http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (10): 1691-1696, 2000

The Bovine Testis ------- I: Pre and Post Natal Development

Laeeq Akbar Lodhi, Zafar Iqbal Qureshi and Farah Ali
Department of Animal Reproduction, University of Agriculture, Faisalabad, Pakistan

Introduction

Testis is a Latin word that means 'witness' or spectator. The mammalian testes perform two functions, the production of male gametes (spermatozoa) and male sex hormones (androgens). By 41 days of fetal life, the bovine fetal gonad is a definable structure and between 3 to 4 months of gestation transcend to scrotum. At birth the testes are small, solid and composed of cords of gonocytes and undifferentiated gonocytes. Testicular growth increases markedly between 7 and 10 months of age. Sequential maturation of spermatogonia through primary and secondary spermatocytes to rounded to elongated spermatids and spermatozoa leads to puberty which occurs between 32 to 44 weeks in Bostaurus bulls. In part I of this review references are made to pre and post natal development of bovine testis starting from their origin, testicular growth, testicular compartments and cell populations. In part II, the role of various hormones in male reproduction and regulation of testicular steroidogenesis is reviewed briefly. Reference is also made to the role of somatomedin-C and Insulin like Growth Factor-1.

Pre-Natal Development of Bovine Testis

Origin of Testis: The bovine testis develops from a thickening of coelomic epithelium on the medio ventral aspect of mesonephros. These thickenings, known as genital ridges, are invaded by primordial germ cells which develop from the yolk sac and migrate through the mesenchyme to the area lateral to the dorsal aorta and ventral to the mesonephric tubules (Gier and Marion, 1970). The migration of these cells to the genital ridge is by ameboid movement (Chretien, 1966; Gier and Marion, 1970; Setchell, 1978). In bovine, these cells can be found in the gonadal ridge region at 26 days of gestation. The primordial germ cells are large, rounded and rich in alkaline phosphatase and glycogen (Setchell, 1978). They settle into coelomic epithelium and cannot be distinguished from the surrounding epithelial cells on day 28 in bovine embryo. The testis further differentiates by the appearance of indentations on the anterior limit of the germinal epithelium which then spread further along the ridge and progressively change from hollow indentations into solid cores of cells called medullary or primary epithelial cords. The primary epithelial cords, after separation from germinal epithelium, will become seminiferous tubules or seminiferous cords. The tunica albuginea covers the seminiferous tubules at about 40 days of gestation in bull fetus (Gier and Marion, 1970). Sustentacular cells and a basement membrane surround the germ cells. This gives primordial germ cells a central location in the cord, which is maintained till the time of onset of spermatogenesis (Courot, 1971). In the three months old bovine fetus, testis weighs 0.5 grams, seminiferous tubule diameter is 34 um and contains two cell types: large gonocytes or primordial germ cells and small indifferent or supporting cells (Hashimoto and Eguchi, 1955; Santamarina and Reece, 1957). These two main cell types are found in the seminiferous tubule of new born bovine calves (Hooker, 1944; Santamarina and Reece, 1957; Courot, 1958). The supporting cells or indifferent cells contain rounded nuclei with course chromatic and have been classified into basal and central indifferent supporting cells depending upon their position in the seminiferous tubule. The gonocytes are large cells, contain large nuclei with one or two globular nucleoli and five chromatic granules and lie between indifferent cells (Abdel-Raouf, 1961). The centrally located gonocytes continue to undergo degeneration until initiation of meiosis which occurs 6 months post-natally in bull (Abdel-Raouf, 1961; Gier and Marion, 1970).

Rate Testis: The bovine rete testis is labyrinth of inter-communicating channels centrally located in the testes and develops as a separate entity of cords.

Interstitium: After the formation of seminiferous cords, a vascular net in the influx area grows into a substantial mass of tissue, separates the seminiferous tubules giving rise to so called interstitial clumps (Gier and Marion, 1970). The interstitial tissue in fetal calves consists of a mesenchymal tissue enveloping the sex cords. The interstitial mesenchymal tissue is continuous with tunica albuginea at the surface of the testis and inwardly with mediastinum testis (Hullinger and Wensing, 1985a). The Leydig cells that are principal source of androgens developed from mesenchymal and are intermingled with the undifferentiated mesenchymal cells that later will become myoid cells and fibroblasts (Hullinger and Wensing, 1985b). At 190 days of pregnancy, the interstitial cells are at peak number and decrease in number towards birth (Santamarina and Reece, 1957; Gier and Marion, 1970). Heterozygous populations of interstitial cells have been reported in rats and boars (Van Straaten and Wensing, 1977; Janszen *et al*., 1976) but have not been observed in the bovine fetus (Hullinger and Wensing, 1985b).

Light microscopy and bichromatic staining of interstitial endocrine cells (IEC's) of bovine fetus during the gubernacula swelling reaction occurring at 14-15 weeks of gestation (see below), revealed a circular nucleus, 6-6.5 um in diameter with a prominent circular nucleolus of 1.2-1.3 um and presence of heterochromatic bodies scattered throughout the nucleoplasm. Histochemical and electron microscopic investigations of the mesenchymal cells at the same time indicated that mesenchymal cells later differentiate into myoid cells and fibroblasts (Hullinger and Wensing, 1985b).

