Bull reproductive development and sperm production enhancement

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Abstract

The onset of puberty and daily sperm production of bulls are fundamental aspects that impact natural breeding and semen production centers. Critical molecular and endocrine aspects of testicular development, spermatogenesis and functions are reviewed. Methods to enhance sperm production (gonadotropin supplementation, immunization against inhibin and induction of temporary hypothyroidism) and findings are discussed.

Keywords: Spermatogenesis, Sertoli cell, endocrinology, puberty, hypothyroidism

Introduction

Important aspects of bull testicular development and sperm production have been reviewed.[1-3] Breeding soundness examinations of thousands of bulls are performed every year before they are used for natural breeding or enter artificial insemination centers. However, on their first examination, approximately 20 - 25% of yearling beef bulls fail to meet minimum standards for testicular size and sperm morphology.[4,5] For high genetic value bulls entering artificial insemination centers for semen collection and cryopreservation, maximum sperm production is required at each collection.

Development of reproductive system and in particular testicular function, starts during fetal life and continues through postnatal period until puberty (Table). These developmental periods are primarily affected by genetics and environmental factors. Testicular function and spermatogenic efficiency are mainly influenced by factors during prenatal period, prepubertal growth phase and post-pubertal maturation phase. Understanding bull gonadal development facilitates optimal management of seedstock dams and young bulls. Objectives are to review bull developmental biology, factors affecting spermatogenesis and potential ways to improve sperm production.

Development of reproductive system in the fetus

Understanding testicular differentiation and development has facilitated substantial improvement in recent years using molecular tools and advancements in genomics. Differences in gene expression can be detected as early as blastocyst stage.[6,7] Differentiation of inner cell mass into somatic germ layers (ectoderm, endoderm, and mesoderm) follows hatching. Hypothalamus, pituitary gland, and penis derive from ectoderm, whereas gonads, epididymides, ductus deferens and urinary system derive from mesoderm. Somatic germ cells are pluripotent except some yolk sac inner lining cells that differentiate into primordial germ cells (PGCs). Undifferentiated gonad forms after passive or amoeboid migration of PGCs into genital/gonadal ridge. This phenomenon is likely controlled by several molecular signals that are not yet completely understood. High mitotic index is recognized in PGCs and they rapidly populate genital ridge; 1000 - 2000 PGCs are already present in genital ridge by 25 days and gonads can be identified at 28 - 29 days of pregnancy.

Several key player genes (Wilms tumor-1 factor, Lim1 transcription factor, and Steroid factor) are identified in gonadal development.[8,9] Wilms tumor-1 factor and zinc finger like transcription factor (produced by Sertoli cells) have important roles in testicular cord assembly and regulating development of fetal Leydig cells and peritubular myoid cells.[10]

Differentiation of fetal testes is determined by SRY gene (Y chromosome sex determining region). Expression of SRY begins on day 37 and peaks on day 39.[11] This gene encodes for a protein of high mobility group and regulates several other genes involved in gonadal differentiation into testis. One of these genes, SOX9, is essential in differentiation of fetal Sertoli cells. Furthermore, GATA4 (required for expression of SRY gene and formation of genital ridge) and DMRT are also implicated.

Table. Chronology of major events during fetal and postnatal development of bull reproductive system

