Testicular Weight, Sertoli Cell Number, Daily Sperm Production, and Sperm Output of Sexually Mature Rabbits after Neonatal or Prepubertal Hemicastration[1]

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ABSTRACT

The present study was conducted to investigate the influence of hemicastration and age at hemicastration on the subsequent testicular development of male rabbits through sexual maturity. Thirty New Zealand white rabbits were left intact or were hemicastrated on Day 35, 49, 77, or 105 postconception. Beginning at 7 mo of age, ejaculates were collected every other day for 1 mo, and the last ten ejaculates were used to quantify daily sperm output. At 8 mo, the rabbits were killed and their carcasses, testes, testicular capsules, and epididymides were weighed. Testicular tissue was processed for quantification of sperm production rates by enumeration of homogenization-resistant spermatids and via histometric evaluation. Regardless of the age at hemicastration, this manipulation did not alter the development of the remaining testis as assessed from testis weight, numbers of Sertoli cells per testis, daily sperm production, or sperm output ($p > 0.05$). On the basis of these findings, it would appear that the development of the spermatogenic capacity of the remaining testis of the rabbit is not altered appreciably by hemicastration at a young age.

INTRODUCTION

In many species, the removal of one testis elicits a compensatory hypertrophy of the remaining testis, which has been characterized chiefly as an increase in testicular weight [1–8]. This response appears to be age-dependent, rarely occurring when hemicastration is performed on adult animals [4, 9–13]. Increases in testicular size after hemicastration have been associated with an increase in daily sperm production (DSP). Thus through an understanding of the mechanisms responsible for compensatory hypertrophy, it may be possible to develop methods for clinical treatment of human infertility or methods of increasing sperm production in outstanding sires of economically important species.

It has been hypothesized that the hemicastration response is Sertoli cell-mediated. For most species, it is thought that Sertoli cell proliferation ceases at some point before puberty, establishing the maximum number of Sertoli cells available for germ cell support for adult reproductive function. If so, this may be in part the reason that hemicastration usually evokes its most profound effects when performed on prepubertal animals. However, it is not clear whether the published reports indicate a true hypertrophic response, in which the individual testis exceeds normal functional capacity, or merely a rapid growth to normal mature size and capacity. This question arises because most studies on hemicastration terminated before the males had attained the ages of peak sexual maturity [1, 5, 14] and also

because of the observation that the magnitude of the compensatory response tended to diminish in young males as the interval from hemicastration to evaluation increased. Furthermore, only a limited number of investigations have actually quantified the Sertoli cell response to hemicastration [5, 7, 11, 12, 15, 16], and this relationship has not been examined in the rabbit. Therefore, the objectives of the present study were 1) to determine whether hemicastration alters the numerical size of the Sertoli cell population in the remaining testis of the New Zealand white rabbit, and if so, to ascertain the effect of age of hemicastration on this response; and (2) to quantify the influence of prepubertal hemicastration on testis size and DSP in the remaining testis of sexually mature New Zealand white rabbits.

MATERIALS AND METHODS

Hemicastration

Thirty male rabbits from the University of New Hampshire breeding colony were supplied with food and water ad libitum. All males were weaned at 6 wk of age and randomly assigned to one of five groups: intact controls or Day 35, Day 49, Day 77, or Day 105 postconception hemicastrates. Hemicastration was performed on a postconception timetable to facilitate scheduling of surgeries and all subsequent activities. This was desirable since gestation length is variable in rabbits, ranging from 28 to 34 days in the present study.

Hemicastrations at Day 35 or 49 vs. 77 or 105 postconception were performed through abdominal or scrotal incisions, respectively. Left testes were removed from all hemicastrates. Surgeries were performed under general (xylazine, 5 mg/kg body weight; ketamine, 44 mg/kg body

weight) and local (lidocaine) anesthesia. Intact control males were maintained under conditions identical to those for the hemicastrates, but were not subjected to sham operations.

Ejaculate Collection

Beginning at 7 mo of age, males were allowed one false mount before ejaculates were collected via an artificial vagina, and ejaculates were collected every other day for a total of sixteen collections. The first six were used to stabilize sperm output and were not included in analyses [17]. Each ejaculate was diluted 1:40 in a 10% formol/0.9% saline solution; spermatozoa were counted independently by each of four observers via hemacytometers.

