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Bull Vet Inst Pulawy 58, 141-147, 2014
DOI: 10.2478/bvip-2014-0022

Cytological image of the endometrium in cows in follicular and luteal phases of the ovarian cycle and in cows with follicular and luteal ovarian cysts

Piotr Brodzki, Adam Brodzki[1], Krzysztof Kostro[2], Łukasz Kurek[3], Jan Marczuk[3], Leszek Krakowski

Department and Clinic of Reproduction,
[1]Department and Clinic of Animal Surgery,
[2]Department and Clinic of Infectious Diseases,
[3]Department and Clinic of Internal Medicine,
University of Life Sciences in Lublin, 20-612 Lublin, Poland
wetdoc@interia.pl

Received: May 28, 2013 *Accepted: October 14, 2013*

Abstract

The experiment was conducted on 30 Holstein-Friesian cows: 10 cows in the follicular phase of the cycle and in the luteal phase 10 d later, 10 cows with follicular cysts, and 10 with luteal cysts. The presence of the ovarian structures was confirmed by ultrasonography. Serum levels of progesterone and 17β-oestradiol were tested with ELISA. Samples for cytological examination were collected from the uterus of all cows using a cytological brush. Following staining, the smears were evaluated in terms of quality and percentages of endometrial cells. In the follicular phase of the oestrous cycle, cells of type A – superficial cells (64.6 ± 4.48) were proportionally the largest group of cells. Cells of type C – basal cells (19.8 ± 2.75) were also present. In the luteal phase, the highest percentage of cells was of type B – intermediate cells (76.9 ± 4.26). When follicular cysts were present on the ovaries, the cytology resembled the follicular phase of the cycle, but with many younger type C cells (33.1 ± 4.11). In the case of luteal cysts on the ovaries, the cytology was similar to that of the luteal phase of the cycle, however with a lower percentage of type B cells (58.1 ± 5.71), and a slightly higher percentage of the other types. The differences in the cytological image of the uterus when different ovarian structures are present, depend on the hormonal activity of those structures. Due to the lack of literature data, the results of the study are important as a model, and may substantially facilitate identification of phases of the oestrus cycle, or the pathologies described, as well as indicate the current status of the endometrium.

Key words: cows, uterus, cytology, hormones, ovarian cycle, ovarian cysts.

Introduction

Endometrial cytology is not a method commonly used in gynaecological practice for the diagnosis of reproductive disorders in cattle. This is due to the unusual structure of the cervix, which can make ~~to~~ reaching the uterus difficult, and also due to the unavailability of equipment for collecting material from the uterus. Recently, however, interest in this type of diagnostic method has significantly increased (13, 14). Proper preparation and evaluation of cytological smears depend not only on the experience of the personnel performing the examination, but also on the selection of an appropriate method for obtaining material from the uterus. One of three well-known methods described in the literature can be selected: a swab, a cytological brush, or a uterine flush (11, 14). According to the published data, cytological examination is performed in cows mainly in order to exclude or confirm subclinical endometritis when ultrasonography reveals no irregularities or only slight changes (1, 15). Cytological examination can also be very useful in monitoring the postpartum period in cows (13, 14, 24). It enables the earliest possible detection and treatment of cows with chronic endometritis. The percentage of leukocytes visible in the smear is determined in this case, and an inflammatory state is ascertained depending on the time after calving. Endometritis is indicated 21-33 d after parturition if leukocytes exceed 18%, and 34-47 d after parturition if the percentage exceeds 10 (17, 24). The

cytological examination in such cases is limited to calculating in various ways the percentage of cells characteristic for inflammatory infiltration, *i.e.* leukocytes, mainly segmented neutrophils and lymphocytes. The evaluation involves counting 100-150 of all cells visible in the smear and determining the percentage of leukocytes among them, or counting all cells visible in 10 fields of view and determining the percentage of leukocytes (14, 15, 22). There is no available information in the literature concerning cytological changes in the uterus of cows without inflammatory changes. It seems interesting to create a cytological model of the endometrium representative of the cyclical and pathological changes that take place on the ovaries, which alter the appearance and functioning of the endometrium by producing the hormones associated with these changes. In addition to the ovarian follicle and corpus luteum, which normally occur on the ovary, other structures such as ovarian cysts may disturb the physiology of the oestrus cycle. This disturbance can also change the functioning of the uterus, particularly of the endometrium (3, 20).

The aim of the study was a comparative evaluation of the cytology of the uterus in cows in different phases of the oestrus cycle, and with the most commonly occurring pathologies of the ovaries extant, such as follicular and luteal cysts, and the aim was also a determination of the differences in cytology in these periods.