Period	Major events
Embryonic and fetal development	
Blastocyst	Differences between male and female embryos in gene expression
Hatching	Primordial germ cells derived from inner lining of yolk sac
Day 25	1000 - 2000 primordial germ cells migrate to genital ridge
Days 28 - 29	Presence of undifferentiated gonad
Days 37 - 39	SRY expression
Days 41 - 42	Differentiation of male gonad, testicular chords, fetal Leydig cells and Sertoli cells
Day 47	Masculinization of external genitalia due to effects of testosterone and androstenedione from fetal Leydig cells
Day 50	Start of paramesonephric ducts regression
Days 56 - 58	Appearance of seminal vesicles and prostate
Day 60	Differentiation of scrotum
Day 70	Branching of seminal vesicles, differentiation of epididymis, stabilization of mesonephric ducts
Day 80	Complete regression of paramesonephric duct; testes begin transabdominal phase of descent
Day 90	Presence of major components of male reproductive system
Day 110	Formation of epididymis, testis begin inguinoscrotal phase of descent
Day 120	Testicular descent into scrotum
Postnatal development	
Month 1	Degeneration of fetal Leydig cells, increased adult Leydig cells and undifferentiated Sertoli cells; proliferation of prespermatogonia
Month 5	Appearance of primary spermatocytes; seminiferous tubule lumens begin to form
Months 5 - 8	Presence of primary and secondary spermatocytes in seminiferous tubules
Months 7 - 9	Complete detachment of penis from prepuce
Months 8 - 10	Presence of mature sperm in seminiferous tubule lumens
Months 8 - 12	Puberty: ejaculate with $\geq$ 50 million sperm and $\geq$ 10% progressive motility
Months 12 - 16	Maturation

Differentiation of bovine testis starts at 41 - 42 days. At this stage, testicular cords lack a lumen and consist of undifferentiated Sertoli cells. By 60 - 70 days, rete testis appears at end of testicular cords and become connected to each other. Concurrently, first generation of fetal Leydig cells (from mesonephros) appear in mesenchyme. After birth, fetal Leydig cells degenerate and are replaced by adult Leydig cells.

External genitalia become masculinized by day 47 under effects of testosterone and androstenedione produced by Leydig cells. By 60 days, scrotum is well developed. Paramesonephric ducts start regressing by day 50 and are completely regressed by day 80. Seminal vesicles and prostate appear by 56 - 58 days.[12] By end of first trimester, all components of reproductive system are present except for epididymis (begins to form at 110 days.[13]) Internal and external genitalia complete development occurs after 100 days. Descent of testes within scrotum occurs at 100 - 120 days of pregnancy. Testicular descent is mediated by insulin-like peptide 3 (from fetal Leydig cells) that initiates transabdominal phase around 80 - 90 days of pregnancy. Inguinoscrotal phase of testicular descent is mediated by androgens. This early maturation of hypothalamo-pituitary gonadal axis in bovine has been confirmed. Concentrations of insulin-like peptide 3 and testosterone in maternal plasma are significantly higher at 4 and 8 months of pregnancy in cows carrying a male versus female fetus.[14]

Prepubertal development

Onset of puberty in bulls is highly dependent on nutrition and body growth. Slow growth is correlated to slower testicular development and delayed puberty. In some breeds, scrotal circumference is highly correlated to body weight.[15] At birth, testes are composed of solid (no lumen) chords comprised of primordial germ cells, fetal Leydig cells and undifferentiated Sertoli cells (Figure 1).[16] Accessory sex glands are not functional and penis is adherent to prepuce. Fetal Leydig cells degenerate

in first postnatal month and are replaced by adult Leydig cells. GnRH receptors in anterior pituitary increase significantly from 6 - 10 weeks.[17] Increased frequency of GnRH pulses and corresponding increases in LH concentrations occur during cell differentiation. Number of undifferentiated Sertoli cells increases rapidly.[16,18] During prepubertal period, serum FSH and inhibin concentrations are high, but decline with onset of rapid testicular growth.[19] FSH has an important role in prepubertal proliferation of Sertoli cells,[20] as it increases at 4 - 25 weeks.[21]

