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A MICROMORPHOLOGICAL STUDY ON THE UTERUS OF EGYPTIAN DOES (CAPRA HIRCUS) DURING THE OESTROUS CYCLE

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SUMMARY

The uteri of 25 Egyptian does at different phases of oestrus and 8 uncycling does were examined microscopically. The endometrium was found to be the most affected layer. It responded to oestrogen by further stratification of the epithelium , increased vas-ularity of the propria and growth of the glands. Desuamation of some of the surface and gland epithelial cells, disappearance of hyperaemia and release of secretion characterized the luteal phase of the cycle. In one doe, invagination of a gland from the caruncular epithelium was considered to be a case report.

INTRODUCTION

eproduction is a complicated process which deends largely on the co-ordination between the fe-ale genital tract and rythmic activity of the hy-othalamus-pituitary-ovarian axis. When Co-perate properly,the uterus is considered to be the ost important target organ. This suggestion is ained since troubles in the oviduct could be over-omed by embryo transfere and unhealthy vagino-estibular segment could be passed by artificial semination and then caeserian section at the roper time . The uterus, as an important repro-uctive organ, hence, directed our attention to udy its structure in one of ruminant animals, the oes.

eports on the structure and changes in the uterus f domestic farm animals during the oestrus cycle re numerous (Asdell 1955, Restall, 1966 Mari-ov and Lovell 1968 Agoub 1973 and Nawar, bul-Fadle and Mahmoud 1979). As the availa-ble literature pointed , studies using goats were comparatively very few (Hamilton and Harrison 1951 and Bareedy, Awad and El-Hariri, 1983) . The present work, therefore, was conducted aiming to fulfill this gap and to establish some basic knowledge which might be useful for veterinarians, breeders and researchers.

MATERIALS AND METHODS

Thirty-three genital tracts of apparantly healthy Baladi Egyptian does were selected during spring; 7 at pro-oestrus, 4 at oestrus ; 6 at metoestrus; 8 at dioestrus and 8 uncycling females. The criteria of Ramachandraiah et al. (1986) was used to judge the different phases. Immediately after slaughter, the internal genitalia were cut out and fixed in 10% buffered neutral formalin. Thin slices at the utero-tubal junction, the middle and posterior parts of the uterine horns, the body and the utero-cervical junction were refixed in Bouin's fluid. Parts from the ovaries, including the most promi-

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nant structures, were also sliced from each animal only to confirm the macroscopic judgment of the ovaries. All parts were embedded in paraffin wax and cut in the transverse direction at 5-7 micrometer . The junctions of the uterus with the oviduct and the cervix were also cut longitudinally . The sections were then stained with Harris haematoxylin and eosin, Crossmon's trichrome stain, Aldehyde fuchsin stain and Gomori's reticulin method (Romeis 1948, and Drury & Wallington 1980)

RESULTS

Pro-oestrus phase:

Longitudinal sections slong the utero-tubal junction and cross sections of the uterine horn tips indicated absence of an intramural part of the oviduct and lack of a muscular sphinctor. The epithelium of the oviduct changed gradually into that of the uterus and uterine glands began to appear in the propria. The muscular and serous coats of both organs joined each other (Fig.1).

Apart from variation in diameter and some other minor differences, the uterine horns and the body possessed nearly the same structural features.
The endometrium was lined with a pseudostratified columnar epithelium (Fig. 2) which was frequently reduced to simple columnar between the mucosal folds . Migrating lymphocytes were rarely seen .

The thick propria was fibro-reticular in nature. The elastic fibers were only present in the blood vessels (Fig. 3) . Two zones of propria were demonstrated; a narrow cellular zone under the epithelium (stratum compactum) and an outer thick fibrous zone (stratum spongiosum) . Whereas fibroblasts occurred in the latter, modified fibroblasts (stroma cells) prevailed in the former. They were larger and their faintly-stained cytoplasm contained oval large vesicular nuclei.

