

## DIFFERENT DIAGNOSTIC METHODS FOR UTERIN INFECUNDITY IN DAIRY COWS

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### Abstract

*Different clinical and paraclinical methods were used to investigate some reproductive indices. In this work 42 Holstein cows, between 21 and 62 days in milk (DIM), were examined by rectal palpation (RP), vaginoscopy (V), endocervical (ECC) and endometrial (EMC) cytological exam. Cervicitis was diagnosed when the second cervical fold was swollen and prolapsed with or without reddening. Cytobrush technique and cotton swab technique were used to take cytology samples at 21-33 DIM (PMN  $\geq$ 8%), at 34-47 (PMN  $\geq$ 6%) DIM and 48-62 DIM (PMN  $\geq$ 4%) and stained using Diff-Quick. The percentage of neutrophils in the uterus and the cervix as indicators of inflammation was determined. Also, for reproductive management it was used uterine histopathological (HPT) exam in another 12 puerperal cows. Conventional microscopic examination of the tissue sections allowed visualization of histological changes such as normal structure, infiltrate of polymorphonuclear neutrophils (PMN), hiperemia, moderate acute endometritis, periferal hemorrhages secundar to biposy and hemosiderosis. In total, 78.57% (11/14) of femals had a normal cervix and, after using cytobrush method it was observed that 5 from 14 cows had over 91.08% of PMN. The incidence of subclinical endometritis was recorded as 60.71% (17/28). Another conclusion is that cervical swab cytology is not a good method compared to cytobrush technique that is reliable. Using cytobrush the incidence of subclinical endometritis was recorded as 60.71% (17/28). Endometrial biopsy in postpartum dairy cows might be useful and accurate procedure for detecting existence and severity of endometritis.*

**Key words:** cytology, dairy cow, histopathology, infecundity.

### INTRODUCTION

In recent years, basic research on degenerative endometrial and endocervical diseases or inflammation is well advanced. In contrast to endometritis, the effect of endocervical inflammation or bacterial contamination of the cervix on reproductive performance has been inadequately investigated in dairy cows (Deguillaume et al., 2012; Hartmann et al., 2015).

Regarding the subclinical endometritis it can say that there are the most important reproductive impairments in dairy cows (Pascottini et al., 2017).

Rectal palpation of the uterus, examination of the vagina and *portio vaginalis cervicis* with a speculum, culture of uterine secretions and evaluation of uterine biopsies are the techniques available for diagnosing uterine infections (Garoussi et al., 2010). Cytology is considered the best technique to diagnose endometrial or endocervical inflammation and

it's more noninvasiv then histopathology. Cytology samples are mainly obtained by cytobrush (Singh et al., 2016), cotton swab, uterine biopsy or low-volume uterine lavage, either of them providing similar results (Melcher et al., 2014; Thomé et al., 2016). Both subclinical endometritits and cervicitis are commonly diagnosed during voluntary weiting period before insemination, usually from 21 to 64 DIM (Hammon et al., 2006; Barlund et al., 2008; Deguillaume et al., 2012; Hartmann et al., 2015).

Uterine biopsies can provide meaningful prognostic information about the reproductive health. Evaluation of uterine biopsy samples is not a common practice in cows as compared to mares. However, it is a useful procedure at 26-40 days postpartum (Garoussi et al., 2010). Histologically, endometritis is characterized by disruption of the surface epithelium, infiltration with inflammatory cells, vascular congestion, and stromal edema, with varying degrees of

lymphocyte and plasma cell accumulation in the superficial layers (Meira Jr. et al., 2012). The present study aimed to evaluate and compare cervical and uterine cytology and uterine biopsy in their capacity to establish the correct diagnosis of infecundity.

## MATERIALS AND METHODS

The study population consisted of 42 Holstein cows from two industrial dairy farms in south of Romania. The females were without any clinical signs. Cows were housed in ties stalls and individually fed a total mixed ration (TMR) twice daily and water ad libitum. A close-up diet was fed beginning 3 weeks prior to expected calving date, a fresh cow diet was fed beginning the day of calving through 3 weeks postpartum, and a lactation diet was fed from 3 weeks postparturition.

