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Increased testicular estradiol during the neonatal interval reduces Sertoli cell numbers

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ABSTRACT

Previous studies have demonstrated that reducing endogenous testicular estradiol in neonatal boars would stimulate increased proliferation of Sertoli cells during the neonatal interval. The objective of this experiment was to determine if increasing testicular estradiol would have the opposite effect of reducing Sertoli cell numbers during the neonatal interval. Five littermate pairs of boars were evaluated with one littermate receiving a silastic implant containing estradiol and the second receiving only silastic at 1.5 weeks of age. Testes were recovered at 6.5 weeks of age and Sertoli cell numbers determined. Littermates treated with exogenous estradiol had approximately two-thirds as many Sertoli cells as their control littermates ($P < 0.001$). This is additional evidence for regulation of Sertoli cell numbers during the neonatal interval by intratesticular estradiol.

1. Introduction

The number of Sertoli cells is a major influence on sperm production capacity (Berndtson et al., 1987a,b; Kumar et al., 2016). Hence, Sertoli cell number is an important parameter contributing to reproductive efficiency of livestock, including pigs. The understanding of how this number is regulated, however, is quite incomplete. Although a stimulatory role is often ascribed to FSH, this hormone appears to have no more than a minimal role in boars (Ford et al., 2001; Lunstra et al., 2003; McCoard et al., 2003) and disparate responses in mice suggest the role of FSH in regulating Sertoli cell numbers may be less convincing than originally thought (Migrenne et al., 2012; O'Shaughnessy et al., 2012). In previous research (Berger and Conley, 2014), proliferation of Sertoli cells of pigs during the first postnatal wave was prolonged when endogenous estrogen production was reduced. This reduction in endogenous estrogens in boars led to an increased number of Sertoli cells at puberty as well.

The absence of a pituitary hormone response following altered systemic estradiol may be unique to the boar (At-Taras et al., 2006b; Wagner et al., 2006). Conveniently, this absence allows separation of a local testicular effect from a systemic response involving pituitary hormones present in rodent models. A feedback loop between pituitary gonadotropins and estradiol also complicates interpretation of testicular responses in species other than the pig. Systemically administered estradiol would be expected to reduce gonadotropins and ultimately reduce testicular steroids including estradiol in these other species. A local testicular effect of reduced estrogens seems most consistent with the collective data on increased Sertoli cell proliferation in pigs following reduction in testicular estradiol synthesis (Berger et al., 2013). The current experiment was designed to determine if increased testicular estrogen would decrease Sertoli cell numbers in juvenile pigs, the converse of previous experiments. Such a result would further support the hypothesis of a local testicular influence on Sertoli cell numbers by estrogens.

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2. Materials and methods

Animal experiments were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching and approved by the University of California, Davis Institutional Animal Care and Use Committee.

2.1. Animals and treatments

Littermate pairs of crossbred boars (Spot, Yorkshire, Hampshire, and Duroc genetic background) from five different litters were assigned to treatment at 1.5 weeks of age. Animals were anesthetized with telazol (4 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA, USA), a single injection site per animal was washed and cleaned with iodine and alcohol wipes, and the implant injected into the testis through the scrotal wall. One littermate received the estradiol silastic implant (approximately 10 mg estradiol and 0.2 g silastic) and the remaining littermate received silastic alone. Implants were prepared as previously described (Conley and Ford, 1989). In a preliminary study, three littermate boars were treated weekly with letrozole (0.1 mg/kg body weight) beginning at 1 week of age to suppress testicular estradiol production. At 2 weeks of age, one littermate received a testicular silastic implant containing estradiol as described above and a second littermate received an implant of silastic only. Testes were collected at 3 weeks of age from these boars and from an untreated littermate at euthanasia and estradiol determined in testicular homogenates.

2.2. Sample collection

Animals were anesthetized with 4 mg/kg telazol at 6.5 weeks of age and a systemic blood sample was obtained from the jugular vein. The scrotum was cleaned with iodine and ethanol wipes, and testes and epididymides removed by normal castration procedures. The epididymidis, tunica vaginalis and additional non-testicular tissues were dissected away from each testis prior to weighing. An approximately 3 mm thick section was removed from the equator of the injected testis, fixed in 4% paraformaldehyde in PBS overnight, rinsed in PBS and dehydrated in an ethanol series prior to embedding in paraffin for subsequent determination of Sertoli cell density. When readily detected, the tissue surrounding the implant was similarly fixed, dehydrated, and embedded. An aliquot of testicular parenchyma was flash-frozen on dry ice and stored at −80 °C prior to further analysis. Serum was separated by centrifugation of the blood and stored at −17 °C.

2.3. Analyses

Testicular tissue was homogenized in 0.1 mM potassium phosphate buffer (pH 7.4) for 3 min followed by sonication for 3 s. Residual tissue was pelleted by centrifugation at 4 °C (15,000 x *g* for 10 min). Supernates were analyzed for protein (Pierce™ Micro BCA assay, Thermo-Fisher #23252). Supernates and serum were extracted with methylene chloride before analysis of estradiol content by ELISA as described by the manufacturer (Cayman Chemical Company, Ann Arbor, MI). Sections (25 μm thick) from the equator of the testis were labeled with the GATA4 antibody (sc-1237, Santa Cruz Biotechnology, Dallas, TX, USA) and the average density of Sertoli cells determined in 100 counting frames, each 17040 $\mu m^2 \times 17$ μm deep (At-Taras et al., 2006a; Berger and Conley, 2014). Sertoli cells per testis were determined from the weight of the testis multiplied by the density of Sertoli cells. Sections (5 μm thick) were stained with hematoxylin and eosin and the proportion of parenchyma occupied by seminiferous tubules and the diameter of the smallest round tubular cross section in each of five fields was determined using ImageJ (Schneider et al., 2012). Coefficients of variation were 3.4% for proportion of parenchyma occupied by seminiferous tubules and 1.2% for diameter of seminiferous tubules. Data were analyzed as a mixed model with litter as a random factor and treatment as a fixed factor using the lmer procedure in R (R Development Core Team, 2014).

3. Results

Adding exogenous estradiol to the testis at 1.5 weeks of age decreased subsequent Sertoli cell numbers at 6.5 weeks of age ($P < 0.001$; Fig. 1A) and similarly decreased testicular weight ($P < 0.01$; Fig. 1B). Body weight was similar in the estradiol-treated littermates and those receiving only silastic (Fig. 1C). Testicular histology was unaffected by the silastic implant (Fig. 2). Sertoli cell density, the percentage of testicular parenchyma occupied by seminiferous tubules, and the diameter of seminiferous tubules were not altered by the testicular estradiol implant (Fig. 3). Weight of the testis receiving the estradiol-containing silastic implant was similar to the weight of its contralateral testis (6.1 g compared with 5.9 g, SEM of 1.3). At 6.5 weeks of age, estradiol content in the estradiol implanted testis was numerically greater than in the contralateral testis or the silastic alone implanted testis (about one third greater) but not statistically different (Fig. 4). In the preliminary trial 1 week after insertion of the implant, testicular estradiol was three-fold greater in the estradiol-implanted testis from the letrozole-treated littermate (38.2 pg estradiol/mg protein) compared with testes from letrozole-treated littermates with or without the silastic only implant (12.1 pg estradiol/mg protein; SD of 5.3). The estradiol implant caused testicular estradiol in a letrozole-treated littermate to be approximately 40% of that present in a vehicle-treated littermate (91.2 pg estradiol/mg protein), suggesting the estradiol implant was providing estradiol in a near physiological range.