Tissue Collection

At 8 mo of age (273 days postconception), males were killed via T-160 euthanasia fluid and the remaining testis or testes (intacts) were removed. The weights of the epididymis, testicular capsule, testis, and body of each animal were recorded. Portions of testicular tissue were frozen to be used later for spermatid reserve counts [18] or processed for quantitative testicular histology.

Tissue Histology and Light Microscopy

Portions of testicular tissue were placed in Zenker-formol solution for 24 h, washed with running tap water for 24 h, and then transferred to 70% ethanol. Subsequently they were embedded in paraffin, sectioned at 4 μm, stained with periodic acid-Schiff reagent, and counterstained with Harris hematoxylin [19]. Stage I tubular cross sections were identified according to Swierstra and Foote [20]. The numbers of Sertoli cell nuclei with nucleoli and germ cell nuclei were recorded for ten round tubular cross sections per animal independently by each of two observers (i.e., 20 tubular cross sections per rabbit). Previous studies have established that the power and sensitivity of experiments are not enhanced appreciably by examinination of more than 8–12 tubular cross sections per rabbit [21]. The resulting crude counts, which represent both whole and sectioned nuclei, were then corrected to true counts (whole nuclei equivalents) through use of Abercrombie's correction factor [22].

Spermatid Reserves

Testicular parenchyma was thawed, minced with scissors in 5 ml of 0.05% Triton-X/0.9% saline homogenization fluid [18], and homogenized in 5 additional ml of homogenization fluid for 3 min. The blender was rinsed with up to 70 ml of homogenization fluid, depending upon the amount of original sample available. Diluted samples were stored at 4°C and counted within 10 h of preparation. Duplicate evaluations within 10% of each other were made independently for each sample by each of four observers with a hemacytometer.

Sertoli Cell Numbers

The numbers of Sertoli cells per testis or per gram of testicular parenchyma were calculated via a previously published method [23–25] on the basis of the following equation:

$$\text{No. of Sertoli cells} = \frac{\text{No. of spermatids}}{\text{No. of spermatids per Sertoli cell}} \times \text{Correction factor}$$

In this equation, the number of spermatids (per gram or per testis) equals the number of elongated spermatids in testicular homogenates (per gram or per testis); the number of spermatids per Sertoli cell equals the number of spermatids per Sertoli cell as determined from the histometric analysis; and the correction factor adjusts for the fact that homogenization-resistant elongated spermatids are not present at all stages of the cycle of the seminiferous epithelium. For the rabbit, we used a correction factor of 3.204, reflecting the fact that elongated spermatids are present for only 3.33 days of the 10.67-day seminiferous epithelial cycle [17] (i.e., 10.67/3.33 = 3.204).

Statistical Methods

Data were examined through one-way analysis of variance using Lotus 1–2–3 and Minitab statistical packages. Where a significant treatment effect was found, differences among individual group means were assessed by Tukey's test [26].

RESULTS

Mean body weights and reproductive organ weights are summarized in Table 1. Hemicastration was without effect ($p > 0.05$) on body weight, right testis weight (actual weight or right testis weight as a percent of body weight), the weight of the testicular capsule, or the weight of the right epididymis.

Hemicastration also was without effect ($p > 0.05$) on the numbers of homogenization-resistant spermatids per testis or per gram of testicular parenchyma (Table 2). In addition, hemicastration did not alter the mean numbers of sperm per ejaculate ($p > 0.05$), whether this was expressed on a per rabbit or on a per testis basis (Table 2).

The mean numbers of Sertoli cells per testis or per tubular cross section (Table 2) also were not altered ($p > 0.05$) by hemicastration. However, the numbers of Sertoli cells per gram of testicular tissue (Table 2) were significantly ($p < 0.05$) lower for hemicastrates than for the intact males. The number of type A spermatogonia per Sertoli cell was not altered via hemicastration at any age ($p > 0.05$). In contrast, the numbers of young primary spermatocytes per Sertoli cell were similar among controls and rabbits hemicastrated on Day 49 postconception, but were increased above control values ($p < 0.01$) for rabbits hemicastrated on Days 35, 77, and 105 postconception. However,

TABLE 1. Mean (±SD) body and reproductive organ weights of male rats.

