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Review: Ontology and endocrinology of the reproductive system of bulls from fetus to maturity

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This review focuses on current understanding of prenatal, prepubertal and post-pubertal development of the male reproductive system of cattle. The critical developmental events occur during the first 3 to 4 months of gestation and the first ~6 to 9 months after birth. The Wilms Tumor-1 and SRY proteins play critical roles in early development and differentiation of the fetal testis, which in turn drives gestational development of the entire male reproductive system. The hypothalamic–pituitary–gonadal axis matures earlier in the bovine fetus than other domestic species with descent of the testes into the scrotum occurring around the 4th month of gestation. An array of congenital abnormalities affecting the reproductive system of bulls has been reported and most are considered to be heritable, although the mode of inheritance in most cases has not been fully defined. Early postnatal detection of most of these abnormalities is problematic as clinical signs are generally not expressed until after puberty. Development of genomic markers for these abnormalities would enable early culling of affected calves in seedstock herds. The postnatal early sustained increase in lutenising hormone secretion cues the rapid growth of the testes in the bull calf leading to the onset of puberty. There is good evidence that both genetic and environmental factors, in particular postnatal nutrition, control or influence development and maturation of the reproductive system. For example, in Bos taurus *genotypes which have had sustained genetic selection pressure applied for fertility, and where young bulls are managed on a moderate to high plane of nutrition puberty typically occurs at 8 to 12 months of age. However, in many* Bos indicus *genotypes where there has been little selection pressure for fertility and where young bulls are reared on a low plane of nutrition, puberty typically occurs between 15 to 17 months. Our understanding of the control and expression of sexual behavior in bulls is limited, particularly in* B. indicus *genotypes.*

Keywords: Bovine, bull, ontology, reproductive system

Implications

A good understanding of prenatal and postnatal development of the reproductive system of bulls and the factors affecting it's development provides the basis for 'best practice' recommendations on the management of pregnant seedstock dams and management of young bulls through to them either, becoming herd sires or semen donors. Both prenatal and postnatal development of the reproductive system is controlled by interactions between genetic and environmental factors. Some disturbances in development may be diagnosed before puberty but some such as unilateral testicular hypoplasia and premature spiral deviation of the penis may not manifest until months to several years after bulls reach puberty.

Introduction

To optimize the management of young bulls and to understand the pathogenesis of subfertility or infertility in young and older bulls knowledge of the sequence and broad timing of developmental and physiological changes in the reproductive system that occur from conception to sexual maturity is required. Disturbances in either prenatal and or postnatal development of the reproductive system of males are associated with varying degrees of subfertility or infertility. Furthermore, it is important to consider development of the reproductive system as part of development of the whole male animal, not in isolation. For example, it is well recognized that at the same time critical peripubertal changes are occurring in development of the testes important maturational changes are occurring in articular cartilage, and disturbances in the latter can predispose to degenerative joint disease which in turn may be associated with reduced libido, mating ability and/or semen quality (Persson *et al.*, 2007). This review will focus on current understanding of prenatal, prepubertal and post-pubertal development of the reproductive system of bulls and highlight some of the key factors affecting this development.

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Prenatal development of the reproductive system

Period of the embryo through to day 100 of gestation

The chromosomal sex of the embryo is determined at fertilization by the sex chromosome carried by the fertilizing spermatozoon. Immediately following fertilization (review: Avella *et al*., 2013) the embryo remains transcriptionally silent until the 8 to 16 cell stage (Graf *et al*., 2014). By the blastocyst stage, when differentiation begins with formation of the inner cell mass (ICM) and the trophectoderm (Reijo Pera and Prezzoto, 2016), differences in genes expressed can be detected between male and female embryos (Heras *et al.,* 2016). At about the time the embryo hatches through the zona pellucida a second phase of differentiation which involves both the ICM and trophectoderm occurs. Cells of the ICM facing the blastocoel flatten and elongate and form the hypoblast whilst the remaining ICM cells become the epiblast. From the epiblast three somatic germ layers develop, the ectoderm, endoderm and mesoderm, and these can be detected before appositional attachment of the conceptus to the endometrium. These layers give rise to different organ systems. With respect to the male reproductive system the hypothalamus, pituitary gland and penis are derived from the ectoderm, and the gonads, epididymis, ductus deferens and urinary system are derived from the mesoderm.

