Histological and histomorphometrical changes of different regions of oviduct during follicular and luteal phases of estrus cycle in adult Azarbaijan buffalo

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Summary

In this study the reproductive organs of adult and apparently healthy female Azarbaijan buffaloes were collected after slaughter from abattoir. Through observation of the ovaries, the luteal and follicular phases of each buffalo were specified. A total number of 36 oviducts at follicular phase and 36 oviducts at luteal phase were collected and 3 tissue samples were taken from 3 regions of infundibulum, ampulla and isthmus of each oviduct. Sections were stained through the use of H&E, PAS, verhoffe and toluidine blue methods. Histological observations revealed that the oviduct consists of 4 layers of mucosa, submucosa, tunica muscularis and serosa. The primary and secondary folds decreased both in number and in height from infundibulum to isthmus. Epithelium of folds was composed of simple columnar, although seems pseudostratified in some areas, and contains ciliated and secretory cells. Histomorphometric examinations of three regions demonstrated that the mean height of primary folds increase and the mean thickness of tunica muscularis decrease at follicular phase. The mean thickness of mucosa-submucosa at follicular phase was slightly similar to luteal phase. More visibility of the ciliated cells and mucosal folds in infundibulum and the increase of their height at follicular phase facilitate the capture of the oocyte; the thick tunica muscularis in isthmus transports sperm cells up; and both require promoting fertilization to occur in ampulla.

Key words: Azarbaijan buffalo, Estrus cycle, Oviduct, Microscopic structure

Introduction

Oviducts are paired convoluted tubes extending from ovaries to the uterus (Pineda, 2003). These tubes are about 20 to 30 cm long and 1.5 to 3.0 mm in diameter in cow which makes them difficult to palpate on rectal examination (Arthur *et al*., 1996). The oviduct may be divided into four functional segments: the fringe-like fimbriae; the funnel-shaped abdominal opening near the ovary, the infundibulum; the more distal dilated, major portion of the uterine tube from the infundibulum to the isthmus is called the ampulla; and the narrow proximal portion of the oviduct connecting the oviduct with the uterine lumen, the isthmus (Arthur *et al*., 1996; Hafez and Hafez, 2000). The oviduct provides the micro-environment for sperm capacitation, fertilization and early cleavage-stage embryonic development, where it undergoes marked morphological and functional changes during the estrus cycle (Thibodeaux *et al*., 1991; Bauersachs *et al*., 2004).

The oviductal mucosa is made of primary, secondary, and tertiary folds. The mucosa in the ampulla is thrown into high, branched folds that decrease in height toward the isthmus and become low ridges in the uterotubal junction. The complex arrangement of these mucosal folds in the ampulla almost completely fills the lumen so that there remains only a potential space. In the ewe, the mucosa consists of one layer of columnar epithelial ciliated and non-ciliated cells (Rajesh *et al*., 1997). The ciliated cells of the oviductal mucosa have slender motile cilia that extend into the lumen. The percentage of ciliated cells decrease gradually in the ampulla toward the isthmus and reach a maximum in the fimbriae and infundibulum. Ciliated cells are noted in large numbers at the apices of the mucosal folds (Hafez and Hafez, 2000).

The secretory cells of the oviductal mucosa are nonciliated and characteristically contain secretory granules (Banks, 1993), the size and number of which vary widely among species and during different phases of the estrous cycle (Cigankova *et al*., 1996). The morphology of the ampullar epithelia in Mouse changes during estrous cycle. At estrus and metestrus most of the ampullar epithelial cells are comprised of non-ciliated secretory cells, whereas at diestrus and proestrus the ciliated cells are dominant among the ampullar epithelial cells (Morita *et al*., 1997).

The thickness of the tunica muscularis increases from the ovarian to the uterine end of the oviduct and constricts in response to hormones (Hafez and Hafez, 2000).

In the present study, an attempt is made to determine the probable histological changes of oviduct during follicular and luteal phases of estrus cycle in Azarbaijan buffalo.

