# CYTOLOGICAL CHARACTERISTICS OF ENDOMETRITIS IN DAIRY CATTLE

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## ABSTRACT

In the last decades, related to increased milk yield, the reproductive performance has rapidly decreased in dairycows, especially in the Holstein breed. Although milk yield is negatively associated with reproductive-performance, there are other additional factors which affect the fertility in dairy cattle, such as animal health-condition, management and balanced rations. Additionally, physiologic dysfunctions, such as uterine infections, areelements which are responsible for decreased reproductive performance and fertility in dairy cattle. The objective of this study was to obtain a clear view over normal cell clusters in cow`s vagina and uterus, so this information will be useful for comparison in future examination related to rapid cytology diagnosis. Neutrophils are the first and most significant inflammatorycell involved in endometritis, but are also foremost duringnormal uterine involution. The inflammatory cell response in cases of subclinical endometritis is widely believed to bequantifiably more severe than that associated with normalinvolution yet milder than clinical endometritis. Such cytological diagnostic approach is useful for both – normal and infected vagina/uterus with or without presence of discharge. Vaginoscopy is a rapid and simple technique for thediagnosis of purulent vaginal discharge. Clear mucus isnormal, whereas purulent and foul-smellingdischarge are indicative of disease. Other ways of detecting uterine dischargehave been studied, including the gloved handand theMetricheck device (Simcrotech, Hamilton, New Zealand).

The results show clear relation between cytological positive diagnosis and affected condition of the reproductive function.

**Key words:** subclincal endometritis, cytological diagnosis, dairy cattle

## Introduction

In the last decades, related to increased milk yield, the reproductive performance has rapidly decreased in dairy cows, especially in the Holstein breed. Although milk yield is negatively associated with reproductive performance, there are other additional factors which affect the fertility in dairy cattle, 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 (Chebel et al. 2007).

The objective of this study was to obtain a clear view over normal cell clusters in cow`s vagina, so this information will be used for comparison in future examination related to rapid cytology diagnosis.

The cytological criteria for the diagnosis of subclinical endometritis continue to be refined, with the postpartum interval for sampling being a key variable (Chapwanaya et al. 2008). Assessments of the severity of inflammation are made by determining the percentage of polymorphonuclear (PMN) cells per 100 cells (PMNs plus endometrial cells) at 400x magnification by method of Barlund et al. They reported that a threshold of more than 8 % PMNs was the lowest proportion of PMNs significantly affecting pregnancy status at 150 days postpartum. Despite the specificity of this threshold at 89.9 %, the sensitivity was poor at 12.9 %, indicating that there are many reasons for nonpregnancy apart from cytological evidence of endometritis.

**Material and methods**

- The specimens were been obtained in two groups of dairy cattle in the town of Troyan in Central North Bulgaria. First group was Mountbeliarde breed with 13 representatives. Second group of 14 Jerseys. Days in milk from 44 to 346. The average BCS in both groups was 2,5 – 3,5 (Mean 2,78±1,41). Age 3 – 8 years. Data for breeding and calving history was taken.