At the time the embryo differentiates into the three somatic germ layers most cells lose their pluripotency except for the primordial germ cells which are derived from the inner lining of the yolk sac. These cells migrate either passively or by amoeboid movement, in response to as yet unidentified molecular cues, into the genital or gonadal ridge to form the indifferent gonad. The understanding of these processes in the bovine lags behind that in smaller mammals (Tarbashevich and Raz, 2010), but the expectation is the processes will be similar.

Primordial germ cells are recognized as large cells which stain positive for alkaline phosphatase and the well-known marker of pluripotency octamer-binding transcription factor 4. Primordial germ cells which do not populate the genital ridge degenerate. Rapid population of the genital ridge occurs because of the high rate of mitotic division shown by these cells, probably in response to Steel Factor and the cytokine Leukaemia Inhibitory Factor (Gu *et al.,* 2009). By day 25 in the bovine fetus there are 1 to 2000 primordial germ cells in the genital ridge.

The indifferent gonad is located on the inner surface of the dorsal body wall medial to the embryonic kidneys. It is first seen at about days 28 to 29 when the fetus has a crown-rump length of 9 to 10 mm. It remains morphologically indifferent for several weeks. There are three cell types in the genital ridge: local mesenchymal cells, cells derived from the coelomic epithelium and cells from the regressing mesonephric tubules that invade the presumptive gonadal tissue (Hyttel *et al.,* 2009). Some of the genes important in this process have been identified from their high levels of expression in the developing gonad. The earliest acting gene is Wilms tumor-1 (*WT-1*). The WT-1protein is a zinc finger transcription factor produced by the Sertoli cells, and is essential for the development of the kidneys and gonads. In the developing gonad it plays a crucial role in testis cord assembly and maintenance. The WT-1 protein also regulates development of the fetal Leydig cells, interstitial progenitor cell lineages and peritubular myoid cell development through Notch signalling, thus facilitating fetal testis compartmentation (Wen *et al*., 2016). The gene *Lim1* encodes a homeobox transcription factor which plays a major role in development of the kidney, but is also important in gonad development because in its absence the gonads do not develop (Davies and Fisher, 2002). Steroid factor 1 (*SF1*) is a nuclear receptor and acts as a regulator of multiple genes (Kohler and Achermann, 2010). It is highly expressed in the early indifferent gonad but its role there is unclear. *SF1* is also highly expressed in steroidogenic cells of the adrenal cortex and gonads, as well as in neurons of the ventromedial nucleus of the hypothalamus (Kohler and Achermann, 2010). The gonad ceases being indifferent when cords of epithelial cells from the mesonephric tubules and regressing glomerular capsule penetrate the mesenchyme of the genital ridge and form the primitive sex chords.

Differentiation of the fetal testis occurs in response to the sex-determining region on the **Y** chromosome (i.e. the *SRY* gene). The SRY protein is a member of the SRY related high mobility group box (Sox) transcription factor family. Expression of *SRY* begins at day 37 and peaks at day 39 in the bovine fetus (Ross *et al*, 2009). The bovine protein encoded by SRY is composed of 229 amino acids (Soleymani *et al*., 2017) and is very similar in size to murine SRY. In both species the encoded protein is a member of the high mobility group (HMG) of proteins. The *SRY* gene regulates a number of other genes which collectively drive differentiation of the indifferent gonad to become a testis. The gene *Sox9* is the direct target for SRY. Expression of *Sox9* leads to differentiation of the foetal Sertoli cells (Gonen *et al,* 2017) which orchestrate testicular morphogenesis. *Sox9* controls a conserved genetic program that involves most of the sex-determining genes. In the fetal testes *Sox9* modulates both transcription, and also directly or indirectly differential splicing of its target genes, through binding to genomic regions with sequence motifs that are conserved among mammals and are called Sertoli cell signatures. Sertoli cell signatures display precise organization of binding motifs for the Sertoli cell reprogramming factors *Sox9*, *Gata4* and *DMRT1*. Recently, a new factor, tripartite motif containing factor 28 which can interact with *Sox9* in the fetal testes, was identified by Rahmoun *et al.* (2017).

