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Cytologic and histologic characteristics of endometritis in postpartum dairy cows

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ABSTRACT

Endometritis is a prevalent uterine infection in postpartum dairy cows that causes some alterations in the endometrium. So, the aim of this study was to determine the comparative cellular and histological characteristics of uterus, especially neutrophils index and cell morphological alterations, in healthy and endometritic Holstein cows. Thirty non-pregnant cows were used. Based on uterine discharge score, they were grouped into healthy (nine) and endometritic (twenty one) cows. Uterine discharges were sampled at day 0 of oestrus cycle for studying cellular characteristics. At second day of cycle, larger horn of uteri were sampled for histological investigations. Also, blood samples were collected at days 0, 3 and 9 for measuring of progesterone concentrations changes. Cytological study of uterine discharge showed that the bacterial and neutrophils population ($P<0.01$), and also cell alterations indices ($P<0.05$) were affected in endometritic group. Histologically, the thickness of endometrium, neutrophils infiltration, glands dispersal and percent of fibrotic regions in endometritic cows increased significantly. In addition, staining density ($P<0.01$) and cellular solidarity ($P<0.01$) in endometrial glands were decreased in endometritic cows. Also, the progesterone concentrations were in higher marginal levels during entire experimental period in affected cows. The present results indicated that uterine cytology and endometrial biopsy in postpartum dairy cows might be useful and accurate procedures for detecting existence and severity of endometritis.

Key words: endometritis, cytology, histology, dairy cattle.

INTRODUCTION

In the last decades, linked to increasing milk yield, the reproductive performance has rapidly decreased in dairy cows, especially in the Holstein breed [8]. Although milk yield is negatively associated with reproductive performance, there are other additive factors which affect the fertility in dairy cattle farms, such as animal health condition, management and balanced rations. Additionally, physiologic dysfunctions, such as uterine infections, are elements which are responsible for decreased reproductive performance and fertility in dairy cattle [1, 5, 8].

Uterine infections could be caused by some bacterial species when they are transited to the uterus by vaginal and cervical ways after calving. The previous reports [20, 22] indicated that an estimated number of 90-100% of bacterial uterine infections is considered within the first week after parturition. However, uterine involution, endometrial reconstruction and immune system responses such as bacterial phagocytosis by neutrophils in 3 weeks postpartum play bactericidal roles commonly, but 15-30% of cows are still infected after this period of time [22]. A balanced response of host immune system to pathogen agents prevents diseases caused by microbes, but retained placenta, dystocia, ketosis, stillbirth, displaced abomasum, hypocalcemia and aging are factors which affect the balance between host immune system and bacterial pathogenesis [10, 25]. In this regard, uterine infections usually cause ovarian activity dysfunction by affecting follicle growth and ovulation [17]. They also cause decreased

conception rate and increased mean of time between calving to pregnancy (open days), number of inseminations to pregnancy and culling percent [21, 23]. Cows and heifers which had retained placenta and metritis, produced 300 to 500 kilograms milk lesser than their herdmates [27], and this leads to economic problems for farmers.

Metritis and endometritis are prevalent uterine infections in dairy cows after parturition [10]. Metritis is an acute inflammatory process caused by bacterial infection of uterus during the first 14 days postpartum [14]. Endometritis is an endometrium-limited infection which affects the uterus after at least 21 days postpartum and it is not associated with any systemic diseases sign such as fever [19, 26]. Until now, more than thirty-five bacteria species are known infecting the genital tract after calving [25]. *Arcanobacterium Pyogenes* and *Escherichia coli* are the commonest reported bacteria in the infected uterus and they are suggested as bacteria which cause infertility [1]. Also, *Tritrichomonas foetus* is a flagellate, pyriform protozoan parasite [3] that can contribute in some fertility problems. After microbial challenge, the immune system uses some mechanisms such as Toll-like receptors [22], acute phase proteins, immunoglobulins [7], and polymorphonuclear leukocytes (PMNs) or neutrophils [20, 24] against these infectious elements. Diagnosis of endometritis is somewhat difficult due to lack of unique definition of disease and simple techniques [6]. Transrectal palpation of uterus, vaginoscopy, uterine biopsy and cytology are different ways to detect endometritis [6, 20]. Although in mare, endometrial biopsy is the gold standard method for the endometritis detection, cytological studies by uterus lavage or cytobrush are less invasive in cattle [20]. The PMN migration to the uterine lumen is one of the first responses of immune system to endometritis [17]. Therefore, determination of PMNs proportion to the another cells (plasma cells, neutrophils and endometrial cells) in uterus [2] could help evaluation of endometritis existence and severity in dairy cows. In this regard, the previous studies reported various thresholds for percentage of PMN in this luminal mucus (5% up to 25%, at 21 to 60 days postpartum) [6, 12, 20, 21]. Additionally, cytological study on uterine smears can use to distinguish some cell alterations such as nuclear contour irregularity (shrinking, destruction or duplicating of nucleus) and presence of cytoplasmic vacuoles (vacuolization) that have direct correlation with existence and severity of infection. These cell alterations are used as a diagnostic criterion in Pap smear test in the women [4], however using of them was not reported in cows before. Furthermore, histologically, endometritis is accompanied with destruction of surface epithelium, vascular congestion, stromal edema and variable degrees of inflammatory cells infiltration such as neutrophils [19].