End point	Age at hemicastration (days postconception)				
	Intact[a]	35[a]	49[a]	77[a]	105[a]
Body wt. (kg)	3.73 ± .38	3.34 ± .40	3.87 ± .54	3.92 ± .67	3.51 ± .29
Right testis					
Actual wt. (g)	2.52 ± .38	3.53 ± .59	3.09 ± .63	3.17 ± 1.48	2.62 ± .65
% of body wt.	0.07 ± .01	0.12 ± .02	0.08 ± .01	0.08 ± .03	0.08 ± .02
Capsule wt. (g)	0.11 ± .03	0.10 ± .03	0.11 ± .03	0.14 ± .06	0.11 ± .04
Epididymal wt. (g)	1.10 ± .22	1.17 ± .17	1.24 ± .22	1.19 ± .20	1.27 ± .24

[a]The numbers of intact males and Day 35, 49, 77, and 105 hemicastrates equalled 7, 5, 6, 6, and 6, respectively.

the numbers of old (pachytene) primary spermatocytes and spermatids per Sertoli cell did not differ among treatment groups (Table 2).

DISCUSSION

Data from the present study suggest that hemicastration did not induce a true compensatory hypertrophy in these rabbits, since neither testis weight nor sperm production was altered ($p > 0.05$). The occasional findings of statistically significant treatment effects (Table 2) are indeed suspect since they are difficult to reconcile with other data and do not fit any apparent general pattern. For example, although hemicastration increased the ratio of young primary spermatocytes to Sertoli cells in Day 35, 77, and 105 hemicastrates, the response was not accompanied by a corresponding increase in the ratios of type A spermatogonia, old primary spermatocytes, or round spermatids to Sertoli

cells or in overall daily sperm output (DSO). Similarly, while the number of Sertoli cells per gram of testicular tissue was lower in hemicastrates ($p < 0.05$), this measure is directly related to testis weight and the number of Sertoli cells per testis, neither of which was altered ($p > 0.05$) by hemicastration.

It should be noted that the focus of the present study was the assessment of potential effects of hemicastration on the spermatogenic elements of the rabbit testis. Potential effects on the interstitial tissue or endocrine functions of the remaining testis were not assessed, but have been examined by others in the rat [16]. In rats, neonatal hemicastration has been found to elicit an increase in plasma levels of FSH [16]. Hypertrophy or hyperplasia of the interstitial elements could result in a slight increase in testis weight without associated changes in numbers of germ cells, Sertoli cells, or various cell ratios. However, an increase in testis weight attributable to the interstitial components alone

TABLE 2. Sperm production, sperm output, and Sertoli cell numbers in intact and hemicastrated rabbits (Mean ± SD).

End point	Age at hemicastration (days postconception)				
	Intact	35	49	77	105
Spermatids[ab]					
Per testis	3.67 ± 1.04	4.64 ± 1.25	3.78 ± 1.17	3.96 ± 2.17	3.35 ± 0.85
Per gram[c]	1.39 ± 0.25	1.30 ± 0.19	1.24 ± 0.31	1.25 ± 0.24	1.28 ± 0.21
Sperm per ejaculate[b]					
Actual	2.28 ± 0.94	1.83 ± 0.48	1.77 ± 0.51	1.49 ± 1.48	1.13 ± 0.30
Per testis[d]	1.14 ± 0.47	1.83 ± 0.48	1.77 ± 0.51	1.49 ± 1.48	1.13 ± 0.30
Sertoli cells					
Per tubular cross section	9.95 ± 0.58	10.07 ± 1.26	10.03 ± 1.22	9.18 ± 0.76	9.46 ± 1.17
Per gram[ef]	44.60 ± 10.25[g]	35.99 ± 6.33[h]	34.45 ± 9.28[h]	30.24 ± 4.38[i]	33.99 ± 6.04[h]
Per testis[f]	111.02 ± 27.78	128.00 ± 34.50	103.94 ± 23.98	95.06 ± 49.22	88.23 ± 21.16
Germ cells per Sertoli cell					
Type A spermatagonia	0.21 ± 0.02	0.21 ± 0.05	0.28 ± 0.08	0.25 ± 0.03	0.24 ± 0.02
Spermatocytes:					
Young 1°	2.84 ± 0.36[j]	3.74 ± 0.59[k]	3.13 ± 0.41[jl]	3.66 ± 0.43[k]	3.43 + 0.39[kl]
Old 1°	2.85 ± 0.38	3.63 ± 0.69	3.22 ± 0.68	3.49 ± 0.35	3.47 ± 0.49
Round spermatids	10.99 ± 1.51	12.39 ± 2.91	11.90 ± 1.98	13.84 ± 1.48	13.32 ± 1.73