Commencement of differentiation of the male bovine gonad occurs at days 41 to 42 of gestation, preceded by several days with the first detection of *SRY*. Initially each testicular cord is lumenless with undifferentiated Sertoli cells around the periphery. They are shaped like a horseshoe with tiny strands connecting the ends which at days 60 to 70 of gestation begin to develop into the rete testis. Also at this time, in the mesenchyme between the testicular chords, the

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first generation of fetal Leydig cells begin to differentiate. These fetal Leydig cells originate in the mesonephros. Interestingly, these cells degenerate postnatally and are replaced by adult Leydig cells, which differentiate only after birth (O'Shaughnessy and Fowler 2014). It is clear that *SRY* and the cascade of genetic events it initiates collectively drive the formation of the testes and through this, ultimately development of the entire male reproductive system.

Vigier *et al.* (1976) describe the key events in development of the male bovine reproductive tract, commencing with masculinization of the external genitalia around day 47 of gestation, driven by testosterone and androstenedione secretion from the newly differentiated fetal Leydig cells. By day 60 the scrotum is well differentiated. Regression of the paramesonephric ducts starts from day 50 (Vigier *et al.,* 1976) and is completed by day 80. The masculinization of the internal genitalia occurs in two distinct phases. In the first phase (days 56 to 58) the early buds of the seminal vesicles and prostate appear, and the bulbourethral gland begins to develop. In the second phase (beyond day 70) branching of the seminal vesicles begins, differentiation of the epididymis begins, and stabilization of the mesonephric ducts occurs (Vigier *et al.,* 1976). At the end of the first trimester the major components of the bovine male reproductive system are all present but not yet fully developed. However, the epididymis only begins to form at day 110 (Alkafafy and Sinowatz, 2012).

The highly complex series of events which occur in differentiation and development of the male reproductive system are underpinned by the program of regulated gene expression described above. However, epigenetic mechanisms are also likely to influence many aspects of development of the male reproductive tract. Although a cell's transcriptional machinery, provides the basis for its differentiation and development, epigenetic marks on its DNA may alter components of this machinery (Rojas-Garcia *et al.,* 2013). These epigenetic marks are subject to both environmental and developmental perturbations which can subsequently impact on early embryonic development (Farin *et al*., 2006) or gametogenesis (Mochizuki *et al*., 2012). Although there is extensive research on the impact of *in-utero* nutrition of the dam and fetus on development and function of the male reproductive system in sheep there has been only a few studies conducted in cattle. Sullivan *et al.* (2010) reported that when tropically adapted heifers were fed a ration formulated to provide an average of 2.4× the recommended energy and protein requirements for the first and second trimester their male calves at 5 months of age had smaller testes and lower serum concentrations of testosterone compared with the calves from heifers fed an average of 1.9× and 0.7× the recommended energy and protein requirements. *In-vitro* manipulation and culture of gametes and embryos have also been shown to affect the normal epigenome of the subsequent fetus or offspring (Ventura-Junca *et al*., 2015; Anckaert and Fair, 2017; Canovas *et al*., 2017). However, it is important to note that with respect to the development of the reproductive system of the bull there are no confirmed reports that the use of assisted reproductive technologies has contributed to abnormal development.

Period from day 100 of gestation through to birth

During this period final development of the internal and external genitalia is completed including descent of the testes into the scrotum. The hypothalamic–pituitary–gonadal (HPG) axis continues to mature and the fetal Leydig cells secrete androgens, testosterone and dihydrotestosterone (DHT), which act to stabilize the mesonephric ducts and to masculinize the external genitalia, and insulin-like peptide 3 (Insl3) which acts with testosterone to induce testicular descent.