All the cows were examined by rectal palpation and 14 of them were vaginoscopically examined between 42 and 51 days postpartum. Transrectal palpation served to assess uterine size and symmetry of the uterine horns as well as uterine fluctuation: uterus retractable and horn diameter <2 cm (score 1), 2–5 cm (score 2) or >5 cm (score 3), uterus not retractable but greater curvature palpable (score 4), uterus not retractable and greater curvature incompletely palpable (score 5), and uterus not retractable and greater curvature poorly outlined (score 6) (Hartmann et al., 2016).

After the vulva was cleaned with dry paper towels, a Polanski speculum and flashlight were used for vaginoscopy. Form and colour of the *Portio vaginalis cervicis* were evaluated (Hartmann et al., 2016). Inclusion criteria for further examinations were absence of abnormal vaginal discharge and abnormalities of the uterus (fluctuation) at rectal palpation. Cows that did not meet these criteria were excluded from the study. Cervicitis was diagnosed when the second cervical fold was swollen and prolapsed with or without reddening. All examinations were made by the same veterinarian.

Cytobrush samples from the endometrium (n=28, 21-62 DIM) and cytology (cytobrush and swab cytology) from the cervical mucosa (n=14) were collected using a modified artificial insemination gun with an attached

sterile brush or swab covered from vaginal contamination with a bovine split universal sheath.

Using rectal guidance, the cytobrush was directed through the cervix, then exposed and rolled twice into the endometrium and then covered again with a protective sheath (Pascottini et al., 2017). Once outside the vagina, the cytobrush was removed from the pistol grip, rotated on a paint-coated microscopic slide and fixed (Madoz et al., 2014; Pascottini et al., 2017). For vaginal cytology it was used the Polanski speculum.

Samples were conventionally evaluated by light microscopy at 400x magnification. Stainings were done using Diff-Quick according to the instructions of the manufacturer (Madoz et al., 2014). All the cells from the nine visual fields were counted and differentiated. Subsequently, the percentage of PMN was determined by evaluation of endometrial and cervical cells and PMN. To assess the repeatability of PMN estimates, slides were counted and differentiated by one person.

A total of 12 animals (4-12 DIM) were edometrial biopsied under anesthesia using a human modified colon biopsy instrument. After the cows were restrained, caudal epidural anesthesia was made at the sacrococcygeal space (S5-Co1) using an 18-gauge, 3.8-cm needle (with no syringe attached). After a local iodine asepsia, the needle was directed perpendicular to the skin surface. Once the skin was penetrated, a drop of local anesthetic solution was placed in the hub of the needle (hanging drop technique). The needle was advanced slowly until the anesthetic solution was drawn into the epidural space by negative pressure. The syringe was attached to the needle, and the anesthetic solution slowly injected with no resistance. The dose of local anesthetic used was 0.5 ml per 45 kg (99 lb) of body weight (Lin and Walz, 2014). At the time of biopsy, the perineal area and vulva were washed with soap and rinsed with water. The vulva was then disinfected with a 2% solution of povidone iodine. The forceps was covered with a bovine split universal sheath then was guided through cervix into the previously gravid horn approximately 3 cm past the uterine bifurcation. The jaws of the forceps were opened and medial wall of the uterine

horns were gently pushed laterally into the jaws by the hand which was in the rectum and jaws were shut gently. The biopsy forceps was withdrawn from the genital system. The uterine specimen was removed from the forceps with a needle, placed in 10% formalin for 24 hours and sent for histopathologic examinations. Six  $\mu\text{m}$  thick tissue sections were stained with hematoxylin & eosin and evaluated using an Olympus light microscope.

The influence of cervicitis and endometritis on reproduction indices were investigated.

Reproductive indices included: days to first service, days open (period between calving and conception) (DO), first service conception rate (number of pregnant cows after first insemination: number of first inseminations x 100) (FCR), total conception rate (number of pregnant animals: number of total insemination x 100) (TCR), total pregnancy rate at 120 days into lactation (number of pregnant animals  $\leq$  120 days into lactation: number of inseminated animals) (TPR120), and total pregnancy rate at 140 days into lactation (number of pregnant animals  $\leq$  140 days into lactation: number of inseminated animals) (TPR140).