Regarding the high rate of uterine infections after parturition in cattle, using accurate and suitable techniques for detection of infection in early stages of postpartum is so necessary. Therefore, the present study was designed to investigate the cytologic and histologic alterations of uterus in endometritic postpartum dairy cows.

MATERIALS AND METHODS

Animals

This experiment was conducted for comparison of cellular and histological characteristics of uterus in healthy and endometritic cows from July to October 2011. Thirty lactating Holstein cows from a commercial dairy farm located in Esfahan, Iran were selected for this study. The mean of days in milk (DIM) for enrolled cows was 57.3 days (26-79 days). The mean of cows body condition scores was 3 (2.5-3.5, scale 1 to 5) and their average milk yield was 42.1 kg/day. All cows were in second parity, too. Firstly, the oestrus cows, detected by estrous detectors, were assessed for pregnancy and ovaries status by rectal palpation and using ultrasonography. These cows had given birth to female calf and were inseminated at least two times after parturition. They didn't receive any antibiotic or hormone therapy after parturition.

Cytological study

For discharge sampling, the vulva of cows was cleaned with paper towel and uterine mucus samples were collected using a plastic pipette of 10 mL in volume and 35 cm long. Based on ultrasonographic examination and uterine discharge score [10], cows were grouped into the healthy (nine cows) and the endometritic (twenty one cows). Then, mucus was rolled onto a glass slide and air dried. Slides were stained using standard Papanicolaou stain [16] in the farm laboratory. Prepared slides were assessed by light microscope at 400× magnification and plasma cells, neutrophils (PMNs) and endometrial cells were counted at three microscopic squares for determination of PMNs proportion. As described by Matytsina et al. (2010), in line with bacterial population, the microscopic background color of uterine discharge slides change clearly. We scored background color as 1: neutral, 2: light gray, 3: gray, and 4: dark gray.

Histological study

At second day of oestrus cycle, larger uterine horn biopsies were performed, as described previously [22], from nine control and nine endometritic cows. Uterine samples were fixed in formalin (10%) for 24 hours. Then, fixed samples were paraffin-embedded, sectioned at about 10 µm thickness with microtome and stained with haematoxylin and eosin (H&E). Prepared slides were studied with light microscope at 400× magnification for diagnosing differences

between healthy and endometritic cows. Samples were scored as 1 to 4 (mild, moderate, severe and very severe, respectively) for some microscopic characteristics such as neutrophil infiltration, glands and epithelial cellular solidarity, endometrial thickness, number of blood vessels (mean of numbers in three microscopic squares with 100× magnification), endometrial glands dispersal and necrotic regions score as indices for assessing health of tissue, percent of fibrotic regions, and staining density.

Endocrinology

Blood samples were collected from caudal vein, at days 0 (estrus), 3 and 9 of oestrus cycle. Samples were centrifuged at 4000 rpm for 15 minutes. Then, serum was separated and refrigerated at -20 °C for measuring progesterone concentrations.

