RELATIONSHIP OF ABSOLUTE NUMBERS OF SERTOLI CELLS TO TESTICULAR SIZE AND SPERMATOGENESIS IN YOUNG BEEF BULLS

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ABSTRACT

Testes were obtained from 34 Hereford or Angus bulls at about 1.5 yr of age and were used to investigate the relationship between the absolute number of Sertoli cells vs testicular size and daily spermatozoal production (DSP). Quantitative determination of DSP was based upon enumeration of elongated spermatids in testicular homogenates. The ratio of step 8 spermatids to Sertoli cells (S:SC) was established by direct counts of these cells in each of 20 round stage VIII seminiferous tubular cross sections for each bull. The number of Sertoli cells per paired testes was calculated as (total spermatids + S:SC)/.394, where total spermatids equaled the number of homogenization-resistant spermatids. The factor of .394 adjusted for the fact that the latter cells are present for only 39.4% of the spermatogenic cycle. All data were subjected to simple linear and second-order regression analyses. A positive linear relationship (P<.005) was found between testicular weight (Y, in grams) and the absolute number of Sertoli cells per paired testes (X, in billions), which was characterized by the equation Y = 315.2 + 10.74X and a coefficient of correlation (r) of .56 (P<.01). A similar relationship was observed between DSP (Y, in billions) and Sertoli cell numbers (X, in billions). This was characterized by the equation Y = 1.36 + .222X (P<.005) and a coefficient of correlation of .70 (P<.01). Daily sperm production was unrelated to the S:SC ratio (P>.05). It was concluded that testes possessing a greater number of Sertoli cells were heavier and produced more sperm than testes with fewer Sertoli cells.

(Key Words: Sertoli Cells, Bulls, Spermatogenesis, Testes, Reproduction.)

Experimental Procedure

General. Testes were obtained from 34 Hereford or Angus bulls that were castrated between mid-October and mid-December at approximately 1.5 yr of age and a mean body weight of 418.6 kg. These bulls were from a larger study involving procedures that do not adversely affect the reproductive system (Berndtson et al., 1979, 1980). The weight of each testis was determined immediately after castration. The tunica albuginea was removed and weighed, and the weight of the testicular
parenchyma was calculated. The left or right testis from alternate bulls was used for quantitative assessments of spermatogenesis and Sertoli cell numbers. For these evaluations, one portion of testicular parenchyma was placed in a pre-weighed vial and frozen. A second portion of the same testis was placed in Zenker formol solution for 24 h, washed in running tap water for 24 h, and transferred to 70% ethanol.

Quantification of Daily Sperm Production (DSP). Testicular spermatic reserves were quantified by enumeration of elongated spermatids in homogenates of testicular parenchyma as described by Amann and Almquist (1961). Counts were performed in duplicate by each of four technicians. The coefficient of variability among technicians averaged 17.2%. Data for each bull were pooled over technician and the mean was used for statistical analysis. Daily sperm production was computed utilizing a time divisor of 5.32 d, which equalled the number of days of sperm production represented by homogenization-resistant elongated spermatids (Amann et al., 1974).

Determination of the Absolute Number of Sertoli Cells. A portion of fixed tissue from each bull was embedded in paraffin, sectioned at 5 μm, stained with periodic acid-Schiff's reagent and hematoxylin and used for quantitative histometric analyses. A sample from a single region of the testis was regarded as representative of the entire testis since uniformity among various regions within a testis has been observed consistently for bulls and other species (Amann, 1962; Kennelly and Foote, 1964; Swierstra, 1966, 1968; Gebauer et al., 1974).

For each bull, the nuclei of round step 8 spermatids and Sertoli cell nuclei with a visible nucleolus were enumerated via direct counts in each of 20 round seminiferous tubular cross sections at stage VIII as defined by Berndtson and Desjardins (1974). The resulting crude counts were converted to true counts by application of Abercrombie's formula, where

\[
\text{true count} = \frac{\text{crude count} \times \text{section thickness}}{\text{section thickness + nuclear or nucleolar diameter}}
\]

and measurements are in microns (Abercrombie, 1946; Berndtson, 1977). Nuclear and nucleolar diameters were determined histometrically by measurement of five nuclei (or nucleoli) of each cell type per bull with an ocular micrometer. For the nuclei or nucleoli of round step 8 spermatids and Sertoli cells the coefficients of variability among the five measurements within bulls (i.e., among individual cells) were 4.1 and 8.5%, respectively. Means based on all five measurements were used for Abercrombie's correction. The precision of the true counts was also high. For example, the average coefficient of variability among duplicate estimates of mean spermatid to Sertoli cell ratios within bulls averaged 6.7% when each mean was based upon true cell counts for only 10 seminiferous tubules. The mean true counts used herein for statistical analyses were based upon 20 seminiferous tubules per bull.

