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HISTOLOGICAL CHANGES IN BUFFALO ENDOMETRIUM DURING ESTROUS CYCLE

(With 4 Figures)

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التغيرات الهستولوجية فى الجدار المبطن للرحم فى الجاموس أثناء دورة الشبق

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تم الحصول على العينات المبطنة لجدار الرحم من الجاموس على فترات معلومة ومحدودة من دورة الشبق لاستيضاح التغيرات المميزة له وقد تم وضع هذه العينات فى شمع البرافين وقطعت وصبغت بالهيماتوكسلين والايوسين وقد وجدت التغييرات الهستولوجية الاتية: أثناء دورة الشبق:- ظهرت الاسترومامتورمة والاوعية الدموية شديدة الاحتقان وظهرت الخلايا المهاجرة نحو التجويف. ظهرت تجاويف بارزة وموزعة بطريقة منتظمة فى الغدد المبطنة لجدار الرحم وهذه التجاويف ازاحت الانوية لاعلى لتخليق نسيج متعدد الطبقات. أثناء فترة ما بعد الشبق:- ظهرت الاستروما كثيفة مع نقص ملحوظ فى السائل الوديمى ويوجد خلايا لمفاوية واسعة الانتشار ومعظم التجاويف فى الغدد المبطنة لجدار الرحم أخرجت أفرازتها وكذلك يوجد سائل متجانس محب للصبغة الحمراء يصل الى قمة افرازة ويتجمع فى تجويف الغدد المبطنة للرحم. أثناء فترة مابين الشبقيين: ظهرت الاستروما على شكل طبقة رقيقة من الخلايا الشديدة التلاصق. تحولت بعض خلايا الاستروما الى خلايا البريدوسيديوال. الخلايا المبطنة للغدد تظهر على شكل صف من الانوية تجاه قاعدة الخلية مع وجود سيتوبلازم محب للصبغة الحمراء (الحامضية) تجاة تجويف الغدة. أثناء فترة ما قبل الشبق: تنقسم الاستروما الى طبقتيين طبقة خاجية كثيفة غير نشطة وطبقة داخلية مفككة يوجد بها قليل من الخلايا المهاجرة. الخلايا المبطنة لغدد الرحم تظهر على شكل خلايا صغيرة مكعبة غامقة الصبغة غير نشطة.

SUMMARY

Endometrial specimens were obtained from normally cyclic buffalo at known stages of their estrous period to show precise characteristic changes. These tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin. The following histological changes were observed: During estrous, the stroma was highly oedematous with highly congested blood capillaries and few migratory cells directed toward the lumen. The endometrial glands showed prominent and regularly distributed vacuolation. These vacuoles displace the nuclei upward to create a pattern of

pseudostratification. During met-estrous: The stroma was denser with a marked decrease in oedematous fluid and highly infiltrated lymphocyte and mast cells. Most of the vacuoles of the endometrial glands have discharged its secretion, and a homogenous acidophilic secretion reached its peak and accumulated in the lumen. During di-estrous: The stroma looked like a solid sheet of closely packed stromal cells. Some stromal cells showed signs of transformation into predecidual cells. The glandular epithelium showed an orderly row of nuclei with homogenous acidophilic cytoplasm above them. During pro-estrous, the stroma divided into outer condensed non functional layer and inner loosed layer with few migratory cells. The endometrial glands lined with inactive, low columnar darkly stained cells.

Keywords: *Histology-buffalo-endometrium-estrous.*

INTRODUCTION

Morphologically the endometrium is one of the dynamic target tissue in which structural changes occur in a rythmic fashion throughout the reproductive life. The histological changes of cyclic endometrium including follicular and luteal stages, have been reported in human (*FINN and PORTER, 1975)* sheep *(SEIDA, 1977 and EZZO, 1989)* and bovine *(PRIEDKALNS, 1987 and OHTANI et al., 1993)*. These changes included the height of the glandular epithelium, the size of the gland lumen, the secretion in the gland lumen and stromal oedema. However, the studies did not demonstrate the characteristic changes that occur in each stage during the estrous cycle which are controlled by internal hormonal signals.

In the present study we undertook to examine whether or not endometrial specimens in buffalo could be used to demonstrate characteristic changes during each stage of the estrous cycle.

