individual branches of the uterine glands, and glandular nests (> 1 in the field of view) were observed. In the overgrown stroma, more than 35% of the glands had cystic lesions, and the glandular epithelium was usually hypertrophic. In this category, there were signs of endometrial oestrogenization in five samples (Fig. 1C, E) and no signs in nine samples (Fig. 1D, F). In samples classified as Category III, multilayer layers (> 2–8 layers) of the periglandular fibrous capsule with myofibroblasts formed around individual branches of the uterine glands, and glandular nests (> 2 in the field of view) were observed. In the overgrown stroma, approximately 80% of gland nests were composed of hypertrophic glandular epithelium that produced secretions and 20% of atrophic glandular epithelium. In addition, more than 35% of the glands showed cystic lesions, and the glandular epithelia were usually hypertrophic. In this

category, there were signs of endometrial oestrogenization in three samples, while no signs were seen in the other three samples (not shown).

Finally, after the exclusion of samples in the anoestrus phase (n = 9) and those in category IIA (n = 3), a total of 46 samples were assigned to one of two groups; (i) with healthy endometria (in total n = 26; follicular phase n = 6; luteal phase n = 20), and (ii) with moderate or severe endometrosis (in total n = 20; follicular phase n = 8; luteal phase n = 12). The detailed distribution of samples between categories of endometrosis and specific ovarian cycle phases is presented in Table 1.

Table 1.
Distribution of evaluated samples between categories of endometrosis (Kenney and Doig classification [1]) and specific ovarian cycle phases

	I	**IIB**	**III**	**Total**
Follicular phase	6	5	3	14
Luteal phase	20	9	3	32
Total	26	14	6	46

Kenney and Doig's classification: category I corresponds to healthy endometrium; category IIA corresponds to mild endometrosis; category IIB corresponds to moderate endometrosis; category III corresponds to severe endometrosis; differentiated by ovarian cycle phases: follicular phase, luteal phase, and anoestrus phase.

Immunohistochemical staining and analysis of mucosa samples

Deparaffinized slides with endometrial and oviduct tissues were stained immunohistochemically (IHC) using a previously described protocol (Profaska-Szymik et al., 2020). Briefly, slides were incubated with primary antibodies: vimentin (1:100; clone V9.(1); Merck, Darmstad, Germany), desmin (1:100; clone D33; Merck, Darmstad, Germany), ERα (1:100; clone 6F11; Dako A⁄S, Glostrup, Denmark) or PRA clone 16 (1:100; clone PR4-12; Novocastra Lab, Newcastle, UK). Next, biotinylated antibodies (1: 400; Vector, Burlingame CA, USA) and avidin-biotinylated horseradish peroxidase complex (ABC/HRP; 1:100; Dako, Glostrup, Denmark) were applied. Bound antibodies were visualized with 3,3′-diaminobenzidine (DAB) (0.05%; v/v; Sigma-Aldrich, Poland). For control sections, primary antibodies were omitted and irrelevant IgG was used in their place.

Two blinded, independent observers performed a light microscopic evaluation (Olympus BX40F4, Olympus, Poland) of the IHC–stained slides of mucosa. An immunoreactive score (IRS) was determined for 100 cells in four to five random fields of the serial sections (4–5 μm thick) which had been removed from each animal.

The reaction intensity was assessed as 0 – no reaction, 1 – weak reaction, 2 – moderate reaction, or 3 – strong reaction, and calculated as the percentage of all cells. Therefore, in every specimen, two variables were evaluated: the number of stained cells and the intensity of the reaction according to the formula IRS = ($_{n1}$x1) + ($_{n2}$x2) +($_{n3}$x3), where: n - number of cells showing the reaction with intensity of 0, 1, 2 or 3.

Statistical analysis

Data were tested using the Shapiro–Wilks W test for normality. The homogeneity of variance was assessed with Levene's test. The Mann–Whitney test was used to assess the differences in the variables between the healthy and endometrosis groups as well as between ovarian cycle phases. Data were considered statistically significant at $*P < 0.05$ and $**P < 0.01$. Statistical analyses were performed using JASP software (v0.11.1; JASP Team 2019).

RESULTS

Histological features of oviduct mucosa

In each mare, specimens from both oviduct sites showed similar histopathological features. Intensive folding of the luminal epithelium was present in the follicular phase (Fig. 2A), while there was a low degree of folding in the luteal phase (Fig. 2B). No differences in the oviduct structure were found between the healthy endometria and the endometrosis groups.

