Development of the testicular interstitium after neonatal hemicastration in the boar*

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Summary. Development of the prepubertal interstitium of the right testes was characterized every 14 days from 10 to 122 days of age in intact boars (I) and boars hemicastrated (HC) at 10 days of age from two herds (Trial 1 and Trial 2). Comparisons were made between the remaining testis of Group-HC boars and one testis in Group-I boars. The relative mass (mass of component/body mass) of interstitium was 151% greater ($P < 0.001$) in Group-HC than Group-I boars by 52 days of age. The relative mass of interstitium was greater ($P < 0.01$) in Trial-1 than Trial-2 boars within each treatment from 80 to 122 days of age. The relative mass of interstitial space was 76% greater ($P < 0.05$) in Group-HC than in one testis of Group-I boars by 52 days of age and greater ($P < 0.05$) in Trial-1 than Trial-2 boars within each treatment from 80 to 122 days of age. The relative mass of Leydig cells was 254% greater ($P < 0.0001$) in Group-HC than Group-I boars by 52 days of age and remained greater ($P < 0.05$) in Group-HC than Group-I boars from 52 to 122 days of age. By 52 days of age the relative mass of Leydig cell nuclei and cytoplasm was 235% and 265% greater ($P < 0.0001$) in Group-HC than Group-I boars, respectively, and both remained greater ($P < 0.05$) in Group-HC than in Group-I boars until 122 days of age. The relative mass of small vessels was 86% greater ($P < 0.01$) in Group-HC than Group-I boars from 24 to 66 days of age and was similar in Group-I and Group-HC boars from 66 to 122 days of age. The relative mass of Leydig cells, nuclei and cytoplasm as well as small vessels was similar between trials of boars within each treatment. Neonatal hemicastration of boars at 10 days of age therefore resulted in an overcompensation in numbers of Leydig cells, measured as nuclear mass, whereas the increase in vascular development and interstitial space did not fully compensate the loss of testicular tissue. The cytoplasm to nuclear ratio reflected the steroid production of the Leydig cells which was related to pubertal tubular development rather than treatment.

Keywords: boar; testis; hemicastration; interstitium; Leydig cell

Introduction

Compensatory development of the remaining gonad occurs after hemicastration in the male rat, rabbit, ram, bull, boar and stallion (Lipschütz, 1922; Land & Carr, 1975; Cunningham *et al.*, 1978; Barnes *et al.*, 1980; Sundby *et al.*, 1981; Ott *et al.*, 1984). Neonatal hemicastration of bulls and rams does not influence plasma testosterone concentration (Boockfor *et al.*, 1983; Waites *et al.*, 1983) because of the increased steroid production by the remaining gonad (Lindner & Rowson, 1961; Lindgren *et al.*, 1976; Moger *et al.*, 1985). In the boar, however, a decrease was observed 1–6 days after hemicastration at 10 days of age (Ruen *et al.*, 1988). This treatment also resulted in a doubling in the mass of the remaining gonad in <28 days which coincided with elevations in plasma FSH

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and GH concentrations (Kosco *et al.*, 1987). The weight increase of the remaining gonad in these boars was mainly caused by an increase in seminiferous tubule development, including a 14-day advancement of the onset of spermatogenesis (Kosco *et al.*, 1989). The Leydig cells occupy a relatively minor portion of the testis and variation in the weight of these cells would not be reflected in the testicular weight. Since virtually nothing is known about the response of the Leydig cells after neonatal hemicastration, the purpose of this paper is to characterize morphometrically the prepubertal development of the testicular interstitium in boars after neonatal hemicastration. Seminiferous tubule development (Kosco *et al.*, 1989) and hormonal changes (Kosco *et al.* 1987) have been described for these boars.

Materials and Methods

The experimental design, care of animals, and castration procedures have been described previously (Kosco *et al.*, 1987). Techniques used for vascular perfusion fixation of the testis, tissue preparation, and morphometric analysis have also been described (Kosco *et al.*, 1989).

Volume densities (the number of points superimposed over a particular type of testicular component divided by the total number of points in the reference area) for the following interstitial structures were determined: cytoplasm and nuclei of Leydig cells, large vessels (small arterioles), small vessels (blood and lymphatic vessels) and interstitial space. No correction factors for tissue preparation were used since all tissues were prepared and handled the same way. Mass of the interstitial components per testis was determined by multiplying the volume density of the components by the parenchymal mass. The relative mass of the parenchyma and interstitial components for each testis was determined by dividing the mass of the parenchyma and interstitial component by the body mass. The ratio of Leydig cytoplasm to nuclei for each testis was determined by dividing the relative mass of Leydig cell cytoplasm by the relative mass of Leydig cell nuclei.

