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Original Article

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

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ABSTRACT

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 mucopolysaccarides 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.

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

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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 slaughter-houses. 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 Trichome 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. Morphometric 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).