Scattered throughout the propria were numerous, simple, branched coiled tubular glands which occasionally extended deeply to lie within the muscular layer (Fig. 4) . Their ducts were characterized by large diameter and their wide lumina were lined by an epithelium similar to that of the surface. The secretory tubules, on the other hand were much smaller in caliber and their narrow lumina were surrounded by extremely thin columnar cells (Fig. 5) . Their cytoplasm was lightly acidophilic and finely-granular but not vacuolated . Migrating lymphocytes were rarely observed between the gland cells . Toward the oviduct and the cervix, the glands were fewer, relatively smaller and less coiled.

Dome-shaped caruncles were observed as circumscribed elevations of the mucosa of uterine body and horns. They were non-glandular highly cellular structures derived from the stratum compactum and lined by a lower pseudo-stratified columnar epithelium. The ducts of glands did not penetrate the caruncles but pass diagonally to open into the inter-caruncular areas. Only in one case, a gland was seen invaginated from the epithelium of one of the caruncles (Fig.6) .Usually the subcaruncular area was more vascular and arterio-venous anastomoses were frequently observed (Fig.7) .

The myometrium formed about 1/4 to 1/3 of the wall thickness . In the body and in the proximal free parts of the horns, the inner circular layer was thicker than the outer longitudinal (Fig.8). Between the two layers, or deeper in the inner layer, loose connective tissue containing tortuous blood vessels, fasicles of myelinated nerve fibers and isolated bundles of smooth muscles, were frequently observed . It has been found that the veins were provided with valves. In the distal parts of the horns where they were partially fused , the musculosa possessed a special arrangement . Each horn was surrounded by a circular layer and both horns shared a common outer longitudinal envelop. In all sections examined, no smooth muscle fibers were seen extending into the lamina propria.

The uterus was found to be wrapped completely by a serosal coat (Fig. 8) except where the neurovascular supply penetrated into the uterus.

The enterance into the cervix through the internal os was narrow and the uterine epithelium extended to line the first part of the cervical canal. The uterine glands disappeared at or proximal to the os.Continuity of the bilayered musculosa and the serosa of the uterus into those of the cervix was

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oticed .

)estrcus phase:

he epithelium was slightly increased in height nd the cells tended to be more crowded . Goblet ells, frequently, manifested themselves (Fig.9). his did not exclude areas where simple columnar ells persisted particularly over the caruncles .

hanges in the propria were characterized by in-reased capillary circulation and appearance of igrating acidophils. Visible oedematous areas ere not met with. Growth of the glands was indi-ated by an increase in their length and diameter. lebbing of the apical parts of some cells and de-achment of others was , sometimes, recorded Fig. 10) .Frequently, secretions mixed with de-aching and migrating cells were seen accumulat-ng in some ducts and in the uterine cavity. The ells included acidophils, lymphocytes, macro-hages, desquamated epithelial cells and also de-ached parts of the surface epithalium.

Metoestrus phase:

The findings were quite similar to those described during oestrus in many respects and more exager-ated in others .

Stratification of the surface epithelium was gener-lly increased, particularly in the inter-caruncular reas. Over the caruncles, only one or two layers f columnar cells were recorded (Fig.11) .

Numerous lymphocytes, and to a lesser, extend cidophil cells, were recorded under the surface pithelium around the secretory tubules and also igrating through them. Many blood capillaries were still opened. Development of glands contin-ued . A greater number of tubule cuts, propably due to increased coiling , was recognized . Indi-vidual tubules also became larger in diameter , lined with taller cells and so many of them con-tained secretory products (Fig. 12).

Mitosis in the surface and gland epithelial cells was frequently recorded.

Di-oestrus phase:

Although the epithelium was generally reduced in height with lesser stratification (Fig. 13) , small areas of thick pseudostratified columnar epithelium with apocrine-secreting cells inbetween still persisted (Fig. 14) .

