Journal of Andrology, Vol. 11, No. 5, September/October 1990

Changing Relationships between Testis Size, Sertoli Cell Number and Spermatogenesis in Sprague-Dawley Rats

WILLIAM E. BERNDTSON AND TERRY L. THOMPSON

From the Department of Animal and Nutritional Sciences, University of New Hampshire, Durham, New Hampshire

Relationships between several reproductive characteristics were investigated in 25 Sprague-Dawley rats aged 60, 150, and 240 days (n = 75). Daily sperm production correlated with body weight (r = 0.63), paired testes weight (r = 0.68), testes weight as a percentage of body weight (r = −0.50), the number of spermatids supported per Sertoli cell (r = 0.51) and the number of Sertoli cells per gram (r = 0.89) or per testis (r = 0.95) among rats pooled across age groups. In general, the number and magnitude of significant coefficients of correlation were decreased when calculated within age groups. The latter often appeared to reflect a statistical consequence of relative homogeneity among rats rather than the absence of a biological relationship. However, the total number of Sertoli cells per testis correlated with daily sperm production within age groups, and could account for 85 to 94% of the variability in sperm production at 150 and 240 days, respectively.

Key words: reproduction, testes, gametogenesis, correlations, variability

J Androl 1990; 11:429–435.

Many important physiological relationships have been identified between factors such as age, testis size, Sertoli cell number, and efficiency of spermatogenesis in large domestic animals and humans by calculating coefficients of correlation (r) between variables (Amann and Almquist, 1962; Swierstra, 1968; Swierstra et al, 1975; Johnson et al, 1984a,b; Jones and Berndtson, 1986; Berndtson et al, 1987a,b; Neaves et al, 1987). Similar investigations using the laboratory rat, which serves extensively as a model for male reproductive research, appear much more limited (Amann, 1970; Robb et al, 1978), and the biological significance of correlations in the rat generally has received little attention.

Because a coefficient of correlation, by definition, measures the extent to which variability in one characteristic is associated with corresponding variability in another, variability must be present for a correlation to be detected. Variability is normal among living things, but most laboratory rats are the product of extensive inbreeding that has increased genetic and phenotypic homogeneity. Homogeneity can restrict opportunities for detecting actual relationships, yet it has rarely been acknowledged as a possible factor contributing to the absence of significant correlations in the rat.

Because the inherent variability of a number of reproductive characteristics among male rats is influenced by age (Berndtson and Thompson, 1990), comparisons of correlations among rats within dif-

Scientific contribution No. 1625 from the New Hampshire Agricultural Experiment Station.

Correspondence to: William E. Berndtson, Department of Animal and Nutritional Sciences, University of New Hampshire, Durham, NH 03824.

Received for publication January 29, 1990; accepted for publication April 10, 1990.

ferent age groups could be useful to distinguish whether small or non-significant correlations reflect an actual lack of a biological relationship, rather than a statistical consequence of extensive population homogeneity. This study was undertaken to examine possible biological relationships between body weight, testis size, Sertoli cell number, sperm production, and spermatogenic efficiency in Sprague-Dawley rats, with attention to the impact of sample population homogeneity on the magnitude of the coefficients of correlation between these variables.

Materials and Methods

Groups of 25 Sprague-Dawley male rats, aged 60, 150, and 240 days, were euthanized and their body weight and testes weights were recorded. These rats were also used in a larger study which, in part, assessed the influence of age on the number of rats needed to provide experiments of a known power and sensitivity for detecting treatment effects on various testicular end points (Berndtson and Thompson, 1990). One testis from each rat was assigned, on a random, alternating basis, for freezing and subsequent determination of daily sperm production (DSP). The other testis was processed for quantitative histometric analyses.

Daily Sperm Production

DSP was quantified via enumeration of homogenization-resistant elongated spermatids, as described by Robb et al (1978). Elongated spermatids were enumerated independently in duplicate by four persons; an evaluator performed additional counts when duplicate estimates differed by >12%. Numbers of elongated spermatids were converted to DSP using a time divisor of 6.1 days (Robb et al, 1978).