[a]Homogenization-resistant spermatids.
[b]×100 million.
[c]Per gram of testicular parenchyma.
[d]Sperm per ejaculate/2 for intact males.
[e]Per gram of testicular parenchyma.
[f]×1 million.
[g-i]Means within the same row that do not bear a similar superscript differ ($p < .05$).
[j-l]Means within the same row that do not bear a similar superscript differ ($p < .01$).

would contribute to a decrease in the number of Sertoli cells per gram of testis. Accordingly, compensatory hypertrophy of the interstitial tissue could also be considered as a factor possibly contributing to some of the observations noted in the present study. Since the interstitial tissue constitutes a relatively small proportion of the testicular parenchyma of normal males (e.g., 10%), substantial hypertrophy of this component would result in only relatively small increases in overall testis weight.

Although differences usually were not statistically significant, it can be noted that actual means for many of the end points appeared larger within some hemicastrated groups. For example, Day 35 postconception hemicastrates had the greatest mean values for the number of spermatids per testis, sperm per ejaculate per testis, Sertoli cells per seminiferous tubular cross section and per testis, and numbers of young and old primary spermatocytes per Sertoli cell (Table 2). Because means tended to be larger for so many end points within this group, it might be tempting to speculate that some compensatory hypertrophy may have occurred. But this line of thought is risky since many of these end points are not independent. For example, DSO is highly correlated with testis weight in rabbits [27], and high correlations have been reported between testis size and Sertoli cell number in bulls [24] and between Sertoli cell number and DSO in humans [28]. Thus, animals with larger testis weights would be expected to have higher DSO, Sertoli cell numbers, and DSP. The issue, therefore, is whether observed differences among the treatment groups arose as a result of treatment or by chance. The absence of any statistical significance indicated that the observed differences in actual means are within the normal ranges to be expected (in the absence of a treatment effect) due to random assignment of 5–7 animals to individual treatment groups (i.e., due to chance).

It should be noted that several objective, quantitative measures were used to assess results in the present investigation. Some, such as testicular weight, have been examined extensively by others. However, few investigators have employed such rigorous assessments of DSP as were used here. Indeed, DSP was assessed via histometric methods, via the enumeration of homogenization-resistant spermatids, and via determination of DSO during intervals of regular frequent ejaculation. It is notable that hemicastration was without effect on DSP as judged through any of these methods. Similarly, few investigators have attempted to quantify Sertoli cell numbers or to examine the germ cell:Sertoli cell ratios as a means of assessing potential mechanisms for any hypertrophic response. With the exception of greater numbers of young primary spermatocytes per Sertoli cell in Days 35, 77, and 105 postconception hemicastrates, no evidence of true compensatory hypertrophy was observed in the present study.

The absence of compensatory hypertrophy of the rabbit testis in the present study was surprising, since substantial hypertrophic responses have been reported frequently with several other species [1, 3, 4, 6, 8, 10, 11, 13, 15, 29–38]. However, many others have seen a relatively consistent and predictable decline in the magnitude of response as the age of hemicastration was increased [2, 4–6, 8, 13, 15], or when hemicastration effects were assessed at longer intervals (i.e., greater ages) post-treatment. Our evaluations were performed on males aged 8 mo, the age previously shown to coincide with maximal testicular development [27]. Evidence from the current and previous studies [14, 32, 39, 40] using adult hemicastrated rabbits suggests that in rabbits, hemicastration may not cause a compensatory hypertrophy in the remaining testis. Not only did we not detect a compensatory hypertrophy, there was also no effect due to increasing age at the time of hemicastration ($p > 0.05$) for most end points.