Statistical analyses

All data were analyzed using the SAS software (SAS Institute, 2001). After testing normality with Shapiro-Wilk test, GLM procedure was used and means were compared with Tukey's test. In the study of neutrophils, bacteria and *Trichomonas foetus* population, celluar alterations index, and progesterone concentrations at days 0, 3 and 9 of oestrus cycle, we used body condition score and DIM as covariables and cows were grouped in two classes; DIM≤60 (n=18) and DIM>60 (n=12). Furthermore, in the study of changes in progesterone concentrations at days 3 and 9, progesterone concentrations at day 0 was used as another covariate. Differences were considered significant at P≤0.05, and P>0.05 but ≤ 0.15 was considered a trend.

RESULTS AND DISCUSSION

Cytologic results

The results of cytologic study on uterine discharge are shown in Table 1. Our findings indicated that the bacterial population scores was significantly higher in endometritic cows than healthy ones (3.28±0.19 vs. 1.20±0.27, P<0.01). *Tritrichomonas foetus* was only observed in endometritic smears, too.

Table 1. Neutrophil and bacterial pouplation scores, cellular alterations, and *Tritrichomonas foetus* numbers in uterine discharge of healthy (n=9) and endometritic (n=21) cows (mean±standard error).

	Neutrophil pouplation (score)	Cellular alterations (score)	Bacterial population (score)	*Tritrichomonas foetus* population (number[c])
Control	1.22±0.24[a]	1.45±0.36[a]	1.20±0.27[a]	0.00±0.17
Endometritic	3.21±0.17[b]	2.68±0.26[b]	3.28±0.19[b]	0.18±0.10
P- value	<0.0001	0.02	0.0001	0.39

[a,b] values with different superscripts within a column are significantly different (P<0.05)
[c]mean of numbers in three microscopic squares with 100× magnification

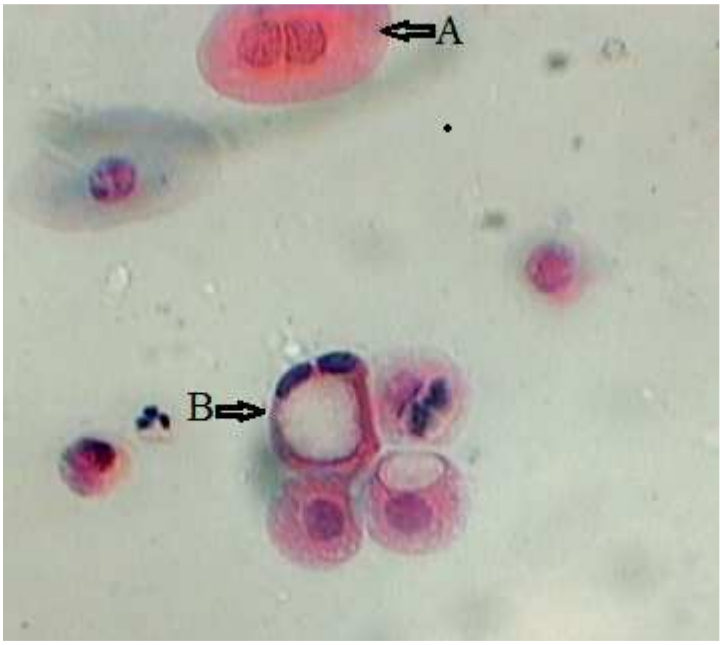

Figure 1. Cellular alterations in uterine discharge of an endometritic cow; A: nucleus duplicating, and B: vacuolization (400× magnification).

On the other hand, neutrophil population was significantly higher in endometritic cows than control ones (3.21±0.17 vs. 1.22±0.24, P<0.0001). As shown in Figure 1, the cell morphological alterations including nuclear contour irregularity and vacuolization that have direct correlation with existence and severity of infection, occurred markedly in endometritic cows (P<0.05), however these alterations existed in the control group slightly.