Material and Methods

The study was conducted on 30 Holstein-Friesian (HF) cows: 10 cows in the follicular phase of the oestrus cycle and in the luteal phase 10 d later, 10 cows with follicular cysts, and 10 with luteal cysts. The cows were kept in the same environmental conditions and were fed and housed in the same manner. All the experimental animals had given birth over 60 d earlier. All cows were at first birth; both birth and postpartum period were normal. Ten cows were given an intramuscular injection of prostaglandin $PGF_{2\alpha}$ (Dinolytic, Zoetis (formerly Pfizer Animal Heath), USA) twice with an 11-day interval, in order to synchronise oestrus in all cows. When signs of oestrus appeared, a sample for cytological examination was collected from the uterus. Ten days after oestrus symptoms subsided, cytological material was collected again from the same cows. In addition to the observation of signs of oestrus, ultrasonography (Honda Electronics Co. Ltd, Japan) was used to confirm the presence of an ovarian follicle in all cows as well as the presence of the corpus luteum. If the cows did not display signs of oestrus following synchronisation, or ultrasonography did not confirm the presence of a follicle or corpus luteum, they were eliminated from the experiment and replaced with cows that had reacted as expected to the pharmaceutical treatment. Follicular and luteal cysts were detected during routine gynaecological examinations of cows with fertility problems. The type of cyst was diagnosed by ultrasonography. Follicular cysts were 3-5 cm in diameter with a thin wall up to 0.3 cm thick, and persisted for at least 10 d. Luteal cysts, 3-5 cm in diameter with a cavity filled with fluid and a thick luteinised wall over 0.5 cm thick, persisted for at least 14 d (3, 20). In addition to the ultrasound diagnosis of the structures appearing on the ovary, serum concentrations of progesterone and 17β-oestradiol were tested to confirm the associated hormonal activity. For this purpose 9 mL of blood was collected from the external jugular vein into standard test tubes (Vacutest Kima srl, Italy). The blood was centrifuged at 2500 g for 10 min., and the serum was stored at -20°C until further analysis. The hormone concentrations were measured by ELISA, using commercial kits (Biomerieux, France) and a Benchmark Plus spectrophotometric microplate reader with Microplate Manager Software v. 5.2.1 (Bio-Rad Laboratories Inc., USA).

The endometrial cytology was performed with a cytological brush (Jiangsu Yada Technology Group Co., China), specially adapted for use in cows. The procedure for collecting the sample for analysis was the same for all experimental animals. After the catheter was passed through the cervix to the base of the uterine horn, the brush was released from the tube and rotated around its long axis within the uterus to collect the sample for analysis. Insertion of the catheter and collection of the sample for analysis were controlled by the other hand through the rectum. After a few rotations, the brush was retracted into the catheter and removed from the uterus in such a way that it did not come into contact with the rest of the genital tract. All elements used to collect the sample for analysis were sterile. Cytological smears were prepared directly from the brushes as imprint specimens on microscope slides. After the smears were completely dried, they were fixed and stained with Hemacolor rapid staining (Merck KGaA, Germany). The samples were then examined under an Olympus CX 41 microscope (Olympus Corporation, Japan), at 1000× magnification.

Cytological evaluation of the smears was based on determination of the type and quality of cells: the presence of cytoplasm, presence of cell nuclei alone, size of cell nuclei, shape of cells and cell nuclei, and presence of additional cells such as leukocytes or erythrocytes, or even mucus or other artefacts visible in the smear. Following an overall qualitative evaluation, the percentages of particular types of endometrial cells were calculated in the samples from different phases of the ovarian cycle and from different pathological statuses in the ovaries.

Statistical analysis. All values are presented as means ± SEM. Statistical analysis was performed using Statistica software (v 6.0) and one-way analysis of variance (ANOVA). The Bonferroni *post-hoc* test was used to compare differences between the investigated groups of cows. The differences between the mean values were considered statistically significant at $P < 0.01$.

