Original Article

Microscopic and histochemical characterization of the bovine uterine tube during the follicular and luteal phases of estrous cycle

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A B S T R A C T

The morphometrical and morphological features of the infundibulum and ampulla of the uterine tubes of adult cattle were studied. The materials used in this study were consisted of 12 pairs of uterine tube of healthy cows at age of 16–36 months, collected from Assiut slaughterhouses. Through observations of the ovaries, follicular and luteal phases of estrous cycle of each cattle were specified. Semithin sections of ampulla and infundibulum at follicular and luteal phases were made and histochemical analysis of the ampulla by use of PAS, Alcian Blue, Sudan Black B was also done. In addition, acid phosphatase activity of the ampullar epithelium was demonstrated. Histological analysis of the epithelium of bovine oviduct revealed that it was consisted of non-ciliated secretory cells, two populations of ciliated cells (CC), basal cells and Peg cells. At the luteal phase, the secretory cells possessed many cytoplasmic protrusions that extended beyond the luminal borders of the ciliated cells and exocytosis of secretory materials was observed. While at the follicular phase, the ciliated cells were predominated. The histochemistry of the ampullar epithelium revealed increase in secretions of neutral, acidic mucopolysaccharides and lipid from the secretory cells at the luteal phase with moderate acid phosphatase activity. Histomorphometric examinations of infundibulum and ampulla indicated that the mean number and height of primary folds as well as the thickness of the epithelium were increased significantly at the follicular phase.

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1. Introduction

The oviduct plays an essential role in reproduction, as it creates an important microenvironment for the final maturation of male gametes, fertilization and early development of embryos [1]. The bovine oviduct could be divided into infundibulum, ampulla and isthmus. Many studies have been done in order to describe the characteristic morphological changes in the oviduct of several domestic species including cows [2], goats [3], pigs [4] and bitches [5] in relation to estrous cycle.

The epithelium lining of uterine tube of mammals is consisted mainly of ciliated and non-ciliated secretory cells. These cells show atrophy and hypertrophy according to the endocrine status, and thus the ratio of these cells may undergo changes during the estrous cycle [2]. Moreover, it is known that the oviductal epithelial cells show marked regional variations in ultrastructural, histochemical and physiological features in many mammals [6].

The ciliated cells aid in transport of both gamete and embryo [7], while the secretory cells may be involved in
secretion of the oviductal fluid that plays an important role in many sperm functions and embryo development [6]. The secretory product is mainly produced by ampulla as the fertilization occurs in it [3].

Not only cattle are important agricultural species but their ovarian follicular dynamics also make them to be an ideal model for different aspects of human reproduction [8], due to distinct similarities between bovine and human ovarian physiology [9]. However, many details about the morphology and morphometry of oviduct of cattle at the critical estrous phases are still lacking [10,11].

A main topic of this study is to describe the histological, histochemical and morphometrical changes in uterine tube of cattle in Egypt (Bos indicus) at follicular and luteal phases of estrous cycle.

2. Materials and methods

2.1. Tissue collection

The material used in this study consists of 12 pairs of bovine uterine tubes of healthy adult cows (B. indicus) at age of 16–36 months. The samples were collected within 30 min after routinely slaughter from Assiut slaughterhouses. The stages of estrous cycle were estimated by the appearance of ovarian follicles and corpora lutea.

2.2. Histological analysis

Specimens from infundibulum and ampulla were washed by physiological saline and immediately fixed in Bouin’s fluid for 20 h. The fixed materials were dehydrated in graded series of alcohols, cleared in methyl benzoate and embedded in paraffin wax. The embedding time was not more than 8 h. Serial longitudinal and transverse sections were obtained at 3 μm and stained with Harris Haematoxylin and Eosin [12], Van Gieson Resorcin Fuchsin [13] and Goldener’s Trichrome stain [14].

2.3. Histochemical analysis

Acidic and neutral mucus were detected by Alcian Blue stain (pH 2.5) and PAS stain, respectively [15,16]. Lipid was demonstrated by Sudan Black B [17]. Acid phosphatase activity of ampullar epithelium was identified with Gomori’ Lead Nitrate [18] at both follicular and luteal phases of the estrous cycle.

2.4. For semithin sections

Small specimens of infundibulum and ampulla at both follicular and luteal phase were fixed in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.3 for 4 h at 4 °C. They were washed in the same buffer used and then post-fixed in 1% osmic acid in 0.1 M Na-cacodylate buffer for further 2 h at room temperature. The samples were then dehydrated in ethanol and embedded in Araldite–Epon mixture. Semithin sections (1 μm in thickness) were cut and stained with Toluidine blue.

