Postnatal changes in testicular gonadotropin receptors, serum gonadotropin, and testosterone concentrations and functional development of the testes in bulls

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Abstract

The primary objectives of this study were to follow the temporal patterns of testicular LH and FSH receptor (LH-R and FSH-R) concentrations and affinity (K_a) during sexual maturation in bulls and to see if such patterns could help explain the control of rapid testicular growth that occurs after 25 weeks of age, when serum gonadotropin concentrations are low. Separate groups of Hereford×Charolais calves ($n=6$) were castrated every 4 weeks from 5 to 33 weeks of age and at 56 weeks of age. A week prior to castrations, from 5 to 33 weeks of age, blood was collected every 15 min for 10 h. The transition from indifferent supporting cells to Sertoli cells in seminiferous tubules was rapid between 13 and 25 weeks and rapid testis growth occurred after 25 weeks of age. Serum LH and FSH concentrations were transiently elevated at 12 weeks of age ($P<0.05$). LH-R concentrations decreased from 13 to 25 weeks of age and increased to 56 weeks of age ($P<0.05$). LH-R K_a decreased from 9 to 17 weeks of age, increased to 29 weeks of age and declined to 33 weeks of age ($P<0.05$). FSH-R concentrations declined from 17 to 25 weeks of age then increased to 56 weeks of age ($P<0.05$). FSH-R K_a increased from 17 to 25 weeks of age ($P<0.05$). High concentrations of gonadotropins and their receptors may be critical to initiate testis growth postnatally and support it after 25 weeks of age in the face of low serum gonadotropin concentrations.

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Introduction

In bulls, puberty has been defined as the time when an ejaculate has at least 5.0×10^7 sperm and a 10% linear motility (Wolf *et al.* 1965). Age at puberty varies with breed: in Hereford bulls it occurs between 39 and 52 weeks of age, in Charolais bulls between 33 and 53 weeks of age, and in Holstein bulls between 39 and 41 weeks of age (Wolf *et al.* 1965, Killian & Amann 1972, Lunstra *et al.* 1978). In bull calves, serum luteinizing hormone (LH) concentrations are transiently increased between 4 and 25 weeks of age, with minimal or no subsequent increase prior to puberty (McCarthy *et al.* 1979, Amann *et al.* 1986, Wise *et al.* 1987, Evans *et al.* 1993, 1996). In developing bulls, the patterns of serum FSH concentrations are variable; with reports of no change with time (McCarthy *et al.* 1979), a slight increase in the early postnatal period (Rawlings & Evans 1995, Aravindakshan *et al.* 2000), and an increase from 4 to 32 weeks of age (Amann & Walker 1983). During the early rise in serum LH concentrations, serum testosterone concentrations are low, increasing markedly

after 28 weeks of age and reaching adult concentrations by 40 weeks of age (Rawlings *et al.* 1972, 1978, Secchiari *et al.* 1976, Amann & Walker 1983).

In males, there are two distinct populations of Leydig cells: fetal and adult Leydig cells, which differentiate from mesenchymal cells prenatally and postnatally respectively (Mendis-Handagama & Ariyaratne 2001). In bull calves, it was suggested that the demise of fetal Leydig cells occurred during the early postnatal period (Hooker 1970). Amann (1983) suggested that the high frequency of LH pulses that occurred in bull calves after 4 weeks of age, initiated differentiation and maturation of adult Leydig cells from progenitor cells around 12–16 weeks of age, and a switch from androstenedione to testosterone secretion. The elevated intratesticular testosterone concentrations, following the onset of Leydig cell differentiation, may initiate differentiation of indifferent supporting cells to Sertoli cells; differentiation is restricted to the period of 16–28 weeks of age (McCarthy *et al.* 1979, Curtis & Amann 1981, Amann 1983). Subsequent increases in serum testosterone concentrations after

28 weeks of age probably reflect enhanced production by Leydig cells or increased Leydig cell numbers (Amann 1983). In bull calves, spermatogenesis is initiated as early as 16 weeks of age and the appearance of elongated spermatids in the seminiferous tubules at 32 weeks of age marks the initial achievement of complete spermatogenesis (Curtis & Amann 1981). Rapid testicular growth that occurs after 25 weeks of age is, therefore, a reflection of the above changes. It is unclear whether the transient early postnatal increase in serum LH concentrations is sufficient to trigger rapid testicular development or how important the subsequent low levels of LH and FSH are. There could also be important regulatory changes in the testis itself from 25 weeks of age to puberty that are critical for rapid testicular growth (Amann *et al.* 1986, Wise *et al.* 1987, Evans *et al.* 1993, 1996, Rawlings & Evans 1995).

