The effect of hemi-orchidectomy on reproductive traits of boars

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Abstract

Large White boars (n = 120) were assigned to hemi-orchidectomy at 60, 100, 140 or 300 days of age or as intact controls throughout the 450 day observation period to evaluate the effects of unilateral testicular hypertrophy on libido (reaction time - RT), ejaculation rate (EJACS) and sperm quality in hemi-orchidectomised males. Hemi-orchidectomy of the experimental males involved the surgical removal of the left testis. Sperm viability tests were performed from aliquots of fresh semen following libido and sexual capacity tests. Hemi-orchidectomy of boars at days 60, 100, 140 and 300 of age caused the weight of the remaining testis to hypertrophise by 15.1%, 124.4%, 346% and 483.2%, respectively, as compared to the uncastrated control at 450 days. All the measurements were taken at the end of the study period (day 450). Total daily sperm production per testis (TDSP) increased to 154%, 160.8, 184.3% and 247%, respectively, with this increase being dramatic (247%) in the 300 day old boars. At day 450 compensatory testicular hypertrophy in boars hemi-orchidectomised at day 300 resulted in the highest number of mounts (15.6 ± 0.8 per 30 min), ejaculation rates (9.7 ± 0.9 per 30 min), total sperm count (94.1 ± 4.7 x 109), sperm motility (90.3 ± 1.7%) and normal acrosome morphology (92.2 ± 2.3%), with the shortest RT (2.2 ± 0.5 min). Mounting frequency, RT, EJACS, total sperm, sperm motility and normal acrosome morphology were correlated with testis weight, TDSP, seminiferous tubular diameter, tubular length, tubular mass and total Leydig cell mass. Results suggest that hemi-orchidectomy induces compensatory testicular hypertrophy, leading to significant improvement in the reproductive output of boars. These responses became more pronounced the older the boars were when orchidectomised.

Keywords: Castration, reproductive compensatory mechanisms, servicing capacity, sperm viability, testicular hypertrophy
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Introduction

Reproductive output in the male comprises the production of semen containing normal sperm (quality) in adequate numbers (quantity), together with the desire (libido) and mating ability (servicing capacity). It is generally accepted that testis size is correlated with the capacity to produce sperm and that testicular hypertrophy determines the testicle size in males of various mammalian species (Johnson & Neaves, 1983; Lustra *et al*., 2002). It is also accepted that hemi-orchidectomy (which is a surgical removal of one testis) is correlated with sperm production in male animals (Johnson & Neaves, 1983; Lunstra *et al*., 2002). Hemi-orchidectomy of prepubertal male mammals caused compensatory hypertrophy and increased the size in the remaining testis, and an increase in sperm production per testis at puberty (Sharpe, 1994; Okwun *et al*., 1996; McCoard, 2001; Lunstra *et al*., 2002). Definitive studies regarding the effect of hemi-orchidectomy on the reproductive efficiency of Large White boars remain to be conducted.

By definition, semen from boars that exhibit frequent mountings, with a relatively quick reaction time and ejaculation rate can be used to inseminate a greater number of females per unit time, compared to semen from males with a poorer servicing capacity (Price, 1987; Umesiobi & Iloeje, 1999; Umesiobi *et al*., 2002; 2004; Umesiobi, 2004; Langendijk, 2005). If these hypotheses are true, then the degree of compensatory testicular hypertrophy may serve as an indicator of the reproductive status of boars. To test such a premise requires the use of hemi-orchidectomised boars, which would also permit the assessment of the effects of hemi-orchidectomy on mounting frequency, reaction time, ejaculation frequency and sperm viability. Ivany *et al*. (2002) reported that unilateral castration returned bulls with unilateral disease of the scrotum or testis to productive service by six months after surgery, indicating that hemi-orchidectomy is an effective treatment for unilateral disease of the scrotum or testes in males. As compensatory testicular response to hemi-orchidectomy is often used as a model for studying factors influencing testicular development, an understanding of the mechanisms responsible for compensatory hypertrophy, may be valuable in developing methods for clinical treatment of human infertility or methods of increasing reproductive efficiency in outstanding sires of economically important species. The present study was thus designed to investigate the ability of boars to compensate for the loss of one testis by modifying sperm production rates in the remaining testis with a resultant influence on reproductive efficiency at different ages.