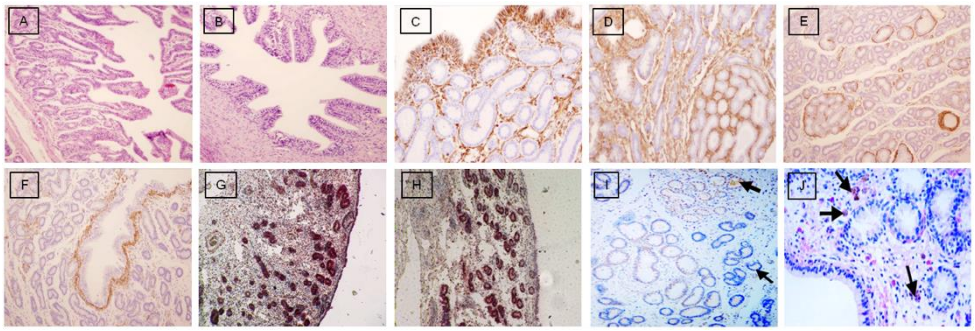


Figure 2. Various views of the fallopian tube mucosa in two ovarian cycle phases: the follicular phase (A) and luteal phase (B). Randomly chosen representative microphotographs: H&E staining, magnification 40×. Various patterns of expression of vimentin (C, D), desmin (E, F), oestrogen receptor α (ERα) (G, H), and progesterone receptor isoform A (PRA) (I, J) in endometrosis. Distribution of vimentin in category IIB – moderate endometrosis in the follicular phase (C) and luteal phase (D). Distribution of desmin in category IIB – moderate endometrosis in the follicular phase (E) and luteal phase (F). Distribution of ERα in category IIB – moderate endometrosis in the follicular phase (G) and luteal phase (H). Distribution of PRA in category IIB – moderate endometrosis in the follicular phase (I) and luteal phase (J). IHC staining, magnification 40×.

Vimentin and desmin expression in the mucosa of the uterus and oviducts

In both groups of mares, healthy and with endometrosis, vimentin-positive cells and desmin-positive cells were observed predominately in endometrial stoma rather than in the epithelia of either group (Fig. 2C-F). Vimentin-stromal positive cells were present in approximately 85% of glandular nests, while these cells were not observed in 15% of the glandular nests. In vimentin-positive nests, hypertrophic glandular

epithelial cells and cystic glandular enlargements were usually noted. Few vimentin-positive cells and desmin-positive cells were observed in the stroma of the oviduct mucosa and luminal epithelium.

In the endometrial stroma, vimentin IRS was higher in the endometrosis group than in healthy mucosa, in both the follicular (P = 0.011) and luteal (P = 0.044) phase (Fig. 3A). This difference was not observed between experimental gropus when samples of oviduct stroma were evaluated (Fig.3D). No difference in vimentin IRS between healthy and affected groups was noted in any of the epithelia, in either the uterus (Fig. 3B, C) or the oviduct (Fig. 3E). Moreover, no difference was found between ovarian cycle phases in any of the tissues (Fig. 3A-E).

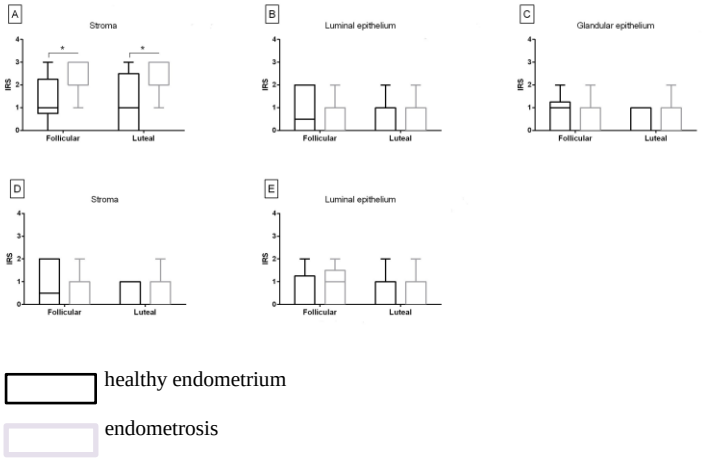
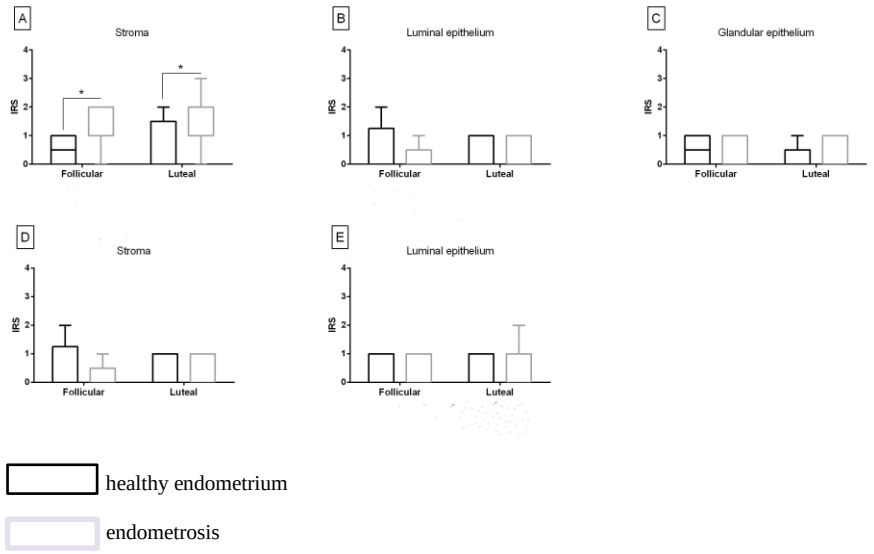


