INTERNATIONAL JOURNAL OF SYSTEMS APPLICATIONS, ENGINEERING & DEVELOPMENT
DOI: 10.46300/91015.2022.16.7 Volume 16, 2022

Evaluation of the cytology and histology of uterus and cervix as predictors of estrous stages in ewes and dairy cows

Benbia S[1]., Yahia M[1]., Boutelis S[1]., Chennaf A[1]., Yahia Massinissa[1]
[1]Department of Biology, University of El Hadj Lakhdar
[1]Biotechnology's laboratory of the bioactive molecules and the cellular physiopathology
Batna, Republic of Algeria

Abstract—The aim of this study was to describe in ewes and dairy cows, uterine, cervical and vaginal aspect in a physiological context, using cytological and histological techniques as a gold standard. Reproductive tracts of slaughtered cows and ewes were collected from a commercial slaughterhouse in the region of batna. The reproductive phase of each tract was estimated by ovarian performance. Cytobrush samples from the uterus and cervix were prepared. The smears were stained and examined for differential cellular counts. Furthermore, biopsy samples fixed in 10% buffered formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin. The results showed no significant difference (P≥0.05) for cellular densities between cervical and uterine in different phases of estrus cycle (follicular and luteal phases) of the ewes and cows. However, there were significant differences in the percentage of neutrophil cells of uterine and cervix smears in tracts with a corpus luteum (CL) or without a CL. in spite of, the following histological changes were observed during the estrous cycle in cows and ewes. Histological examination was a useful diagnostic method that provided rich detail on endometrial inflammation, and allowed evaluation of different components of the tissue such as the epithelium, lamina propria, endometrial glands, and blood vessels. Therefore, in cows and ewes cervical smears may be used for evaluation of uterine condition.

Key words: ***ewes, dairy cows, cytology, histology, reproductive tracts, estrous.***

I. INTRODUCTION

The bovine uterus, as with all ruminants, is bicornuate. The so-called body of the uterus is short, mostly being formed by incomplete fusion of the caudal parts of the horns that lie side by side, enclosed within a common serosa and muscular coat. Cranially, the two uterine horns, or cornua, have an inverted comma shape, held within the abdomen by the broad ligament, so that the ovaries come to lie adjacent to the bifurcation of the two cornua near the body of the uterus.

During the early stages of pregnancy, secretions from the endometrial glands provide nourishment for the embryo. Hence the anatomy of endometrial glands in cattle at different stages of the oestrous cycle, and the inflammatory changes associated with microbiological contamination of the uterine lumen and endometrium in the postpartum period, has been a frequent object of study [1].

Reproductive tract evaluation in ewes is not generally performed clinically and there are only a few reports on the use of laboratory methods in ewes for the evaluation of the clinical condition of the tract [2].

Uterus is vulnerable to trauma and infection during and after parturition in cows and sheep. Pathogenic bacteria pass through the cervix and contaminate the uterus [3] resulting endometritis. this Pathological changes in the endometrium have been implicated in lowered fertility for several domestic animal species; mares [4] and cattle [3], Many methods are used for the diagnosis of endometritis, including inspection of vaginal discharge, transrectal palpation, transrectal ultrasonography, uterine bacterial culture, uterine biopsy and endometrial cytology [3] [5] [6] [7].

Since the early 1970s, histological examination of endometrial biopsy specimens has been an essential part of investigating lowered fertility in mares , but the technique has been used rarely in cattle and ewes.

Cytological examination of reproductive tract is commonly used to evaluate reproductive lesions in humans and domestic animals [3] [5]. Endometrial samples are collected by using, including cotton swabs, uterine biopsy, uterine lavage and cytobrush technique for cytological examination as an aid in the diagnosis of acute and subclinical endometritis in cows [3] [5] [8].

The objective of this study was to describe in ewes and dairy cows, uterine and cervical aspect in a physiological context, using cytological and histological techniques in cows and ewes.

1. MATERIALS AND METHODS

2. *Animals and source of material*

Animals were obtained from apparently healthy, mature dairy cows and ewes. Intact uteri, cervix and ovaries were collected at a commercial slaughterhouse. The parity of these Animals was unknown.The phase of the oestrous cycle that influenced each animal when it was slaughtered was assessed by the gross appearance of the ovaries. Based on the presence or absence of a corpus luteum (CL) or follicle and their respective sizes, the specimens were divided in a non-luteal group and a luteal group. Selection of specimens for the non-luteal group was based on the following parameters: at least one ovary with a small and hard CL or no CL, a preovulatory follicle or ovulation stigma, a soft swollen

cervix and abundant clear mucus in the vagina. Selection of specimens for the luteal group was based on the following parameters: at least one ovary with a large and soft CL, with either follicle(s) present, a h ard and firm cervix and no mucus present in the vagina. Immediately after slaughter, reproductive tract were removed, p laced o n i ce an d transported to the laboratory.

A. Cytobrush technique

Endometrial and cervical samples for cytologic examination were collected by using a cytobrush (Fishers Scientific). Two slides were prepared by rotating the cytobrush in a clockwise direction while in contact with the uterine and cervix wall. Slides for cytologic examination were prepared by rolling the cytobrush onto a cl ean glass microscope slide, air dried and fixed in methanol 20% for 2 min and stained with May- Grunwald- Giemsa stain for 30 min.