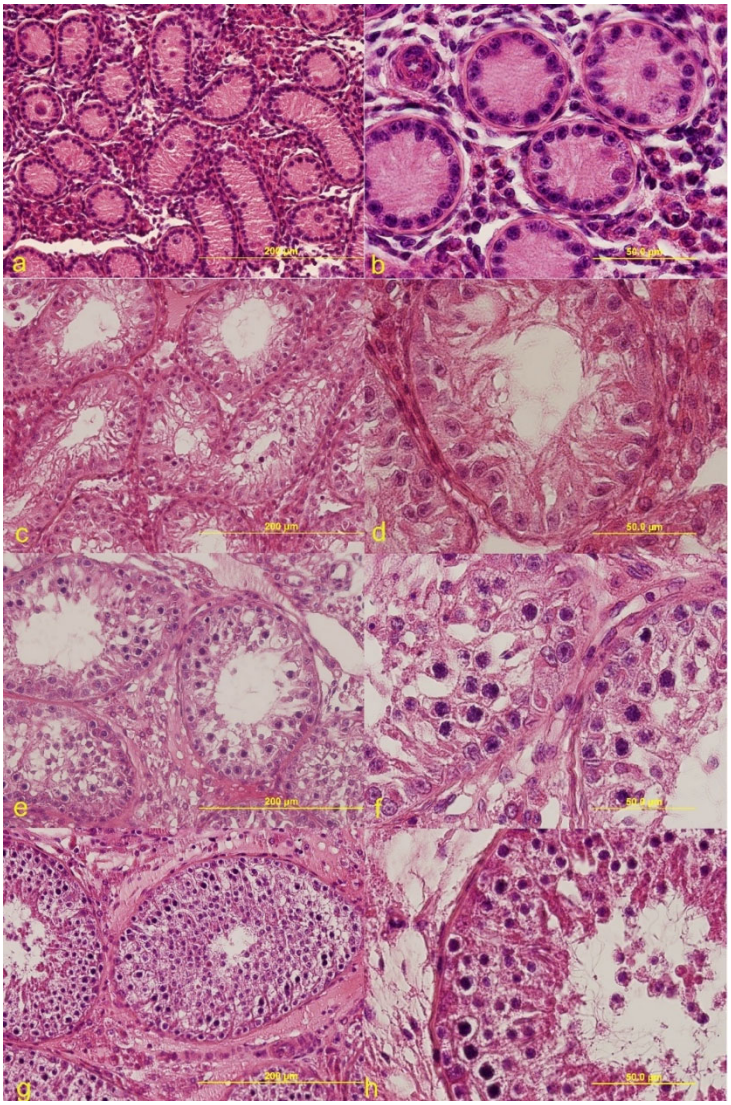

Figure 1: Histological appearance of bull testis at 1 month (a, b), 5 months (c, d), 8 months (e, f), 10 months (g, h); note: development of a lumen at 5 months, initiation of spermatogenesis at 8 months, appearance of sperm in lumen by 10 months. (Left column x 400, Right column x 1000)

Proliferation of prospermatogonia began at 1 month, and primary spermatocytes appeared ~ 5 months.[22] Age of puberty was negatively correlated with magnitude of LH secretion in first 5 months of life,[23] a highly heritable trait.[24]

Detachment of the peno-preputial adhesions occurs ~ 6 weeks prior to puberty. As puberty approaches, testicular and epididymal growth accelerate and become almost linear. Low-frequency LH pulses increase serum testosterone concentrations produced by a rapidly increasing number of adult Leydig cells, promoting establishment of spermatogenesis.[16] Primary and subsequently secondary

spermatocytes are detected 5 - 8 months (Figure 1). Mature sperm are present in seminiferous tubules from 8 - 10 months. Puberty (ejaculate with at least 50 million sperm and at least 10% progressive motility) occurs when scrotal circumference reaches 28 - 30 cm in *Bos taurus*[2] and 27 - 28 cm in *Bos indicus*.[25]

Following puberty, bulls enter a phase of maturation characterized by increases in accessory sex gland weights, sperm production and proportion of morphologically normal sperm. This phase of maturation varies considerably amongst bulls, lasting 3 - 4 months in *Bos taurus*.[26]

Effect of nutrition on early development

Nutrition of the dam profoundly affects testicular development.[27] Heifers fed 2.4 x recommended energy and protein requirement in the first 6 months of pregnancy produced calves that had smaller testes and lower serum testosterone concentrations compared to calves from heifers fed 1.9 x or 0.7 x recommended ration.[27] Santa Gertrudis bull calves from dams fed low-protein diet had delays in onset of puberty and sexual maturity than those from dams fed high-protein diet.[28] However mechanism of this action remains unclear.