Material and Methods

One hundred and twenty Large White boars were randomly chosen from the commercial pig unit of Umesiobi Farm-Agro Clinics Ltd., Isuikwuato, Nigeria. These boars were weaned at 30 days of age and placed in weaner pens. At approximately 40 days of age, the boars were moved to grower pens in groups of five pigs per pen. Boars were fed a diet *ad libitum* containing 160 g CP/kg until an average body weight of 63 kg. Thereafter they were fed 2.5 kg/day of a diet containing 140 g CP/kg until an average body weight of 120 kg, as recommended by Rathje *et al*. (1995). Diets and full description of the management practices of the experimental boars have been reported by Umesiobi (2000) and Umesiobi *et al*. (2004).

Hemi-orchidectomy was performed at 60, 100, 140 or 300 days of age on the respective groups, and a control was left intact for the duration of the study, which extended to 450 days of age. All the measurement were taken at the end of the study period (day 450). Hemi-orchidectomy involved the surgical removal of the left testis. Hemi-orchidectomy was conducted one day after a sexual capacity test. The one testis was exposed through unilateral proximal scrotal incisions and surgically removed. Boars were then returned to their individual pens for the subsequent recovery period. All surgical procedures conformed to approved guidelines for humane treatment of animals (FASS, 1999). Further tests regarding sexual competence and sperm viability were performed at day 450.

At day 450 total daily sperm production per testis was determined. Oestrus was induced in teaser gilts by a single subcutaneous injection of P.G. 600® (400 IU PMSG with 200 IU HCG/5 mL dose/animal; Intervet Inc., DE) 72 h prior to libido testing. Each boar was exposed individually to at least two oestrous induced gilts during the libido test. Mounting and prompt ejaculation into an artificial vagina (AV) provided a definite, clearly recognizable climax, for establishing that a boar was sexually stimulated.

After these tests, at day 450, the remaining testis was removed as well as those of the intact boars. The epididymis was trimmed immediately, and the testis was weighed. Each testis was cut into three sections longitudinally with the aid of a scalpel, and each sample (1 g) of parenchymal tissue from the proximal, mid, and distal regions were snap-frozen for subsequent homogenization. Parenchyma samples were thawed, weighed, and 1 g was placed in 32 mL ice-cold physiological saline (0.9% NaCl), containing 0.1% Triton X-100 (Scintillation grade, Research Product International, Mount Prospect, IL). Each sample was then homogenized in a Sorvall Omnimixer for 30-sec, at maximum speed. One mL of the suspension was removed and mixed with 1 mL Trypan blue stain (0.4% in phosphate-buffered saline, Gibco/BRL, Grand Island, NY) according to the procedures of Johnson & Neaves (1983), Rathje *et al.* (1995) and Lunstra *et al*. (1997; 2002). Total daily sperm production (TDSP) was determined from the testis slices by dividing the number of maturation phase spermatids in the homogenates (determined by phase contrast cytometry) with 4.37 (the estimated lifespan of the spermatids). Based on their morphology and chromatin condensation, these spermatids corresponded to maturation phase spermatids which have the lifespan of 4.37 days (Johnson & Neaves, 1983; Rathje *et al*., 1995; Lunstra *et al*., 2002).

Data were analyzed using the general linear model procedure of SAS (1996). For analyses of compensatory hypertrophy from 60 through to 450 days of age, the model included the fixed effects and interactions of age groups and testicular changes. All testicular composition data were transformed (log^{10}) prior to statistical analysis to adjust for heterogeneity of the variances (Sokal & Rohlf, 1981). Data were reported as least-square means (± s.e.). As sexual capacity tests data for mounts, reaction time and ejaculations were discrete these were analyzed using the Wilcoxon signed rank test, and presented as least-square means (± s.e.). Differences between treatment means were tested for significance using the statistical package, SAS (1996).