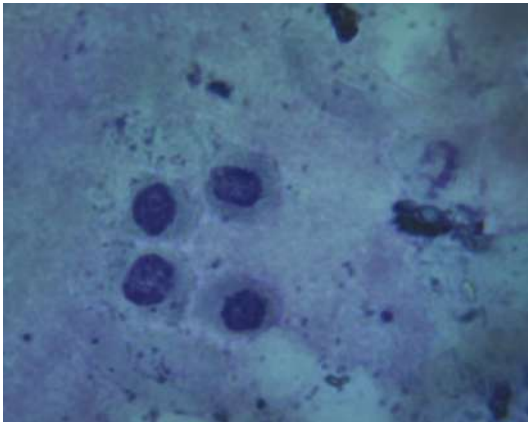The examination protocol was equal to both groups including the following:
- Fixing in metal chute
- Wipe up the external genitalia
- Metricheck sampling and vaginal discharge scoring (Mc Dougall et al 2007)
- Smear preparation on a microscopE slide
- SMEAR Dry off on room temperature
- Staining a Diff Quick dye
- Microscope examination under magnification 40x and 400x with immersion
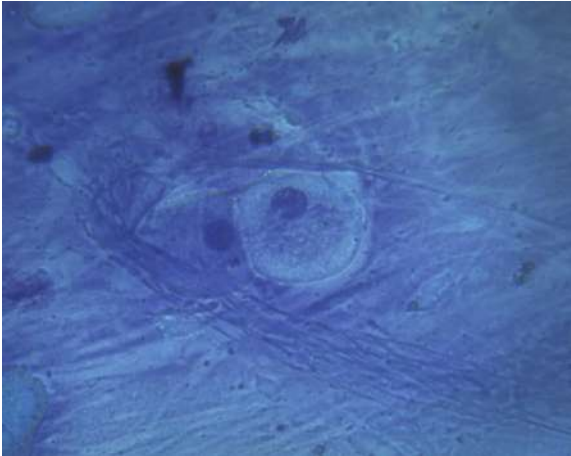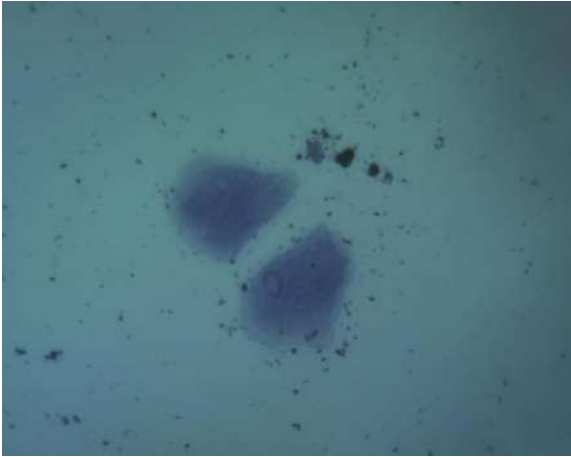
One clinician took all the specimens. Moreover, different 2 pathologist checked all the smears without knowing history of the animals.
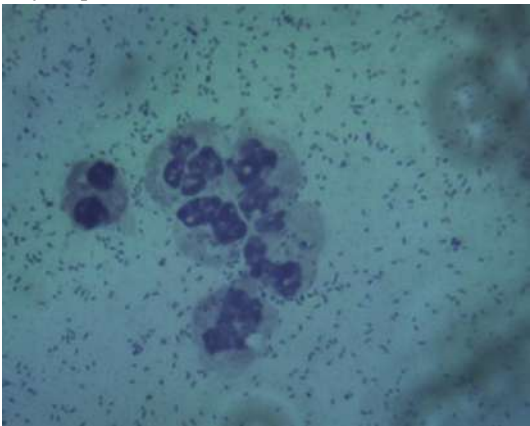
**Results**

Firstly a microscopic findings are presented. The results show the cells of different type in the smears. The different clusters are clearly distinct with specific features summarized in the table 1 – based on the microscopic results.

**Table 1: Vaginal cells characteristics in dairy cattle**

| Cell type | Characteristics |
|---|---|
| **Parabasal cells** | |



| | Parabasal cells are one of the smallest epithelial cells seen in routine vaginal cytologic samples. These cells have a high nuclear-to-cytoplasmic ratio. They have round nuclei and basophilic cytoplasm. These cells are typical for diestrus and anestrus. Large numbers of parabasal cells may be seen normally in vaginal smears of prepubertal animals. |

| Cell type | Characteristics |
| --- | --- |

**Intermediate cells**



The Intermediate cells have variety in size, but in general they have to be twice larger than parabasal cells. Their nuclear-to-cytoplasmic ratio is decreased. These cells have abundant amounts of blue to bluegreen (keratinized) cytoplasm. Depending on the amount of cytoplasm, there are two types of intermediate cells – small intermediate cells and large intermediate cells. Large intermediate cells are sometimes called superficial intermediate cells or transitional intermediate cells.

**Superficial Cells**



The superficial cells are one of the largest epithelial cells seen in vaginal smears. These are dead cells, whose nuclei become pyknotic and then faded, often progressing to anucleate forms. The superficial cells have abundant amounts of light blue to bluegreen (keratinized) cytoplasm, and angular to folded cell borders. Superficial cells with pyknotic nuclei and anucleated superficial cells have the same physiologic significance. Superficial epithelial cells are commonly called cornified cells.

**Mucin**



The Mucin is a neutral polysaccharide comprising glikoproteid. Mucin is a major part of the mucus, which is produced by the mucous glands and the epithelial cells of the mucosa. Mucus with mucin is normally found in routine vaginal cytologic samples from healthy animals.

| Cell type | Characteristics |
|---|---|
| **Polymorphonuclear cells (PMN)**  | The neutrophil nucleus is elongate and separated into multiple lobules by invaginations of the nuclear border. Cytoplasm is clear, pale eosinophilic to faintly basophilic with a fine grainy texture and, rarely, contains a few small vacuoles. |

Secondly the relationship between PMN%, VDS and pregnancy rate was calculated. The results are presented in table 2.