The ratio of step 8 spermatids to Sertoli cells (S:SC) for each bull was computed from the true cell count data. The absolute number of Sertoli cells per paired testes (Y) was calculated by the procedure of Jones and Berndtson (1986), with modification to reflect the appropriate time divisor for elongated spermatids in the bull. The calculation was based on the equation 

\[
Y = \frac{(\text{total spermatids} + \text{S:SC})}{.394}
\]

where total spermatids equalled the number of homogenization-resistant spermatids per paired testes. The factor of .394 adjusted for the fact that the homogenization-resistant spermatids are present for only 5.32 d (Amann et al., 1974), or 39.4%, of the 13.5-d cycle of the seminiferous epithelium (Courrot et al., 1970).

Statistical Analyses. All data were subjected to simple linear and second-order polynomiial regression analyses. The statistical significance of relationships between variables was judged by F-tests for the sums of squares attributed to the regression in an analysis of variance, and by the t-ratio for the regression coefficients (Steel and Torrie, 1960). Because all second-order components of the regression equations were non-significant, all significant relationships described subsequently are linear. All coefficients of determination (r²) that follow represent values adjusted for degrees of freedom.

Results and Discussion

A positive relationship (P<.005) was found between paired testes weight and the absolute number of Sertoli cells, as illustrated in figure 1. This relationship was linear and was characterized by the equation 

\[
Y = 315.2 + 10.74X
\]

where Y equals testes weight in grams and X denotes the number of Sertoli cells per paired testes in billions. The mean number of Sertoli cells per paired testes was 10.9 billion. The coefficient of correlation between testes weight and the absolute number of Sertoli cells was .56.
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Figure 1. The relationship between paired testes weight (Y, in grams) and the total number of Sertoli cells per paired testes (X, in billions) was characterized by the equation

\[ Y = 315.2 + 10.74X \]  

\((P<.005, R = .56)\).

(P<.01). A virtually identical relationship was found between the total weight of testicular parenchyma (Y, in grams) and number of Sertoli cells (X), as evident from the equation

\[ Y = 285.8 + 9.99X \]  

\((P<.005)\) and a coefficient of correlation of .56 \((P<.01)\). Thus, it was concluded that testes containing a larger total number of Sertoli cells were heavier than testes with fewer Sertoli cells. Although the relationship between Sertoli cell number and testes weight was highly significant, differences in Sertoli cell number could account for only 30.2% of the variation in paired testes weight \((r^2= .302)\); other factors in addition to Sertoli cell number must influence the testes weight of young bulls. These might include precise age (Coulter et al., 1975), environmental influences (including nutrition) on rates of testicular growth and development (Coulter et al., 1975; Latimer et al., 1982) and body weight (Coulter and Foote, 1977).

One way by which the Sertoli cell is thought to contribute to spermatogenesis is by regulating the milieu within which the germ cells develop. The blood-testis barrier, formed by tight junctional complexes between adjacent Sertoli cells, is important for this function (Dym and Fawcett, 1970). Obviously, larger testes would contain a greater volume of seminiferous tubules, characterized by either greater total tubular length and/or diameter; the total physical area over which the blood-testis barrier was required would also be greater in such testes. In addition, if Sertoli cell formation is completed before puberty, as is widely believed (Clermont and Morgentaler, 1955; Clermont and Perey, 1957; Oakberg, 1959; Lino, 1971; Steinberger and Steinberger, 1971), the demands on individual Sertoli cells for maintaining the blood-testis barrier would increase during post-pubertal testicular growth. Thus, one might anticipate that Sertoli cell numbers would become an increasingly critical factor as a bull attained sexual maturity.

Differences in DSP among bulls are widely recognized (Elliott, 1967). Such variation was also noted among bulls in the present study, for which DSP ranged from 1.99 to 7.86 billion (mean: 3.79 billion). The causes(s) of such variation are not fully understood. However, given the known functions of Sertoli cells, such differences might result from: 1) differences in the absolute number of Sertoli cells where similarity exists among bulls in the number of germ cells supported per Sertoli cell, 2) differences in the relative number of germ cells supported per Sertoli cell while total Sertoli cell number remained similar among bulls or 3) some combination of these. To assess these possibilities, DSP was examined in relation to the absolute number of Sertoli cells and to the ratio of spermatids to Sertoli cells.

Daily sperm production was related to the absolute number of Sertoli cells in the present study. The relationship between these two variables was linear, as depicted in figure 2, and as described by the equation

\[ Y = 1.36 + 0.222X \]  

\((P<.005)\). The correlation \((r)\) between the number of Sertoli cells and DSP was \(.70\) \((P<.01)\). Interestingly, a 3.8-fold range in total Sertoli cell numbers was noted for these bulls, which was similar to the 4.0-fold range in DSP. From the present results, it is clear that DSP was highest in those bulls possessing the greatest number of Sertoli cells. This finding was similar to that observed in the human (Johnson et al., 1984), for which the correlation between DSP and the absolute number of Sertoli cells was also \(.70\).