Results

The least-square means (± s.e.) for testicular weight, total daily sperm production (x 10^9 per testis), seminiferous tubular diameter, tubular length, total tubular mass and total Leydig mass per testis following hemi-orchidectomy in the boars at different ages are set out in Table 1. The influence of age at hemi-orchidectomy on compensatory testicular hypertrophy of the boars' testes, sexual competence and sperm viability tests are summarized in Table 2. At the end of the trial (day 450) testis weight differed significantly

Table 1 Mean (± s.e.) testicular characteristics after hemi-orchidectomy at different ages in Large White boars at 450 days of age

Parameters at 450 days	Intact Control	Day on which hemi-orchidectomised			
	450	60	100	140	300
Testis weight (g)	13^{a} ± 0.3	106^{b} ± 3.1	295^{c} ± 2.6	412^{d} ± 5.3	508^{e} ± 3.5
Total daily sperm production (X 10^9 per testis)	7.9^{a} ± 2.2	8.2^{a} ± 0.3	9.4^{b} ± 0.4	12.6^{c} ± 0.8	15.3^{d} ± 1.1
Tubular diameter (µm)	211^{a} ± 1.1	220^{b} ± 1.0	241^{c} ± 0.3	260^{d} ± 0.7	277^{e} ± 1.5
Tubular length (mm)	455^{a} ± 23.8	490^{b} ± 1.8	1583^{c} ± 37.6	4018^{d} ± 2.2	4235^{e} ± 2.7
Total tubular mass (g)	8.3^{a} ± 2.2	47.3^{b} ± 5.3	85.4^{c} ± 7.3	231.3^{d} ± 16	244.4^{d} ± 26.7
Total Leydig mass (g)	5.5^{a} ± 0.7	35.5^{b} ± 3.5	61.5^{c} ± 2.7	90.6^{d} ± 2.3	99.1^{e} ± 10.9

$^{a, b, e}$ within rows means followed by different superscripts differ significantly (P < 0.01)

Table 2 Mean (± s.e.) sexual capacity and sperm viability tests after hemi-orchidectomy at different ages in Large White boars at 450 days of age

Parameters at 450 days	Intact Control	Day on which hemi-orchidectomised			
	450	60	100	140	300
Reaction time (min)	35.1^{a} ± 6.6	21.7^{b} ± 2.8	14.5^{c} ± 0.8	8.4^{d} ± 0.3	2.2^{e} ± 0.5
Ejaculation frequency/30 min	1.5^{a} ± 0.4	2.9^{b} ± 0.6	4.3^{c} ± 0.4	5.9^{d} ± 0.2	9.7^{e} ± 0.9
Total sperm (x 10^9)/mL	55.8^{a} ± .04	42.8^{b} ± 5.2	60.3^{c} ± 0.4	69.5^{d} ± 7.4	94.1^{e} ± 4.7
Sperm motility (%)	45.2^{a} ± 2.1	51.9^{b} ± 2.2	68.9^{c} ± 2.6	72.5^{c} ± 3.0	90.3^{d} ± 1.7
Normal acrosome (%)	41.6^{a} ± 3.8	52.9^{b} ± 4.9	62.5^{b} ± 5.9	75.8^{c} ± 7.8	92.2^{d} ± 2.3

$^{a, b, e}$ within rows means followed by different superscripts differ significantly (P < 0.01)

(P < 0.01) between boars hemi-orchidectomised at days 60, 100, 140 and 300. At day 450, testis weight was heavier in boars hemi-orchidectomised at older ages compared to those hemi-orchidectomised at younger ages (Table 1). Testis weight values in the 300 day (507.9 ± 3.5 g) boar group was heaviest when compared with those of day 60 (106.0 ± 3.1 g), 100 (294.8 ± 2.6 g), 140 (411.9 ± 5.3 g) and the control (intact) (12.9 ± 0.3) boars, respectively. Compared to the control boars, hemi-orchidectomy on day 60, 100, 140 and 300 of age caused the remaining testis to hypertrophise by 160.8%, 184.3, 247% and 300%, respectively (P < 0.01) (Table 1). Testes weight in the respective hemi-orchidectomised groups was approximately 2.5-fold heavier than in the control boars, regardless of the age at hemi-orchidectomy.