It has been speculated that the compensatory hypertrophic response seen in most species may be Sertoli cell-mediated, and that neonatal or prepubertal hemicastration may be altering the normal proliferative processes of Sertoli cells [41]. In support of this, Orth et al. [16] found that incorporation of [³H]thymidine by Sertoli cell nuclei is elevated during the interval from 8 h to 4 days after hemicastration of 3-day-old male rats. In most species, Sertoli cell proliferation is thought to cease early during neonatal or prepubertal development, establishing the maximal number of Sertoli cells available thereafter. In the rabbit, Sertoli cell proliferation essentially ceases by the seventh week of life [42, 43]. In our study, the first three treatments were within the time frame of this proliferative period, at Days 35 (3 days old), 49 (2 wk old), and 77 (6 wk old) postconception. If hemicastration alters Sertoli cell proliferation, responses should have occurred in rabbits hemicastrated during this time period.

There are several possible explanations for the differences between our findings and those of others. First, the differences in response to hemicastration could reflect differences among species. However, there are no physiological species differences known to us that would allow for the lack of effect in rabbits when there are reportedly such dramatic responses to hemicastration in many other species. Another possible explanation is that the hypertrophic response in rabbits may have been too small to have been detectable with 5–7 rabbits per treatment group. In that regard, Table 3 summarizes the replication requirements for detection of statistically significant differences for various end points. These requirements were estimated by use of previously published procedures [44] from the coefficients of variability (CV) among the intact rabbits in this study. For example, a minimum of 5 animals per treatment group would be needed to ensure a 90% probability for detecting a large (40%) statistically significant change in testis weight (CV = 15.4%). The numbers of animals in our study should have been sufficient for detection of increases in testis weight of the magnitude reported in the literature.

TABLE 3. Number of rabbits needed per treatment group to provide a 90% chance for detecting a statistically significant difference ($p < .05$) from the control.[a]

End point	CV(%)	Difference from control (%)				
		10	20	30	40	50
Body wt.	10.2	23	7	4	3	3
Testis wt.	15.4	55	15	8	5	4
Epididymal wt.	20.2	85	23	11	7	5
Capsule wt.	23.4	85	23	11	7	5
Spermatids/testis	28.4	190	49	23	13	9
DSO[b]/rabbit	41.4	337	85	39	23	13
Sertoli/cross section	5.9	7	3	3	—	—
A[c]/Sertoli	11.4	32	9	5	4	3
YP[d]/Sertoli	12.8	32	9	5	4	3
OP[e]/Sertoli	13.4	43	12	6	4	3
Spermatid/Sertoli	13.7	43	12	6	4	3
Sertoli/testis	25.0	132	34	16	10	7

[a]Replication requirements were estimated from the coefficient of variability (CV) among control rabbits via the procedure of Berndtson [44].
[b]DSO = daily sperm output.
[c]Type A spermatagonia.
[d]Young 1° spermatocytes.
[e]Old 1° spermatocytes.

However, for the detection of a 10% change in testis weight, for example, at least 55 rabbits would have been needed per treatment group. Therefore, if there were a small increase in testicular weight as a result of hemicastration, it is unlikely that our experiment would have been sensitive enough to detect it. One should also note the very large number of rabbits needed per group for detecting changes in daily sperm output (DSO, Table 3). This undoubtedly explains why an anticipated 50% decrease in sperm output after hemicastration was not detectable in the absence of compensatory hypertrophy. For such reasons, one must be cautious in regarding the data in the present study as proof that compensatory hypertrophy does not occur. At the same time, the data in Table 3 do indicate that relatively small-to-moderate treatment effects should have been detectable with only 5–7 rabbits per treatment group if manifested for certain end points (e.g., Sertoli cells per tubular cross section and germ cell-to-Sertoli cell ratios). On that basis, and given the findings discussed above, it seems reasonable to conclude that compensatory hypertrophy either does not occur in the rabbit or, if it does, the magnitude of the response is probably quite limited.