Histological results

Endometrial histological characteristics in endometritic and healthy cows are shown in Table 2. Endometrial thickness was significantly higher in endometritic than healthy cows (28.11±2.65 µm vs. 16.05±2.65 µm, P<0.01). Endometrial glands dispersal score showed remarkable differences between control and endometritic groups (1.88±0.21 vs. 2.55±0.21, P<0.05). Although the cellular solidarity score of endometrial glands was lower in endometritic cows (1.77±0.24 vs. 3.11±0.24, P<0.01), the percent of fibrotic regions of endometrium increased in this group (2.55±1.51 vs. 7.33±1.51, P<0.05). Significant decreases in score of endometrial staining density was observed in the endometritic cows (3.16±0.25 for healthy and 2.05±0.25 for endometritic cows, P<0.01). However, there was no difference between control and affected cows on endometrium epithelial solidarity score, the numbers of endometrial blood vessels and necrotic regions were slightly more in the endometritic cows (P=0.15). Also, neutrophil infiltration score in comparison with endometritic ones (2.05±0.20) was lower in the endometrium of healthy cows (1.44±0.20, P<0.05)

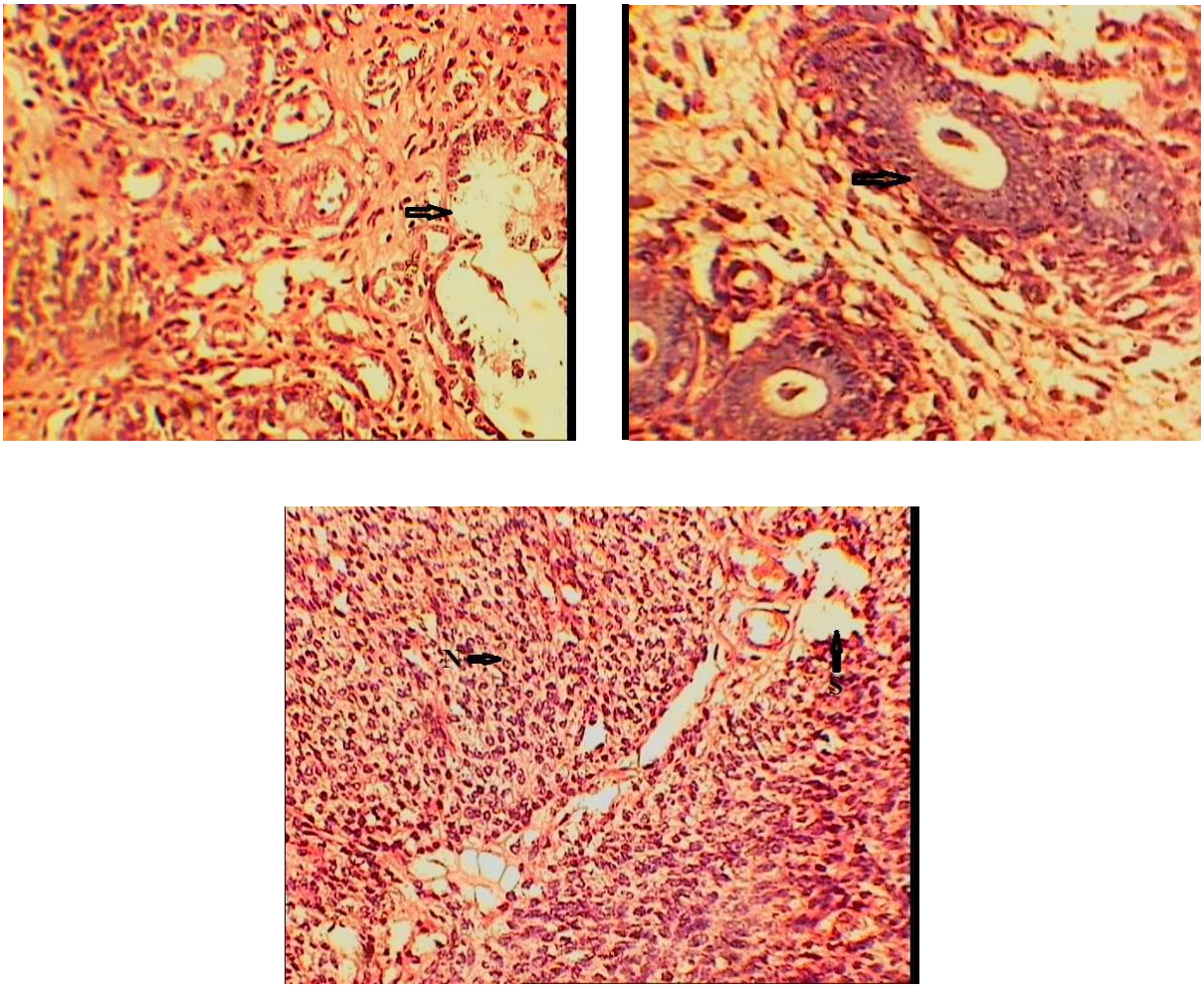

Figure 2. *Above:* Differences between staining density and glands cellular solidarity between healthy (right) and endometritic cows (left), glands are shown with arrows. *Below:* Severe endometrial neutrophil (N) infiltration, and necrotic regions space (S) in an endometritic cow (score 3.5).