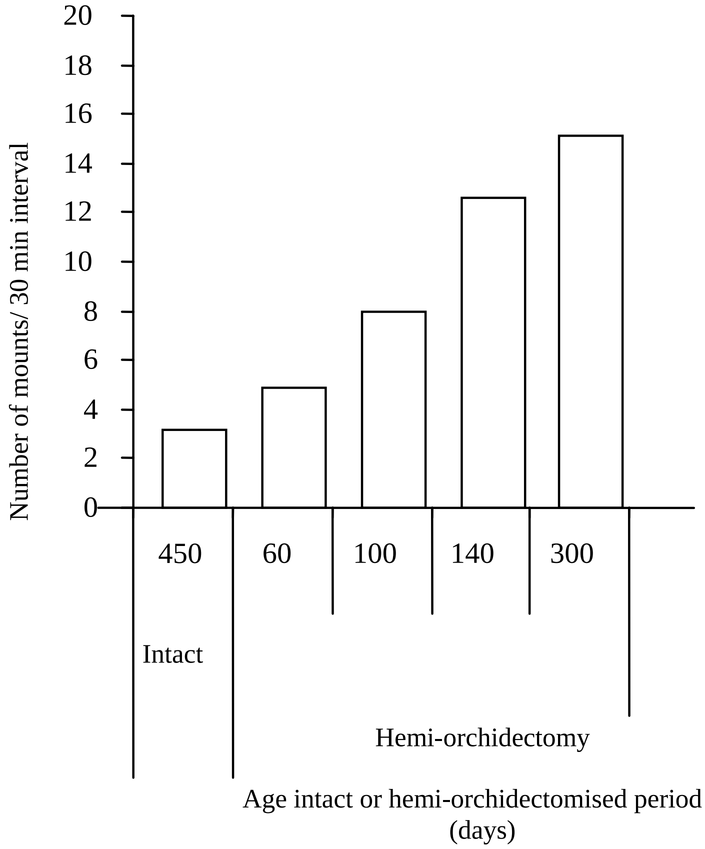

Figure 1 Comparison of mounting frequency (at 450 days of age) between intact boars and boars hemi-orchidectomised at different ages

Sperm production per testis was significantly higher in boars hemi-orchidectomised at the various ages, and this increase was dramatic in boars hemi-castrated on day 300, (P < 0.01) (Table 1). Testes weight was highly correlated to sperm production (r = 0.94). The diameter of the seminiferous tubules was significantly affected (P < 0.01) in boars subjected to hemi-orchidectomy, and regardless of the age at hemi-orchidectomy increased by approximately 107.7% in the day 60, 100, 140, and 300 groups of boars (P < 0.01), compared to the uncastrated controls that were bilaterally castrated at 450 days of age, (Table 1). The tubular diameter was highly correlated to testicle weight (r = 0.99) and sperm production (r = 0.88). Hemi-orchidectomy increased the total tubular length in all boars, and this increase was most prominent when hemi-orchidectomy was performed between days 140 and 300 (P < 0.01). A strong relationship was recorded between tubular length and testes weight (r = 0.95). Hemi-orchidectomy had a substantial effect on the total mass of the tubules and total mass of the Leydig cells in boars hemi-orchidectomised at days 60, 100, 140 and 300, but mostly from those hemi-orchidectomised at 300 days of age (Table 1). The increase in total tubular mass and Leydig cell numbers in hemi-orchidectomised boars was most pronounced when hemi-orchidectomy was performed at day 300 (Table 1). In keeping with Leydig cell proliferation, the average volume per Leydig cell was increased by compensatory testicular hypertrophy in the treated boars at every castration age, compared to the control (intact) group (Table 2).