Materials and Methods

Reproductive tracts of 72, 5–8-year-old and apparently healthy female cyclic Azarbaijan buffaloes (36 at follicular and 36 at luteal phase) were collected immediately after slaughter from abattoir. Tracts with different abnormal infections, pregnant and from recently delivered buffaloes were eliminated. In each tract by precise inspections both the ovaries for the presence of corpus luteum and or growing follicles, the luteal and follicular phases were specified and 3 tissue sections collected from three regions of infundibulum, ampulla and isthmus of each oviduct. Tissue samples were preserved in 10% neutral buffered formalin solution. After complete fixation of tissues, specimens were processed through routine paraffin embedding. Transverse sections were cut at 5-7 µm thickness and stained with haematoxylin and eosin for routine fibrocellular architecture, verhoffe for elastic fibers, toluidine blue for mast cells and periodic acid schiff (PAS) reaction for carbohydrate compounds of epithelial cells (Gretchen, 1979).

Graduated (scaled) microscopic lens device was used to measure the height of primary folds; the thickness of primary, secondary and tertiary folds and the thickness of epithelium, mucosa-submucosa, tunica muscularis and serosa in 3 regions of infundibulum, ampulla and isthmus of each oviduct during follicular and luteal phases of estrus cycle. Results show mean height and mean thickness, and significance was assigned at $P<0.05$. One-way ANOVA test was used to compare differences between mean height or thickness of each parameter in each region of oviducts during two follicular and luteal phases of estrus cycle.

Results

Histological studies

Microscopic evidence reveals that the infundibulum at follicular phase consists of large primary folds and their branches inside. These primary folds can be identified with secondary and sometimes tertiary folds in some areas (Fig. 1). The epithelium is simple columnar or pseudostratified columnar with both ciliated and non-ciliated cell types. Morphologic signs of secretory activity are evident only in the non-ciliated cells. Ciliated cells are more identified during follicular phase in the infundibulum and decrease gradually in the ampulla toward the isthmus. The mucosa-submucosa consists of loose connective tissue with many plasma cells, mast cells, neutrophils and other leucocytes with blood vessels. The mucosa continues with the submucosa. Tunica muscularis is very thin and consists mainly of an inner circular layer and a few outer longitudinal bundles of smooth muscle. Infundibulum is covered by serosa, which consists of loose connective tissue with many blood vessels. During luteal phase, the secretory cells become higher than the ciliated cells (Fig. 2).

Distribution of the primary folds decreases in the ampulla. The number of the primary folds is more than the secondary and tertiary folds. Compared with the luteal phase, the ampulla at follicular phase is highly folded. The mucosa continues with the submucosa and consists of loose connective tissue. The tunica muscularis increases remarkably in thickness and consists mainly of smooth muscle bundles, but isolated outer longitudinal bundles are also present. The last layer of the ampulla is serosa. The epithelium of the ampulla during follicular phase is higher than the luteal phase, which consists of long cells with secretory activity (Fig. 3). Other morphologic signs are similar to those of the follicular phase.

In the isthmus, during follicular phase, the number of the primary folds decreases, where only a few secondary folds are present and tertiary folds disappear. The epithelium is pseudostratified columnar or simple columnar. The number of ciliated cells decreases in this region of oviduct, but the tunica muscularis is very thick and consists mainly of inner circular smooth muscle with thin outer longitudinal bundles (Fig. 4). The isthmus is covered by serosa with loose connective tissue. There is no noticeable difference in the microscopic structure of the isthmus at follicular and luteal phase.

Through the application of verhoffe staining method, very thin elastic fibers are visible in the folds, connective tissue, mucosa-submucosa, tunica muscularis and especially around the blood vessels (Fig. 5). There are no important differences between the elastic fibers distribution in the infundibulum, ampulla and isthmus at the follicular and luteal phase of estrus cycle.

Through the use of PAS staining method, in the infundibulum, during follicular phase, the secretory cells could be identified in the epithelium. Also, reticular fibers are visible as a flexible network in the mucosa-submucosa, tunica muscularis and around the blood vesicles. It seems that during luteal phase the secretory cells and reticular fibers decrease in the epithelium. The number of these cells and fibers in the ampulla are more than those in the infundibulum (Fig. 3). With the increase of distance from the ampulla, the secretory cells and reticular fibers gradually disappear and in the isthmus-uterus junction, they are not present.