Results

Visible differences in the cytological image and pathological changes of the ovaries in different phases of ovarian cycle were observed. The endomet-rial cytology (Table 1) performed during the follicular phase of the oestrus cycle revealed mainly the presence cells of type A – superficial cells (64.6 ± 4.48%), the nuclei of which differed in their degree of degeneration and in size: large (Ad) or small (Am) (Fig. 1a). The second largest group comprised cells of type C – basal cells (19.8 ± 2.75%), having an appearance as shown in Figs 1b and 1c. Cells of type B – intermediate cells, accounting for the smallest percentage of cells (10.4 ± 3.0) were also observed. These cells are illustrated in Figs 2a, 2b, and 2c. Leukocytes, mainly segmented neutrophils (PMN) and single lymphocytes were also observed in the oestrus cycle. During the luteal phase, the cytology of the uterus was completely different, as type B cells were the most numerous (76.9 ± 4.26). Statistical compari-son of the two phases of the oestrus cycle demonstrated that the number of cells of type A, type C, and PMN in the follicular phase was statistically significantly higher ($P < 0.001$), and the number of type B cells lower ($P < 0.001$) than those in the luteal phase of the cycle. Only in the case of lymphocytes was there no statistically significant difference. When follicular cysts were present on the ovary (Table 1), cells of type A were the largest group (50.5 ± 5.23%), type C cells were less numerous (34.5 ± 5.08%), and cells of type B (6.7 ± 4.12%) were the least numerous group. The highest percentage of PMN was noted in the case of these pathological changes (7.4 ± 2.20%). The cytology of the uterus when follicular cysts were present, is shown in Figs 3a, 3b, and 3c. When luteal cysts were on the ovaries, the percentage of different types of cells in the cytological smears was completely different (Table 1 and Figs 4a, 4b, and 4c). The number of type A and C cells, and PMN in the cows with follicular cysts was statistically significantly higher ($P < 0.001$), and the number of type B cells statistically significantly lower ($P < 0.001$), than in cows with luteal cysts. Table 1 presents a comparison of the endometrial cytology in the follicular phase of the oestrus cycle and when follicular cysts were present. In the follicular phase, the number of type A cells was statistically significantly higher ($P < 0.001$), the number of B cells was also higher, but at $P = 0.021$, the percentage of type C cells and PMN was statisti-cally significantly lower ($P < 0.001$). Table 1 presents the comparison of the results of cytology in the luteal phase of the cycle with cytology when luteal cysts are present on the ovary. The values obtained show that in the luteal phase the number of type A cells, type C cells, and PMN was statistically significantly lower (at $P < 0.001$, $P = 0.010$, and $P = 0.008$, respectively). The number of type B cells was statistically significantly higher ($P < 0.001$).

Table 2 presents mean values and standard deviation for progesterone and 17β-oestradiol concentrations in the experimental cows, tabulated by phase of the oestrus cycle and pathological changes of the ovaries. The data indicate a high level of 17β-oestradiol in the follicular phase of the cycle and when follicular cysts were present on the ovary, and a low level of progesterone. In the luteal phase and when luteal cysts were present, a high progesterone level and low values for 17β-oestradiol were noted.

Table 1. Percentages of cell types in the cytological samples from cows in the follicular or luteal phase and cows with follicular or luteal cysts

Cows	Type of cells observed in cytological samples from the uterus					
	A (%)	B (%)	C (%)	PMN (%)	Lymphocytes (%)	Artefacts small (-) or large (+) amount
Cows in the follicular phase (n = 10)	64.6*,# ± 4.48	10.4* ± 3.00	19.8*,# ± 2.75	4.0*,# ± 1.67	1.2 ± 0.87	+
Cows in the luteal phase (n = 10)	13.9## ± 1.92	76.9## ± 4.26	6.7## ± 1.27	1.2## ± 1.46	1.3 ± 0.9	-
Cows with follicular cysts (n = 10)	50.5** ± 5.23	6.7** ± 4.12	33.1** ± 4.11	7.4** ± 2.20	2.3 ± 1.68	++
Cows with luteal cysts (n = 10)	27.1 ± 3.70	58.1 ± 5.71	9.7 ± 3.32	3.0 ± 1.41	2.1 ± 1.58	+

A – cells with atrophic cytoplasm and round nuclei, or cell nuclei alone – round or oval with atrophic changes (superficial cells). B – cells or cell nuclei alone – conical, cylindrical or comma-shaped (intermediate cells). C – cells with distinctly stained cytoplasm and small or large nuclei, from the deeper layers of the endometrium (basal cells). PMC – polymorphonuclear cells. * – statistically significant differences between cows in the follicular phase *versus* cows in the luteal phase. ** – statistically significant differences between cows with follicular cysts *versus* cows with luteal cysts. # – statistically significant differences between cows in the follicular phase *versus* cows with follicular cysts. ## – statistically significant differences between cows in the luteal phase *versus* cows with luteal cysts. $P \leq 0.01$

Table 2. Mean serum levels of progesterone and 17β-oestradiol in the experimental cows

Hormone	Oestrus cycle phase or type of change on ovary			
	Cows in the follicular phase of the oestrus cycle (n = 10)	Cows in the luteal phase of the oestrus cycle (n = 10)	Cows with follicular cysts (n = 10)	Cows with luteal cysts (n = 10)
Progesterone (ng/mL)	1.35** ± 0.83	10.35 ± 2.16	0.89** ± 0.70	8.73 ± 2.16
17β-oestradiol (pg/mL)	19.58** ± 2.44	3.59 ± 1.36	21.23** ± 3.66	6.24 ± 3.90

± standard deviation (SEM)

** – statistically significant differences between cows in the follicular phase *versus* cows in the luteal phase and between cows with follicular cysts *versus* cows with luteal cysts $P < 0.01$