During sexual development, an increase in testicular gonadotropin receptor (LH-R) concentrations was reported in rats (Purvis *et al.* 1977, Hardy *et al.* 1990, Shan & Hardy 1992) and dogs (Inaba *et al.* 1994). In the latter, the increase coincided with rapid testicular growth. In rams, testicular LH-R concentrations increase from 90 to 150 days of age (Yarney & Sanford 1989). In the two studies with bulls, testes were either collected from one separate individual Norwegian red calf every 4 weeks from 12 to 32 weeks of age, and at 40, 52, and 68 weeks of age (Sundby *et al.* 1984) or from Holstein calves at 1, 7, 14, and 56 days of age ($n=5$); 12 weeks of age ($n=4$) and 24 weeks of age ($n=3$); however, no samples were collected during the period of rapid testicular growth or just prior to puberty (Dias & Reeves 1982). In these studies, gonadotropin receptor concentrations decreased with age, while binding affinities remained the same. Sample size and timing precluded a careful examination of gonadotropin receptor changes around the time of the early postnatal increase in serum gonadotropin concentrations or the period of rapid testicular growth.

The objectives of the present study were to determine if changes in gonadotropin receptor concentrations and affinity were related to the early postnatal increase in serum LH and FSH concentrations, differentiation of Sertoli cells, the onset and progression of spermatogenesis, and the period of rapid testicular growth, which occurs while serum LH and FSH concentrations are low.

Materials and Methods

Animals and experimental procedures

Fifty-four spring-born, age matched (± 3 days) bull calves (Hereford $\times$ Charolais) were divided into nine groups of six calves each. Calves were suckled at pasture until they were weaned at 26 weeks of age. After weaning, calves were kept in corrals and provided with water and a standard feed ration *ad libitum* (Evans *et al.* 1995). Calf body weights were measured bi-weekly until castration. All experimental procedures were done in

accordance with the regulations of the Canadian Council for Animal Care.

Blood and testes collection

Separate groups of calves ($n=6$) were castrated every 4 weeks from 5 to 33 weeks of age, and at 56 weeks of age. A week prior to castration, from 5 to 33 weeks of age, blood samples (5 ml) were collected every 15 min for 10 h from the jugular vein by an i.v. catheter, starting at 0800 h (Evans *et al.* 1995). Blood samples were left to clot for at least 12 h at room temperature and then serum was harvested and frozen at -20°C until assayed. At castration, the testes were weighed and a tissue sample from the mid section of the right testis of each bull was fixed in Helly's reagent (HgCl_2 (70 g/l) and $\text{K}_2\text{Cr}_2\text{O}_7$ (25 g/l) (VWR International Ltd, Edmonton, Alta, Canada) were dissolved in warm distilled water and formaldehyde (40%) was then added immediately prior to fixing the testis (VWR International Ltd)). The rest of the right and left testes were plunged into liquid nitrogen for approximately 20 min and stored at -70°C until homogenization.

RIAs

Serum LH and FSH concentrations were determined using previously validated double-antibody RIAs (Rawlings & Evans 1995). The LH concentrations are expressed in terms of NIDDK-bLH4. Sensitivity of the LH assay, defined as the lowest concentration of LH capable of significantly displacing labeled LH from the antibody (*t*-test), was 0.1 ng/ml. Intra- or interassay coefficients of variation (CV values) for the LH assay were 7.1 and 8.6% or 12.7 and 11.9% for reference sera with LH concentrations of 0.4 or 0.8 ng/ml respectively. The sensitivity of the FSH assay was 0.1 ng/ml and concentrations are expressed in terms of USDA-bFSH-11. Intra- or interassay CV values for the FSH assay were 6.4 and 5.7% or 17.5 and 18.8% for reference sera with FSH concentrations of 1.9 or 6.8 ng/ml respectively. Serum testosterone concentrations were determined in a single pool of samples collected from each intensive bleed using a commercial, double-antibody RIA kit (Coat A-Count total testosterone; Diagnostics Product Corporation, Los Angeles, CA, USA). The testosterone assay sensitivity was 0.04 ng/ml. The intraassay CV values were 19.7, 12.7, and 6.6% for reference sera with testosterone concentrations of 1.2, 2.7, and 7.0 ng/ml respectively.