With toluidine blue staining, mast cells with centrally located nucleus and secretory granules in the cytoplasm are present in the connective tissue of the primary folds, between the muscular fibers in tunica muscularis and serosa (Fig. 6). They are especially abundant around blood vessels.

Morphometric studies

The results of the morphometric study indicate that the mean epithelial thickness of infundibulum in both phases is less than that of the two other regions ($P<0.05$). Also, its thickness in three different regions at follicular phase is relatively more than the luteal phase.

In three different regions the mean thickness of mucosa-submucosa at follicular phase is relatively higher than the luteal phase, whereas the mean thickness of tunica muscularis at luteal phase is significantly more than the follicular phase. They increase gradually as the distance from the infundibulum increases, and become higher in isthmus than in the other two regions ($P<0.05$).

The mean height of primary folds decreases from infundibulum to isthmus in both phases ($P<0.05$). Also, the primary folds at follicular phase are higher than those in the luteal phase in three different regions ($P<0.05$).

There are no remarkable changes in the mean thickness of primary, secondary and tertiary folds in the three regions of oviduct (Tables 1, 2 and 3).

Discussion

There are species differences in the histological structures of the different parts of the tubular genital tract during the estrus cycle. These differences probably reflect different secretion rates for the ovarian hormones (Ayen and Shahrooz, 2002; Shahrooz *et al*., 2009). Uterine tube is a part of the female genital tract which picks up the oocyte and makes a suitable situation for fertilization, directing the ovum to the uterus. The epithelial structure of the oviduct has a basic role in oocyte nutrition and further embryonic development and survival (Hartigan, 1992). In the present paper, a

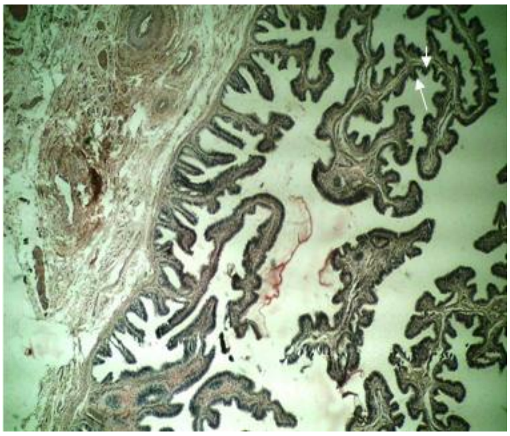

Fig. 1: Photomicrograph showing section of epithelium of the infundibulum with primary, secondary (long arrow) and tertiary (short arrow) folds at follicular phase of estrus cycle, (H&E, ×40)

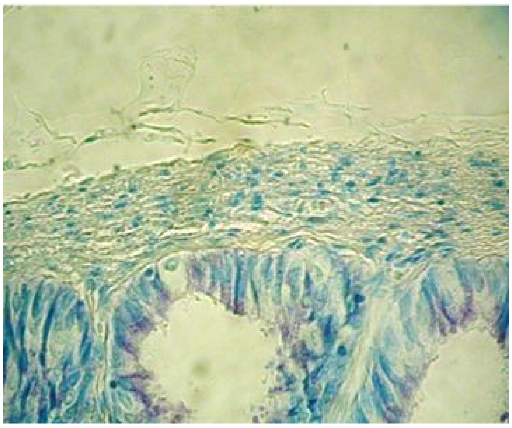

Fig. 2: Photomicrograph showing section of epithelium of the infundibulum with secretory cells at luteal phase of estrus cycle, (toluidine blue, ×400)

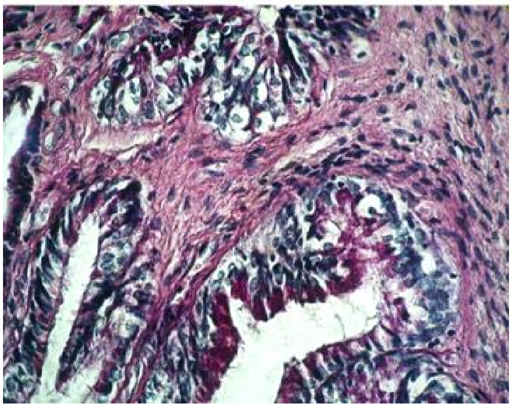

Fig. 3: Photomicrograph showing section of epithelium of the ampulla with long secretory cells at luteal phase of estrus cycle, (PAS, ×400)

