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Endometrial Cells Morphology Depending on Estrous Cycle and Histologic Layers in Cows: Morphometric Study

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Abstract: The goal of our study was to establish the effect of the estradiol (E2) and progesterone (P4) levels on endometrium morphology changes in cyclic cows. Uteri were collected from 50 healthy cows at BATNA abattoir (Algeria). Animals were classified into two groups as: follicular (FP) and luteal (LP). Blood samples were collected at the time of slaughter. The height of epithelial cells, density, perimeters and area of superficial (SG) and deep (DG) glands were measured using both Panoramic Viewer and Image Pro-Plus version 6. All morphometrical parameters was not influenced by estrous phases in the DG. However, a greatest height of SG and luminal epithelium (LE), perimeters, area and density of superficial glands were higher at LP. Furthermore, no difference was observed between the LE and SG. Significant positive relationship was found between E2 and height of epithelium cells in LE and SG at the follicular phase. During luteal phase, P4 correlated positively with almost all morphometrical parameters.

Key words: Follicular · Luteal · Morphometry · Endometrium · Cows · Cells

INTRODUCTION

For reproductive success, an intact uterine state is of pivotal importance. During every estrous cycle and for receiving the next embryo, the endometrium undergoes a temporally and locally synchronized remodeling to rejoin the needs of next blastocyst and the early conceptus elongation and developing. In the same way, the excretion and synthesis of the nutritious fluid namely histotroph by the glandular and luminal endometrium epithelium varied throughout oestrus cycle [1-3]. In buffalo, the lower success in embryo recovery and transfer was associated with lower circulating progesterone concentration and uterine development [4].

Histological endometrial changes during different stages of reproductive cycle were described in immature ewes and cows [5]; during the oestrus cycle and the early pregnancy in heifer and cows [6 , 7], ewe [8, 9], mare [10] and during implantation window in human [11].

Growth of uterus was quantified during pregnancy in the pig, sheep, bitch and mare [10-12]. In pregnant cows, morphometrical and stereological analysis of endometrium had been quantified at different trimesters of pregnancy [13]. For almost mammalian species, a cyclic variations in the morphology and function of the uterus were synchronized mainly by ovarian steroids levels [14-17]. Additionally, evaluation of ovarian steroid hormone effect on uterus histological variation through estrus cycle as described previously in cows and ewes using cell cultures and ovarectomised ewe respectively [15-18]. Estrogen promoted endometrial cells proliferation. However, the main function of progesterone was to establish development and secretory activity of endometrium [19]. To reveal the great importance to assess the variations in endometrium cells compartments individually, This study aimed to describe, simultaneously, morphometrical changes, namely height of luminal and glandular epithelium cells, density, areas and perimeters of DG and

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SG, occurring in the endometrium of cows during follicular and luteal phases and their correlation with ovarian steroids levels as well as E2 and P4.

MATERIALS AND METHODS

Animals and Samples: 50 apparently healthy lactating multiparous cows were used in our study. Uteri were collected shortly after slaughter from the commercial abattoir, Batna, Algeria. A Jugular blood samples were also obtained immediately to determine E2 and P4 levels. Cows were classified as described by Mokhtar [20], into two follicular (n =25) and luteal (n=25), groups. Endometrial samples were obtained from the body, fixed and conserved in formaline10% before embedding.

Histological Technique: After fixation, all samples were dehydrated in ethanol, clarified with xylene and embedded in Paraplast plus. Sections of 3-4 µm were mounted on SuperFrost slides (Menzel-Glaser, Freiburg, Germany) and stained with hematoxylin - eosin (H&E) using standard histological protocol [15, 21, 22].

Morphomertical Analysis: Image analysis was executed by two observers who were not informed of animal's cycle stage. Different morpho-metrical parameters were assessed in various endometrium compartments (LE, SG and DG), including: height of epithelial cells (from the basement membrane to the tip of epithelial cell, cilia or secretory droplets were not included), density (gland number /1mm^2), perimeter and area of SG and DG. Sections were examined and viewed under a Nikon Eclipse E400 microscope using both Image Pro-Plus version 6 (Media Cybernatics Inc., MD, USA) and image analysis software panoramic viewer (3D Histech Ltd, Budapest, Hungary). Ten randomly selected areas of each compartment were evaluated per sample [13- 23].