MATERIAL and METHODS

Female genital tracts of 55 non-pregnant Egyptian buffaloes were collected from El-Monib slaughter house. Immediately after evisceration of the animal, the ovaries were carefully, examined for the physiological cyclic alteration, while the uterus were prepared for histological examination.

The macroscopic physiological changes indicating the normal ovarian cyclicity were carefully described from the naked eye appearance of ovarian follicles and/or corpus luteum according to the scheme adopted by *JAINUDEEN (1986)*. Grouping of uterine specimens was then undertaken according to the phase of the oestrous cycle indicated from the ovarian cyclic

status (follicular phase: pro-estrous, estrous and luteal phase: estrous and di-estrous).

The aforementioned specimens were fixed in Bouin's then processed by the usual histological techniques to obtain paraffin sections of 5-7 um thick. There after, the paraffin sections were stained by Harris haematoxylin and eosin *(DRURY and WALLINGTON, 1980)* and Toluidin blue stain for detection of sulphated mucopolysaccharides in mast cells *(LAYDEN, 1971)*.

RESULTS

The examination of cross sections of the uterus revealed some cyclic variations appeared in the form of mitoses of stromal and glandular cell, vacuolation of the glandular epithelia cells, stromal oedema, pseudodecidual reaction and metrorrhagia.

At estrous, metrorrhagia (destruction of the surface epithelium) considered to be a characteristic feature of this phase and shreds of mucosa were observed in the uterine lumen. At the end of estrous, the endometrium was formed of high columnar epithelium with clear infranuclear vacuolation (Fig. 1-a). The stroma was very loose, highly oedematous and the amount of tissue fluid increased so that the cells were more widely separated from each other. The stromal tissue was highly vascular with congested blood capillaries. The stromal cells hypertrophied and resembled mature fibroblasts with scanty cytoplasm. Few wandering lymphocyte were infiltrated in the subepithelial tissue, migrated at different levels among the epithelial cells and desquamated in the lumen. The endometrial glands showed maximal activity appeared in the form of increased diameter of glands which accompained with dilated lumen. Vacuolation of the glandular epithelium was the most distinct histological change in this phase and it was more prominent and regularly distributed. These vacuoles displace the nuclei upward to creat a pattern of pseudostratification (Fig. 1-b). The lightly stained secretory cells resemble goblet cells, it might contain glycogen or lipoid granules which dissolved when immersed in the fixing solution, sometimes it projected beyond the surface epithelium (the terminal bars). Glandular mitosis were commenly seen during this phase. A homogenous acidophilic secretion accumulated in the lumen. The glandular epithelium was shed into the lumen and could be recognised as a central cluster of cells with intact basal cell layer (Fig. 1-c).

At met-estrous, the surface epithelium was consisted of high columnar epithelium with complete disappearance of the infranuclear vacuoles (Fig.2.-a) The stroma become denser with a marked decrease in oedematous fluid and congested blood capillaries. The migratory cells were highly infiltrated beneath and between the epithelial cells and appeared to be migrated toward

have been mentioned by *OHTANI et al (1993)*. These vacuoles are seen to represent the spaces from which glycogen has been leached out. The secretory vacuoles is proportional to estrogen profiles because estrogen stimulates glycogen synthesis in the endometrium through stimulation of glycogen synthetase activity *(BUGALIA and SHARMA, 1990)*. The pseudostratification of the glandular epithelium considered to be a characteristic feature during estrous phase. It may be due to high mitotic activity and high cell proliferation in the glands at the time of ovulation as have been mentioned by *FINN and PORTER (1975)*. During di-estrous, there were a complete disapearance of secretory vacuoles and pseudostratification of the gland. Similar results have been mentioned by *FINN and PORTER (1975)*, that a homogenous acidophilic secretion which contain sulphate observed in the supranuclear cytoplasm of the endometrial gland and in the lumen. The luteal phase consider to be a secretory phase as have been mentioned by *DAVID and CORMACK (1987)* in human, *WILLIAM (1984) and EZZO (1989) in EWES and OHTANI et al. (1993)* in cow. The secretory activity could be associated with increasing progestrone concentration because it influences glycogen secretion to nourish the blastocyst during the preimplantation period *(BUGALIA and SHARMA, 1990)* The secretion of the uterine glands during luteal phase contains mucopolysaccharide *(HEAP and LAMMING, 1962)* and carboxymucins *(HESTER et al., 1970)* while the secretion during estrous phase consists mainly of protein which are absent from plasma *(SQUIRE, BAZER and MURRAY, 1972)*.

