Stereological evaluation of Sertoli cell ontogeny during fetal and neonatal life in two diverse breeds of swine

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Abstract

Chinese Meishan (MS) boars have smaller testes due to fewer Sertoli cells compared with White Composite (WC) boars. The objective was to describe Sertoli cell development relative to circulating FSH concentrations in fetal and neonatal MS and WC boars. Testes and blood samples were collected on days 60, 75, 90 and 105 postcoitum (dpc) and 1, 7, 14 and 25 postpartum (dpp). One testis was immunostained for GATA4 or Ki67 antigen to evaluate total and proliferating Sertoli cell numbers respectively. Testicular size was greater ($P < 0.01$) in WC than MS boars at all ages, associated with a greater mass of interstitial tissue. Tubular mass ($P < 0.01$) was greater in prenatal WC boars, but postnatally increased more rapidly ($P < 0.001$) in MS boars, exceeding WC boars by 25 dpp. Sertoli cell numbers increased with age, was greater ($P < 0.001$) in WC than MS boars during prenatal development but increased rapidly ($P < 0.01$) by 1 dpp in MS and thereafter was similar in both breeds. The proportion of Ki67-

positive Sertoli cells was maximal at 90 dpc, declining thereafter, did not differ between breeds through 7 dpp, but was greater ($P < 0.05$) in WC than MS boars at 14 and 25 dpp. Plasma FSH concentrations were greater ($P < 0.05$) in WC than MS boars at 75 dpc. FSH concentrations were elevated at 105 dpc (MS) and 1 dpp (WC) but declined thereafter with advancing postnatal age in both breeds. This study illustrates that late gestation represents the period of maximal Sertoli cell proliferation. Despite asynchronous Sertoli cell population growth between breeds during early postnatal life, differential mature Sertoli cell numbers and testicular size are probably due to differences in duration of the proliferative period after 25 dpp, potentially regulated by Sertoli cell maturation and blood-testis barrier formation. These events were not associated with fetal or early postnatal changes in FSH secretion.

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Introduction

Sertoli cell numbers determine adult testicular size (Orth 1984, Russell *et al.* 1990, Chubb 1992), thereby establishing an upper limit for total daily sperm production. Thus boars with large testes provide an opportunity to decrease production costs for artificial insemination while simultaneously allowing use of genetically superior sires. Identification of factors that control adult Sertoli cell population size is necessary to develop methodologies that either predict adult testicular size at a young age, such as by marker-assisted selection, or enhance adult testicular size and thus sperm production.

Male sexual differentiation in pigs is initiated at 26 days postcoitum (dpc) (Pelliniemi 1975, McCoard *et al.* 2001b) and is characterized by differentiation of Sertoli cells from precursor somatic cells and formation of testicular cords (Tung *et al.* 1984, Tung & Fritz 1987). Leydig cells in the interstitial compartment differentiate shortly thereafter,

initiating androgen-stimulated development of the male phenotype. Sertoli cell numbers increase during fetal and neonatal life, reaching maximum numbers prior to terminal differentiation of the Sertoli cell population. Sertoli cell maturation is characterized by acquisition of a characteristic shape and nuclear features (Fawcett 1975), blood-testis barrier formation (Tindall *et al.* 1975) and cessation of mitotic activity (Gondos & Berndtson 1993). Coincident with Sertoli cell maturation, lumen formation occurs within seminiferous tubules and germ cells proliferate rapidly followed by onset of spermatogenesis.

Several indirect studies of Sertoli cell development in pigs indicate that the early postnatal period corresponds to the most rapid rates of Sertoli cell proliferation (Tran *et al.* 1981, Putra & Blackshaw 1985, Kosco *et al.* 1987, Swanlund *et al.* 1995). During the first month of life there is a 6-fold increase in the number of Sertoli cells (Franca *et al.* 2000) and between 1 and 14 days of age approximately 15–30% of the total Sertoli cell population are

undergoing proliferation (McCoard *et al.* 2001a). Increased follicle-stimulating hormone (FSH) secretion (Lunstra *et al.* 1997) during this time has been assumed to be the stimulus for this proliferation as occurs in rats (Meachem *et al.* 1996, Baker & O'Shaughnessy 2001). In the present study, two genetically diverse breeds of swine, Meishan (MS) and White Composite (WC) were compared because they differ in adult testicular size, Sertoli cell number and sperm production (Okwun *et al.* 1996, Lunstra *et al.* 1997). The objective was to describe patterns of Sertoli cell development in purebred MS and straightbred WC boars during late fetal and early neonatal life and to relate these developmental patterns to circulating FSH concentrations.