Sexual competence as exemplified by mounting, libido (reaction time - RT) and ejaculation frequency was consistently different ($P < 0.01$) in hemi-orchidectomised boars at all ages, compared to the control group (Table 2; Figure 1). Mounting frequency of the boars was increased by compensatory testicular hypertrophy, with the highest number of mounts (15.6 ± 0.8 per 30 min period) recorded at 450 days of age, from boars hemi-orchidectomised at day 300 (Figure 1). The intact and hemi-orchidectomised boars differed significantly in sexual drive (RT) at 450 days, with hemi-castrated boars exhibiting shorter reaction times than the control (intact) group. The shortest reaction time (2.2 ± 0.5) of boars was observed ($P < 0.01$) in the day 450 group of males that exhibited greater frequencies of mounting (Table 1; Figure 1). The two post-copulatory behaviours measured in this trial, namely mounting and reaction time, were correlated with the ejaculation rate in the hemi-orchidectomised boars at the various ages. Testicular hypertrophy tended to increase the ejaculation frequency ($P < 0.01$), irrespective of age. Ejaculation frequency was highly correlated with testis weight ($r = 0.98$), sperm production ($r = 0.96$), seminiferous tubular diameter ($r = 0.99$), seminiferous tubular length ($r = 0.93$), seminiferous tubular mass ($r = 0.94$) and total Leydig cell mass ($r = 0.97$).

Furthermore, strong correlations existed between reaction time and the ejaculation frequency ($r = 0.98$), testis weight ($r = 0.99$), seminiferous tubular diameter ($r = 0.97$), tubular length ($r = 0.96$), tubular mass ($r = 0.91$), total Leydig mass ($r = 0.99$) and sperm production ($r = 0.98$). In evaluating the effect of hemi-orchidectomy of boars on sperm viability, the sperm quality of hemi-orchidectomised boars at the different ages was clearly superior ($P < 0.05$) to those of intact (control) boars (Table 2). The 300 days of age boars produced a substantially largest volume of sperm (94.1 ± 4.7 $x10^9$/mL), the highest sperm motility (90.3 ± 1.7%) and recorded normal acrosome morphology (92.2 ± 2.3%), compared to the 60, 100, 140 day, and the control boars groups.

Discussion

As envisaged, hemi-orchidectomy of boars on days 60, 100, 140 or 300 of age caused the mass of the remaining testis to hypertrophise. Thus, the effect of compensatory hypertrophy in the seminiferous compartments (tubules and interstitium) may be accomplished via Leydig cell proliferation (Berndtson & Igboeli, 1989; Borg *et al*., 1993; Lunstra *et al*., 2002). The magnitude of hypertrophy was most prominent in boars hemi-orchidectomised at 300 days of age (Table 1). These findings are in agreement with the testicular compensatory effects reported to occur in boars (Putra & Blackshaw, 1985; Kosco *et al*., 1989; Umesiobi, 2000; Lunstra *et al*., 2002) and other species (Leidl *et al*., 1980; Johnson & Neaves, 1983; Waites *et al*., 1983; Orth, 1984). Likewise, the total daily sperm output increased by 50% after hemi-orchidectomy in 60-day old boars and peaked 3-fold by day 450, compared to intact boars. Testicular characteristics of hemi-orchidectomised boars were comparable to those previously reported in boars of different age groups (Borg *et al*., 1993; Nonneman *et al*., 2005).

In agreement with these findings, compensatory testicular hypertrophy in boars at different ages (Table 1) was associated with an increase in the number of germ cells. The number of maturation phase spermatids per testis increased with testicular weight. This is in accordance with studies on boars of different age groups in which: i) the number of mature spermatids per g testicular weight increased ($P < 0.0001$) by 220 days following hemi-castration (Willenburg *et al*., 2003); ii) daily production of spermatids increased after hemi-castration (Hochereau-de-Reviers *et al*., 1976); and iii) testicular hypertrophy of the remaining testis was due to seminiferous tubular expansion *per se* (Pinckard *et al*., 2000; Umesiobi, 2000). It is likely that the changes in the total Leydig mass and germ cells were sufficient to account for most of the increase in testicular weight.