Although there are several studies on the structure of the bovine endometrium during estrous cycle, only a few of them discuss mitotic activity in the glandular epithelium. In the present study, glandular mitoses were observed during proestrous and estrous. Similar finding was given in the cow by *PRIEDKALNS (1987) and OHTANI et al. (1993)*. In human, the glandular epithelial mitosis is frequent during the proliferative phase of the cycle, reaching a peak at the time of ovulation *(FINN and PORTER, 1975)*. All these authors confirm that estrogen causes an increase in glandular mitosis.

Mitotic activity appeard in stromal cells during the luteal phase. These results were similar to those of *PRIEDKALNS (1987)*, while *OHTANI et al (1993)* observed stromal mitosis in estrous and mid-luteal phases, with results similar to that found in human (*FINN and PORTER 1975)* that stromal mitosis shows two peaks, one in the regenerative period as a response to the destruction and removal of tissue at menstruation and a further one during the luteal phase just before the expected time of implantation. In mice, progesterone induced stromal mitosis if priming

plasma cells and macrophages), as have been mentioned by *FINN and PORTER (1975)* in human and *SEIDA (1977) and EZZO (1989)* in sheep, as a normal defence mechanism of genital tract. They are though to be involved in the destruction and phagocytosis of introduced sperm and bacteria because the leucocytes contain peroxidases and hydrolytic enzymes *(KLEBONOFF, 1966)* During luteal phase highly lymphocytic infiltration in addition to a peculiar number of mast cells which reached the maximum during di-estrous. *PRIEDKALNS (1987)* described highly agranulocyte invasion in the basal region of the endometrium of the cow on days 3 to 5, while *OHTANI et al. (1993)* described highly leukocyte invasion on days 7 to 8, Similar finding were given by *FINN and PORTER (1975)* that the number of mast cell appear to decrease during oestrous, this reduction is apparently induced by the high levels of estrogen. More extensive depletion takes place just before implantation *(SHELESNYAK, 1960)*. This has been put forward as evidence for the involvement of histamine in implantation although it might indicate mobilisation of sulphated mucopolysaccharides in preparation of decidual growth *(FINN, 1971)*. The method employed to show depletion of mast cells (Toluidine blue) depend on the presence of sulphated mucopolysaccharides.

FINN and PORTER (1975) have demonstrated pseudodecidual cells within stromal cells in human in the late luteal phase of the cycle, showing englarged nuclei and condensed cytoplasm. *OHTANI et al. (1993)* also observed pseudodecidual cells around the arterioles on day 4 and larger sheets of pseudodecidual cells were observed in the functional layer on days 5 to 6. In the present study, pseudodecidual cells were observed around the arterioles and in between stromal cells during luteal phase. All those authors confirmed that the pseudodecidal reaction usually associated with increasing progesterone concentration.

Stromal oedema observed during the follicular phase. This oedema resulted from the effect of estrogen hormone which enhanced the permeability of the cell membrane and capillary *(PRIEDKALNS, 1987 and FINN and PORTER, 1975)*. The interstitial oedema begins to subside until it completely disappears during the luteal phase. These results were in agreement with those reported in sheep by *SEIDA (1977) and EZZO (1989)*.

Vacuolation of the uterine gland was very prominent and regularly distributed during estrous phase. These results were in agreement with the endometrial glands of human which hypertrophy and become pale staining because of the accumulation of considerable amounts of glycogen as have been mentioned by *ROBERT and NELSON (1983)* or may be due to their abundant content of storage product as have been mentioned by *DAVID and CORMACK (1987)*. These results were similar to the supranuclear vacuolation which apear in bovine between 3 and 7 days after ovulation as

the lumen. There were a peculiar increase in mast cells infiltrated in-between the stromal cells (Fig. 2-b). Most of the vacuoles of the endometrial glands have discharged its secretion. Most of the nuclei returned to the base of the cell which lead to marked decrease in pseudostratification (Fig. 2-c). A homogenous acidophilic secretion reached its peak and accumulated in the lumen and the central cluster could be recognized also.