B. Cytological assessment

Cytological assessment was performed by counting a minimum of 100 cells at 100x, 400x and 1000x magnification to determine the percentage of neutrophils (%PMN). Initially the whole slide was assessed and a r epresentative area was selected to determine the %PMN. Slides for cytologic examination were assessed twice. Cows with >5% PMN in the uterine samples were regarded as affected by endometritis (Drillich *et al*., 2005)

C. Tissue preparation and histological techniques

Endometrial and cervical biopsies were placed in fresh buffered formalin for fixation, washed and dehydrated in an ethanol series (70%, 80%, 96%, and 100%), cleared in xylene, and embedded in paraffin wax. Sections 5 mm thick were cut and, after dewaxing with xylene and hydration in an ethanol series of descending concentrations, were then stained with hematoxylin and eosin.

D. Statistical analysis

Differences between cytological percentagein the different phase of oestrous cycle either in the cows and ewes were tested by Student test using Graph pad prism 5 for Window. Data were presented as (mean ±SEM) and $p<0.05$ were considered significant.

II. RESULTS AND DISCUSSION

The objectives of this study were to compare the cyclic changes of the uterus and cervical sample in the cows and ewes used the cytobrush technique and histological methods for the diagnosis of endometritis, which adversely effects reproductive performance and causes economic loss.

A. Cytology

Slides prepared for endometrial and cervical mucosa cytologic examination were all readable and assessed successfully. The counted cells were epithelial cells (EC), large vacuolated epithelial cells (LVEC) and neutrophil (PMN) and lymphocyte. Data showing cell percentages of dairy cows samples are presented in table I.

TABLE I
MEAN (±SEM) UTERINE AND CERVICAL MUCOSA CELLS AT ESTRUS CYCLE PHASES OF DAIRY COWS

Sample position	Cows No.	EPC (%)	LVEP (%)	PMN (%)	Lymphocyte (%)
Cerixe (FP)	6	97.00 ±1.46	1.83± 0.30	0.50± 0.22	0.66 ±0.21
Uteri (FP)	6	97.50 ±0.67	2.00± 0.25	0.33± 0.21	0.16 ±1.16
Cerixe (LP)	6	96.17 ±0.40	1.00 ± 0.36	2.33± 0.42	0.5± 0.22
Uteri (LP)	6	96.00 ±0.44	2.66 ± 0.49	2.33± 1.11	0.33 ±0.21

EPC= epithelial cell, LVEP= large vacuolated epithelial cell, PMN =neutrophil, FP= follicular phase, LP= luteal phase

This study examined cervical and uterine cellular presence and compared them at different estrus cycle phases of table I. Evaluation of smears showed that There was no significant difference between cervical and uterine smears, which is in agreement with [9].

There was, however, a significant difference ($p<0.05$) in the neutrophil cells in the uterine and cervical mucosa slides of cows between follicular and luteal phase. the decrease of progesterone in proestrus and the probable increase in uterine blood flow caused increased migration of neutrophils to the reproductive tract lumen [5] .

TABLE II
MEAN (±SEM) UTERINE AND CERVICAL MUCOSA CELLS AT ESTRUS CYCLE PHASES OF DAIRY EWES

Sample position	Ewes No.	EPC (%)	LVEP (%)	PMN (%)	Lymphocyte (%)
Cerixe (FP)	12	97.88 ±0.79	1.25± 0.32	0.31± 0.15	0.56 ±0.15
Uteri (FP)	12	97.109 ±0.85	2.12± 0.44	0.56 ± 0.18	0.18±0.10
Cerixe (LP)	12	95.44 ±0.90	1.31 ± 0.21	2.62± 0.28	0.62± 0.17
Uteri (LP)	12	95.25 ±0.83	2.56 ± 0.28	2.87± 0.32	0.31 ±0.11

EPC= epithelial cell, LVEP= large vacuolated epithelial cell, PMN =neutrophil, FP= follicular phase, LP= luteal phase

The results for biopsy specimens in ewes are presented in table I. these ewes showed the same cytological changes during estrous phase as dairy cows.

B. Histology

The uterine wall can be divided functionally into the endometrium and myometrium.The adult endometrium of ruminants (sheep, cattle) consists of two epithelial cell types, luminal epithelium (LE), glandular epithelium (GE), stratified stromal compartments that include a d ensely organized adluminal zone of fibroblasts (stratum compactum) extending into a more loosely organized zone in the deeper or basal endometrium (stratum spongiosum), blood vessels, and

immune cells. Grossly, the adult ovine endometrium is divided into raised, aglandular caruncular areas and intensely glandular intercaruncular areas [11].

There were a s ignificant histoarchitectural changes in the uterine and cervix in cows and ewes in our studies.

An increase of the entire thickness of the myometrium and endometrium was observed in non-luteal group of cows and ewes. The thickness of the longitudinal and circular muscle layers of the myometrium was increased due to estrogen level [12]. Epithelial height in the folllicular phase groups was significantly greater luteal phase groups. Marked changes were observed in the vascular bed. However, The vascularization becomes extremely dense when the CL is absent. Endometrium gland density appears greater during the luteal phase due to a reduction in oedema in the lamina propria .

It was demonstrated in our study, that The height of the cervical epithelium varied with the stage of the estrous cycle in cows and sheep,being lowest during the luteal phase and highest shortly after the onset of estrus . This was in agreement with other studies that examined fewer stages in cattle [13].

III. Conclusion

This study has shown that there is no difference in cytological evaluation between cervix and uterus.

Some of the main changes that occur during the estrous cycle in uteri and cervix include histological changes, such as changes in height of cells, proportion of ciliated and nonciliated cells. Most of these changes are thought to occur in response to variations in the concentration of ovarian steroids. Although more research is needed, it is probable that cytological evaluation of the cervix can be used for the evaluation of uterine status of sheep and cattle particularly during the estrus phase and before mating, then collection of cervical mucosa is easy and there is no damage to the endometrium.

It is important to consider cervical cytology and hormonal assay together for the evaluation of dairy cow and ewe reproductive tract function, and more studies are necessary on these characters in relation with other factors

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