The Indian Journal of Veterinary Sciences & Biotechnology ***(2018) Volume 14, Issue 1, 38-42***
ISSN (Print) : 2394-0247 : ISSN (Print and online) : 2395-1176, abbreviated as IJVSBT
10.21887/ijvsbt.v14i1.12995

Scanning Electron Microscopic Studies on Uterus of Jaffarabadi Buffalo during Follicular and Luteal Phases of Estrous Cycle

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Publication Info

Article history:

Received : 03-05-2018
Accepted : 15-06-2018
Published : 20-07-2018

Key Words:

Follicular phase, Luteal phase, Jaffarabadi buffalo, Uterus, SEM.

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Abstract

The study was conducted on uterus of 20 adult Jaffrabadi buffaloes. Scanning electron microscopic observation showed that the surface of endometrium was folded. Lining epithelium of horn and body consist of ciliated and nonciliated cells. During follicular phase cells were flat, formed hexagonal structure with numerous microvilli, whereas, cells were narrow and polygonal in shape during luteal phase. More number of well-developed ciliated cells were found in follicular phase as compared to luteal phase. Numerous secretory blabs were observed on surface of secretory cells during luteal phase. The folds in the cervix were narrow and deep. The lining epithelium of cervix had ciliated and nonciliated cells. The cilia were more in cranial part than the caudal part of the cervix and present in the form of bunch, which were overlapping the secretory cells. Ciliated cells were more in number during follicular phase. Cervical glands were distributed on the cervical mucosa and ciliated cells were present around the openings of these glands.

Introduction

In mammals, the endometrium of the uterine horns plays a critical role in normal fertility and also represents different features in order to adapt through various phases of estrus cycle. The uterus not only has an influence on ovarian structure but also can participate in different physiological events by producing uterine milk. Therefore, it can provide an appropriate environment for sperms and blastocyst. Finally, the implantation process can be done completely (Shahrooz *et al.,* 2013). Generally, the most important function of the uterus in ruminant is involved in sperm transport, implantation, pregnancy and parturition which is associated with adjustment of cellular structures and microenvironments (Barnes, 2000). To better understand the basic structure and physiology of the buffalo uterus, more information is needed regarding the differences between the segments associated with the stages of estrus cycle. Therefore, the aim of the present study was to investigate the follicular and luteal phases of estrus cycle in the uterus of Jaffarabadi buffalo through the scanning electron microscopic approaches.

Materials and Methods

The present study was conducted on horn body and cervix of uterus from 20 adult Jaffrabadi buffaloes (10 at follicular and 10 at luteal phases). The uterus collected fresh from local abattoir, after examining the status of ovaries. Tissue samples were collected from horn, body, and uterus region. These tissues were thoroughly washed in chilled 0.1 M phosphate buffer (pH 7.2) and were subjected to fixation in 2.5% glutaraldehyde in 0.1 M phosphate buffer at 4°C for 4-6 hrs. Followed by washing in buffer. Thereafter, the samples were dehydrated in ascending grade of acetone solutions at 4°C.The specimens were mounted on aluminium stubs, coated with gold in sputter coater. The processed tissue samples were viewed under Zeiss EVO-18 (Germany) scanning electron microscope to take photographs.

Results and Discussion

Scanning electron microscopic (SEM) observation showed that the surface of endometrium of horn and body was folded. In follicular phase mucosal folds were thick and tall (Fig. 1), while in luteal phase they were thin and short. Two patterns of mucosal folds viz., mosaic-like in body and wave like in horn part were observed by Hafez and Kanagawa (1973) in rabbit. Similar observation were reported by Pathak and Bansal (2012) in Indian buffalo.