Figure 3. Vimentin IRS in the endometrial stroma (A), luminal epithelium (B), and glandular epithelium (C) and in the oviductal stroma (D) and epithelium (E) in two phases of the ovarian cycle.

Similarly, in the endometrial stroma, desmin IRS was higher in the endometrosis mucosa than in the healthy mucosa. Differences were observed in both the follicular (P = 0.036) and luteal (P = 0.028) phase (Fig. 4A). Desmin IRS did not differ between healthy and affected groups in mucosa epithelia, in either the uterus (Fig. 4B, C) or the oviduct (Fig. 4E), and also did not differ between ovarian cycle phases (Fig. 4A-E).

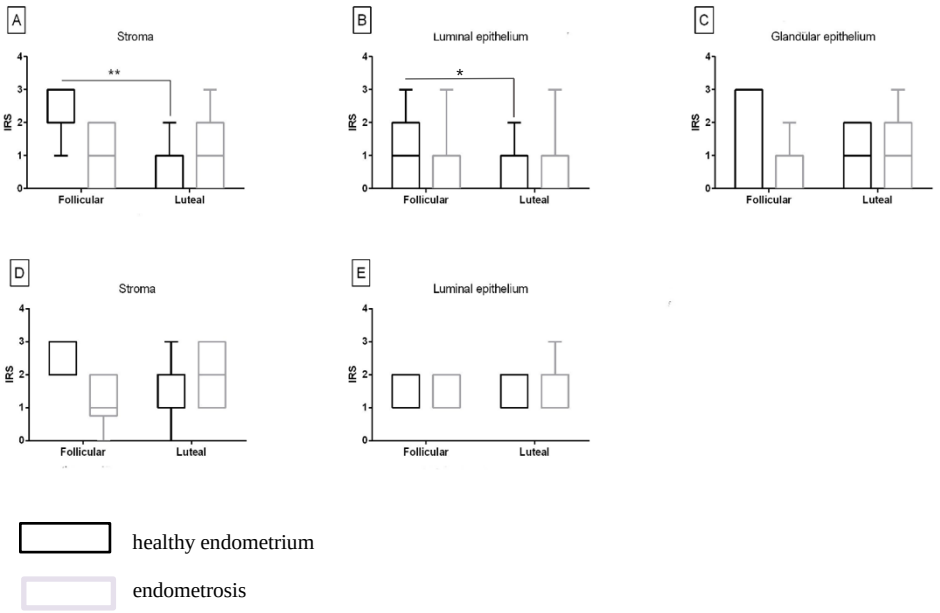


*statistically significant difference $P < 0.05$

Figure 4. Desmin IRS in the endometrial stroma (A), luminal epithelium (B), and glandular epithelium (C) and in the oviductal stroma (D) and epithelium (E) in two phases of the ovarian cycle

