During fetal life, the morphogenesis of ovary include colonization by primordial germ cells, interaction of primordial germ cells with somatic cells, formation and disappearance of ovigerous cords and concomitant establishment of a population of definitive primordial follicles. Some of the studies have been conducted on the formation of ovigerous cords and ovarian follicles during prenatal life buffalo (Bhardwaj, 1996) and sheep (Sawyer et al., 2002).

The present study was undertaken to observe the development of ovarian follicles from the ovigerous cords during early stages of gestation period.

**MATERIALS AND METHODS**

The study was conducted on sixteen buffalo foetii ranging from 2-25 cm curved crown rump length (CVRL) collected from abattoir. The age of foetii was determined by measuring the CVRL by using following formula given by Soliman (1975).

\[ Y = 28.66 + 4.496X \quad (CVRL < 20 \text{ cm}) \]
\[ Y = 73.544 + 2.256X \quad (CVRL > 20 \text{ cm}) \]

Where Y is the age in days and X is the CVRL in cm.

The tissue samples fixed in 10 per cent neutral buffered formalin and Bouin’s fixatives were processed for paraffin blocks by acetone benzene schedule (Luna, 1968). The sections of 5-6 µ were stained with haematoxylin and eosin for routine morphology and Masson’s trichrome for connective tissue.

For transmission electron microscopy, tissue samples were collected and thoroughly washed in phosphate buffer saline (pH 7.4) solution. These samples were fixed for 2h in 2.5% gluteraldehyde and then secondary fixation was done for 2 h in 2% OsO4. Subsequently tissue samples were subjected to dehydration in ascending grades of acetone (30% to absolute). Subsequently, infiltration was carried out and the tissues were embedded in pure embedding media. Semi thin sections (0.5-2.0 µ) were cut for selection of area. The ultrathin sections (70-90 nm) were cut and lifted on copper grids (100 mesh size) and stabilized by coating with carbon film of 50 Å thickness. The grids were then stained with uranyl acetate (15 min) followed by lead citrate (10 min) and examined under TEM (Morgagni) at All India Institute of Medical Sciences, New Delhi.

**RESULTS AND DISCUSSION**

**Differentiation of ovarian cortex and medulla:** At 10.0 cm CVRL, the ovarian tissue was differentiated into an outer dark zone as cortex and inner lighter zone as medulla (Fig. 1). The clusters of epithelial cells around the oocytes were observed in the cortex whereas the medulla had only mesenchymal tissue. There was an increased vascularity...
in the developing cortico-medullary junction. The germinal epithelium of the ovary was consisted of flat cells with oval nuclei in the centre. At 15.0 cm CVRL, the lining epithelium contained squamous and cuboidal cells as observed in prenatal swamp buffalo (Mungkornkarn, 1980) and Surti buffalo (Baishya and Vyas, 1998).

**Formation of ovigerous cords:** The ovigerous cords were visible at 12.0 cm CVRL (Fig. 2). With the advancement of foetal age, the ovigerous cords became more extensive, convoluted, and elongated. With the penetration of medullary tissue, the inner half of the cortex was consisting of several germ cells surrounded by a layer of pregranulosa cells. At 18 cm CVRL, the germ cells (oogonia) were spherical in shape and appeared towards the periphery of the ovary (Fig. 3), whereas pregranulosa cells were located in the central part. At this stage, the oogonia were located in superficial part and oocytes in deeper part of the ovigerous cords. At 23 cm CVRL, there was degeneration of the oocytes which were darkly stained and appeared as Z-cells. These oocytes showed different stages of prophase I of meiosis. The developing oocytes showed a close contact with each other. The number of Z cells increased with the depletion of oogonia, which disappeared with the increase in foetus age. At early stages of development, the oocytes were located near the oogonial clusters which moved towards deeper part of cortex after maturation.

The electron microscopic studies revealed that the germ cells had large, round nuclei with prominent nucleolous in buffalo fetus at 20 cm CVRL (Fig. 4). Their cytoplasm contained circular mitochondria and free ribosomes, whereas granular endoplasmic reticulum and Golgi apparatus were not prominent. These features corresponded to the premeiotic stage of the germ cells. Pregranulosa cells were ellipsoid or spindle shaped and had ellipsoid nuclei with electron-dense cytoplasmic granules. The outer half of the cortex remained homogeneous and was not divided by the penetration of medullary fibro vascular tissue at this stage. The medulla was composed of blood vessels, spindle shaped fibroblasts and extracellular matrix. The cytoplasmic organelles were not well differentiated in these cells.

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**Fig. 1.** Differentiation of cortex (Cx) and Medulla (Md) in buffalo fetus ovary at 10 cm CVRL. H. & E. × 20

**Fig. 2.** Formation of ovigerous cords (Og) in the outer part of cortex at 12 cm CVRL. H. & E. × 40

**Fig. 3.** Differentiation of pregranulosa cells at the periphery of ovigerous cords at 18 cm CVRL. H. & E. × 200

**Fig. 4.** Electron-micrograph showing differentiation of pregranulosa cells (Pg) and germ cells (G) at the periphery of ovigerous cords at 20 cm CVRL. × 1000

**Fig. 5.** Formation of primordial follicle at corticomedullary junction at 25 cm CVRL. H. & E. × 100

**Fig. 6.** Electron-micrograph showing penetration of interstitial tissue at 20 cm CVRL. × 1000
**Differentiation of primordial follicles:** The aggregates of undifferentiated primordial follicles were first observed at 25 cm CVRL at the cortico-medullary area (Fig. 5). The primordial follicles contained oocyte surrounded by a layer of flattened granulosa cells. Similarly, Kurilo et al. (1986) in bovine foetus and Baishya and Vyas (1998) in the buffalo foetus identified the primordial follicles at 4 months and 124 days, respectively, however Sharma and Luuktuke (1988) observed the formation of primordial follicles in buffalo foetus ovary at 600 mm of CRL. The primordial follicles were separated from each other by interstitial cells (Fig. 6). These may be differentiated from the fibroblast like cells of the medulla (Konishi et al., 1986).

At 33 cm CVRL, most of primordial follicles were present in the inner part of the cortex, whereas the outer part had ovigerous cords. The cortex was clearly demarcated into two zones; zone-I containing ovigerous cords and zone-II was characterized by the presence of newly formed follicles at 37 cm CVRL. Similar observations have been reported in the sheep ovarian cortex which was divided into two distinct zones at 90 days of foetal age (Sawyer et al., 2002). In the buffalo foetus at 50 cm CVRL, these cords were regressing with an increase in the formation of ovarian follicles. The cords were separated from the surface epithelium by the development of an intervening basement membrane. At later stages of development, there was complete regression of ovigerous cords and formation of primordial follicles which occurred centrifugally from the inner region of the cortex toward the periphery.

**REFERENCES**