Bovine testicular LH and FSH receptor membrane fractions

A frozen section (6 g) from the right testis of each of the bull calves castrated at 4 weeks intervals from 5 to 33 weeks of age and at 56 weeks of age ($n=6$ per age

group), was homogenized to obtain membrane fractions using a published procedure (Sairam 1978, Yarney & Sairam 1991). The testicular parenchyma was weighed, diced, and homogenized at maximum speed for 2.5 min, using a Brinkmann polytron (PT 10203500, Steinhof-halde 22, Switzerland) in 25 mM Tris-HCl buffer, containing 100 mM sucrose (pH 7.5). Five milliliters of buffer were used per gram of tissue. All procedures, unless otherwise indicated, were carried out at 4 °C. The homogenate was centrifuged at 500 *g* for 30 min and the supernatant decanted into a fresh polystyrene tube and recentrifuged at 39 000 *g* for 1 h. The pellet was resuspended in 25 mM Tris-HCl buffer (pH 7.5), containing 10 mM MgCl₂, at a concentration of 1 g original tissue per milliliter. Aliquots of these fractions were stored at -70 °C. Prior to assay, the membrane fractions were gently pulsed to obtain a uniform suspension. The protein concentration in the testicular membrane fractions was determined by a Bio-Rad Assay (Bio-Rad) using BSA as a standard.

Radio-receptor assay

The radio-receptor assay was a modification of published methods (Sairam 1978, Yarney *et al.* 1988, Yarney & Sairam 1991). Bovine LH (USDA-bLH-I1) or FSH (NIDDK-AFP5332B) was iodinated (¹²⁵I) (Amersham Biosciences) using published methods (Sairam 1978, Yarney & Sanford 1989). When 730 ng ¹²⁵I-bLH and 491 ng ¹²⁵I-bFSH were incubated with an excess of bovine testicular membrane preparations total binding ranged from 12 to 13.7% and 17 to 19% respectively. The specific activity of ¹²⁵I-bLH or ¹²⁵I-bFSH was 0.162 or 0.192 mCi/μg respectively. To determine the affinity constant (*K_a*) and receptor concentrations by Scatchard analysis, three serial dilutions of 120 ± 2, 243 ± 3, and 364 ± 4 CPM/μl of ¹²⁵I-bLH (USDA-bLH-I1) and 99 ± 2, 194 ± 1, and 291 ± 2 CPM/μl of ¹²⁵I-bFSH (NIDDK-AFP5332B) were used in the assay with each testicular homogenate. All incubations for the radio-receptor assays were done in triplicate in borosilicate glass test tubes (12 × 75 mm; VWR, West Chester, PA 19380, USA). To determine the total binding, 300 μl of ¹²⁵I bLH or ¹²⁵I bFSH was added to 100 μl of testicular membrane fractions, the volume was made-up to 500 μl with 100 μl buffer (25 mM Tris-HCl buffer (pH 7.5), containing 10 mM MgCl₂ and 0.1% BSA). In order to determine the non-specific binding (NSB) for each of the testicular membrane fractions, 100 μl buffer containing 1500 ng non-radiolabeled bLH or bFSH was added. Specific binding was defined as the difference between the total binding and NSB and was approximately 37 ± 3 and 61 ± 1% of total binding for LH and FSH respectively. The assays were incubated 24 h at 4 °C. Incubation was terminated by adding 2.5 ml of 25 mM Tris-HCl buffer (pH 7.5, containing 10 mM MgCl₂, 0.1% BSA and 8% polyethylene glycol) and test tubes were immediately

centrifuged at 4000 *g* for 25 min in a Beckman JS-5.2 Rotor (Beckman Instruments, Inc., Palo Alto, CA, USA). The supernatant was decanted and the radioactivity in the pellet determined using a gamma counter (efficiency 80%; Apex gamma counter, Titertek Instruments, Inc., Huntsville, AL, USA). Affinity constants (*K_a*) and binding capacities were determined by analyzing the binding data using a non-linear regression analysis or Scatchard analysis (Sigma Stat for Windows, version 1.0; Jadel Corporation, San Rafael, CA, USA) similar to Munson & Rodbard (1980). After the ratio of receptor bound radiolabeled hormone to free radiolabeled hormone (B/F) was plotted against the receptor bound radiolabeled hormone (B), a non-linear curve was fitted to determine the slope or the binding affinity (*K_a*) and x-intercept (binding capacity). The receptor concentrations were then expressed as moles per milligram of protein (specific binding capacity). The interassay CV for the binding capacity and *K_a* were determined by replicating pooled testicular membrane fractions collected at 56 weeks of age, in all assays, as an internal control. The interassay CV for LH-R with a mean specific binding capacity and *K_a* of 4.8 ± 0.5 pM/mg protein and 1.82 ± 0.1 × 10⁻¹⁰ M⁻¹ were 16.2 and 13.5% respectively. The interassay CV for FSH-R with a mean specific binding capacity and *K_a* of 9.8 ± 0.7 pM/mg protein and 2.0 ± 0.1 × 10⁻¹⁰ M⁻¹, were 12.8 and 7.4% respectively.

