HISTOLOGICAL STUDY OF THE OVARY IN FOLLICULAR PHASE
WITH SPECIAL REFERENCE TO PREOVULATORY FOLLICLE
IN
INDIAN BUFFALOES
(Bos bubalis)

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INTRODUCTION

Cockrill (1967) while describing water buffalo said "This gentle beast is a source of power and food for a substantial fraction of humanity. It has much to recommend it for these uses yet it has been studied far less than many other domestic animals".

India being an essentially agriculture country bears about 48 millions of domestic buffaloes of River type which is the largest concentration for any one country. Of the total milk production in the country, 55.9% comes from buffalo, though they constitute 30% of milch cattle. (Bhattacharya 1965) Dairy Industry especially in tropical country like India, depends much on selection of a suitable type of Dairy animal, having high milking capacity and tolerance to severe climatic conditions, disease and defective nutrition. The above prerequisites of a dairy animal are fully found in buffalo, as a suitable dairy animal of this country.

Reproductive performance of a herd is directly related to its productivity and is of importance in Dairy production. The successive changes in the morphology and function of the female sexual organs, recognised as " estrous Cycle" is undoubtedly an important phenomenon which is mainly controlled by hormones of pituitary and ovary. The two major functions of the ovary, the endocrine and the reproductive, are bound up with Graafian follicles, without the formation
of which ova cannot be released nor can Corpora lutea be
formed. Examination of the Graafian follicles, then, is
a valuable source of information, on the sexual physiological
status of the ovaries. (Sajakoski 1965)

On scanning the pertinent literature, it is observed
that, very little information is available as regards the
histological changes occurring in the ovary of buffalo, during
various phases of reproductive cycle.

Hence, the present study is undertaken to study the
microscopic changes occurring in the follicle before ovulation.
MORPHOLOGY

Gross appearance and size of the follicles: Hammond (1927) noticed the large follicle of 1.1 cms. diameter during three days before heat and of 1.5 cms. before ovulation in cow.

McNutt (1927) observed the rapid maturation growth of the follicle in cows during heat and reported the size of the follicle at ovulation as 15-19 or 20 mm. in diameter.

Moss et al (1954) observed the transparency and bluish colour of the preovulatory follicle in cow.

Hafez (1955) examined 24 pairs of ovaries from nonpregnant Egyptian buffaloes in different phases of the oestrus cycle and determined the late dioestrus or prooestrus phase by the presence of either large Graafian follicles or a pale corpus luteum. He recorded the diameter of the largest follicle during late dioestrus and prooestrus in buffaloes ranging from 1.2 - 2.0 cms. with a mean value of 1.8 cms. present on the ovary along with the presence of corpus luteum. The diameter of the largest follicle in the same phase varied from 0.3 - 0.9 cms. with a mean value of 0.7 cms. present on the ovary without the presence of corpus luteum.

Hansel (1959) observed the rapid follicular growth during 3 days before estrus and reported the average diameter of largest follicle of 20 mm at 18-20 days of cycle in 18-50
week old Holstein heifers.

Rajakoski (1960) reported the presence of a clear spot of about the size of a pin head, bulging over the follicular wall on the surface of the ovary in preovulatory follicle in two heifers.

Zemjanis (1962) observed 12-15 mm as the size of the follicle during proestrus and 20-22 mm as the size on 21st day of the cycle in cow. He observed that the size of the follicle increased and reached its peak at the height of physical oestrus. The follicle appeared large when it was developing on the same ovary with the presence of corpus luteum.

Cier and Marion (1967) studied the ovaries of 600 dairy cows and reported that normal follicles over 12 mm in diameter were found only as preovulatory.

Choudary et al (1968) studied nineteen normal cycling cows and heifers and observed that normal follicles above 5 mm in size were not present during the entire luteal phase, however, they were consistently present during the follicular phase. They noticed the presence of normal follicles from 8-18 mm exclusively during follicular phase. They further concluded that follicles above 5 mm in size were competent and subjected to the action of gonadotrophin which was in optimum balance during follicular phase.

Marion et al (1968) made an extensive study of
bovine follicular system on 700 dairy cows with known reproductive histories and observed that, follicular growth to 1.00 mm size was rapid and development from 1-12 mm appeared continuous without periods of acceleration. The preovulatory follicle observed by them varied from 10 to 2 mm in diameter which increased to 16-18 mm during 24 hours before ovulation.

**Frequency of occurrence of Graafian follicle and ovulation in right and left ovaries:**

Hafez (1955) found the frequency of formation of corpora lutea more on right ovary than left and also observed the ovulation in 75% cases in the right ovary in Egyptian buffaloes during his study.

Reddy, B. (1960) on rectal diagnosis of pregnancy in 27 buffaloes observed 136 pregnancies in right horn and 111 in the left and concluded that, the right ovary of the buffalo was functionally more active than left. He further emphasised that, normally pregnancy occurred in the right cornua when there was ovulation from the right ovary.

Rajakoski (1960) noticed a significantly greater number of normal follicles ≥ 5 mm in diameter in the right ovary (37 in the right and 20 left) during his study. He also observed the greater number of newly formed corpora lutea on right ovary than left, which he attributed for greater frequency of ovulation on right side than left.

Marrow et al (1968) during their study of 139...
series of parturition in cows, observed the corpus luteum of pregnancy on right ovary in 82 cows (59%) and in 55 cows (39.6%) on left ovary.

Regressing Corpus luteum:

Folding and Lall (1945) studied small number of genitalia of Indian buffaloes (Murrah buffaloes) and reported that colour changes in corpus luteum were absent.

Laing (1955) described the corpus luteum in cow becoming firmer in consistency from 12-19 days and reported rapid shrinkage with a diameter of 1 cm during prooestrus and oestrus period.

Sane et al (1964) & (1965) studied 4,000 Murrah buffalo genitalia and 214 of Jaffri buffalo genitalia and observed the colour of corpus luteum from bluish red to dirty grey on regression. They remarked that the dark bluish red colour was maintained for the major part of the oestrus cycle and did not turn lighter until approach of subsequent oestrus.

Bhattacharya (1965) observed the corpus luteum in buffalo as pinkish grey in colour, veined with red, which faded during the regressing phase. As the corpus luteum regressed, the red veining disappeared and whole tissue turned white and sunk into the stroma of ovary.

Roy (1966) made a study on corpus luteum of 25 experimental buffaloes and 142 slaughter house animals and classified the 16-24 days old corpus luteum as "Regressing"
and that older than 24 days as "Regressed Corpus luteum". He observed the colour of Corpus luteum at the regressing stage becoming red, pink or flesh in colour.