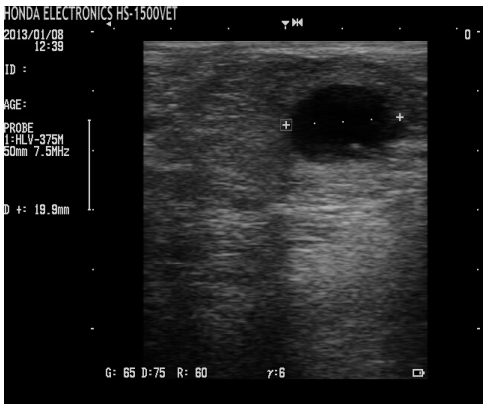


Fig. 1. USG of the ovary with ovarian follicle



Fig. 2. USG of the ovary with corpus luteum

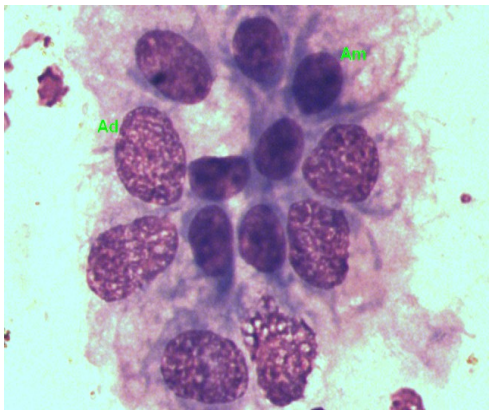


Fig. 1a. Cytology of the uterus in follicular phase, cell type A with atrophic cytoplasm and round small (Am) or large (Ad) cell nuclei

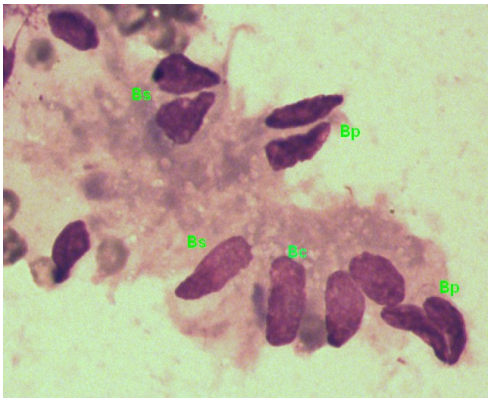


Fig. 2a. Cytology of the uterus in luteal phase, cell type B with comma- shaped (Bp), conical (Bs), or cylindrical (Bc) cell nuclei

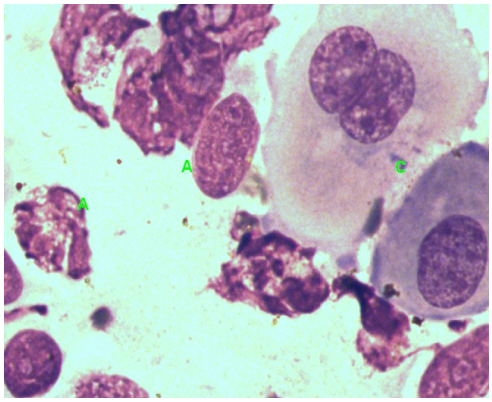


Fig. 1b. Cytology in follicular phase, cell type A (A) with atrophic cytoplasm and round cell nuclei and cell type C (C) with cytoplasm

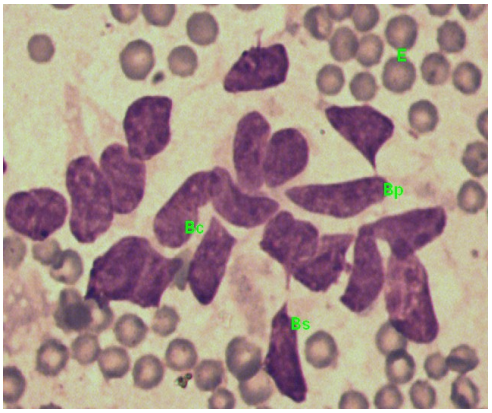


Fig. 2b. Cytology of the uterus in luteal phase, cell type B with comma- shaped (Bp), conical (Bs), or cylindrical (Bc) cell nuclei and erythrocytes (E)

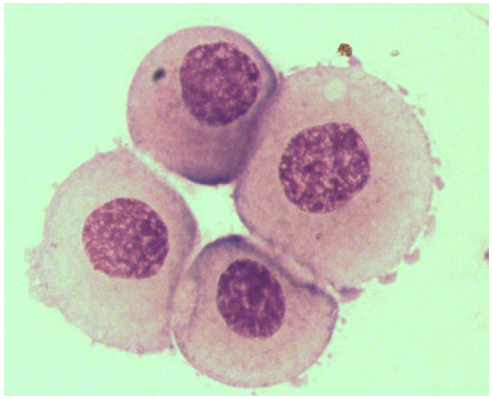


Fig. 1c. Cytology of the uterus in follicular phase, cell type C with clearly visible cytoplasm and round cell nuclei