In the bovine LH-R assay, 21 or 45% displacement of ¹²⁵I-bLH was caused by 10 or 100 ng of bLH per tube (USDA-bLH-I1) respectively. No displacement was seen with 100 ng of either bovine prolactin (AFP 4835b) or bovine growth hormone (bGH AFP 11182B); however, 100 ng/tube of bovine FSH (USDA-bFSH-I-1) and bovine TSH (NIADDK bTSH I3) caused 14 and 16% displacement respectively. In the bovine FSH-R assay, 30 or 51% displacement of ¹²⁵I bFSH was caused by 10 or 100 ng of bFSH/tube respectively (USDA-bFSH-I-1). No displacement was seen with 100 ng of either bovine prolactin (AFP 4835b) or bovine growth hormone (bGH AFP 11182B); however, 100 ng/tube of bovine LH (USDA-bLH-I1) and bovine TSH (NIADDK bTSH I3) caused 24 and 23% displacement respectively.

Histology

Sections of testicular parenchyma were stored in Helly's reagent for 24 h, rinsed in water for 48 h, washed in 70% ethanol, then dehydrated in alcohol and embedded in paraffin wax. A 5 μm thick section from each of the bull calves castrated at 4 week intervals from 5 to 33 weeks of age and at 56 weeks of age (*n*=6 per age group) was stained with hematoxylin and periodic acid Schiff's (H-PAS) and evaluated for development of spermatogenesis. A systematic, uniform, random sampling technique as described by Curtis & Amann (1981) and Evans *et al.* (1995), was used to select 20 round seminiferous tubules

in the section from each bull. In each seminiferous tubule, at 1000 $\times$ magnification, Sertoli cells and their progenitor indifferent supporting cells were enumerated and the most mature germ cell identified; the tubule was classified according to the most mature germ cell in it. This classification was based on a scheme of seven groups of germ cell types: (1) gonocytes, (2) pre-spermatogonia, (3) A- and B-spermatogonia, (4) primary spermatocytes, (5) secondary spermatocytes, (6) round spermatids, and (7) elongating spermatids. The percentage of tubules with a particular germ cell type as the most mature germ cell was determined by multiplying the number of seminiferous tubules with a particular germ cell type as the most mature germ cell by 100% and then dividing the product by 20 (the total number of seminiferous tubules evaluated from the section of each bull). Sertoli cells and their precursor cells were only evaluated in sections collected from at 5, 13, 21, 25, 33, and 56 weeks of age. Testicular germ cells and somatic cells were identified according to published descriptions (Berndson & Desjardins 1974, Curtis & Amann 1981). All the histological evaluations were done by a single operator in a random order without knowledge of the age groups.

Data analysis

Serum LH and FSH secretory characteristics in blood samples collected every 15 min for 10 h were determined by the PC-pulsar program (J Gitzen and V Ramirez, University of Illinois, Urbana, IL, USA). Serum LH pulse frequency and amplitude as well as basal and mean serum concentrations are presented. The secretory patterns of FSH were judged to be non-pulsatile, therefore mean concentrations are presented. The LH pulses were defined using s.d. criteria of height (G values) and duration (Merriam & Wachter 1982). Data for LH pulse amplitude, LH pulse frequency, basal and mean LH concentrations, mean FSH concentrations, mean testosterone concentrations, testicular weight, testicular gonadotropin receptor concentration per milligram of protein, gonadotropin receptor K_a , the percentage distribution of the most mature germ cell in each of the 20 seminiferous tubules, indifferent supporting cell counts per seminiferous tubule, Sertoli cell counts per seminiferous tubule, and body weight were analyzed for effects of age by one-way ANOVA (one-ANOVA, Sigma Stat for Windows, version 1.0; Jadel Corporation, San Rafael, CA, USA). If main effects were significant, paired comparisons were made using the Fisher's least significant difference method for postANOVA multiple comparisons ($P < 0.05$). Pearson correlations were used to examine the relationships between mean serum LH concentrations, serum LH pulse frequency, mean serum FSH concentrations, mean serum testosterone concentrations, testicular LH-R concentrations, LH-R affinity constant, testicular FSH-R concentrations, testicular FSH-R affinity constant,

testicular weight, indifferent supporting cell counts per seminiferous tubule, and Sertoli cell counts per seminiferous tubule.

