Ovarian histopathological changes in mares with uterine endometrosis

Maria Katkiewicz, Maciej Witkowski¹, Sylwester Zając²

¹Department of Animal Reproduction and Anatomy, Faculty of Animal Breeding and Biology
University of Agriculture in Krakow, 30-059 Krakow, Poland
²Veterinary Clinic, 05-500 Piaseczno, Poland
maria_katkiewicz@sggw.pl

Received: November 19, 2013  Accepted: August 07, 2014

Abstract

The aim of the study was to find out the relationship between the progression of endometrosis and the appearance of the structural microscopic changes in mares’ ovaries. The investigation was performed on slaughtered mares of various age and breed. Four groups each received a portion of the specimens, and designation to group was according to the stage of endometrosis determined on the basis of Kenney’s classification. Uterine and ovarian sections were stained with haematoxylin and eosin. The results of the microscopic evaluation were compared between mares classified into specific Kenney’s categories. It was shown that an increase in ovarian follicular cysts was related to mares’ ages and correlated with significant progression of the endometrosis. These observations suggest that the same aetiological factors may take part both in triggering disorders of ovarian oo/folliculogenesis and in spurring uterine endometrosis. Further more detailed methods of investigation are needed to elucidate the mechanisms of both disease processes.

Keywords: mare, endometriosis, ovary, pathology.

Introduction

For decades, mare endometrosis has been well known, but similarly to endometriosis in women, the aetopathogenesis of its pathological processes is still not precisely defined. In contrast to endometriosis in women, whose salient feature is the pathological proliferation of the interstitial tissue as well as basal endometrial glands and expansion of the endometriotic tissue into various internal organs (the pathway of this process is still under discussion), in endometrosis in the mare, glands proliferate into the entire uterine mucosa and are encapsulated by fibrotic tissue. Hence in mares the term endometrosis is used, in contrary to endometriosis in woman; however, there are similarities in both pathological processes indicated by their both affecting the uterine endometrial cells. When comparing the changes observed in bovine adenomyosis/endometrosis (6) to mare endometrosis, similarities in the pathological processes of both diseases can be found (7, 8) as well. This information may suggest that in these animal species the cause of the disease is due to the same or little-different types of pathological disorders.

In mares a biopsy is used for the diagnosis of the health status of the uterus. In 1978 Kenney divided the stages of pathological changes found in the biopsy specimens into four categories, according to their progression (8). This description of the stages of advancement of the disease observed in the endometrial specimens included further prognosis of the mares’ fertility.

In women, endometriosis is a very serious gynecological problem, which in many cases can lead to infertility through ovarian dysfunction, mostly connected with the failure of oo/folliculogenesis and/or the ovulation process (4). Pathological changes have also been found in the ovaries of cows with uterine adenomyosis/endometriosis (5). There are numerous data concerning mare endometrosis; however, there are no data concerning the potential correlation between the progression of endometrosis and ovarian pathological changes.
The aim of this study was to investigate the microscopic structure of the ovaries in mares with previous diagnosis of uterine endometrosis.

Material and Methods

Specimens of the ovaries and uterus were collected from 58 slaughtered mares. The animals were of various breeds and different ages. The specimens were fixed in 10% buffered formalin and then embedded in paraffin. The microtome slices were stained with haematoxylin and eosin (HE), and by alcian blue periodic acid (AB/PAS).

By applying Kenney’s classification method, the examined mares were divided into four experimental groups according to the extent of pathological changes in the uterus. Healthy mares formed group 1; group 2 was formed by mares matching Kenney’s category IIa; group 3 was formed by mares classified as category IIb; and mares with the lesions typical for category III were assigned to group 4.

Results

Results of the microscopic examination are presented in Table 1. The particular types of lesions were totalled for each group of mares and their percentages were calculated to find possible correlations between particular lesions and endometrial health status. The types of ovarian pathological changes were divided as follows: features of oo/folliculogenesis disorders, pathological changes in ovarian interstitial tissue, and lesions in blood and lymphatic vessels.

In mares of group 1 (n=7), no signs of uterine disease were found (Fig. 1). In two cases, however, secondary interstitial cells were observed. In a few cases, cysts with walls of luteinised cells were also noted. Remnants of the thickened luteinised cells were seen in the ovarian interstitial tissue (Fig. 2). In one mare, the early phase of arteriosclerosis in the gonadal blood vessels was found.