Differentiation of Gonads: Although primordia for both sexes are present during indifferent state of gonads, the differentiation of the male gonads and their excurrent ducts depends upon the secretion of testosterone by the fetal Leydig cells. Concomitantly indifferent supporting cells secrete Muellerian inhibiting hormone, mueflerian, which causes involution of female sex organs (Jost *et al*., 1970, 1972). Differentiation of mesonephric tubules into ductule efferentes and that of mesonephric duct into epididymis and ductus deferens are known to begin at 40 days in the bovine fetus (Amann and Walker, 1983). Unlike the testis and epididymis whose differentiation depends upon the testosterone, the differentiation of prostate, bulbourethral gland, male urethra and phallus depends on

dihydrotestosterone which is a 5% reductase reduced metabolite of testosterone.

Testicular Migration to the Scrotum: The testicular migration or descent is completed in bulls well before birth and has been divided into three stages. In the first stage, also called "nephric displacement", the testis stays closer to the caudal end of the abdominal cavity while, the rest of the animal grows. The second stage called "transabdominal passage", is characterized by the pull of gubernaculum by some obscure forces. It has been suggested that as the rest of the body grows, gubernaculum does not grow and therefore, the testis changes its relative position (Setchell, 1978). The final stage is the "inguinal passage", which is characterized by formation of the processes vaginalis around the posterior attachment of the gubernaculum which enlarges to make the inguinal canal. In 11 week old calf fetus, the gubernaculum ceases to grow and the testis is moved to the inguinal opening. Between 14 and 15 weeks, the gubemaculum swells and by 22 weeks the testis has made ingress into the scrotum (Hullinger and Wensing, 1985a).

Post-Natal Development of Bovine Testis

Testicular Growth: Bovine testes increase 10 fold in weight from birth to 3 months of age and two-folds from four to seven months of age (Michatach, 1933). On the contrary Lagerlof (1934), reported slow increase in testicular size in the early pest-natal life until 16 weeks of age, then a rapid increase with the onset of puberty between 16 and 32 weeks and a still greater gain between 32 and 48 weeks in Swedish Red and White bulls. Testicular weight gain, though slower between 48 and 64 weeks was still more than that of relative increase during first 16 weeks after birth (Abdel-Raouf, 1960; Killian and Amann, 1972). Similar observations were also observed in beef bulls (Rawlings *et al*., 1978) and Bos Indices bulls (Raja and Rao, 1983; Wildeus and Entwistle, 1983). Lodhi (1987) reported on the bases of single testis weight an increase of 183% between 10 to 70 days of age, 86% between 70 to 130 days, 160% between 130 to 190 days and 104% between 190-250 days of age in Holstein bull calves. Weight difference between the right and left testis within the same. animal were reported (Abdel-Raouf, 1960). Howeyer, such differences were less in new born and young animals and become more obvious close to the time of puberty at about 24 weeks of age (Abdel-Raouf, 1960). Season of birth did not influence the growth of testis in Holstein bulls (Curtis and Amann, 1981).

Testicular Compartments: The testes have three functional compartments. The interstitial tissue compartment contains Leydig cells responsible for the production of testosterone, fibroblasts and myoid cells. This compartment surrounds the seminiferous tubules and keeps them bathed with a fluid rich in testosterone. The myoid cells which along with the non-cellular material, make the boundary walls of nerves, blood vessels and lymphatics are located in this compartment.

Seminiferous Tubules: The other two compartments within the seminiferous tubules consist of a basal and an adluminal compartment. The basal compartment contains spermatogonia that divide mitotically and the adluminal compartment provides environment for meiotic division of spermatocytes and differentiation of spermatids into spermatozoa (Amann and Walker, 1983). Sertoli cells extend from basal compartment to the adluminal compartment but both the compartments are separated from each other by junctional complexes that are major constituent of blood testis barrier (Fawcett *et al.,* 1970; Dym and Fawcett, 1971; Setchell, 1982; Amann and Walker, 1983).

Interstitial Tissue Compartment

Leydig Cells: The Leydig cells, first described by Leydig in 1850, are large, polyhedral cells occurring in clusters and randomly scattered in loose connective tissue in the interstitial compartment of bull testis (Setchell, 1978). These cells originate from the mesenchymal cells (Hooker, 1944). The fetal interstitial cells have declined in numbers before birth (Bascom, 1923), increase again at 90 days of age reducing the area occupied by intertubular spaces (Abdel-Raouf, 1960). The Leydig cells contain large amounts of smooth endoplasmic reticulum, lipid filled vacuoles, mitochondria and prominent Golgi apparatuses (Setchell, 1978; Zirkin *et al*., 1980).