Ovarian Steroids Determination: Blood samples were collected from jugular vein, placed in ice (4°C), centrifuged and serum was stored at -20°C until analysis. Progesterone concentrations were determined using Progesterone II kit (Cobas®, Roche). The intra- and inter-assay coefficient of variations was under 20 % and the sensitivity of the assay was 0•03 ng/ml. Follicular phase was confirmed if animals had a low progesterone level (0.00-0.38 ng/ml progesterone). However, progesterone concentrations during the luteal phases ranged from 2.11 to 11.51 ng/ml [24]. The blood serum concentrations of estradiol were carried out using Estradiol II kit (Cobas®, Roche). The intra- and inter-assay coefficient of variation was under 20% and the sensitivity of the assay was 5 pg/ml.

Statistical Analysis: A graph pad prism 6 (ver. 5.02, GraphPad Software, Inc., CA, USA), was used to analyzed All data. The data were then analyzed using two way analysis of variance (mixed model). The factors in the procedure were stage of estrous cycle at the two phase of cycle (LP and FP) and histological compartment at three cells types (LE, SG and DG).The interaction between all factors was also analyzed. Therefore, additional one way analysis of variance studies within each endometrial cells types were performed . Tukey's post hoc for multiple comparison tests, or student's T-test was used. Pearson's correlation was calculated to study the relationship between hormonal levels and all morphometrical measurements. Results were expressed as mean ± standard error of mean(SEM). Probabilities less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Hormonal Concentration: The results of plasma ovarian steroid hormone concentration were shown in (Figure 1).

A high plasma concentration of estradiol (10.58 ± 0.72 pg/ml) was found when a developing follicle was revealed, while the level of progesterone raised during LP(10.35 ± 0.57pg/ml). Data on luteal phase showed that the luteolytic drop of progesterone or increase of oestradiol had not yet began in cows at the time of endometrium collection. Our results were approximately similar to those reported by Hozyen *et al.* [25]. In cows, the concentration of progesterone was less than 1.0 ng/ml during the late follicular and early luteal phase. Whereas at 4 days after estrus as the corpus luteum is growing, the concentration of progesterone increased dramatically [4].

Histologic Changes: Generally, in the present study, the endometrium of dairy cows was consisted by two regions as previously described by Tienthaiand Sajjarengpong [23].

A thick protrusion tissue without gland in superficial stroma act for caruncules and a thinner depressive area with numerous shallow and deeper glands, intre-caruncular part. Main endometrium samples

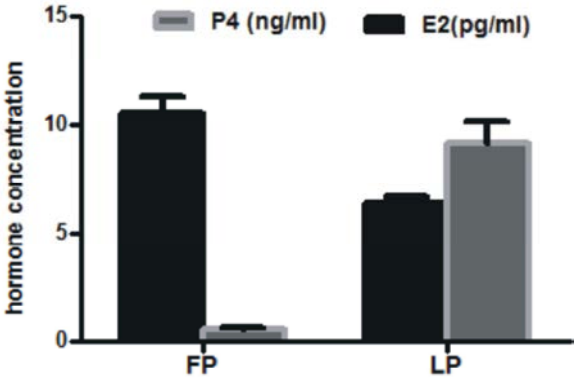

Fig. 1: Oestradiol (E2) and Progesterone (P4) levels during follicular (FP) and luteal (LP) phases in dairy cows (mean ± SEM)

