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Quantitative Studies of Compensatory Testicular Hypertrophy Following Unilateral Castration in the Boar

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Abstract

Unilateral castration of Large White × Landrace boars at monthly intervals up to 5 months of age, with the remaining testis being removed 2 months later, resulted in compensatory hypertrophy of the testis which decreased with age. In pigs 3 and 4 months old there was significant hypertrophy of the testis but at 5 and 7 months of age testicular weight of the hemicastrates did not differ significantly from control values. The increase in the testicular weight of unilaterally castrated pigs was correlated with an increase in the number of Sertoli and germ cells at 3 months of age and germ cells at 4 months of age occupying the seminiferous epithelium. This was correlated with increased total seminiferous tubule length and larger cross-sectional area of the tubule. Sertoli cell occupancy did not differ significantly between unilaterally castrated and intact boars.

Introduction

It has long been known that unilateral castration of immature animals results in hypertrophy of the remaining testis. Although this phenomenon has been examined mainly in the rat a few studies of unilateral castration of the boar have been made, and compensatory hypertrophy of the remaining testis demonstrated (Hauser *et al.* 1952; Sundby *et al.* 1981). In these studies hypertrophy was judged principally from an increase in testicular weight. In other species, however, work has indicated that enlargement of the remaining testis was associated with increased numbers of Sertoli cells (Hochereau-de Reviers 1975; Cunningham *et al.* 1978) or germ cells (Santolaya and Burgos 1978; Barnes *et al.* 1980) within the seminiferous epithelium, and with an increase in diameter (Voglmayr and Mattner 1968; Walton *et al.* 1980) or length (Hochereau-de Reviers *et al.* 1976; Leidl *et al.* 1980) of the seminiferous tubule. Moreover, hemicastration performed in prepubertal rats and calves resulted in an increase in Sertoli cell and stem spermatogonial numbers, whereas in post-pubertal rats, it led to an increase in stem spermatogonia but not in Sertoli cell numbers (Hochereau-de Reviers and Courot 1978). It had been found earlier that Sertoli cells do not divide in the adult animal (Steinberger and Steinberger 1971).

As the nature of hypertrophy of the boar testis occurring after hemicastration does not appear to have been adequately described, the present study was designed to examine whether compensatory testicular hypertrophy following unilateral castration of the boar was due to an increase in both Sertoli and germ cell numbers in young animals or to an increase in germ cell numbers only in the mature boars.

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Materials and Methods

Large White × Landrace males were maintained on commercial pig pellets, with free access to water. Hemicastration was performed through a scrotal incision under general anaesthesia (intramuscular azaperone, 2 mg/kg, followed by intravenous thiopentone, 11 mg/kg body weight). Five animals were allotted to each group and alternate left and right testis were removed from entire boars at 1, 2, 3, 4 and 5 months of age. Testes collected at hemicastration were considered as intact or control. Two months later the remaining testis was removed, and these animals were designated as hemicastrates at 3, 4, 5, 6 and 7 months of age. The testes taken from hemicastrates were compared with those from entire boars of the same age.

Testicular samples were immediately fixed in Susa's fixative for at least 24 h. The tissue was embedded in paraffin, sectioned at 7 μm and stained with haematoxylin and eosin (Humason 1972).

Quantitative morphological studies were performed using stereological techniques (Putra and Blackshaw 1982). The 42-point graticule of Weibel *et al.* (1966) which was used has an area containing 21 lines of constant length arranged in seven equidistant and parallel rows. Calibration of the area of the graticule for each magnification used enabled tubular length and cross-sectional area to be estimated. The slides were assessed for seminiferous tubule length (×125) (Elias *et al.* 1971), cell number (×1250) (Aherne 1967; Underwood 1970) and the volume fraction (%) of the testicular components (×125) (Weibel *et al.* 1966). Cross-sectional area of the seminiferous tubule (Weibel *et al.* 1966) was assessed at magnifications depending on the size of the tubule. The occupancy of Sertoli cells by germ cells was determined by the ratio of germ cell and Sertoli cell numbers.

The results were transformed to angles (volume fractions) or to logarithms for analyses of variance. Tabular means are either angles retranslated to percentages (volume fractions) or geometric. Standard errors are derived from the error mean squares of the analyses of variance and in the case of the geometric means are expressed as factors. When a significant difference between treatments was obtained the method of least significant difference (L.S.D.) was applied.

