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Short Paper

Histomorphometrical study of the cervix during the oestrous cycle in adult Azarbaijan buffalo

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Summary

In this study, genital tracts of 20 healthy non-pregnant buffaloes were collected from Urmia abattoir. These genital tracts were selected based on their ovaries conditions, half of them were in follicular phase and the other half were in luteal phase. The samples were taken from anterior, middle and posterior regions of the cervix and fixed in 10% buffer formalin. Then, histological sections of 5-7 μm thickness were prepared and stained with haematoxylin and eosin for histomorphometrical study and toluidine blue for study of mast cells. Histomorphometrical study was accomplished by graded and latticed objective lens device. The results revealed that the thickness of epithelium significantly ($P<0.05$) increased in the luteal phase. Mean thickness of mucosa-submucosa layers in the middle (290.4 ± 12.69 μm) and posterior (283.14 ± 16.49 μm) regions of the cervix in the follicular phase was significantly more than the luteal phase ($P<0.05$). Mean thickness of tunica muscularis increased significantly ($P<0.05$) during the follicular phase in the anterior region of the cervix (3325.28 ± 286.69 μm). This study also revealed that the mean distribution of mast cells in the luteal phase (0.53 ± 0.02) was significantly more than the follicular phase ($P<0.001$). Generally, this study showed that the histomorphometrical changes in the cervix of buffalo occur in the follicular and luteal phases of oestrous cycle. These changes may be related to the fluctuation of oestrogen and progesterone hormones and distribution of mast cells.

Key words: Cervix, Histomorphometry, Buffalo, Oestrous cycle

Introduction

Histologically, wall of the cervix from lumen to outside is composed of tunica mucosa, submucosa, muscularis, and serosa. Lamina muscularis is not existent in the cervix so that the mucosa and submucosa layers are not well separated (Eurell and Brian, 2006). Cervix has a sphincter-like structure which projects into the anterior region of vagina. This organ is structured by fibrous connective tissue and muscles, and changes a lot in oestrous cycle. The luminal wall of the cervix has lockable folds and this organ is always close except in the oestrous phase and parturition (Hafez and Hafez, 2000). The cervix relaxes enough in the oestrous phase for artificial insemination and discharges lucent fluid in this phase that changes into less concentrated and viscous substance after ovulation (Arthur *et al*., 1996). The propria-submucosa consists of dense irregular connective tissue, which becomes edematous and changes to a loose areolar structure during oestrous (Eurell and Brian, 2006).

This study was designed to address the hypothesis that histomorphometrical changes may occur in different regions of cervix during different phases of oestrous cycle.

Materials and Methods

Genital organs including ovaries of twenty 4–8-year-old healthy and non-

pregnant buffalo were collected from abattoir for histomorphometrical study of the cervix. Twenty samples were selected on the basis of ovaries condition, luteal and follicular phases (10 in each group). Then, the samples (0.5 cm thickness) were taken from the anterior, middle and posterior regions of the cervix and fixed in 10% buffer formalin. The samples were cut into sections of 5-7 µm thickness. After tissue preparation using paraffin embedding technique, they were stained with haematoxylin and eosin for routine histomorphometry and toluidine blue for study of mast cells (Humason, 1979). Histomorphometrical study was accomplished by graded and latticed lens device. The changes in thickness of epithelium, tunica mucosa-submucosa, primary and secondary folds, tunica muscularis and also distribution of mast cells were studied in different regions of the cervix in both follicular and luteal phases of oestrous cycle. Data were statistically analyzed by Student t-test, one-way ANOVA and Duncan's test by SPSS software (Table 1).

Results

Histomorphometrical study of buffalo cervix in different regions (anterior, middle and posterior), revealed that the thickness of epithelium in the follicular phase was not significantly different, but the thickness of epithelium in the anterior region (16.15 ± 0.81 µm) and posterior region (15.47 ± 0.91 µm) in the luteal phase was significantly more than the follicular phase ($P<0.05$). Generally, mean thickness of epithelium of the cervix in the luteal phase was significantly ($P<0.05$) more than the follicular phase (Table 1).