Histometric Analyses

Zenker–formol-fixed tissues were embedded in paraffin, sectioned at 5 μm, and stained with periodic acid-Schiff's reagent and hematoxylin. Sertoli cell nuclei with nucleoli and the nuclei of stage VII (Leblond and Clermont, 1952) germ cells were quantified by one individual (WEB) in 10 round, seminiferous tubular cross-sections per rat. This combination of rats × observations per rat had been estimated to provide a 90% chance for detecting a treatment (ie, age) difference ($P < 0.05$) of ≥10%, if such a difference actually existed (Berndtson et al, 1989). The resulting crude counts, which included whole nuclei (or nucleoli) and fragments produced by sectioning, were converted to true counts (whole cell equivalents) via Abercrombie's procedure (Abercrombie, 1946; Berndtson, 1977). Yields of various germ cells from their less advanced progenitors (a measure of spermatogenic efficiency) and the ratio of various germ cells to Sertoli cells (one measure of relative sperm production rates) were calculated from true count data.

Sertoli Cell Number

Sertoli cell numbers were estimated using the equation of Jones and Berndtson (1986), modified to reflect the appropriate time factor for the rat, as follows:

$$\text{Sertoli cell number} = \frac{(\text{Total spermatids} \div [\text{Spermatids/Sertoli cell}])}{0.473}$$

For this equation, "total spermatids" represents the number of spermatids in testicular homogentates, "spermatids/Sertoli cell" represents the ratio of spherical spermatids to Sertoli cells in stage VII (Leblond and Clermont, 1952) seminiferous tubular cross-sections, and "0.473" represents a time divisor for the presence of elongated spermatids for only 6.1 days of the 12.9 day (Clermont and Harvey, 1965) seminiferous epithelial cell cycle.

Statistical Analyses

Incomplete data were available for one of the 240-day-old and for two of the 150-day-old rats; all data for these rats were omitted. Since heteroscedasticity was noted due to age (Berndtson and Thompson, 1990), all data were transformed to $\log_{10}$ before conducting analyses of variance to test for age effects on individual characteristics (Steel and Torrie, 1960). Since some tubular cross sections did not contain spermatogonia, it was necessary to use individual rat means for each cell ratio (rather than all 10 observations per rat) for the transformation and subsequent analysis of the spermatid:spermatogonium and spermatogonium:Sertoli cell ratios. Where an F ratio was significant, differences among individual treatment (ie, age) means were examined by Tukey's w procedure (Steel and Torrie, 1960). Nontransformed data are presented in the text and tables, and were used to calculate all coefficients of correlation.

Results

The characteristics of rats of each age group are summarized in Table 1. Means were similar ($P > 0.05$, Table 1) for rats aged 150 and 240 days, and generally reflected more advanced development than of 60 days of age. Estimates of Sertoli cell numbers per testis at 60 days (not shown) were about 25% lower than those for the two older groups (Table 1), but must be considered inaccurate. As discussed by Jones and Berndtson (1986), Sertoli cell numbers per testis would be underestimated via their approach if spermatogenesis was inefficient. Although spermiogenic efficiency was not quantified, some advanced steps of spermatogenesis were less efficient or, more likely, had not yet attained mature levels by 60 days. For example, only 79% of the theoretical yield of four spermatids per preleptotene spermatocyte was observed in 60-

Table 1. *Mean (± SE) body weight, testis weight, germ cell and Sertoli cell numbers in 60, 150, and 240-day-old rats**