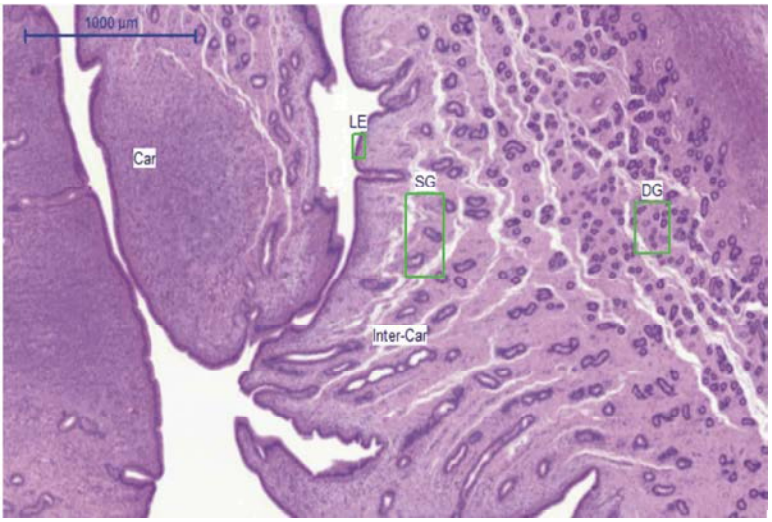

Fig. 2: Histological aspect of endometrium during luteal phase in dairy cows. LE: Luminal Epithelium, SG: superficial gland, Car: carncular region, Inter-car: inter- carncular region. (x20)

Table 1: Morphometric measurements of luminal and glandular epithelial cells in endometrium samples from cows during the estruos cycle (mean ± sem)

	Epithelial Cells height		
Oestrus phases	LE	SG	DG
Luteal (n=25)	24.23 ± 1.92$^{a\,c\,*}$	29.65±2.02$^{b\,c**}$	17.79±0.80^{a*}
Follicular (n=25)	23.22+ 1.80$^{a\,*}$	23.09± 1.67^{a*}	15.98 ± 1.69^{b*}

Different letter superscripts in the same row indicate difference statistically significant (p□0.05).
Different asterisk *, ** in the same column indicate difference statistically significant (p□0.05).
n: number of cows.

Table 2: Morphometric measurements of glandular density, perimeters and area in endometrium samples from cows in oestrus cycle (mean ± sem)

	Glands density		Glands Area		Glands Perimeters	
Oestrus phases	SG	DG	SG	DG	SG	DG
LP	29.39 ± 1.39^{a*}	37.61± 3.19^{b*}	9630±918.2*	492.0 ±72.87*	583 ±33.76^{**a}	317.7 ± 30.04^{**b}
FP	16.72±3.6^{a**}	34.11± 4.18^{b*}	4891±882.17^{a**}	251.8± 28.93 ** ^{b}	326.3 ±37.8^{*b}	202.0 ± 11.92^{*b}

SG: superficial gland; DG: deeper gland
Different letter superscripts in the same line indicate difference statistically significant between the same parameters (p?0.05)
Different asterisk in the same column indicate difference statistically significant (p? 0.05)

were devoid of the caruncules area, for these reason, our histo-morphetrical analysis was focused only on the intercaruncular region as shown in (Figure 2). When the endometrium are under oestrogen level dominant, our histological findings revealed more samples with discontinuity of surface epithelium, stroma edema, metrrorhagia , inflammatory cells infiltration, Vascular dilatation. Although some sign of degeneration were marked in the superficial gland, stroma in both estrous cycle phases.

Our research showed that SG and LE presented the same cells morphology, both were columnar and pseudo-stratified during estrous cycle, while a few region exhibited simple tall columnar or transitional epithelium. This template is in contrast to an recent histomorphological description in the swamp buffalo indicating that endometrium was lined by pseudo-stratified columnar epithelium during estrus and low simple columnar type at the mid luteal phase [23]. Our microscopic analysis showed that main of the ciliated cells were marked the LE during the follicular phase. Likewise, their number decreased when the endometrium is under a progesterone dominant. A similar finding was observed already by Tienthai and Sajjarengpong [23]. These authors observed the raise of the secretory cells in both luminal and glandular epithelium in the mid- luteal phase.

Morphometric Changes: The morphometrical changes occurred throught estrous cycle in cows are summarized in (Table 1 and 2).

Statistical analysis showed a significant effect of stage of estrous cycle (p□0.01), histologic compartments (P□0.01) on the epithelial cells height (ECH).

In general, irrespective of the histologic compartments, the ECH was lower on follicular than in luteal phase. The interaction between estrous stage and histologic compartments was significant (p□0.001).