Testosterone regulates three main aspects of male phenotypic development, directly or through DHT: the genital tubercle develops into the penis; the urogenital sinus forms the urethra, the prostate gland and the bulbourethral glands; and the mesonephric duct is converted into the epididymis, vas deferens, ampulla and seminal vesicles. When considering the pathogenesis of developmental and congenital abnormalities of the male reproductive tract it is important to understand that both testicular descent and the formation of the internal and external genitalia involve multistep developmental processes influenced by many factors, including specific genetic factors (Klonisch *et al.,* 2004), and environmental factors. In humans increased incidence of abnormalities such as delayed preputial separation, hypospadias, cryptorchidism and reduced semen quality (WHO, 2012) have been speculated to be associated with *in-utero* exposure to endocrine-disrupting compounds, however the impact of these compounds on development of the reproductive system of bulls has not been determined. In sheep there is experimental evidence demonstrating that the reproductive axis of male lambs born to DES and testosterone treated pregnant ewes is adversely affected with subsequent reduced testicular development and semen quality in mature rams (Recabarren *et al.,* 2008; Rojas-Garcia *et al.,* 2013).

Congenital disorders of development of the reproductive tract in the bull are well described, particularly disorders related to testicular development and descent, and for structures originating from the mesonephric ducts and genital tubercle (Barth, 2013). Many of the described congenital disorders are considered to be heritable, however neither the causal mutation(s) nor the molecular etiology of these phenotypes have been definitively identified. However, ongoing advances in genomics are likely to significantly improve our understanding of the underlying cause of these abnormalities (Han and Peñagaricano, 2016).

By 100 to 120 days of gestation, the testes of the bovine fetus have passed through the inguinal canal and entered the scrotum, which is derived from the urogenital folds. This is early in comparison with other domestic species and is preceded by an earlier maturing HPG axis in this species. There are two critical phases in descent of the testes, the transabdominal and inguinoscrotal phase. These phases are essential to move the testes into the scrotum (Klonisch *et al*., 2004). In the bovine, testicular descent begins relative early in gestation with the transabdominal phase beginning around days 80 to 90 and the inguinoscrotal phase around day 112. Insl3 produced by the fetal Leydig cells mediates

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transabdominal descent, and secreted androgens mediate the inguinoscrotal descent. Both Insl3 and testosterone are necessary for normal development and reorganization of the gubernaculum during the inguinoscrotal descent. In cattle plasma concentrations of Insl3 and testosterone at 4 to 8 months of gestation have been shown to be significantly higher in dam's carrying a male fetus compared with a female fetus. In the bovine fetus plasma testosterone peaks at day 125. Measurement of these hormones have been used for mid-gestation determination of the sex of the fetus (Kibushi *et al*., 2016).

The descent of the testes into the scrotal sac is a complex multifactorial process, and a variety of environmental and genetic factors have been shown to affect the process. Although mutations in the *Insl3* gene or LGR8/GREAT, acting as ligand and receptor respectively, have been found to be associated with cryptorchidism in humans (Foresta and Ferlin, 2004), similar mutations have not been identified in cattle. In cattle the prevalence of cryptorchidism has been reported to be 0.17% (St.Jean *et al*., 1992) with left-sided retention occurring twice as frequently as right-sided retention. Interestingly in the bull inguinal hernia occurs most frequently on the left side (Foster 2016). There may be a heritable predisposition to cryptorchidism in some breeds such as Polled Herefords and Shorthorns (St.Jean *et al*., 1992).

Differentiation of the external genitalia commences around day 60 in the bovine fetus under the influence of DHT. The glans penis originates from the apex of the genital tuberculum and a cord of epithelia cells moves into the genital tubercles to fuse with the urethral groove. The cord forms the distal part of the penile urethra (Hyttel *et al.,* 2009). Hypospadias is an abnormal opening of the urethra due to failure or incomplete closure of the embryonic urethral groove. It is often considered a mild form of psedo-hermaphrodism reported to result from an inadequate response of the distal urethral fold to DHT. In cattle, the reported prevalence of hypospadias is very low, around 0.3% (Saunders and Ladds 1978), and in some cases is accompanied by other abnormalities of the reproductive tract such as penile aplasia and cryptorchidism. Familial clustering has been reported (Kumi-Diaka and Osori, 1979) indicating a potential genetic etiology.