Mesenchymal cell differentiation into Leydig cells occurs at about 4 months of age and becomes very obvious at 5 months of age (Abdel-Raouf, 1960; Hooker, 1944). Lodhi (1987) reported that during 70-130 days of age in Holstein bull calves, the development of interstitial mesenchymal like cells in to leydig cells took place. A process called "cytoplasrnic thread formation" was observed in the interstitial mesenchymal cells at 45 days of age which thereafter, gradually become granular till 90 days when the cells have taken the appearance of a Leydig cell. The process of granulation continued until 2 years of age (Hooker, 1944; Bascom, 1923). At 5 years of age in bulls, the intertubular spaces increase and contain large vacuolated Leydig cells. With advancing age, vacuolation and the number and size of the Leydig cells decreases. These changes can be readily observed in the testis of 7 years old or older bulls (Hooker, 1944).

Leydig cells are the main source of testicular androgens of which the most important one is testosterone (Hall, 1979). Regulation of testosterone production therefore depends upon the Leydig cell division, growth and differentiation (Ewing and Zirkin, 1983). Leydig cell volume and/or number is reflected in the testosterone concentration of peripheral blood (Pahnke *et al*., 1975; Pirke *et al*., 1978; Gondos *et al*., 1980; Nussdorfer *et al*., 1980; Bedair and Thibier, 1979). The best correlation between plasma testosterone concentration and morphological parameters was, however, obtained with the endoplasmic reticulum volume (Ewing and Zirkin, 1983).

Other Components of Interstitial Tissues: The interstitial tissue, in addition to Leydig cells, contains myoid cells, blood and lymphatic vessels and nerves (Setchell, 1978, 1982). Macrophages in large numbers can also be observed in the interstitium. The lymphatic vessels are discrete ducts in bulls and the relative volume of total vessels increases with age (Humphrey and Ladds, 1975). The myoid cells that line the boundary wall of the seminiferous tubule have been suggested to play a possible role in the peristalsis like movement of the seminiferous tubules (Setchell, 1982).

The seminiferous Tubule Development: In Normandy bulls the length of the seminiferous tubule has been reported to be 400 meters per testis at birth and 2700 meters at 2 years of age. The length of seminiferous tubule increases rapidly until the testicle weighs 33 grams and at a slower rate thereafter till 400 grams of testicular wt. (Attal *et al*., 1963). In Holstein bulls, Curtis and Amann (1981) reported the tubule

of one testis to be approximately 830 meters long at 12 weeks of age, 1280 meters at 16 weeks and 1510 meters at 24 weeks and 1540 meters at 28 weeks of age. At 32 weeks of age, the seminiferous tubule made up 81% of testicular parenchyma and the length was 2010 meters. Al-Haboby (1986) reported this length to be 3128 meters at the age of 36 weeks in Holstein bulls.

A four fold increase in the diameter of seminiferous tubule from birth to 18 months of age occurred in Jersey and Holstein bulls (Fossland, 1954) and Abdel-Raouf (1960) reported a five fold increase in the same period for Swedish Red and White bulls. A variation of 170-300 um was reported in tubular diameters of adult Swedish Red and White bulls. At one month of age, the tubules are small and have no lumen which first appears in the age of 52 months and is indicated by the vacuolation of the tubule centers (Hooker, 1944). At birth, the tubule diameter is 45 um (Santamarina and Reece, 1957; Attal *et al*., 1963), 65 um at 12 weeks, 110 um at 20 weeks and 208 um at 32 weeks of age (Curtis and Amann, 1981). Lodhi (1987) reported the seminiferous tubule diameter of 49.4 micrometer at 10 days, 60.9 um at 70 days, 85.5 um at 130 days, 123.2 um at 190 days and 178.7 urn at 250 days of age in Holstein bull calves. He further reported the height of seminiferous tubule to be 24.6 micrometers at the age of 10 days, 30.5 urn at 70 days, 42.70 um at 130 days, 54.40 at 190 days and 53.30 um at the age of 250 days.

Tubule diameter increases in form of an S-shaped curve with age and the rate of increase in testis weight and tubular diameter are closely related (Abdel-Raouf, 1960). Al-Haboby (1986) reported a linear increase in the tubule diameter from day 10 to day 250 in Holstein bulls.

Lumen appeared at the age of 20 weeks (Curtis and Amann, 1981) and increased from 2% to 98 um by the age of 32 weeks. The lumen formation is initiated by the cracking of tubular cytoplasm radiating from the center of the tubule followed by star shaped or roughly circular lumen formation, the size of which may vary in the same animal (Abdel-Raouf, 1960).

Somatic Cells of the Seminiferous Tubules: Originating from the sex cord, the indifferent supporting cells are the only somatic cells of seminiferous tubule and differentiate into Sertoli cells. These cells rest upon a basement membrane. The indifferent supporting cells have large nuclei (6-7 um in diameter) with coarse chromatic granules. Occasionally, gonocytes are intermingled among the supporting cells.

Central indifferent supporting cells appear as a result of mitotic division of basal indifferent supporting cells and appear first at the age of 1 week in bull calves after which both cells undergo several mitotic divisions before they differentiate into mature Sertoli cells (Abdel-Raouf, 1960). The highest number of basal indifferent supporting cells was observed at the age of 12 weeks and stayed constant till 16 weeks in Swedish Red and White bulls (Abdel-Raouf, 1960); 20 weeks (Curtis and Amann, 1981) and 27 weeks (Al-Haboby, 1986), in Holstein bulls followed by a decline. Attal *et al*. (1963), reported 970 million indifferent supporting cells per testis at birth increasing to 4560 million at 3 months of age. Indifferent cells possess dark cytoplasm and elongated spaces surrounded by membranes exhibiting small dense granules on their outer surface. These granules were also seen inside the cytoplasm. Electron microscopy also showed the presence of small elongated mitochondria with dark matrix and sparse cristae (Nicander *et al*., 1961).

