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TESTICULAR DEVELOPMENT, HISTOLOGY, AND HORMONE PROFILES IN THREE YEARLING ANGUS BULLS WITH SPERMATOGENIC ARREST

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ABSTRACT

This article discusses the interactions between testis criteria and hormone profiles in Angus bulls with spermatogenic arrest. From 2 to 12 months (mo), testis diameter and hormone concentrations (basal and GnRH-stimulated) were evaluated in 27 bulls. At 12 mo, testes were excised. The z statistical test was used to determine whether parameters in three infertile bulls were different ($P < 0.05$) from those in 24 bulls with normal spermatogenesis. Bull 1 had Sertoli cell-only syndrome and Bull 2 had 90% of the tubules without germ cells and only A1 spermatogonia in the remaining. In Bull 3, germ cells did not advance beyond the primary spermatocyte stage. At 12 mo, testes of Bull 1 (99 g), Bull 2 (105 g) and Bull 3 (32 g) weighed less than those of normal bulls (251.5 ± 56 g). Sertoli cell numbers/testis in Bull 1 (3.8×10^9) and Bull 2 (4.3×10^9) were not different from those in normal bulls ($4.9 \pm 0.3 \times 10^9$), but were reduced in Bull 3 (1.6×10^9). The number of Leydig cells per gram of testis parenchyma was higher in Bull 1 (5.4×10^7), Bull 2 (7.3×10^7) and Bull 3 (19×10^7) than in normal bulls ($3.6 \pm 0.2 \times 10^7$). In Bulls 1 and 2, basal and GnRH-stimulated LH, FSH, testosterone (T), androstenedione (Δ_4A) and estradiol 17-β (E_2) were within normal ranges at most ages. However, basal FSH and LH were greater in Bull 3 than in normal bulls, probably the causes for higher Leydig cell density. Also in the same animal, GnRH induced lower responses in LH and FSH, consequence of low basal T and E_2 at some ages. Basal and GnRH-stimulated Δ_4A in Bull 3 were greater than in normal bulls after 6 mo, indicating impairment of Leydig cell differentiation. Deficiency in hormone secretion did not appear to be the cause of infertility, which points toward impaired gonadal responses or secretion of intratesticular factors, or genetic defects. Moreover, infertile animals may not always show pronounced changes in hormone secretion, but evaluation of testis growth around puberty can help identify those animals that do not have proper gonadal development.

Key words: bull, testes, hormones, spermatogenic arrest, Sertoli cell

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INTRODUCTION

Testicular development is the result of a complex interaction between GnRH, gonadotropins, steroids and locally-secreted growth factors and the production of a fertile male is a consequence of normal mitosis and meiosis of germ cells and proper function of the Leydig and Sertoli cells. An interruption of germ cell differentiation, spermatogenic arrest, leads to subfertility or complete infertility and may be associated with either altered hormone secretion or genetic abnormalities (29, 34). Several studies described cases of spermatogenic arrest in men (6, 10, 15, 34), mice (16, 36) and rats (38). Also, some investigations dealt with cases of impaired spermatogenesis in adult bulls (13, 23, 40, 42, 49), but no detailed description of prepubertal and pubertal testicular and hormone changes have been reported. Because of the lack of diagnostic tools or indicators, usually farm animals with spermatogenic impairment are not identified until the age they are expected to breed. In the present work, we had a unique opportunity to study, from an early age (up to 1 year), aspects of growth, testicular development, hormone profiles and histology of bulls with spermatogenic arrest, as compared to the same criteria in normal bulls.

MATERIALS AND METHODS

General Procedures

Twenty seven Angus calves were weaned at 8 months and kept thereafter in feedlots with free access to hay and corn silage. The group of cows were bred with only two bulls. Genetic linkages on the maternal side were unknown. From 2 to 12 months, all bulls were bled monthly from the jugular vein three times at 1.5-h intervals to establish basal hormone concentrations. On the next day, bulls received a subcutaneous injection of GnRH (des-gly,^{10} [D-ala^{6}]-GnRH-ethylamide, Sigma Co., St. Louis, MO) and blood samples were taken 1.5 and 3 h later. Doses of GnRH were based on an average ratio of 0.05 μg/Kg body mass. Monthly, animals were also weighed and the diameter of the right testis was measured using a caliper. At the age of 12 months (361 ± 15 days; 433.3 ± 6.5 Kg), all bulls were surgically castrated by a veterinarian using approved animal-care practices.

Histological Analysis

At castration, testes were weighed after removal of the tunica vaginalis and epididymis. Two 4-mm thick segments were taken near the poles of one testis and placed in Bouin's fixative for 24 h, rinsed with water and washed in three changes of 70% ethanol. Thereafter, the tissue was dehydrated in alcohol, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin. To estimate the degree of seminiferous tubule development at 12 months of age, 400 tubule cross sections from each of two testicular segments were evaluated and placed in one of the following categories, based on the most advanced germ cell type: tubules without germ cells (ST0), tubules with A1 spermatogonia, Intermediate or B spermatogonia (STB), pachytene spermatocytes (STP), round spermatids (STR), elongate spermatids (STE) and mature spermatids (STM).

Estimate of total number of Sertoli cells per testis (S) was described (21, 35). Testicular volume (V) was determined dividing testis weight (g) by testis density of 1.052 g/cm^3 (30). The volume occupied by 10 seminiferous tubule cross sections (Vst) was calculated by the formula $Vst = \Pi \times h \times (d^2/4)$, where h was the section thickness (5 μm) and d was tubule diameter (μm). The percentage of

testicular volume occupied by seminiferous tubules (%ST) and interstitium was determined by Chalkley's method (14), which is based on 600 "hits" taken at random within a cross section of a testis. Crude numbers of Sertoli cells per testis was determined by: Crude number = (V x %ST x C) / (Vst x 10), where C represented the true number of Sertoli cells with and intact nucleolus counted in 10 round cross sections. The resulting crude numbers were converted to true counts according to Abercrombie's formula (1): True number = Crude number x (section thickness / (section thickness + average nucleolar diameter in microns). Section thickness in this case was 5 μm.

The relative volume of the Leydig cells in the intertubular tissue (VLc%) was based on the percentage of 30 "hits" taken at random in each of 20 fields of view of interstitium space (600 "hits" per animal). The volume of the intertubular compartment was estimated by testis volume x percentage of the testis occupied by the interstitium. Total volume of the Leydig cells per testis (TVLc) was determined by multiplying VLc% times intertubular volume. The volume of an individual Leydig cell was based on the formula (assuming it is spherical): $Lc = (4/3) \times \Pi \times D^3$, where D is the average diameter (in μm) taken from 25 Leydig cells. Therefore, the number of Leydig cells per testis was determined by dividing TVLc by the volume of a single Leydig cell. Dividing this number by testis mass (in g) gave the numerical density of the Leydig cell population (29).