El, Sheikh et al. (1967) studied the histological and functional changes of 31 Corpora lutea of Egyptian buffaloes during various phases of the oestrus cycle obtained from slaughter house and assessed the age of Corpus luteum during different phases of cycle on the basis of diameter, weight, volume, colour and histological appearance. According to them, 16-20 days old Corpus luteum was called as "Regressive Corpus luteum" which was of a diameter varying from 8-13 mm. They observed the colour of regressive Corpus luteum as pale yellow.

Gross appearance of Uterus and Cervix:-
Murphay (1924) observed slight oedema of uterus with straw coloured longitudinal folds in endometrium, with light coloured cotyledons and noted the light red coloured cervix with mucus discharge, during the proestrus period in a Jersey cow.

Roberts (1956), Zemjanis (1962) and Hafez (1968) observed the tone in uterus with slight oedema and congested endometrium with mucus secretion and dilated cervix with mucus discharge, during oestrus period in cow.

HISTOLOGY

Cervical epithelium:-
It consisted of flattened cells present on the
surface of the ovary in cow. (Asdell 1955, Cole and Cupps 1959, and Hafez, 1968)

Trautmann and Förbiger (1957) reported the germinal epithelium consisting of a single layer of cuboidal or columnar cells in young animals while the cells were flattened in the adult.

Settergren (1964) found the germinal epithelium in the cow consisting of squamous to cuboidal type, but in the depths of shallow grooves present on the surface of the normal ovary, the cells were sometimes columnar. The shape of the nuclei varied from round to flat according to type of covering epithelium. The nuclei were rich in chromatin and the cytoplasm was sparse causing the nuclei to lie close together.

Tunica Albugenia:-

Asdell (1955), Cole & Cupps (1959) & Hafez (1968) observed the connective tissue of Tunica albugenia consisting of somewhat denser fibers, and the cells arranged roughly parallel to the surface of the ovary.

Trautmann and Förbiger (1957) mentioned the Tunica albuginea richer in Collagen fibers and somewhat poorer in cells than cortical stroma. The fibers were irregularly interwoven but occasionally were arranged in lamellae as in man. The Tunica albuginea lacked in elastic and reticular fibers.
Settergren (1964) observed in cow the thick connective tissue layer of the Tunica albuginea consisting of the fibers running in various directions.

Cortex:

Asdell (1955), Cole & Cupps (1959) and Hafez (1968) described the Cortex as the thick peripheral layer containing stroma mainly of fibroblast cells. Developing follicles, atretic follicles, and functioning and degenerating Corpora-lutese were found in Cortex. This layer surrounded the medulla.

Trautmann & Fleibiger (1957) described cortical stroma rich in cells but free from elastic tissue. The cellular structure was reinforced by reticular fibers which could not be demonstrated by usual staining technique. The connective tissue cells which were probably not ordinary fibroblasts, had elongated nuclei and occurred in dense strands. Their direction was parallel to the surface of the ovary or the follicles and blood vessels which they enclosed.

Follicular system:

Trautmann and Fleibiger (1957), Rajakoski (1960), Erickson et al (1966) and Hafez (1968) classified 3 types of follicles present in the cortical stroma of ovary in cow as detailed below.

Primary follicles consisted of an oocyte, surrounded by a single layer of squamous cells. Rajakoski (1960) observed the cell membrane of oocyte thin and cytoplasm...
finely granular and appeared as weakly eosinophilic reticulum.

In growing or secondary follicles, surrounding cells were in several layers and cells were cuboidal in nature. Oocyte grew with homogenous envelope (Zona pellucida) and assumed an eccentric position. The basement membrane between follicular layer and the stroma became more distinct.

Later on the follicular cells separated to form clefts, which became confluent and thus the cavity or antrum formed was surrounded by several layers of follicular cells, the membrana granulosa. Hence, the follicle was called vesicular or Graafian follicle. Such Graafian follicles were then enveloped by stroma cells, the theca folliculi, which later differentiated into an inner theca interna and outer theca externa.

McRutty (1924) described the theca externa as simply a portion of ovarian stroma, the cells of which were arranged parallel with follicular wall. It contained larger blood vessels and smooth muscles. According to him, theca interna contained elongated and frequently spindle shaped connective tissue cells with a large, oval and granular nucleus. Few cells were more oval with spherical nuclei. Endoplasm of cells contained varying number of lipid granules of fairly uniform size. Those cells increased in size and became epitheloid cells amongst which was a rich capillary network.

Trautmann and Fitbiger (1957) described the theca...
interna cells, well supplied with capillaries, became epitheloid, lipid containing spheroid or poly morphic cells. According to him, the theca externa consisted of fusiform stroma cells, which were arranged concentrically around the follicle.

Bajakoski (1960) described two types of cell in theca interna. One type resembled epitheloid cells, oval or round with light vesicular and distinct nucleoli. The other cell type represented the majority of cells in theca interna which resembled connective tissue cells and were arranged in a loose network. The theca externa was made up of relatively few elongated connective tissue cells which was loosely arranged in the medium size and large follicles.

Trautmann and Piebiger (1957) described the membrana granulosa composed of a layer of columnar cells next to the thin basement membrane present between theca interna and membrana granulosa and of several layers of spheroid to polyhedral cells.

He described the oocyte with a thick Zona pellucida with a granular cytoplasm and large vesicular nucleus containing nucleolus.

Moss et al (1954) observed primordial follicles present in all stages of oestrus cycle. Cluster of oocytes were observed during oestrus. At later stage of cycle no cluster of oocytes were seen, though individual oocytes
were always present in variable numbers and in various developmental stages. He observed that some primordial follicles contained one or more Call Exner bodies in the granulosa layer.

Bloom and Fawcett (1957) observed the eccentric nucleus in the oocyte. They described the Call Exner bodies as round, darkly stained bodies, surrounded by follicular cells present in the growing follicles. They regarded them as new centres of secretion of follicular fluid.

Harrison (1962) mentioned about the small cavities present in the granulosa layer either before or after formation of antrum. He considered them as Call Exner bodies, described by Call Exner (1875) in rabbit follicle. Such bodies were also seen in human follicles. They contained a reticulated coagulum, sometimes surrounded by a fine membrane, with a radially arranged granulosa cells around it.

Marion et al (1968) noticed Call Exner bodies in the early atretic follicles.

**PREOVULATORY FOLLICLE**

McNutt (1924) described the granulosa cells having more spherical and somewhat smaller nuclei than the cells of the inner theca, in the mature ovarian follicle. Those cells lying in inner theca were cuboidal and others were chiefly polygonal. Fine lipoid granules smaller than those of theca cells were seen throughout in granulosa cells.
The cumulus in mature Graafian follicle was not a solid clump of cells but contained many small antra. The blood vessels in the theca interna were distended with blood and erythrocytes were present in the outer portion of liquor folliculi and few (R.B.C.s) scattered in the granulosa in the mature follicle during heat. Leucocytes, especially eosinophiles were present in large number throughout theca and granulosa in mature follicle. Eosinophiles were present in outer theca also in few follicles.