Since individual interstitial components may or may not develop at a rate equal to the rate of body growth, masses are expressed as relative mass to emphasize growth in relation to the animal as well as marked changes in growth rate.

The results were analysed using SAS (Statistical Analysis System, 1982) as a completely random design in a factorial arrangement. All values were analysed for treatment, age and trial, as well as for interactions between the variables. No significant treatment- $\times$ -trial interaction occurred for any parameter. Treatment- $\times$ -age and age- $\times$ -trial interactions were observed and were apparently due to a later onset of pubertal weight increase in Trial-2 boars. Therefore, Trial 1 and Trial 2 were represented separately whenever a significant interaction occurred, although interactions never involved the hemicastration effect. When significant differences occurred ($P < 0.05$) means were separated using Duncan's Multiple Range test.

Results

Response of the interstitium

Percentage of the parenchymal mass (testicular mass less estimated weight of the testicular capsule) comprised by the interstitium was related to the stage of pubertal testicular development and not to treatment or trial within a treatment. The interstitium comprised 80% of the parenchymal mass at 10 days of age and decreased ($P < 0.05$) to approximately 50% of the parenchymal mass by 80 days of age. In Trial-1 Group-I and Group-HC boars and in Trial-2 Group-HC boars, all of which exhibited an increase in relative mass of the parenchyma from 80 to 122 days of age (Kosco *et al.*, 1989), the portion of parenchymal mass consisting of interstitium decreased ($P < 0.05$) to $\sim 25\%$ by 122 days of age. In Trial-2 Group-I boars, which had no increase in relative mass of the parenchyma from 80 to 122 days of age (Kosco *et al.*, 1989), the portion of parenchyma comprised by interstitium remained at $\sim 50\%$ from 80 to 122 days of age.

The relative mass of interstitium (Fig. 1a) in Group-I boars changed little from 10 to 80 days of age. From 80 to 122 days of age it increased by 58% in Trial-1 Group-I boars ($P < 0.05$) but remained the same in Trial-2 Group-I boars. By 52 days of age Group-HC boars exhibited a 151% greater ($P < 0.0001$) relative mass of the interstitium compared to Group-I boars. From 80 to 122 days of age Trial-1 Group-HC boars exhibited an increase ($P < 0.05$) in relative mass of the interstitium, but Trial-2 Group-HC boars did not.

There was little change in the relative mass of interstitial space (Fig. 1b) of Group-I boars from 24 to 80 days of age. From 80 to 122 days of age there was an increase ($P < 0.05$) in the relative

Fig. 1. Relative mass (component mass/body mass $\times 10^{-5}$) of interstitium (a) and interstitial space (b) every 14 days from 10 to 80 days of age for all Group-I (○—○) and Group-HC (○—○) boars and from 80 to 122 days of age for Trial-1 (●—●) and Trial-2 (■—■) Group-I (—) and Group-HC (---) boars. All values mean $\pm$ s.e.

mass of interstitial space in Trial-1 Group-I boars, but not in Trial-2 Group-I boars. By 52 days of age the relative mass of interstitial space in Group-HC boars had increased ($P < 0.05$) to 76% over that in Group-I boars. The difference was maintained throughout the study. Trial-1 Group-HC boars exhibited a rapid increase ($P < 0.05$) in the relative mass of the interstitial space from 80 to 122 days of age, but Trial-2 boars did not.

The relative mass of Leydig cells (Fig. 2a) of Group-I boars did not change from 10 to 24 days of age, decreased ($P < 0.05$) from Day 24 to Day 52, remained unchanged from Day 52 to Day 80, and increased ($P < 0.05$) from 80 to 122 days of age. By 52 days of age the relative mass of Leydig cells in Group-HC boars was 254% greater ($P < 0.0001$) than in single testes of Group-I boars. The difference ($P < 0.05$) was maintained from 52 to 122 days of age. There was no difference in relative mass of Leydig cells between trials of boars within each treatment.

The relative mass of small vessels of Group-I boars increased ($P < 0.05$) from 10 to 24 days of age, decreased ($P < 0.05$) from Days 24 to 52 and remained essentially the same from 52 to 122 days of age. In Group-HC boars there was an 86% increase ($P < 0.01$) in relative mass of small vessels by Day 24, but Group-HC and Group-I boars were similar from 66 to 122 days of age. The relative mass of small vessels was similar between groups of boars within each treatment.