Radioimmunoassays

Procedures for RIAs were described previously in detail (35). Briefly, concentrations of FSH were determined in 200 μL of serum using a double antibody assay (13). Both the first antibody (USDA-5-0122) and the purified FSH used for iodination and reference curve (USDA-bFSH-I-1) were provided by Dr. D. J. Bolt (USDA, Beltsville, MD). The detection level of the FSH RIA was 0.25 ng/mL and the intra- and interassay coefficient of variation (CV) were 7% and 12%, respectively. Concentrations of LH were quantified in 100 μL of serum using a method first described by Niswender (37) and modified by Bolt (12). The LH first antibody (15 antiovine LH) was obtained from Dr. G. Niswender (Colorado State University, Fort Collins, CO) and the purified hormone was provided by Dr. L. E. Reichert (Rochester Medical School, Albany, NY). The detection level was 31.3 pg/mL and the intra- and interassay CV were < 9% and < 12%, respectively.

Samples of serum (150 μL) were extracted with 1.5 mL of benzene before being analyzed for testosterone (T), androstenedione (Δ_4A) and estradiol 17-β (E_2). Concentrations of T and Δ_4A were estimated based on a single-antibody method (18). The Δ_4A antibody (X - 322 Rao) was purchased from Dr. P. N. Rao (Southwest Foundation for Biomedical Research, San Antonio, TX) and the T antibody was provided by Dr. G. Niswender. The detection level for the T and Δ_4A assays were 10 pg/mL and 2.5 pg/mL, respectively. The intra- and interassay CV were 10% and 12%, respectively, for the T assay, and 8% and 12%, respectively, for the Δ_4A assay. Concentrations of E_2 were quantified according to a procedure described by Cox et al. (18) and later modified by Britt (personal communication). The antibody was supplied by Dr. N. Manson (Lilly Research Laboratories, Indianapolis, IN). The detection level of the assay was 0.15 pg/mL and the intra- and interassay CV were 8% and 15%, respectively. Assays were validated by adding known quantities of hormone to previously characterized serum and estimating the recovery thereof (35).

Statistical Analysis

The z statistical test (20) was used to determine whether testis criteria and hormone concentrations measured in infertile bulls were significantly different from the average of the other 24 bulls considered as having normal spermatogenesis. In the case of hormone data, the average of the samples taken 1.5 and 3.0 hours after GnRH were used to make the comparisons.

RESULTS

Testicular Development and Histology of the Seminiferous and Interstitial Compartments

The group of normal bulls was formed by 24 bulls (Figure 1) that had all the 12 stages of the cycle of the seminiferous epithelium, as described by Berndtson and Desjardins (9). On average, in the 12-month old testes of these bulls, 10.8 ± 3% of the sections of seminiferous tubules had no germ cells, 2.5 ± 0.9% of the sections had A1 spermatogonia, 0.7 ± 0.3% had B or Intermediate spermatogonia, 5.5 ± 1.4% had pachytene spermatocytes, 37.5 ± 2% had round spermatids, 33 ± 3% had elongate spermatids and 10 ± 2% of the sections had mature spermatids as the most advanced germ cell type.

Among the three bulls with abnormal spermatogenesis, Bull 1 had no germ cells (Figure 2) and Bull 2 had 90% of the tubules without germ cells and only A1 spermatogonia were present in the remaining cross sections (Figure 3, A and B). In Bull 3, analysis of seminiferous tubules revealed that 32% of them had no germ cells, 30% of the sections had spermatogonia A1, 25% had either B or intermediate spermatogonia and 12% of the tubules had primary spermatocytes as the most developed germ cell. Some of the spermatocytes observed in the tubule sections showed signs of degeneration (Figure 4, A and B).

Testis diameter of Bulls 1 and 2 was smaller than those of normal bulls from the age of 4 months, but differences became significant only after 11 months ($P < 0.05$, Figure 5). From 4 to 12 months, the testis diameter of Bull 3 was smaller ($P < 0.05$) than that of normal bulls. Testicular parenquima of Bulls 1, 2 and 3 weighed much less (99 g, 105 g and 32 g, respectively) than those of normal bulls (251.5 ± 55.8 g, $P < 0.01$). Body weights of Bull 1 (396 Kg), Bull 2 (434.3 Kg) and Bull 3 (392 kg) were reduced but not significantly different from the average of normal bulls (437 ± 31.7 kg, $P > 0.05$).

Sertoli cell numbers per testis in Bulls 1 and 2 were 3.8×10^9 and 4.3×10^9, respectively, but these values were not different from the average counts in bulls with normal spermatogenesis ($4.9 \pm 0.3 \times 10^9$, $P > 0.05$). Total number of Leydig cells per testis of Bull 1 (5.3×10^9) and Bull 2 (7.7×10^9) were numerically reduced when compared to that of normal bulls (10.9×10^9). However, the number of Leydig cells per gram of testicular parenchyma was higher ($P < 0.05$) in those bulls (5.4×10^7 and 7.3×10^7, respectively) than in bulls with normal spermatogenesis ($3.6 \times 10^7 \pm 0.2$). Cross sections of the seminiferous tubules of these two animals were vacuolated and had diameters of 169 μm and 174 μm, respectively, which were smaller ($P < 0.05$) than the average tubule diameter of normal bulls (200 ± 3.08 μm). As observed by light microscopy, the Sertoli cell nuclei of Bull 1 and Bull 2 did not show major morphological differences from that of

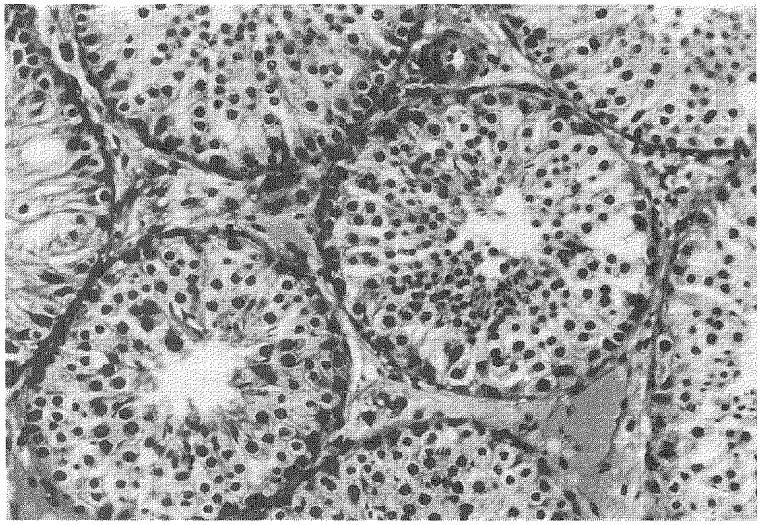

Figure 1. Seminiferous tubules and interstitial space of yearling Angus bulls with normal spermatogenesis (400 x).