Hammond (1927) described the connective tissue of theca interna consisting of a dense mass of cells with their nuclei close together, but that, just before ovulation the connective tissue was drawn out and fibrous and nuclei were long away apart. At 18 days before ovulation the capillaries of theca interna were described as hardly observable and the large blood vessels were rounded in section. At about 3 days before oestrus, the capillaries became more evident and larger vessels were flattened by pressure and from this time until ovulation, capillaries continued to enlarge, became congested and came to lie close beneath the granulosa cells.

Asdell (1956) (1955) & (1960) confirmed the observations of McNutt (1924) and Hammond (1927) on the distension of capillaries in mature follicle during heat and observed slight leakage of erythrocytes into granulosa cells and in outer part of liquor folliculi. He also observed enlargement of granulosa cells with dark nuclei and ...
infiltration of many leucocytes in the follicle as observed
by McCutt (1924). His study on 36 heifers in all stages of
proestrus cycle revealed the mitosis as rare in granulosa cells
during proestrus. He observed the granulosa cells became
separated to some extent by liquor folliculi giving the
"foamy aspect" to the layer during the day of oestrus. This
change was especially apparent in cumulus and as ovulation
approached, the cells tend to shrink, to stain poorly while
nuclei stained heavily and were pyknotic. At the time of
oestrus, the layer of cells near the granulosa margin increased
in size and became epitheloid in nature. During his
observation on two ovaries nearing ovulation, slight collapse
of largest follicle was noted, which was shown by a distinct
orination of the theca interna.

Moss et al (1954) studied the histology of ovaries
obtained from 18 Holstein and crossbred heifers, during
different phases of oestrus cycle. Their data of study on
3 preovulatory follicles, suggested that, preovulatory changes
might occur at least three days before the onset of oestrus.
They agreed with the observations of McCutt (1924), on the
preovulatory follicle except that they did not find erythrocytes
in the granulosa, although extravasated erythrocytes and highly
dilated capillaries and blood vessels were seen in theca.
They remarked that, mature follicle in contrast to growing
follicle was characterised by marked vascularisation of the
theca and lack of mitosis in both the granulosa and theca.
Cupps et al. (1956) studied the histology of Pituitary, Adrenals, Ovaries and Uteri of Dairy cattle and observed 8-10 layers of granulosa cells in the Graafian follicle of normal animal during proestrus and oestrus phase. The granulosa cells were described as large with well defined cell walls, intermediate amount of cytoplasm and vascicular nuclei.

Cole and Cupps (1959) described the nuclei of granulosa cells becoming densely chromatin and cytoplasm vacuolated, in heifers, in estrus and prior to ovulation.

Rajakoski (1960) studied the ovarian follicular system in 36 virgin heifers of the Swedish Red and White Breed (S.B.B) and concurred with the histological observations of McNutt (1924), Asdell (1946)(1955) & (1960), and Moss et al. (1954), of preovulatory follicle. He observed eosinophiles and hyperaemia in the follicle. He expressed the loosening of the granulosa layer as impressive and the cells were thinly scattered with the nuclei at considerable distance giving spongy appearance to the cumulus. The liquor folliculi in the antrum showed the layers of varying staining intensity.

Marion et al. (1968) made an intensive study on micromorphology of bovine follicular system on ovaries from 700 dairy cows of known reproductive history. They observed the stratum granulosum of two to seven cell thick in the Graafian follicle of 0.7-0.8 mm in diameter. The theca was...
a thin layer of oriented stroma cells surrounding the follicle. The membrana propria was distinct at the primary follicle stage and continuous thereafter. The oocyte became surrounded by an increasingly thick layer of cumulus oophorus, five to six cells thick in a 2 mm follicle. The increase in stratum granulosum to four cells thick was due to mitosis, and the outer layer was low columnar resting on the membrana propria. The cells of the inner most layer had become somewhat flattened forming an incomplete squamous lining of follicular cavity. The Glandular theca interna cells were identifiable at that time differentiating within the theca layer immediately surrounding the follicle. Those Glandular cells constituted approximately one fifth of the total interna and had oval nuclei and lightly stained cytoplasm. The stratum granulosum increased in thickness as the Graafian follicle enlarged to attain size of 10 mm in diameter. The cumulus oophorus was 8-10 cell thick, whereas the basal layer was distinctly columnar, placed perpendicularly to the membrana propria. Inner layers were of polyhedral cells, whereas the inner most layer, lining the estrum was of continuous layer until few days before ovulation. Mitosis was frequently observed. Capillaries were numerous in theca interna. The theca externa consisted of fibrocytes and blood vessels with no clear demarcation between externa and surrounding stroma.

During preovulation, rapid expansion of both granulosa & interna, continued exhibiting mitosis in both layers. The stratum granulosum thinned to five to
seven cells thick, with the basal columnar layer becoming polyhedral, much like the remaining granulosa cells. Nuclei in granulosa cells remained uniformly plump, spherical and hyperchromatic. There was no indication of pyknosis, vacuolization, separations or any other characteristics of degeneration in any of five, 15-18 mm follicles in final hours of oestrus or post oestrus preovulatory period in cow. The theca interna was stretched and glandular interna cells were distinct among the fibrocytes.

ATRETIC FOLLICLE

Moss et al (1954) noticed the Classy membrane formation as a characteristic of early stages of follicular atresia, during his histochemical studies of bovine ovary. They observed the viscous nature of the substance inside the folds of collapsed follicles, resulting from the liquefaction of pyknotic nuclei in the granulosa of atretic follicles. Follicles undergoing such degeneration had poorly vascularized theca and had no mitosis.

Roberts (1956) stated that any ova that did not mature and ovulate, became atretic during proestrus and estrus.

Trautmann and Fiebigter (1957) described atresia setting at any stage of development of follicles characterised by degenerative changes like fatty changes and pyknotic nuclei in the oocyte and membrana granulosa.
Rajakoski (1960) classified mainly two types of atresia in follicles in heifers during his study of follicular system, as detailed below.

I. Atresia with primary oocyte degeneration:
   1) Without hyalinisation in Zona pellucida:
      This form was seen in primordial follicles and smallest of the growing follicles. Degenerative changes appeared throughout oocyte, characterised by blurred nuclear structure and disappearance of nuclear membrane. The cytoplasm of oocyte became coarsely granular, opaque, vacuolated and shrunk.

   ii) With hyalinisation of the Zona pellucida:
      This type was encountered in growing follicles with well developed Zona pellucida and multilayered follicular epithelium and also in Graafian follicles. Due to hyalinisation the Zona pellucida became thick and opaque. The follicular epithelium shrunk with cell border indistinct. The extreme hyalinisation of the Zona pellucida showed peripheral concentric hyaline layers.