A number of congenital penile anomalies have been described in the bull including hypoplasia of the penis, diphallus and premature spiral deviation of the penis (Walker, 1964; Foster, 2016). In bulls penile hypoplasia is often described as *congenital short penis*, and in some cases may be due to congenital shortening of the retractor penis muscle. This results is penile protrusion being restricted to <25 cm from the penile tip to preputial orifice (Gilbert, 1989). Barth (2013) estimates the annual incidence of this abnormality to be 0.0002%, and cases have been diagnosed in both *Bos taurus* and *Bos indicus* genotypes (Gilbert, 1989). Also described in bulls is partial or complete absence of the sigmoid flexure of the penis (Foster, 2016). Premature spiral deviation of the penis is the most commonly diagnosed penile deviation and in most cases is thought to be due to abnormal development of the dorsal apical ligament of the penis predisposing to progressive post-pubertal degeneration of the ligament (Ashdown, 2006). It has been reported in most breeds and there is a higher prevalence in polled than horned bulls (Ashdown and Pearson, 1973), however this is unlikely to be linked to the polled gene. The observed prevalence of premature spiral deviation of the penis in a population of British breed bulls was 16% for polled bulls compared with 1% in horned bulls (Blockey and Taylor, 1984). In a later study (Norman *et al*., 2008) involving both *B. taurus* and *B. indicus* genotypes, approximately twice as many cases of premature spiral deviation of the penis were observed in polled-breed bulls (13.5%) than in horned-breed bulls (5.6%). Although Blockey and Taylor (1984) concluded from their pedigree analysis that the condition was likely to be heritable, Norman *et al*. (2008) concluded that it was not associated with the polled gene *per se*. The major problem with this abnormality is that expression is often delayed until bulls are 3 to 6 years of age and it can only be conclusively diagnosed by observing a bull attempting to serve multiple times (Norman *et al*., 2008).

Another common abnormality of the penis and prepuce is persistent frenulum which results when there is incomplete breakdown of the preputial attachment to the glans penis around the time of puberty. Throughout gestation the penis is attached to the penile prepuce by a lamella of ectodermal cells and a frenulum of connective tissue. The preputial cavity subsequently develops as the ectodermal lamella keratinizes and splits into two epithelial surfaces. This keratinization commences at the tip of the penis in the calf shortly after birth (around 4 weeks), but protrusion of the penis does not occur until just before puberty. Studies have indicated that keratinization of the ectodermal lamella is controlled by androgens, which may be the case during both fetal and prepubertal development (Ashdown, 1960). The reported prevalence in both *B. taurus* and *B. indicus* genotypes is 0.5%, however the author (M. M.) has frequently observed 2% to 4% affected bulls in large groups of 1- to 2-year-old bulls. It is considered a heritable abnormality but the mode of inheritance has not been determined (Barth, 2013).

Differentiation of the seminal vesicles commences around days 56 to 58 as small out-growths of the posterior mesonephric ducts and after day 70 these simple diverticula become branched and grow rapidly. On day 110 the seminal vesicles are ~7 mm in length and at the same time the epididymis, begins to form when the mesonephric duct lengthens and coils forming the three distinct epididymal regions (*caput*, *corpus* and *cauda*) (Alkafafy and Sinowatz, 2012). Hypoplasia of the epididymis and/or the accessory sex glands has been described in bulls (Williams *et al.,* 2010; Foster, 2016). Segmental aplasia or hypoplasia of the mesonephric duct is a sporadically reported defect. The condition is characterized by partial or complete absence of structures derived from the mesonephric duct, including the epididymis, ductus deferens, ampullae and seminal vesicles (Foster 2016). Both uni- and bilateral aplasia of the mesonephric duct has been described in the bull

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(Saunders and Ladds, 1978; Campero *et al*., 1989). These abnormalities are considered to be heritable but the mode of inheritance is poorly understood (Saunders and Ladds, 1978). A pedigree analysis of 18 Simmental bulls with segmental aplasia of the epididymis indicated an autosomal recessive mechanism as the mode of inheritance (Konig *et al*., 1972). Spermatic granuloma of the head of the epididymis is related to failure of one or more of the efferent ductules to join with the head of the epididymis (Foster, 2016). This condition will in most cases only become apparent after puberty. The prostate and bulbourethral glands arise from endodermal epithelial buds from the middle or pelvic part of the urogenital sinus. Congenital abnormalities of the prostate gland and bulbourethral glands are very uncommon in the bull (Campero *et al*., 1989; Foster, 2016).

