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Assessment of pubertal development of boars derived from ultrasonographic determination of testicular diameter§

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Abstract

At the onset of puberty, seminiferous tubules rapidly increase in diameter, thereby occupying a greater proportion of the testis, resulting in a rapid increase in testicular size. The objective of the current studies was to evaluate ultrasonography for assessing testicular diameter, as a basis for ranking boars relative to their extent of pubertal development. In the initial study, prior to castration at 4, 5, 6, or 7 mo of age, testicular length and diameter were assessed by ultrasonography in 160 anesthetized boars. After castration, testes were weighed. Mean diameter of seminiferous tubules and percentage of the testis occupied by tubules were determined by histological evaluations of all testes. Testicular volume was calculated from length and diameter and was correlated with testicular weight ($P < 0.001$; $r \geqq 0.78$) within each of the four age groups. At 4 and 5 mo of age, testicular diameter correlated positively ($P < 0.001$) with diameter of seminiferous tubules; this relationship was not significant at older ages. In two subsequent studies, testicular diameter determined ultrasonographically in conscious boars was highly correlated ($r > 0.8$) when assessed twice on the same day, or when diameter of the right was compared with diameter of the left testis. Similarly, testicular diameter obtained initially at 92 d of age correlated positively ($P < 0.001$) with the diameter observed at older ages, but the magnitude of the relationship decreased as time between evaluations increased. These findings supported ultrasonographic determination of testicular diameter during early pubertal development, as a means to rank boars of similar chronological age for extent of pubertal development.

Keywords: Boar; Puberty; Testis; Seminiferous tubule; Ultrasonography

1. Introduction

Onset of pubertal development in mammalian males involves rapid expansion of seminiferous tubules, accompanied with markedly increasing testicular size [1,2]. Within the testis of pubertal males, proliferation of Sertoli cells ceases in association with formation of the blood-testis barrier; luminal development occurs within the seminiferous tubules, and secretion of fluids into tubular lumens increases. These changes are followed by release of mature spermatids into the lumen and their movement into the mediastinum. Evaluation of scrotal circumference in bulls and rams with their pendulous scrotum offers an effective method to estimate testicular development in pubertal males [3–6]. However, assessment of scrotal circumference in pubertal boars is not practiced routinely, due to their nonpendulous scrotum with its broader attachment. No reports were identified that comprehensively examined the association of development of

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seminiferous tubules in pubertal boars with the diameter of their testes.

Ultrasonography has been used to estimate testicular dimensions in many species, including boars [7,8], thereby providing potential to develop a means to estimate stage of pubertal development in boars. For maternal lines, younger pubertal age is desirable, whereas in terminal sire lines, sexual maturity at older ages has an advantage of delaying onset of sexual behavior during rearing. The objectives of the first study were to: 1) characterize testicular development of boars by evaluating testicular weight and morphological changes at defined ages throughout pubertal development; 2) estimate correlations of mean diameter of seminiferous tubules with testicular diameter determined by ultrasonography; and 3) estimate the correlations of mean diameter of seminiferous tubules with testicular weight. The objective of the second and third studies was to use ultrasonography to estimate the relationships within boars of testicular diameter at successive ages.

2. Materials and methods

2.1. Animals, management, and sample collection

Boars were a four-breed composite [9] maintained by *inter se* mating and reared at the U.S. Meat Animal Research Center using standard production and experimental practices, in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [10]. All boars were weaned at 15 to 21 d of age, reared in confinement buildings, and fed standard diets formulated to meet NRC requirements varying with increasing age to accommodate changing nutritional needs. Water was available *ad libitum*. Boars in the first (N = 160) and second study (N = 25) were progeny of 12 sires. Boars in the third study (N = 63) were progeny of 12 different sires. Body weights were obtained at birth, weaning, and at 2 and 5.5 mo of age.

