Haryana Vet. (April, 2019) 58 (S.I.), 76-79

Research Article

LIGHT AND ELECTRON MICROSCOPIC STUDIES ON BUFFALO OVARY DURING EARLY FOETAL LIFE

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Received : 12.03.2019; Accepted : 26.03.2019

ABSTRACT

The present study was conducted on the ovaries of 20 buffalo foetuses ranging from 2.5 to 20.0 cm CVRL. The tissue samples were collected and processed for light and electron microscopic studies. The results indicated that differentiation of ovarian cortex and medulla started at 8.0 cm CVRL. At 15.0 cm CVRL, the formation of ovigerous cords were observed in the cortex, but at 20 cm CVRL the cortex was divided into an outer zone of ovigerous cords and an inner zone of differentiating primordial follicles whereas the medulla contained number of blood vessels and intraovarian rete cords. Interstitial cells located within the medullary tissue penetrating cortex were first differentiated at 15 cm CVRL and became maximum at 20.0 cm CVRL. The ovigerouscords contained germ cells and pregranulosa cells. The germ cells were spherical in shape and appeared towards the periphery of the ovary, whereas pregranulosa cells were located in the central part. The aggregates of undifferentiated primordial follicles were first observed in the buffalo foetus at 20 cm CVRL at the corticomedullary junction and contained an oocyte surrounded by pregranulosa cells. Electron microscopic studies showed that the germ cells had large, round nuclei with one or two prominent nucleoli and their cytoplasm contained circular mitochondria with tubular cristae and free ribosomes, whereas pregranulosa cells were ellipsoid or spindle shaped, with oval nuclei and abundance of prominent cytoplasmic organelles. Micrometrical data showed that the size of oocyte became almost double from 8.0 cm CVRL to 20.0 cm CVRL buffalo foetus, whereas the size of ovarian follicles did not vary significantly in early foetal life.

Key words: Buffalo, Electronmicroscopy, Fetus, Histology, Ovary

Ovary is responsible for both exocrine and endocrine functions. The structure of ovary varies with the species, age and phase of reproductive cycle. During foetal life, the morphogenesis of ovary include colonization by primordial germ cells, interaction of primordial germ cells with somatic cells, formation of ovigerous cords, 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 in human (Konishi *et al.,* 1986), buffalo (Bhardwaj, 1996 and Gill, 2000) and sheep (Sawyer *et al.,* 2002). The present study was undertaken to observe the formation of ovigerous cords and ovarian follicles during early stages of gestation period.

MATERIALS AND METHODS

The study was conducted on 20 buffalo foetuses ranging from 2.5 to 20.0 cm CVRL. The samples were collected from abattoir and age of foetuses was determined by measuring the CVRL as a curved line in cm using an inelastic thread along the vertebral column between the most anterior parts of frontal bone to the rump at ischiatic tuberosity (Edward, 1965).The approximate age of the foetii was calculated by using following formula given by Soliman (1975).

$Y = 28.66 + 4.496\ X$ (CVRL < 20 cm)

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

Immediately after collection the issue sample were fixed in 10 percent neutral buffered formalin (NBF) and Bouin's fixatives and processed for paraffin blocks preparation by acetone benzene schedule (Luna, 1968). . These paraffin sections of 5-6 were stained with haematoxylin and eosin for routine morphology, 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 and subsequently trimmed of 1 mm^3 size. These samples were fixed for 2 hour in 2.5% gluteraldehyde and then secondary fixation was done for 2 hour in 2% OsO_4. Subsequently tissue samples were subjected to dehydration in ascending grades of acetone (30% to absolute). The dehydration in dry acetone was done at room temperature. The clearing of the samples was accomplished by treatment with toluene. Subsequently infiltration was carried out and the tissues were embedded in pure embedding media using beam capsule. After polymerization the blocks were prepared and trimmed by block trimmer (Reichert TM 60). Semi thin sections (0.5-2.0 µm) were cut to scan the tissues under light microscope for selection of area for ultra thin sectioning. The ultra thin sections (70-90 nm) were cut and lifted on copper grids (100 mesh size) and stabilized by coating with carbon film of 50 Aº thickness. The grids were then stained with uranyl acetate (15 min) followed by lead citrate (10 min). The grids thus prepared were examined under TEM (Morgagni) for detailed study and required photographs were taken.