Materials and Methods

Sample collection and histological methods

Fetal and early neonatal boars were obtained from either purebred MS or WC (Yorkshire × Landrace) sows (*Sus scrofa*). Estrus was checked once daily, and sows were bred on the first and second day of detected estrus. Day 0 (for fetal samples) corresponds to the first day of mating, and 1 day postpartum (dpp) corresponds to 12–24 h following parturition. Pregnant sows (60, 75, 90 and 105 dpc) were slaughtered by electrical stunning followed by exsanguination. Fetal boars were produced by nine WC and eight MS sires. Fetal blood samples were obtained from the umbilical artery; body weight was recorded, and testes were collected from fetal boars with at least three to five litters represented per age group per breed ($n=6$ –10 boars per breed per age). Neonatal blood samples were obtained by vena caval puncture, prior to castration on 1 dpp, from ten MS boars, produced by six sires, and ten WC boars, produced by seven sires, each from different litters. Littermates to each of these boars were castrated on 7, 14 and 25 dpp. These age groups represent early gonadotropin secretion in the porcine fetus (Liwska 1975, Goxe *et al.* 1993) and the neonatal peak in gonadotropin secretion (Lunstra *et al.* 1997). Procedures for handling all animals in this study complied with those specified in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS 1999).

The left testis from each boar was trimmed of epididymides and excess connective tissue and weighed. Fetal testes were processed intact and postnatal testes were cut into two pieces longitudinally. Tissues were fixed overnight in two changes of 4% paraformaldehyde in PBS (pH 7.6) at 4 °C with gentle agitation. Each sample was washed in PBS (1 × 1 h), dehydrated through graded percentages of ethanol (50, 70, 80, 90, 100%, 2 × 1 h each), cleared in xylene (Sigma) (2 × 1 h), infiltrated with paraffin wax (60 °C, 4 × 1 h), and embedded in paraffin wax. Four longitudinal serial sections (5 µm) were made from the middle of the left testis of each animal. Approximately the same region of each testis was used. Sections were dried

overnight onto glass slides at 37 °C and stored at room temperature until staining. Sections were stained immunohistochemically for GATA4, a marker for Sertoli cells, and Ki67 antigen as described previously (McCoard *et al.* 2001a).

Stereological evaluation of testicular composition

Sections stained for GATA4 or Ki67 antigens were examined under brightfield microscopy. Briefly, Sertoli cell and germ cell morphological characteristics including total number, nuclear volume and proportion undergoing proliferation (Ki67-positive) were determined from four randomly selected regions (quadrants) of each longitudinal section. Approximately $5 \times 10^6 \mu\text{m}^2$ were evaluated per testis ($1.2 \times 10^6 \mu\text{m}^2$ per quadrant). At least 250 seminiferous tubules were measured at ×200 per testis. For determination of nuclear size, four round tubules were selected per quadrant and Sertoli cell number, germ cell number and nuclear volumes within each tubule were determined at a magnification of ×1000. At least 600 Sertoli and 40 germ cell nuclei were measured per testis in each age group. Detailed stereological methods are described elsewhere (McCoard *et al.* 2001b). All measurements were made using computerized morphometric planimetry (Bioquant Nova 2000 Advanced Image Analysis; R&M Biometrics, Nashville, TN, USA); calculations for testicular parameters were conducted as described previously (McCoard *et al.* 2001a).