The Indifferent Cells Transformation into Senor' Cells: This maturation was reported to occur at the age of 28 weeks in Swedish Red and White bulls (Abdel-Raouf, 1960); 24 weeks in Normandy bulls (Attal *et al*., 1963) and 20 weeks in Holstein bulls (Curtis and Amann, 1981; Al-Haboby, 1986). Differentiation of indifferent cells into mature Sertoli cells is first observed as a lightening of the cytoplasm, loss of density in the mitochondria and irregular distribution of the endoplasmic reticulum, This is followed by development of folds of the nuclear membrane making the shape of nucleus irregular and dense vesiculation of the nucleoli (Nicander *et al*., 1961). The size of the Sertoli cell nucleus and the number of indentations in the nucleus membrane then increase from 16 to 32 weeks in Holstein bulls (Curtis and Amann, 1981). Sertoli cells are the main architectural component of the seminiferous tubule. They extend from outer wall of tubule to the lumen and have contact with all other cell types (Setchell, 1982; Waites and Gladwell, 1982). A positive correlation between the total number of Sertoli cells and stem cells per testis exists (De Reviers *et al*., 1978). The number of Sertoli cells per cross section of seminiferous tubules is reported to be 5.2 cells and 3.5 cells at the age of 28 weeks in Holstein and Swedish Red and White bulls respectively (Curtis and Amann, 1981; Abdel-Raouf, 1960), 0.70 at 32 weeks and 15 cells at 44 weeks in Swedish Red and White bulls (Abdel-Raouf, 1960). The relative volume of Sertoli cells per cross sectional area in the seminiferous tubule was 2-2.75 cells in younger bulls (Swierstra, 1966; Humphrey and Ladds, 1975), With the completion of Sertoli cell differentiation from indifferent cells, their number stabilizes (Steinberger and Steinberger, 1977; Nagy, 1972; De Reviers *et al*., 1978). At this stage, the number was reported to be 7×10^9 cells/testis in Holstein bulls (Curtis and Amann, 1981) and a 6×10^9 in Normandy bulls (Attal *et al*., 1963). Since the transformation into Sertoli cells occurs during puberty and the number of stem cells or Aspermatogonia renewing in the seminiferous tubule is positively correlated to the number of Sertoli cells (De Reviers *et al*., 1978), it is possible that factors modifying the number of Sertoli cells may also modify the sperm production in the male (Curtis and Amann, 1981). Electron microscopy showed various cellular structures responsible for cellular metabolism.

The Sertoli cells make a remarkable range of specialized contacts or junctions with germ cells and with each other. A greater morphological and a less physiological difference between the terminal segment and rest of the tubule exists. The terminal segment is further subdivided into transitional region, middle portion and terminal plug. The Sertoli cells of the terminal segment in bulls have well developed Golgi apparatus, abundant mitochondria, long cisternae of mitochondria, large membrane bound spaces and intercellular dilations. Sertoli-Sertoli junctions are mainly found in the transitional region, rarely in middle portion and absent in terminal plug where in the basolateral position, the Sertoli cells form intracellular junctions (Osman and Ploen, 1979; Bielanska-Osuchowska and Sysa, 1981; Wrobel *et al.,* 1982). Sertoli-Sertoli cells junctions of the terminal segment form a narrow luminal cleft thus facilitating the phagocytic activity of these cells geared mainly towards the discarded or degenerated products of spermatogenesis (Sinowatz *et al*., 1979; Wrobel *et al.,* 1982).

The Sertoli cells are the only source of communication across the blood testis barrier. The intercellular junctional complexes divide the intercellular spaces into basal and adluminal compartments. The blood testis barrier has been suggested to be functional between the age of 24 to 28 weeks in Holstein bulls (Curtis and Amann, 1981), evidenced by the lumen formation at this age, the fluid secreted by the Sertoli cells is believed to cause formation of the lumen and occurs at the same time as tight Sertoli junctions are formed (Setchell, 1980). Spermatogonia are present in the basal compartment whereas the primary spermatocytes and other spermatogenic cells are found in adluminal compartment. In the basal compartment of the bull seminiferous tubule, the spermatogonia are attached to the Sertoli cells with spot-like, single tight junctions supported by microfilaments on both sides. Thin projections arising from Sertoli cells in the region of adluminal compartment support the spermatocytes instead of any junctional complexes possessed by the cells themselves. Membranes of spermatozoa also attach to the Sertoli cells by means of microfilaments and cisternae of smooth endoplasmic reticulum (Bielanska Osuchowska and Sysa, 1981). Follicle stimulating hormone (FSH) induces morphological and biochemical changes in the Sertoli cells and has specific binding sites on Sertoli cells (Schanbacher, 1979).