During follicular phase the luminal portion of the surface epithelial cells was flat and the cells were often formed hexagonal structure with numerous microvilli (Fig. 2) as earlier reported in African buffalo (Schmidt *et al.,* 2006) and in sheep (Pathak *et al.,* 2008). Whereas, the luminal portion of the surface epithelial cells was narrow and the cells were polygonal in shape during luteal phase (Fig. 3). However, Tienthai and Sajjarengpong (2010) reported that the epithelial cells were convex and separated from each other by the cell borders located on deep level in Thai swamp buffalo. The endometrium of the horn and body was lined by ciliated and nonciliated cells in both follicular and luteal phases of estrus cycle. More number of well-developed ciliated cells were found in follicular phase as compared to luteal phase (Fig. 4). Similar observations were reported by Pathak *et al.* (2008) in sheep and Tienthai and Sajjarengpong (2013) in swamp buffalo. Contrary to our findings Fathalla *et al.* (1975) observed non-ciliated epithelium in bovine uterus.

The apical border of the ciliated and nonciliated cells have microvilli. As earlier reported in bovine (Guillomot and Guay, 1982) and in Thai swamp buffalo (Tienthai and Sajjarengpong, 2010). Longer and numerous microvilli were observed during follicular phase but these microvilli were short during luteal phase.

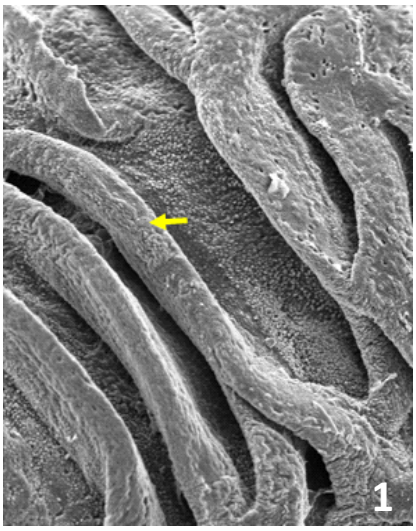

Fig-1. Scanning electron micrograph of uterine body showing presence of mucosal folds (arrow). X 335.

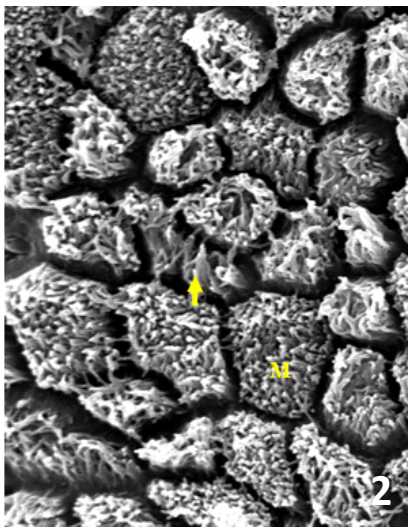

Fig-2. SEM micrograph of uterine horn in follicular phase showing hexagonal cells with microvilli (M) and few ciliated cells (arrow). X 7.74K.

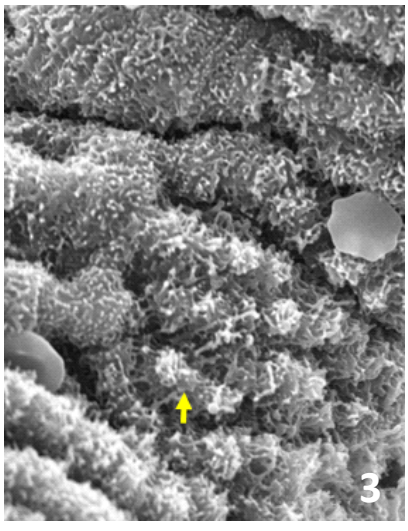

Fig-3. SEM micrograph of uterine horn in luteal phase showing narrow polygonal cells (arrow). X 5.09K.

The cilia were well developed in follicular phase but disintegrated during luteal phase, which indicated that the ciliated cells showed physiological response to the reproductive hormones. These cilia are thought to be important for the transportation of secretory material. The more number of secretory blabs were observed on surface of secretory cells during luteal phase (Fig. 5). Similar observation were reported by Pathak *et al.* (2008 and 2017) in sheep and in buffalo respectively. Tienthai and Sajjarengpong (2013) in swamp buffalo.