II. Atresia with primary follicular wall degeneration:
   1) Obliterative atresia:
      This was more commonly encountered in Graafian follicles of sexually mature heifers. Cessation of mitotic activity and appearance of degenerative changes like pyknosis, chromatolysis and hyperchromatosis of nuclear membrane were first observed. Degenerative changes were
first apparent in the mural portion of the granulosa, while in the cumulus mitosis occurred long after the point when the remainder of the granulosa was practically completely degenerated.

The oocyte with finely granular cytoplasm and translucent zona pellucida was observed in cumulus. Numerous pyknotic nuclei often gathered into large clumps were found floating in liquor folliculi. This was described as obliterative atresia of first degree.

The atresia of second degree was characterised by complete degeneration of granulosa without pyknotic nuclei in the lumen. Thecal connective tissue cells extended inwards and began to fill the lumen. The entire follicle became oval after shrinkage. The cumulus persisted about the oocyte but degenerative changes, like pyknotic nuclei appeared. The zona pellucida became opaque and its oocyte cytoplasm got shrunk and stained irregularly.

Atresia of third degree was characterised by the disappearance of cumulus to leave the oocyte naked and the development of glass membrane.

ii) Cystic Atresia:

The characteristic of this type of atresia was the presence of 2-3 layers of granulosa cells with fairly large nuclei, which appeared as "String of beads" staining purple with haematoxylin and eosin.
Pyknotic nuclei were seen and the theca interna was found atrophic.

Erickson (1966) studied 116 ovaries in Bovine and called the vesicular follicle as atretic wherein degenerative changes among or absence of granulosa cells were noticed. They considered the atresia as complete if fibroblastic invasion of follicular cavity had started.

Sier and Marion (1967) during the study of size changes in bovine atretic Graafian follicles, studied on ovaries from 600 dairy cows with known reproductive histories. They observed collapsing follicles decreased by compressive folding of theca interna and stratum granulosa. In most common type of follicular atresia found in bovine ovaries they observed, rapid expansion with thinning of theca interna and disappearance of glandular theca cells. Contraction of follicles was then observed causing a thickening of the theca interna and shortening and plumping of fibrocytes.

Marion et al (1968) also studied the atresia in follicles and classified as Early, Definite & Late, which was noticed in any stage of follicular development.

Early atresia was noticed by 1) Loosening and sloughing of granulosa cells lining the antrum. Atretic bodies were noticed in the antrum and among granulosa cells. These bodies were described as clump of pyknotic nuclei floating in the antrum by Rajakoski (1960) in atretic follicle.
2) Disappearance of membrana propria and loss of orientation of basal layer of granulosa. 3) Shortening & rounding of theca interna cells and 4) Appearance of Call Exner bodies.

Definite atresia was characterized by more advanced stages of regression in follicles and was further described as collapsing, Contracting, and Cystic atresia.

Late atresia was considered to be final stage of regression common to all types of atretic vascicular follicles. The size was found to be reduced with disorganization of cell layers. The antrum was filled with fibrous granulosa remnant and theca layer was hyalinized. These structures might remain for several cycles before becoming indistinguishable later on.

Hafez (1968) agreed with the description of Rajakoski (1960) on the follicular atresia and mentioned that cysts underwent cyclic changes with alternate growth and regression. According to him cystic follicles might be associated with Anoestrus, prolonged oestrus behaviour, or cyclic oestrus period, which appeared normal.

B) Corpus luteum

McNutt (1924) studied the morphology and histology of Corpus luteum in cow, in various stages of cycles obtained from the ovaries of 19 animals out of which 12 were virgin and 7 cows. He observed that involutionary changes started at 14 days and continued gradually upto...
20 days which then became more marked. Two types of cells have been described by him, one type the Theca lutein cells, which were found near the centre of original fold and were oval to polygonal in shape. Some cells were found to be mixed with Granulosa lutein cells. The other type was Granulosa lutein cells, which contained more lipid than that of Theca lutein cells during early stage. He noticed the beginning of degenerative changes in Granulosa lutein cells, shown by faintly stained nuclei or Karyolysis, in 14 days, and numerous fibroblasts in 18 days. At 19 days degenerative changes of same degree were shown by both type of cells. The connective tissue septae were heavier and prominent in 19 days and at 20 days large vacuoles were present in the endoplasm and the nuclei stained lightly and frequently, no distinct membrane was visible in the nucleus. The blood vessels and connective tissue appeared prominent.

Moss et al (1954) described the retrogressive changes starting from 14 days in Corpus luteum of cow and at 19 days pyknotic nuclei were seen in the Theca lutein cells. At 21 days they observed that, granulosa lutein cells were reduced in size and contained round vacuoles at periphery of cells. One day after onset of oestrus there were numerous dilated blood vessels in the stroma near the retrogressing Corpus luteum, which he considered the probability of overall hyperaemia of the ovary at that stage.

Rajakoski (1960) observed the lobulated
appearance of the Corpus luteum in heifers on 9th day due to
growth of connective tissue trabeculae. The connective tissue
became more abundant and walls of the blood vessels thickened
from 13th to 16th day of the cycle. During 17th & 18th day
lutein cells appeared with shrinkage of cytoplasm and pyknotic
nuclei which were more evident on 19th and 20th day, and
leucocytes were also noticed. During 21st day and first day
of succeeding cycle, the Corpus luteum underwent great
structural modification with complete degeneration of lutein
cells in some, and lutein cells persisted in others.

Mares et al (1962) mentioned that, luteal cell
degeneration was prominent on the 17th & 19th day old
Corpora lutea of cow, as evidenced by pyknotic and shrunken
nuclei with dark staining cytoplasm.

Donaldson and Hansel (1965) made the histological
study of bovine Corpora lutea, obtained from 53 Holstein
heifers in normal cycle, slaughtered at different stages.
Some additional Corpora lutea were also studied. During their
study of development of Corpus luteum, they reported that, the
basement membrane between Theca interna and Membrana granulosa
disappeared within 24-48 hours after estrus and distinction
between two layers (Granulosa and Theca interna) was lost at
that time. They observed a distinct thickening of the walls
of the arteries, as the Corpus luteum aged and continued
until 21 days, while the lumina of arteries were obliterated.
The first sign of degeneration noticed by them was a decrease
in cytoplasmic stippling and rounding of the cell outline.
Vacuolation occurred around the periphery of the cell, which was
followed by condensation of the cytoplasm which stained darkly,
giving stellate outline to the cells. Lighter staining of
nuclei and loss of nucleolus and indistinct nuclear membrane
was also noticed by them along with shrinkage and darkly stained
pyknotic nuclei. It was further stated that, some large lutein
cells were distinguishable on 21st day but no small luteal
cells could be positively identified. The large cells had lost
all cytoplasmic stippling at that stage.