FSH assay

Plasma concentrations of FSH were determined by RIA using anti-porcine FSH (AFP2062096Rb) and porcine FSH (AFP10640B) (from Dr A F Parlow, National Hormone and Peptide Program, National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD, USA) for iodination and for the reference preparation. All samples were included within a single assay. Data from fetuses collected at 60 dpc were not included in the statistical evaluation because FSH concentrations were below the limits of sensitivity, 0.2 ng/ml, for 44% of the WC and 30% of the MS fetuses. The intra-assay coefficients of variation for three serum pools that assayed 1.2, 2.1 and 3.6 ng/ml were 5.2, 9.0 and 4.9% respectively.

Statistical analysis

Differences between breeds in all components estimated were tested using mixed model procedures; the repeated measure procedure was used with all morphological data (SAS 1999). For fetal samples, the model included fixed effects of breed, age and breed × age interactions and random effects of litter nested within breed. For postnatal samples, the model included fixed effects of breed, age and breed × age interaction and random effects of litter.

Figure 1 (A) Body weight and (B) testis weight of Meishan (MS) and White Composite (WC) boars during fetal and neonatal life. Data are LSMs $\pm$ S.E. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; dpc, days postcoitum; dpp, days postpartum; Brd, breed; NS, not significant.

Quadrant was the repeated measure. Use of a covariate was used to adjust for fetal weight within breed and age or for birth weight within breed in the statistical models that assessed testicular weight, mass of tubules and interstitium, and number of Sertoli and germ cells. Paired comparisons were made using the Tukey–Kramer procedure. Data were transformed to logarithms to adjust for heterogeneity of variance when required but are presented as least square means (LSMs) with standard errors.

Results

Body weight increased with advancing age in both breeds ($P < 0.001$); WC boars were heavier than MS boars (Fig. 1A). Likewise, testicular weight increased with age ($P < 0.001$), and WC boars had larger testes than MS boars (Fig. 1B). No differences were observed in any testicular morphological parameters between different quadrants of the testis sampled. The mass of interstitial tissue was

Figure 2 (A) Interstitial mass, (B) seminiferous tubule mass and (C) proportion of the testis occupied by seminiferous tubules of the left testis in Meishan (MS) and White Composite (WC) boars during fetal and neonatal life. Data are LSMs $\pm$ S.E. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; dpc, days postcoitum; dpp, days postpartum; Brd, breed; NS, not significant.

greater in WC boars at all ages (Fig. 2A) and increased more rapidly during early postnatal development in WC than in MS boars. Tubular mass was greater in WC boars

Table 1 Characteristics of testicular structures (LSM $\pm$ S.E. during fetal and early postnatal life in Meishan (MS) and White Composite (WC) boars