2.2. Experiment 1—Testicular traits during pubertal development

In the first study, the right testis of each boar was removed at 3, 4, 5, 6, 7 or 10 mo of age, to evaluate weight and morphological changes during pubertal development; the number of boars per each age was 24, 24, 21, 33, 24, and 34, respectively. Space restrictions and requirements of other experiments did not allow age at unilateral castration to be randomized across the three farrowing seasons. Boars in Season 1 (May farrowing) were unilaterally castrated at 6 mo of age; boars in Season 2 (July farrowing) were unilaterally castrated at 4, 5, or 7 mo of age, and boars in Season 3 (January farrowing) were unilaterally castrated at 3 mo of age, or were bilaterally castrated at 10 mo of age. Boars were given 2.5 mg/kg of xylazine (RX Veterinary Products, Westlake, TX, USA) im, 15 to 20 min before they were given thiopental sodium (Hospiara, Inc., Lake Forest, IL, USA) iv, followed with closed circuit anesthesia of isoflurane (Halcarbon Laboratories, North Augusta, SC, USA) and oxygen. The scrotal area was cleaned thoroughly with surgical soap, and then 70% ethanol. After expulsion of the testis, the spermatic cord was ligated and the testis removed. The left testis of these boars was removed at 10 mo of age using the protocol described above. Likewise, 34 additional boars were bilaterally castrated at 10 mo of age to define testicular weight at this older postpubertal age.

Boars that were unilaterally castrated at 4, 5, or 7 mo of age (Season 2) and boars unilaterally castrated at 3 mo, or bilaterally castrated at 10 mo of age (Season 3) were assigned at random, stratified within sire, to age of castration using all vigorous boars within a litter. In contrast, boars that were unilaterally castrated at 6 mo of age (Season 1) were the larger boars within a litter and were selected prior to weaning only from large litters balanced within sire.

Before hemicastration at 4, 5, 6, or 7 mo of age, length and width of the right testis of anesthetized boars were determined by B-mode ultrasonography (Model SSD-500V with UST-5011-3.5 linear transducer and standoff formed to fit curvature of testes; Aloka, Wallingford, CT, USA). Water-soluble gel (LiquaSonic Ultrasound Gel; Chester Labs Inc, Cincinnati, OH, USA) was applied to the transducer and to the boar's scrotum before acquiring images, as described previously [8]. Ultrasonographic images were captured when there was clear visibility of the mediastinum and the tunica albuginea on both sides of the testis. At each age, volume was calculated using the length and diameter estimated by ultrasonography. The right testis was removed at the specified ages of hemicastration.

After castration at all ages, testes were trimmed, weighed, and split longitudinally. Two aliquots of tissue, <1 cm^3, were placed immediately into 10% formalin (neutral, phosphate buffered; Polysciences, Inc., Warrington, PA, USA) for approximately 5 h with gentle agitation followed by placement into fresh fixative with additional agitation for approximately 22 h. Thereafter, blocks of tissue were dehydrated with increasing concentrations of ethanol and imbedded in paraffin [11,12].

Proportion of testis occupied by seminiferous tubules and diameter of seminiferous tubules were determined for each boar on 5 μm sections that were deparaffinized and stained with hematoxylin [12]. The morphology of four random fields from one section of each aliquot of tissue per boar was evaluated via bright-field microscopy using computerized morphometric planimetry (Bioquant Nova Advanced Image Analysis 2000; R&M Biometrics, Inc., Nashville, TN, USA [12]). Weight of parenchyma was estimated by multiplying testicular weight by 0.91 to adjust for weight of the tunica albuginea [13].

2.3. Experiments 2 and 3—Repeatability of testicular diameter at successive ages

In the second and third studies, diameter of testes was estimated by ultrasonography at sequential ages while conscious boars were confined within a scale pen using a Model SSD-900V with UST-5524-7.5 linear transducer (Aloka). Water-soluble gel was applied to the transducer prior to monitoring and capture of the ultrasonographic image. The second study estimated testicular diameter at 114 ± 0.6 d of age. Ten of these boars were re-evaluated a second time the same day, with knowledge of their first estimated diameter concealed from the evaluator. All boars were re-evaluated at 134 ± 0.7 d of age and were castrated at 300 d of age. In the third study, diameters of the left and right testes were estimated at a mean age of 96 ± 0.4 d. Additional estimates of diameters of the left testis of these same boars were recorded at mean ages of 122 ± 0.3, 144 ± 0.3, and 164 ± 0.3 d.

2.4. Statistical analyses

Mixed Models procedures of SAS were used to evaluate season of rearing upon body weight at birth, weaning, 2 mo, and at 5.5 mo of age [14]. Body weight at each age was evaluated in a separate analysis, with season as the main effect, age on the day weight was recorded was a covariate, and sire was a random effect.