Item	Days of age		
	60	150	240
Body wt (g)	223.7 ± 2.1†	532.7 ± 4.3‡	548.2 ± 10.0‡
Testes wt			
Paired (g)	2.47 ± 0.04†	3.57 ± 0.05‡	3.62 ± 0.06‡
Paired (% of BW)	1.11 ± 0.02†	0.67 ± 0.01‡	0.66 ± 0.01‡
DSP (millions)			
Per gram	12.1 ± 0.58	13.8 ± 0.56	13.0 ± 0.79
Per testis	14.4 ± 0.83†	23.2 ± 0.83‡	22.4 ± 1.38‡
Germ cells/Sertoli cell			
Spermatogonia	0.12 ± 0.006	0.13 ± 0.006	0.12 ± 0.005
Preleptotene	2.4 ± 0.07	2.4 ± 0.03	2.5 ± 0.04
Pachytene	2.1 ± 0.07§	2.3 ± 0.04§,‖	2.3 ± 0.1‖
Round spermatid	7.6 ± 0.3†	9.1 ± 0.1‡	9.3 ± 0.1‡
Total germ cells	12.2 ± 0.4†	13.9 ± 0.2‡	14.1 ± 0.2‡
Spermatids/spermatogonium	64.9 ± 2.7§	75.0 ± 3.5‖	77.1 ± 2.9‖
Sertoli cells (millions)			
Per gram	¶	19.8 ± 0.9	18.1 ± 1.2
Per testis	¶	33.2 ± 1.4	31.1 ± 2.0

* n = 25, 23, and 24 for 60, 150, and 240-day-old rats, respectively.
†,‡ Means with unlike superscripts in the same row differ ($P < 0.01$).
§,‖ Means with unlike superscripts in the same row differ ($P < 0.05$).
¶ Not available.

day-old rats, vs. 95 and 93% at 150 and 240 days, respectively. Accordingly, data for total Sertoli cell numbers based on 60-day-old rats were excluded.

Coefficients of correlation between selected variables and paired testes weight are summarized in Table 2. For rats pooled across ages, paired testes weight was negatively correlated with testis weight as a percentage of body weight. In contrast, the corresponding correlations either were large and positive or non-significant when calculated within age groups. Also, testis weight was positively correlated with body weight for 240-day-old rats and for rats pooled across ages, but these variables were not correlated at 60 and 150 days. Except for a moderate, negative correlation between Sertoli cells per gram and paired testis weight at 150 days, Sertoli cell number was not correlated with testis weight for rats among or within age groups.

Correlations among selected variables and daily sperm production are shown in Table 3. Daily sperm production was correlated with the body weight of rats pooled across, but not within, age groups. Daily sperm production also was correlated with testes weight and testes weight as a percentage of body weight for rats pooled across age groups and at 60 days of age, but these variables were not correlated within groups of rats aged 150 or 240 days. In general, daily sperm production

Table 2. *Coefficients of correlation (r) between selected end points and paired testes weight*

Variable	Across ages*	Within age (days)		
		60	150	240
Paired testes wt as % BW	−0.76‡	0.87‡	0.87‡	0.37
Body wt	0.92‡	0.21	0.08	0.58‡
Sertoli cells/g	−0.12	—	−0.45†	0.12
Sertoli cells/testis	0.14	—	−0.15	0.32

* Data pooled across all ages, excluding data for Sertoli cells per gram or per testis in 60-day-old rats.
†,‡ Significant at $P < 0.05$ and $P < 0.01$, respectively.

Table 3. *Coefficients of correlation (r) between selected end points and daily sperm production*

Variable	Across ages*	Within age (days)		
		60	150	240
Body wt	0.63‡	−0.13	−0.16	0.27
Paired testes wt	0.68‡	0.66‡	−0.00	0.39
Testes wt as % BW	−0.50‡	0.72‡	0.08	0.13
Spermatids/ spermatogonium	0.08	0.08	−0.10	−0.40†
Spermatids/Sertoli cell	0.51‡	0.38	−0.24	0.26
Sertoli cells/g	0.89‡	—	0.84‡	0.92‡
Sertoli cells/testis	0.95‡	—	0.92‡	0.97‡

* Data pooled across all ages, excluding data for Sertoli cell number per gram or per testis in 60-day-old rats.
†,‡ Significant at $P < 0.05$ and $P < 0.01$, respectively.

was unrelated to the efficiency of spermatogenesis, as judged by the yield of spermatids per spermatogonium. Sperm production was only moderately correlated with the spermatid:Sertoli cell ratio among rats pooled across age groups, and these variables were unrelated within age groups. However, daily sperm production was highly correlated with Sertoli cell number, whether correlations were determined within or between age groups. In fact, total Sertoli cell number could account for 85 to 94% of the variability in daily sperm production among these rats (ie, $r^2 = 0.85$ to 0.94 for rats within or pooled across age groups).