Response of Leydig cells

The number of Leydig cells measured as the relative mass of Leydig cell nuclei (Fig. 2b) of Group-I boars decreased ($P < 0.05$) from 10 to 52 days of age before increasing ($P < 0.05$) from 52 to 122 days of age. The relative mass of Leydig cell cytoplasm (Fig. 2c) of Group-I boars doubled

Fig. 2. Relative mass (component mass/body mass $\times 10^{-5}$) of Leydig cells (a), Leydig nuclei (b), and Leydig cytoplasm (c) every 14 days from 10 to 122 days of age for Group-I (—) and Group-HC (---) boars. All values mean $\pm$ s.e.

($P < 0.05$) from 10 to 24 days of age, decreased ($P < 0.05$) from Days 24 to 80 and increased ($P < 0.05$) from 80 to 122 days of age. By 52 days of age Group-HC boars exhibited a 235% and 263% greater ($P < 0.05$) relative mass of Leydig cell nuclei and cytoplasm, respectively, than did Group-I boars (Figs 2b, 2c). These differences ($P < 0.05$) were maintained from 52 to 122 days of age. There were no differences between trials within each treatment.

The activity of the Leydig cells measured as the ratio of the mass of Leydig cell cytoplasm to nuclei (Fig. 3) of Group-I boars decreased ($P < 0.05$) by 85% from 24 to 80 days of age, but by 122 days of age it had regained ($P < 0.05$) more than half its value at Day 24. The ratio of the mass of Leydig cell cytoplasm to nuclei was similar between treatments and trials of boars within each treatment during the study.

Histological observations

The morphology of the Leydig cells (Figs 4 & 5) agreed with descriptions by van Vorstenbosch *et al.* (1984) and Lunstra *et al.* (1986). There were no differences detected between Group-I and

Fig. 3. Ratio of the mass of Leydig cell cytoplasm to nuclei every 14 days from 10 to 122 days of age for Group-I (—) and Group-HC (---) boars. All values mean $\pm$ s.e.

Group-HC Leydig cells in testes at a similar stage of development. Degenerating Leydig cells were never detected in any section.

Discussion

Development of Leydig cells in the normal boar occurs at the time of gonadal differentiation, late gestational-neonatal development and at puberty (Allen, 1904; Whitehead, 1904; van Straaten & Wensing, 1978; Peyrat *et al.*, 1981; van Vorstenbosch *et al.*, 1982, 1984). Leydig cell development is characterized by an increase in number and cytoplasmic volume. The increase in cytoplasmic volume of Leydig cells occurs in response to a proliferation of smooth endoplasmic reticulum (Belt & Cavazos, 1967; Aquas, 1981; van Vorstenbosch *et al.*, 1984) which coincides with increased testicular steroidogenesis (Raeside & Sigman, 1975; Colenbrander *et al.*, 1978; Ford *et al.*, 1980; Herrera *et al.*, 1983; Lunstra *et al.*, 1986). The ratio of the mass of Leydig cell cytoplasm: nuclei, therefore, is a measure of the cytoplasmic volume and an estimate of steroidogenesis of individual Leydig cells. The mass of Leydig cell cytoplasm of the testis of Group-HC boars was double that of one testis in Group-I boars by 52 days of age, yet the cytoplasmic volume of individual Leydig cells remained similar for Group-HC and Group-I boars throughout the study. Therefore, the proliferation in the mass of Leydig cell cytoplasm that occurred in Group-HC boars appears to be the result of an increase in Leydig cell numbers. In the male mouse, human and boar, Leydig cells originate primarily by differentiation from mesenchymal cells, and mitosis is rarely observed among Leydig cells of these species (Russo & Rosas, 1971; Mancini *et al.*, 1963; Moon & Hardy, 1973). Changes in the number of Leydig cells of boars have been reported to be indirectly related to the number of mesenchymal cells (Moon & Hardy, 1973). Therefore, proliferation of Leydig cell numbers in Group-HC boars may have been due to increased mitotic activity among testicular mesenchymal cells which then differentiate into Leydig cells, or to an increased recruitment of existing mesenchymal cells for differentiation into Leydig cells.