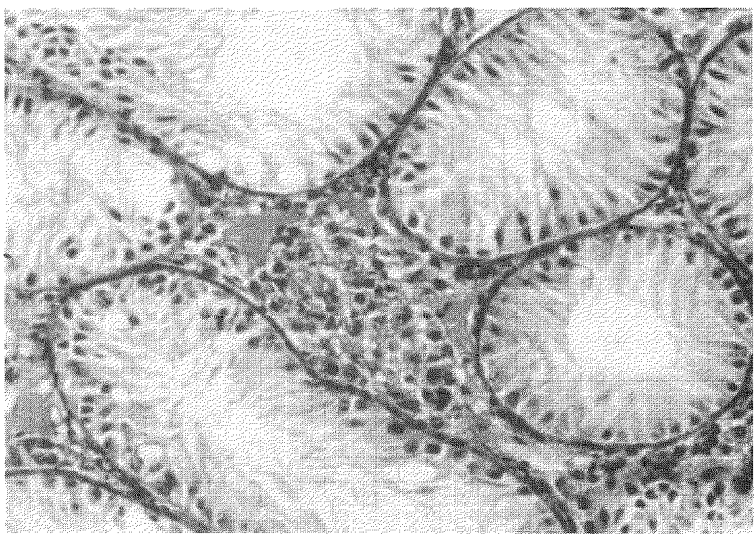

Figure 2. Seminiferous tubules and interstitial space from yearling Angus bulls with Sertoli-cell only Syndrome (400x).

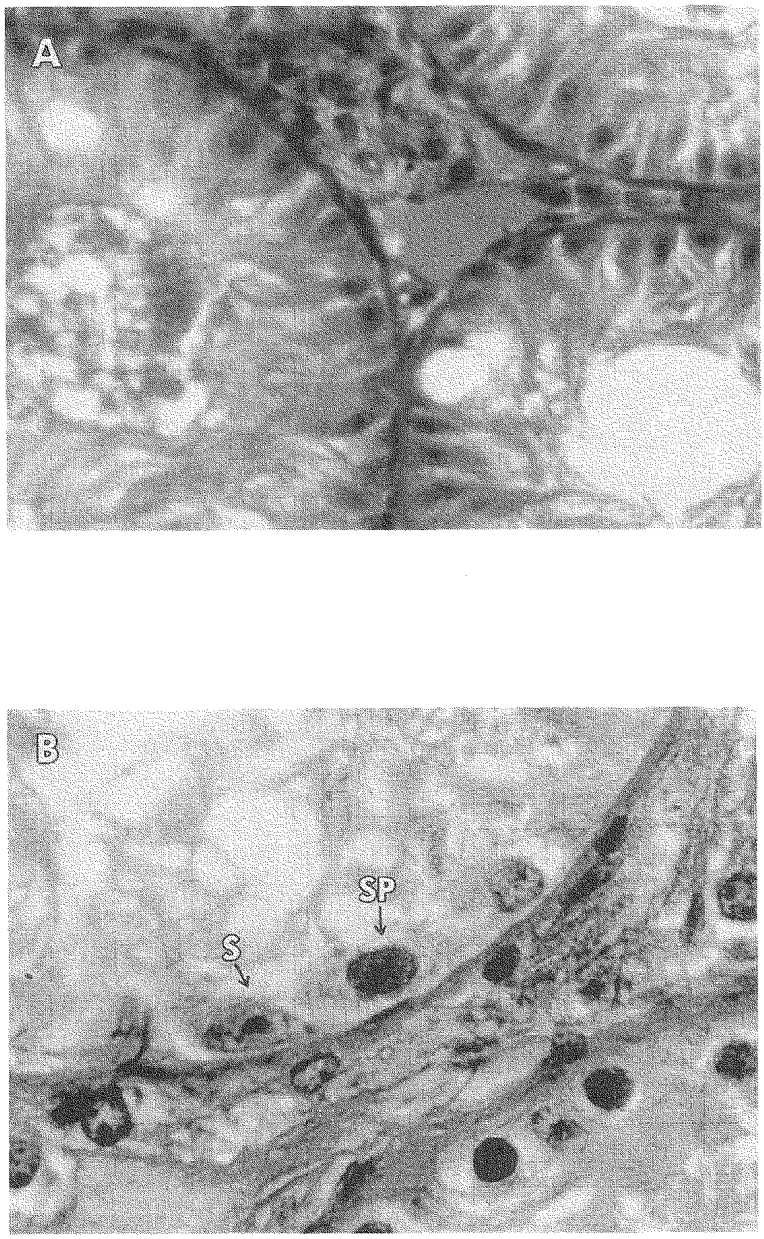

Figure 3. Seminiferous tubules and interstitial space from yearling Angus bulls with arrest of A spermatogonial development (A: 400; B: 800 x). SP: A spermatogonia; S: Sertoli cell