RESULTS AND DISCUSSION

Differentiation of cortex and medulla

The cortical stroma of the ovary was consisted of fibro cellular component of mesenchymal cells and few fibroblasts. The amount of collagen fibres increased from the peripheral region to the medulla of the ovary. The medulla constituted the central part and contained large

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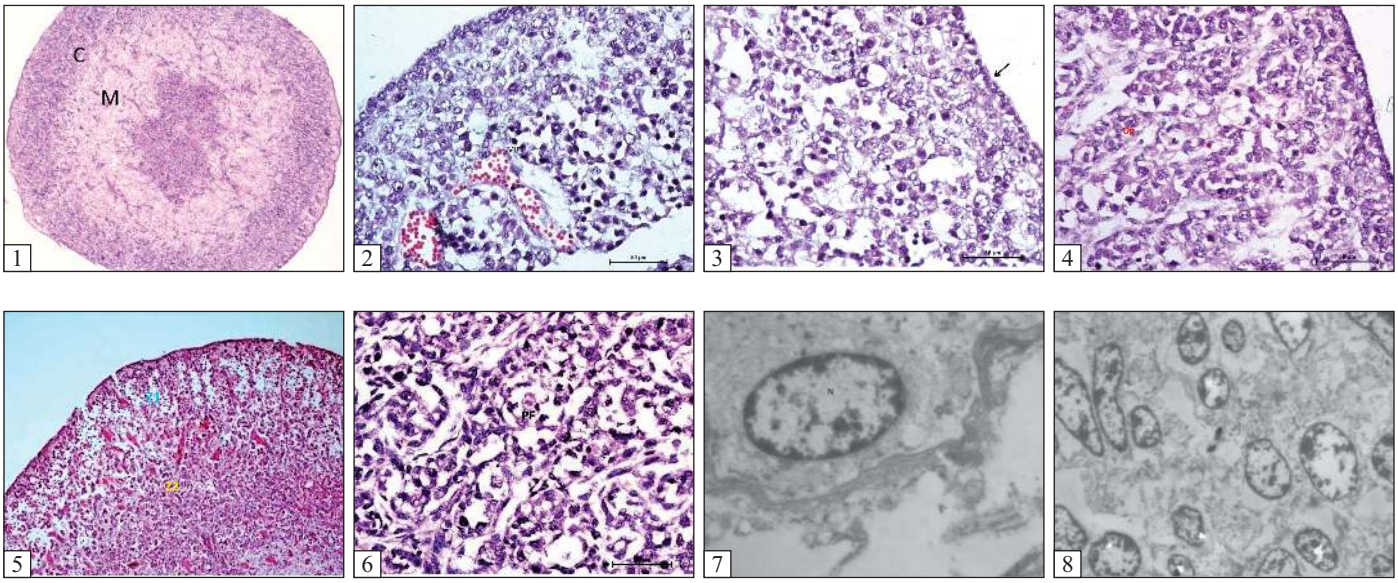

Fig. 1: Differentiation of cortex (C) and Medulla (M) in buffalo foetus ovary at 11.5 cm CVRL. Hematoxylin and Eosin X 100. Fig. 2: higher magnification of Fig 1 showing formation of surface epithelium (arrow), undifferentiated cortex with penetration of medullary rays (MR) in buffalo foetus ovary at 11.5 cm CVRL. Hematoxylin and Eosin X 400. Fig. 3: Simple cuboidal surface epithelium (arrow) followed by tunica albuginea in buffalo foetus ovary at 15.0 cm CVRL. Hematoxylin and Eosin X 400. Fig. 4: Formation of ovigerous cords (Og) in the outer part of cortex at 15 cm CVRL. Hematoxylin and Eosin X 400. Fig. 5: Formation of Zone 1 of ovigerous cords in the outer part of cortex and Zone 2 of undifferentiated primordial follicles at 20 cm CVRL. Hematoxylin and Eosin X 100. Fig. 6: Formation of primordial follicle (PF)at corticomedullary junction at 20 cm CVRL. Hematoxylin and Eosin X 400. Fig. 7: Electron micrograph showing Simple cuboidal surface epithelium with oval nucleus (N) in buffalo foetus ovary at 15.0 cm CVRL. X 2000.Fig. 8: Electron micrograph showing mesenchymal cells in ovarian stroma. X 1000.