As discussed above, few researchers have allowed animals to reach full sexual maturity before evaluating the testis remaining after hemicastration. By evaluating our rabbits at full sexual maturity (8 mo of age or 273 days postconception), we could have missed any transient hypertrophy that might have occurred (i.e., a response limited merely to a more rapid attainment of normal mature testis size and function). Indeed, for several species examined by others [6, 15, 31], it seems most likely that at least some portion of the compensatory hypertrophy of the remaining testis is transient. This view is supported by the trend in numerous studies [1, 4, 8, 13] to record substantially less hypertrophy

as the age of hemicastration is increased or when the response to unilateral castration at an early age is evaluated at a time closer to that of normal male sexual maturity. Whereas additional studies would be needed to determine whether a transient hypertrophy of the testis occurs in the rabbit, our findings indicate that a true, permanent compensatory hypertrophy either does not occur or is likely to be of very limited magnitude in the rabbit. Furthermore, the large number of rabbits needed to enable detection of true hypertrophy would seem to render the hemicastrated rabbit an unsuitable model for evaluation of factors that affect testis size, sperm production, or Sertoli cell numbers.

REFERENCES

1. Boockfor FR, Barnes MA, Kazmer GW, Halman RD, Bierley ST, Dickey JF. Effects of unilateral castration and unilateral cryptorchidism of the Holstein bull on plasma gonadotropins, testosterone and testis anatomy. J Anim Sci 1983; 56:1376–1385.
2. Brown JL, Chakraborty PK. Comparison of compensatory pituitary and testicular responses to hemicastration between prepubertal and mature rats. J Androl 1991; 12:119–125.
3. Brown JL, Stuart LD, Chakraborty PK. Testicular hypertrophy after hemicastration of prepubertal rams is not associated with altered spermatogenic efficiency. Biol Reprod 1986; 34(suppl 1):58 (abstract 17).
4. Hoagland TA, Ott KM, Dinger JE, Mannen K, Woody CO, Riesen JW, Daniels W. Effects of unilateral castration on morphologic characteristics of the testis in one-, two-, and three-year-old stallions. Theriogenology 1986; 26:397–405.
5. Hochereau-de Reviers MT, Pelletier J, de Reviers MM. Effect of hemicastration on young and adult male rats. J Reprod Fertil 1972; 31:519 (abstract).
6. Jenkins N, Waites GMH. Effects of hemicastration at various ages and of oestradiol-17β on plasma concentrations of gonadotrophins and androgens, testicular growth and interstitial cell responses in prepubertal lambs. J Reprod Fertil 1983; 67:325–334.
7. Kosco MS, Loseth KJ, Crabo BG. Development of the seminiferous tubules after neonatal hemicastration in the boar. J Reprod Fertil 1989; 87:1–11.
8. Pierce RA, Riesen JW, Woody CO. Compensatory hypertrophy of guinea pig testes. J Anim Sci 1972; 35:251 (abstract 334).
9. Bergh A, Damber JE, Lindgren S. Compensatory hypertrophy of the Leydig cells in hemiorchidectomized adult rats. Experientia 1982; 38:597–598.
10. Hochereau-de Reviers MT, Loir M, Pelletier J. Seasonal variations in the response of the testis and LH levels to hemicastration of adult rams. J Reprod Fertil 1976; 46:203–209.
11. Mirando MA, Hoagland TA, Woody CO, Reisen JW. Sertoli cell number remains constant following testicular compensatory hypertrophy in the ram. Biol Reprod 1986; 34(suppl 1):105 (abstract 112).
12. Mirando MA, Hoagland TA, Woody CO Jr, Riesen JW. The influence of unilateral castration on testicular morphology and function in adult rams. Biol Reprod 1989; 41:798–806.
13. Ott KM, Mannen KA, Dinger JE, Hoagland TA, Woody CO, Riesen JW. Effects of unilateral castration (UC) on the physiological and morphological characteristics of stallions testes. J Anim Sci 1984; 59(suppl 1):355 (abstract).
14. Lipschutz A. The so-called compensatory hypertrophy of the testicle after unilateral castration. J Physiol (Lond) 1922; 56:451–458.
15. Cunningham GR, Tindall DJ, Huckins C, Means AR. Mechanisms for the testicular hypertrophy which follows hemicastration. Endocrinology 1978; 102:16–23.
16. Orth JM, Higginbotham CA, Salisbury RL. Hemicastration causes and testosterone prevents enhanced uptake of [³H]thymidine by Sertoli cells in testes of immature rats. Biol Reprod 1984; 30:263–270.
17. Amann RP. A critical review of methods for evaluation of spermatogenesis from seminal characteristics. J Androl 1981; 2:37–58.
18. Amann RP, Lambiase JT Jr. The Male Rabbit. III. Determination of daily sperm production by means of testicular homogenates. J Anim Sci 1969; 28:369–374.
19. Berndtson WE, Desjardins C. The cycle of the seminiferous epithelium and spermatogenesis in the bovine testis. Am J Anat 1974; 140:167–180.
20. Swierstra EE, Foote RH. Duration of spermatogenesis and spermatozoan transport in the rabbit based on cytological changes, DNA synthesis and labeling with tritiated thymidine. Am J Anat 1965; 116:401–411.
21. 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.