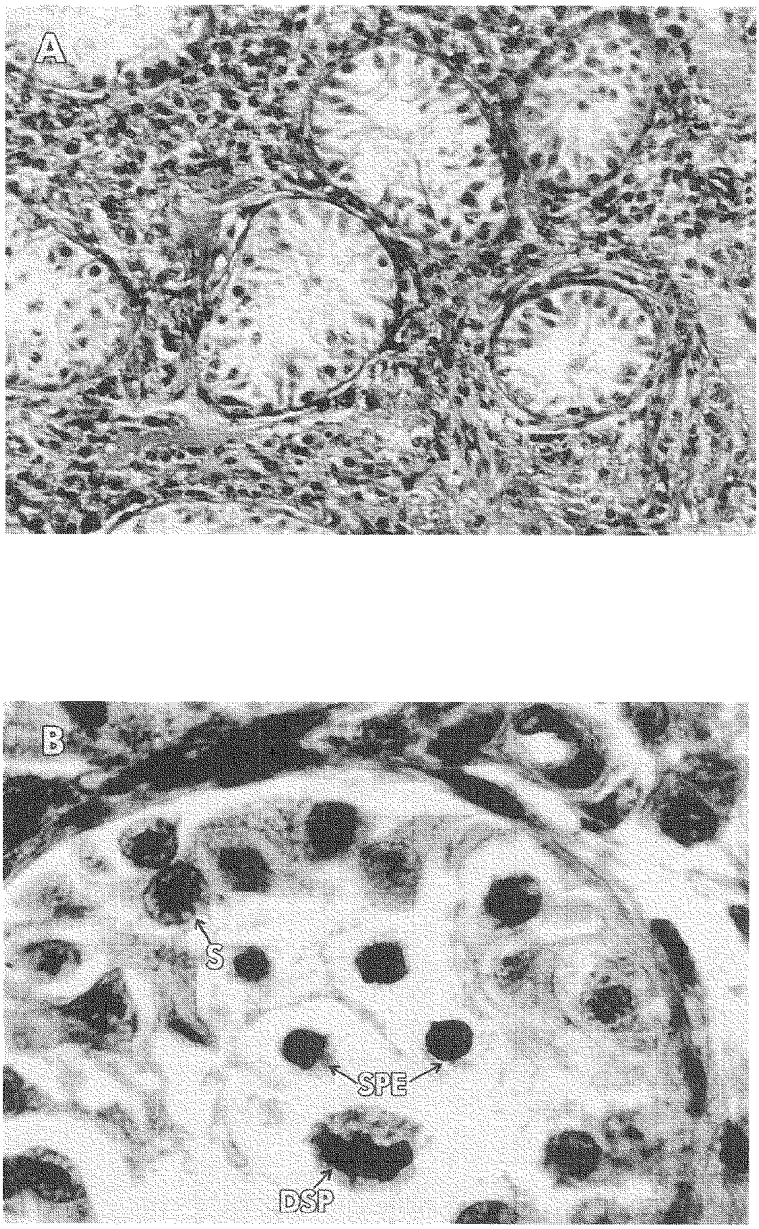

Figure 4. Seminiferous tubules and interstitial space from yearling Angus bulls with spermatogenic arrest at the level of primary spermatocytes (A: 400; B: 800 x). SPE: spermatocyte; S: Sertoli cell. DSP: degenerating spermatocyte.

normal bulls, i. e., they were located near the basement membrane, irregularly-shaped with indentations and the nucleolus appeared as a single structure with little chromatin surrounding it.

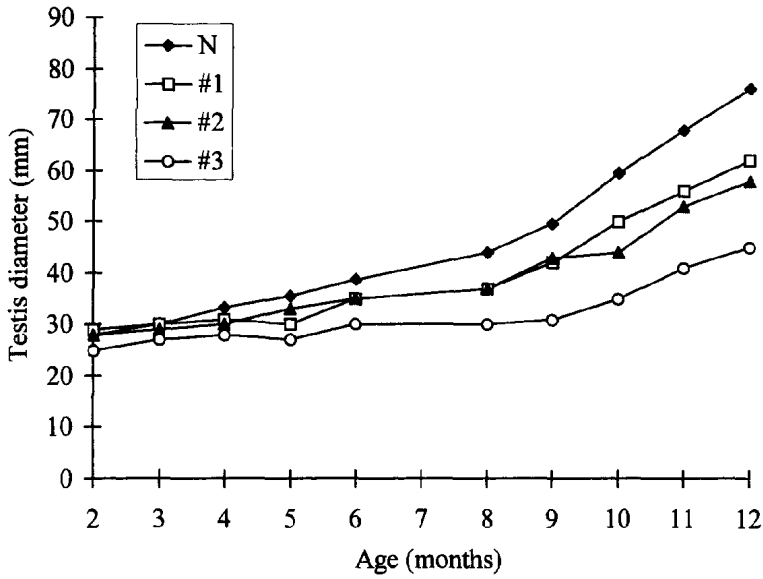

Figure 5. Testicular development in normal Angus bulls (N) and bulls with Sertoli-cell only syndrome (Bull 1), and spermatogenic arrest at the level of A1 spermatogonia (Bull 2) and primary spermatocytes (Bull 3).

In Bull 3, numbers of Sertoli cells (1.6×10^9) were 33% of those measured in normal animals ($P < 0.01$) and the average diameter of the tubules (98.5 μm) in Bull 3 was also lower ($P < 0.01$). Moreover, Sertoli cell nuclei were round or oval with few of them resembling those of mature cells. There was limited occurrence of indentations on the nuclear membrane and the nucleoli appeared to be split and often associated with large aggregates of heterochromatin. The population of Leydig cells per testis (4.9×10^9) was reduced, but the numerical density of Leydig cells was 5.3-fold higher (19×10^7 cells per g of testicular parenchyma) than in normal bulls ($3.6 \pm 0.2 \times 10^7$ cells, $P < 0.05$).

Peripheral Hormone Concentrations

In the case of Bulls 1 and 2, basal and GnRH-stimulated FSH were within normal range at most ages (Figure 6, A and B). Only at 9 and 10 months, basal FSH in Bull 1 was higher than in normal bulls and GnRH-stimulated at 11 and 12 months were also increased ($P < 0.05$). At 11 and 12 months, GnRH-stimulated FSH in Bull 2 was higher ($P < 0.05$) than that measured in normal bulls. Concentrations of basal and GnRH-stimulated FSH in Bull 3 were, on average, 5-fold and 3.2-fold higher ($P < 0.01$) than the respective values for normal bulls throughout the

study. However, the average increase in FSH secretion caused by GnRH injections was lower in Bull 3 (36%) than in normal bulls (110%).

At none of the ages studied was basal LH in Bulls 1 and 2 significantly different from that of normal bulls (Figure 7A). GnRH-stimulated LH secreted by Bull 1 between 2 and 12 moths was lower than the secretion of normal bulls (Figure 7B), but differences were significant only at 3 and 5 months ($P < 0.05$). Concentrations of GnRH-stimulated LH in Bull 2 were significantly lower only at 2 months (16.58 ng/mL versus 23.75 ± 6.23 ng/mL), and hormone levels became close to those found in normal bulls after 5 months. Basal LH in Bull 3 increased with age and differences related to normal bulls were significant from 2 months (0.76 ng/mL versus 0.49 ± 0.03 ng/mL, $P < 0.05$) to 12 months (4.1 versus 2.85 ± 0.15 ng/mL, $P < 0.01$). Throughout the year, the LH response to GnRH in Bull 3 was lower than in normal bulls, but differences became significant ($P < 0.05$) from 3 to 5 months and from 9 to 12 months of age.

Basal testosterone in Bull 1 (Figure 8A) was lower than that of normal bulls, but differences approached statistical significance only from 10 to 12 months ($P < 0.10$). In Bull 2, basal testosterone was similar to that found in normal bulls ($P > 0.05$). Testosterone in Bull 3 was reduced at most ages but differences were significant only at 8 and 9 months (0.64 and 1.27 ng/mL), when compared to normal bulls (3.45 ± 1.56 ng/mL and 4.23 ± 1.88 ng/mL). However, while testosterone in normal bulls decreased after 10 months, that in Bull 3 continued to increase, reaching a peak of 6.08 ng/mL at 12 months. From 2 to 12 months, GnRH-stimulated testosterone in Bulls 1 and 2 was lower than that in normal bulls, but differences were significant only from 6 to 12 months in Bull 1 and between 9 and 11 months in Bull 2. In Bull 3, GnRH-stimulated testosterone was lower only between 8 and 11 months (Figure 8B).