The endometrial caruncular areas were rounded to ovoid elevated structures without any gland openings. The caruncular surface appeared net-like due to elongated cellular ridges. These elongated cells were well differentiated from the surrounding epithelial cells in both the phases of estrous cycle. These cellular ridges were interconnected by surrounding cells. The surface of intercaruncular region was characterized by glandular openings which were round, smooth surfaced and unevenly distributed (Fig. 6). Similar observation were recorded in African buffalo by Schmidt *et al.* (2006).

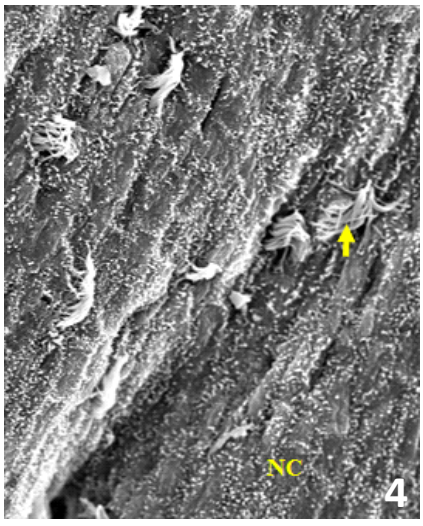

Fig-4. SEM micrograph of uterine body in follicular phase showing non-ciliated (NC) and few ciliated cells (arrow). X 2.60K.

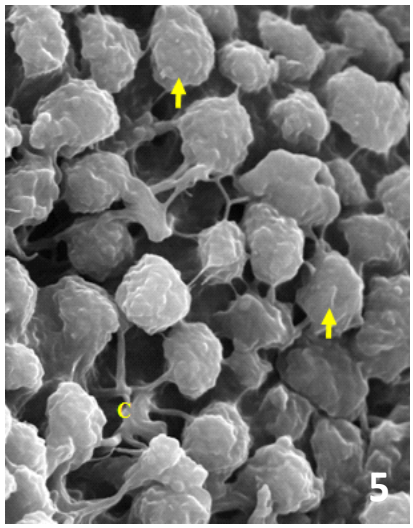

Fig-5. SEM micrograph of uterine body in luteal phase showing more number of secretory cells (arrows) with secretory material and few ciliated cells (C). X 7.12K.

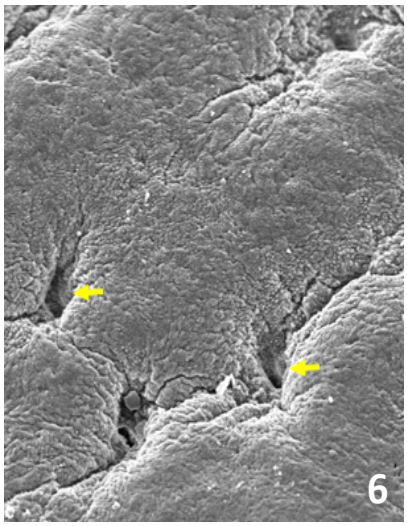

Fig-6. SEM electron micrograph of uterine horn showing intercaruncular region with glandular openings (arrows). X 416.

The glands were observed on the intercaruncular region of horn and body. These endometrial glands were formed from the invagination of the endometrial epithelium. The secretory activity of uterine glands was more during the luteal phase. (Fig. 7). However, Hafez (1980) reported that the uterine glands were more active during estrus in cow. The opening of these glands were surrounded by ring of ciliated epithelial cells. These openings were appeared as depression on the surface of uterus. Secretory material was found at the openings of glands in the luteal phase. However, Pathak *et al.* (2008) reported in sheep that the secretory material was oozing out through the glandular opening in follicular phase, while inactive endometrial glands were observed in late luteal phase. The lumen and the interior of the gland could not be observed in all the phases of estrus cycle. Similar observations were reported by Fathalla *et al.* (1975) in bovine.