Fig. 4: Photomicrograph showing section of the isthmus with thick tunica muscularis, (H&E, ×100)

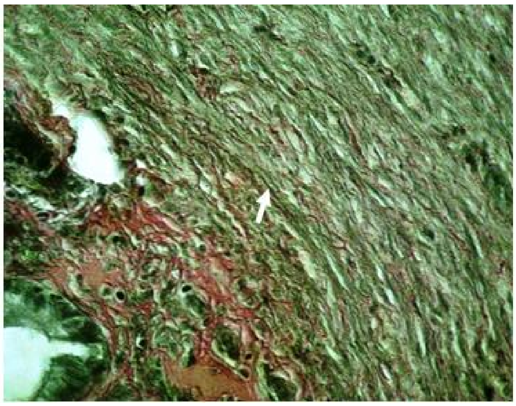

Fig. 5: Photomicrograph showing section of the isthmus with thin elastic fibers (arrow) in mucosa-submucosa, (verhoffe, ×400)

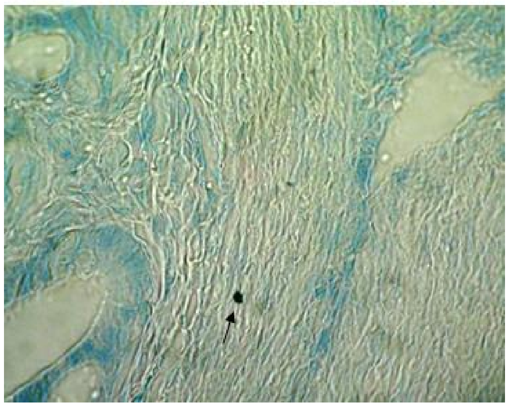

Fig. 6: Photomicrograph showing section of the isthmus with a mast cell (arrow) in submucosa, (toluidine blue, ×400)

comparative histological study of uterine tube at follicular and luteal phases of estrus cycle in three distinct regions of oviduct of the Azarbaijan buffalo has been carried out

Table 1: Mean (±SE) morphometric values for different parameters of infundibulum at follicular and luteal phase

Parameter (μm)	Luteal phase	Follicular phase
Height of primary folds	965.7 ± 28.9*	1390.6 ± 250*
Thickness of primary folds	75.6 ± 13.7	79.8 ± 25
Thickness of secondary folds	70.8 ± 11.5*	79.8 ± 12.5*
Thickness of tertiary folds	76 ± 14.7	83.4 ± 12.2
Thickness of tunica muscularis	78.7 ± 12.1*	43.5 ± 10.8*
Thickness of serosa	75.9 ± 14.1	72.9 ± 38.9
Thickness of epithelium	14.9 ± 3.4	17.8 ± 2.5
Thickness of mucosa-submucosa	19.5 ± 1.9	20.4 ± 2.5

Means with superscript * within each row are significantly different (P<0.05)

Table 2: Mean (±SE) morphometric values for different parameters of ampulla at follicular and luteal phase

Parameter (μm)	Luteal phase	Follicular phase
Height of primary folds	345.2 ± 20.6*	610.5 ± 20.7*
Thickness of primary folds	60.8 ± 5.2*	65.7 ± 2.5*
Thickness of secondary folds	ND	ND
Thickness of tertiary folds	ND	ND
Thickness of tunica muscularis	210.5 ± 35.7*	105.2 ± 7.4*
Thickness of serosa	176.7 ± 42.6*	110.1 ± 20.4*
Thickness of epithelium	20.4 ± 0	27.2 ± 0
Thickness of mucosa-submucosa	25.9 ± 1.1	33.9 ± 5.2

Means with superscript * within each row are significantly different (P<0.05). ND: Not determined

Table 3: Mean (±SE) morphometric values for different parameters of isthmus at follicular and luteal phase