The Germ Cells of the Seminiferous Tubule: After the migration to genital ridge is completed, the primordial germ cells occupy central positions in the sex cords. After transformation of the sex cords into seminiferous tubules, the germ cells are surrounded by supporting or pre-Sertoli cells and the basement membrane (Setchell, 1982). The only germ cells present at the time of birth are the gonocytes (Abdel-Raouf, 1960; De Reviers *et al*., 1978) which are the precursors of spermatogonia. The gonocytes are large cells with spherical nuclei of up to 9 urn in diameter exhibiting scattered rough endoplasmic reticulum, well developed mitochondria and Golgi apparatus containing small vesicles (Nicander *et el.,* 1961; Attal *et al*., 1963). If the germ cells fail to enter the embryonic testis, the adult animal lacks any germinal epithelium. The gonocytes remain inactive until shortly before puberty at which time they migrate to the boundary tissue of the tubule and undergo a period of multiplication (Setchell, 1982). Some gonocytes degenerate during early post-natal life (Abdel-Raouf, 1960; Santamarina and Reece, 1957) and the reason for this degeneration has been suggested to be lack of sufficient nutrition and hormonal stimulus as the indifferent supporting cells that are rapidly multiplying at this stage consume most of the nutrition and still lack the capability of nourishing the gonocytes (Abdel-Raouf, 1961). The highest number of gonocytes per cross section of seminiferous tubule was found at 12 weeks of age (Curtis and Amann, 1981). The highest relative weight of gonocytes in Holstein bulls was reported to be at the age of 100 days (Al-Haboby, 1986). The gonocytes develop into type Aspermatogonia through mitosis and the appearance of Aspermatonia alongwith the formation of Sertoli cells is considered the end of prepubertal period and onset of spermatogenesis (Curtis and Amann, 1981). Spermatogonia were first observed at 8 weeks of age in Swedish Red and White bulls (Abdel-Raouf, 1961) and at 70 days in Holstein bull calves (Al-Haboby, 1986). Basing on the presence of spermatogonia in all the bull testes in a study at the age of 16 weeks in Holstein calves and joining this information with the presence of Sertoli cells in all testis at the age of 24 weeks, the period for the initiation of spermatogenesis was reported to be between 16 and 24 weeks (Curtis and Amann, 1981). Type A0-spermatogonia that are the immediate product of gonocytes have slightly smaller and more elongated nuclei than those of gonocytes and divide mitotically producing type A-I spermatogonia, which have pale and ovoid nuclei, centrally located nucleoli and dusty chromatin (De Reviers *et al*., 1978; De Reviers, 1976). The type Aspermatogonia transform into intermediate spermatogonia (Type In-Spermatogonia) that have comparatively coarser chromatic granules as compared to those in type Aspermatogonia. The type B-spermatogonia have spherical nuclei and the amount of chromatic granules is very little in bulls (Setchell, 1982). A significant increase in the number of type In and type B-spermatogonia between 28 to 32 weeks was reported in Holstein bulls (Curtis and Amann, 1981). The total number of spermatogonia per seminiferous tubules was maximum at 44 weeks of age that was about 25 cells per cross section of the tubule (Abdel-Raouf, 1960). The maximum volume of spermatogonia was achieved at 64 weeks of age (Humphrey and Ladds, 1975). The total spermatogonia relative weight was reported highest at 190 days of age in Holstein bull calves (Al-Haboby, 1986). Six spermatogonial generations have been reported in bulls before the last generation of spermatogonia produces primary spermatocytes through meiosis (Cardoso and Godinho, 1983; Curtis and Amann, 1981; De Reviers, 1976). Spermatogonia after last mitotic division undergo meiosis to produce primary spermatocytes. The primary spermatocytes are large, spherical cells and can be observed in different stages of cell division. These cells can be observed in different layers above the spermatogonia (Abdel-Raouf, 1960). The preleptotene stage is characterized by active DNA synthesis leading to leptotene stage that ends by the formation of long, slender chromosomes. In Zygotene stage, the corresponding pairs of chromosomes come together and linear synaptonemal complexes appear. This is followed by pachytene and diplotene stages leading to diakinesis when separation of homologous chromosomes from paired doubled chromosomes occurs and each primary spermatocyte gives rise to two secondary spermatocytes (Setchell, 1982). The primary spermatocytes were observed first at the age of 130 days in Holstein bulls (Al-Haboby, 1986); 20 weeks in Holstein bulls (Curtis and Amann, 1981) and in Swedish Red and White bulls (Abdel-Raouf, 1960). Another report indicated the appearance of primary spermatocytes as early as 70 days in bulls (Santamarina and Reece, 1957). The number of primary spermatocytes continues to increase until the age of sixty weeks when their number is 40 to 45 spermatocytes per tubule cross section. The total number of primary spermatocytes depends upon the rate of division of Bspermatogonia and the appearance of new crop of primary spermatocytes and the rate of maturation of spermatids (Abdel-Raouf, 1960). Second mitotic division of primary spermatocytes results in the formation of secondary spermatocytes that are seldom seen in a tubular cross section for their short appearance. These are smaller cells than primary spermatocytes, spherical in shape, have round nucleus containing karyosomes (Abdel-Raouf, 1960). Secondary spermatocytes appear for the first time between 20-28 weeks in Swedish Red and White bulls (Abdel-Raouf, 1960), at 28 weeks in Holstein bulls (Al-Haboby, 1986) and increase by 100 folds in the next 4 weeks (Curtis and Amann, 1981). Each secondary spermatocyte produces two haploid