2.5. Morphometrical and statistical analysis

Morphometrical measurements to infundibulum and ampulla at follicular and luteal phases were performed by using Image Analysis Tools (IT system). Measurements included the number of primary mucosal folds/cross section as well as height and thickness of primary mucosal folds. In addition, height of epithelium and number of secretory to ciliated cells at follicular and luteal phases were assessed and all respective data analyzed statistically and significance was assigned at P < 0.05. Student’s t test Graph pad Software was used to compare differences between each parameter.

3. Results

3.1. Infundibulum

The mucosa of the infundibulum was highly folded with primary and secondary folds. The primary folds were tall and somewhat irregular that gave rise to many secondary folds and sometimes tertiary folds in some areas (Fig. 1A). The mean number of the mucosal folds at the follicular phase was 52 and that at the luteal phase was 46 (Table 1). The epithelium was of simple columnar type and was consisted of two main cell types: ciliated and non-ciliated secretory cells. Two types of ciliated cells (CC) in infundibulum were observed. The first type characterized by its large size and pale staining cytoplasm with enlarged rounded to ovoid nucleus located at the apical third of the cell. These cells were provided with few short cilia or even completely lacking of cilia. The second type was smaller in size, possessed long cilia with basal bodies and its nucleus was more compressed than the first type and centrally located (Fig. 1B and C).

At the luteal phase, the mean number of secretory to ciliated cells was 57:51 and the secretory cells were demonstrated by increase of cellular activity by the way of apical cytoplasmic projections. Merocrine mode of secretion was identified in many secretory cells that were characterized by irregular apical surfaces and little released materials were detected in the lumen (Fig. 1C). Few “Peg cells” or “Intercalary cells” were interspersed between the ciliated cells of the infundibulum that appeared as rod-like slender cells with a dark compressed nucleus. These cells were more frequently distributed at the basal portion of the mucosal folds and possessed no cilia (Fig. 1B and C). At the follicular phase, the ciliated cells were more demonstrated than the secretory ones as the mean number of secretory to ciliated cells was 48:56 and these ciliated cells were characterized by its rectangular shape and centrally oval vesicular nucleus (Fig. 1D).

In semithin sections, the exocytosis or eccrine mode of secretion was detected in many secretory cells by broken surfaces on some secretory cells with irregular apical cytoplasmic processes and deeper invaginations of plasma membrane (Fig. 2A and B). While at the follicular phase, the ciliated cells were predominated with its clear cilia and light colored cytoplasm. The secretory cells possessed few secretory granules with no cytoplasmic protrusions (Fig. 2C).
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Fig. 1. Infundibulum of cattle during follicular and luteal phases stained by H & E. (A) General view of the infundibulum showing highly folded mucosa with primary folds (arrow) and secondary ones (arrowhead). (B and C) Infundibular epithelium at luteal phase was consisted of dark ciliated cells with clear cilia (white arrowhead), light ciliated cells with few or no cilia (stars), secretory cells with apical secretory protrusions (two stars) and narrow slender peg cells (black arrowhead). (D) Infundibular epithelium at follicular phase characterized by predominance of ciliated cells (arrowheads) and absence of secretory activity. Note presence of light ciliated cells (star).

The cytomorphometric data revealed significant increase in the mean number and length of the primary mucosal folds at the follicular phase ($P < 0.05$). There are no remarkable changes in the mean thickness of primary folds during follicular and luteal phases. Significant increase in the epithelium height at the follicular phase was recorded (Table 1).

3.2. Ampulla

The mucosa of ampulla was thrown into numerous elaborately branched leaf-like folds that formed of primary, secondary and tertiary ones (Fig. 3A). These folds may be interconnected with one another in a complex manner (Fig. 3B). The mean number of the primary folds of the ampulla at the follicular phase was 42, while at luteal phase was 36 and thus, the number of the folds decrease from infundibulum to ampulla (Table 1). The lamina propria–submucosa was consisted of loose connective tissue with abundant collagenous fibers (Fig. 3C and D). Tunica muscularis was consisted of smooth muscle bundles with many blood vessels were interspersed between them. Serosa was the outer connective tissue layer (Figs. 3C, D and 4A). The lining epithelium was of simple columnar type that consisted of two types of cells: ciliated and non-ciliated secretory ones (Fig. 4B). The ampullar epithelium during the follicular phase was significantly higher than that at the luteal phase (Table 1). The secretory
Table 1
Morphometrical and statistical analysis of the uterine tube at the luteal and follicular phases of estrous cycle.