**Table. 2: Relation between clinical condition, PMN percentage and pregnancy rate**

| Clinical condition | PMN % | VDS | PR |
|---|---|---|---|
| Subclinical endometritis | $\leq 7.7$ | $\leq 2$ | not affected |
| Subclinical endometritis | $\geq 8^*$ | $\leq 2$ | affected |
| Clinical endometritis/metritis | $\geq 20$ | $\geq 3$ | affected |
| Normal | $\leq 1$ | $\leq 1$ | not affected |

*p≤0,01
PMN – plymorphonuclear cells; VDS – Vagina Discharge score; PR – pregnancy rate.

## Discussion

Neutrophils are the first and most significant inflammatory cell involved in endometritis, but are also foremost during normal uterine involution. The inflammatory cell response in cases of subclinical endometritis is widely believed to be quantifiably more severe than that associated with normal involution yet milder than clinical endometritis. Such cytological diagnostic approach is useful for both – normal and infected vagina/uterus with or without presence of discharge (LeBlanc 2008).

Vaginoscopy is a rapid and simple technique for the diagnosis of purulent vaginal discharge (PVD). The use of vaginoscopy for the diagnosis of clinical endometritis is based on the premise that purulent exudate present in the cranial vagina is probably the result of drainage from the uterus. The nature of the discharge is important. Clear mucus is normal, whereas purulent (>50 % pus) and mucopurulent (approximately 50 % pus and 50 % mucus) and foul-smelling discharge are indicative of disease. By utilizing vaginoscopy after 26 days postpartum, 44% of cows with an abnormal discharge are been identified that would have otherwise gone unnoticed if only palpation and external examination were used. By delaying the vaginal examination until approximately 1 month after calving, false positives (i.e., cows undergoing normal involution) will be less likely. Other ways of detecting uterine discharge have been studied, including the gloved hand and the Metricheck device

decribed by McDougall 2007 (Simcrotech, Hamilton, New Zealand). These alternatives are at least as efficacious as vaginoscopy and may offer the advantage of detecting exudate that would otherwise go unnoticed, especially in cases where the cranial vagina slopes ventrally. Another practical advantage is that it is much easier for the examiner to avoid being soiled. Those with larger hands and arms may find the gloved hand technique difficult to employ, whereas the Metricheck device is easy to insert and easy to clean between cows. Vaginoscopy, or a similar procedure, offers an immediate result, but fails to identify all cows at risk of poor reproductive performance due to endometritis. Subclinical endometritis cannot be diagnosed by inspection of vaginal exudate; however, if no other screening tests are being used, routine vaginal examination to detect mucopurulent or purulent exudate is a simple, reliable, and cost-effective way to identify cows at risk of impaired reproductive performance. Endometrial cytology, based on the presence of cellular evidence of inflammation, is currently considered to be the most accurate way to diagnose endometritis in cattle, both clinical and subclinical. Inflammatory cells may be recovered by either of two techniques: uterine lavage or cytobrush (LeBlanc 2002).

The microscopic examination supplied and easy and clear approach to the examined organs (vagina, uterus). All the cells discussed were clearly identified by two pathologists with 100 % agreement. Such success could be accepted as a proof for the value of this simple and cheap diagnostic method (McDougall et al. 2011).

The clinical significance of this diagnostic approach was visualized by the results of for the pregnancy rate, VDS and PMN % (table 2). Affected pregnancy rate is associated with both types of endometritis. Cthe clinical cases are easy to be identified with or without additional examination of the discharge. Opposite to the latter most of the subclincal cases persist unidentified. The threshold of 8% PMN in the smear is highly correlated ($p \leq 0,01$) low pregnancy rates in the examined animals.

### Summary of the results

1. A distinct clusters of cell types are produced by cattle`s vagina and uterus.
2. Successful sampling and staining is possible to recognize the cell clusters in cattle`s vagina.
3. Cytology is valuable and inexpensive tool to diagnose the presence of inflammatory cells in cattle`s uterus/vagina.
4. Future examinations are need to develop successful confidential intervals for PMN in the EM cases.

### Conclusion

The represented results are good basement for development of modified cytological methods for subclinical endometritis diagnostics based on the clear distinction in cell clusters and high correlation between PMN% and pregnancy rate.

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