Roy (1966) made a study on morphological and
histological structure of Corpus luteum, during normal and
abnormal oestrus cycle in buffaloes. His study included the
material obtained from a group of 25 experimental buffaloes
and 142 slaughter house material. He observed the average
size of lutein cells larger in buffaloes than that of cows and
noticed high percentage of type III, IV and V cells with
regression of Corpus luteum. The lutein cells type I and II
in the regressing Corpus luteum were found to be 8.9%.

El Sheikh et al (1967) studied the histological
and functional changes in 31 Corpora lutea of Egyptian
buffaloes, during various phases of the oestrus cycle and
observed the luteal cells smaller and compact with a stained
reticulum of residual cytoplasm frequently seen in the
degenerated cells. They observed increase in fibroblastic
cells in the regressive Corpus luteum with disappearance of
luteal cells.
Hafez and Armstrong (1968) histologically revealed typical large lutein cells associated with progesterone synthesis in the Corpus luteum of cow. Those large lutein cells were rounded in appearance on 18th day. Arterioles of Corpora lutea on days 18, and 20, possessed thickened walls and endothelia appeared serrated. Corpora lutea on day 18, 20, and 0 (Day of ovulation) revealed extensive degenerative changes of large lutein cells characterised by striking shrinkage of the cytoplasm and pyknotic nuclei.

**MEDULLA**

Asdell (1955), Cole & Cupps (1959) & Hafez (1968) described the medulla as the portion of the ovary near the mesorchium or attachment to the broad ligament and consisted of loose fibroelastic tissue with short canals & spaces which were vestigial remnants of the male ducks. Blood vessels & nerves entered and left the ovary through this route.
MATERIALS AND METHODS

For the purpose of the present study 244 genital organs of buffalo cows were collected from Bandra Abattoir. As the genitalia were collected at random, the buffaloes slaughtered were not of specific age and breed but they were mostly adult. The genital organs were then wrapped in a cloth moistened with normal saline and were immediately transported to the laboratory where they were washed under running water to remove all blood clots and tissue debris. Out of the total organs collected, 21 genital organs representing the morphological structure of proestrus phase of the cycle were selected for the study.

The proestrus phase of the cycle or otherwise the follicular phase of the cycle was determined by the presence of largest Graafian follicle on either of the ovaries as observed by Hafez (1955), in buffaloes. The presence of regressing Corpus luteum and other characters like tone of uterus, pale or congested endometrium with slimy discharge and dilated cervix with mucus discharge, associated with the proestrus phase were also considered.

The diameter of the largest visible follicle present on either of the ovaries was recorded.

The ovaries were then immediately cut longitudinally into two pieces and were fixed in Bouin's fluid.
other fixatives used were

1) Susa
2) Carnoy’s fluid
and 3) Formaline 10%

Fixation in Bouin’s fluid for 12-24 hours was found to be satisfactory.

Before subjecting them for further processing of dehydration etc., the tissues were cut into smaller pieces of not more than 0.5 cms. The tissues were then subjected to conventional routine methods of dehydration, clearing etc. as described by Carleton (1957).

The paraffin blocks were sectioned at 5-6 microns thickness. The sections were stained with

1) Delafield’s Haematoxylin and Eosin.
2) Harri’s Haematoxylin and Eosin.

Special stain like Van Gieson’s stain was used to reveal certain particular details.
OBSERVATIONS

As indicated earlier, the observations made in the present study were based on the ovaries obtained from 21 genital organs, but 33 ovaries presented required material for the purpose of study.

Out of 33 ovaries selected for study, 10 right and 2 left ovaries were having largest mature Graafian follicle without Corpus luteum and 4 right and 7 left ovaries were having largest mature Graafian follicle and regressing Corpus luteum. The Corpus luteum was alone present without the presence of largest Graafian follicle on 2 right and 10 left ovaries.

(Table No. I)
(Plate No. I)

Out of 33 ovaries examined, 21 showed largest Graafian follicles out of which, it was noticed on 14 right ovaries and on 7 left ovaries. The percentage of presence of largest Graafian follicle on right ovary was 42.42% while on left side 33.33%. The largest Graafian follicle of the diameter of 1.9 cms. was also noticed on the right ovary only.

The diameter of the largest Graafian follicle without Regressing Corpus luteum on the same ovary ranged from 0.6 cms. to 1.9 cms. with an average of 1.10 cms. (Table No. II)

The diameter of the largest Graafian follicle having the Regressing Corpus luteum on the same ovary ranged from
0.70-1.45 cms. with an average of 1.14 cms. (Table No. II)

The bluish tinge over the transparent surface of largest Graafian follicle was noticed in almost all cases, however, pinhead like protrusion over the surface of the largest mature Graafian follicle was observed in 2 cases only.

The colour of the Corpus luteum was varying from light pink to grayish white and the Corpus luteum was deeply embedded in the ovarian stroma and firm in nature. The Corpus luteum appeared slightly protruded over the surface of the ovary with a transparent membrane covering the protruded part.

The body and horn of the uterus in each 17 cases presented a tone on palpation, whereas in four cases tone was not so distinct. The endometrium in 15 cases was pale in colour covered with a thin layer of slimy discharge along with pale coloured caruncles. Slight congestion of endometrium and caruncles was noticed in five cases whereas it was pink in one case. Cervix, in 19 genitalia was dilated with copious mucus discharge. However in two cases the dilatation of cervix was incomplete but mucus discharge was present.

HISTOLOGICAL OBSERVATIONS

The Germinal epithelium of a single layer of flattened cells with rod shaped nuclei was present on the surface of the ovary. (Plate No. I)
The Tunica albuginea consisted of dense connective tissue fibers arranged roughly parallel to the surface of the ovary, while few fibers were running in different directions. (Plate No.II)

The Cortex consisted mainly of stroma cells with round or oval nuclei, with more of collagen fibers, and presented different types of follicles, Corpora lutea and blood vessels.

Priming follicles were observed in large number in the periphery of Cortex. They consisted of single oocyte with a thin membrane surrounded by a single layer of squamous cells. (Plate No.III)

Growing follicles were noticed which were characterised by a central oocyte surrounded by 3-4 layers of squamous cells but without the formation of antrum. (Plate No.IV)

Graafian follicles characterised by the presence of antrum, with distinct Zona pellucida around oocyte, the membrana granulosa with cumulus oophorus, theca interna and externa, were present in various stages of development. The granulosa cells were cuboidal in nature with vesicular nuclei. The cells of the theca interna and externa were spindle shaped with round or oval nuclei, but the cells of theca externa were loosely arranged and blended with stroma of ovary. The theca interna was compact in nature. There was a distinct basement membrane between the theca interna and membrana granulosa. (Plate No.V)
In two cases, Call Exner bodies which were either round or oval in shape with cytoplasm enveloped by thin membrane, were present among granulosa cells. Call Exner bodies were surrounded by a single layer of radiating follicular cells.