	60 dpc	75 dpc	90 dpc	105 dpc	1 dpp	7 dpp	14 dpp	25 dpp
Tubule area (μm^2)								
WC	3086 $\pm$ 382	3021 $\pm$ 296	2894 $\pm$ 296	2662 $\pm$ 382	2916 $\pm$ 239	3388 $\pm$ 239	2757 $\pm$ 239	2546 $\pm$ 252***
MS	3130 $\pm$ 331	2891 $\pm$ 331	2895 $\pm$ 382	3822 $\pm$ 296	3149 $\pm$ 252	2744 $\pm$ 239	2851 $\pm$ 252	4052 $\pm$ 239
Average SC nuclear volume (μm^3)								
WC	447 $\pm$ 39	411 $\pm$ 31	478 $\pm$ 31	429 $\pm$ 39	412 $\pm$ 31	666 $\pm$ 31***	565 $\pm$ 31*	482 $\pm$ 33*
MS	490 $\pm$ 34	459 $\pm$ 34	432 $\pm$ 39	458 $\pm$ 30	432 $\pm$ 33	483 $\pm$ 31	487 $\pm$ 33	563 $\pm$ 31
Average Ki67-positive SC nuclear volume (μm^3)								
WC	652 $\pm$ 44	561 $\pm$ 42	575 $\pm$ 50	509 $\pm$ 49	510 $\pm$ 34	702 $\pm$ 34**	708 $\pm$ 34†	509 $\pm$ 36
MS	608 $\pm$ 69	594 $\pm$ 49	454 $\pm$ 72	483 $\pm$ 49	583 $\pm$ 36	570 $\pm$ 34	622 $\pm$ 36	524 $\pm$ 49
GC no. ($\times 10^6$)								
WC	0.7 $\pm$ 0.63	1.3 $\pm$ 0.50	2.8 $\pm$ 0.49	6.8 $\pm$ 0.63	9.0 $\pm$ 3.4**	10.2 $\pm$ 3.4	14.6 $\pm$ 3.5**	28.5 $\pm$ 3.6***
MS	0.8 $\pm$ 0.55	1.6 $\pm$ 0.55	2.7 $\pm$ 0.63	6.7 $\pm$ 0.50	6.2 $\pm$ 3.5	8.2 $\pm$ 3.4	21.1 $\pm$ 4.0	48.5 $\pm$ 3.5
GC nuclear volume (μm^3)								
WC	951 $\pm$ 109	931 $\pm$ 84	1083 $\pm$ 84	1035 $\pm$ 109	1105 $\pm$ 106	1932 $\pm$ 106***	1676 $\pm$ 106**	1445 $\pm$ 112†
MS	914 $\pm$ 94	1024 $\pm$ 94	874 $\pm$ 109	868 $\pm$ 86	1087 $\pm$ 111	1245 $\pm$ 106	1245 $\pm$ 112	1179 $\pm$ 106
GC no./SC								
WC	0.16 $\pm$ 0.03†	0.13 $\pm$ 0.02**	0.13 $\pm$ 0.02*	0.10 $\pm$ 0.03	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01
MS	0.24 $\pm$ 0.03	0.23 $\pm$ 0.03	0.21 $\pm$ 0.03	0.14 $\pm$ 0.02	0.08 $\pm$ 0.01	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.07 $\pm$ 0.01

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; dpc, days postcoitum; dpp, days postpartum; SC, Sertoli cell; GC, germ cell.

during prenatal development, but tubular mass increased more rapidly in postnatal MS boars such that MS boars had a greater tubular mass than WC boars at 25 dpp (Fig. 2B). Similarly, seminiferous tubule size remained relatively constant until 25 dpp, when MS boars had considerably larger tubules compared with WC boars (Table 1; Fig. 3A and B). The proportion of the testis occupied by tubules declined with advancing age ($P < 0.001$) until 14 dpp, followed by a small increase in both breeds by 25 dpp (Fig. 2C). Volume % of tubules was consistently higher in MS than WC testes (Fig. 2C). Conversely, the proportion of the testis composed of interstitial tissue increased with age ($P < 0.001$) and was consistently greater in WC than MS testes (data not presented). General morphology of seminiferous tubules was similar between breeds. However, extremely large tubules with considerably more Sertoli cells and germ cells, reminiscent of egg cell nests in the porcine fetal ovary, were often observed in testes from MS boars (Fig. 3C).

The total number of Sertoli cells per testis increased with age ($P < 0.001$), and was greater prenatally in WC than in MS boars (Fig. 4A). Postnatally, the number of Sertoli cells increased rapidly in MS to be greater ($P < 0.01$) on 1 dpp than observed in WC boars. The average nuclear volume of Sertoli cells remained constant during fetal life but increased during the postnatal period ($P < 0.001$); this increase was asynchronous between the two breeds ($P < 0.001$; Table 1). WC boars had larger Sertoli cell nuclei compared with MS boars at 7 and 14 dpp but the reverse was observed at 25 dpp (Table 1). The average nuclear volume of Ki67-positive Sertoli cells

tended to be larger than the average nuclear volume of the total Sertoli cell population (Table 1). WC boars had larger Ki67-positive Sertoli cell nuclei at 7 dpp with a similar trend at 14 dpp. No other breed differences were observed.

The proportion of Ki67-positive Sertoli cells increased ($P < 0.001$) from 75 dpc, was maximal around 90 dpc (Fig. 4B) and declined thereafter in both breeds ($P < 0.01$). Breed differences were not observed from 60 dpc through 7 dpp, but WC boars had a greater proportion of proliferating (Ki67-positive) Sertoli cells at 14 and 25 dpp compared with MS boars (Fig. 4B).