At di-estrous, the surface epithelium was pseudostratified columnar types with migratory cells directed toward the lumen (Fig. 3-a). The stroma become denser with a complete disappearance of the oedematous fluid and congested blood capillaries. It looked like a solid sheet of closely packed stromal cells which were elongated with condensed nuclei and abundant cytoplasm. Some stromal cells arranged around the arterioles and began to show signs of transformation into predecidual cells (cell with enlarged nuclei and abundant cytoplasm). The stroma was highly infiltrated with lymphocytes and mast cells. The uterine glands were hypertrophied with highly coiling appearance and a marked increase in height of the glandular cells which lead to narrowing of the lumen. The glandular epithelium showed an orderly row of nuclei with homogenous acidophilic cytoplasm above them, all the nuclei have returned to the bases of the cells and there were a complete disappearance of secretory vacuoles and pseudostratification (Fig. 3-b).

At pro-estrous, the surface epithelium was formed of high columnar epithelium with no infranuclear vacuoles resembled those of met-estrous. The endometrial stroma divided into outer condensed non functional layer and inner loosed layer with few migratory cells (Fig. 4-a). The stromal cells mostly of mesenchymal. nature. The endometrial glands lined with inactive, low columnar epithelial cells which had relatively dark cytoplasm and dark neucleus (Fig. 4-b).

DISCUSSION

The changes occuring in the endometrium were more pronounced in the stroma and uterine glands than in the free epithelial surface. The epithelial lining of the uterus was columnar and pseudostratified at all times. Infranuclear vacuolation in the surface epithelium act as characteristic feature of the estrous phase. These vacuoles are seen to represent the space from which glycogen has been leached out as have been mentioned by *FINN and PORTER (1975)* in human and *OHTANI et al. (1993)* in bovine.

The leukocyte invasion beneath the surface epithelium was useful in determining the phase of the estrous cycle. During follicular phase, few migratory cells took a way to the uterine and glandular lumens through their lining epithelia. These may be mononuclear inflamatory cells (lymphocytes,

estrogen was given *(FINN and MARTIN, 1973)*. By contrast, estrogen alone caused an increase in stromal mitosis in rabbits *(KOSEKI and FUJIMOTO, 1974)*.

The usefulness of histological changes (in normal cyclic buffalo) for diagnosing uterine disorders is not yet definitive. To advance the understanding of histological changes in the normal and infertile animals, will need to be compared throughout the estrous cycle. In addition, physiological roles of histological changes during estrous cycle will also need to be addressed.

REFERENCES

Bugalia, N.S. and Sharma, R.D. (1990): Endometrial Glycogen, protein, nucleic acids and phosphatases during oestrous cycle in buffaloes (babulus bubalis). Br. Vet.J. (1990). 146, 559.

David, H. and Cormack, Ph.D. (1987): Ham's Histology-9th Ed. Part four the systems of the body the female reproductive system.

Drury, R. and Wallington, E. (1980): Carleton's histological technique 5th Ed. Oxford Univ. Press New York, Toronto.

Ezzo, H.O. (1989): Some aspects of reproduction in Barki ewes. Ph.D. thesis Cairo Univ.

Finn, C.A. and Porter, D.G. (1975): The uterus. Reproductive Biology Handbooks. Vol. I. 1st. Ed. Paul Elek (Scientific books) Ltd., London.

Finn, C.A. (1971): Biology of decidual cells, in adv. Reprod. Physiolog. 5, Ed.M.W/.H. Bishop, Elek, London,pp. 1-26.

Finn, C.A. and Martin, L. (1973): Endocrine control of gland proliferation in the mous uterus. biol. Reprod. 8: 585-588 (1973).

Heap, R.B. and Lamming. G.E. (1962): The influence of ovarian hormones on some chemical constituents of the uterine washing of the rat and rabbit. J. Endocr. 25, 57-68.

Hester, L.L.; Kellet, W.W.; Spicer, S.S.; Williamson, H.O. and Pratt-Thomas, H.R. (1970): Effects of intra-uterine contraceptive device on endometrial enzyme and carbohydrate histochemistry. Amer.J.Obst. Gynec. 106, 1144-1151.

Jainudeen, M.R. (1986): Reproduction in water buffaloes in current therapy in theriogenology. W.B. Saunders Company, Philadeliphia, USA.