Discussion

Coefficients of correlation often differed as a function of rat age and when calculated within vs. across age groups (Tables 2, 3). Consequently, generalized conclusions regarding possible biological relationships between variables must be made with caution. The presence of large positive or negative correlations is consistent with, but does not prove, the existence of a cause and effect relationship. The absence of a significant correlation may reflect the actual absence of a relationship or, alternatively, difficulty in detecting a correlation when variables are relatively homogeneous. Coefficients of variability for characteristics examined in this study are presented in Table 4 to assist in distinguishing among these possibilities. Also, it should be noted that these rats (Table 1) probably are representative of the laboratory rat population at large, since they were comparable to others of similar age for each characteristic examined (Mills et al, 1977; Robb et al, 1978; Johnson et al, 1980; Wing and Christensen, 1982).

Correlations with Paired Testes Weight

Age-related development undoubtedly contributed to the high correlation between body weight and testis weight for animals pooled across ages (Table 2). However, increases in body weight were proportionally greater than the increases in testis weight (Table 1). Therefore, the negative correlation between paired testes weight and testis weight as a percentage of body weight reflects the fact that the heaviest testes were present in older rats, for whom testis weight constituted a lower percentage of total body weight (Table 1).

Age-related differences are apparent for correlations between body and testes weights (Table 2). The differences at 150 vs 240 days were especially surprising, since mean body and testicular weights were similar at these ages (Table 1). It is notable that, within age groups, paired testes weight was positively correlated with body weight or with testis weight as a percentage of body weight, but not with both. In fact, these correlations are mutually exclusive. For example, paired testis weight and body weight were positively correlated at 240 days (Table 2). Therefore, at 240 days, larger testes were associated with greater body weights, rather than with increased testis weight as a percentage of body weight. The opposite situation was noted for 60- and 150-day-old rats.

The reason for the aforementioned age-related differences, rather than the actual differences, constitutes a biologically intriguing issue. Clearly the existence of a biological relationship between body weight and paired testes weight was supported by the finding of large positive correlations for rats at 240 days of age and for rats pooled across ages, but was not supported by the absence of correlations at 60 and 150 days. These inconsistencies may be attributed to differences in sample population homogeneities. Large among-animal differences were present within data pooled from all age groups (Table 4). Less variability was present within age groups, especially at 60 and 150 days (Table 4). Because body weight was very homogeneous among rats at 60 or 150 days, one would expect difficulty in detecting correlations with this variable even if a biological relationship did exist. Although laboratory rats are especially homogeneous, the tendency for correlations to be reduced when calculated within vs across age groups is probably applicable to most other species. For example, scrotal circumference was highly correlated ($r = 0.80$) among 411 6- to 72-month-old Holstein bulls, but the correlation was reduced to 0.58 when age effects were statistically removed (Coulter and Foote, 1977). Given the presence of a positive relationship at 240 days and among rats pooled from all age groups, we conclude that a positive biological relationship does exist between body weight and testes weight in the rat. At the same time, it is clear that this relationship is limited and that another factor(s) must contribute more substantially to differences in testis size, especially among rats of similar age.

The general lack of correlation between testes weight and Sertoli cell number was unexpected; these variables are highly correlated in the bull

(Berndtson et al, 1987a,b). The lack of significant correlation in the rat, and especially among rats pooled from the 150- and 240-day age groups (Table 2), cannot be attributed to excessive homogeneity of Sertoli cell number (Table 4). Although we are unable to explain these species differences, we must conclude that, in general, differences in the testicular weight of normal, untreated, sexually mature rats are attributable to a factor(s) other than Sertoli cell number.

