



Differentiation of bipotential gonads in buffalo foetus: A histomorphological study

INDERPREET SINGH¹, NEELAM BANSAL², VARINDER UPPAL³ and ANURADHA⁴

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

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ABSTRACT

The present study was conducted on buffalo fetuses during early prenatal life to determine the developmental stages from the gonadal ridge to form bipotential gonads. The present study was conducted on the gonads of buffalo fetuses from 2.5 to 4.2 cm CVRL to determine the development of bipotential gonads from the gonadal ridge. The gonadal ridge appeared as a globular or rectangular thickening on the ventromedial surface of mesonephros at 2.5–3.0 cm CVRL (40–42 days). It was lined by simple cuboidal type of epithelium and contained mesenchymal cells, immature RBC's, differentiating fibroblasts and primordial germ cells. The formation of indifferent gonad started at 3.5 cm CVRL as paired rounded structures, which were attached to the mesonephros by a thin stalk and were present completely separated from the mesonephros at 4.0 cm CVRL. At 3.5 cm CVRL, these gonads were lined by simple cuboidal type of germinal epithelium with some interspersed PGC's. The PGC's and epithelium proliferated and penetrated into the underlying mesenchymal tissue to form primitive sex cords. In the centre of indifferent gonads the blastema was observed at 4 cm CVRL. The blastema had variable shape and prominent nucleus.

Key words: Buffalo foetus, Gonadal ridge, Indifferent gonads

During early prenatal life, a genital ridge is formed on the sides of the embryonic tissue, ventral to the mesonephros. Initially, the genital ridge is made of coelomic epithelium with mesenchyme tissue. Subsequently, this area is invaded by germinal cells originating in the yolk sac. These clusters of cells are the indifferent gonads which will evolve into testis or ovary. The name indifferent gonads or bipotential tissue is given because at this developmental stage the gonads have the potential to be either ovaries or testis (Wilhelm *et al.* 2007). Formation and differentiation of a gonad depend on finely controlled interactions between germ cells and various types of somatic cells. These interactions begin when the germ cells start migrating towards the gonadal ridge. Reaching the presumptive gonadal area on the mesonephros, the germ cells join with the mesonephric-derived cells, which are probably the precursors of the steroid-producing cells (Byskov 1986). Thus, the development of the gonads may be divided into different stages, viz. arrival of germ cells at the gonadal ridge, formation and proliferation of blastema to form indifferent gonads, formation of cords of cells from the blastema which surrounds the germ cells and differentiation and development into a testes or an ovary. The mechanisms

directing the gonad to develop into a testis or an ovary are still obscure, so the present research work was conducted on the gonads of buffalo fetuses during early foetal life.

MATERIALS AND METHODS

The present study was conducted on 7 buffalo fetuses ranging from 2.5 cm to 4.2 cm curved crown rump length (CVRL), collected from abattoirs located in New Delhi and Meerut. The age of fetus 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 fetus was calculated as per 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 whole fetuses were fixed in 10% neutral buffered formalin (10% NBF) and were processed for paraffin blocks preparation by acetone benzene schedule (Luna 1968). The paraffin sections of 5–6 μ m were obtained on glass slides with the help of rotary microtome and were stained with Hematoxylin and eosin for routine morphology and for Masson's trichrome for connective tissue (Luna 1968) in the developing gonads.

RESULTS AND DISCUSSION

The development of bipotential gonads may be divided into 2 sub stages, viz. first stage is arrival of germ cells at

Present address: ¹M.V.Sc student, ²Professor cum Head (bansal.neelam@rediffmail.com), ^{3,4}Associate Professor (v.uppal@yahoo.co.in, anuradha71@in.com), Department of Veterinary Anatomy.