Table 1. Histopathological changes in the ovaries of mares with various categories of endometriosis

<table>
<thead>
<tr>
<th>Kenney’s category</th>
<th>I</th>
<th>IIa</th>
<th>IIb</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mares</td>
<td>7</td>
<td>17</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>Mean age of mares (years) ± SD</td>
<td>0.8 ± 0.16</td>
<td>5.9 ± 4.7</td>
<td>11.3 ± 3.4</td>
<td>15.1 ± 4.7</td>
</tr>
<tr>
<td>Pathological changes in follicles (n/%)</td>
<td>Follicular cyst</td>
<td>-</td>
<td>5/29</td>
<td>7/30</td>
</tr>
<tr>
<td>Granulosa cells luteinisation in preovulatory follicles</td>
<td>-</td>
<td>1/14</td>
<td>2/12</td>
<td>3/12</td>
</tr>
<tr>
<td>Pathological changes in ovarian interstitium (n/%)</td>
<td>Secondary interstitial cells</td>
<td>2/28</td>
<td>5/29</td>
<td>6/25</td>
</tr>
<tr>
<td>Thickened basement membranes</td>
<td>-</td>
<td>2/12</td>
<td>-</td>
<td>1/10</td>
</tr>
<tr>
<td>Remnants of thickened basement membranes</td>
<td>1/14</td>
<td>-</td>
<td>3/18</td>
<td>-</td>
</tr>
<tr>
<td>Pathological changes in blood/lymphatic vessels (n/%)</td>
<td>Thickened blood vessel walls</td>
<td>1/14</td>
<td>-</td>
<td>6/35</td>
</tr>
<tr>
<td>Dilated lymphatic vessels</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1/10</td>
</tr>
</tbody>
</table>

Fig. 1. Mare’s ovary. Secondary interstitial cells with brown cytoplasmic granules disseminated in ovarian interstitial tissue. HE, 10×

Fig. 2. Mare’s ovary. Remnants of thickened follicular basement membranes located in interstitial tissue. AB/PAS, 20×
Mares belonging to group 2 (n = 17) demonstrated endometrosis as for Kenney’s category IIa. As shown in Table 1, pathological lesions indicated disturbances in oo/folliculogenensis, i.e. follicular cysts (Figs. 3 and 5.), cysts with follicular cell luteinisation (Fig. 4), and thickening of the follicular basement membranes in the ovarian follicles.

The ovarian follicular cysts were of various diameters, from 2 to 5 mm. Their microscopic structure was typical for the follicular origin. As seen in Fig. 3, one layer of flattened granulosa cells is located on the usually thickened basement membrane. The cyst is surrounded by cells similar to the cells of the theca folliculi interna.

The disturbances of interstitial cell homeostasis were evinced by the appearance of secondary interstitial cells. The cells possessed brown cytoplasm granules in HE stained sections, and purple-blue granules in sections stained with AB/PAS (Fig. 6).

The pathological changes seen in the blood vessels were manifested by the thickening of vessel walls, due to fibrosis. Dilated lymphatic vessels were observed in one mare.

In the mares of group 3 (n = 24) classified to Kenney’s category IIb, the pathological changes in the ovaries were almost identical to the lesions in mares belonging to category IIa (Table 1).

In the mares of group 4 (n = 10), the extent of uterine pathological changes qualified them for Kenney’s category III. Among the changes, an increase in the follicular cyst index (Table 1) was the one of the most significant features, as compared to the remaining experimental groups. Fibrosis of the blood vessel wall was also noted, but the magnitude of these lesions did not change significantly in comparison to other groups.

**Discussion**

The main task of this research was to find pathological lesions in the mares’ ovarian microscopic
structure which may reflect the disturbances in the oo/folliculogenensis and ovulation processes, and possibly correlate them to the progression of uterine endometrosis. According to previously published data (9), as well as to our previous work (5), there was evidence of the correlation between ovarian function disturbances and simultaneously developing uterine diseases. The results of this research may suggest such a relationship to some degree as well. However, difficulties in drawing final precise conclusions are caused by the type of mare population being investigated. They were of various races, ages, and breeds. Probably these factors were partly responsible for the results’ variability among the groups of mares. To simplify the interpretation of the results, the lesions were presented according to their frequency and shown in percentage. It enabled the data to disclose an increase in follicular cyst formation in the ovaries of mares with a simultaneous increase in the magnitude of pathological changes in the uterus. However, it should be mentioned that the progress of both disease types could be age-related, and that the predisposition to forming ovarian follicular cysts can also be connected with the age of the mare without the prerequisite of progressing endometrosis.

The general opinion presented in the professional literature is that there is no cyst formation in mares’ ovaries, which is the main sign of ovarian function disorders in women and other mammalian species. Mc Cue (11) precisely reviewed ovarian abnormalities in mares’ “cystic ovaries”. According to the cited paper, they are bilateral and reflect some genetic failure, which is also observed in women and many mammals. On the other hand, so-called anovulatory follicles were discussed in literature (11, 13, 14) together with the aetiology and clinical symptoms of other ovarian functional disorders, which can be diagnosed on the basis of the characteristic pathological cell lesions. They were haemorrhagic follicles and anovulatory follicles with luteinisation of the follicular wall (luteinised unruptured follicle (LUF) syndrome). In the cited reviews, diagnoses of ovarian changes were based on the results of ultrasound examination. So, it is evident that the ovarian pathology descriptions cannot be precise. It must be stressed that the ovarian pathological conditions diagnosed in those mares have their counterparts in the specific microscopic structural changes which were described in our paper.