Correlations with Daily Sperm Production

Since body growth and maximal spermatogenic activity develop progressively with age, up to a point, the positive relationship between these variables in rats pooled from all age groups was anticipated. However, because body weight varied minimally within age groups (Table 4), it is not clear whether the absence of a correlation within age groups is a statistical or a biological phenomenon. The former must be suspected due to the relationships between body weight and testis size and between testis size and daily sperm production described in this report.

Age-related development of testes weight and daily sperm production undoubtedly contributed to the correlation between these variables among rats pooled from all age groups. However, the observation that these variables were highly correlated among 60-day-old rats, but not among those aged 150 or 240 days (Table 3), was of some interest. These age-related differences cannot be attributed to different sample population homogeneities (Table 4). We suspect the correlation at 60 days may reflect relative differences in sexual maturity among rats; some 60-day-old rats may be more precocious than others and, therefore, possess heavier testes with more highly developed spermatogenesis. In contrast, testis size and spermatogenesis are maximal in all rats by 150 days; clearly the differences in sperm production within our 150- and 240-day-old populations must be attributed to a factor(s) other than testis size. This conclusion is not intended to imply the lack of a biological relationship between testis weight and sperm production in sexually mature rats. The minimal variability among testes weights within each age group (Table 4) would render detection of significant correlations unlikely. Thus, the age-related differences in the coefficients of correlation attest to the strength of the relationship in young rats, and not necessarily the total absence of such a relationship in older males. Indeed, an obligatory relationship would seem to exist between sperm production and space requirements for developing germ cells. Consistent with this view is the fact that positive correlations have been reported for many large domestic species, within which testis size and daily sperm production appear much more variable than among rats (Amann and Almquist, 1962; Swierstra, 1968; Swierstra et al, 1975; Berndtson et al, 1987a,b). Given these facts and the existence of correlations among 60-day-old rats and among rats pooled from all age groups, we believe that a relationship does exist between testis weight and sperm production of rats. However, since this relationship was not strong among sexually mature rats of the same age, it is evident that differences in sperm production within such populations are largely attributable to a factor(s) other than testis size.

Table 4. *Coefficients of variability (%) among rats for selected characteristics*

Variable	Across ages*	Within age (days)		
		60	150	240
Body wt	35.4	4.8	3.9	9.2
Testes wt				
Paired	19.5	9.1	11.4	12.7
Paired (% of BW)	29.1	9.6	11.3	13.6
DSP				
Per gram	54.4	23.8	18.7	36.7
Per testis	69.0	28.7	17.3	37.3
Spermatids/Sertoli cell	44.6	17.5	7.0	7.7
Spermatids/spermatogonium	21.5	20.7	22.2	18.3
Sertoli cells				
Per gram	27.1	—	22.7	31.2
Per testis	25.9	—	19.7	31.4

* Data pooled across all ages, excluding data for Sertoli cell number per gram or per testis in 60-day-old rats.

Spermatogenic efficiency is high among normal, untreated, sexually mature rats (Russell and Clermont, 1977), but did differ substantially among rats within age groups (spermatids/spermatogonium, Table 3). Since variability seemed adequate to permit the detection of significant correlations with daily sperm production if a relationship did actually exist, we must conclude from the absence of such correlations that spermatogenic efficiency (yield of spermatids per type A spermatogonium) was not an important factor contributing to differences in daily sperm production among normal, untreated rats. Spermatogenic efficiency has been identified as an important factor contributing to seasonal changes in equine

spermatogenesis (Berndtson et al, 1983), but could account for only 11.6% of the variability in sperm production among sexually mature Holstein bulls (Berndtson et al, 1987a).