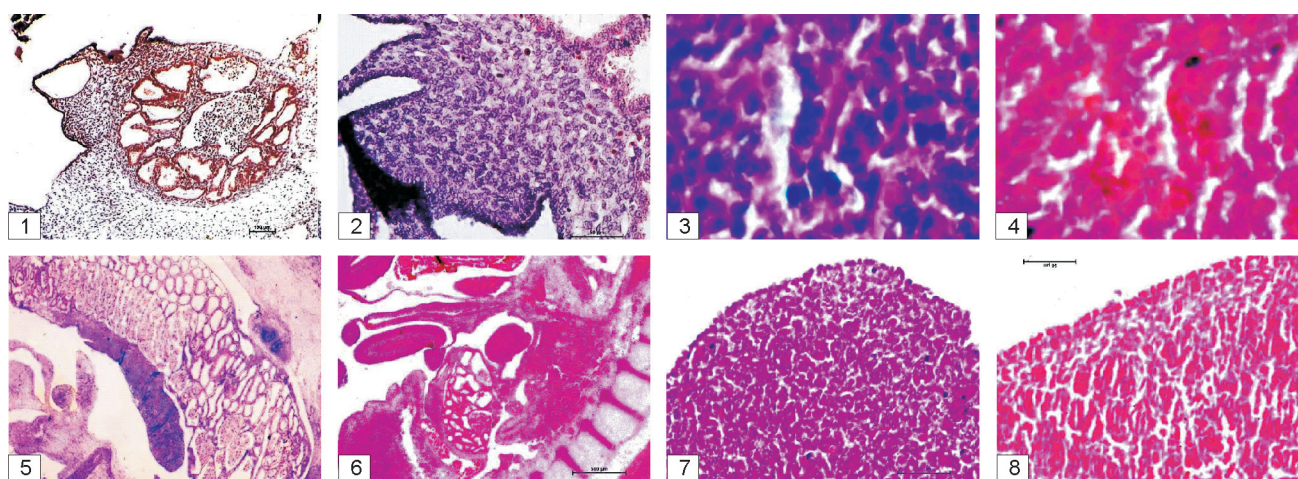
the gonadal ridge and the second stage includes formation and proliferation of blastema to form indifferent gonads.

Formation of gonadal ridge: The gonadal ridge appeared as a globular or rectangular thickening on the ventromedial surface of mesonephros in the buffalo foetus at 2.5–3.0 cm CVRL (40–42 days) (Fig.1). It was lined by a simple cuboidal type of coelomic epithelium forming a dense outer lining of presumptive gonads in buffalo foetus at 2.5 cm CVRL. At 3.0 cm CVRL, the size of gonadal ridge increased due to an increase in amount of mesenchymal tissue between the epithelium and mesonephric tubules (Fig.1). The coelomic epithelium was of simple cuboidal type forming a dense outer lining of presumptive gonads in buffalo foetus at 2.5–3.0 cm CVRL. Similar findings were reported in bovines, pig and dog embryos at 28, 26 and 24 days post conception respectively (Gier and Marion 1970), between 17–23 days post conception in goat embryos (Harshan 1986) and at 76 days of gestation in embryos of African elephant (Stansfield *et al.* 2012).

At 2.5 cm CVRL only mesenchymal cells and primordial germ cells (PGC's) were observed (Fig.3). At 3.0 cm CVRL, the genital ridge was made up of mesenchymal cells, immature RBC's, differentiating fibroblasts and PGC's (Fig.4). The PGC's were larger with acidophilic cytoplasm and a darker nuclei having concentrated chromatin material (Fig.3). The number of PGC's increased with the age of the foetus and these cells migrated towards the germinal epithelium at 3.2 cm CVRL. According to Carlson (1985) PGC's in the germinal epithelium is responsible for the formation primitive sex cords in the gonadal mesenchyme during sexual differentiation. PGC's were also responsible for the initiation of the process of gonadal ridge formation and its differentiation into testis or ovaries. The

mesenchymal cells were spherical or oval in shape having rounded nuclei with granulated nuclear chromatin. Some of the spindle shaped cells were also observed in the mesenchymal tissue, may be designated as future fibroblast cells. These cells show slightly eosinophilic cytoplasm with darker nuclei. Few immature RBC's with larger nuclei were also reported in the present study. Some of the PGC's showed the mitotic activities. Few cells exhibited binucleated type indicating the intermediate status of the cellular components (Figs 3,4). Stansfield *et al.* (2012) also showed the binucleated PGC's in the embryos of the African elephant. Similar cell types were also reported in goat embryos at 23 days of gestation (Farooqui *et al.* 2012). With the advancement of the foetal age these cells proliferated to increase the size of gonadal ridges. Similarly Guigon and Magre (2006) observed the proliferation of germ cells and somatic cells responsible for rapid enlargement of genital ridges. Similarly Dapena (1979) stated that the proliferation of germinal epithelium and underlying mesenchymal cells responsible to form the gonadal ridge in 5 weeks old human embryos.