Because both testicular size and DSP were positively correlated with the total number of Sertoli cells, it was not surprising that DSP and testicular weight were positively correlated \((P<.005)\) with each other (figure 3). The coefficient of correlation between DSP and parenchymal weight of \(.72\) for these young bulls \((P<.01)\) agrees closely with previously reported values (Amann and Almquist, 1962; Swierstra, 1966; Hahn et al., 1969).
Daily sperm production was unrelated to the S:SC ratio (P>.05, figure 4); the regression equation that best characterized the relationship was only significant at P<.10 and the coefficient of determination (R²) was only 6.5%. Interestingly, the S:SC ratio (Y) declined slightly in a linear manner as the total number of Sertoli cells (X, in billions) increased (P<.025, figure 5), as evident from the equation Y = 6.14 − .119X. However, the slope of the regression was small and the coefficient of correlation was only .42 (P<.05). Whereas DSP was associated with the absolute number of Sertoli cells, differences in DSP could not be attributed to the number of spermatids supported per individual Sertoli cell within this population of bulls.

To our knowledge, this is the first demonstration of a relationship between DSP and the absolute number of Sertoli cells in the bull. This finding may be of considerable importance to those investigators seeking to develop procedures for enhancing the sperm-producing capabilities of valuable breeding males, since it is consistent with the possibility that success will depend upon the ability to increase the absolute number of Sertoli cells. Moreover, since many researchers (Clermont and Morgentaler, 1955; Clermont and Perey, 1957; Oakberg, 1959; Lino, 1971; Steinberger and Steinberger, 1971) believe that Sertoli cell proliferation is confined to the prepubertal period, the present findings may stimulate a shift in the focus of research aimed at increasing DSP towards the prepubertal rather than the adult male.

These findings are consistent with the hypothesis that the numerical size of the Sertoli population might establish an upper limit for DSP of any given male. Nonetheless, the activity of individual Sertoli cells as an ultimate contributing factor must not be totally discounted. The bulls in this study were about 1.5 yr of age at castration. A marked progressive increase has been noted in sperm output from around puberty until at least 2 yr of age in Angus and Hereford bulls maintained on a frequent seminal collection schedule (Almquist and Cunningham, 1967; Almquist et al., 1976). Thus, it is clear that the testes of Angus and Hereford bulls are undergoing significant development at this age. Although the stability of the Sertoli cell population has recently been questioned in another species (Johnson and
with proportional increases in the number of germ cells prior to puberty and do not divide thereafter (Clermont and Morgentaler, 1955; Clermont and Perey, 1957; Oakberg, 1959; Lino, 1971; Steinberger and Steinberger, 1971). If so, age-related increases in DSP would coincide with proportional increases in the number of germ cells per Sertoli cell. It is possible that DSP increases up to the point at which individual Sertoli cells simply cannot support additional developing germ cells, at which time the absolute number of Sertoli cells would be more critical than at an earlier age. In this regard, it is of interest to note that the S:SC ratio was higher in those bulls having lower numbers of Sertoli cells (figure 5). If the inherent potential of each Sertoli cell is independent of the absolute numerical size of the Sertoli population, those young bulls with the highest S:SC ratio would already be functioning closer to their maximum potential, and their opportunity for increased DSP would be more limited.

Clearly, investigations similar to that reported herein, but with more mature bulls, would be of considerable interest. Nonetheless, the present findings amply demonstrate a high degree of association between the absolute number of Sertoli cells per paired testes and both testicular size and daily sperm production. Furthermore, they are consistent with the possibility that the absolute number of Sertoli cells may be an important factor in establishing the maximal rate of sperm production by any given bull.

**Figure 5.** The relationship between the number of step 8 spermatids per Sertoli cell at stage VIII of the cycle of the seminiferous epithelium, expressed as the spermatid to Sertoli cell ratio (Y), and the absolute number of Sertoli cells per paired testes (X, in billions) was described by the equation $Y = 6.14 - .119X$ ($P<.025$, $R = .42$).

Thompson, 1983; Berndtson and Jones, 1984), most investigators have held that Sertoli cells form prior to puberty and do not divide thereafter (Clermont and Morgentaler, 1955; Clermont and Perey, 1957; Oakberg, 1959; Lino, 1971; Steinberger and Steinberger, 1971). If so, age-related increases in DSP would coincide with proportional increases in the number of germ cells per Sertoli cell. It is possible that DSP increases up to the point at which individual Sertoli cells simply cannot support additional developing germ cells, at which time the absolute number of Sertoli cells would be more critical than at an earlier age. In this regard, it is of interest to note that the S:SC ratio was higher in those bulls having lower numbers of Sertoli cells (figure 5). If the inherent potential of each Sertoli cell is independent of the absolute numerical size of the Sertoli population, those young bulls with the highest S:SC ratio would already be functioning closer to their maximum potential, and their opportunity for increased DSP would be more limited.

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