As reported in an earlier study (Kosco *et al.* 1987), plasma luteinizing hormone (LH) and prolactin concentrations were similar in these same Group-HC and Group-I boars. Changes in plasma prolactin and LH concentrations of Group-HC boars from 24 to 52 days of age (Kosco *et al.*, 1987) appeared unrelated to the proliferation of Leydig cell numbers. Plasma growth hormone (GH) and follicle-stimulating hormone (FSH) concentrations for these same Group-HC boars (Kosco *et al.*, 1987) were higher than the concentrations in Group-I boars from 16 to 38 and 24 to 38 days of age, respectively, but became similar to the concentrations in the Group-I boars 14 days

Fig. 4. Light micrographs of Leydig cells for Group-I boars at 24 days (a) and 52 days (b) of age and for Group-HC boars at 24 days (c) and 52 days (d) of age. Leydig cells (LC) at 24 days of age are polygonal in shape and have large cytoplasmic volumes. Each cell contains a prominent eccentrically positioned nucleus (N) and small osmium stained droplets. From 24 to 52 days of age, cytoplasmic volume of Leydig cells decreases and nuclei become less pronounced. Poly/Bed embedding, staining 1% toluidine blue; $\times 400$.

before the completion of Leydig cell proliferation. These observations suggest that none of the pituitary hormones measured may be directly involved in the proliferation of Leydig cell numbers in Group-HC boars. Sertoli cells from immature rats and boars produce a somatomedin C/insulin-like growth factor-I (Sm-C/IGF-I) *in vitro* (Tres *et al.*, 1983; Smith *et al.*, 1984; Perrad-Sapori *et al.*, 1987a) under the influence of FSH and GH (Tres *et al.*, 1983; Perrad-Sapori *et al.*, 1987b). Leydig cells of immature boars also have Sm-C/IGF-I receptors (Perrad-Sapori *et al.*, 1987a). Administration of purified human Sm-C/IGF-I to immature pig Leydig cells *in vitro* enhances LH binding and steroidogenesis (Benahamed *et al.*, 1987; Perrad-Sapori *et al.*, 1987a). Whether proliferation of Leydig cells occurred in response to elevated FSH and GH concentrations in Group-HC boars is unknown. However, it is possible that elevated plasma FSH and GH concentrations in Group-HC boars may have induced Leydig cell proliferation by altering the intratesticular concentration of Sm-C/IGF-I which was then responsible for the cytodifferentiation of existing mesenchymal cells into Leydig cells. However, it is also possible that GH acts via the liver, since in rats a pubertal increase in Sm-C/IGF-I has been shown to be at least partly independent of the gonads (Handelsman *et al.*, 1987).

Fig. 5. Light micrographs of Leydig cells for Group-I boars at 80 days (a) and 122 days (b) of age for Group-HC boars at 80 (c) and 122 (d) days of age. Leydig cells of Group-HC boars are more developed than those of Group-I boars at 80 days of age. From 80 to 122 days the cytoplasmic volume of Leydig cells increases. Lipid droplets in the cytoplasm become more prominent. Poly/Bed embedding; staining 1% toluidine blue; $\times 400$.

Plasma testosterone concentration did not differ in these same Group-HC and Group-I boars from 24 to 122 days of age under the sampling interval used (15 days) (Kosco *et al.*, 1987), indicating that the testosterone production per testis in Group-HC boars was increased compared to Group-I individuals. Change in the cytoplasmic volume of Leydig cells is directly related to the change in the amount of smooth endoplasmic reticulum (Belt & Cavazos, 1967; Aquas, 1981; Ewing & Zirkin, 1983; van Vorstenbosch *et al.*, 1984) and directly coincides with changes in testicular steroid production (Raeside & Sigman, 1975; Colenbrander *et al.*, 1978; Ford *et al.*, 1980; Herrera *et al.*, 1983). Cytoplasmic volume of Leydig cells of Group-HC and Group-I boars was similar during the study, and the plasma testosterone profile (Kosco *et al.*, 1987) resembles that of the relative mass of Leydig cytoplasm of Group-I boars given in Fig. 2(c) and the diameter of Leydig cells in normal boars as determined by Peyrat *et al.* (1981). Proliferation of Leydig cells was not complete until 28 days after similar plasma testosterone concentrations were first observed for Group-HC and Group-I boars, suggesting that each Leydig cell in Group-HC boars is capable of increasing steroidogenesis or that steroid synthesis may occur in cell types other than Leydig cells. In prepubertal human males, mesenchymal cells have cytology indicative of steroidogenesis (Mancini *et al.*, 1963; Pelliniemi *et al.*, 1981). Exposure of human prepubertal mesenchymal cells to human chorionic gonadotrophin *in vitro* results in testosterone production (Chemes *et al.*,

1985). Further studies are needed to characterize the effect of hemicastration on testicular steroid production in the boar.

In summary, neonatal hemicastration of boars at 10 days of age results in a compensatory increase in the mass of Leydig cells due to an increase in Leydig cell numbers. Proliferation of Leydig cells in neonatally hemicastrated boars is not directly related to changes in plasma LH and testosterone concentrations and appears to be regulated by mechanisms other than the one stimulated by LH.

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