Exploration of these interaction showed that during follicular phase the greater ECH was observed in LE and SG than in deep glands (P□0.001), difference between LE and SG was not significant (P□0.05). Nevertheless, ECH in DG was not changed during estrous cycle. There was a positive correlation between circulating estrogen levels and ECH in LE and SG (r=0.57, =0.02) during follicular phase, but in DG a tendency correlation was revealed (r= 0.39, *P*=0.054). During luteal phase, ECH correlated positively with both circulating progesterone (r =0.62, P□0.05) and estrogen (r =0.40, *P* =0.05) of almost all endometrium compartments (P <0.05). Earlier work demonstrated that the LE was lower at estrus than during luteal phase in cattel [26, 27].

The greater height of superficial epithelia compartments during luteal phase in this study could be explained by the greater secretary activities in epithelial cells as the corpus luteum grow and that if the animal is bred and conceived such epithelia will support implantation. Recently, Grazul-Bilskaa *et al.* [28] describe that progesterone caused increase in area occupied by Lipid droplets in luminal epithelium and superficial glands cells in ewes and its greater in LE than in SG but in DG lipid droplets were not detected. In addition, E2 and P4 could modify the size, height, morphology, density and function of cells as well as the morphology of endometrium cells in sex steroid-responsive [14].

Statistical analysis revealed a significant effect of estrous cycle (p□0.01), histologic compartments (P□0.05) on the gland density, area and perimeters.

Mean of SG and DG density, area and perimeters were significantly higher at the luteal than follicular phase (p□ 0.05) (Table 2). Whereas, these changes were mainly pronounced in superficial than in the deeper glands (P <0.05).

In addition, the density of basal glands appeared unchanged through estrous cycle (P=0.5). In agreement with our results, previous studies demonstrated that histo-morphological parameters did not vary throughout the oestrous cycle in the deeper gland [15-23].

In the present work, significant positive relationship was found, between E2 levels and height of epithelium cells in luminal surface and superficial glands (r=0.59; P <0.05). Whereas, greatest concentration of progesterone during luteal phase correlated positively (P<0.05) with almost all morphometrical parameters of our investigation except gland density, which correlated negatively and positively with estrogen and progesterone respectively.

There was an evidence that overall morphometrical parameters in the current work were higher at luteal than follicular phase and it was higher in the superficial gland than other compartments. These changes could be due to the enhanced cells activities in superficial epithelia, which is the critical site for embryo development and early implantation in ruminant [5-29].

Other studies in ruminant indicated the critical role of superficial glandular cells in secretion of uterine histotroph, during luteal and early pregnancy, for conceptus elongation and development [16-29]. Furthermore, on the basis of hormonal changes, profile the morphological and functional of superficial gland can be divided into two patterns: proliferation and growth during follicular phase correlated with a highest estrogen level. Moreover, as progesterone concentration rise, high secretion activity of epithelium gland was observed,

suggesting that progesterone act as a regulator of secretary epithelium cells function mainly in superficial gland [5- 30]. This idea was supported by Ford [31, 32] who showed that progesterone up regulate epithelium glandular genes encoded transport, secretion and cell proliferation during mid- luteal phase and early pregnancy in cows and heifer.

Previous studies analyzed the effect of progesterone, at day 5 and 8 on the bovine endometrium glands morphology, without separating the superficial and deeper glands [15]. Overall, the comparison between former works with our report concerning Histo-morphometrical parameters undergoes spatial and temporal changes in almost of all cells compartments of the endometrium especially in superficial gland and surface epithelium; however, the deeper gland appeared unchanged.

To our knowledge, this is the first report describing simultaneous the histo-morphometrical changes, namely height of luminal and glandular epithelium cells , density, areas and perimeters of deeper and superficial glands, occurring in the endometrium of cows during follicular and luteal phases and their correlation with ovarian steroid level as well as oestradiol (E2) and progesterone (P4). In general, dramatic spatio-temporal changes occurred in SG and LE, which coincided with development of the CL. However, in DG almost morphometrical parameters were constant.

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