(Plate No.VI)

The preovulatory follicle was surrounded by the theca interna and externa consisting of spindle shaped cells with round or oval nuclei, but the theca interna appeared loosely arranged.

(Plate No.VII)

In few cases, the marginal cells of the theca interna towards the membrana granulosa, were epitheloid (granular) in nature with round or oval nuclei. The arterioles in the theca interna were rounded in appearance packed with blood cells and were present in more number throughout theca interna.

The capillaries were congested and more evident in the theca interna. In two cases, the capillaries packed with R.B.Cs. were found lying close to the membrana granulosa.

(Plate No.VIII)

In five cases, extravasation of R.B.Cs. was noticed in theca interna only, while in one case R.B.Cs. were found scattered among granulosa cells. R.B.Cs. were also noticed in one case in both theca interna and membrana granulosa.

Lymphocytic infiltration was observed in both theca interna and membrana granulosa in 16 cases. (Plate No.XII)

Membrana granulosa consisted of cuboidal cells with vesicular nuclei and the cells were arranged in many layers.
The cumulus oophorus was well developed surrounding the oocyte. The oocyte was round in shape with a well developed Zona pellucida and granular cytoplasm with eccentric nucleus.

In one case, the cumulus oophorus appeared slightly lengthened with tendency of cells of granulosa to move towards the centre of antrum. The distance of layer of cumulus oophorus from the oocyte to the peripheral layer of granulosa cells was considerable. The separation of cells was observed by formation of small antra in cumulus oophorus. The peripheral granulosa cells also showed tendency to move towards the centre which was observed by the distortion of basement membrane with slight crination of theca interna. (Plate No.IX)

The cells in the cumulus oophorus were vesicular in nature while as cells near periphery were hyperchromatic. The granulosa cells were arranged in many layers and were cuboidal in nature with vesicular nuclei. The cells of the theca interna and externa were spindle shaped with round or oval nuclei, but were loosely arranged. The epitheloid (glandular) cells of theca interna were distinct.

Apart from the presence of normal Graafian follicles, a few Graafian follicles showing the degenerative changes in oocyte or in the granulosa cells or in both were also encountered. In a few cases, oocyte degeneration was shown by the presence of thick, opaque, slightly distorted Zona pellucida, with vacuolations in oocyte cytoplasm. However, the degenerative changes were not observed in either
cumulus oophorus or in the peripheral cells of granulosa lining the antrum. (Plate No. X)

In a few other cases degenerative changes manifested by pyknotic nuclei, slight shrinkage in cytoplasm, with uneven staining characters were noticed among granulosa cells. The darkly stained bodies were found among the degenerated granulosa cells. The oocyte degeneration characterised by thick zona pellucida and hyalinisation was also noticed. In a few follicles, the number of layers of granulosa cells appeared few and pyknotic nuclei with shrinkage of cytoplasm was encountered among granulosa cells.

In a few other follicles, connective tissue cells of theca interna were noticed with reduction in the antrum of follicle which was containing hyalinised material.

CORPUS LUTEUM

The Corpus luteum presented a lobulated appearance with thick connective tissue septa of collagen fibers entering the substance. (Plate No. XI)

It showed two types of cells one large and other small. Both types of cells showed extensive degenerative changes marked by pyknotic nuclei, shrinkage of cytoplasm, giving stellate appearance, and large peripheral vacuolation. In a few cells, chromatolysis of nucleus was noticed, while in some, vesicular nuclei, were encountered. The cell outline was not distinct in all cases. The connective tissue was abundant in the Corpus luteum throughout. (Plate No. XII)
The dilated blood vessels full of blood cells were present in the stroma near the regressing Corpus luteum and mature Graafian follicle, in most of the cases.

(Plate No. XIX)

The medulla of the ovary contained loose fibroelastic tissue and blood vessels.
DISCUSSION

The proestrus phase of the cycle was determined by the macroscopic appearance of genitalia, mainly by the presence of largest well developed Graafian follicle present on either of the ovaries, according to the method adopted by Hafez (1955), in determining the proestrus phase of the cycle, in Egyptian buffalo.

The presence of largest Graafian follicle on right ovary in 14 cases, out of 21 ovaries (66.67%) as against 33.33% of left ovaries, tallied with the observations of Hafez (1955) and B. Reddy (1960) in buffaloes, Rajakoski (1960) and Narow et al (1968) in cows. Hafez (1955) found more percentage (75%) of ovulation on right side and B. Reddy (1960) observed more number of pregnancies in the right cornua. Rajakoski (1960) observed more number of large Graafian follicles on right ovary, whereas Narow et al (1968) noticed more number of pregnancy Corpora lutea in right ovary (59%). The present observation indicated more activity of right ovary which was in agreement with similar conclusions of above mentioned authors.

The diameter of the largest Graafian follicle present on ovary having Corpus luteum varied from 0.70-1.45 cms. with an average of 1.14 cms. The diameter of largest Graafian follicle present on ovaries without the presence of Corpus luteum varied from 0.6-1.90 cms. with an average of 1.10 cms. indicated no marked variation between the two conditions.
The average diameter of the largest Graafian follicle and the diameter of the largest Graafian follicle of more than 12 mm. in majority of the cases, was in agreement with the observations of McNutt (1928) and Zamjens (1962).

In most of the cases the surface of the largest Graafian follicle was observed to be transparent and with bluish tinge. Similar findings were reported by Moss et al (1954) in cows.

The presence of a small protuberence of a pinhead size, observed over the surface of the largest Graafian follicle in two cases during the present study exhibited one of important characters of a preovulatory follicle. Similar observation was reported by Rajakoski (1960) in a preovulatory follicle in two heifers.

The colour of Corpus luteum was observed to vary from light pink to greyish white whereas Sane et al (1964) and (1965) reported it to be dirty grey on regression. However, Roy (1966) observed the colour of the Corpus luteum at the regressing stage becoming red, pink or flesh in colour.

In almost all cases the Corpus luteum was deeply seated in the stroma of ovary. The same type of findings were observed by Bhattacharya (1965), in buffaloes.

The Corpus luteum was firm in consistency which corroborated the findings of Laing (1965).
In many cases the uterus presented a tone on palpation which agreed with the observations made by Roberts (1956), Zamjenis (1962) and Hafes (1968).