Basal concentrations of Δ_4A (Figure 9A) in Bull 1 were lower than those detected in normal bulls at most ages, but differences were not significant ($P > 0.05$). Levels of Δ_4A in Bull 2 were similar to those found in normal bulls. In Bull 3, basal Δ_4A from 3 to 5 months was not different from that in normal animals ($P > 0.05$). However, between 6 and 12 months, basal Δ_4A increased from 231.5 to 767.5 pg/mL, reaching a peak of 801 pg/mL at 10 months, while concentrations of Δ_4A during the same period in normal bulls were lower ($P < 0.05$), with values from 181 ± 15 to 243 ± 15 pg/mL. GnRH-stimulated Δ_4A in Bull 1 (Figure 9B) remained within normal ranges, showing a small decrease after the age of 9 months only ($P < 0.05$). GnRH-stimulated Δ_4A in Bull 2 was within normal ranges at all ages ($P > 0.05$), despite numerical differences at 3 and 4 months. The GnRH-stimulated Δ_4A in Bull 3 increased with age and became higher ($P < 0.05$) than in normal bulls from 8 to 12 months.

Basal concentrations of E_2 in Bulls 1 and 2 (Figure 10A) presented only small variations in comparison to those values measured in normal bulls throughout the study ($P > 0.05$). However, at 2 and 3 months, basal E_2 in Bull 3 (1.35 and 1.3 pg/mL) was lower ($P < 0.05$) than in normal bulls. Also, from 8 to 10 months, basal estradiol was lower in Bull 3 (2.13 to 6.0 pg/mL) than in normal bulls (3.79 ± 0.29 to 9.26 ± 0.64 pg/mL). GnRH-stimulated estradiol in Bulls 1 and 2 (Figure 10B) was within normal ranges, although there were some numerical differences after 10 months. GnRH-stimulated E_2 measured in Bull 3 (1.48 to 5.70 pg/mL) were lower than those in normal bulls (2.88 ± 0.15 to 10.68 ± 1.5 pg/mL), but differences were significant only at the ages of 3, 6 and 8 months ($P < 0.05$).

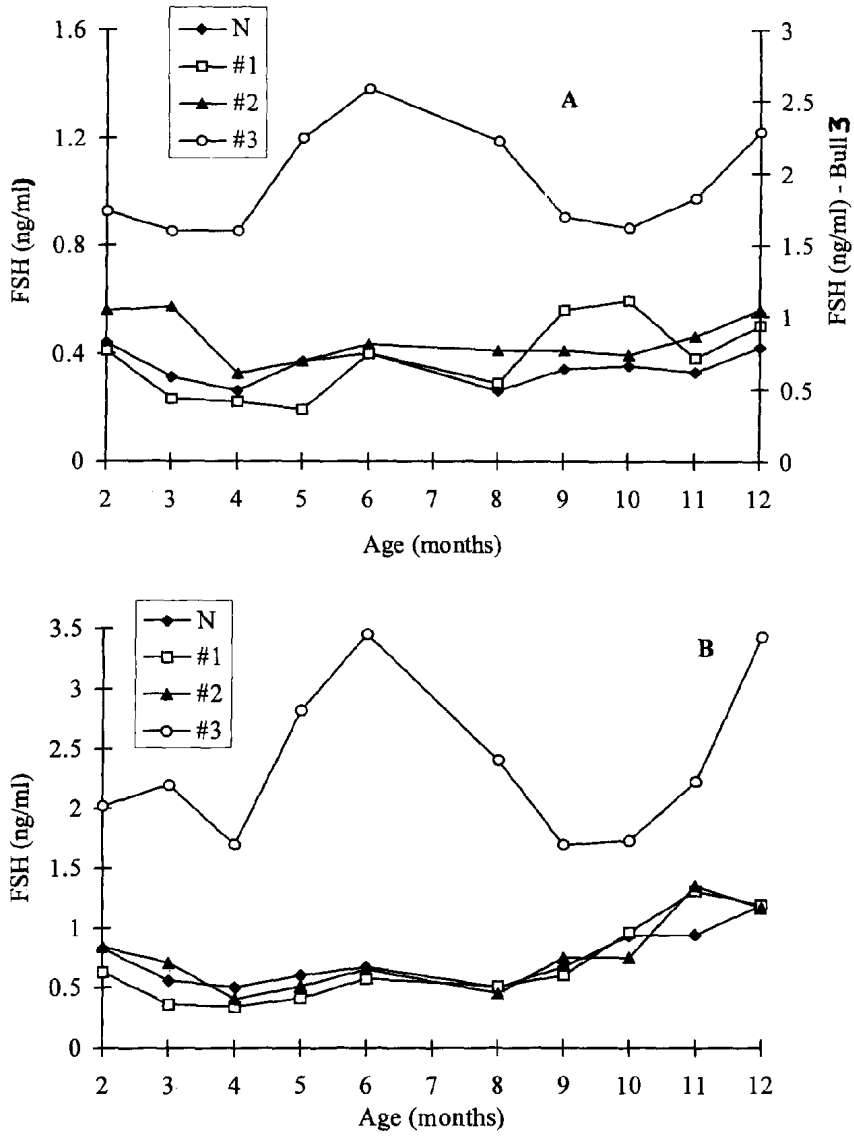

Figure 6. Basal (A) and GnRH-stimulated (B) concentrations of FSH in normal Angus bulls (N) and bulls with Sertoli-cell only syndrome (Bull 1), and spermatogenic arrest at the level of A1 spermatogonia (Bull 2) and primary spermatocytes (Bull 3). Basal values: average of 3 samples taken at 1-h intervals. GnRH: average of samples collected 1.5 and 3 h after a GnRH injection.