So many capillaries in the propria were collapsed and the number of lymphocytes and acidopils infilterating the surface epithelium was greatly reduced even in areas where a lymph noduls was present (Fig. 13). Growth of uterine glande was further increased by budding from preexisting glands. Pale-stained cells appeared first which then grew to form new side adenomeres (Fig. 15). Late in the period, the secretory adenomeres became smaller in size and the lumina of many of them were narrowed or even obscured. Widening and even sacculation of some parts of the glands were frequently observed (Fig.16) .

Uncycling does:

The height of the surface epithelium reached its lowest level. One or two layers of crowded columnar or cuboidal cells prevailed (Fig. 17).

Marked regression of the glands was established. They became fewer , shorter, less coiled and smaller (Fig. 18) . Moreover, the cells were shorter with ill-distinct boundaries and many of their nuclei showed degenerative changes. Marked regression of the glands rendered the propria more compact, more fibrous and less cellular. Migrating cells were of rare occurrence.

DISCUSSION

Goats and ewes are two species of ruminant animals whose reproductive pattern differs greatly from primates **(Thibault and Levasseur, 1974)**. Therefore, discussion of the results would be confined largely to domestic farm animals, particularly ewes. The oestrus cycle is generally longer in gaots (20-21 days) than in ewe (16-17 days), although the follicular phase was the same in both animals (2-3 days). Ovulation tended to occur near the end of the oestrous phase in most ewes **(Thibault and Levasseur 1974)**. Furthermore,it was found that the uterus of ewe shares in regression of the corpus luteum by releasing a luteolytic

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factor ; prostaglandin $F_{2\alpha}$ (Bland, Horton and Poysen 1971), since removal of the uterine horn ipsilateral, but not contralateral to the corpus luteum prolonged the luteal phase and total hysterectomy maintained the corpus luteum for a period about the length of gestation (McCracken, et al.1972).

Seasonal variations in sexual activity is known to occur in many animals . Although it is clear cut in ewes and goats, yet domestic breeds of ewes varied from being seasonal polyoestrus to polyoestrus animals.In tropical and subtropical countries, ewes breed throughout the year (Terril,1974). Similarly, the goats in Egypt were found to be polyoestrus but sometimes, for one season or other, the frequency of oestrus cycles was interrupted by a phase in which the ovaries and the uteri were closely similar to those in seasonally polyoestrus animals during the anoestrus phase (Ramachandraiah et al., 1986). In these uncycling does, the ovaries did not contain growing structures and the endometrium showed signs of inactivity . The glands were markedly regressed. They were short, slightly coiled and the existing few adenomeres were lined with small inactive cells.

In cycling goats, fluctuating levels of oestrogen and progesterone were found to act primarily on the endometrium . Several investigators reported that the ruminant's uterus was lined by a pseudostratified columnar epithelium whose thickness varied during the different phases of oestrus cycle (Casida and Mckenzie 1932; Marinov and Lovell 1968; Bareedy et al.1983 and Jabbar 1986). A quite similar result was recorded in Egyptian does in which stratification, and consequently thickness , of the epithelium was gradually increased during the follicular phase before it was reduced again at dioestrus. Stratification of the epithelium was attributed to the stimulating effect of oestrogen (Ramachandraiah et al.,1986) which possessed an anabolic action (Dupont-Mairesse and Galand 1974).What was seen during the dioestrus was attributed to desquamation and even detachment of some parts of the epithelium, a finding which was ascribed to the action of progesterone on a uterus sensitized previously by oestrogen (Orji and Steinbach 1979).

The pesence of macrophages in the uterine lumen may be needed for their protective role (Dellmann and Brown 1976) . By engulfing desquamated cell debris , they could maintain the integrity of the uterine epithelium and the physico-chemical composition of the uterine secretion within normal . As in other ruminants (Marinov and Lovell 1968, Seida 1977 and Bareedy et al. 1983), the doe's endometrium was foumd to be infiltrated with lymphocytes when the epithelium was desquamated . It might be suggested that lymphocytes, as immuno-compatent cells, act by increasing the resistance of the endometrium which was stressed by desquamation.