ERα and PRA expression in the mucosa of the uterus and oviducts

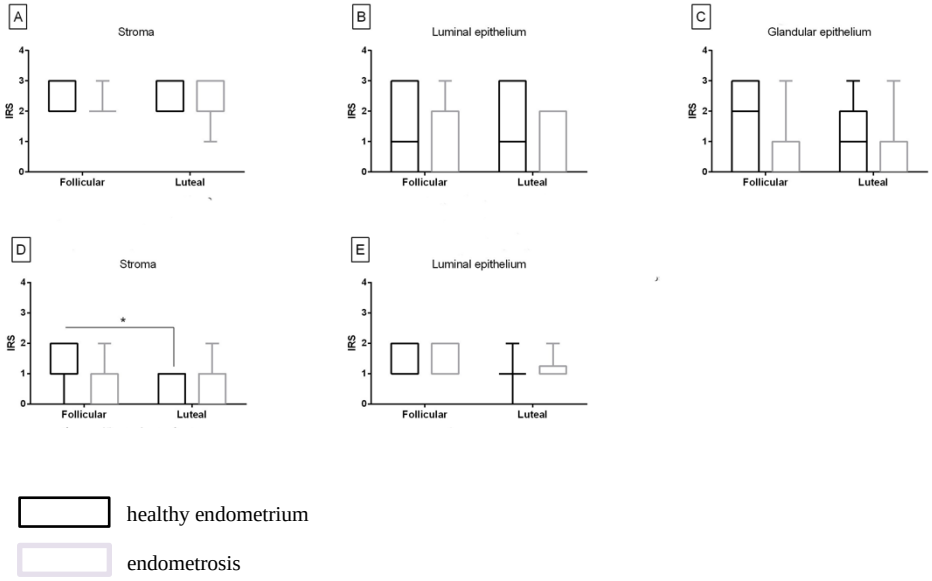
In both groups of mares (healthy and with endometrosis), ERα-positive cells and PRA-positive cells were observed predominately in the endometrial stroma around the uterine glands. More ERα-positive cells were found in the stroma around nested glands in the luteal phase than in the follicular phase. Similarly, more ERα-positive cells were found in unaffected glands in the follicular phase than in the luteal phase (Fig. 2G-H). However, the ovarian cycle-related differences in ERα IRS were observed only in the stroma and luminal epithelium of healthy endometria. In the endometrial stroma, ERα IRS was higher in the follicular phase than in the luteal phase in the healthy group ($P = 0.002$), but not in the affected group ($P > 0.05$) (Fig. 5A). Similarly, in endometrial luminal epithelia, ERα IRS was higher in the follicular phase than in the luteal phase in the healthy group ($P = 0.049$), but not in the affected group (Fig. 5B). No difference was found between ovarian cycle phases in the endometrial glandular epithelium (Fig. 5C) or in the stroma (Fig. 5D) or luminal epithelium (Fig. 5E) of the oviducts.



*statistically significant difference P<0.05
**statistically significant difference P < 0.01

Figure 5. ERα IRS in the endometrial stroma (A), luminal epithelium (B) and glandular epithelium (C) and in the oviductal stroma (D) and epithelium (E) in two ovarian cycle phases.

In the endometrial stroma (Fig. 6A), luminal epithelium (Fig. 6B), and glandular epithelium (Fig. 6C), no differences in PRA IRS were found between the healthy and affected groups in either the follicular or the luteal phase. In the oviduct stroma, PRA IRS was higher in the follicular phase than in the luteal phase in the healthy group ($P = 0.046$), but not in the affected group ($P < 0.05$) (Fig. 6D). No similar difference was found in the oviduct luminal epithelium (Fig. 6E). Moreover, PRA IRS did not differ between healthy and affected groups in either layer of the oviduct (Fig. 6D-E).



*statistically significant difference $P < 0.05$

Figure 6. ERα IRS in the endometrial stroma (A), luminal epithelium (B), and glandular epithelium (C) and in the oviductal stroma (D) and epithelium (E) in two ovarian cycle phases.

DISCUSSION

In horse breeding, endometrosis is diagnosed in about 30% of problematic mares (Mowry et al., 1958). However, just one study has focused on endometrosis and its association with oviduct pathology (Pinto-Bravo et al. 2018). The authors analysed collagen transcriptase distribution in the uterus and oviduct and proposed a possible correlation between oviduct fibrosis and endometrosis. They suggested that the changes observed were most likely to be age-related and concluded that there was no clearly defined relationship between these processes. Therefore, the current study may partially fill a gap in existing research on endometrosis-related oviduct pathologies by studying the expression of the intermediate filament (composed of vimentin and desmin) and steroid hormone receptors (ERα and PRA) in the uterine and oviduct mucosa and comparing them between healthy and endometrosis-affected mares.

The analyses conducted in the present study revealed that changes in the distribution and/or expression of intermediate filament and sex hormone proteins may be one important factor contributing to endometrosis, without affecting the functional status of the oviducts in the disease. We found no histopathological signs of any pathology in either the stroma or luminal epithelium in any of the oviduct samples. The immunohistochemical comparison revealed expected differences in intermediate filament

and sex hormone receptor protein expression between the endometrium and oviduct tissues during endometrosis.