Results

Body weights and testicular growth

Mean body weight increased from 5 to 21 weeks of age and from 25 to 29 weeks of age then subsequently from 33 to 56 weeks of age ($P < 0.05$; Fig. 1). Mean testicular weight increased from 9 to 21 weeks of age and then from 25 to 56 weeks of age ($P < 0.01$; Fig. 1).

Hormonal patterns

Mean serum LH concentrations increased from 4 to 12 weeks of age then declined to 20 weeks of age ($P < 0.05$; Fig. 2a). Basal serum LH concentrations increased from 8 to 12 weeks of age then declined to 16 weeks of age ($P < 0.05$; Fig. 2b). The LH pulse frequency increased from 4 to 12 weeks of age then declined to 16 weeks of age ($P < 0.05$; Fig. 2c). The LH pulse amplitude declined from 24 to 28 weeks of age ($P < 0.05$; Fig. 2d). Mean serum FSH concentrations declined from 12 to 20 weeks of age ($P < 0.05$; Fig. 3). Mean serum testosterone concentrations increased from 8 to 20 weeks of age, declined to 28 weeks of age and then subsequently increased from 28 to 32 weeks of age ($P < 0.01$; Fig. 4).

Testicular gonadotropin receptor patterns

The mean testicular LH-R concentrations (picomolar per milligram of protein) decreased from 13 to 25 weeks of age and then increased to 56 weeks of age ($P < 0.05$; Fig. 5a). Mean testicular LH-R concentrations (picomolar per milligram of protein) were positively correlated with LH pulse frequency and mean serum concentrations of LH and FSH ($P < 0.05$; r -values of 0.47, 0.51, and 0.51 respectively). Testicular LH-R K_a decreased from 9 to 17 weeks of age, increased to 29 weeks of age and then declined to 33 weeks of age ($P < 0.05$; Fig. 5b). Testicular LH-R K_a was negatively correlated with mean serum LH concentrations, LH pulse amplitude, and mean testicular LH-R concentrations (picomolar/milligram of protein; $P < 0.05$; r -values -0.32 , -0.32 , and -0.31 respectively). The mean testicular FSH-R concentrations (picomolar/milligram of protein) declined from 17 to 25 weeks of age then increased to 56 weeks of age ($P < 0.05$; Fig. 6a). Mean testicular FSH-R concentrations (picomolar/milligram of protein) were positively correlated with mean testicular LH-R concentrations (picomolar/milligram of protein) and mean testicular weight ($P < 0.05$; r -values of 0.53 and 0.63 respectively). The testicular FSH-R K_a increased from 17 to 25 weeks of age ($P < 0.05$; Fig. 6b). Testicular FSH-R K_a was positively correlated with mean testicular weight ($P < 0.05$; r -value

Figure 1 Mean ($\pm$ S.E.M.) (a) body weight and (b) testicular weight of bull calves. Separate groups of bull calves ($n=6$) were weighed and then castrated at 4 weeks intervals from 5 to 33 weeks of age and at 56 weeks of age. The testes (right and left) were weighed at castration. The differences between ages within variables are indicated by different superscripts ($P<0.05$).

0.4); however, it was negatively correlated with mean testicular FSH-R concentrations (picomolar/milligram of protein), from 5 to 33 weeks of age ($P<0.01$; $r = -0.43$).

Sertoli cell development

The mean indifferent supporting cell (immature Sertoli cell) count per seminiferous tubule decreased from 13 to 33 weeks of age ($P<0.05$; Fig. 7). As the indifferent supporting cells differentiated into mature Sertoli cells, the mean Sertoli cell count per seminiferous tubule increased from 13 to 33 weeks of age ($P<0.05$; Fig. 7).