Table 2. Histological alterations of uterus in healthy (n=9) and endometritic (n=9) cows (mean±standard error).

	Endometrial thickness (µm)	Endometrial glands dispersal (score)	Cellular solidarity of endometrial glands (score)	Fibrotic regions in endometrium (Percent)	Endometrial blood vessles (number[c])	Endometrial staining density (score)	Epithelial solidarity of endometrium (score)	Necrotic regions (score)	Neutrophil infiltration (score)
Control	16.05±2.65[b]	1.88±0.21[b]	3.11±0.24[a]	2.55±1.51[b]	2.44±0.79	3.16±0.25[a]	2.66±0.28	1.38±0.22	1.44±0.20[b]
Endometritic	28.11±2.65[a]	2.55±0.21[a]	1.77±0.24[b]	7.33±1.51[a]	0.55±0.79	2.05±0.25[b]	2.33±0.28	2.00±0.22	2.05±0.20[a]
P-value	0.005	0.04	0.001	0.04	0.11	0.008	0.41	0.07	0.04

[a,b] values with different superscripts within a column are significantly different (P<0.05)
[c]mean of numbers in three microscopic squares with 100× magnification

Progesterone concentrations

The progesterone concentrations had no significant differences between healthy and endometritic cows at days 0, 3 and 9 of oestrus cycle. However, the progesterone concentrations were in higher marginal levels during entire experimental period in endometritic cows (P=0.15, Figure 3).

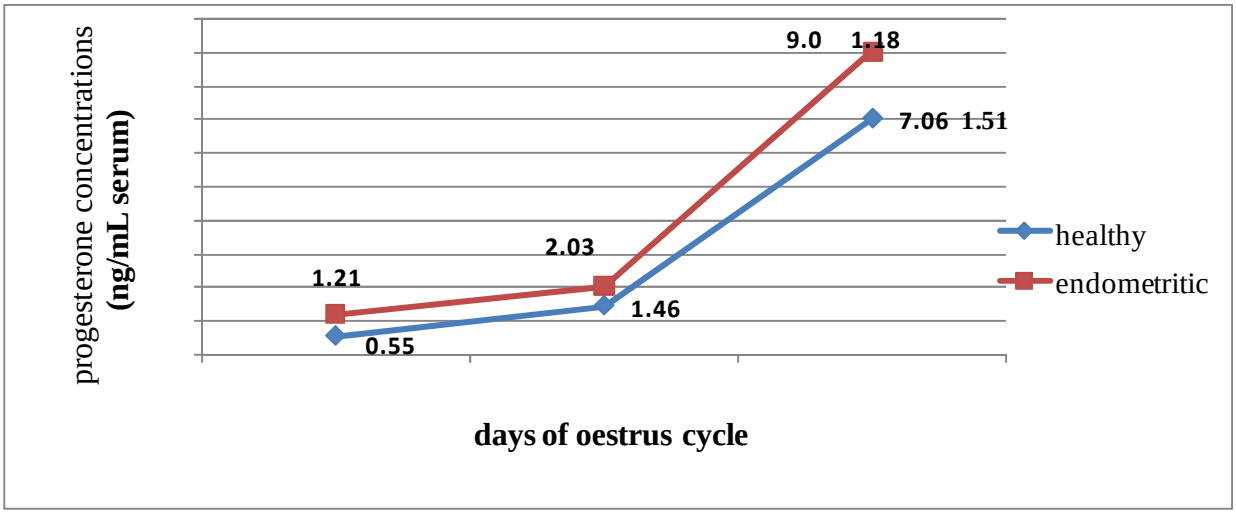

Figure 3. Progesterone concentrations of healthy (n=9) and endometritic (n=21) cows at days 0, 3 and 9 of oestrus cycle (mean±standard error).