In majority of cases thin layer of slimy discharge was observed on pale coloured endometrium and caruncles, which was in agreement with the observation of Murphey (1924) in a cow in proestrus phase. The congestion of the endometrium observed in a few cases tallied with similar findings of Roberts (1956), Zamjenis (1962) and Hafes (1968).

Dilated cervix with mucus discharge was observed in the present study, which was in accordance with similar observations of Roberts (1956), Zamjenis (1962) and Hafes (1968).

**HISTOLOGY**

In all the cases a single layer of Germinal epithelium consisting of flattened cells with rod shaped nuclei confirmed the findings of Asdell (1955), Trautmann & Fiebigcr (1957), Cole & Cupps (1959) and Hafes (1968). But Trautmann & Fiebigcr (1957) particularly observed the single layer of Germinal epithelium consisting of flattened cells in the ovary of adult animal as compared to columnar cells in the young animal. Since the ovaries collected in the present study belonged to adult animals, the above finding corroborated with similar observation of Trautmann & Fiebigcr (1957).

The lay out of connective tissue fibers in Tunica albuginea was roughly parallel to the surface of the
ovary in most of the cases which was in agreement with the observations of Asdell (1955), Cole & Cupps (1959) and Hafez (1968). Some of the fibers of tunica albuginea were found to be running in different directions which was in accordance with the findings of Sattergren (1964).

The Cortex was mainly composed of stroma cells with round or oval nuclei, with more of collagen fibers. Normal follicles of various types, atretic follicles, Corpora lutea and blood vessels were present in the Cortex, as described by Asdell (1955), Cole and Cupps (1959) and Hafez (1968).

The primordial follicles and growing follicles noticed in many cases during the present study correlated with the observation of Moss et al (1954), Trautmann & Fiebiger (1957), Rajakoski (1960) and Hafez (1968).

The theca interna and externa consisted of spindle shaped cells with round or oval nuclei as reported by McNutt (1924) in cow. But the cells in theca externa were loosely arranged as described by Rajakoski (1960).

The cells of granulosa were cuboidal in nature with vesicular nuclei, and were arranged in many layers with a distinct cumulus oophorus surrounding the oocyte. Similar observations were made by Cupps et al (1956). The presence of a distinct basement membrane between theca interna and membrana granulosa was in agreement with the observation of Trautmann & Fiebiger (1957) and Donaldson & Hansel (1965).
In the preovulatory follicle, the oocyte was characterised by zona pellucida with granular cytoplasm and vasicular nucleus, which was according to the descriptions given by Trautmann & Piebig (1957). The nucleus was eccentric in position, which was in accordance with the finding of Bloom & Fawcett (1957).

The theca interna cells were loosely arranged as reported by Hammond (1927) in cow. The few marginal cells of theca interna were observed as epitheloid(glandular) in nature with round or oval nuclei which was in agreement with similar observation of Asdell (1946a) & (1955) and Marion et al (1968). In almost all cases, the capillaries were more evident and congested, and the arterioles were full of blood cells in theca interna which concurred with observations of McVitt (1924), Hammond (1927), Asdell (1946a) & (1960), Moss et al (1958) and Sajakoski (1960). In a few cases, the capillaries were lying close to the granulosa cells which was similar to the observation of Hammond (1927). In majority of the cases, the blood vessels were more evident and dilated with blood cells, present in the ovary which was in agreement with the similar findings of Moss et al (1958). This exhibited the overall hyperaemia of ovary during the oestrous period.

The extravasation of R.B.C.s. was observed in a few cases in theca interna whereas in a few other cases R.B.C.s. were noticed in membrana granulosa. The presence of R.B.C.s. in theca interna correlated with findings of Moss et al (1958).
while extravasation of R.B.C.s. in membrana granulosa was in
corroboration with observation of McNutt (1924) and
Asdell (1946) & (1960).

McNutt (1924) and Rajakoski (1960) observed the presence
of eosinophiles in large number throughout theca interna and
membrana granulosa in mature follicle in cow. Asdell (1946) &
(1960) also reported the presence of leucocytes in the follicle
of cow, but he did not specify the type of leucocyte. But
during the present study, the lymphocytic infiltration in large
number was fairly common in theca interna and membrana granulosa.

The arrangement of granulosa cells in many layers
observed during the present study was mostly similar to the
observation of Cupps et al (1956) and Marion et al (1968) in
cow. The presence of distinct basement membrane between the
theca interna and membrana granulosa was similar to the
findings of Donaldson & Hansel (1965) and Marion et al (1968).

In one preovulatory follicle small atra in cumulus
ophorus causing separation of its cells, observed in the
present study was similar to the findings of McNutt (1924),
Asdell (1960) and Rajakoski (1960) in a preovulatory follicle
in cow. The distorted basement membrane with slight crination
of theca interna observed in the same case was also reported by
Asdell (1960). The hyper-chromatosis of granulosa cells in
peripheral layer observed in the same ovary was in accordance
with similar findings of Marion et al (1968) in preovulatory
follicle in cow.
Besides normal Graafian follicles few other Graafian follicles showed oocyte degeneration characterised by the presence of thick, distorted, opaque Zona pellucida and vacuolated cytoplasm, was mostly in agreement with the findings of Rajakoski (1960) of oocyte degeneration. In a few other follicles, follicular cell degeneration was shown by pyknotic nuclei in granulosa cells, with a darkly stained round bodies scattered in the granulosa layer, which was in agreement with the description of early atresia given by Marion et al (1968). However, degeneration in the oocyte characterised by thick hyalinised Zona pellucida with hyalinised cytoplasm, was also observed along with follicular cell degeneration.

**CORPUS LUTEUM**

The thick connective tissue septa of collagen fibers observed in the present study corroborated with similar findings of McNutt (1924), and Rajakoski (1960) in the regressing Corpus luteum.

The extensive degenerative changes characterised by the presence of pyknotic nuclei, shrinkage of cytoplasm, observed in both, large granulosa lutein cells and small theca lutein cells, was in agreement with similar observations of McNutt (1924), Moss et al (1954), Rajakoski (1960) and Donaldson & Hansel (1965) in regressing Corpus luteum.

Stellate appearance of degenerating cells accompanied by peripheral vacuolation was similar to the findings of
Moss et al (1954) and Donaldson & Hansel (1965).

Thick walled arterioles in the regressing Corpus luteum in cow, reported by Donaldson & Hansel (1965) and Hafe and Armstrong (1968), were also noticed in the present study.
SUMMARY AND CONCLUSION

The available literature pertaining to gross appearance of uterus and ovary along with the microscopic structure of ovary of cow and buffalo, was reviewed.

Macroscopic and microscopic studies of 33 buffalo ovaries in proestrus phase, were carried out.