## RESULTS AND DISCUSSIONS

At transrectal palpation the uterine horns size ranged between scores one and two. In 7.14% (1/14) of cows, the uterus was retractable and horn diameter  $<2$  cm (score1) and 92.85% (13/14) had a uterine horn diameter of 2-5 cm (score 2).

In total, 78.57% (11/14) of femals had a normal cervix. Cervicitis without reddening was diagnosed in 14.28% (2/14) and 7.14% (1/14) of cows displayed cervicitis with reddening of the two cervical fold of the *Portio vaginalis cervicis*. All three cases of cervicitis had all reproductive indices in limit with the group. One of the hypotheses to test in this study was that a correlation exists between diagnostic outputs obtained by vaginoscopy and endocervical cytology. Contrary, it was found a low degree of agreement between the two diagnostic methods. Regarding another similar study, (Hartmann et al., 2016), cervicitis in dairy cows is an independent disease and could have negative effects on reproductive performance. In that article 60.80% of cows

examined had cervicitis between 42 and 50 days post-partum while in previous studies it was estimated a prevalence of cervicitis from 15.60% to 56.90% (9, 17-20 of cervicitis).

In all, 28 endometrial and 7 cervical cytology slides were assessed successfully. Cervical swab cytology was not a good method for cellularity examination because in 7 cows was identified just mucus and rare epithelial cells. After using cytobrush on cervix it was observed that 5 from 14 cows had over 91.08% of PMN. On the other hand, after endocervical cytobrush in seven femals it was identified that 71.42% (5/7) of cows had PMN  $\geq$  5% and 28.57% (2/7) had PMN  $<$  5%. Endometrial cytology was applied on cows 3 times : at 21-33 DIM (PMN  $\geq$  8%) , at 34-47 (PMN  $\geq$  6%) DIM and 48-62 DIM (PMN  $\geq$  4%) (Madoz et al., 2014). In first interval 100% (11/11) of cows had PMN  $\geq$  8%, between 34-47 DIM 50% (4/8) of cows had PMN  $\geq$  6% and in the last interval 22.22% (2/9) of bovines had PMN  $\geq$  4%. The incidence of subclinical endometritis was recorded as 60.71% (17/28). This result recorded in the present study is higher then others from previous studies (Carneiro et al., 2014; Singh et al., 2016).

Table 1. Reproductive indices regarding cervical and endometrial cytobrush technique

Reproductive indices	Cervical cytology	Endometrial cytology
DO	82.21 days	70.10 days
FCR	64.28 %	60.71 %
TCR	100 %	100 %
TPR120	42.85 %	32.14 %
TPR140	92.85 %	89.28 %

Regarding endometrial cytological results there were no correlation with reproductive indices. In contrast, cows with cervical cytology had a increased percent of reproductive indices (DO, FCR, TPR120, TPR140) (Table 1) then cows with endometrial cytology.

In the current study, all cows selected for endometrial biopsy were in good health, and no cow showed any signs of uterine or systemic illness after biopsy.

Conventional microscopic examination of the tissue sections allowed visualization of histological changes such as normal structure, infiltrate of PMN, hyperemia, moderate acute

endometritis, periferal hemorrhagies secundar to biposy and hemosiderosis.

Endometrial biopsy facilitates the collection of tissue samples from an individual cow allowing detailed profiling of cellular changes in response to tissue remodeling and/or pathology in the postpartum period. Even if some authors raported that endometrial biopsy perturb fertility or induce pathology, in this study no side effects on health, estrous cycle or fertility were registered. All these are in agreement with other studies (Chapwanya et al., 2010).

## CONCLUSIONS

As first conclusion, the present results indicated that rectal palpation, vaginoscopy and cytology evaluation of cervix are good methods for cervicitis diagnosis. Another conclusion is that cervical swab cytology is not a good method compared to cytobrush technique that is more reliable. Using cytobrush the incidence of subclinical endometritis was recorded as 60.71% (17/28). Endometrial biopsy in postpartum dairy cows might be useful and accurate procedure for detecting existence and severity of endometritis.

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