The lamina propria in does was thickened progressively from proestrus to dioestrus , an observation which was demonstrated also in sheep (Nalbandov 1964 and Ramachandraiah et al, 1980) . Thickening of the propria during oestrus and metoestrus phases was found to be due to growth of uterine glands, increased fibrocellular content and also increased capillary circulation. So many capillaries were opened to supply nutrients to the surface and gland epithelia and also to the stroma cells of the propria. Exudation of tissue fluid to induce oedema, as in cattle (Asdell, 1955), was not observed. The arterio-venous anastomoses, however, did not show any noticeable activity during the different phases of the oestrous cycle. It might be suggested that their activity is preserved to the time of pregnancy when large amounts of blood are required to reach the fetus through the placentomes.

Secetory gland cells within the endometrium were represented by the unicellular glands in the epithelium and the tubular glands in the lamina propria. The former could be demonstrated in progressively increasing numbers during oestrus and early metoestrous phases. This finding in goats, which occurred also in ewes (Cloud and Casida 1969) and cattle (Marinov and Lovell 1968), was attributed to the ovarian hormones which possessed anabolic and secretory sequences on these cells (Orji and Steinbach 1979) . The uterine glands were found also to be hormone dependant structures, since they were developed progressively during the oestrous cycle and were regressed markedly in uncyclic does. As in other animals,

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the criteria of gland development included the number of acini (Ghannam and Deeb 1966), acinar diameter (Hamilton and Harrison 1951) height of cells (Ramachandraiah et al. 1986) and increased secretory activity of the cells (Ayoub 1973). The present investigation showed also the development of new side branches from pre-existing ones. Early, Asdell (1955) decided that growth of glands was due to the stimulating action of progesterone but recently it has been found that the action of progesterone on an oestrogen-sensitized uterus was the cheif stimulus (Orji and Steinbach 1979).

It was demonstrated that the secretion of glands was usually mixed with some detached and migrating cells. It appeared that this mixture formed only a part of uterine milk since Hafez (1974) added the low molecular derivatives of proteins, polysaccharides and mucopolysaccharides digestion as well as glycogen and fat. The author claimed that the volume of genital secretion was generally increased by oestrogen. It could be suggested that genital secretion is required to various reproductive processes including transport, survival and capacitation of sperm cells, maintenance, transport and fertilization of the ovum and nourishment of the developing zygote. In sheep Thibault and Levasseur (1974) reported that uterine milk provided nutrients for the zygote from the 9 day blastocyst stage onward.

Caruncles in does, as in other ruminants (Dellmann and Brown 1976). were described as non glandular but highly cellular structures projecting from the endometrium. Unlike this generalization, one of the examined does showed a gland invaginating from the epithelium of one of the caruncles, a finding which was not recorded previously. This finding shifted our attention to two possible explanations. The first is to consider the non-glandular nature of caruncles as a relative condition rather than an absolute. The other alternative is to consider that the gland was developed from an abnormal site (ectopic gland), and this doe, therefore, could be considered as a case report. Confirmation of one of these sugestions then, depends upon the demonstration of a similar structure in does and other aniamls.

As a new trend to increase the livestock of seasonally polyoestrus ewes, attempts were tried so that lambs can be produced at any season of the year. Shortening of the day length and cooling have both been shown to be effective. Also, oestrus, ovulation and fertilization may be induced in anoestrus ewes by some hormonal schedules (Terril 1974). When all or some of these attempts were tried in gosts to abolish uncycling females, probably will lead to increasing thir livestock.