spermatids, their shape dictated by the stage of the seminiferous epithelium cycle. Initially, these cells are spheroidal with rounded nuclei, exhibiting 2 to 4 granules (Abdel-Raouf, 1960) and are characteristically in cap phase (Leblond and Clermont, 1952). In the acrosome phase, the cells move towards the Sertoli cells, are elongated, with more oval nuclei that embed into Sertoli cell cytoplasm. Most of the spermatid cytoplasm is engulfed by Sertoli cells and spermatids are repositioned towards the lumen of seminiferous tubules (Amann and Walker, 1983) engulfed by Sertoli cells and spermatids are repositioned towards the lumen of seminiferous tubules (Amann and Walker, 1983). The spermatids first appeared at 28 weeks of age (Curtis and Amann, 1981; Abdel-Raouf, 1960, 1961). After the age of 40 weeks, the number of spermatids is highest compared to any other cells in corresponding age but may vary greatly from one tubule to the other (Abdel-Raouf, 1961). The elongated spermatids transform into spermatozoa that are first observed at the age of 7-8 months in Holstein bulls (Curtis and Amann, 1981; Schanbacher, 1979; Macmillan and Hafs, 1968); at 81/2 months (Hooker, 1944); at 10 months (Fossland, 1954), in Holstein bulls, 9 months in Jersey bulls (Knudsen, 1954) and 32 weeks in Swedish Red and White bulls (Abdel-Raouf, 1960). The number of sperm in the testis is said to be dependant on the rate of metamorphosis of spermatids and the rate of output of sperms (Abdel-Raouf, 1960). The sperms are released from the testis as soon as they are formed an ripened.

Epididymal Development: The epididymis is composed of a single convoluted tube about 33-35 meters in length in adult bulls. The weight of epididymis was reported to be 9 grams at 7 days of age increasing to 27.2 grams at the age of 16 months in Holstein bulls. The maturation of spermatozoa takes place in the epididyrnis where they are stored afterwards until ejaculation. The epididymis has three physiological, i.e. caput, corpus and cauda and three functional divisions, i.e. initial, middle and terminal segment. It can be divided into six regions based upon the histological features (Nicander *et al*., 1961; Abdel-Raouf, 1960). The first three regions can be included in caput, 4th and 5th in corpus and sixth region is made up of cauda epididymis (Nicander *et al*., 1961). The transformation of infantile epididymis to the adult stage is divided into two processes. First, stage is characterized by the increase in the height of epithelial lining and the other by the transformation of simple epithelium to pseudostratified epithelium. Both the processes start at birth but complete at different ages. The differentiation of epithelium is completed earlier and the maximum height is achieved later in a specific area or region. Various regions exhibit differences in the rate of differentiation of epithelium and rate of increase in the height and both these processes take place in ascending order (Abdel-Raouf, 1960), which might be attributed to the sensitivity of various regions to the factors involved in their development. Epididymal weight increases in the same fashion as testicular weight but the onset of this process takes place much later and the rate is slower than that for testis (Raja and Roa, 1983; Wildeus and Entwistle, 1982; Humphrey and Ladds, 1975; Macmillan and Hafs, 1968; Abdel-Raouf, 1960). The epididymal weight gain varies in various segments where caput increases twice in weight compared to corpus and cauda in first year of life in bull (Abdel-Raouf, 1960; Wildeus and Entwistle, 1983).

References

Abdel-Raouf, M., 1960. The postnatal development of the reproductive organs in bulls with special reference to puberty (incluing growth of the hypophysis and the adrenals). Acta Endocr., 49: 1-109.

Abdel-Raouf, M., 1961. The proliferation of germ cells in the testes of bull calves and young bulls. Acta Vet. Scand., 2: 22-31.

Al-Haboby, A.H., 1986. Influence of neonatal hemiorchidectomy on bovine testicular development and plasma hormone levels. Ph.D. Thesis, University of Minnesota, USA.

Amann, R.P. and O.A. Walker, 1983. Changes in the pituitary-gonadal axis associated with puberty in Holstein bulls. J. Anim. Sci., 57: 433-442.

Attal, J., M. Courot, C. Richetin and C. Pisselet, 1963. Developpement testicu-laire et etablissement de la spermatogenese chez le taureau. Annales de Biologie Animale Biochimie Biophysique, 3: 219-241.

Bascom, K.F., 1923. The interstitial cells of the gonads of cattle with especial reference to their embryonic development and significance. Am. J. Anat., 31: 223-259.

Bedair, G.A. and M. Thibier, 1979. Peripheral plasma androstenedione and testosterone concentrations in bulls before and during puberty. J. Reprod. Fertil., 56: 7-10.

Bielanska Osuchowska, Z. and P.S. Sysa, 1981. Ultrastructure of the bull seminiferous tubule supporting cells with special consideration of the cell junctions. Anat. Histol. Embryol., 10: 370-387.