Results

The age at which unilateral castration was performed and the effects of hemicastration on the remaining testis of the boar are shown in Table 1. Hemicastration increased raw testicular weight, the response being significant at 3, 4 and 6 months of age but there was no significant response at 5 and 7 months. However, the normalized testicular weight (weight per 100 kg of body weight) for hemicastrated boars was significantly higher at all ages, except at 7 months, than that for intact animals. The percentage of hypertrophy of the remaining testis, based on the normalized testis weight, decreased with age, falling from 145% at 3 months of age to 17% at 7 months (Table 1).

Quantitative histological data (Table 2) showed that Sertoli cell number per testis increased following hemicastration ($P < 0{\cdot}01$). The increase was highly significant in animals at 3 months of age, but in older animals there was no significant change in the number of Sertoli cells. The number of germ cells per testis was also increased in unilaterally castrated animals observed at 3 and 4 months of age. The diminishing response of the change in number of germ cells to hemicastration was clearly shown by the highly significant linear interaction with age. Sertoli cell occupancy, on the other hand, was unaltered by hemicastration.

The cross-sectional areas of the seminiferous tubules of hemicastrated boars at 3 and 4 months of age were larger ($P < 0{\cdot}05$) than those of controls. The length of the seminiferous tubules per testis was only significantly increased in 3-month-old pigs and not at any other time. However, there was no significant difference in relative volumes of seminiferous tubules and interstitial cells between the two groups.

Discussion

The present study has confirmed the occurrence of compensatory testicular hypertrophy after unilateral castration performed on young and maturing boars up

to 7 months of age. The extent of compensatory hypertrophy of the remaining testis was inversely related to sexual maturation, as has previously been demonstrated in the rat (Ojeda and Ramirez 1972; Cunningham *et al.* 1978; Putra and Blackshaw 1982).

Table 1. Weight changes in the remaining testis of the boar following unilateral castration
Mean values for the remaining testis in boars hemicastrated at different ages are compared with means for the equivalent testis in intact animals; a,b, = significantly different from the corresponding value for intact group: a, $P < 0{\cdot}05$; b, $P < 0{\cdot}01$; $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$

Age (months)	Body wt (kg)	Testis wt (g)	Testis wt/100 kg body wt (g)	Compensatory hypertrophy (%)[A] Testis wt	Testis wt/100 kg body wt
		Intact boars (5 animals in each group)			
3	32·4	14·4	45·4		
4	41·5	43·2	84·2		
5	70·6	106·3	150·4		
6	70·8	173·7	245·4		
7	101·6	220·5	217·0		
		Boars hemicastrated 2 months previously (5 animals in each group)			
3	26·0	29·0[b]	111·5[b]	101·4	145·6
4	47·8	80·8[b]	169·0[b]	87·0	100·7
5	57·9	135·7	234·3[b]	27·7	55·7
6	75·7	272·1[a]	359·3[b]	56·6	46·4
7	94·1	239·4	254·2	8·6	17·1
Standard errors	1·26	1·33	1·24		

Summary analyses of variance

Source of variation	d.f.	Variance ratio Body wt	Testis wt	Testis wt/100 kg body wt
Effect of hemicastration	1	0·745	26·209**	72·550**
Age	(4)			
Linear	1	155·25**	452·624**	238·79**
Quadratic	1	—	38·703**	35·116**
Cubic	1	—	—	8·686**
Interaction	(4)			
Linear	1	—	5·923*	17·686**
Error mean square	40	0·0104	0·0157	0·0086

[A] Compensatory hypertrophy (%) $= \dfrac{\text{Mean hemicastrated testis wt} - \text{mean intact testis wt}}{\text{mean intact testis wt}} \times 100.$

In animals aged 3–4 months, the increase in weight of the testis after unilateral castration was due to an increase in Sertoli and germ cell numbers. Although there was a significant overall response of Sertoli cell number to hemicastration, the principal effect occurred only when the operation was performed at 1 month of age as Sertoli cell numbers did not increase significantly at 4, 5, 6 and 7 months of age. These results are partly in agreement with our previous results for the rat (Putra and Blackshaw 1982) which showed that increased germ cell number following hemicastration was not accompanied by an increase in number of Sertoli cells, but rather of Sertoli cell

Table 2. Histological changes in the remaining testis of the boar following unilateral castration