In the cervix narrow and deep folds with small crypts were observed. The cervical crypts near internal os were more complex than the external os. The primary cervical crypts were arranged in parallel rows and secondary cervical crypts were on the longitudinal folds (Fig. 8). Similar findings were reported by Hafez and Kanagawa (1973) in cervix uteri of cattle, Wergin (1979) in the cervix of ewe and Pathak *et al.* (2017) in buffalo. Kanagawa *et al.* (1972) has suggested that the surface pattern of mucosal folds possibly act as a sperm passage to the site of fertilization or as a sperm barrier or sperm reservoir.

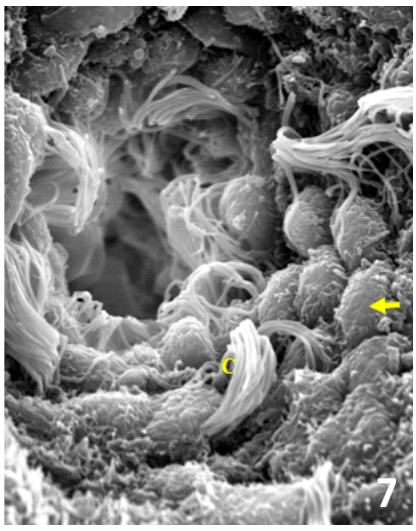

Fig-7. SEM micrograph of uterine body in luteal phase showing number of secretory cells (arrow) and few ciliated cells (C). X 7.48 K.

Fig-8. SEM electron micrograph of cervix in follicular phase showing presence of mucosal folds (F). X 160.

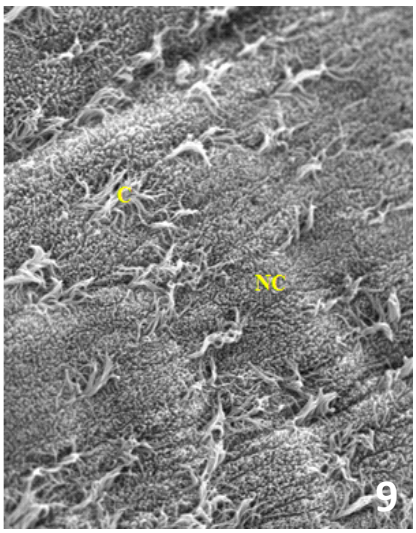

Fig-9. Scanning electron micrograph of cervix during follicular phase showing number of ciliated (C) and non-ciliated cells (NC). X 5.25K.

The lining epithelium of cervix was consisted of ciliated and non-ciliated secretory cells. Ciliated cells having cilia present on their surface in the form of bunch, which overlapping the secretory cells (Fig. 9). The cilia were more in cranial part than the caudal part of the cervix as earlier reported by Wordinger *et al.* (1973) and Hafez and Kanagawa (1972) in bovine, Riches *et al.* (1975) in rabbit Pathak *et al.* (2017) in buffalo. However, Hafez *et al.* (1971) reported that no ciliated cells were present in the bovine cervical epithelium.

Ciliated cells were more in number during follicular phase as compared to luteal phase in the cervix, the variation in morphological structure in different phase may be due to specific functions. Kanagawa *et al.* (1972) suggested that in the rabbit cilia present in endometrium may facilitate the release of secretory granules adhering to the surface of adjacent secretory cells. Hafez and Kanagawa (1972) reported that kinocilia in the cervix uteri beat rhythmically towards the vagina creating a directional flow of luminal fluids in the bovine reproductive tract. Pathak *et al.* (2008) suggested that the cilia may help the release of secretory granules present on the surface of adjacent secretory cells. Cervix uteri may prevent the transport of abnormal and inactive spermatozoa and acts as a sperm selector.