At 60 dpc, plasma FSH concentrations were undetected in 37% of the fetuses; if these were assigned the limits of detection, 0.2 ng/ml, then mean concentrations were 1.2 and 1.3 ng/ml in WC and MS male fetuses. At 75 dpc, plasma FSH concentrations were greater ($P < 0.05$) in WC than in MS boars (Fig. 5). FSH concentrations decreased through 105 dpc in WC but increased during this period in MS boars ($P < 0.01$, Fig. 5). From 105 dpc to 1 dpp, FSH increased in WC but decreased in MS boars (Fig. 5). From 1 to 25 dpp, plasma FSH concentrations decreased in boars of both breeds ($P < 0.001$).

Total numbers of germ cells increased with advancing age during fetal and neonatal life ($P < 0.001$). Germ cell numbers did not differ between breeds during fetal development, WC boars had greater germ cell numbers than MS boars at 1 dpp, but MS boars had substantially more germ cells at 14 and 25 dpp compared with WC boars (Table 1). Breed differences in germ cell nuclear volume were not observed during fetal life, but WC boars

Figure 4 (A) Total Sertoli cell number per testis and (B) percentage of proliferating (Ki67-positive) Sertoli cells in Meishan (MS) and White Composite (WC) boars during fetal and neonatal life. Data are LSMs $\pm$ S.E. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; dpc, days postcoitum; dpp, days postpartum; Brd, Breed; NS, not significant.

had larger germ cell nuclei at 7, 14 and 25 dpp (Table 1). The germ cell to Sertoli cell ratio was greater in MS than WC fetal boars, but breed differences were not observed at postnatal ages (Table 1).

Discussion

Sertoli cell number is the primary determinant of mature testicular size and capacity to produce sperm (Orth *et al.* 1988, Russell *et al.* 1990, Chubb 1992), and thus therein resides potential to decrease production (artificial

Figure 3 Brightfield photomicrographs of (A) Meishan and (B) White Composite boar testes at 25 days postpartum, and (C) Meishan testis at 105 days postcoitum immunohistochemically stained with GATA4. In (C), note the enlarged seminiferous tubules (est) containing many Sertoli cells (sc) and germ cells (gc) as compared with normal seminiferous tubules (st).

Figure 5 FSH concentrations. Data are LSMs $\pm$ S.E. * $P < 0.05$; dpc, days postcoitum; dpp, days postpartum; Brd, breed; NS, not significant.

insemination) costs by producing boars with large testes and sperm cells containing superior genes. Potential modification of testicular size is thus dependent on identification of factors that control Sertoli cell population size. This is the first study to describe Sertoli cell population ontogeny in boar testes during fetal and neonatal life using direct stereological methods.

Mature MS boars have smaller testes resulting from fewer Sertoli cells, a greater percentage of interstitial structures but a lower percentage of seminiferous tubules and reduced total daily sperm production compared with European breeds (Okwun *et al.* 1996, Lunstra *et al.* 1997). Reduced testicular size in MS boars was evident in this study by 60 dpc and remained lower through 25 dpp compared with WC boars. The mass of seminiferous tubules and interstitial structures both increased with advancing age in each breed during fetal and neonatal life; however, interstitial mass increased at a faster rate in WC than MS testes, accounting for differential testicular size. These breeds were reversed in the rate of seminiferous tubule growth during fetal and early neonatal life, whilst MS boars had a greater mass of tubules by 25 dpp compared with WC boars.

Sertoli cell number during fetal and early neonatal life showed a pattern of development similar to that of tubular mass, as predicted by the strong correlation between tubular mass and Sertoli cell number in prepubertal and mature boars (Russell *et al.* 1990, Lunstra *et al.* 1997, Franca *et al.* 2000). From 60 to 90 dpc, tubular mass and number of Sertoli cells were greater in WC than in MS boars and increased synchronously in both breeds. However, after 105 dpc and during early postnatal life, this synchrony was lost. Notably, MS boars had a significantly greater mass of tubules with greater diameter compared with WC boars at 25 dpp despite similar numbers of Sertoli cells.