In cattle, most heifers born as co-twins with males exhibit the intersexual syndrome commonly known as free-martinism. The effects on the male born co-twin to a freemartin is however less evident. Chimerism is readily detected in the male, but the effects on the reproductive system have been much debated (Long, 1979). Spermato-gonial chimerism was demonstrated in three bulls born as freemartins (Rejduch *et al*., 2000). In this study a low number of spermatogonia (10%) were shown to be carrying XX chromosomes, which could affect the sex ratio of offspring sired by these bulls. The fertility of bulls born as a co-twin to a freemartin varies with some being normally fertile whilst others are subfertile or infertile due to reduced percentages of motile and morphologically normal sperm (Padula, 2005)). In a study of 22 bulls born co-twin to freemartins, and with evidence of chimerism, a higher proportion (58%) of co-twin bulls were culled because of poor fertility than normal controls (5%; Dunn *et al*., 1979). However, Long (1979) did not detect any difference in fertility between chimeric and non-chimeric bulls.

Postnatal development of the reproductive system

Rawlings *et al*. (2008) have provided an excellent review of studies describing postnatal development of the reproductive system of bulls. However, it should be noted that most of the reported studies involved *B. taurus* breed cattle managed on a moderate to high plane of nutrition from birth to sexual maturation (average daily live weight gain of ~1 kg/day). This contrasts with the situation commonly encountered in tropical rangelands where *B. indicus* genotypes predominate, and very marked seasonal variation in rainfall restricts post-natal average daily live weight gain to only 0.3 to 0.4 kg/day. The primary impact of low prepubertal growth rate is delayed onset in puberty and slower rate of testicular development. Age guidelines for the occurrence of critical events such as puberty should always be interpreted with body weight (BW) and growth rate data. McGowan *et al*. (2012) demonstrated that testicular development (as defined by measurement of scrotal circumference) in a population of young tropically adapted bulls was better described when weight rather than age was used in a standard non-linear model.

Using Rawlings *et al*. (2008) review of development of the reproductive system of the bull three periods of development should be considered, prepubertal, peripubertal and post pubertal.

Prepubertal development

This is the period from birth through to the onset of rapid increase in testicular size at ~ 6 months of age. At birth the testes of the bull calf are fully descended and are primarily composed of lumenless chords of primordial germ cells, fetal Leydig cells and undifferentiated Sertoli cells (Wrobel, 1990; Rawlings *et al*., 2008). The penis is firmly attached to the prepuce and although present the accessory sex glands are essentially non-functional. Interestingly, the seminal vesicular glands begin to rapidly increase in size several months after birth (Chandolia *et al*., 1997) well before the period of rapid growth of the testes.

However, commencing about a month after birth the fetal Leydig cells degenerate and numbers of adult Leydig cells and undifferentiated Sertoli cells begin to increase rapidly (Sinowatz and Amselgruber, 1986; Wrobel 1990). This critical growth and differentiation of cells begins around the time of the early sustained postnatal increase in lutenising hormone (LH) secretion which is driven by an increase in the frequency of pulses of GnRH (Rawlings *et al*., 2008). Although serum concentrations of LH remain elevated for several months serum concentrations of testosterone are low (Evans *et al*., 1996). Rawlings *et al*. (2008) concluded that the duration of increased LH secretion is controlled by negative enhanced feedback suppression of gonadotropin secretion by testes-derived androgens and oestradiol, but changes in central opiodergic tone may also play a role in regulating GnRH secretion. Both serum concentrations of follicle stimulating hormone (FSH) and inhibin are also high during the prepubertal period but decline around the time of onset of rapid growth of the testes (Miyamoto *et al*., 1989; Evans *et al*., 1996). Although the role of the prepubertal increase in LH in cueing the onset of puberty is clear the role of FSH is unclear. Evans *et al*. (1995) found no differences in the prepubertal FSH concentrations of calves which had a mean difference in onset of puberty of 6 weeks. However, there is some evidence (Bagu *et al*., 2004) that FSH secretion is a key driver of prepubertal proliferation of Sertoli cells, which in turn is a critical determinant of daily sperm production in the bull (Berndtson *et al*., 1987). The role of inhibin in regulating the pattern of secretion of FSH is unclear (Rawlings *et al*., 2008).