Statistical evaluation of changes in testicular traits were restricted to boars unilaterally castrated at 4, 5, or 7 mo of age (boars reared within a common season) using Mixed Models procedures, with age of castration as the main effect, birth weight as a covariate, and sire as a random effect. When the main effect was significant ($P < 0.05$), means were compared with the PDIFF option.

Correlation coefficients (r) among testicular traits within boars of a common age group were derived from linear regression procedures of SAS [15]. Data are presented as mean ± SEM.

3. Results

3.1. Experiment 1

Weaning weight and 5.5 mo weight differed ($P < 0.05$) across the three seasons of rearing (Table 1). The percentage of the testis occupied by seminiferous tubules increased ($P < 0.01$) after 3 mo and reached a maximum by 5 mo of age, whereas mean diameter of seminiferous tubules was greater ($P < 0.01$) at 7 than at 4 or 5 mo of age (Fig. 1).

Testicular volume calculated from ultrasonographic estimates of diameter and length was correlated ($P < 0.001$) with testicular weight at 4 to 7 mo of age (Table 2). Testicular dimensions were not taken at 3 mo of age, whereas at 10 mo, testicular length was beyond the limits of the instrument. The correlation coefficients of mean diameter of seminiferous tubules with testicular weight and with testicular diameter decreased with advancing age becoming nonsignificant at 6 mo for testicular diameter and at 7 mo for testicular weight (Table 2). The coefficient of variation (CV) for mean diameter of seminiferous tubules within each age group of boars, an assessment of variation in pubertal development, decreased from 21.6% at 3 mo and 20.6% at 4 mo to 11.2, 10.9, and 6.6% at 5, 6, and 7 mo of age, respec-

Table 1
Mean (± SEM) body weights at defined stages of development of boars born in different seasons.

Season (Farrowing)	No.	Weight (kg)			
		Birth	Weaning	2 mo	5.5 mo
1 (May)	33	1.60 ± 0.05	5.9 ± 0.15^{a}	19.9 ± 0.73	91.0 ± 2.21^{a}
2 (July)	69	1.65 ± 0.04	5.4 ± 0.13^{b}	21.4 ± 0.57	99.4 ± 2.05^{b}
3 (January)	58	1.55 ± 0.04	5.5± 0.15^{b}	20.1 ± 0.53	93.0 ± 1.93^{a}

^{a,b} Within a column, means without a common superscript differ ($P < 0.05$).

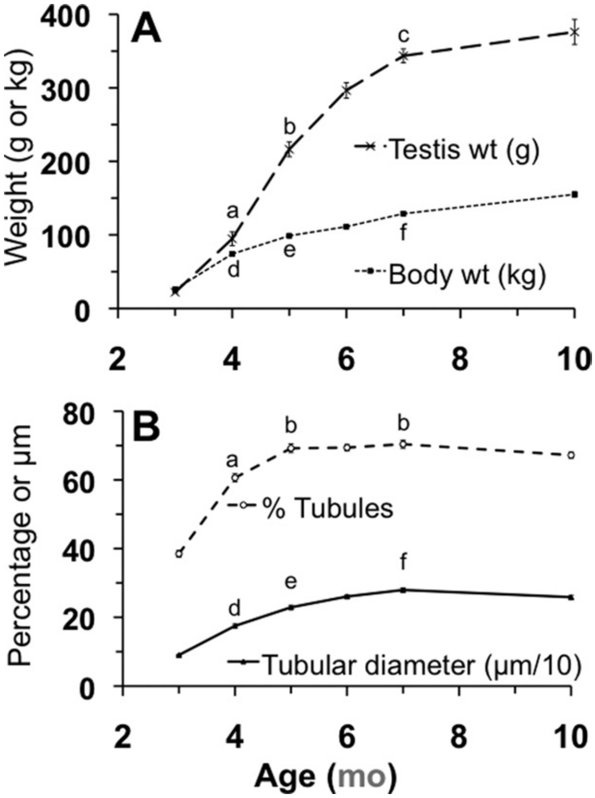

Fig. 1. Developmental changes in testicular trait of boars. (A) Increases in body weight and right testicular weight with age ($P < 0.001$). (B) Percentage of the testis occupied with seminiferous tubules and diameter of seminiferous tubules. Diameter was divided by 10 to better fit the data to the scale of the figure. Statistical comparisons were limited to boars that were unilaterally castrated at 4, 5, or 7 mo of age due to their common season of rearing.