In the endometrial stroma, both vimentin and desmin were expressed in all types of endometrosis, with the expression of vimentin always stronger than that of desmin (Hoffmann et al., 2009). According to findings by Aupperle et al. (1997), vimentin and desmin have higher expression in moderate and severe periglandular fibrosis compared to mildly abnormal fibrotic nests. In the present study, vimentin and desmin expression in the endometrial stroma were higher in mares with endometrosis than in healthy mares. Nests expressing vimentin were composed of hypertrophic glandular epithelial cells and often had cystic glandular enlargements. In contrast, endometrial epithelia did not reveal similar differences. Some studies have shown very high expression of vimentin in the fibrous capsule of the nests as well as in the cells of the epithelial glands in the nests compared to other endometrial cells (Aupperle et al., (1997). Hoffmann et al. (2009a) also observed high expression of vimentin in biopsies from mares with endometrosis. On the other hand, desmin expression in the endometrial stroma has been observed in mild endometrial alterations (Ellenberger et al., 2002) and in endometrosis (Raila et al., 1998, Aupperle et al., 2004), but not in healthy endometria during physiological changes in the oestrus cycle (Aupperle et al., 1997). Our results are consistent with the previous studies, as we found no differences in vimentin and desmin expression between phases of the ovarian cycle in the tissues analysed. The authors cited (Aupperle et al., 1997, 2004, Raila et al., 1998, Ellenberger et al., 2002, Hoffmann et al. 2009a) suggested that vimentin and desmin overexpression in affected endometria may be due to the loss of contact between various types of endometrial cells, including basal lamina cells. Thus, it is believed that the increased expression of the intermediate cellular filaments observed in the endometrial foci plays an important role in the initiation and progression of mare endometrosis. Therefore, Royas et al. (2020) postulated that the expression of the intermediate filament could be used as a diagnostic tool for predicting predisposition to endometrosis, as expression of this marker is highest in mares in the initial stages of this disease. The lack of differences observed in intermediate filament expression in the oviduct indicates that contact between mucosal cells may be maintained. Thus, there is no evidence of alteration of intercellular contact propagated by the uterus and the oviduct microenvironment, where interactions of several different types of signalling molecules take place. Therefore, the two reproductive organs studied here appear to be effectively separated (Pinto-Bravo et al., 2018).

Fibrosis leads to the formation of glandular nests of diverse histological appearance (destructive and non-destructive), with varying secretion activity. This is probably due to the varied sensitivity of cells to endogenous steroid hormones during phases of the ovarian cycle in the nests (Aupperle et al., 1997, Jasiński et al., 2022b, Hoffmann et al., 2009a). The correlation between oestrogens and P4 levels and uterine fibrosis was demonstrated by Ricketts and Barrelet (1997) and confirmed by further research (Watson et al., 1992, Aupperle et al., 1997, Hoffmann et al., 2009a, Jasiński et al., 2022). Ricketts and Barrelet (1997) assumed an association between increases in connective tissue stromal fibrosis in the endometrium and symptoms of hyperoestrogenism. However, other researchers have reported quite different observations related to ERα and PRA expression in endometrial disorders. Özgen et al. (1997) showed that ERα expression in the endometria of mares with hydromucometra was higher than in healthy

mares. Hoffmann et al. (2009a) observed a considerable decrease in the expression of ERs and PRs in endometria affected by fibrosis, except in mares with destructive endometrosis, in which their immunoexpression was higher than in healthy mares. Jasiński et al. (2022b) confirmed a decrease in ER and PR expression associated with the severity of endometrial fibrosis at the gene level, noting low transcription of steroid hormone genes in both inactive and active types of destructive endometrosis. In contrast, Rojas et al. (2020) observed no significant correlation between PRs in the endometrium and mare endometrosis. Similarly, the present study also showed no significant endometrosis-related PRA differences in any of the examined tissues. The only differences observed in the expression of ERα and PRA were associated with the phases of the ovarian cycle and were noted in both the healthy endometrial stroma and luminal epithelium as well as in the healthy stroma of the oviduct mucosa.

Regarding the phases of the ovarian cycle in healthy endometria, our results are in line with previous reports (Jasiński et al., 2022b) indicating that transcription of the ERα gene was higher in the follicular phase than in the luteal phase. This finding was confirmed in the current research on protein levels in the endometrial stroma and luminal epithelium. Jasiński et al. (2022b) also reported changes in ERα expression in mild endometrosis (IIA) as well as the absence of ovarian cycle-related differences in advanced endometrosis (IIB and III). This was also confirmed by our research on the level of this protein (the IIA group was excluded). These ovarian phase-related changes in ERα levels confirm the dysregulation of ERα in the course of endometrosis. Neither Jasiński et al. (2022b) nor the present study found ovarian phase-related differences in PR expression in either healthy or affected endometria. Examination of ERα and PRA expression in the stroma and luminal epithelium of the oviduct in successive ovarian cycle phases showed no differences in ERα levels and only a slight increase in PRA in the follicular phase in healthy mares. However, the diversity observed in the functional state of the glandular epithelial cells in nests suggests a disturbance in differentiation of the glands, probably due to impairment of cell sensitivity to hormonal stimuli (Bigsby, 2002). The altered heterogeneous differentiation of endometrial gland cells, independent of the phase of the ovarian cycle, is a dominant feature of endometrosis, during which damage to the physiological function of the glandular and superficial epithelium can affect embryo development and implantation (Woods et al., 1985, Shlafer et al., 1987, Ricketts and Barrelet 1997, Katila et al., 2022).