Bull calves fed diets to achieve 1.4 - 1.5 kg/day average daily gain reach puberty earlier with greater paired testes weight and daily sperm output.[2,29,30] In general, with an average daily gain of 1 kg/day, puberty occurs at 8 - 12 months.[31] Preweaning early development of bulls was largely dependent on maternal milk production. Bull calves born to heifers and aged dams (> 9 years) had smaller scrotal circumference due to lower hypothalamo-pitutary activity.[2] However, effects of diet on onset of puberty are confounded by genetics and greater feed intake does not consistently result in earlier puberty.[32]

It is important to note that a high-energy diet, such as often fed in bull testing stations, may have adverse effects on testicular development and spermatogenesis. Young bulls fed high energy diets have better average daily gain and thicker backfat, but their scrotal circumference may not be altered. [2,3,34] However, these bulls have more morphologically abnormal sperm due to poor testicular thermoregulation attributed to increased scrotal fat.[2,33,34]

Enhancement of sperm production

Enhancement of sperm production in valuable bulls is important to satisfy the demand for more doses of semen (particularly sexed semen) for artificial insemination. In addition to care and nutrition of the pregnant dam, several other postnatal strategies were investigated to increase bull sperm production. Unilateral castration increased the contralateral testicular size but failed to increase sperm production. In recent years, our understanding of the role of Sertoli cell as support cell for spermatogenesis, provides new approaches to enhance sperm production.

The role of Sertoli cells in spermatogenesis was studied in a variety of species.[20,35] Strong positive correlation between Sertoli cell number and sperm production capacity in bulls was established.[20] Sertoli cell number correlated with number of spermatogonial stem cell niches in mice, critical for spermatogenic activity and sperm production.[36,37]

Postnatal expansion of Sertoli cell population was described in several mammalian species as a 2 wave linear increase in Sertoli cell population.[37] Termination of Sertoli cell proliferation appears to be an extremely important biological point for manipulating spermatogenesis.[37] Increases in testicular estrogen concentrations arrest Sertoli cell expansion in swine but apparently not in bulls.[35] In the latter, based on early cytological studies, Sertoli cell number plateaus at 6 - 7 months.[22] However, based on cell proliferation marker KI67 and expression of Sertoli cell marker SOX9, Sertoli cell proliferation ceases at 4.5 - 5 months (Figure 2).[38]

During prepubertal development, Sertoli cell proliferation is stimulated by FSH but inhibited by thyroid hormone concentrations. This relationship between Sertoli cell proliferation and thyroid hormone concentrations was demonstrated in boars[39] and rams.[40] In bulls, thyroxine concentrations were negatively correlated with testicular size at puberty.[41] These observations led to development of hormonal methods during early development to enhance sperm production. These hormonal methods

include immunization against inhibin, GnRH injections, FSH injections, and establishment of a transient hypothyroidism.

Immunization against inhibin in early postnatal period increases serum FSH concentrations, testicular size, and daily sperm production in bulls.[42] However, it was transient and required multiple vaccinations. There was also a great variability of the response among individuals. Administration of GnRH to prepubertal bulls (120 µg/kg) BID from 4 - 8 weeks hastened puberty by 6 weeks compared to untreated bulls.[43]