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

23. 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.

24. 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 1987; 37:60–67.

25. Berndtson WE, Thompson TL. Changing relationships between testis size, Sertoli cell number and spermatogenesis in Sprague-Dawley rats. J Androl 1990; 11:429–435.

26. Steel RGD, Torrie JH. Principles and Procedures of Statistics, 2nd ed. New York: McGraw-Hill Book Co., Inc.; 1980: 191–192.

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

28. 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 1984; 31:785–795.

29. Al-Haboby AH, Loseth KJ, Crabo BG. Influence of neonatal hemiorchidectomy of bulls on compensatory development of tubular and interstitial testicular tissues. In: 11th Int Congr Anim Reprod Artif Insem; 1988; Dublin, Ireland. Abstract 365.

30. Barnes MA, Kazmer GW, Boockfor FR, Wade RJ, Halman RD, Dickey JF. Testosterone, luteinizing hormone, follicle stimulating hormone, and prolactin response to unilateral castration in prepubertal Holstein bulls. Theriogenology 1982; 19:635–646.

31. Putra DKH, Blackshaw AW. Quantitative studies of compensatory testicular hypertrophy following unilateral castration in the boar. Aust J Biol Sci 1985; 38:429–434.

32. Paufler SK, Foote RH. Semen quality and testicular function in rabbits following repeated testicular biopsy and unilateral castration. Fertil Steril 1969; 20:618–625.

33. Brown JL, Stuart LD, Chakrabory PK. Endocrine profiles, testicular gonadotropin receptors, and sperm production in hemi-castrated ram lambs. J Anim Sci 1987; 65:1563–1570.

34. Hochereau-de Reviers MT, Land RB, Perreau C, Thompson R. Effect of season of birth and of hemicastration on the histology of the testis of 6-month-old lambs. J Reprod Fertil 1984; 70:157–163.

35. Voglmayr JK, Mattner PE. Compensatory hypertrophy in the remaining testis following unilateral orchidectomy in the adult ram. J Reprod Fertil 1968; 17:179–181.

36. Waites GMH, Wenstrom JC, Crabo BG, Hamilton DW. Rapid compensatory hypertrophy of the lamb testis after neonatal hemiorchidectomy: endocrine and light microscopal morphometric analyses. Endocrinology 1983; 112:2159–2167.

37. Moger WH. Endocrine responses of the prepubertal male rat to hemiorchidectomy. Biol Reprod 1977; 17:661–667.

38. Putra DKH, Blackshaw AW. Morphometric studies of compensatory testicular hypertrophy in the rat after hemicastration. Aust J Biol Sci 1982; 35:287–293.

39. Edwards J. The effect of unilateral castration on spermatogenesis. R Soc Lond Proc B 1940; 128:407.

40. Mirando MA, Sopelak VM, Woody CO, Hoaglund TA, Riesen JW. Influence of unilateral castration on FSH and LH in serum and testosterone (T) and dihydrotestosterone (DHT) in rete testis fluid of mature rabbits. Biol Reprod 1988; 38(suppl):169 (abstract 363).

41. Orth JM. Proliferation of Sertoli cells in fetal and postnatal rats: a quantitative autoradiographic study. Anat Rec 1982; 203:485–492.

42. Sun EL, Gondos B. Proliferative activity in the rabbit testis during postnatal development. In: Byskov AG, Peters H (eds.), Development and Function of Reproductive Organs. Amsterdam: Exerpta Medica; 1981: 140–148.

43. Gondos B, Byskov AG. Germ cell kinetics in the neonatal rabbit testis. Cell Tissue Res 1981; 215:143–151.

44. Berndtson WE. A simple, rapid and reliable method for selecting or assessing the number of replicates for animal experiments. J Anim Sci 1991; 69:67–76.