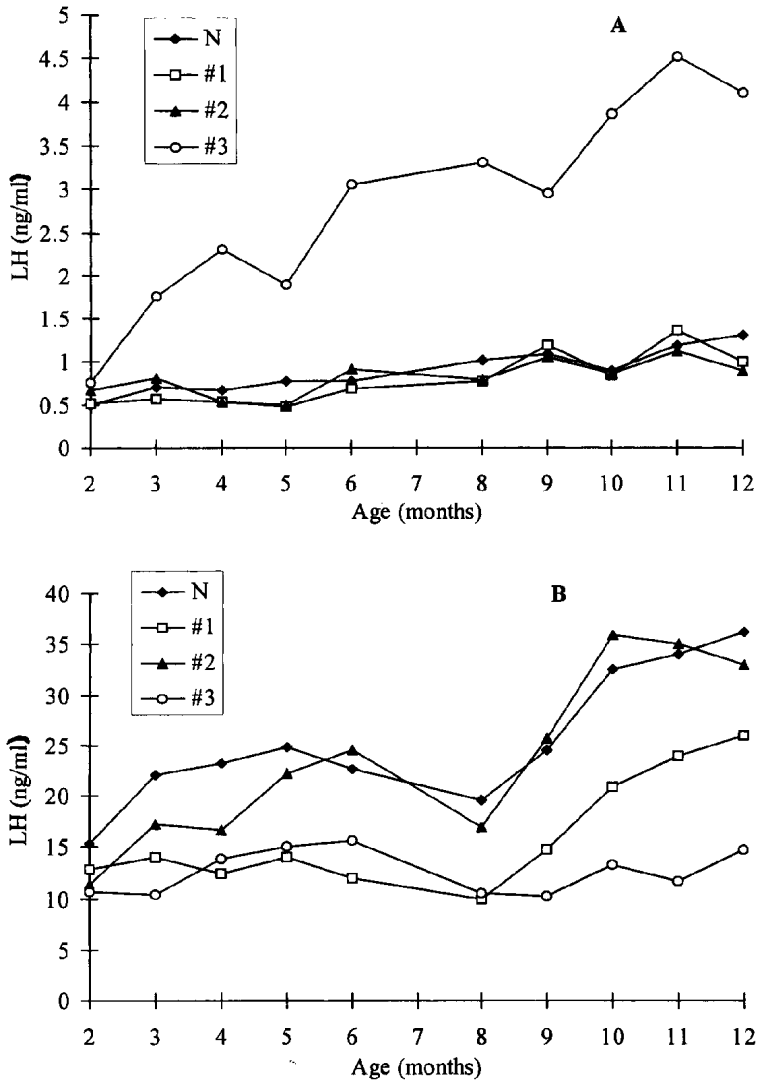

Figure 7. Basal (A) and GnRH-stimulated (B) concentrations of LH in normal Angus bulls (N) and bulls with Sertoli-cell only syndrome (Bull 1), and spermatogenic arrest at the level of A1 spermatogonia (Bull 2) and primary spermatocytes (Bull 3). Basal values: average of 3 samples taken at 1-h intervals. GnRH: average of samples collected 1.5 and 3 h after a GnRH injection.

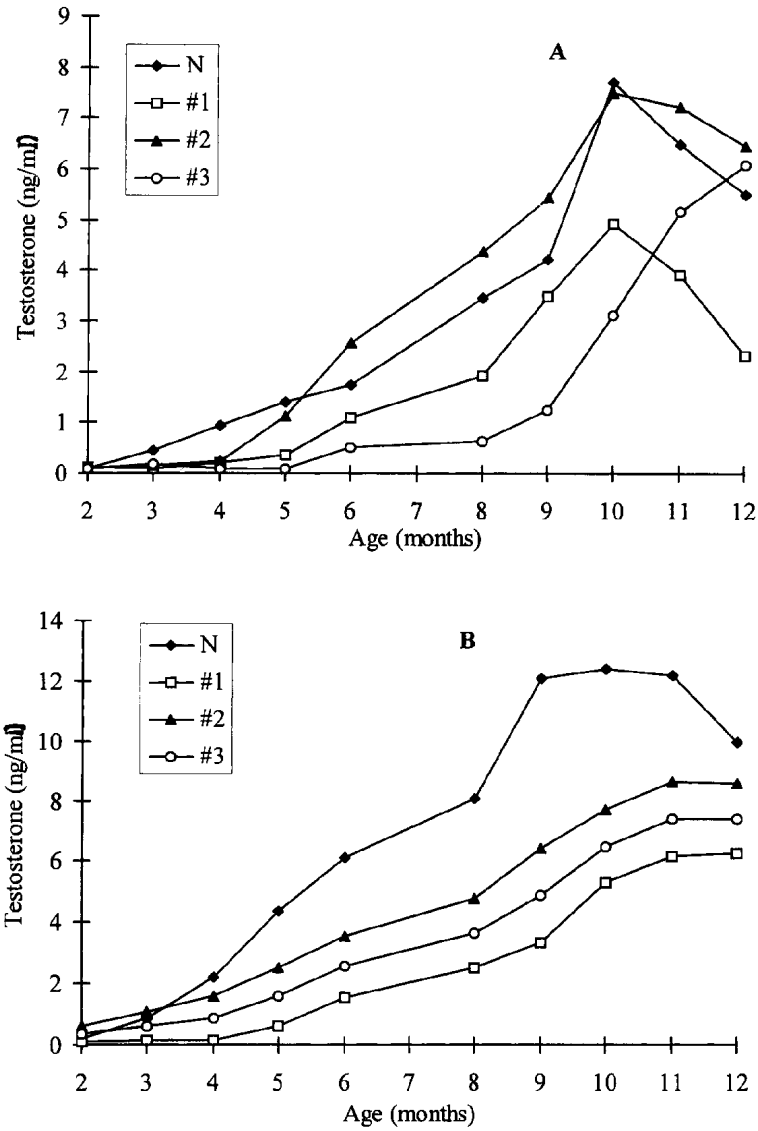

Figure 8. Basal (A) and GnRH-stimulated (B) concentrations of testosterone in normal Angus bulls (N) and bulls with Sertoli-cell only syndrome (Bull 1), and spermatogenic arrest at the level of A1 spermatogonia (Bull 2) and primary spermatocytes (Bull 3). Basal values: average of 3 samples taken at 1-h intervals. GnRH: average of samples collected 1.5 and 3 h after a GnRH injection.