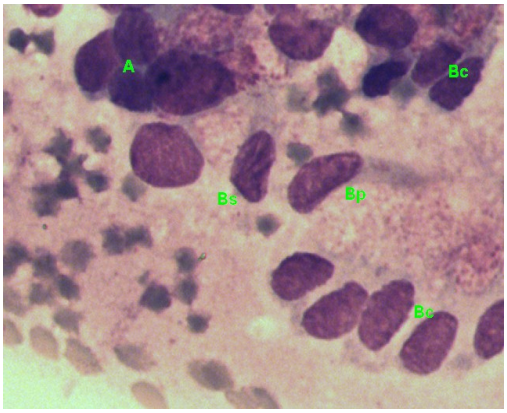


Fig. 2c. Cytology of the uterus in luteal phase, cell type B with comma- shaped (Bp), conical (Bs), or cylindrical (Bc) cell nuclei and cell type A (A)

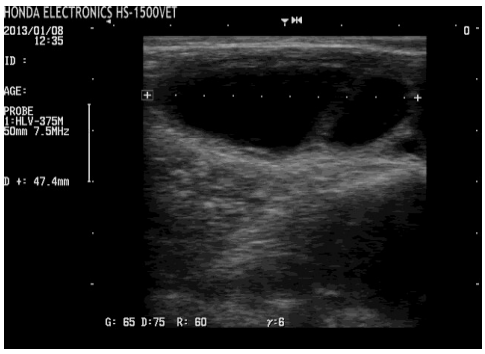


Fig. 3. USG of the ovary with follicular cysts

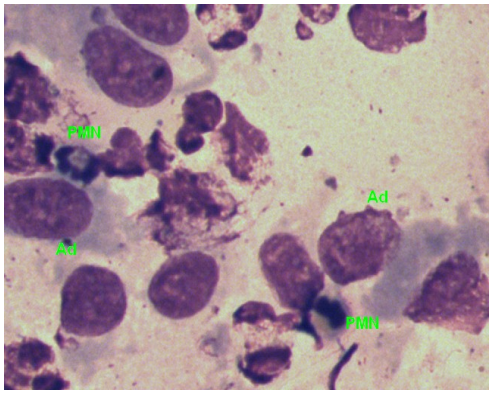


Fig. 3a. Cytology of the uterus in the presence of follicular cysts in the ovary, cell type A with atrophic cytoplasm and round small (Am) or big (Ad) cell nuclei and polymorphonuclear cells (PMN)

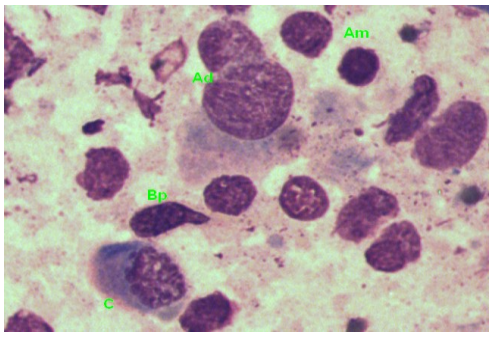


Fig. 3b. Cytology of the uterus in the presence of follicular cysts in the ovary, cell type A atrophic cytoplasm and round small (Am) and large (Ad) cell nuclei, cell type B with comma-shaped (Bp) cell nuclei and cell type C (C)

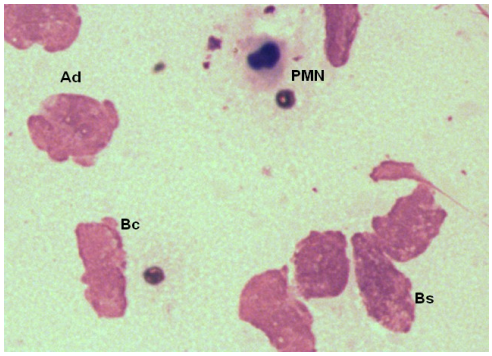


Fig. 3c. Cytology of the uterus in the presence of follicular cysts in the ovary, cells type A with round, large cell nuclei (Ad), cells type B with cylindrical (Bc) and conical (Bs) cell nuclei, and polymorphonuclear cells (PMN)