Pre-spermatogonia begin to proliferate and some sper-matogonia appear about a month after birth with primary spermatocytes appearing at about 5 months of age (Curtis and Amann, 1981). However, spermatogenesis only really begins to progress rapidly at the end of the early postnatal increase in LH secretion, and as serum concentrations of FSH and inhibin decrease markedly (Rawling *et al*., 2008).

The magnitude of LH secretion between 1 to 5 months after birth in bull calves directly affects the pattern of growth and differentiation of the testes and thus age of onset of puberty. Evans *et al*. (1995) demonstrated that the

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prepubertal increase in LH secretion was greater in bull calves that had an early onset of puberty compared with those that had a later onset. This difference maybe under significant genetic control as evidenced by the moderate heritability (0.3 to 0.5) of GnRH induced LH secretion at 4 months of age in *B. indicus* (Brahman) and *B. indicus* cross bull calves (Corbet *et al*., 2013). The high heritability (0.7) of serum concentrations of inhibin at 4 months of age in both genotypes in this study requires further investigation to determine its significance.

There has been considerable interest not only in the AI industry, but also amongst seedstock producers selling yearling bulls, in developing to advance the onset of puberty. Feeding high energy and protein diets to young beef and dairy calves to achieve average daily weight gains (ADG) of ~1.4 to 1.5 kg/day through to 16 months of age has been shown to significantly reduce age of onset of puberty, increase paired testes weight and increase total daily sperm production without any adverse effects on semen quality (Brito *et al*., 2007; Dance *et al*., 2016). These impacts are driven by enhanced secretion of GnRH and LH during the period of early postnatal increase in LH, and are directly influenced by increased concentrations of circulating insulin and in particular IGF-1. Although there is a strong association between increased plane of nutrition and increased concentrations of IGF-1, it is also important to recognize that IGF-1 secretion is under significant genetic control as reported by Corbet *et al*. (2013). Thus, the response to nutritional manipulation is likely to be significantly influenced by genetics, and may explain why Harstine *et al*. (2015) observed that Holstein bull calves which managed to achieve ADG of 1.5 kg/day did not have an earlier onset of puberty than those fed to grow at 0.75 kg/day, despite significant differences in testes size.

Peripubertal development

This is the period encompassing the rapid almost linear growth of the testes and epididymides, including establishment of a lumen in each seminiferous tubule (Evans *et al*., 1996), through to puberty. It is interesting to note that Wolf *et al*. (1965) observed that protrusion of the penis with complete separation from the prepuce precedes the onset of puberty by ~ 1.5 months. The continuing rapid increase in adult Leydig cells (Wrobel, 1990) and low frequency pulses of LH, result in a rapid increase in serum concentrations of testosterone which drives this rapid growth of the testes (Rawlings *et al*., 2008) and spermatogenesis. However, the trajectory of growth of the testes between about 6 to 12 months varies considerably between bulls which adversely affects the accuracy of selection of bulls at an early age which will have small testes at 18 to 24 months of age (Barth, 2013). Spermatogenesis is also supported by the differentiation of Sertoli cells which occurs between about the 4th and 10th month after birth (Abdel-Raouf, 1960; Curtis and Amann, 1981). Primary then secondary spermatocytes are detected between the 5th and 8th month after birth, and between the 8th and 10th month mature spermatozoa are present in the lumen of the seminiferous tubules (Curtis and Amann, 1981; Evans *et al*., 1996). Also during this period the seminal vesicular glands become functional with amounts of fructose and citric acid increasing markedly after about the 5th to 6th month (Abdel-Raouf, 1960).