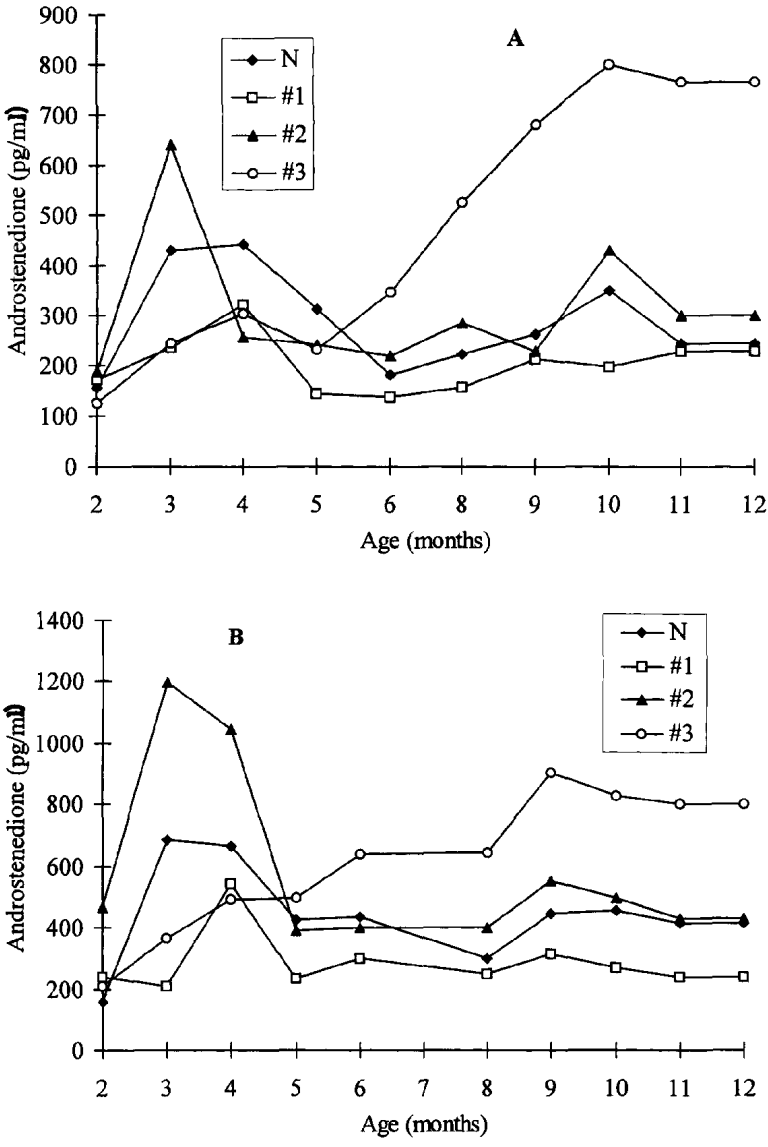

Figure 9. Basal (A) and GnRH-stimulated (B) concentrations of androstenedione in normal Angus bulls (N) and bulls with Sertoli-cell only syndrome (Bull 1), and spermatogenic arrest at the level of A1 spermatogonia (Bull 2) and primary spermatocytes (Bull 3). Basal values: average of 3 samples taken at 1-h intervals. GnRH: average of samples collected 1.5 and 3 h after a GnRH injection.

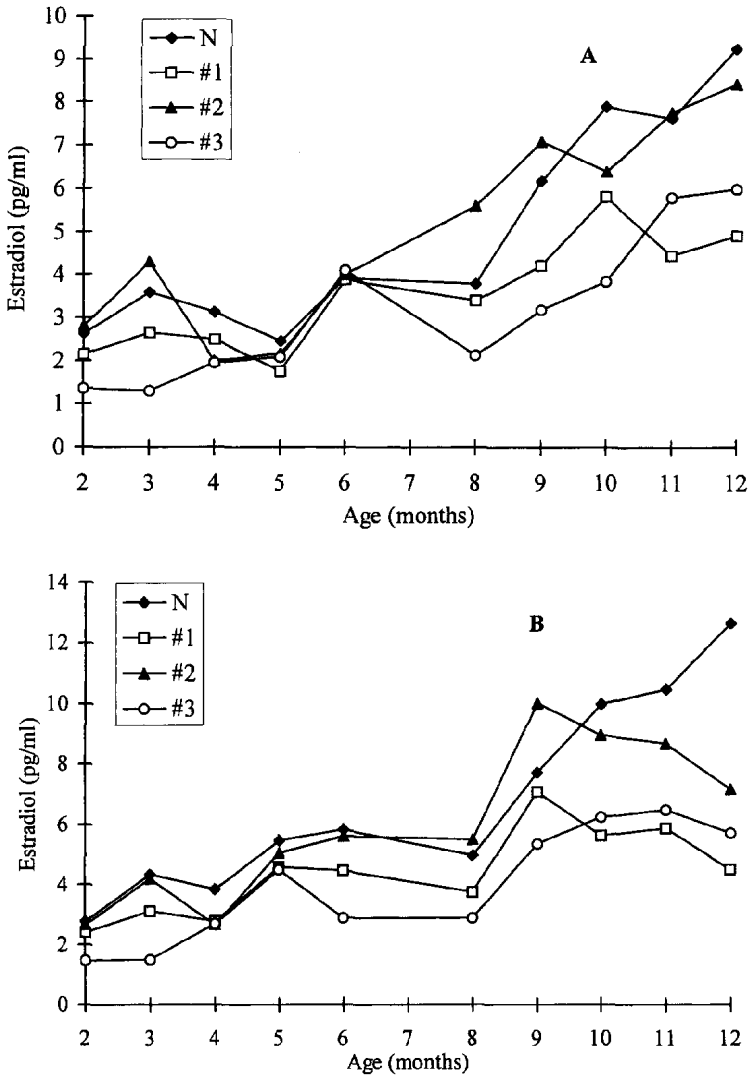

Figure 10. Basal (A) and GnRH-stimulated (B) concentrations of estradiol 17-β in normal Angus bulls (N) and bulls with Sertoli-cell only syndrome (Bull 1), and spermatogenic arrest at the level of A1 spermatogonia (Bull 2) and primary spermatocytes (Bull 3). Basal values: average of 3 samples taken at 1-h intervals. GnRH: average of samples collected 1.5 and 3 h after a GnRH injection.

DISCUSSION

In this study, three distinct cases of spermatogenic arrest are characterized by Sertoli-cell only syndrome (Bull 1), impaired differentiation of A1 spermatogonia (Bull 2) and interruption of meiosis in spermatocytes (Bull 3). The number of Sertoli cells per testis in Bulls 1 and 2 was not different from that of normal bulls, which shows that the difference in testis weight and seminiferous tubule diameter was mainly due to the absence of germ cells.

In Bull 3, the reduction in testis size was detected earlier than in the other infertile bulls not only because of low numbers of germ cells, but also as a consequence of a small population of Sertoli cells. Given that there were high peripheral concentrations of FSH, a hormone known to stimulate Sertoli cell proliferation both in vivo (4, 48) and in vitro (26), the decrease in Sertoli cell population is a consequence of impaired response to gonadotropins and/or secretion of intratesticular factors that may have been altered since the early stages of testicular development.

Morphological features of the nucleus and nucleolus of Sertoli cells in Bulls 1 and 2 resembled those of differentiated cells present in normal bulls. However, in the mutant mouse testes lacking germ cells, the volume of the cytoplasm, nucleus and smooth endoplasmic reticulum of the Sertoli cell were reduced significantly, as revealed by electron microscopy (19). Also, several studies showed that loss of germ cells caused a decrease in the surface area of the apical region of the Sertoli cell (24, 45). Thus, although the Sertoli cells in the testis of Bulls 1 and 2 appeared normal under light microscopy, it is possible that ultrastructural changes may have occurred as a consequence of the absence of germ cells.

Sertoli cells of Bull 3 appeared undifferentiated, similar to those in normal prepubertal bulls (46). In men, prepubertal Sertoli cells, as characterized by continued expression of anti-Mullerian hormone, also were associated with spermatogenic arrest (47). It is known that FSH is important for Sertoli cell mitosis and differentiation (5) but studies suggested that testosterone is also involved in this process. Immunization of 18-month old Holstein calves against testosterone delayed the differentiation of the Sertoli cells and allowed them to proliferate for a longer period of time (50). In hemicastrated neonatal rats, exogenous testosterone prevented the mitosis of Sertoli cells in the remaining testis (40). In the case of Bull 3, testosterone was low before the age of 10 months, but increased afterwards and reached normal levels at 11 and 12 months, suggesting that reduced androgen secretion is not the reason why Sertoli cells still appeared immature at the age of 1 year. Thus, in Bull 3, interrupted differentiation of these cells is probably related to their inability to respond to FSH and testosterone or altered secretion of intratesticular factors.