number of blood vessels, lymphatics and connective tissue fibres. It mostly contained collagen, reticular and nerve fibres but elastic fibers were located only in the walls of the blood vessels. Similar findings have been reported by Bashiya and Vyas (1998) and Bhardwaj (1996) in buffalo ovary. The differentiation of cortex and medulla occurred in the buffalo foetus at 8 cm CVRL. It was observed that at 11.5 cm CVRL, cortex was hypercellular layer due to presence of the germ cells whereas the medulla was centrally located and consisted of fibrovascular tissue (Fig. 1). At the cortico-medullary junction, large numbers of blood vessels were observed having darkly stained endothelial cells along with elongated fibroblasts running parallel to the surface of ovary. Similarly, the presumptive cortico-medullary border was observed in African elephant foetus by Stansfield *et al.* (2012). At 15 cm CVRL, the formation of ovigerous cords were observed in the cortex whereas medulla was seen in the centre. At 20 cm CVRL the cortex was divided into an outer zone of ovigerous and an inner zone of differentiating primordial follicles whereas the medulla contained number of blood vessels and intraovarian rete cords as reported by Gill (2000) and Bhardwaj (1996) in buffalo foetus. At 11.5 cm CVRL the fibrovascular tissue from the medulla started penetrating into the cortex (Fig. 2). At this stage the inner part of the cortex was divided into ovigerous cords whereas the outer part remained homogenous and was divided by penetration of medullary fibrovascular tissue. The inner half of the cortex had large, rounded or oval cells with rounded nuclei and abundant cytoplasm. According to Konishi *et al.* (1986) these cells are associated with the steroid production in the ovaries. The outer half of the cortex showed frequent mitosis in the germ cells. From 15 cm CVRL onwards, the medullary fibrovascular tissue penetrated into the outer part of the cortex and formation of ovigerous cords were observed.

Differentiation of surface epithelium

The differentiation of surface epithelium was observed in the buffaloes at 8 cm CVRL. It was consisted of low cuboidal type of epithelium with prominent rounded nucleus. The ovary was lined by one or two layers of low cuboidal mesothelial cells resting on a basement membrane which is interrupted at places. At 11.5 cm CVRL the cells from the inner layer showed degenerative changes and the epithelium was simple cuboidal with multilayered at places (Fig. 2). At 15 cm CVRL, the ovary was lined by simple cuboidal type of epithelium with spherical to oval nucleus (Fig. 3). These cells contained lesser amount of cytoplasm and their boundaries were not clearly differentiated. At 20 cm CVRL, some of the simple squamous type of cells was also observed into the surface epithelium. This indicated that the surface epithelium was transforming from simple cuboidal to simple squamous type with age of the foetus. Similar type of observations have also been reported in Surti buffalo (Baishya and Vyas, 1998), sheep (Sawyer *et al.,* 2002) and African elephant foetus (Stansfield *et al.,* 2012). Beneath the surface epithelium, a well-developed tunica albugenia was observed in the buffalo ovary from 8.0 cm onwards. There was abundance of collagen fibres present in the tunica albuginea with few reticular and elastic fibres. Micrometrical data showed that the thickness of tunica albugeniavaried from 18.16±0.60m to 9.88±0.29m in buffalo foetuses of 8.0 cm CVRL to 20 cm CVRL, respectively. The thickness increased upto 11.5 cm CVRL (24.850.27m) and then decreased at 20 cm CVRL

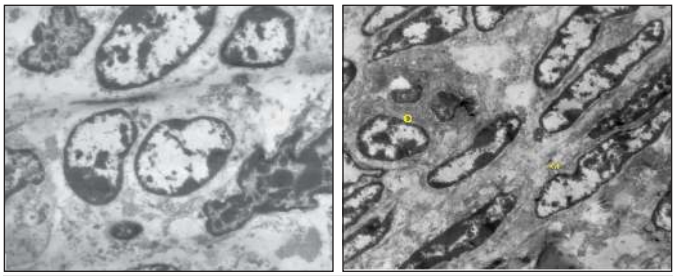

Fig. 9: Electron micrograph showing pregranulosa cells and germ cells in the ovigerous cords at 20 cm CVRL X 1000; Fig. 10: Electron micrograph showing the formation of primordial follicle with an oocyte (O) surrounded by pre granulosa cells (PGC). X 1000

(9.880.29 m), which may be due to the condensation of mesenchymal tissue.