These observations on numbers of Sertoli cells contrast with those of our earlier study (McCoard *et al.* 2001a) that identified greater numbers of Sertoli cells in WC than MS boars at 14 dpp. Differences in birth weight of the boars in these two experiments potentially account for this inconsistency. In the earlier study, WC boars were disproportionately heavier relative to newborn boars of this genetic line, and their birth weights were 17% heavier than WC boars of the current study. Whereas, MS boars had similar birth weight in these two studies, 1.05 vs 1.00 kg. These observations emphasize the significant influence of birth weight on testicular size and Sertoli cell population size in boars and support findings of a related study with MS $\times$ WC crossbred boars that identified a significant influence of birth weight on mature testicular size (J J Ford, unpublished observations). Thus, the current observations should better reflect differences between boars of these two breeds than that which was observed in our earlier study.

An enigma in the current findings is that the greater number of Ki67-positive Sertoli cells in WC boars on 14 and 25 dpc did not translate into a greater total number of Sertoli cells relative to MS boars. The presence of Ki67 identifies cells in all phases of the cell cycle except G_0 and early G_1 ; thus, Sertoli cells of MS boars from 14 to 25 dpp may have a shorter cell cycle with reduced duration of the proliferative phases. Alternatively, cell cycle length and thus proliferation rate of Sertoli cells increased in WC boars during this stage of development, or there may be differences within each breed of boars in Sertoli cell longevity.

Sertoli cell proliferation peaks during late fetal life, declines during postnatal life with the adult complement reached prior to puberty in rats (Means *et al.* 1976, Orth 1982, Gondos & Berndtson 1993), mice (Kluin *et al.* 1984), sheep (Monet-Kuntz *et al.* 1984) and New World monkeys (Rey *et al.* 1993). Maximal Sertoli cell proliferation during late fetal life observed in this study illustrates that porcine Sertoli cell population growth is consistent with that in other species, contrasting with earlier reports in pigs employing indirect measures of Sertoli cell population growth (Tran *et al.* 1981, Putra & Blackshaw 1985, Kosco *et al.* 1987, Swanlund *et al.* 1995). However, in Old World monkeys (Marshall & Plant 1996) and a strain of wild pigs (Franca *et al.* 2000) Sertoli cell proliferation continued through pubertal development.

Gonadotropins have been implicated in the regulation of Sertoli cell proliferation in rodents (Orth 1984, Meachem *et al.* 1996, Baker & O'Shaughnessy 2001) and monkeys (Marshall & Plant 1996). In contrast to observations of the current study relative to FSH, Goxe *et al.* (1993) reported no difference in serum FSH concentration and FSH receptor levels between MS and a White breed of pigs. However, these investigators also observed no difference in gonadal weights between the two breeds. In the present study, maximal proportions of Ki67-positive

Sertoli cells (~90 dpc) occur prior to the period of maximal FSH concentrations in both breeds. Further, during the first 25 dpp, Sertoli cell numbers increase at a more rapid rate in MS than in WC boars, but during this period plasma FSH secretion subsides (Fig. 5; Lunstra *et al.* 1997, Franca *et al.* 2000, Ford *et al.* 2001). This further supports the observation that Sertoli cell proliferation in boars is not accelerated by greater FSH secretion (Ford *et al.* 2001), in contrast to earlier claims that exogenous FSH had a stimulatory effect (Swanland *et al.* 1995); however, this study reported on length of seminiferous tubules rather than enumeration of numbers of Sertoli cells. Furthermore, in WC boars, proliferation of Sertoli cells continues until 100 dpp (Tran *et al.* 1981), a period of reduced FSH secretion, and the magnitude of the neonatal increase in FSH secretion is not related to adult testicular size in boars (Ford *et al.* 2001). Also, the increase in FSH secretion that occurs after unilateral castration has minimal effect on Sertoli cell proliferation (Lunstra *et al.* 2003).