Mean values for the remaining testis in boars hemicastrated at different ages, compared with means for the equivalent testis in intact animals. a,b, = significantly different from the corresponding value for intact group; a, $P < 0{\cdot}05$; b, $P < 0{\cdot}001$; $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$

Age (months)	Tubule length per testis (m)	Tubular area (μm^2)	10^{-9} × No. of Sertoli cells per testis	10^{-9} × No. of germ cells per testis	Sertoli cell occupancy	Volume fractions (%) Tubules	Interstitial cells
Intact boars (5 animals in each group)							
3	2312	2678	9·02	2·21	0·2	57·1	26·7
4	4426	10233	8·14	22·49	2·7	71·8	18·6
5	5370	22428	5·55	151·98	27·1	76·9	14·2
6	7834	27797	10·98	237·46	27·1	81·4	10·3
7	8356	34088	12·96	403·46	29·1	82·1	9·0
Boars hemicastrated 2 months previously (5 animals in each group)							
3	4487[b]	3885[a]	20·54[b]	6·07[b]	0·3	54·5	26·4
4	5834	15382[a]	9·03	79·18[b]	8·7	75·8	13·5
5	6730	26327	7·42	203·89	24·5	81·8	11·7
6	10139	32900	15·71	479·95	30·5	81·2	9·2
7	7656	36074	20·36	375·49	31·4	75·5	12·6
Standard errors	1·32	1·40	1·58	1·92	1·77	18·7	4·6

Summary analyses of variance

Source of variation	d.f.	Variance ratios Tubule length	Tubular area	Sertoli cell No.	Germ cell No.	Occupancy	Volume fractions Tubules	Interstitial cells
Effect of hemicastration	1	11·361**	7·159**	9·878**	14·255**	3·689	0·003	0·669
Age	(4)							
Linear	1	72·219**	281·262**	2·840	292·321**	388·842**	44·455**	68·407*
Quadratic	1	7·416**	47·804**	18·942**	36·067**	119·628**	21·185**	15·418*
Cubic	1	—	—	—	—	4·214*	—	—
Interaction	(4)							
Linear	1	7·356**	—	—	10·255**	—	—	—
Error mean square	40	0·0148	0·0214	0·0394	0·0801	0·0610	25·60	12·43

occupancy, being an increased capacity of the Sertoli cell to accommodate germ cells. A significant increase in the total number of Sertoli cells per testis was obtained after hemicastration of 1-day-old rats (Hochereau-de Reviers 1975). However, the significant increase in the number of Sertoli cells observed in these prepubertal rats could not be demonstrated in post-pubertal animals (Hochereau-de Reviers and Courot 1978). Sertoli cells, occupying less than a third of the volume of the seminiferous tubules of mammals with active spermatogenesis, do not divide in the adult (Steinberger and Steinberger 1971). Therefore, as the age at hemicastration approaches that at which the Sertoli cell mitotic activity terminates, less compensatory hypertrophy develops in the remaining testis. In the present study, it is likely that the mitotic activity of the Sertoli cells terminated relatively early, so that a Sertoli cell response to hemicastration fell off quickly after 3 months of age.

The cytoplasm of the seminiferous tubules has germ cell and Sertoli cell components. Associated with increased numbers of cells, the length and the cross-sectional area of the seminiferous tubules in young hemicastrated boars were significantly greater than those in intact animals. These findings parallel the results of others which demonstrated that hemicastration resulted in an increase in diameter of the tubules (Voglmayr and Mattner 1968; Riesen *et al.* 1977; Johnson 1978; Hochereau-de Reviers *et al.* 1980; Walton *et al.* 1980). However, when seminiferous tubule diameter reached maximum size, or nearly so, no effect of hemicastration was observed (Hauser *et al.* 1952). Increased length of the seminiferous tubule following hemicastration has been reported in the bull (Leidl *et al.* 1980) and the ram (Hochereau-de Reviers *et al.* 1976).

Intertubular tissue volume of the adult ram testis (Hochereau-de Reviers *et al.* 1976) and the total Leydig mass in the remaining testis of adult rats (Bergh *et al.* 1982) were increased by hemicastration. In the present study neither the volume fraction of the seminiferous tubules nor of the interstitial cells were significantly affected by hemicastration. Nevertheless, the increase in testicular volume after hemicastration showed that the total volume of hormone-producing tissue increased proportionally with that of the seminiferous tubules.

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