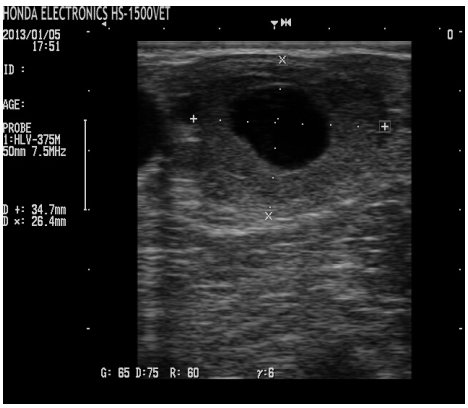


Fig. 4. USG of the ovary with luteal cysts

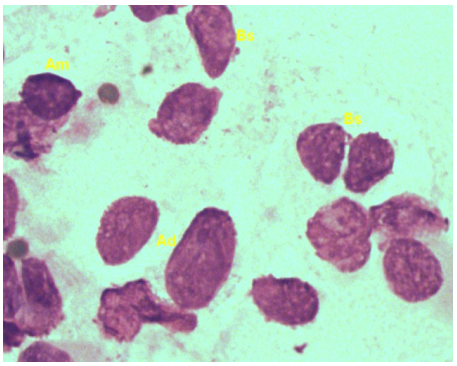


Fig. 4a. Cytology of the uterus in the presence of luteal cysts in the ovary, cell type A with round, small (Am) and big (Ad) cell nuclei and cell type B with conical cell nuclei (Bs)

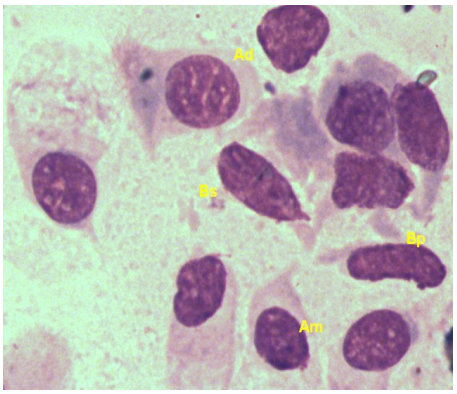


Fig. 4b. Cytology of the uterus in the presence of luteal cysts in the ovary, cell type A, small (Am), and large (Ad) cell nuclei and cell type B with conical (Bs) and comma-shaped (Bp) cell nuclei

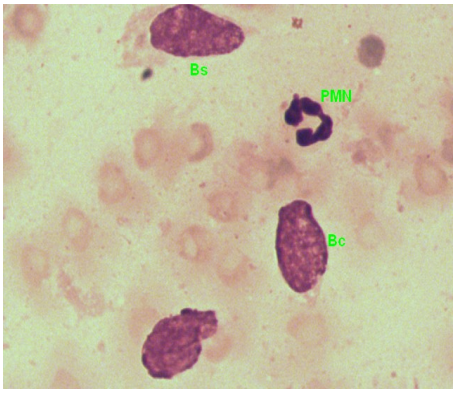


Fig. 4c. Cytology of the uterus in the presence of luteal cysts in the ovary, cell type B with conical (Bs) and cylindrical (Bc) cell nuclei and polymorphonuclear cells (PMN)