Bacterial population in the uterine discharge of endometritic cows was clearly higher than healthy ones (about 2.5 times). Our findings were consistent with the results of Herath et al. (2009) that showed the cows with uterine disease had more bacterial isolates than the fertile cows during the postpartum period (median 4.5 vs 1.0 isolates). Also, *Tritrichomonas foetus* was observed only in endometritic group. It is well demonstrated that this single-celled protozoan may lead to endometritis, pyometra, abortion and some reproductive diseases [11].

Neutrophil population score of uterine discharge was higher (P<0.0001) in endometritic cows than control ones. The PMNs are the most prevalent inflammatory cells in uterine discharge and the main effective cells for removing bacteria from the uterus after calving [10, 20]. Different thresholds for PMN% was reported by previous studies (5-25% at 21 to 60 days postpartum) [6, 12, 20, 21]. Although, the results of Dubuc et al. (2010) suggested that the PMN≥4% could use in dairy cows as an endometritis index for DIM ≤60, there are not appropriate reports for estimating of PMNs proportion for DIM≥60. Therefore, we considered scoring (1 to 4) to use similar method for both periods. In this way, scores 1, 2, 3 and 4 were used for mild, moderate, severe and very severe neutrophils presence, respectively. However, the previous reports [6, 12, 20, 21] suggested that the neutrophil immigration to uterus occur mainly in DIM<60, our data showed that this cytological alterations could continue even after 60 days postpartum. On the other hand, as described above, the microbial populations were in the higher levels in the endometritic cows compared to the healthy ones. This increment could be a reason for enhancing of leukocyte infiltrations as described by other studies [15, 22].

As shown in Table 1, the cytological studies indicated that the cell alterations such as vacuolization and nuclear contour irregularity, as uterine infection criteria, increased in endometritic cows significantly (P<0.05). This finding suggested that not only these indices can be used in the detection of human uterine infections [4], but also they may be exert beside another cytologic criteria in endometritis diagnosing of dairy cows.

On the other hand, endometritis is characterized by mild to moderate neutrophil and lymphocyte infiltration in lamina propria, epithelial disruption, degeneration and necrosis in epithelial cells of some endometrial glands [13, 18, 19]. We used these criteria for histological investigation as described by Chapwanya et al. (2009) In this categorized method, the scores considered as grade 1: an inert uterus with no evidence of inflammation, grade 2: minimal inflammation, few fibrotic areas, and cystic glandular degeneration, grade 3: moderate inflammation, and grade 4: severe inflammation characterized by high infiltration of polymorphonuclear cells, uterine gland atrophy, necrosis and areas with epithelial disruption or loss. Reinforced the previous findings [1, 13, 18] endometrial thickness, neutrophil infiltration, endometrial glands dispersal and percent of fibrotic regions increased markedly in endometritic cows, however the staining density and glands cellular solidarity decreased in this group. In fact, our data showed that the bacterial infection disrupts endometrial structure and function, as mentioned by Sheldon et al. (2009) They indicated that the endometrial cells could secrete cytokines and chemokines for PMNs and macrophages attraction to eliminate the bacteria, and consequently endometrial disruption leads to lasting infection.
In the endometritic cows,.progesterone concentrations were in the higher marginal levels during entire experimental period. There is an association between the occurrence of endometritis and elevated circulating progesterone concentrations in postpartum dairy cows [9]. Luteal phases are often extended in endometritic cows, because the bacteria switch the endometrial epithelial secretion of prostaglandins from the F series to the E series and it causes disruption of luteolysis markedly [10]. Furthermore, it has been demonstrated that the progesterone have strong immunosuppressive properties, by affecting the expression and function of some local regulatory proteins [9, 10]. These alterations might be another reason for persistence of uterine infections in postpartum affected cows.

CONCLUSION

In conclusion, the present results indicated that uterine cytology and endometrial biopsy in postpartum dairy cows might be useful and accurate procedures for detecting existence and severity of endometritis.