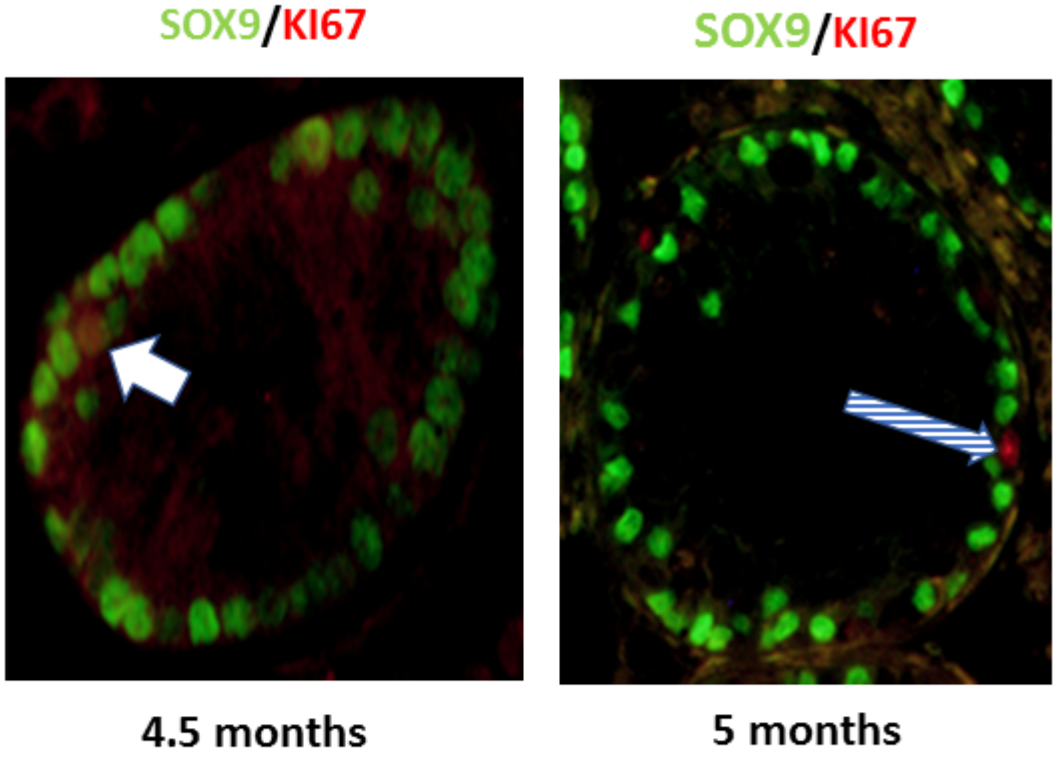

Figure 2. Cross-sectional view of seminiferous epithelium.[38] Green cells are SOX9 positive Sertoli cells, red cells are ki67 positive dividing cells. White arrow points to a dividing Sertoli cell stained with both SOX9 and KI67 at 4.5 months. Pattern filled arrow indicates a KI67 positive dividing germ cell at 5 months; note: at 5 months none of the sox9 positive Sertoli cells express KI67.

Bull calves given exogenous FSH every 2 days for 4 - 8 weeks had more Sertoli cells number at 56 weeks and attained puberty 5 weeks earlier than nontreated calves.[44] In a more recent study, administration of porcine FSH (30 mg, Folltropin-V in 2% hyaluronic acid) from 35 - 91 days increased endogenous FSH at 70 days and Sertoli cell numbers per seminiferous tubule at 93 days,[45] with increase in endogenous FSH due to a positive feedback loop through Activin A produced by Sertoli cells. FSH-treated bulls had on an average 4 more Sertoli cells per seminiferous tubule section than untreated bulls.[46] However, there was no difference in spermatogonial cell number.[46]

Potential for enhancement of sperm production after induction of transient hypothyroidism in bulls (based on negative relationship between Sertoli cell proliferation and circulating thyroid hormones concentrations) was investigated.[38] To induce hypothyroidism, bull calves received Methimazole (2

mg/kg BW, BID) from 4 - 6 months. Age of puberty was delayed with increased sperm production. Bulls subjected to transient hypothyroidism had 30 - 180% more sperm per ejaculate than control bulls, with no effect on sperm motility, morphology, post-thaw survivability or rates of IVF cleavage and blastocyst development. Treated bulls had a 22% higher mean paired testes weight and 50% higher epididymal weight compared to untreated bulls. Finally, treated bulls had a 2.3-fold increase in number of Sertoli cells per cross section of seminiferous tubule than control bulls. These promising results are impetus for further studies.

Conclusion

In the current era of genomic testing, sire selection can be performed very early in life and bull development and sperm production are increasingly important for efficient use of elite sires. In this context, studies are needed to dissect Sertoli cell-specific role in sperm production and methods to enhance it. Current knowledge on prenatal and postnatal bull development suggests that these phases can be modulated through judicious nutritional supplementation of dams and postnatal hormonal treatments of bull.

Conflict of interest

There are no conflicts of interest to declare.

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