Proestrus phase of the cycle was determined mainly by the presence of largest Graafian follicle on either of the ovaries. Other characters associated with the proestrus phase, like the colour of the regressing Corpus luteum, tone of the uterus, colour of the endometrium showing discharge and dilatation of cervix showing discharge, were also taken into consideration.

Out of 33 ovaries examined, 21 ovaries showed largest Graafian follicle. Out of 21, 14 right ovaries (66.67%) were having largest Graafian follicle as against 7 (33.33%) on left ovaries.

The diameter of the largest Graafian follicle present in the ovary without the presence of Corpus luteum ranged from 0.6-1.9 cms. with an average of 1.19 cms. The diameter of largest Graafian follicle in the ovary having regressing Corpus luteum ranged from 0.78-1.15 cms. with an average of 1.21 cms.

The Graafian follicles of more than 1.2 cms. could be considered as mature follicle (in buffaloes) nearing...
ovulation on the basis of histological findings.

The colour of the regressing Corpus luteum varied from light pink to greyish white.

**HISTOLOGY**

The single layer of Germinal epithelium of flattened cells with rod shaped nuclei was present on the surface of the ovary.

Primordial follicles and Growing follicles were present in the follicular phase.

The Graafian follicles in various stages of development were present. The cells of the theca externa and interna were spindle shaped with round or oval nuclei. But the cells of the theca externa were loosely arranged and blended with the stroma of Cortex. Distinct basement membrane was observed between theca interna and membrana granulosa. The membrana granulosa consisted of cells of cuboidal type with vesicular nuclei and the cells were arranged in many layers. It was differentiated into cumulus oophorus surrounding the oocyte having Zona pellucida and granular cytoplasm with eccentric nucleus.

The mature follicle nearing ovulation was characterised by the cells of theca interna loosely arranged with epitheloid (glandular) cells on the marginal area near membrana granulosa. The theca interna showed increased vascularity with the distended and congested capillaries.
The arterioles in theca interna were rounded in appearance packed with blood cells. Capillaries were lying close to the granulosa layer in a few cases. The hyperaemia of the ovary was noticed by the presence of dilated blood vessels in the stroma.

The presence of RECs in theca interna in a few cases and in membrana granulosa in a few other cases were encountered.

The lymphocytic infiltration in theca interna and membrana granulosa was fairly common.

The separation among the cells in Cumulus oophorus by formation of small antra and distortion of basement membrane with slight erosion of theca interna were noticed in one preovulatory follicle. The granulosa cells were cuboidal with vesicular nuclei, whereas peripheral cells showed hyperchromatosis. The oocyte was round with well developed Zona pellucida enveloping the granular cytoplasm and eccentric nucleus.

Oocyte atresia marked by the presence of thick distorted Zona pellucida and vacuolated cytoplasm was observed in atretic Graafian follicles. Early atresia in Graafian follicles characterised by pyknotic nuclei and darkly stained bodies, in the granulosa layer, was also noticed.

The Corpus luteum in regressing stage presented
a lobulated appearance with thick connective septa, entering the substance. Two types of cells viz., large granulosa lutein cells and small theca lutein cells were present. Both types of cells showed extensive degenerative changes marked by pyknotic nuclei, or chromatolysis, shrinkage of cytoplasm and peripheral vacuolation. Cell outline was not distinct. Connective tissue was abundant in the Corpus luteum and thick walled arterioles were present.
TABLE NO. I

Showing the presence of Graafian follicle and regressing Corpus luteum on right and left ovaries

<table>
<thead>
<tr>
<th>No. of ovaries with largest Graafian follicle</th>
<th>No. of ovaries with largest Graafian follicle and regressing Corpus luteum</th>
<th>No. of ovaries right</th>
<th>No. of ovaries left</th>
<th>Percentage of right</th>
<th>Percentage of left</th>
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<tr>
<td>Percentage</td>
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<td>33.33%</td>
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Right Left Total Left Left Total Right Left Total
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<th>Sr. No.</th>
<th>No. of observations</th>
<th>Range (cm.)</th>
<th>Average</th>
<th>Ovaries with largest Graafian follicle and regressing Corpus luteum</th>
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PLATE I

Internal genitalia of the buffalo showing a mature graafian follicle only on left ovary and a regressing Corpus luteum on right ovary.
PLATE-II

Longitudinal section of the ovary showing the single layer of germinal epithelium of flattened cells with rod shaped nuclei and the tunica albuginea with connective tissue fibers running in different direction.

H.E. Stain x 400.

PLATE-III

L.S. of ovary showing the single primordial follicle, with oocyte and nucleus and surrounded by the single layer of squamous cells.

H.E. Stain x 400
PLATE IV
L.S. of ovary showing the growing follicle consisting of oocyte and surrounded by 3rd layers of squamous cells.
H.E. Stain x 400

PLATE V
L.S. of ovary showing the graffian follicle with antrum, oocyte, membrana granulosa, theca interna and theca externa. The basement membrane is seen between theca interna and and membrana granulosa. The theca interna is compact.
H.E. Stain x 100
PLATE-VI

L.S. of ovary showing two oval shaped Call Exner bodies with cytoplasm and thin capsule, present in the granulosa layer of the graafian follicle. The Call Exner bodies are seen surrounded by a single layer of radiating cells.

H.E. Stain x 100

PLATE-VII

L.S. of ovary showing the preovulatory follicle with a loosely arranged theca interna.

H.E. Stain x 100
PLATE VIII

L.S. of ovary showing the congested capillary in theca interna, lying close to membrana granulosa in preovulatory follicle. Few lymphocytes are also seen.

C = Capillary
L = Lymphocyte
H.E. Stain x 400

PLATE IX

L.S. of ovary showing preovulatory follicle with small antrum in cumulus oophorus and distorted basement membrane with slight crimation of theca interna. The cells in the granulosa layer are showing a tendency to move towards the centre of antrum.

H.E. Stain x 100
HII A

L.S. of ovary showing oocyte degeneration characterized by thick, distorted and opaque zona pellucida, with vacuolated cytoplasm, in the atretic granulian follicle.

H.E. stain x 100

HII A

L.S. of ovary showing lobulated appearance of regressing corpus luteum with thick connective tissue septa.

H.E. stain x 100
PLATE-XXXI

L.S. of ovary showing the lutein cells of regressing corpus luteum, with degenerative changes, marked by pyknotic nuclei, shrinkage of cytoplasm with stellate appearance and peripheral vacuolation.

H.S. Stain x 400

PLATE-XXXII

L.S. of ovary showing few lutein cells of regressing Corpus luteum, with vesicular nuclei, whereas others with pyknotic nuclei.

H.E. Stain x 400
PLATE-XIV

Low power view of ovary with dilated blood vessels filled with blood cells, present in the cortex close to the regressing corpus luteum and granulosa follicle.

H.E. Stain x 100