| Data                                      | Infundibulum at luteal phase | Infundibulum at follicular phase | Ampulla at luteal phase | Ampulla at follicular phase |
|-------------------------------------------|------------------------------|---------------------------------|-------------------------|----------------------------|
| Number of primary mucosal fold/cross section | 46 ± 3                      | 52 ± 4                          | 36 ± 2                  | 42 ± 3                     |
| Length of primary mucosal fold            | 1212.01 ± 54.02             | 1412.09 ± 68.21                 | 859 ± 40.74            | 932.12 ± 51.98             |
| Thickness of the primary mucosal folds    | 96.13 ± 1.7                 | 98.04 ± 2.1^ss                  | 142.89 ± 1.26          | 140.10 ± 1.32^ss           |
| Height of epithelium                     | 24.14 ± 2.5                 | 30.71 ± 3.1^1                   | 26.42 ± 0.17           | 28.04 ± 1.0^1              |
| Number of secretory to ciliated cells/primary fold | 57:51                      | 48:56                           | 42:31                   | 33:45                      |

The values were represented by mean ± SE. (ns) means that the differences were not significant.

^ Data analyzed statistically and be significant at P < 0.05.

Fig. 2. Semithin section of infundibulum at follicular and luteal phase stained by Toluidine blue. (A) Exocytosis of secretory products is evident at the luteal phase by broken surfaces of secretory cells and invaginations of plasmalemma (black arrowheads). Notice presence of ciliated cells with nucleus at the apical third (white arrowheads). (B) High secretory activity of the epithelium at luteal phase by means of cytoplasmic protrusions (black arrowheads) beyond the borders of ciliated cells (white arrowhead). (C) Large distribution of ciliated cells (arrowheads) at follicular phase with fewer darkly stained secretory cells with some apical secretory granules (stars). Lamina propria (LP) consisted of collagenous fibers and many connective tissue cells.

activity was very clear in the luteal phase, the bulging apices of the slender secretory cells exhibited constrictions and some secretory products were detached from the cell surfaces. Nuclei were situated at varying levels within the cell and may be extruded into the lumen (Fig. 4C). The follicular phase was characterized by predominance of ciliated cells with their numerous and prominent cilia (Fig. 4D).

The secretory cells were predominated in the luteal phase with various degrees of numerous apical cytoplasmic protrusions as the mean number of secretory to ciliated cells was 42:31 (Fig. 5A and Table 1). The nuclei of secretory cells were observed in the basal position and also were observed in the cytoplasmic protrusions (Fig. 5A). Basal cells were observed in the basal portion of the ampullar epithelium and were characterized by rounded dark nucleus surrounded by scant and lightly stained cytoplasm that were similar in appearance to lymphocytes (Fig. 5A). Cellular integrity disruption was markedly observed in the luteal phase (Fig. 5A). The ampullar epithelium at the follicular phase was characterized by an extensive distribution of ciliated cells as the mean number of secretory to ciliated cells was 33:45 (Table 1) and was characterized by its prominent cilia that were protruded into the uterine lumen with decrease in the number of secretory cells. Two populations of ciliated cells were observed in the epithelium of the ampulla: light staining
and large CC with apical rounded nucleus as well as darker and smaller ones with central oval nucleus (Fig. 5B). In semithin sections, the secretory cells of the luteal phase were characterized by their narrow slender shape with basal elongated nucleus and more densely stained cytoplasm than that of the ciliated cells. The shape of ciliated cells ranged from rectangular to pear shape, the nucleus was large ovoid, vesicular and more superficial than the nuclei of secretory cells (Fig. 5C). Reduction in size or even loss of cilia at the luteal phase was observed. Mast cells were demonstrated in the lamina propria of ampulla with their characteristic purple metachromatic granules by Toluidine blue (Fig. 5C). In the follicular phase, the secretory cells appeared dark in color, slender in shape, narrow in diameter and possessed apical microvilli. Also the ciliated cells were characterized by large number of apical tall cilia (Fig. 5D).

3.3. Histochemistry of the ampullar epithelium

Large amount of cidic mucopolysaccaridies were identified in the secretory cells of the ampulla by Alcian Blue (pH 2.5) at the luteal phase (Fig. 6A) and little amount of acidic mucopolysaccharides were identified in the follicular phase (Fig. 6B). The cytoplasm of secretory cells was granular in nature and characterized by positive reaction to PAS at both follicular and luteal phases (Fig. 6C and D). But, the reaction increased in the luteal phase as the number of secretory cells increased and was abundant patricularly in the crypts (Fig. 6C). The lipid droplets were identified by Sudan Black B in the apical portion of the ampullar epithelium at the luteal phase more than those present in follicular phase (Fig. 6E and F). Also, moderate acid phosphatase activity was demonstrated during the luteal phase at the apical region of the secretory cells by Geison’s Lead Nitrate method and weak activity was recorded in the follicular phase (Fig. 6G and H).