Cardoso, F.M. and H.P. Godinho, 1983. Cycle of the seminiferous epithelium and its duration in the zebu, *Bos indicus*. Anim. Reprod. Sci., 5: 231-245.

Chretien, F.C., 1966. Etude de la migration et de las multiplication des cellules germinates chez 1 embryon de lapin. J. Embryo. Exp. Morpho., 16: 591-607.

Courot, M., 1958. Cited by Ortavant, R. (1959). In: Reproduction in Domestic Animals, Vol. 2, Chapter 1, Cole, H.H. and P.T. Cupps (Eds.)., Academic Press, London.

Courot, M., 1971. Etablissement de la spermatogenese chez l'agneau (*Ovis aries*). Etude experimentale de son controle gonadotrope: Importance des cellules de la lignee sertolienne. Ph.D. These, University of Paris, France.

Curtis, S.K. and R.P. Amann, 1981. Testicular development and establishment of spermatogenesis in Holstein bulls. J. Anim. Sci., 53: 1645-1657.

De Reviers, M.T.H., 1976. Variation in the stock of testicular stem cells and in the yield of spermatogonial divisions in ram and bull testes. Andrologia, 8: 137-146.

De Reviers, M.T.H., M. Courot, C. Perreau and C. Pisselet, 1978. Sertoli cells and development of seminiferous epithelium. Ann. Biol. Anim. Biochim. Biophys., 18: 573-583.

Dym, M. and D.W. Fawcett, 1971. Further observations on the numbers of spermatogonia, spermatocytes and spermatids connected by intercellular bridges in the mammalian testis. Biol. Reprod., 4: 195-215.

Ewing, L.L. and B. Zirkin, 1983. Leydig cell structure and steroidogenic function. Recent Prog. Horm. Res., 39: 599-635.

Fawcett, D.W., L.V. Leak and P.M. Jr. Heidger, 1970. Electron microscopic observations on the structural components of the blood-testis barrier. J. Reprod. Fertil. Suppl., 10: 105-122.

Fossland, R.G., 1954. Histological development in the postnatal bovine testis. J. Dairy Sci., 37: 669-669.

Gier, H.T. and G.B. Marion, 1970. Development of the Mammalian Testis. In: The Testis I. Development, Anatomy and Physiology, Johnson, A.D., W.A. Gomes and N.I. Vandemark (Eds.). Academic Press, New York, pp: 2-45.

Gondos, B., A. Rao and J. Ramachandran, 1980. Effects of antiserum to luteinizing hormone on the structure and function of rat Leydig cells. J. Endocrinol., 87: 265-270.

Hall, P.F., 1979. Endocrinology. In: Degroot, L.J., G.F. Cahill Jr., L. Martini, D.H. Nelson and W.D. Odell (Eds.)., Grune and Stratton, New York, pp: 1511-1519.

Hashimoto, Y. and Y. Eguchi, 1955. Histological observations on the gonads in the cattle and the horse fetus. I. The cattle fetus. Jpn. J. Zootech. Sci., 26: 259-265.

Hooker, C.W., 1944. The postnatal history and function of the interstitial cells of the testis of the bull. Dev. Dyn., 74: 1-37.

Hullinger, R.L. and C.J.G. Wensing, 1985a. Descent of the testis in the fetal calf: A summary of the anatomy and process. Acta Anat., 121: 63-68.

Hullinger, R.L. and C.J.G. Wensing, 1985b. Testicular organogenesis in the fetal calf: Interstitial endocrine (Leydig) cell development. Acta Anat., 121: 99-109.

Humphrey, J.D. and P.W. Ladds, 1975. A quantitative histological study of changes in the bovine testis and epididymis associated with age. Res. Vet. Sci., 19: 135-141.

Janszen, F.H.A., B.A. Cooke, M.J.A. van Oriel and H.J. van der Molen, 1976. Purification and characterization of Leydig cells from rat testes. J. Endocr., 70: 345-359.

Jost, A., B. Vigier and J. Prepin, 1972. Freemartins in cattle: The first steps of sexual organogenesis. J. Reprod. Fertil., 29: 349-379.

Jost, A., D. Price and R.G. Edwards, 1970. Hormonal factors in the sex differentiation of the mammalian foetus [and discussion]. Phil. Trans. R. Soc. Lond. B: Biol. Sci., 259: 119-131.

Killian, G.J. and R.P. Amann, 1972. Reproductive capacity of dairy bulls. IX. Changes in reproductive organ weights and semen characteristics of holstein bulls during the first thirty weeks after puberty. J. Dairy Sci., 55: 1631-1635.

Knudsen, O., 1954. Cytomorphological investigations into the spermiocytogenesis of bulls with normal fertility and bulls with acquired disturbances in spermiogenesis. Acta Pathol. Microbiol. Scand. Suppl., 101: 1-79.

Lagerlof, N., 1934. Morphologische Untersuchungen Uber Veranderungen im Spermabild und in den Hoden bei Bullen mit Verminderter Oder Aufgehobener Fertilitat. Acta Pathologica et Microbiologica Scandinavica Series, Supplementum 19. Almqvist and Wiksell, Uppsala.