Germ cell development

The percentage of seminiferous tubules with gonocytes as the most mature germ cell type decreased from 5 to 13 weeks of age and from 17 to 21 weeks of age ($P<0.01$; Table 1). The percentage of seminiferous tubules with pre-spermatogonia as the most mature germ cell type decreased from 5 to 9 weeks of age and from 17 to 21 weeks of age ($P<0.01$). The percentage of seminiferous tubules with A- and B-spermatogonia as

Figure 2 Mean ($\pm$ S.E.M.) (a) serum LH concentrations, (b) basal LH concentrations, (c) LH pulse frequency, and (d) LH pulse amplitude in blood samples collected every 15 min for 10 h, at 4 weeks intervals from 4 to 32 weeks of age, from separate groups of calves ($n=6$). The differences between ages within variables are indicated by different superscripts ($P<0.05$).

the most mature germ cell type increased from 5 to 13 weeks of age and decreased from 13 to 25 weeks of age ($P<0.01$; Table 1). The percentage of seminiferous tubules with primary spermatocytes as the most mature germ cell type increased from 17 to 25 weeks of age and then decreased from 33 to 56 weeks of age ($P<0.01$; Table 1). The percentage of seminiferous tubules with secondary spermatocytes as the most mature germ cell type increased from 21 to 25 weeks of age ($P<0.01$; Table 1). The percentage of seminiferous tubules with round spermatids as the most mature germ cell type increased from 21 to 25 weeks of age and from 29 to 33 weeks of age, then subsequently decreased from 33 to 56 weeks of age ($P<0.01$; Table 1). The percentage of seminiferous tubules with elongated spermatids as the most mature germ cell type increased from 21 to 25 weeks of age and from 33 to 56 weeks of age ($P<0.01$; Table 1).

Figure 3 Mean ($\pm$ S.E.M.) serum FSH concentrations in blood samples collected every 15 min for 10 h, at 4 weeks intervals from 4 to 32 weeks of age from separate groups of calves ($n=6$). The differences between ages are indicated by different superscripts ($P<0.05$).

Discussion

In the present study, between 4 and 25 weeks of age, serum concentrations of LH and FSH were transiently elevated confirming previous reports (Wise *et al.* 1987, Evans *et al.* 1993, 1996, Aravindakshan *et al.* 2000). As was previously suggested, the early postnatal increase in LH secretion, noted in the present study, was caused by an increase in LH pulse frequency (McCarthy *et al.* 1979, Amann & Walker 1983, Evans *et al.* 1993, 1996). It is probable that the high frequency discharge of LH between 4 and 25 weeks of age influenced Leydig cell differentiation and maturation, and led to increased secretion of testosterone after 12 weeks of age (Rawlings *et al.* 1972, 1978, Secchiari *et al.* 1976, Amann 1983, Amann & Walker 1983, Rawlings & Cook 1986, Mendis-Handagama & Ariyaratne 2001). The marked increase in mean serum testosterone concentrations from 16 to 20 weeks of age, noted in the present study, probably terminated the early postnatal increase in

Figure 4 Mean ($\pm$ S.E.M.) serum testosterone concentrations in pooled blood samples collected every 15 min for 10 h, at 4 weeks intervals from 4 to 32 weeks of age from separate groups of calves ($n=6$). The differences between ages are indicated by different superscripts ($P<0.05$).

Figure 5 Mean ($\pm$ S.E.M.) (a) testicular luteinizing hormone receptor (LH-R) concentrations per milligram of protein; (b) testicular LH-R affinity (K_d) for samples collected from separate groups of bull calves ($n=6$) castrated at 4 weeks intervals from 5 to 33 weeks of age and at 56 weeks of age. The differences between ages within variables are indicated by different superscripts ($P<0.05$).

serum concentrations of LH and FSH (Rawlings *et al.* 1978, Evans *et al.* 1995).

In males, there are two distinct populations of Leydig cells: fetal and adult Leydig cells, which differentiate from mesenchymal cells prenatally and postnatally respectively (Mendis-Handagama & Ariyaratne 2001). In bull calves, it was suggested that the demise of fetal Leydig cells occurred during the early postnatal period (Hooker 1970). Differentiation and maturation of adult Leydig cells in bulls is initiated around weeks 12–16 and continues actively up to 28 weeks of age (Amann 1983). This phase of differentiation and maturation would appear to be reflected in the increased testosterone production from 8 to 20 weeks of age in the present study. Amann (1983) suggested that in bull calves the enhanced testosterone production after 28 weeks of age involved increased cellular synthesis or increased Leydig cell numbers. The peaks in testosterone production seen at 20 and 32 weeks of age in the present study, with the intervening trough, have previously been noted (Rawlings *et al.* 1972, 1978, Secchiari *et al.* 1976). Progenitor Leydig cells and Leydig cells resident in the testicular interstitium are the target cells for LH action (Shanbacher 1979, Mendis-Handagama & Ariyaratne

Figure 6 Mean ($\pm$ S.E.M.) (a) testicular follicle stimulating hormone receptor (FSH-R) concentration per milligram of protein; (b) testicular FSH-R affinity (K_a) for samples collected from separate groups of bull calves ($n=6$) castrated at 4 weeks interval from 5 to 33 weeks of age and at 56 weeks of age. The differences between ages within variables are indicated by different superscripts ($P < 0.05$).