This study showed that the mean thickness of mucosa-submucosa between the folds of different regions of the cervix was

Table 1: Different parameters studied in the buffalo's cervix in different phases of oestrous cycle (Mean ± SE)

Parameters	Phases of oestrous cycle	Anterior region	Middle region	Posterior region	Entire of the cervix
Thickness of epithelium (µm)	Follicular	13.6±1.13	12.58±0.8	11.05±0.73	12.41±0.53
	Luteal	16.15±0.81	13.6±0.81	15.47±0.91	15.07±0.5
Thickness of mucosa-submucosa (µm)	Follicular	254.05±10.8	290.4±12.69	283.14±16.49	275.86±7.99
	Luteal	283.0±38.12	252.64±15.14	257.0±23.25	264.26±15.49
Thickness of primary folds (µm)	Follicular	1409.88±524.38	868.29±87.1	1032.36±115.8	1103.51±180.47
	Luteal	799.31±62.23	951.77±69.37	816.75±117.62	855.94±49.94
Thickness of secondary folds (µm)	Follicular	82.03±13.76	118.33±11.92	94.38±12.28	98.25±7.5
	Luteal	83.49±7.33	75.5±7.54	113.02±14.44	90.67±6.3
Thickness of tunica muscularis (µm)	Follicular	3325.28±286.69	3441.24±125.65	3136.32±382.32	3300.94±162.03
	Luteal	2468.4±83.45	4056.6±489.52	3499.32±181.56	3344.44±198.96
Distribution of granular mast cells in mucosa-submucosa (0.25 mm²)	Follicular	0.26±0.11	0.33±0.12	0.33±0.12	0.31±0.06
	Luteal	0. 6±0.19	0.73±0.2	0.86±0.27	0.73±0.12
Distribution of degranular mast cells in mucosa-submucosa (0.25 mm²)	Follicular	0.00±0.00	0.00±0.00	0.06±0.06	0.02±0.02
	Luteal	0.4±0.16	0.2±0.1	0.4±0.13	0.33±0.07
Distribution of granular mast cells in tunica muscularis (0.25 mm²)	Follicular	0.06±0.06	0.13±0.09	0.33±0.12	0.8±0.04
	Luteal	0.26±0.15	0.26±0.11	0.33±0.12	0.28±0.07
Distribution of degranular mast cells in tunica muscularis (0.25 mm²)	Follicular	0.00±0.00	0.06±0.06	0.00±0.00	0.02±0.02
	Luteal	0.33±0.12	0.2±0.1	0.13±0.13	0.22±0.07
Distribution of mast cells in mucosa-submucosa and tunica muscularis (0.25 mm²)	Follicular	ND	ND	ND	0.41±0.02
	Luteal	ND	ND	ND	0.53±0.02

ND: not determined

not significantly different. Mean thickness of this part of the cervix in the middle region (290.4 ± 12.69 µm) and posterior region (283.14 ± 16.49 µm) in the follicular phase was significantly higher than the luteal phase ($P<0.05$). Generally, the thickness of mucosa-submucosa in the follicular and luteal phases was not significantly different (Table 1).

Mean thickness of tunica muscularis in the anterior region of the cervix (3325.28 ± 286.69 µm) in the follicular phase was significantly ($P<0.05$) more than the luteal phase (2468.4 ± 83.45 µm). Mean thickness of tunica muscularis in the posterior region (3499.32 ± 181.56 µm) was significantly ($P<0.01$) increased compared with anterior region (2468.4 ± 83.45 µm) in the luteal phase. In conclusion, there were not significant differences in mean thickness of tunica muscularis in different phases of oestrous cycle (Table 1).

This study revealed that the mean thickness of primary folds (Fig. 1) in different regions of the cervix and in different phases of oestrous cycle was not significantly different. Mean thickness of secondary folds (Fig. 1), in the middle region of the cervix in the follicular phase (118.33 ± 11.92 µm) and in the posterior region in the luteal phase (113.02 ± 14.44 µm) increased significantly ($P<0.05$). Nevertheless, in entire of the cervix, the difference between mean thickness of secondary folds in the follicular phase (98.25 ± 7.5 µm) and the luteal phase (90.67 ± 6.3) was not significant (Table 1).