Koseki, Y. and Fujimoto, G.I. (1974): Progesterone effects contrasted with 17-B estradiol on DNA synthesis in epithelial unclear proliferation in the castrated rabbit uterus. Biol. Reprod. 10: 596-604 9(1974).

Layden, E.D. (1971): Practical section cutting and staining 5th Ed. churchill Livingstone Edinburgh and London.

Ohtani, S.Okuda, K. Nishrimura, K. and Mohri, S. (1993): Histological changes in bovine endometrium during the estrous cycle. Theriogenology 39: 1033-1042, 1993.

Priedkalns, J. (1987): Female reproductive system. In: Dellman, H.D. and Brown, E.M. (eds.), Text book of Veterinary Histology. Lea & Febiger, Philadelphia, 1987, pp. 332-333.

Robert, L. and Nelson, R. (1983): Medical Histology A text-Atlas with introductory Pathology.

Ross, R. and Klebanoff, S.J. (1966): The eosinophilic leucocyte: fine structure, studies of changes in the uterus during the oestrous cycle. J. Exp. Med. 124, 653-660.

Seida, A.A. (1977): Histological and Histochemical studies on the endometrium of the ewe. M.V.Sc. thesis, Cairo Univ.

Shelesnysk, M.C. (1960): Nidation of the fertilized ovum. Endeavour, 19, 81-86.

Squire, G.D.; Bazer, F.W. and Murray, F.A. (1972): Electrophoretic patterns of porcine uterine secretions during the oestrous cycle. Biol. Reprod. 7, 321-325.

William, H.L. (1984): The reproductive performance of two British breeds of sheep in contrasting photoperiodic environments. J. Agri. Sci, 82: 377-381.

Fig. 1: Photomicrographs of the endometrium during estrous phase:

(1-a) The surface epithelium was formed of high columnar epithelium with clear infranuclear vacuolation -Few migratory cells directed toward the lumen. Stain : H&E X 400.

(1-b) Vacuolation of the glandular epithelium was very prominent and regularly distributed Stain : H&E X 400.

(1-c) Central cluster of the glandular epithelium, notice that the basal layer remained intact Stain : H&E X 400.