2001). In the present study, the high concentrations of testicular LH-R in the early postnatal calves and the decline in concentrations from 13 to 21 weeks of age probably reflected high numbers of fetal Leydig cells and undifferentiated Leydig progenitor cells followed by a decline in numbers of both. It is interesting that as adult

Figure 7 Mean ($\pm$ S.E.M.) Sertoli cell numbers (open circles) and their progenitor indifferent supporting cells (solid circles) per section of seminiferous tubule. Twenty randomly selected tubules were examined from one testis per bull at 1000 $\times$ magnification. Counts were done for separate groups of bull calves ($n=6$) castrated at 5, 13, 21, 25, 33, and 56 weeks of age. The differences between ages within variables are indicated by different superscripts ($P < 0.05$).

Leydig cells differentiated and matured, as reflected in enhanced testosterone production from 8 to 20 weeks of age, testicular LH-R concentrations declined but LH-RK_a was maintained. However, the early postnatal increase in mean serum LH concentrations rising to a peak at 12 weeks of age is clearly positioned to initiate the differentiation and maturation of adult Leydig cells. At least LH drives the latter stages of Leydig cell differentiation in rats, mice, and humans (Mendis-Handagama & Ariyaratne 2001). It is likely that the increases seen in LH-R concentration in the testis beyond 25 weeks of age, in the present study, especially at 56 weeks of age, reflected further Leydig cell maturation or increased numbers of Leydig cells as suggested by Amann (1983). In the present study, the positive correlation of testicular LH-R concentrations with testicular FSH-R concentrations suggested FSH affected Leydig cell development, acting via Sertoli cells. Administration of FSH to *in vitro* co-cultured purified pig Leydig and Sertoli cells increased Leydig cell LH/human choriogonadotropin binding sites and their capacity to secrete testosterone (Tabone *et al.* 1984). In the present study, LH-R concentrations increased from 25 to 56 weeks of age along with the Leydig cell testosterone production.

In the present study, the high mean testicular FSH-R concentrations from 5 to 13 weeks of age were probably a result of the high number of indifferent supporting cells per seminiferous tubule (Curtis & Amann 1981). In bull calves, indifferent supporting cells and Sertoli cells resident in the seminiferous tubules are the target cells for FSH action (Shanbacher 1979). The drop in mean testicular FSH-R concentrations from 17 to 25 weeks of age accompanied the precipitous decline in numbers of indifferent supporting cells as they differentiated into mature Sertoli cells and may have also been influenced by the rapid onset of spermatogenesis, particularly increased spermatocytes. The latter would have effectively decreased FSH-R concentrations per mg of protein. The increase in testicular FSH-R concentrations from 25 to 56 weeks of age was probably due to maturation of the immature Sertoli cells. In bull calves, it was suggested that testosterone stimulates the differentiation of indifferent supporting cells (immature Sertoli cells) to mature Sertoli cells (Amann 1983).

In the present study, mean serum FSH concentrations and FSH-R concentrations were high when the seminiferous tubules were occupied primarily by immature germ cells (gonocytes and prespermatogonia). This may have facilitated the FSH-dependent proliferation and differentiation of the immature germ cells to primary spermatocytes (Means *et al.* 1976, Amann 1983, Jegou *et al.* 1983). In the present study, the percentage of seminiferous tubules with secondary spermatocytes as the most mature germ cell increased from 21 to 25 weeks of age and coincided with the period of low serum LH concentrations and testicular LH-R concentrations, from

Table 1 Mean ($\pm$ S.E.M.) percentage of seminiferous tubules with a particular germ cell type as the most mature germ cell (gonocytes, pre-spermatogonia, A- and B-spermatogonia, primary spermatocytes, secondary spermatocytes, round spermatids, and elongating spermatids) in 20 randomly selected round seminiferous tubules per histological section of testis samples collected from separate groups of bull calves ($n=6$) castrated at 4 weeks intervals from 5 to 33 weeks of age and at 56 weeks of age.