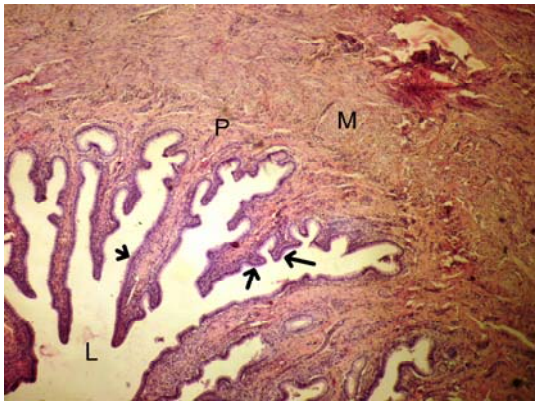

Fig. 1: Section through the cervix wall showing primary (short arrow), secondary (medium arrow), and tertiary (long arrow) folds and Lamina propria, submucosa (P), tunica muscularis (M) and lumen (L), (H&E, ×40)

Mean distribution of degranular mast cells of mucosa-submucosa (Fig. 2) in the posterior region in the follicular phase was significantly ($P<0.05$) more than the other regions. In the luteal phase, mean distribution of granular mast cells in the middle region (0.73 ± 0.2) was significantly ($P<0.05$) more than the other regions. Throughout the cervix, mean distribution of granular (0.73 ± 0.12) and degranular (0.33 ± 0.07) mast cells of mucosa-submucosa in the luteal phase was significantly ($P<0.01$, $P<0.001$) increased compared with the follicular phase.

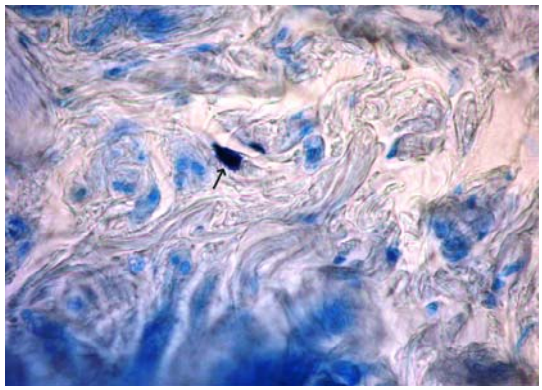

Fig. 2: Tunica submucosa of the cervix. A mast cell with dark purple granules that fills its cytoplasm (arrow), (toluidine blue staining method, ×400)

Mean distribution of granular mast cells in tunica muscularis in the luteal phase (0.33 ± 0.12) and follicular phase (0.33 ± 0.12) in the posterior region was significantly ($P<0.05$) more than the other regions. Mean distribution of granular (0.28 ± 0.07) and degranular (0.22 ± 0.07) mast cells in tunica muscularis in the luteal phase was significantly ($P<0.05$) more than the follicular phase.

Throughout the cervix, mean distribution of mast cells was significantly ($P<0.001$) more in the luteal phase (0.53 ± 0.02) than follicular phase (Table 1).

Discussion

The uterine cervix is a complex organ that undergoes extensive changes to allow its successful adaptation to different

physiological conditions (Wang *et al*., 2000). Oestrogens and progestins regulate many of the physiological processes occurring in the female reproductive tract, mostly through intranuclear receptors that function as steroid-modulated transcription factors (Evans, 1988). There is a need to establish the normal range of physiological changes in the cervix in order to facilitate the accurate interpretation of hormone replacement models.

This study showed that the height of the epithelium in anterior and posterior regions of the cervix increased significantly ($P<0.05$) in the luteal phase. This is in agreement with the study by Singh and Sharma (1985) who reported that the thickness of uterine epithelium in buffalo in the luteal phase was more than follicular phase. Generally, the average thickness of cervix epithelium in both phases of oestrous cycle was significantly ($P<0.05$) different from each other. Administration of a weak oestrogen, genistein, to ovariectomized rats results in significant increases in the height of the luminal epithelial cells of uterine and vaginal tissues (Diel *et al*., 2001).

In oestrus and dioestrus, oestrogen receptor α (ERα) and progesterone receptor (PR) expression exhibited variations according to the region evaluated. Proliferation and apoptosis showed a reciprocal pattern, the epithelium being the region with more cell turnover (Jorge *et al*., 2002). Cell death occurs in all cellular compartments during the oestrous cycle, indicating that apoptosis is a physiological phenomenon in rat reproductive tracts (Suzuki, 1996). This study revealed that thickness of mucosa-submucosa in different regions of the cervix was not significantly different, but during the follicular phase it was more than the luteal phase. Proliferation of connective tissue cells and increasing the collagen fibers of human's cervix under the effect of oestrogen in follicular phase increased the thickness of mucosa – submucosa and its primary folds (Gorodeski, 1998). In fibroblastic stroma of rat's uterine cervix, low proliferation was observed throughout pregnancy; however, there was a net increase in cell number because very few cells underwent apoptosis.