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REFERENCES

[1] A. Chapwanya, K. G. Meade, M. L. Doherty, J. J. Callanan, J. F. Mee, C. O. Farrelly, *Theriogenology*, **2009**, 71, 1396.
[2] A. Dourey, M. G. Colazo, P. P. Barajas, D. J. Ambrose, *RES VET SCI*, **2011**, 91, 149.
[3] A. Warton, B. M. Honigberg, *J PROTOZOOL*, **1979**, 26, 56.
[4] B. Smith, G. K. Nguyen GK, Essentials of Pap smear and breast cytology, Vancouver, BC, Canada, **2012**, 1, 1.
[5] C. Fourichon, H. Seegers, X. Malher, *Theriogenology*, **2000**, 53, 1729.
[6] C. S. Barlund, T. D. Carruthers, C. L. Waldner, C. W. Palmer, *Theriogenology*, **2008**, 69, 714.
[7] G. S. Dhaliwal, R. D. Murray, Z. Woldehiwet, *ANIM REPROD SCI*, **2001**, 67, 135.
[8] H. Rodriguez-Martinez, J. Hultgren, R. Bage, A. S. Bergqvist, C. Svensson, C. Bergsten, L. Lidfors, S. Gunnarsson, *Sustained fertility in dairy cows: problems and suggestions*, **2008**, 1.
[9] I. M. Sheldon, H. Dobson, *ANIM REPROD SCI*, **2004**, 82-83, 295.
[10] I. M. Sheldon, J. Cronin, L. Goetze, G. Donofrio, H. J. Schuberth, *BIOL REPROD*, **2009**, 81, 1025.
[11] I. Serin, O. S. Aldemir, A. Ceylan, G. Serin, *J ANIM VET ADV*, **2010**, 9, 1175.
[12] J. Dubuc, T. F. Duffield, K. E. Leslie, J. S. Walton, S. J. LeBlanc, *J DAIRY SCI*, **2010**, 93, 5225.
[13] J. Singh, P. Singla, G. S. Dhaliwal, A. Kumar, H. S. Banga, *INDIAN J ANIM SCI*, **2008**, 78, 710.
[14] K. N. Galvão, N. R. Santos, J. S. Galvão, R. O. Gilbert, *Theriogenology*, **2011**, 76, 290.
[15] L. A. Matytsina, D. E. Greydanus, Y. A. Gurkin, *World J Pediatr*, **2010**, 6, 32.
[16] L. G. Koss, Diagnostic cytology and its histopathologic bases, In: Lippincott JB, **2005,** 4, 1452.
[17] M. G. Colazo, A. Dourey, P. Ponce-Barajas, D. J. Ambrose, *WCDS Advances in Dairy Technology*, **2011**, 23, 367.
[18] M. T. Javed, M. Z. Khan, *IAS*, **1991**, 4, 242.
[19] R. H. Bondurant, *J ANIM SCI*, **1999**, 77, 101.
[20] R. Kasimanickam, T. F. Duffield, R. A. Foster, C. J. Gartley, K. E. Leslie, J. S. Walton, W. H. Johnson, *Theriogenology*, **2004**, 62, 9.
[21] R. O. Gilbert, S. T. Shin, C. L. Guard, H. N. Erb, M. Frajblat, *Theriogenology*, **2005**, 64, 1879.
[22] S. Herath, S. T. Lilly, N. R. Santos, R. O. Gilbert, L. Goetze, C. E. Bryant, J. O. White, J. Cronin, I. M. Sheldon, *REPROD BIOL ENDOCRIN*, **2009**, 7, 55.

[23] S. J. LeBlanc, T. F. Duffield, K. E. Leslie, K. G. Bateman, G. P. Keefe, J. S. Walton, W. H. Johnson, *J DAIRY SCI*, **2002**, 85, 2223.
[24] S. J. LeBlanc, *VET J*, **2008**, 176, 102.
[25] S. McDougall, H. Hussein, D. Aberdein, K. Buckle, J. Roche, C. Burke, M. Mitchell, S. Meier, *Theriogenology*, **2011**, 76, 229.
[26] S. Westermann, M. Drillich, T. B. Kaufmann, L. V. Madoz, W. Heuwieser, *Theriogenology*, **2010**, 74, 1248.
[27] T. Goshen, N. Y. Shpigel, *Theriogenology*, **2006**, 66, 2210.