Discussion

The results obtained are very difficult to interpret due to the lack of published work dealing with cytological examinations of the uterus in its normal state in animals, particularly cows. Published data only describe cytological examinations used to confirm or exclude endometritis in cows. In these studies, the presence or absence of endometritis was ascertained on the basis of the percentage of leukocytes during different periods after calving (13, 17, 24, 25). The results of the study confirm the authors' assumption that the cytology of the endometrium differs during different phases of the oestrus cycle in cows. The change in the appearance of uterine epithelial cells is undoubtedly linked to hormonal activity, which is dependent on the changing structures on the ovary during the course of the repeating ovarian cycle. It was documented that through their positive or negative effect on the uterine blood supply, hormones indirectly influence the proliferation, necrosis, exfoliation, or regeneration of the endometrial epithelium (7, 9). Oestrogens improve the uterine blood supply and, as cytoprotective hormones, stimulate multiplication of endometrial cells (5, 6). This leads to rapid exfoliation and necrosis of existing epithelial cells and the appearance of young cells in the lumen of the uterus. This was confirmed in the present study. In the follicular phase, when the 17β-oestradiol concen-tration is high and the progesterone concentration is low, the most varied cytology was obtained, with about 20% of young epithelial cells from the deeper layers of the endometrium (type C cells). Various types of epithelial cells exfoliated from the superficial layer, in varying degrees of degeneration (type A cells), were also observed. The endometrial cells with the lowest percentage during this period were type B cells, accounting for only about 10%. The results of cytological examination are typical for the follicular phase of the oestrus cycle, and always depend on the hormonal status described above. During the oestrous cycle in cows, oestrogen acts for only a few days, but the changes occurring in the endometrium are clearly visible. During prolonged oestrogen activity, *e.g.* in the case of follicular cysts on the ovary, these processes become even more accelerated. This was confirmed in the study, as in the endometrial cytology of cows with follicular cysts about 35% of the cells were young epithelial cells, an increased percentage compared to both other types of cells, A and B, due to the rapid divisions in the constantly regenerating endometrial epithelium. Studies by Deligdisch (5, 6), Suvanto-Lukkonen *et al.* (27) and Rutanen (23) have shown that prolonged use of steroid hormones in women can lead to pathological proliferative changes within the epithelium and even in the uterine wall. Sit *et al*. (26) describe a loss of thickness in the endo-metrium in women during menopause, visible even in ultrasonography, which, according to the author, correlates closely with the decreasing oestrogen concentration in the blood. As the corpus luteum gradually develops following ovulation, producing increasing quantities of progesterone, the proliferation processes in the endometrium gradually cease. The very low percentage of young epithelial cells (only about 7%) is the visible effect at this time in the cytological examination, while the percentage of exfoliated epithelial cells typical for this period, with polymorphic cell nuclei, is the highest (about 77%). In the case of luteal cysts present on the ovary, the cytology was reminiscent of the luteal phase, which is probably linked to the dominant role of progesterone in this type of pathological changes (2). However, the effect of oestrogens and an increasing number of young endometrial cells (type C) was visible, as well as exfoliated degenerating cells (type A), with a smaller number of the type B cells characteristic of progesterone activity. It is unknown, however, if this cytological image in the case of luteal cysts was caused by a higher level of 17β-oestradiol or a reduced level of progesterone in comparison with the luteal phase, because both a higher oestradiol level and a lower concentration of progesterone were confirmed by the hormone tests during this period. This pattern in the concentration of the hormones is mostly likely linked to the mechanism by which luteal cysts are formed, through the luteinisation of persistent ovarian follicles or follicular cysts, which is confirmed in the literature (3, 21). The present study and the literature data also show that hormonal activity affects the presence of immune cells in the endometrium itself and in the uterine cavity (12, 18). The elevated level of PMN cells during the follicular phase was found to be highly statistically significant in comparison with the luteal phase. During prolonged oestrogen activity, which occurs in the case of follicular cysts, the number of PMN and lymphocytes increases even more. In the study, this may be linked to the slight inflammation appearing in the uterus, which became more severe as the cystic changes on the ovary persisted. Literature data indicate that some defence mechanisms are weakened during prolonged oestrogenisation in animals (4, 12), but also in the case of elevated progesterone, which makes the uterus more susceptible to infection (10, 18).

Identification of phases of the ovarian cycle and pathological changes occurring on the ovaries in cows is mainly based on rectal examinations, ultrasono-graphy, and hormone tests (2, 16, 19, 28). The present study suggests that these diagnostic methods can be supplemented by endometrial cytology. The results presented constitute model data confirming the phases of the oestrus cycle or the ovarian pathological changes described. Additionally these data indicate the current state of the endometrium. This is significant from a clinical perspective, particularly in cases where diagnosis is doubtful, *e.g.* when signs of oestrus are indistinct or absent, or when the cow fails to become pregnant despite several insemination procedures. Then, ultrasonography detects the presence of the corresponding structure on the ovary, while cytology is used to ascertain whether the endometrium is

responding properly to hormonal activity and its epithelium is exhibiting characteristic changes. It also ensures that the uterus is free of inflammation. A biopsy and histopathological examinations are more precise, but they are also more invasive and time-consuming. Moreover, they must be performed in a specialised laboratory by experienced personnel (8, 24). As the cytological examination described in this paper can be performed quickly and is not highly invasive, it can be done even in the field. Cytological examinations should be conducted routinely, enabling more precise diagnosis through the observation of the differences between the normal state of the endometrium and pathological changes. In clinical practice dealing with reproduction in cattle, confirmation of the normal state of the uterus is the basis for the search for other causes of infertility, and reduces the loss of time, as well as economic losses, associated with unnecessary treatment of animals with suspected endometritis.