Parameter (μm)	Luteal phase	Follicular phase
Height of primary folds	196.1 ± 8.2*	251.3 ± 15*
Thickness of primary folds	67.2 ± 3.5	68.9 ± 12.1
Thickness of secondary folds	ND	ND
Thickness of tertiary folds	ND	ND
Thickness of tunica muscularis	350.7 ± 42.6*	268.6 ± 24*
Thickness of serosa	75.2 ± 25.6*	192.25 ± 25*
Thickness of epithelium	23.2 ± 2.1	26.3 ± 3.8
Thickness of mucosa-submucosa	38.1 ± 9	40.5 ± 7.8

Means with superscript * within each row are significantly different (P<0.05). ND: Not determined

to evaluate the possible changes related to different regions and phases. The results indicate that the folds increase in number and height during follicular phase and decrease from infundibulum to isthmus. These findings in Azarbaijan buffalo are consistent with some other investigations in ewe (Rajesh *et al.*, 1997), rabbit (Kuhnel and Busch, 1979), cow and pig, which may confirm the role of infundibulum in receiving and transporting the oocyte from ovary to ampulla (Eurell and Brian, 2006).

The present study demonstrates that the oviductal mucosa consists of simple columnar or pseudostratified columnar with both ciliated and non-ciliated cell types. The percentage of ciliated cells is more identified during follicular phase when the estrogen is dominant and decreases gradually in the ampulla toward the isthmus and reaches maximum in the infundibulum. The action of cilia is thought to be the primary mechanism for transporting the oocyte rapidly from the infundibulum to the site of fertilization in the ampulla (Cupps, 1991; Hafez and Hafez, 2000; Eurell and Brian, 2006). Ciliation of the oviduct is hormonally controlled. Cilia disappear almost completely after hypophysectomy and develop in response to administration of exogenous estrogens. The oviducts atrophy and deciliate during anestrous and pregnancy, hypertrophy and become reciliated during proestrus and estrus (Hafez and Hafez, 2000). Estrogen dominance during follicular phase of estrus cycle enhances the activity of cilia in the upper oviduct and facilitates the rapid transport of oocytes from the fimbria to the ampulla of the oviduct (Pineda, 2003).

In some species, the isthmus is thought to serve as a sperm reservoir, which could be one of the suitable parts of the female tract for spermatozoal capacitation. It has been observed in cow, sheep, pig and rabbit that spermatozoa accumulate in the isthmus for a few hours after mating or insemination and move up the oviduct as the time of ovulation closed. Spermatozoa have been described as being relatively quiescent in the isthmus but active when they move up into the ampulla (Cupps, 1991). Transport of sperm cells through the isthmus is accomplished primarily through muscular contractions (Hartigan, 1992). The present study demonstrates a significant increase in the thickness of tunica muscularise in isthmus which is required for sperm transportation to ampulla. After the final stages of fertilization in ampulla, the embryos remain in this middle part of the oviduct, but near the ampulla-isthmus junction for 2 to 3 days. Muscular contractions of isthmus probably displace the embryos from the remaining distance into the uterus (Pineda, 2003).

In this study, the presence of epithelial non-ciliated secretory cells in the ampulla is more than the other regions of the oviduct. These cells are also more visible during the follicular phase. These finding are consistent with reports which state that the volume of oviductal fluid from secretory cells changes in relation to the estrus cycle phase, and becomes higher during the period of estrus. Oviductal fluid has several functions: sperm capacitating, sperm hyperactivation, fertilization and early preimplantation development (Hafez and Hafez, 2000).

In conclusion, in adult Azarbaijan buffalo as in some other species, the more presence of different folds and ciliated cells in infundibulum are compared with the other regions of oviduct, where, easily the oocytes are directed down to the ampulla; and the increase in the thickness of tunica muscularis in isthmus transports the sperm cells up to the ampulla. This histological structure of the oviduct in Azarbaijan buffalo employs a carefully controlled mechanism whereby oocyte and spermatozoa are transported in opposite directions at the same time during estrus to ampulla, where fertilization has to occur. Therefore, in this species the oviduct contributes to histological and functional mechanisms, ions and fluid for transporting spermatozoa, oocytes, or embryos, and provides a favorable microenvironment for fertilization and initial embryonic development, and these are affected by the levels of ovarian hormones alternations during follicular and luteal phases of estrus cycle.

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