Formation of indifferent gonads: In the present study, the genital ridge developed into the indifferent gonad at 3.2–4.2 cm CVRL in buffalo foetus. At 3.2–3.5 cm CVRL, the indifferent gonads protruded into the coelomic cavity as paired elongated to rounded structures on either side of dorsal mesentery of the hindgut medial to the mesonephros (Fig.5). These gonads were attached to the mesonephros by a thin stalk, which is presumed to be the precursor of mesovarium (Stansfield *et al.* 2012) or mesorchium (Fig.6). The gonads got separated from the mesonephros and presented into the coelomic cavity as a separate organ in the vicinity of mesonephros at 4.2 cm CVRL. The degeneration



Figs 1–8 **1.** 2.5 cm CVRL buffalo fetus showing formation of gonadal ridge (GR) near mesonephros (M). Masson's trichrome X40. **2.** 3.0 cm CVRL buffalo fetus showing gonadal ridge with simple cuboidal epithelium (Epi) and mesenchyme tissue (Mt). H & E X40. **3.** 2.5 cm CVRL buffalo fetus showing darkly stained PGC, Mesenchymal cells (Mc) and differentiating fibroblast (Fb) in gonadal ridge. H & E X100; **4.** 3.0 cm CVRL buffalo fetus showing PGC, Mesenchymal cells (Mc) and immature RBC in gonadal ridge. H & E X100; **5.** 3.2 cm CVRL buffalo fetus showing formation of bipotential gonads (BG), mesonephros (M) & Metanephros (MT). H&EX40; **6.** 3.5 cm CVRL buffalo fetus showing attachment of bipotential gonads (BG) with mesonephros (M) by a thin stalk. H&EX40; **7.** 3.5 cm CVRL buffalo fetus showing Simple cuboidal epithelium (SE), darkly stained PGC, Mesenchymal cells (Mc) in bipotential gonads (BG). H&EX40; **8.** 4.2 cm CVRL buffalo fetus showing Simple cuboidal epithelium (SE), Primitive sex cords (PSC), Mesenchymal cells (Mc) in bipotential gonads (BG). H&EX40

of mesonephros was reported at 4.0 cm CVRL with the formation of metanephros. With the increase in foetal age, the size of mesonephros decreased due to the degenerative changes, but the size of gonads and metanephros increased due to the increase in cellular components and connective tissue elements. Similar observations were reported by Abd-Elmaksoud (2005) in bovine, Kaur *et al.* (2010) in buffalo and Farooqui *et al.* (2012) in goat foetuses.

At 3.5 cm CVRL, the bipotential gonads were lined by simple cuboidal type of germinal epithelium with some interspersed PGC's (Fig.7). Three types of tissues were distinctly differentiated in the buffalo foetus at 3.5 cm CVRL as mesenchymal tissue, primitive cell cords and germinal epithelium. A distinct basement membrane was observed underneath the lining epithelium. The PGC's and epithelium proliferated and penetrated into the underlying mesenchymal tissue to form primitive sex cords at 4.0 cm CVRL (Fig.8). Similarly Langman (1983) explained the formation of primitive sex cords attached to the surface epithelium. Stansfield *et al.* (2012) observed the penetration of coelomic epithelium to form cortical cords between the mesothelial stromal cells.

Large number of mesenchymal cells of variable shapes were observed in the gonads of the buffalo foetus at 3.5 cm CVRL. These cells had eosinophilic cytoplasm with rounded nuclei. A finely granulated chromatin material and a prominent nucleus was observed in most of the mesenchymal cells. Few connective tissue fibers were observed at this stage. With the advancement of foetal age, the amount of connective tissue fibres increased and the cellular components decreased. This may be due to the formation of clusters of cells responsible for formation of future cortex and medulla in the testis or ovary. Similar findings were reported in goat embryos by Farooqui *et al.* (2012).

In the centre of bipotential gonads, the blastema was observed in the buffalo foetus at 4 cm CVRL. The blastema had variable shape and prominent nucleus. According to Roy *et al.* (2009) the blastema cells develop either from surface epithelium or from mesenchyme. Pelliniemi (1975) stated that the centre of indifferent blastema represented the region of future rete testis in pig embryos. However Guraya (1980) reported that some of the blastema cells were morphologically identical to the presumptive sertoli cells.

It may be concluded from the present study that the establishment of gonadal ridge occurred at 2.5 cm CRVL in buffalo foetus with the formation of germinal epithelium and mesenchymal tissue. The genital ridge developed into the bipotential gonads at 3.5–4.0 cm CVRL with the

differentiation of mesenchymal tissue, primitive cell cords and germinal epithelium. The cellular components were differentiated as mesenchymal cells, immature RBC's, differentiating fibroblasts and primordial germ cells both in the bipotential gonads.

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