References

1. Barański W., Zduńczyk S., Janowski T.: Reproductive performance of cows with subclinical endometritis diagnosed by different cytological thresholds in the postpartum period. Tierärztliche Praxis Grottiere 2013, 1, 15–19.
2. Bartolome J.A., Silvestre F.T., Kamimura S., Arteche A.C.M., Melendez P., Kelbert D., McHale J., Swift K., Archbald L.F., Thatcher W.W.: Resynchronization of ovulation and timed insemination in lactating dairy cows. I. Use of the Ovsynch and Heatsynch protocols after non–pregnancy diagnosis by ultrasonography. Theriogenology 2005, 63, 1617–1627.
3. Brito L.F.C., Palmer C.W.: Cystic ovarian disease in cattle. Large Anim Vet Rounds 2004, 4, 10–12.
4. Brodzki P., Wrona Z., Piech T., Brodzki A.: Effect of cytostatics on the selected parameters of cell–mediated immunity in dogs. Bull Vet Inst Pulawy 2011, 55, 87–93.
5. Deligdisch L.: Effects of hormone therapy on the endometrium. Mod Pathol 1993, 6, 94–106.
6. Deligdisch L.: Hormonal pathology of the endometrium. Mod Pathol 2000, 13, 285–294.
7. Fraser H.M., Wilson H., Silvestri A., Morris K.D., Wiegant S.J.: The role of vascular endothelial growth factor and estradiol in the regulation of endometrial angiogenesis and cell proliferation in the marmoset. Endocrinology 2008, 149, 4413–4420.
8. Garoussi M.T., Sasani F., Hovareshti P.: The histopatological survey of tissue in Holstein dairy cows with or without recorded reproductive disorders. Iranian J Vet Sci Tech 2010, 2, 101–108.
9. Groothuis P.G., Dassen H.H.N.M., Romano A., Punyadeera C.: Estrogen and the endometrium: lessons learned from gene expression profiling in rodent and human. Human Reprod Update 2007, 13, 405–417.
10. Hansen P.J.: Regulation of uterine immune function by progesterone – lessons from the sheep. J Reprod Immunol 1998, 40, 63–70.
11. Hässing M., Steinmann B., Wittenbrink M.M.: Benefit of clinical and laboratory parameters for the diagnosis of endometritis in dairy cows. Schweitz Arch Tierheilkd 2010, 152, 411–416.
12. Hussain A.M.: Bovine uterine defense mechanisms: a rewiev. J Vet Med, Series B 1989, 36, 641–651.
13. Jeremejeva J., Orro T., Valdmann M., Kask K.: Bacteriological and cytological findings during the late puerperal period after two different treatments of retained placenta followed by acute puerperal metritis. Acta Vet Scand 2010, 52, 41–44.
14. Kasimanickam R., Duffield T.F., Foster R.A., Gartley C.J., Leslie K.E., Walton J.S., Johnson W.H.: Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 2004, 62, 9–23.
15. Kasimanickam R., Duffield T.F., Foster R.A., Gartley C.J., Leslie K.E., Walton J.S., Johnson W.H.: A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. Can Vet J 2005, 46, 255–259.
16. Khan F., Nabi S., Pande Megha, Das G., Sarkar M.: Bilateral follicular cysts in a water buffalo. Trop Anim Health Prod 2011, 43, 539–541.
17. LeBlanc S.J., Duffield T.F., Leslie K.E., Bateman K.G., Keefe G.P., Walton J.S., Johnson W.H.: Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci 2002, 85, 2223–2236.
18. Lewis G.S.: Steroidal regulation of uterine immune defenses. Anim Reprod Sci 2004, 82, 281–294.
19. López–Gatius F., López–Bejar M.: Reproductive performance of dairy cows with ovarian cysts after different GnRH and cloprostenol treatments. Theriogenology 2002, 58, 1337–1348.
20. Nelson S.T., Martin A.D., Osteras O.: Risk factors associated with cystic ovarian disease in Norwegian dairy cattle. Acta Vet Scand 2010, 52, 60–64.
21. Peter A.T.: An update on cystic ovarian degeneration in cattle. Reprod Domest Anim 2004, 39, 1–7.
22. Prieto M., Barrio M., Quintela L.A., Perez–Martin C.C., Becerra J.J., Vigo M., Diaz C., Cainzos J., Prieto A., Fernandez F.I., Martinez D., Herradon P.G.: Validation of simple method for the interpretation of uterine cytology in cows. Veterinarni Med 2012, 57, 360–363.
23. Rutanen E.M.: Insulin–like growth factors and insulin–like growth factor binding proteins in the endometrium. Effect of intrauterine levonorgestrel delivery. Human Reprod 2000, 15, 173–181.
24. Sheldon I.M., Levis G.S., LeBlanc S., Gilbert R.O.: Defining postpartum uterine disease in cattle. Theriogenology 2006, 65, 1516–1530.
25. Sheldon M., Cronin J., Goetze L., Donofrio G., Schuberth H.J.: Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol Reprod 2009, 81, 1026–1032.
26. Sit A.S.Y., Modugno F., Hill L.M., Martin J., Weissfeld J.L.: Transvaginal ultrasound measurement of endometrial thickness as a biomarker for estrogen exposure. Cancer Epidemiol Biomarkers Prev 2004, 13, 145–149.
27. Suvanto–Lukkonen E., Sundström H., Penttinen J., Kauppila A., Rutanen E.M.: Insulin–like growth factor–binding protein. 1. A biochemical marker of endometrial response to progestin during hormone replacement therapy. Maturitas 1995, 22, 255–262.
28. Vanholder T., Opsomer G., de Kruif A.: Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a rewiev. Reprod Nutr Dev 2006, 46, 105–119.