[a–c]Means without a common superscript differ ($P < 0.01$).

tively. Similarly, the CV for testicular diameter decreased from 16.1% at 4 mo to 12.6, 8.1, and 5.6% at 5, 6, and 7 mo of age, respectively.

Testicular weight at 10 mo of age was 571 ± 22, 512 ± 19, 546 ± 19, 498 ± 17 and 495 ± 19 g (mean ± SEM) for boars unilaterally castrated at 3, 4, 5, 6 and 7 mo of age, respectively. For boars unilaterally castrated at 4, 5 or 7 mo of age, percentage of seminiferous tubules (69.5 ± 1, 69.5 ± 1, 71.7 ± 1 %, respectively) and weight of seminiferous tubule (323 ± 13, 345 ± 13, 326 ± 13 g, respectively) at 10 mo of age were similar ($P > 0.2$). The correlation coefficients within each boar of weight of the testis removed at hemicastration with weight of the second testis removed at 10 mo of age were low ($r < 0.3$) for boars hemicastrated at 3, 4, 5, or 7 mo of age ($P > 0.10$). In contrast, this relationship was positive ($r = 0.73$) for boars that were hemicastrated at 6 mo of age ($P < 0.001$).

3.2. Experiment 2

At 114 d of age, testicular diameter of conscious boars determined by ultrasonography was 3.2 ± 0.09 cm (range, 2.4 to 4.6 cm; N = 25). The correlation coefficient of the first estimate with the second estimate on the same day was 0.91 ($P < 0.001$). Diameter at 134 d of age was 4.7 ± 0.11 cm (range, 3.5 to 5.8 cm), and the within boar correlation of diameter at 114 d with that at 134 d was 0.85 ($P < 0.001$). Mean total testicular weight at 10 mo of age was 740 ± 0.3 g; the correlations of diameter at 114 or 134 d of age with total testicular weight at 10 mo were 0.2 and 0.17, respectively ($P > 0.5$).

3.3. Experiment 3

At 96 d of age, the correlation of the testicular diameter, determined by ultrasonography of conscious boars, of left testis with that of the right testis was 0.87 ($P < 0.001$). Within boar correlations of left testis diameter at successively older ages were significant for these 63 boars; however, the magnitude of the correlations was reduced as the number of days between evaluations increased (Table 3). Mean testicular diameter was 2.8 ± 0.06, 4.1 ± 0.12, 5.1 ± 0.12, and 6.2 ± 0.11

Table 2
Within-boar correlation coefficients (r) of testicular traits at specified ages.

Age (mo)	No.	Volume[a] versus testis weight	P	Testis weight versus tubular diameter	P	Testis diameter versus tubular diameter	P
3	24			0.79	0.001		
4	24	0.95	0.001	0.63	0.001	0.71	0.001
5	21	0.93	0.001	0.66	0.001	0.75	0.001
6	33	0.85	0.001	0.35	0.025	0.15	0.20
7	24	0.78	0.001	0.10	0.27	0.14	0.48
10	34			0.09	0.27		

[a] Prolate sphere; volume, $4/3\pi$*(length/2)*(width/2)^2.

Table 3
Within-boar correlation coefficients (r) of testis diameters at specified ages.[a]

Age (d)	122 ± 0.3	144 ± 0.3	164 ± 0.3
96 ± 0.4	0.78	0.73	0.56
122	—	0.87	0.66
144	—	—	0.83

[a] ($P < 0.001$) for all correlations.

cm at mean ages of 96, 122, 144, and 164 d, respectively.

4. Discussion

The current studies established that, within groups of boars of similar chronological age from 3 to 5 mo, stage of pubertal development as defined by mean diameter of seminiferous tubules, was highly correlated with testicular weight and testicular diameter determined by ultrasonography. The CV of mean seminiferous tubule diameter within age groups decreased from 3 to 7 mo, indicative of less variation in testicular development as boars progressed through the pubertal process. Likewise, the decrease in the CV for testicular diameter decreased with age reflecting the significant correlation of seminiferous tubule diameter with testicular diameter in pubertal boars. These findings supported use of ultrasonographic determination of testicular diameter at a defined early age of pubertal development as a means to rank boars of similar chronological age for extent of pubertal development (if selection for this trait had merit).