Leblond, C.P. and Y. Clermont, 1952. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann. N. Y. Acad. Sci., 55: 548-573.

Lodhi, L.A., 1987. Prepubertal development of bovine testis following hemicesteration at various ages. Ph.D. Thesis, University of Minnesota, USA.

Macmillan, K.L. and H.D. Hafs, 1968. Gonadal and extra gonadal sperm numbers during reproductive development of Holstein bulls. J. Anim. Sci., 27: 697-700.

Michatach, G., 1933. Die entwicklung des rinderhodens vender geburt bis zur geschlechtareife. Inaug. Diss., Berlin.

Nagy, F., 1972. Cell division kinetics and DNA synthesis in the immature Sertoli cells of the rat testis. J. Reprod. Fertil., 28: 389-395.

Nicander, L., M. Abdel-Raouf and B. Crabo, 1961. On the ultrastructure of the seminiferous tubules in bull calves. Acta Morphol. Neerl. Scand., 4: 127-135.

Nussdorfer, G.G., C. Robba, G. Mazzocchi and P. Rebuffat, 1980. Effects of human chorionic gonadotropins on the interstitial cells of the rat testis: A morphometric and radioimmunological study. Int. J. Androl., 3: 319-332.

Osman, D.I. and L. Ploen, 1979. Fine structure of the modified Sertoli cells in the terminal segment of the seminiferous tubules of the bull, ram and goat. Anim. Reprod. Sci., 2: 343-351.

Pahnke, V.G., F.A. Leidenberger and H.J. Kunzig, 1975. Correlation between hCG (LH)-binding capacity, Leydig cell number and secretory activity of rat testis throughout pubescence. Acta Endocrinol., 79: 610-618.

Pirke, K.M., H.J. Vogt and M. Geiss, 1978. *In vitro* and *in vivo* studies on Leydig cell function in old rats. Acta Endocrinol., 89: 393-403.

Raja, C.K.S.V. and A.R. Rao, 1983. Postnatal growth of testis and epididymis of Brown Swiss cross-bred bulls. Indian J. Anim. Sci., 53: 73-76.

Rawlings, N.C., P.W. Fletcher, D.M. Henricks and J.R. Hill, 1978. Plasma luteinizing hormone (LH) and testosterone levels during sexual maturation in beef bull calves. Biol. Reprod., 19: 1108-1112.

Santamarina, E. and R.P. Reece, 1957. Normal development of the germinal epithelium and seminiferous tubules in the bull. Am. J. Vet. Res., 18: 261-278.

Schanbacher, B.D., 1979. Relationship of *in vitro* gonadotropin binding to bovine testes and the onset of spermatogenesis. J. Anim. Sci., 48: 591-597.

Setchell, B.P., 1978. Development of the Testis. In: The Mammalian Testis, Setchell, B.P. (Ed.). Cornell University Press, Ithaca, New York, ISBN: 9780801411403, pp: 30-43.

Setchell, B.P., 1980. The functional significance of the blood-testis barrier. J. Androl., 1: 3-10.

Setchell, B.P., 1982. Spermatogenesis and Spermatozoa. In: Reproduction in Mammals 1. Germ Cells and Fertilization, Austin, C.A. and A.V. Short (Eds.). Cambridge University Press, Cambridge, pp: 63-101.

Sinowatz, F., K.H. Wrobel, S. Sinowatz and P. Kugler, 1979. Ultrastructural evidence for phagocytosis of spermatozoa in the bovine rete testis and testicular straight tubules. J. Reprod. Fertil., 57: 1-4.

Steinberger, A. and E. Steinberger, 1977. The Sertoli Cells. In: The Testis, IV. Advances in Physiology, Biochemistry and Function, Johnson, A.D. and W.R. Gomes (Eds.). Academic Press, New York, pp: 371-399.

Swierstra, E.E., 1966. Structural composition of Shorthorn bull testes and daily spermatozoa production as determined by quantitative testicular histology. Can. J. Anim. Sci., 46: 107-119.

Van Straaten, H.W.M. and C.J.G. Wensing, 1977. Histomorphometric aspects of testicular morphogenesis in the pig. Biol. Reprod., 17: 467-472.

Waites, G.M.H. and R.T. Gladwell, 1982. Physiological significance of fluid secretion in the testis and blood-testis barrier. Physiol. Rev., 62: 624-671.

Wildeus, S. and K.W. Entwistle, 1982. Postpubertal changes in gonadal and extragonadal sperm reserves in *Bos indicus* strain bulls. Theriogenology, 17: 655-667.

Wildeus, S. and K.W. Entwistle, 1983. A quantitative histological study of testicular and epididymal development in *Bos indicus* cross bulls. Anim. Reprod. Sci., 6: 1-10.

Wrobel, K.H., F. Sinowatz and R. Mademann, 1982. The fine structure of the terminal segment of the bovine seminiferous tubule. Cell Tissue Res., 225: 29-44.

Zirkin, B.R., L.L. Ewing, N. Kromann and R.C. Cochran, 1980. Testosterone secretion by rat, rabbit, guinea pig, dog, and hamster testes perfused *in vitro*: Correlation with Ley dig cell ultrastructure. Endocrinology, 107: 1867-1874.