Spermatogenic arrest affected the population of Leydig cells in different ways. In Bulls 1 and 2, the increase in Leydig cell density was small, but given that such increase was not associated with deficient gonadotropin secretion, it may be related to local regulation of Leydig cell growth. Similarly, relative abundance of Leydig cells have been found in the testis of XXY bulls with no germ cells (33, 42). In the Bull with impairment of spermatocyte development, the hyperplasia of Leydig cells was more pronounced and probably a consequence of high concentrations of LH, because proliferation of those cells is dependent on LH (17). High FSH since early life may have also contributed to the hyperplasia. Increases in Leydig cell numbers were observed when juvenile primates were treated with both LH and FSH (41).

Testosterone and FSH are critical for germ cell survival (11, 22, 27), but the cases of infertility reported here were unlikely to be caused by deficiency of gonadotropins because concentrations of FSH and LH were either normal or elevated at ages from 2 to 12 months. Concentrations of testosterone were close to normal in Bull 2 and reduced, at some ages, in Bulls 1 and 3. Qualitatively normal spermatogenesis can be maintained with low levels of testosterone (32, 44). Also, in rats immunized against GnRH, spermatogenesis can proceed with 20 to 40 % of the normal concentrations of intratesticular testosterone and, in the presence of FSH, the requirements for testosterone are much lower (51). Thus, the change in the pattern of testosterone secretion in the Bull with Sertoli-cell only syndrome and even the delayed increase in the Bull with spermatocyte arrest, did not appear to be sufficient to cause dramatic germ-cell failure.

The bull with Sertoli-cell only syndrome had low testosterone responses to GnRH at some ages and the small LH-response to GnRH, also at some ages, contributed to this effect. However, the absence germ cells causes functional changes in the Sertoli cell (44) and Sertoli cell-secreted factors, stimulated by FSH, can enhance steroidogenesis in rats and human cell cultures (43, 44). In Bull 2 (with arrest of spermatogonia development), concentrations of T stimulated by GnRH were reduced between 9 and 10 months, showing that lack of germ cells in this case also may have impaired some aspects of Leydig cell function, probably through an effect on the Sertoli cell.

A case of Sertoli cell-only syndrome in the bovine was reported, where a 2-year old Hereford Bull with no germ cells had normal peripheral concentrations of FSH but reduced frequency of LH and testosterone pulses (40). Also, a Klinefelter bull with no germ cells had low testosterone concentrations at 17 months of age (42), but an XXY adult bull without germ cells had normal basal and GnRH-stimulated testosterone (33). Bulls with Sertoli cell-only syndrome showed in our study also had normal FSH at most ages and a tendency to have low basal testosterone after 10 months. Significant differences in testosterone and LH secretion were seen only after GnRH challenges, at some ages but it is difficult to conclude whether or not these differences in testosterone reflect physiological events or other factors such as age, sampling periods or breed.

In our study, the bull with spermatogonia (Bull 2) arrest had normal FSH at most ages. There are no similar cases described in bulls. However, male mice homozygous for the mutant gene termed juvenile spermatogonial depletion had also normal testosterone, but high FSH. In these animals, testis weight was reduced to 1/3 and the few germ cells in the seminiferous tubules failed to advance beyond the A1 spermatogonial stage (7). Species differences and/or causes of the spermatogenic arrest may account for the differences in the pattern of hormone secretion.

In Bull 3, high LH concentrations at early ages were caused by reduced secretion of steroids. However, it is uncertain why only LH increased linearly with age, especially after the age of 9 months, when testosterone and estradiol also increased. It seems that the hypothalamus became less sensitive to the negative feedback of the steroids as the animal aged and this would lead to an increase in the activity of the GnRH neuron. This way, the pattern of LH secretion, more than that of FSH, would follow the increase in GnRH. Basal concentrations of FSH in this bull were always high since the age of 2 months and this suggest a different kind of control by the testis. In rats and humans, the absence of elongate spermatids was associated with a decrease in inhibin and an increase in FSH (8, 44, 45). Also, in the rat, high FSH is associated with arrest of primary spermatocytes (25, 38). However, Bulls 1 and 2 did not have spermatids and showed much

smaller changes in FSH and LH, showing that absence of germ cells have different effects on testicular hormone secretion and control of gonadotropins. It is possible that Bull 3, besides having reduced serum levels of steroids at early ages, also secreted less inhibin, affecting the control of FSH secretion. Moreover, the responses of FSH to GnRH, and particularly those of LH, were very low in Bull 3. High basal gonadotrophin and reduced stimulation by GnRH may indicate that, due to decreased inhibition from the testis, most of the hormone synthesized was promptly released and less was stored in the gonadotrophs.

From 2 to 5 months, basal and GnRH-stimulated Δ_4A in Bull 3 were normal but those of testosterone were reduced. Moreover, after the age of 5 months, concentrations of testosterone was still low while those of Δ_4A became substantially higher. In the normal bulls used in this study, levels of Δ_4A decreased after 4 to 5 months of age while those of testosterone continually increased. It is accepted that this shift from Δ_4A to T as the main androgen produced by the testis is an important indicator of Leydig cell maturation (2). It can be concluded, therefore, that Leydig cells did not differentiate properly after the age of 5 months and continued to proliferate due to high LH in the bull with spermatocyte arrest. In addition, these immature Leydig cells failed to convert the Δ_4A into testosterone and released high levels of Δ_4A in response to high basal LH or induced by exogenous GnRH. Also, the low synthesis of testosterone could be one of the causes for the reduced secretion of estradiol by these Leydig cells, especially after the age of 8 months.

Few cases of spermatocyte arrest have been described in bovines. An 18-month old bull was found with arrest at the stage of primary spermatocyte, but no information is given about hormone status and genetic map (23). Ansari et al. (3) also described an infertile Holstein bull with degenerative spermatocytes due to a balanced autosomal reciprocal translocation but, contrary to what was observed in our study, this animal had testes slightly smaller than the average of fertile bulls and the diameter of the seminiferous tubules was normal. Homozygosis for the growth and reproduction complex in rats was associated with sterility, because of an arrest of primary spermatocyte development, and reduced testis size and body weight (12 % and 65 % of normal values, respectively) (25, 31). Also, both FSH and LH were higher but testosterone was lower in affected rats, similarly to what was observed in Bull 3.

In summary, this study describes, for the first time, testicular development and hormone concentrations, and their associations with the degree of germ cell loss in bulls with Sertoli cell-only syndrome and spermatogenic arrest at the level of spermatogonia and pachytene spermatocyte. Deficiency in gonadotropin and steroid secretion did not appear to be the cause of infertility, which points towards other abnormalities, including impaired gonadal responses to those hormones or secretion of intratesticular factors, or genetic defects. Such cases of seminiferous tubule anomaly could be useful models for the study of cell-cell interaction in the testis.

The cases of infertility were difficult to identify while the animals were still young. Although there was only one bull of each case, comparison made with normal bulls show that some infertile animals do not always have pronounced changes in hormone secretion during pubertal development. However, evaluation of testis size around puberty may help identify those animals with abnormal gonadal development.

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