Sertoli cells do not proliferate after formation of the blood–testis barrier (Gondos & Berndtson 1993). The appearance of a lumen within seminiferous tubules coincides with formation of tight junctional complexes and the blood–testis barrier (rat: Tindall *et al.* 1975, Setchell *et al.* 1988, Russell *et al.* 1989, rabbit: Sun & Gondos 1986, bull: Curtis & Amann 1981) signifying Sertoli cell maturation. In the present study, decline in Sertoli cell proliferative activity from 14 to 25 dpp coupled with increased seminiferous tubule diameter and Sertoli cell nuclear size, indicators of maturation (Steinberger 1976), signals earlier termination of mitosis and advanced Sertoli cell maturation in MS compared with WC boars. Whilst total Sertoli cell numbers at 25 dpp are the reciprocal of mature Sertoli cell numbers, early termination of mitosis in MS boars would result in a smaller adult Sertoli cell population and smaller testes compared with WC boars. These observations are consistent with the appearance of lumina by 28 dpp and early onset of spermatogenesis (56 dpp) in MS boars (Lunstra *et al.* 1997), contrasting initiation of lumen formation by 94 dpp (Kosco *et al.* 1989), and completion by 120 dpp (Tran *et al.* 1981) in WC boars. These observations further illustrate the considerable breed differences in timing of Sertoli cell maturation and blood–testis barrier formation, and thus duration of the Sertoli cell proliferation period. Compensatory hypertrophy of testes in boars is not accompanied by a large increase in Sertoli cell numbers, irrespective of time of hemicastration (Lunstra *et al.* 2003), unlike young rams and male rats (Waites *et al.* 1983, Hochereau-de Reviers *et al.* 1984, Orth *et al.* 1984), suggesting mature Sertoli cell population size is genetically determined or pre-programmed prior to birth in boars. Morphological signs of early Sertoli cell maturation by 14 dpp in MS boars in the present study are consistent with this notion.

FSH has been associated with Sertoli cell maturation (Steinberger *et al.* 1964, Griswold 1993). However, the

role for gonadotropins in blood–testis barrier formation is controversial. Absence of germ cells, hypophysectomy and estrogen treatment produce a slight delay but do not prevent or reverse blood–testis barrier formation (Johnson 1973, Vitale *et al.* 1973, Hagenas *et al.* 1978), indicating an uncoupling of tight junction formation from gonadotropins. However, tight junctions that lead to the formation of the blood–testis barrier do not form in hypogonadotropic hypogonadic men, but form following gonadotropin treatment (de Krestser & Burger 1992). Gonadotropin-dependency of tight junction formation is also supported by others (Tung *et al.* 1975, Bressler 1976, Janecki *et al.* 1991a,b), but treatment of rats during postnatal life (0–32 days of age) with antisera to luteinizing hormone or FSH fails to modify Sertoli cell junctional complexes (Chemes *et al.* 1979). Thus, it is likely that species differences exist in the dependency of blood–testis barrier formation on gonadotropins. Early morphological signs of Sertoli cell maturation observed for MS boars in the present study were not coupled to changes in FSH secretion. Further, blood–testis barrier formation in MS boars occurs at ~28–42 dpp (Lunstra *et al.* 1997) and WC boars at 94–120 dpp (Tran *et al.* 1981), preceding the pubertal increase in gonadotropins and testosterone (Lunstra *et al.* 1997), supporting the phenomenon that mature testicular size of boars is not correlated with pubertal increases in gonadotropins and testosterone (Ford *et al.* 2001).

In summary, this study reports that maximum Sertoli cell proliferation occurs during late fetal life in the pig and is not tightly coupled to FSH secretion. Rather, the number of Sertoli cells in adult testes is probably determined by length of the proliferative period, potentially regulated by Sertoli cell maturation and/or timing of tight junction formation leading to blood–testis barrier formation. Mechanisms that regulate duration of Sertoli cell proliferation in boars remain to be elucidated, but MS and WC boars differ in these.

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