In adult animals, ovaries are exposed to hormonal stimuli, which are responsible for the constant cell activity reflected in the cyclic structural changes. Ovarian cells respond to any disorders present in the physiological stimulation, which is transferred to cells by their hormone-specific receptors. The effects of any hormonal stimulation disorders may be marked in structural signs of the imbalance in ovarian cell homeostasis. Depending on the hormonal stimulation failure, various pathological lesions in ovarian cell structure may develop. The interpretation of these lesions is difficult. The lesions may undergo spontaneous regression or they may persist for a long period, which is then reflected in abnormal ovarian cycles or lack of cycling and infertility in the mare.

Mare endometrosis may be the result of a hormonal imbalance. This uterine disease may be also associated with ovarian changes manifested by disorders in oo/folliculogenensis, ovulation, and formation of the corpus luteum. Interstitial ovarian changes were one of the signs of the affected folliculogenensis in the investigated mares. They were manifested by the thickening of the follicular basement membranes and the presence of dispersed secondary interstitial cells in the interstitial ovarian tissue. The follicular basement membrane is a part of a specialised extracellular matrix, which is a highly organised membrane playing multiple roles in oo/folliculogenensis. In the examined ovaries, the thickened follicular basement membranes were found in follicles, follicular cysts, and their remnants, deposited in the interstitial ovarian tissue. It may indicate some types of follicular injury, as well as the impaired phagocytosis process of the follicular basement membranes. During physiological follicular atresia, basement membranes are phagocytised by interstitial mesenchymal cells. Basement membranes are the product of the epithelial and mesenchymal cells. Under pathological conditions, as observed in the investigated mares, the thickened basement membranes were not phagocytised. Therefore, mentioned types of lesions clearly prove the presence of changes in the cell tissue environment, which can play a role in causing the ultimately necrobiosis-destined follicular lesions.

Pathological changes present in follicular basement membranes were the most significant and easiest to diagnose feature of the affected ovarian oo/folliculogenesis. In these cases it can be assumed that firstly the follicle basement membrane is thickened, so the follicle cannot further differentiate, nor undergo physiological elimination, so it can develop into a cystic follicle. Using these criteria, anovulatory follicles in mares are nothing other than classic follicular cysts, which are a well-known type of ovarian lesion in other mammalian species. If cystic fluid becomes absorbed, the thickened basement membrane remnants remain in the interstitial tissue.

The secondary interstitial cells observed in the examined mares’ ovaries indicated disorders in the interstitial cell homeostasis. There is very scant knowledge concerning the pathological events present in the ovarian interstitial tissue. The secondary interstitial cells have been observed in ovaries in other animal species and their presence was usually related to uterine diseases. Erickson et al. (1) have classified ovarian stromal cells in women, but have not presented an opinion on their significance for the eventual affection of the ovarian function. According to one of the earliest pieces of data (3), the secondary interstitial cells appeared as the result of experimental treatment of
mice with stilboestril, and they remained in the ovary for the animals’ whole lives, causing functional disorders. Uterine endometriosis observed in the investigated mares argues for the pathological character of the ovarian secondary interstitial cells. The noxious activity of oestrogens is considered as one of the most important etiological factors in human endometriosis. These hormones induce various effects on the metabolism of sensitive cells. On the one side, they stimulate proliferation of cells in the reproductive organs, and on the other side, are able to suppress physiological cell apoptosis. It is worth considering if the appearance of secondary interstitial cells reflects a defect in the mechanism of physiological gonad cell apoptosis. However, it also cannot be excluded that they reflect pathological transformation of interstitial cells into cells capable of secreting steroid hormones. Research undertaken so far has left the question of the origin of the interstitial ovarian cells in mares’ ovaries unanswered.

Besides the ovarian lesions, there were microscopic changes, which may suggest LUF-syndrome, a condition described in women with endometriosis (4).

The different endocrine status in the examined mares could be also the cause of follicle haemorrhage, which were found in the investigated ovaries. Ginther et al. (2) described haemorrhagic follicles, which were considered physiological structures in mares, especially during transitional periods of reproductive seasonality. Microscopically, their morphology is similar to LUF-syndrome follicles but they are filled with blood.

Pathological changes observed in the ovarian blood vessels were small and were not significant among the ovarian lesions observed.

In conclusion, it is important to note that of significance was only an increase in the follicular cyst percentage among pathological lesions observed in the mares’ ovaries. This disease process showed a tendency to be age-related. It remains to be determine whether it is in fact connected with the mares’ aging process, especially given that ovaries of immature mares were examined, or is significant for some correlations between ovarian follicular cyst formation and increase in pathological changes in uterine endometriosis. As far as other types of ovarian pathological changes are concerned, there were no signs of their increased frequency, neither with the mares’ age nor with the increase in endometriosis intensity. The main aim of the study was to find out the correlation between the development of the ovarian pathological changes and endometriosis. Additionally, it must be emphasised that in this study the uterine disease was diagnosed in very young mares. This was incongruent with most papers, which indicate that endometriosis is the disease of older multiparous mares (12); however, some authors also diagnosed endometriosis in young mares (10).

References