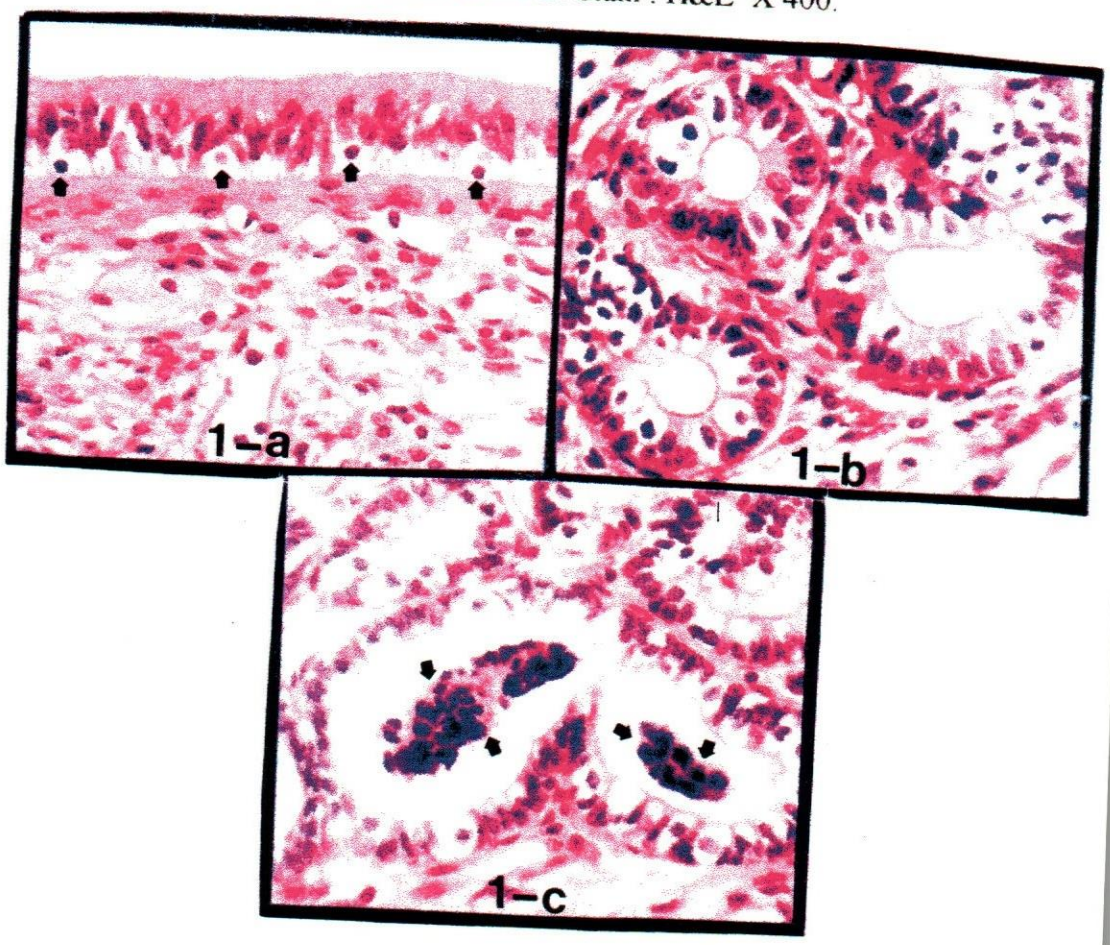

Fig. 2: Photomicrographs of the endometrium during met-estrous phase:

(2-a) The surface epithelium was high columnar with complete disappearance of infranuclear vacuoles. Stain: H&E X 400.

(2-b) The stroma was highly infiltrated with lymphocytes and mast cells (arrows) Stain : Toluidine blue X 400.

(2-c) Most of vacuoles of the endometrial glands have discharged its secretion (arrows) Stain : H&E X 400.