In late follicular phase secretions were very high and secretory materials formed a layer of mucous covering the ciliated cells and the secretory cells. The secretory cells were covered with microvilli and various sizes of ovoid or spherical protrusions on the apical surface of cells were observed (Fig. 10) as earlier reported by Hafez and Kanagawa (1972) in cervix of cattle and Wergin (1979) in the cervix of ewe. Cervical glands were distributed on the cervical mucosa. Ciliated cells were present around the openings of these glands, which indicate the importance of cilia in transport of secretions.

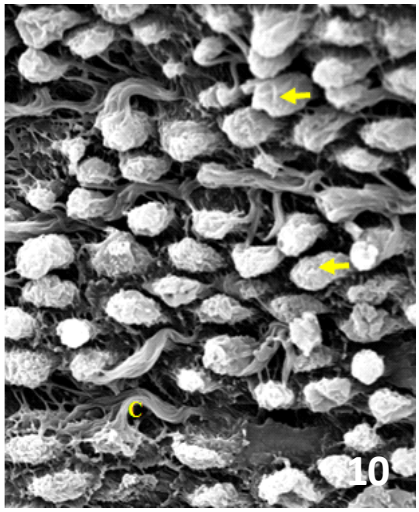

Fig-10. Scanning electron micrograph of cervix in luteal phase showing more secretory cells (arrows) covered with microvilli. X 7.72K.

Acknowledgment

Authors are thankful to the Professor & Head and staff of Department of Biotechnology, College of Agricultural, JAU, Junagadh for providing

necessary facility of scanning electron microscopy.

Conflict of Interest:

All authors declare no conflict of interest.

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The Indian Journal of Veterinary Sciences & Biotechnology ***(2018) Volume 14, Issue 1, 43-46***
ISSN (Print) : 2394-0247 : ISSN (Print and online) : 2395-1176, abbreviated as IJVSBT
10.21887/ijvsbt.v14i1.12996

Influence of Seasons on Seminal Attributes of Crossbred Bulls and its Implications on Fresh, Diluted and Cryopreserved Semen

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Publication Info

Article history:

Received : 20-05-2018
Accepted : 15-06-2018
Published : 20-07-2018

Key Words:

Holstein Friesian bull, Season, Seminal attributes, Cryopreservation.

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Abstract

The present study was conducted on eight crossbred bulls (HFxGir, 50-75% HF inheritance) under Konkan Development Corporation Ltd. of Maharashtra. Semen ejaculates obtained at monthly interval were used for this study. Semen samples were subjected to routine macroscopic and microscopic evaluation. The study was planned for three seasons, viz., summer, winter and monsoon. The ejaculates were divided into two parts: one part was used for analysis as fresh semen and second part was diluted using egg yolk extender and then cryopreserved. It was observed that the sperm motility and live sperm percentage were significantly low during summer season as compared to winter and monsoon season in fresh semen samples. The semen volume was significantly higher during summer season whereas the sperm concentration was higher in winter season. The colour, consistency and density were not affected by season. Thus, it was concluded that summer season adversely affects the seminal attributes which may result in low quality semen production.

Introduction

High quality bull semen is required for successful fertilization and improved herd health. The semen from individual bull may be used for many females and poor quality semen may affect herd fertility and productivity of the farm by lengthening calving intervals. In the breeding systems, their bio-economical effectiveness depends on bull fertility and performance in the field.

The bulls are subjected to environmental variations that interfere with their fertility and herd reproductive effectiveness (Berry *et al.,* 2011). Therefore, the successful evaluation of seasonal effects on thermoregulation and reproductive changes is crucial to identify these alterations in bovine physiology and health. Under tropical conditions the exotic breeds (*Bos taurus*) showed significant seasonal fluctuations in semen characteristics with higher sperm cell abnormalities, lower percentage of live sperm cells and lower sperm concentration during the hot periods (Bhorsekar *et al.,* 1980 and Parkinson, 1985). Hence, the present study was undertaken to assess the influence of seasons on seminal