The spermatid:Sertoli cell ratio has been used by numerous researchers to estimate relative changes in spermatogenesis due to treatment. It is equivalent to the more traditional method of expressing numbers of germ cells per seminiferous tubular cross-section, where germ cell counts are reported per a standard (but often unstated) number of Sertoli cells (Clermont and Morgentaler, 1955; Berndtson, 1977). The spermatid:Sertoli cell ratio is based on the assumption that changes in daily sperm production would alter the ratio of spermatids to a numerically stable Sertoli cell population (Clermont and Morgentaler, 1955; Berndtson, 1977; Russell and Peterson, 1984). Although this ratio is used as a method for quantifying spermatogenesis, the finding that these two variables were not correlated among rats within age groups (Table 3) should not be disturbing since this cell ratio is intended to measure relative, rather than absolute, rates of sperm production. Though one would expect parallel changes in the spermatid:Sertoli cell ratio and daily sperm production in response to experimental treatments, all rats in the present study were untreated. Indeed, the presence of a positive correlation between these two variables among rats pooled from all age groups (Table 3) attests to the similar direction of change in both variables with age (Table 1). It was of interest, however, to note that spermatid:Sertoli cell ratios were considerably more homogeneous than the absolute rates of sperm production (Table 4). Therefore, differences in sperm production among normal, untreated rats appear to be associated more with greater numbers of all cells (ie, germ cells and Sertoli cells) than with differences in germ cell:Sertoli cell ratios.

Large, positive correlations had been reported previously between total Sertoli cell number and the daily sperm production of bulls (0.70 and 0.83, Berndtson et al, 1987a,b) and humans (0.70, Johnson et al, 1984b). This study extends observations from two genetically and phenotypically diverse populations to a more highly inbred and homogenous one. Because population homogeneity tends to minimize the potential for obtaining large correlations, the fact that the corresponding correlations for rats across and within age groups equalled or exceeded 0.92 (range 0.92 to 0.97) is especially notable. The presence of such large correlations and the fact that daily sperm production was more highly correlated with Sertoli cell number than with any other variable constitute compelling evidence that Sertoli number is a key determinant of sperm production rates.

Significance of the Findings

As stated previously, correlations among reproductive characteristics of male rats had only occasionally been reported within the published literature. We suspect that this may reflect the preponderance of small, non-significant values which probably have aroused only limited interest. By discriminating, as much as possible, among correlations that may or may not have been limited due to extensive sample population homogeneity, several relationships resembling those reported for less homogeneous species have been exposed. Because the rat is used extensively as an experimental model, it is worth noting that the homogeneity among individuals of the same strain and age can be an advantage or disadvantage, depending on the purpose of a given experiment. The principal advantage of homogeneity is that as it increases, fewer replicate animals are needed for detecting treatment-induced alterations of a given magnitude (Berndtson, 1989; Berndtson et al, 1989, 1990). However, homogeneity makes it more difficult to identify natural factors that might explain inherent differences in sperm production, etc between individuals from observations of normal, untreated males. Recognition of normal sample population characteristics, including the anticipated coefficient(s) of variability among individuals, should facilitate the selection of the most suitable animal models for any experiment. Such information, when used as described herein, may help resolve what otherwise might appear to constitute inconsistencies among findings due to the age or species of the animals studied.

Acknowledgments

The authors are grateful to Sara Berndtson and Jeffrey Berndtson for skilled technical assistance, to Chris Neefus (Office of Biometrics) for statistical consultation and to Melanie Banker for typing this manuscript.

References

Abercrombie M. Estimation of nuclear population from microtome sections. *Anat Rec.* 1946; 94:239–247.

Amann RP. Sperm production rates. In: Johnson AD, Gomes WR, VanDemark NL, eds. *The Testis*. New York: Academic Press; 1970; 1:433–482.

Amann RP, Almquist JO. Reproductive capacity of dairy bulls. VIII. direct and indirect measurement of testicular sperm production. *J Dairy Sci*. 1962; 45:774–781.

Berndtson WE. Methods for quantifying mammalian spermatogenesis: a review. *J Anim Sci*. 1977; 44:818–833.

Berndtson WE. Sampling intensities and replication requirements for detection of treatment effects on testicular function in bulls and stallions: a statistical assessment. *J Anim Sci*. 1989; 67:213–225.