The morphometric and statistic data of the ampulla revealed increase in number of mucosal folds and length of the primary folds at follicular phase (P < 0.05). There are no remarkable changes in the mean thickness of primary folds at follicular and luteal phases. Significant increase in the epithelium height at the follicular phase was recorded (Table 1).

4. Discussion

Uterine tube is a part of the female genital tract, which picks up the ovum 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 [19]. The aim of the present study is to identify the histological, histochemical and morphometrical features of uterine tube of
cattle during the follicular and luteal phases. My results revealed differences in the histomorphometric structure of the different parts of the uterine tube during phases of the estrous cycle. These differences probably reflect different secretion rates of the ovarian hormones \([20]\). It is well known that the mammalian oviduct is a target organ for the sex steroid hormones, estrogen and progesterone. These hormones cause various morphological changes in oviductal epithelial cells. In particular, estrogen induces hypertrophy and formation of secretory granules \([21]\).

The results indicate that the mucosal folds were significantly increased in number and height during follicular phase and decreased from infundibulum to ampulla. These findings are consistent with some other investigations in ewe \([6]\) and rabbit \([22]\), which may be confirmed the role of infundibulum in receiving and transporting the oocyte from ovary to ampulla \([23]\).

This study revealed that the epithelium of cattle \((B.\ indicus)\) was lined by four types of cells: ciliated, non-ciliated secretory cells, few Peg cells that were non-ciliated slender cells wedged in between them and basal cells resembling lymphocytes near the basement membrane. The number of ciliated cells reached maximum in the infundibulum and decreased gradually in the ampulla. The number of ciliated cells increased in the follicular phase and was greater in infundibulum compared to the ampulla, this may be due to action of estrogen that cause active ciliation of epithelial cells \([24]\). It seems that the cyclic changes observed in the present study reflect the function of the cilia in the infundibulum and ampulla of the...
cattle. 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 [25].

The present study suggests that the secretory product is mainly produced by the secretory cells in the ampulla. Moreover, the mucus content of the oviduct of cattle contains various mucopolysaccharides, which could be stained by PAS and Alcian Blue. In addition, numerous lipid droplets and moderate acid phosphatase activity were recorded in the ampulla at the luteal phase. I suggest that these products are capable of retaining, nutrition and protection of the spermatozoa until ovulation, and the increase in the amount of secretions during the follicular phase may provide spermatozoa with motility and fertilization capacity. Concerning the oviductal secretions, oviductin is a glycoprotein secreted by secretory cells around the time of ovulation, bind to the zona pellucida of postovulatory follicles during their transit to oviduct [26]. Oviductal fluid has several functions: sperm capacitating, sperm hyperactivation, fertilization and early preimplantation development [25]. These findings provide insight into the difference in cellular function.

The apocrine secretion seemed to be the main way by which the secretion of secretory cells was released and this was identified by apical protrusions of secretory materials and their construction and detaching from the cell surfaces. Exocytosis of secretory products also observed in oviduct epithelial cells. Some glycoproteins secreted by secretory cells of oviductal epithelium associate with ovulated ova and developing embryos and may play important roles in early embryonic development [27,28]. Immunohistochemical studies on the bovine uterine tube epithelium are recommended.

Basal cells were demonstrated at this study and also called reserve or indifferent cells that were described by [29]. Ozen et al. [22] suggested that the basal cells are undifferentiated cells that can be transformed to secretory and ciliated ones. Cells with similar appearance had been referred to be leukocytes or lymphocyte-like cells [30]. The present study recorded presence of “Peg” or “Intercalary” cells for the first time in the oviduct of cattle (B. indicus). These cells were first demonstrated in oviduct of some farm animals by [1] who described them as depleted secretory cells and their secretions were consisted of mucoprotein and mucopolysaccharides. Finally, this detailed histological study of normal bovine oviduct will not only help to understand clearly the physiology of reproduction but also will assist in evaluating the pathological processes in the oviduct.
Fig. 6. Histochemical analysis of the ampullar epithelium at follicular and luteal phases. (A) The secretory granules at luteal phase showing strong positive reaction to Alcian Blue (arrowhead). (B) The fewer secretory cells at follicular phase showing positive reaction to Alcian Blue (arrows). (C) The secretory cells at luteal phase showing strong positive reaction to PAS (arrow). (D) The secretory cells at follicular phase showing moderate positive reaction to PAS (arrowhead). (E and F) The lipid droplets (arrowheads) were identified by Sudan Black B in the apical portion of the ampullar epithelium at both luteal and follicular phase respectively. (G) Moderate acid phosphatase activity (arrowheads) was demonstrated during the luteal phase at the apical region of the secretory cells by Gomori’s Lead Nitrate method. (H) Weak activity of acid phosphatase (arrowhead) was recorded in the follicular phase.
Conflict of interest

There is no conflict of interest to declare.

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