In line with Pinto-Bravo et al. (2018), we found here that the ovarian-cycle-related changes in ER and PR expression are less evident in the oviduct than in the uterus. While we found no oviduct pathologies, the pathologies noted in severe endometrosis could be associated with a reduced reaction of the oviducts to pathological hormonal stimulation. Thus there is still an open field for further discussion regarding whether the oviduct is less sensitive to hormonal changes than the uterus. Protein expressional changes and mislocalization can lead to asynchrony in the physiology and diseases of the oviduct and uterus [according to Jasiński et al., (2022a, b)]. In addition, a connection between fibrosis in the oviduct and endometrosis [according to Pinto-Bravo et al., (2018)] is also possible. These mechanisms require further multilevel studies.

This study is limited by the fact that the samples were obtained post mortem, and the reproductive history of the mares was unknown. However, a definitive diagnosis of endometrosis must be based on

histopathological examination, irrespective of whether the tissue material is obtained from a living or dead animal.

In the future, it will be interesting to analyse and compare mRNA expression of vimentin and desmin as well as ERα and PRA in the endometria and oviducts of healthy mares and those with endometrosis in other parts of the uterus and oviduct. Moreover, future research should include an analysis of the interactions of oestrogen and P4 with those proteins, to provide more precise answers regarding the role which these proteins and hormones constituting the local microenvironment play in mare endometrosis induction and progression.

CONCLUSION

This manuscript describes, for the first time, the expression of intermediate filament proteins (vimentin and desmin) and steroid hormone receptors (ERα and PRA) in the equine oviduct in connection with moderate and severe endometrosis. It can be concluded that contact between mucosal cells in the oviduct may be maintained even while signs of moderate and severe endometrosis appear in the endometrium. Moreover, the oviduct seems to be less sensitive to hormonal changes than the uterus. Therefore, a lower reaction to pathological hormonal stimulation may be suspected in the oviduct as compared to the uterus. Based on our results, we postulate that changes in intermediate filament and steroid hormone receptor protein expression and protein mislocalization in endometrosis are some of the leading dysregulating factors, but may contribute to the 'independence' of the oviduct in the course of this disease.

REFERENCES

1. Alm, Y.H., Sukjumlong, S., Kindahl, H., Dalin, A.M. (2009) Steroid hormone receptors ERα and PR characterised by immunohistochemistry in the mare adrenal gland. Acta Veterinaria Scandinavica 51: 31. https://doi.org/10.1186/1751-0147-51-31.
2. Alpoim-Moreira, J., Fernandes, C., Rebordão, M.R., Amaral, A., Pinto-Bravo, P., Bliebernicht, M., Skarzynski, D.J., Ferreira-Dias, G. (2019) Collagens and DNA methyltransferases in mare endometrosis. Reproduction in Domestic Animals 3: 46–52. https://doi.org/10.1111/rda.13515.
3. Aupperle, H., Özgen, S., Schoon, H.A., Schoon, D., Hoppen, H.O., Sieme, H., Tannapfel, A. (2000) Cyclical endometrial steroid hormone receptor expression and proliferation intensity in the mare. Equine Veterinary Journal 32: 228–232. https://doi.org/10.2746/042516400776563554.
4. Aupperle, H., Schoon, D., Schoon, H.A. 2004. Physiological and pathological expression of intermediate filaments in the equine endometrium. Research in Veterinary Science 76: 249–55. https://doi.org/10.1016/j.rvsc.2003.11.003.
5. Aupperle, H., Schoon, H.A., Özgen, S., Klug, E., Bartman, C.P. (1997) Immunohistological methods for characterization of physiological and pathological endometrial functions. Pferdeheilkunde 13: 551.
6. Bigsby, R.M. (2002) Control of growth and differentiation of the endometrium: the role of tissue interactions. Annales New York Academy of Sciences 955: 110-17; discussion 118: 396-406.