Formation of ovigerous cords

After the penetration of medullary fibrovascular tissue the ovigerous cords were clearly differentiated at 15 cm CVRL (Fig. 4). There was increase in the number, size, convolution and elongation of these cords at 20 cm CVRL (Fig. 5). Similar findings have been observed in the human foetus at 15 weeks onwards (Konishi *et al.,* 1986) and in rat foetal ovaries after day 14.5. At this stage, group of oogonia arranged in clusters forming the egg nests were observed. It contained darkly stained large cells called oogonia and lightly stained small mesenchymal cells forming future pregranulosa cells. The oogonia of the ovigerous cords were separated from each other by epithelial cells present in the cords. The solid cords were present in the peripheral part of the cortex was referred as cortical cords in buffalo foetus by Gill (2000) and ovigerous cords in human foetus(Konishi *et al.,*1986). These cords appeared after the disappearance of oogonia clusters. At 20 cm CVRL, the oogonia were located in the superficial part and the oocyte was present in the deeper part of the ovigerous cords. Some of the oocytes showed degenerative changes and these cells were darkly stained to form Z cells. The number of Z cells increased with the depletion of oogonia and it disappeared with the age of the foetus. Similar findings have been reported in buffalo foetus by Gill (2000) and Bansal *et al*. (2014). At early stages of development, the oocytes were located near the oogonial clusters which moved towards deeper part of cortex after maturation. The micrometrical observations of ovigerous cords showed that the diameter of these cords increased significantly with the age of the foetus i.e. 40.300.28 m to 61.070.22 m.

Formation of primordial follicles

At 20 cm CVRL, the cortex was clearly demarcated into two zones; Zone 1 was formed by outer part of the cortex containing ovigerous cords whereas the inner part of cortex formed into zone 2 and contained the differentiating granulosa cells and oocytes (Fig. 5). 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). Some of the oocytes at the cortico-medullary junction were surrounded and enclosed by somatic epitheloid cells of the ovary to form the granulosa cells which would form the primordial follicles. The aggregates of undifferentiated primordial follicles were first observed in the buffalo foetus at 20 cm CVRL (Fig. 6). These follicles were present at the corticomedullary junction and contained an oocyte surrounded by pregranulosa cells. Similarly, Kurilo *et al.* (1986) in bovine foetus and Bashiya and Vyas (1998) in the buffalo foetus identified the primordial follicles at 4 months and 124 days respectively; however, Sharma and Luktuke (1977) observed the formation of primordial follicles in buffalo foetus ovary at 600 mm CRL. The primordial follicles were separated from each other interstitial cells. These may be differentiated from the fibroblast like cells of the medulla (Konishi *et al.,*1989). Micrometrical observations showed that the diameter of undifferentiated ovarian follicles varied from 28.230.58 to 29.721.18 m whereas differentiated ovarian follicles showed 32.230.87 m diameter at 20 cm CVRL. From present observations the size of developing oocytes varied from 6.700.26 to 13.570.27 m in diameter. From the present data it was interpreted that the size of oocyte become almost double from 8.0 cm CVRL to 20.0 cm CVRL buffalo foetus whereas the size of ovarian follicles did not vary much with the foetal age.

Electron microscopic study

Five different types of cells were observed in buffalo foetal ovary at 20 cm CVRL. The surface epithelium was consisted of low cuboidal cells which had oval nucleus and were anchored laterally by tight junctions and desmosomes (Fig. 7). Mesenchymal cells in the ovarian stroma were of different shapes (rounded, oval or elongated) with euchromatic nuclei (Fig. 8). Some of these cells showed cytoplasmic extensions to contact with surrounding cells as reported by Sawyer *et al.* (2002) in sheep ovaries.

The ovigerous cords were surrounded by germ cells and pregranulosa cells (Fig. 9). The germ cells (oogonia) had large, round nuclei with one or two prominent nucleoli and their cytoplasm contained circular mitochondria with tubular cristae and free ribosomes. Some of the oogonia developed into oocyte with elongated pregranulosa cells in the deeper part of the ovarian cortex (Fig. 10). Pregranulosa cells were ellipsoid or spindle shaped, with ellipsoid nuclei present in the ovarian follicle. Desmosome-like junctions were seen both between pregranulosa cells and between pregranulosa cells and the germ cells. Pregranulosa cells had abundance of prominent cytoplasmic organelles, whereas the stromal cells had well-developed rough endoplasmic reticulum. The oogonia and pregranulosa cells contained lipid droplets and smooth endoplasmic reticulum. Similar findings have been reported by Sawyer *et al.* (2002) in sheep and Konishi *et al.* (1986) in human foetus.

It may be concluded from the present study that the formation of ovarian follicles occurred in buffalo

foetus at 20 cm CVRL containing an oocyte surrounded by pregranulosa cells.

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