Wolf *et al*. (1965) has defined puberty in the bull as the age at which an ejaculate containing a minimum of 50×10^6 total sperm with at least 10% showing progressive motility is first collected. These authors have also proposed that a scrotal circumference (SC) of $\geq$28 cm is indicative that a bull has reached puberty. However, in bulls managed on a low plane of nutrition post weaning a more appropriate SC threshold is $\geq$26 cm. In a study of tropically adapted bulls (Chase *et al*., 2001) mean SC when an ejaculate containing 50×10^6 sperm was collected varied by only 1 cm (27 to 28 cm) between genotypes and between years, but mean age and BW when this was achieved varied by ~2months and 100 kg, respectively. Furthermore, mean SC when spermatozoa were first detected in an ejaculate were 2 to 3 cm less than mean SC at puberty using Wolf *et al*. (1965) definition. Overall, for breeds of cattle which have had sustained genetic selection pressure applied for fertility and where young bulls are managed on a moderate to high plane of nutrition (i.e. have achieved ADG since birth of ~1 kg/day), puberty typically occurs at 8 to 12 months of age (Barth, 2013). However, for breeds where there has been little selection pressure for fertility, for example many *B. indicus* genotypes, and particularly where these young bulls are reared on a low plane of nutrition, puberty typically occurs between 15 and 17 months and there is large variation among individual bulls in timing of onset of puberty (Holroyd *et al*., 2005).

Post-pubertal development

This is the period during which maturation of spermatogenesis is completed, testicular growth begins to plateau and bulls develop normal sexual behavior. Also maturational changes in the accessory sex glands and their secretions occur, and it is also likely that the profile of seminal plasma proteins changes during this period. In this period the majority (about 70%) of bulls transition from being markedly subfertile to attaining normal fertility. This is primarily because the ejaculates of pubertal bulls contain a high proportion of morphologically abnormal sperm and sexual behavior is a 'learned' phenomena. The interval from onset of puberty to when bulls are producing an ejaculate containing at least 70% normal sperm has been estimated to be 3 to 4 months in *B. taurus* breed bulls (Lunstra and Echternkamp, 1982) but varies considerably between bulls. Analysis of the findings of beef bull breeding soundness examination in Western Canada have demonstrated that whilst only 45% of 12 month old bulls produced an ejaculate containing >70% normal sperm this had increased to 75% for 14 months old bulls (Barth, 2013). Holroyd *et al*. (2005) reported that the mean percent normal sperm for *B. indicus* bulls (Brahman) aged 14 months was 42%, and this increased to 67% when they reached 16 months of age. Also

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during the post-pubertal period Price and Wallach (1991) and Holroyd *et al*. (2005) both observed marked increase in the expression of normal sexual behavior by bulls exposed to either restrained or unrestrained female cattle in a yard test.

Conclusions

Despite significant advances in our understanding of the genetic control of prenatal and postnatal development of the male reproductive system, the genomic basis for many suspected heritable abnormalities has not been determined. This is important because for many of the abnormalities of the reproductive system of bulls clinical signs are only expressed after puberty (e.g. unilateral testicular hypoplasia), they may spontaneously resolve (e.g. rupture of a persistent frenulum during first mating), or they can only be detected by observing the bull attempting to serve repeatedly (e.g. premature spiral deviation of the penis). Furthermore, in contrast to sheep, in cattle we still have only a limited understanding of the impact of environmental factors on both *in-utero* and longer term development and function. Finally, the major area of deficiency in our understanding of development of the reproductive system of the bulls is development and endocrine control of sexual behavior.

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Declaration of interest

The authors declare that there is no conflict of interest in this review.

Ethics statement

No original data are presented in this review and hence no ethics approval was sought or required.

Software and data repository resources

No software or data were deposited anywhere as part of this review.

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