7. Can A, Tekelioğlu M, Baltaci A. (1995) Expression of desmin and vimentin intermediate filaments in human decidual cells during first trimester pregnancy. Placenta 16: 261-75. doi: 10.1016/0143-4004(95)90113-2.
8. Ellenberger, C., Aupperle, H., Bartmann, C.P., Hoppen, H.O., Schoon, D., Schoon, H.A. (2002) Endometrial maldifferentiation caused by ovarian disorders in the mare – morphological and immunohistochemical results. Theriogenology 58: 499–502.
9. Evans, T.J., Miller, M.A., Ganjam, V.K., Niswender, K.D., Ellersieck, M.R., Krause, W.J., Youngquist, R.S. (1998) Morphometric analysis of endometria periglandular fibrosis in mares. American Journal of Veterinry Research 59: 1209–14. PMID: 9781449.
10. Freeman, D.A., Weber, J.A., Geary, R.T., Woods, G.L. (1991) Time of embryo transport through the mare fallopian tube. Theriogenology 36: 823. doi: 10.1016/0093-691x(91)90348-h.
11. Hartt, L.S., Carling, S.J., Joyce, M.M., Johnson, G.A., Vanderwall, D.K., Ott, T.L. (2005) Temporal and spatial associations of oestrogen receptor alpha and progesterone receptor in the endometrium of cyclic and early pregnant mares. Reproduction 130: 241–50. https://doi.org/10.1530/rep.1.00596.
12. Hoffmann, C., Bazer, F.W., Klug, J., Aupperle, H., Ellenberger, C., Schoon, H.A. (2009b) Immunohistochemical and histochemical identification of proteins and carbohydrates in the equine endometrium: Expression patterns for mares suffering from endometrosis. Theriogenelogy 71: 264–74. https://doi.org/10.1016/j.theriogenology.2008.07.008.
13. Hoffmann, C., Ellenberger, C., Mattos, R.C., Aupperle, H., Dhein, S., Stief, B., Schoon, H.A. (2009a) The equine endometrosis: new insights into the pathogenesis. Animal Reproduction Science 111: 261–78. https://doi.org/10.1016/j.anireprosci.2008.03.019.
14. Jasiński, T., Zdrojkowski, Ł., Ferreira-Dias, G., Kautz, E., Juszczuk-Kubiak, E., Domino, M. (2022b) Molecular Mechanism of Equine Endometrosis: The NF-κB-Dependent Pathway Underlies the Ovarian Steroid Receptors' Dysfunction. International Journal of Molecular Sience 23: 7360. https://doi.org/10.3390/ijms23137360.
15. Jasiński, T., Zdrojkowski, Ł., Kautz, E., Juszczuk-Kubiak, E., Ferreira-Dias, G., Domino, M. (2021) Equine endometrosis pathological features: Are they dependent on NF-κB Signaling pathway. Animals 11: 3151. https://doi.org/10.3390/ani11113151.
16. Jasiński, T., Zdrojkowski, Ł., Kautz, E., Juszczuk-Kubiak, E., Ferreira-Dias, G., Domino, M. (2022a) The NF-κB-signalling pathway in mare's endometrium infiltrated with the inflammatory cells. Reproduction in Domestic Animals 57: 598–610. https://doi.org/10.1111/rda.14099.
17. Katila, T., Ferreira-Dias, G. (2022) Evolution of the Concepts of Endometrosis, Post Breeding Endometritis, and Susceptibility of Mares. Animals 12: 779. https://doi.org/10.3390/ ani12060779.
18. Kenney, R.M., Doig, P.A. (1986) Equine endometrial biopsy. In: Current Therapy in Theriogenology: Diagnosis, Treatment, and Prevention of Reproductive Diseases in Small and Large Animals. Philadelphia: WB Saunders; 723–729.
19. Lehmann, J., Ellenberger, C., Hoffmann, C., Bazer, F.W., Klug, J., Allen, W.R., Sieme, H., Schoon, H.A. (2011) Morpho-functional studies regarding the fertility prognosis of mares suffering from

equine endometrosis. Theriogenology 76: 1326–36. https://doi.org/10.1016/j.theriogenology.2011.06.001.