No difference in ERα was observed in fibroblastic cells during pregnancy and postpartum period; however, a great decrease of this receptor in the epithelial compartment was observed after delivery. Unlike cervical epithelium, PR was highly expressed in stromal cells (Jorge *et al*., 2002). Present study showed that the thickness of secondary folds which are located on the primary folds, in the middle region of the cervix was significantly ($P<0.05$) increased in follicular phase. Oestrogen-sensitive tissues such as cervix and uterus, are affected by the injection of large doses of the phytoestrogen genistein in the ovariectomized gilt. The sensitivity of the gilt uterus to oestrogenic substances makes it a potential model to examine the impact of environmental endocrine modulators on reproductive tissues (Ford *et al*., 2006).

Thickness of tunica muscularis in anterior region of the cervix during follicular phase was significantly ($P<0.05$) more than luteal phase. Location of oestrogen receptors on the nucleus of epithelial cells, stromal cells in lamina propria and smooth muscle cells of the uterus and cervix of human in both immunohistology and immunoelectron microscopy studies were determined (Press *et al*., 1986). The uterine cervix is a dynamic structure with a high capacity to adapt to different, even opposing roles during the sequence of physiological events of gestation (acting as a barrier to retain the fetus during pregnancy and afterwards dilating to allow a normal delivery). Also, it has differential biological responses to modifications of the hormonal milieu (Garfield *et al*., 1998; Luque *et al*., 1998; Challis *et al*., 2000; Shi *et al*., 2000). These different responses imply many cellular and extracellular events, e.g. E_2-mediated eosinophil infiltration (Luque *et al*., 1996; Ramos *et al*., 2000), steroid-hormone receptor expression (Wang *et al*., 2000), collagen metabolism (Vasilenko and Mead, 1987; Luque *et al*., 1998, Winkler and Rath, 1999), and fibroblastic cell plasticity (Varayoud *et al*., 2001). The influence of ovarian steroid hormones on these events is still not fully understood (Jorge *et al*., 2002).

This study showed that the increase in the number of granular and degranular mast cells in mucosa-submucosa during the luteal

phase was highly significant ($P<0.01$). However, presence of these cells may cause the elevation of local immune reaction in cervical tissue of non-gravid mares during dioestrus (Wherend *et al*., 2005). Bosquiazzo *et al*. (2005) showed that there is a correlation between macrophages and connective tissue mast cells in the pregnancy period. The population of macrophages in the cervix of rats was controlled by degranulation of mast cells (Bosquiazzo *et al*., 2005). Results of the present study revealed that mast cells often appear around the vessels, and their distribution in superficial regions of the cervix is more prominent than deep regions. Angiogenesis refers to the growth of new blood vessels from pre-existing microcirculation and mast cells have been associated with this process (Varayoud *et al*., 2004). This assumption is partially supported by the close anatomical association between mast cells and the vasculature, and the recruitment of these cells during tumour growth, wound healing and inflammation (Benítez-Bribiesca *et al*., 2001; Fukushima *et al*., 2001; Norrby, 2002). Mast cells which accumulate around vessels and new capillary sprouting sites, have been implicated in angiogenesis (Hiromatsu and Toda, 2003).

A recent study showed that mast cells and their mediators are capable of regulating cervical contractility in animals in mid-pregnancy, and possibly contribute to cervical competence during pregnancy in guinea pigs (Bytautiene *et al*., 2002). Another study revealed that the number of mast cells increased in oestrogenic phase in women's genital organ (Bree Veld-Dwarkasing *et al*., 2003). Our comparative study of mean distribution of mast cells in tunica muscularis showed that the number of granular and degranular mast cells in the luteal phase was significantly ($P<0.05$) more than the follicular phase. The number of mast cells throughout the cervix in the luteal phase was significantly ($P<0.001$) increased.

Generally, this study showed that the histomorphometrical changes of cervix in buffalo occur in the follicular and luteal phases of oestrous cycle and these changes may be related to oestrogen and progesterone hormones and distribution of mast cells.

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