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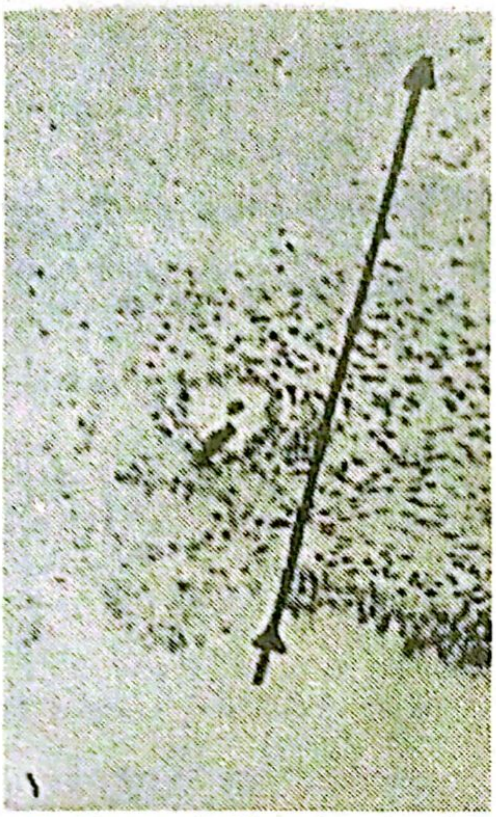

Fig. (1): Utero-tubal junction showing the continuity of the mucosa and the musculosa of the oviduct into the uterine horn indicating absence of an intramural part of the former. H&E stain x16.

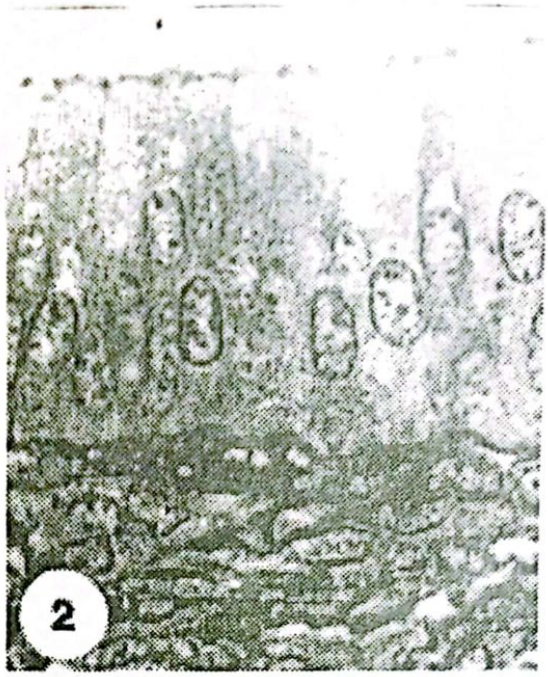

Fig. (2): Mucosa of a doe at pro-oestrus showing a pseudostratified columnar epithelium overlying a highly cellular propria (stratum compactum).Note intact apical parts of cells. Crossmon's trichrome stain x 250

g. (3): Endometrium showing elastic fibers only the supplying coiled blood vessels. Aldehyde chsin stain x 16.

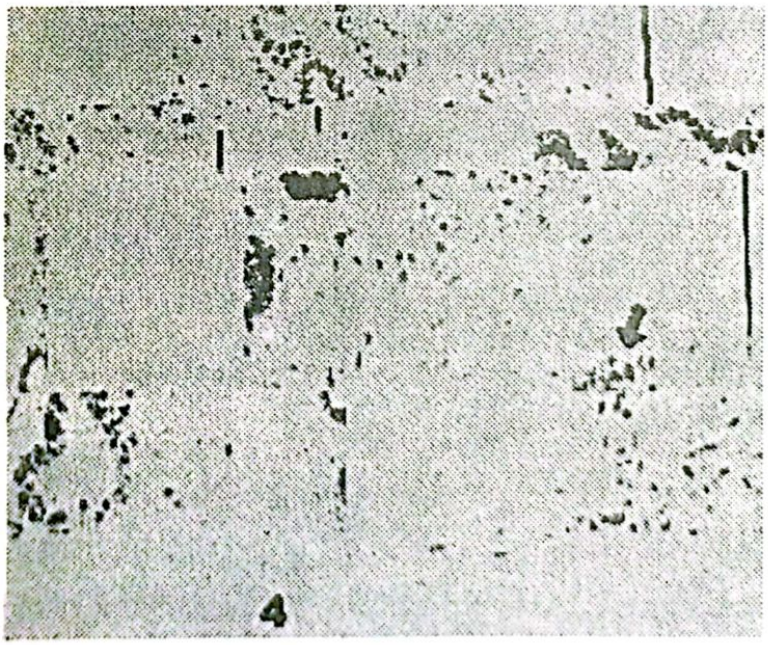

Fig. (4): Uterus of a doe at pro-oestrus showing a gland adenomere within the inner muscular coat (arrow). H&E stain x 40.