Berndtson WE, Thompson TL. Age as a factor influencing the power and sensitivity of experiments for assessing body weight, testis size and spermatogenesis in rats: Recommended replication for future studies. *J Androl*. 1990; 11:325–335.

Berndtson WE, Squires EL, Thompson DL Jr. Spermatogenesis, testicular composition and the concentration of testosterone in the equine testis as influenced by season. *Theriogenology*. 1983; 20:449–457.

Berndtson WE, Igboeli G, Parker WG. The numbers of Sertoli cells in mature Holstein bulls and their relationship to quantitative aspects of spermatogenesis. *Biol Reprod*. 1987a; 37:60–67.

Berndtson WE, Igboeli G, Pickett BW. Relationship of absolute numbers of Sertoli cells to testicular size and spermatogenesis in young beef bulls. *J Anim Sci*. 1987b; 64:241–246.

Berndtson WE, Neefus C, Foote RH, Amann RP. Optimal replication for histometric analyses of testicular function in rats or rabbits. *Fundam Appl Toxicol*. 1989; 12:291–302.

Clermont Y, Harvey SC. Duration of the cycle of the seminiferous epithelium of normal, hypophysectomized and hypophysectomized-hormone treated albino rats. *Endocrinology*. 1965; 76:80–89.

Clermont Y, Morgentaler H. Quantitative study of spermatogenesis in the hypophysectomized rat. *Endocrinology*. 1955; 57:369–382.

Coulter GH, Foote RH. Relationship of body weight to testicular size and consistency in growing Holstein bulls. *J Anim Sci*. 1977; 44:1076–1079.

Johnson L, Petty CS, Neaves WB. A comparative study of daily sperm production and testicular composition in humans and rats. *Biol Reprod*. 1980; 22:1233–1243.

Johnson L, Petty CS, Neaves WB. Influence of age on sperm production and testicular weights in men. *J Reprod Fertil*. 1984a; 70:211–218.

Johnson L, Zane RS, Petty CS, Neaves WB. Quantification of the human Sertoli cell population: its distribution, relation to germ cell numbers, and age related decline. *Biol Reprod*. 1984b; 31:785–795.

Jones LS, Berndtson WE. A quantitative study of Sertoli cell and germ cell populations as related to sexual development and aging in the stallion. *Biol Reprod*. 1986; 35:138–148.

Leblond CP, Clermont Y. Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Ann NY Acad Sci*. 1952; 55:548–573.

Mills NC, Mills TM, Means AR. Morphological and biochemical changes which occur during postnatal development and maturation of the rat testis. *Biol Reprod*. 1977; 17:124–130.

Neaves WB, Johnson L, Petty CS. Seminiferous tubules and daily sperm production in older adult men with varied numbers of Leydig cells. *Biol Reprod*. 1987; 36:301–308.

Robb GW, Amann RP, Killian GJ. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. *J Reprod Fertil*. 1978; 54:103–107.

Russell LD, Clermont Y. Degeneration of germ cells in normal, hypophysectomized and hormone treated hypophysectomized rats. *Anat Rec*. 1977; 187:347–366.

Russell LD, Peterson RN. Determination of the elongate spermatid–Sertoli cell ratio in various mammals. *J Reprod Fertil*. 1984; 70:635–641.

Steel RGD, Torrie JH. *Principles and Procedures of Statistics*. New York: McGraw-Hill Book Co, Inc; 1960.

Swierstra EE. A comparison of spermatozoa production and spermatozoa output of Yorkshire and Lacombe boars. *J Reprod Fertil*. 1968; 17:459–469.

Swierstra EE, Gebauer MR, Pickett BW. The relationship between daily sperm production as determined by quantitative testicular histology and daily sperm output in the stallion. *J Reprod Fertil*. 1975; 23(suppl):35–39.

Wing T-Y, Christensen AK. Mophometric studies on rat seminiferous tubules. *Am J Anat*. 1982; 165:13–25.