Harder et al [16] reporteded that boars from a line selected for large testes at 5 mo of age also had more rapid pubertal development of their testes than boars of the randomly selected control line. However, these correlated responses to selection should not be accepted as evidence that early puberty establishes large testicular size at maturity. Meishan and Piau are breeds of boars that have small testicular size as adults, relative to breeds commonly used in commercial swine production. However, Meishan boars undergo pubertal testicular development at a much younger age than Piau boars. Growth of seminiferous tubules to a mean diameter of 100 μm occurs before 50 d of age in Meishan boars [17–19], but the seminiferous tubules of Piau boars do not achieve this diameter until after 120 d of age [20, 21], and the size of postpubertal testes of Piau boars is greater than in Meishan boars. Furthermore, our second study provided no support for a correlation of testicular diameter early in pubertal development with testicular size of adult boars. Likewise, in the first study in which boars were unilaterally castrated at 4, 5, or 7 mo of age, the weight of the first testis was not associated with the weight of the remaining testis at 10 mo. Based on these observations, early onset of pubertal development, relative to later pubertal development, will not be associated with greater sperm production of adult boars in all genetic lines.

The blood-testis barrier is formed by 4 mo of age in commercial breeds of boars; therefore, a compensatory increase in number of Sertoli cells should not occur after unilateral castration at this age or older [19,22]. As a consequence, unilateral castration at 4 mo or later should not modify the genetic potential for testicular growth of individual boars. This was supported by the similar seminiferous tubular weight observed at 10 mo for boars unilaterally castrated at 4, 5 or 7 mo. In contrast, unilateral castration a 7 d of age would produce a compensatory increase in number of Sertoli cells, and this is reflected by greater mass of seminiferous tubules (384 ± 15 g; unpublished data from [19]). The significant correlation of testicular weight of boars unilaterally castrated at 6 mo of age with weight of the testis present at 10 mo cannot be explained; however, these boars were from a more uniform population than the boars that were evaluated at the other ages. They were the larger boars from large litters, rather than randomly selected boars as was the situation for boars unilaterally castrated at 4, 5, or 7 mo of age. Rate of sexual development and testicular size were correlated traits in the group of boars that was unilaterally castrated at 6 mo, as was the case with the population of boars at the University of Nebraska that was selected for testicular size [16].

The four-breed composite line of boars that was evaluated in the current studies had growth of seminiferous tubules to 100 μm at approximately 90 d of age (Fig. 1) [19] compared with approximately 110 d of age for Landrace × Yorkshire boars [23] and Landrace × Duroc boars [24] and at sometime after 120 d of age in Hampshire × Duroc × Yorkshire boars [25]. Between 3 and 4 mo of age, diameter of seminiferous tubules increased rapidly to 175 μm and the tubular composition of the testis increased from 38 to 61%. Also within these ages, the CV for tubular diameter was the greatest indicating maximum variation in pubertal development. After 4 mo, the CV decreased, but the correlation of testicular diameter, determined by ultrasonography, with seminiferous tubule diameter remained significant at 5 mo of age. Consequently, determination of testicular diameter from 3 to 5 mo of age would provide a method to rank boars of this genetic line relative to their stage of pubertal development.

Based on the current studies, we infer that boars can be selected for either early or late testicular development without compromising testicular size when appropriate technology is used to estimate testicular size of adult boars. It is well established that daily sperm production of adult boars increases with testicular weight [26–28]. Consequently, there is economic value in producing boars with larger testes for artificial insemination studs. Furthermore, sperm production is moderately heritable [29–31]. In order to predict testicular volume, technology is required to economically estimate both width and length of testes of late pubertal boars (i.e., an age when testicular size correlates with the size of adult testes). A proposed protocol would rank boars for testicular diameter at ~4 mo of age, a period of greatest variation in diameter of seminiferous tubules. Then boars would be ranked a second time for testicular volume after 8 mo of age. At both evaluations, testicular traits would be adjusted for age and weight of the boar within a defined genetic line.

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