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Fig. (5): Endometrium of a doe at pro-oestrus showing the wide ducts which lying under the simple columnar epithelium (arrow). Note the difference between the ducts (D) and the secretory tubules (T).H&E stain x 16.

Fig. (6): Endometrium of a doe at pro-oestrus showing a gland invaginating from the surface epithelium of one of the caruncles (arrow). Otherwise, the glands lie at its periphery and open at the inter-caruncular area. Note the rich blood supply at the caruncular base. H&E stain x 16.

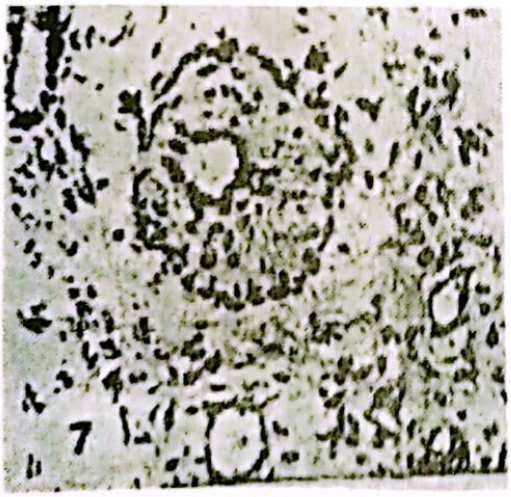

Fig. (7): Endometrium of a doe at pro-oestrus showing an arterio-venous anastomoses (arrows). H&E stain x 16.

Fig. (8): A cross section of a uterine horn showing the bilayered myometrium. the inner circular (C) was thicker than the outer longitudinal (L). The stratum vasculare did not extend to this region. H&E stain x 16.

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Fig. (9): Endometrium of a doe at oestrus showing one of the secretory cells in the surface epithelium (arrow). Note the subepithelial blood capillary (B). Crossmon's trichrome stain x 250.

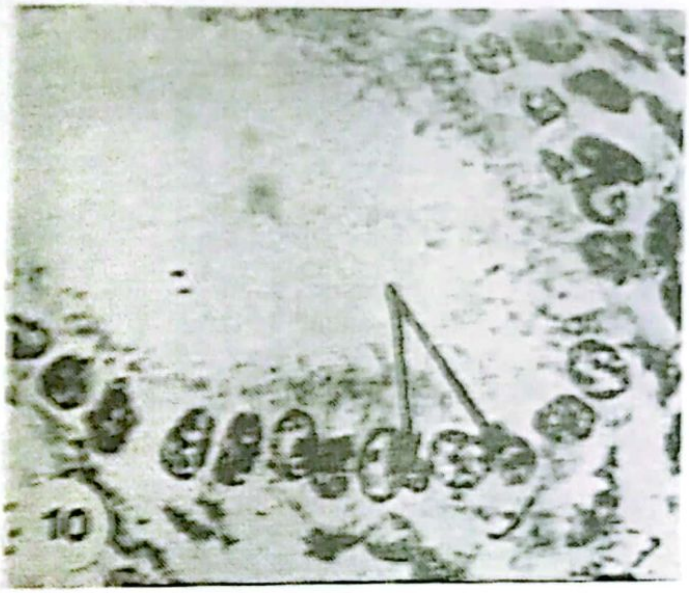

Fig. (10): An adenomere of a doe at oestrus showing the apocrine mode of secretion. Migrating lymphocyte and acidophil cells were also seen(arrows). H&E stain x 250.