Age (weeks)	Gonocytes	Pre-spermatogonia	A- and B-spermatogonia	Primary spermatocytes	Secondary spermatocytes	Rounded spermatids	Elongated spermatids
5	41 $\pm$ 5 ^a	49 $\pm$ 4 ^a	10 $\pm$ 3 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a
9	31 $\pm$ 2 ^b	29 $\pm$ 5 ^b	36 $\pm$ 4 ^b	2 $\pm$ 1 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a
13	13 $\pm$ 4 ^{cd}	32 $\pm$ 4 ^b	51 $\pm$ 4 ^c	3 $\pm$ 3 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a
17	19 $\pm$ 6 ^d	27 $\pm$ 6 ^b	38 $\pm$ 9 ^{bc}	15 $\pm$ 6 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a
21	5 $\pm$ 3 ^{ce}	6 $\pm$ 2 ^c	35 $\pm$ 13 ^{bcd}	37 $\pm$ 15 ^{ab}	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a
25	2 $\pm$ 1 ^e	1 $\pm$ 1 ^c	18 $\pm$ 12 ^{ad}	40 $\pm$ 11 ^b	8 $\pm$ 4 ^{bc}	18 $\pm$ 8 ^b	14 $\pm$ 7 ^b
29	0 $\pm$ 0 ^e	0 $\pm$ 0 ^c	13 $\pm$ 6 ^a	50 $\pm$ 8 ^b	9 $\pm$ 3 ^{bc}	18 $\pm$ 7 ^b	9 $\pm$ 5 ^b
33	0 $\pm$ 0 ^e	0 $\pm$ 0 ^c	6 $\pm$ 6 ^a	44 $\pm$ 9 ^b	3 $\pm$ 2 ^{ab}	31 $\pm$ 7 ^c	17 $\pm$ 9 ^b
56	0 $\pm$ 0 ^e	0 $\pm$ 0 ^c	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	3 $\pm$ 3 ^{ab}	18 $\pm$ 3 ^b	76 $\pm$ 6 ^c

The differences between ages within columns are indicated by different superscripts a, b, and c ($P<0.05$).

20 to 33 weeks of age. In the present study, the LH-dependent release of testosterone, essential for the progression of primary spermatocytes to secondary spermatocytes, could have been facilitated by the increased LH-RK_a noted from 21 to 29 weeks of age (Purvis *et al.* 1977). In the present study, elongated and rounded spermatids were the most mature germ cells in the seminiferous tubules between 25 and 33 weeks of age, during the period of high serum testosterone concentrations. A similar association between stages VII and VIII of spermatogenesis and elevated serum testosterone was seen in rats (Pearson & Tubbes 1967). In the present study, during the period of increase in the percentage of seminiferous tubules with elongated spermatids as the most mature germ cell type, from 29 to 56 weeks of age, mean serum FSH concentrations were low but testicular FSH-RK_a and FSH-R concentrations were high. This implied that the increased Sertoli cell sensitivity to FSH ensured the progression and sustenance of spermatogenesis during low serum FSH concentrations (Means *et al.* 1976, Amann 1983, Jegou *et al.* 1983, Orth 1984).

In the present study, a rapid increase in testicular weight occurred after 25 weeks of age and coincided with increased serum testosterone concentrations, gonadotropin receptor concentrations and affinity. It is likely that testicular growth was initiated by the high postnatal serum gonadotropin concentrations and testicular gonadotropin receptor concentrations and was maintained by the increased sensitivity of the Sertoli and Leydig cells to low serum FSH and LH concentrations respectively as reported for the ram (Yarney & Sanford 1989) and rat (Dufau & Catt 1978). The increase in gonadotropin receptor concentrations from 25 to 56 weeks of age differs from previous reports in bull calves (Dias & Reeves 1982, Sundby *et al.* 1984); however, low sample number and lack of sampling during the period of rapid testicular growth and just prior to puberty could have confounded trends in those studies.

We concluded that high concentrations of serum gonadotropins and testicular gonadotropin receptors during the early postnatal period in bull calves may be critical to the initiation of rapid testis growth; however, increased gonadotropins receptor concentrations and affinity in the face of low circulating gonadotropin concentration and high testosterone concentrations may support rapid testicular growth after 25 weeks of age. The high testicular gonadotropin receptor concentration and affinity during low serum gonadotropin concentrations suggested a high Leydig and Sertoli cell sensitivity to low serum LH and FSH concentrations respectively.

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