20. Losinno, L., Woods, G.L., Miragaya, M.H. (1997) Uterine and fallopian tubeal inflammation in anovulatory mares with ultrasonographicaly – detected intrauterine luminal fluid. Theriogenology 46: 1361–68. https://doi.org/10.1016/S0093-691X(97)00377-4.
21. Mowry, R.W. (1958) Improved procedure for the staining of acidic mucopolysaccharides by Muller's colloidal (hydrous) ferric oxide and its combination with the Feulgen and the periodic acid-Schiff reactions. Lababoratory Investments 7: 566–76. PMID: 13599446.
22. Özgen, S., Rasch, K., Kropp, G., Schoon, H.A., Auperlle, H., Sieme, H., Klug, E. (1997) Aetiopathogenesis and therapy of equine hydromucometra. Pferdeheilkunde 13: 533–34.
23. Pinto-Bravo, P., Rebordão, M.R., Amaral, A., Fernandes, C., Cuello, C., Parrilla, I., Martínez, E., da Costa, R.P., Skarzynski, D.J., Ferreira-Dias, G. (2018) Is mare endometrosis linked to fallopian tube fibrosis? Pferdeheilkunde 34: 43–46. https://doi.org/10.21836/PEM20180107.
24. Profaska-Szymik, M., Galuszka, A., Korzekwa, A.J., Hejmej, A., Gorowska-Wojtowicz, E., Pawlicki, P., Kotula-Balak, M., Tarasiuk, K., Tuz, R. (2020) The G-Protein-Coupled Membrane Estrogen Receptor Is Present in Horse Cryptorchid Testes and Mediates Downstream Pathways. International Journal of Molecular Science 19: 6888. doi: 10.3390/ijms21186888.
25. Raila, G., Aupperle, H., Menger, H., Schoon, D., Schoon, H.A. (1999) Ultarstructural charakterization of equine endometrial stromal cells during the cycle and endometrosis. Reproduction in Domestic Animals 34: 28–35.
26. Raila, G., Aupperle, H., Schoon, H.A., Menger, S., Schoon, D., Mülling, C., Sieme, H., Klug, E. 1998. Endometrosis in the mare: immunohistological and ultrastructural investigations. Reproduction in Domestic Animals 5: 115.
27. Rebordão, M.R., Amaral, A., Lukasik, K., Szóstek-Mioduchowska, A., Pinto-Bravo, P., Galvão, A., Skarzynski, D.J., Ferreira-Dias, G. (2019) Impairment of the antifibrotic prostaglandin E_2 pathway may influence neutrophil extracellular traps-induced fibrosis in the mare endometrium. Domestic Animal Endocrinology 67: 1–10. https://doi.org/10.1016/j.domaniend.2018.10.004.
28. Ricketts, S.W., Barrelet, A. (1997) A retrospective review of the histopathological features seen in a series of 4241 endometrial biopsy sample collected from UK Thoroughbred mares over a 25 year period. Pferdeheilkunde 13: 525–530.
29. Rojas, D., Cabezas, J., Ramírez, G., Saravia, F., Rodriguez-Alvarez, L., Ovidio, Castro, F. (2020) Complimentary Diagnostic Tools for Endometrosis in Biopsies of Mares with Clinical Subfertility. Acta Scientiae Veterinariae 48: 1717, 1–8. https://doi.org/10.22456/1679-9216.98312.
30. Schöniger, S., Schoon, H.A. (2020) The healthy and diseased equine endometrium: A review of morphological features and molecular analyses. Animals 10: 625. https://doi.org/10.3390/ani10040625.
31. Schoon, H.A., Schoon, D., Klug, E. (1997) The endometrial biopsy in the mare with regard to clinical correlations. Pferdeheilkunde 13: 453–64.

32. Shlafer, D.H., Dougherty, E.P., Woods, G.L. (1987) Light and ultrastructural studies of morphological alteriations in embryos collected from maiden and barren mares. Journal of Reproduction and Fertility 35: 695.
33. Szóstek-Mioduchowska, A.Z., Baclawska, A., Okuda, K., Skarżyński, D.J. (2019) Effect of proinflammatory cytokines on endometrial collagen and metallopeptidase expression during the course of equine endometrosis. Cytokine 123: 154767. https://doi.org/10.1016/j.cyto.2019.154767.
34. Walter, A.J., Hentz, J.G., Magtibay, P.M., Cornella, J.L., Magrina, J.F. (2001) Endometriosis: Correlation between histologic and visual findings at laparoskopy. American Journal Obstetrics Gynecology 184: 1407–13. https://doi.org/10.1067/mob.2001.115747.
35. Watson, E.D., Skolnik, S.B., Zanechosky, H.G. (1992) Progesterone and estrogen receptor distribution in the endometrium of the mare. Theriogenology 38: 575–80. https://doi.org/10.1016/0093-691x(92)90020-r.
36. Witkowski, M., Katkiewicz, M., Zając, S., Kochan, J. (2015) Effect of Long-term Hyperimmunization on the Presence of Mast Cells in the Endometrium of the Mare. Journal Equine Veteterinary Science 35: 569–72. https://doi.org/10.1016/j.jevs.2015.03.003.
37. Witkowski, M., Pardyak, L., Pawlicki, P., Galuszka, A., Profaska-Szymik, M., Plachno, B.J., Kantor, S., Duliban, M., Kotula-Balak, M. (2021) Implication of Membrane Androgen Receptor (ZIP9) in Cell Senescence in Regressed Testes of the Bank Vole. International Journal of Molecular Science 1: 7131. doi: 10.3390/ijms22137131.
38. Woods, G.L., Baker, C.B., Hillman, R.B., Schlafer, D.H. 1985. Recent studies relating to early embryonic death in the mare. Equine Veterinary Journal 3: 104–7. https://doi.org/10.1111/j.2042-3306.1985.tb04609.x.