Fig. (11): Endometrium of a doe at metoestrus showing a simpler epithelium over one of the caruncles. H&E stain x 40.

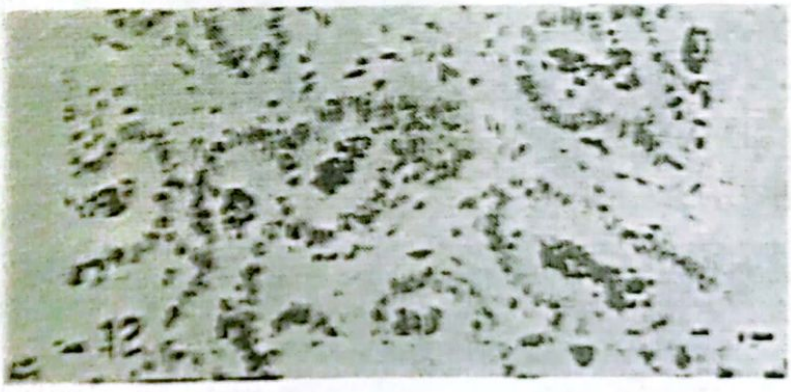

Fig. (12): Endometrial glands of a doe at metoestrus showing a greater accumulation of detached and migrating cells in the lumina of a greater number of glands. H&E stain x 40.

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Fig. (13): Endometrium of a doe at dioestrus showing reduced height of the pseudo-stratified columnar epithelium. Note the great number of lymphocytes in the underlying propria and only few of them migrated through the epithelium. H&E stain x 65.

Fig. (14): Endometrium of a doe at dioestrus showing a part where higher pseudostratified columnar epithelium still persisted. Note the pinching of secretion and their detaching from the apical parts of secretory cells (arrows). H&E stain x 65.

Fig. (15): Endometrium of a doe at dioestrus showing one of the ducts from which several new adenomeres were budding (arrows). Note the greater number of lymphocytes close to them.H&E stain x 65.

Fig. (16): Endometrium of a doe at dioestrus showing that the proximal parts of some glands might be enlarged or even sacculated (arrows). Crossmon's trichrome stain x 16.

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g. (17): Endometrium of a doe at anoestrus owing the lowest and simplest epithelium. H&E in x 40.

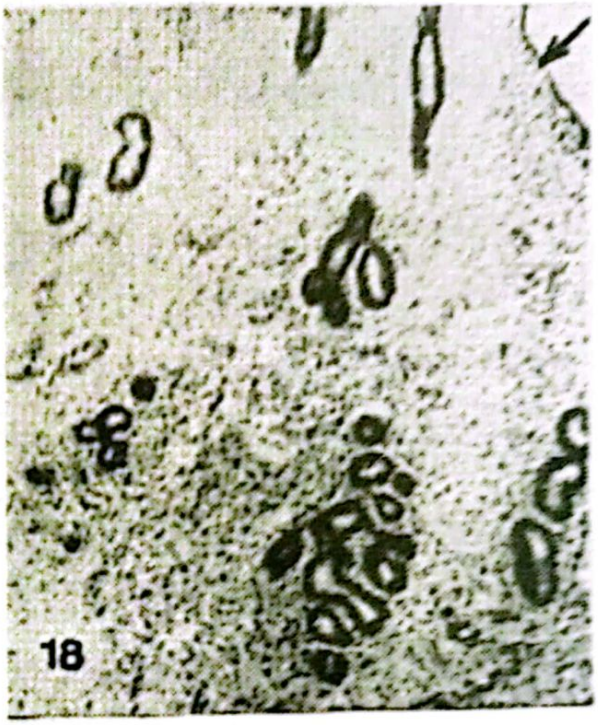

Fig.(18): Endometrium of a doe at anoestrus showing the thinnest epithelium (arrow), absence of subepithelial stroma cells and the uterine glands became short, small and less coiled. The inter vening propria was abundant, compact and less vascular. H&E stain x 16.

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