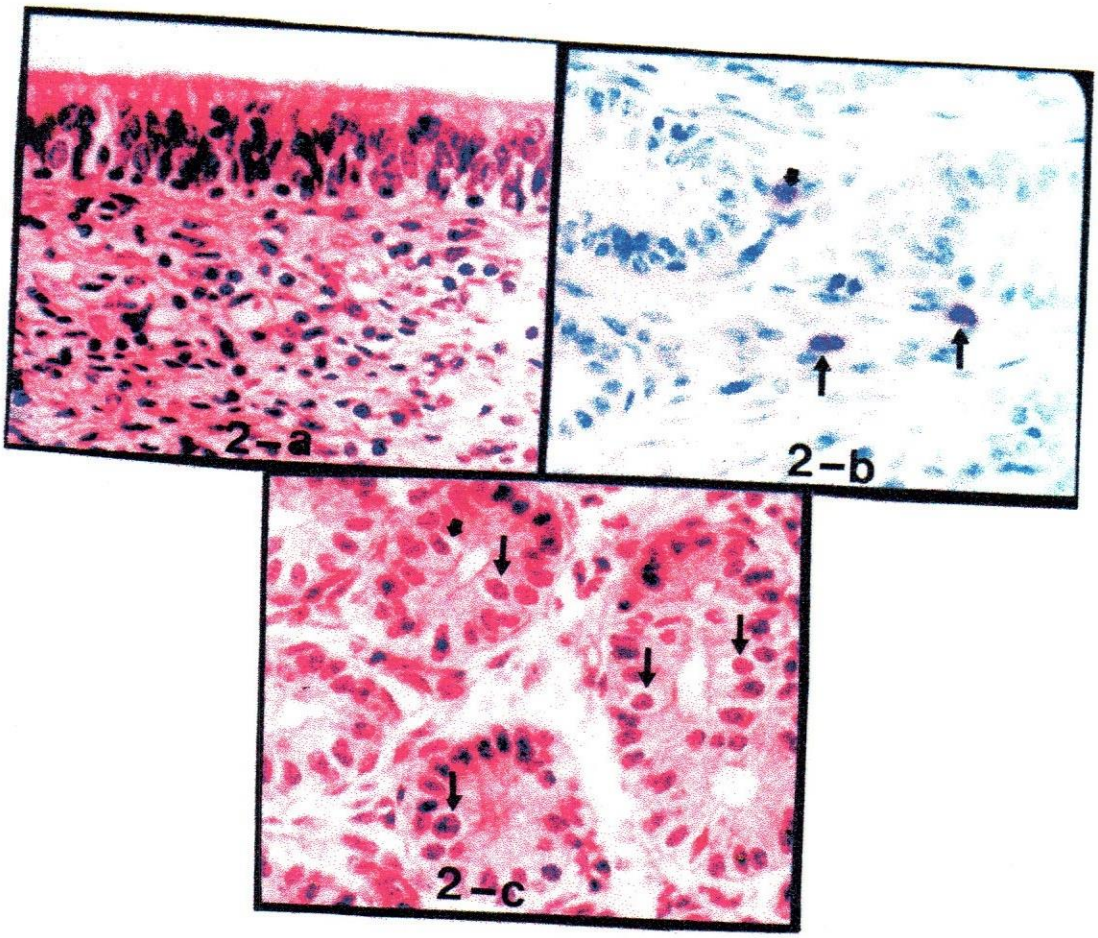

Fig. 3: Photomicrographs of the endometrium during di-estrous phase:

(3-a) The surface epithelium was pseudostratified with migratory cells (arrows). The stromal looked like a solid sheet of closely packed cells. Stain : H&E X 400.

(3-b) The endometrial glands hypertrophied and showed an orderly row of nuclei with homogenous acidophilic cytoplasm above the. Stain : H&E X 400.

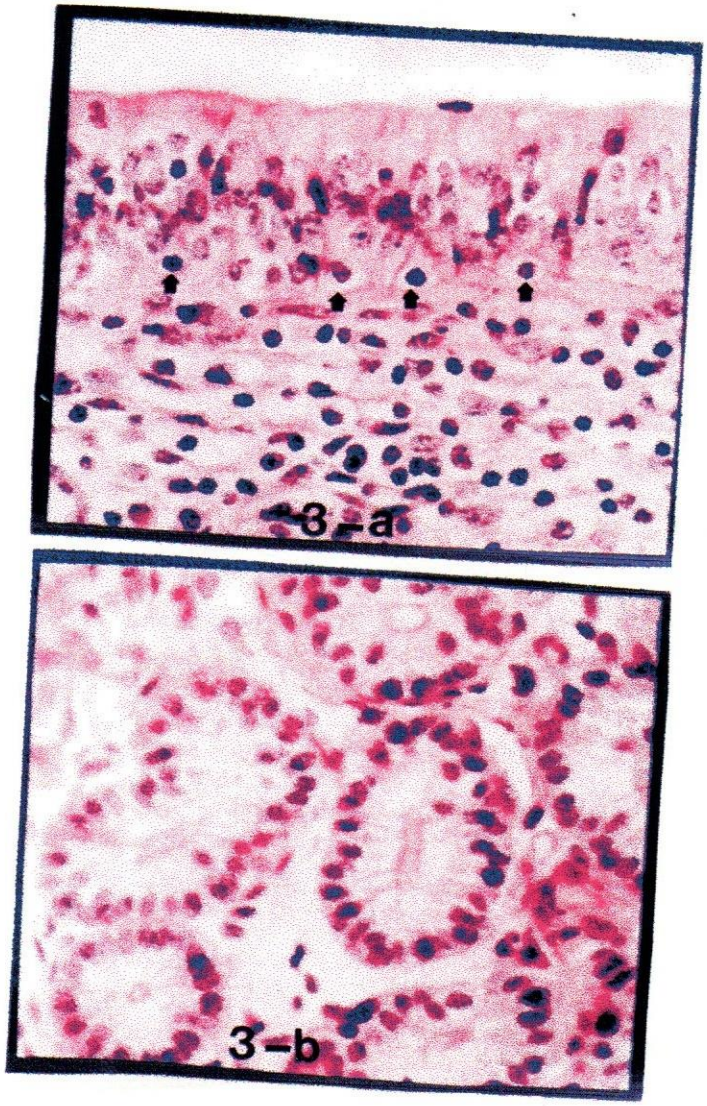

Fig. 4: Photomicrographs of the endometrium during pro-estrous phase:

(4-a) The endometrial stroma was denser in the outer layer and looser in the inner layer. Stain : H&E X 100.

(4-b) The endometrial glands lined with low columnar cells which hand darkly stained cytoplasm and nucleus. Stain : H&E X 400.