It has been suggested that hemi-orchidectomy of males resulted in an increase in libido and sexual competence in boars (Umesiobi, 2004; Langendijk, 2005). In the present study, the efficiency of mounting, reaction time and ejaculation rates that were established in individual animals at days 60, 100, 140 and 300 of age were maintained. Several tangible explanations exist for this phenomenon. The frequency of mounting (pre-copulatory behaviour) in the experimental boars reflected the underlying sexual motivation, or libido, and provided a meaningful prediction of their sexual performance. Lunstra *et al*. (1986) reported oestradiol and testosterone output of boar Leydig cells *in vitro* to be increased during puberty to a peak at approximately 160 days of age following hemi-castration. It therefore stands to reason that the increasing levels of gonadotropins in circulation, or increased sensitivity to gonadotropins following hemi-orchidectomy, could have stimulated the rate of spermatogenesis more in hemi-orchidectomised boars at different ages than in intact boars. Perhaps of more significance is the fact that compensatory testicular hypertrophy resulted in the production of sperm of high quality and quantity. Since sperm production rates depend on (a) the number of stem cells per testis; (b) the number of spermatogonial divisions for that species; (c) the process of stem-cell renewal for that species; (d) the output of spermatogonial divisions (Hochereau-de-Reviers, 1975; Johnson *et al*., 1980); and (e) the level of germ cell loss during meiosis (Barr *et al*., 1971; Balthazard *et al*., 1992), complex processes (such as plasma FSH concentration) in spermatogenesis could be implicated in the induction of sperm production after hemi-orchidectomy. For instance, Johnson *et al*. (1980) found that one week after hemi-castration in aged rats, the plasma FSH levels were elevated and remained high for four weeks after hemi-castration. Thus, it was suggested that FSH was largely responsible for testicular compensatory hypertrophy after hemi-castration.

In response to hemi-orchidectomy of boars, tubular hypertrophy produced an overall advantage in improving the quality of total sperm, sperm motility and normal acrosome morphology approximately 2-fold, compared to the intact (control) groups. Thus, compensatory hypertrophy that caused a substantial increase in total mass of testicular tissue (seminiferous tubules and interstitium) and Leydig cell proliferation may be responsible for the enhancement of sperm quality (Johnson *et al*., 1980; Berndtson *et al*., 1987; Berndtson & Igboeli, 1989; Mani, 1991; Pinckard *et al*., 2000; Flowers, 2002; Umesiobi, 2004). Likewise, in agreement with Umesiobi & Iloeje (1999), Umesiobi (2002), Willenburg *et al*. (2003) and Umesiobi *et al*. (2004), sexual capacity of hemi-orchidectomised boars was positively correlated to sperm quality, which consequently reduced the inherent effects of fructolysis.

Conclusion

This study indicated that differences in reproductive efficiency of boars are presumably a consequence of compensatory testicular hypertrophy following hemi-orchidectomy at different ages. Compensatory hypertrophy which caused a substantial increase in total mass of testicular tissue (seminiferous tubules and interstitium) and Leydig cell proliferation appeared to be the primary factor contributing to the substantial improvement in the reaction time, ejaculation rates and sperm quality of hemi-orchidectomised boars. In addition, the effects of compensatory testicular hypertrophy were expected, as hemi-orchidectomy caused dramatic testicular hypertrophy and a concomitant increase in sperm production, coupled with total Leydig cell proliferation. This hypertrophy was more pronounced by day 450 following hemi-orchidectomy of boars at 300 days of age. Compensatory testicular hypertrophy provided accurate tests for assessing the sperm quality, as well as libido and mating potentials of boars at different ages. However, further investigations are required to establish whether these differences would extend into adulthood, i.e. the long-term consequences of hemi-orchidectomy.

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