Animal Reproduction Science 182 (2017) 134–137

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Response to gonadotrophins differs for gilts from female- and male-biased litters

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ARTICLE INFO

Keywords:
Litter sex-bias
Ovarian function

ABSTRACT

In several species, females masculinised by abnormal androgen exposure in utero have poor reproductive performance and gilts born into litters with a male bias are likely exposed to greater androgen concentrations prenatally than gilts born into female-biased litters. At 24 h of age, piglet plasma testosterone concentrations in gilts from male-biased litters (> 60% male; n = 22) or female-biased litters (> 60% female; n = 27) were not different. At 18 wks of age, all gilts received an injection of 400 IU equine chorionic gonadotrophin plus 200 IU human chorionic gonadotrophin to stimulate oestrus. Two weeks after the injection gilts were slaughtered and ovaries collected for determination of numbers of corpora lutea (CL). Compared to gilts from female-biased litters, gilts from male-biased litters were more likely to ovulate (86.0% vs 59.5%, P = 0.047) and had more CL (13.1 ± 1.5 vs 7.2 ± 1.7, P = 0.015). The present data indicate an effect of birth litter sex-bias on pre-pubertal physiological development, possibly involving organisational effects at the ovarian cellular level impacting on future ovarian function. Potential impacts on subsequent fertility remain to be determined.

1. Introduction

Female lifetime reproductive performance can be affected by exposure to abnormal androgen concentrations in utero (Veiga-Lopez et al., 2009), with abnormally high exposure to androgens in utero causing masculinisation of females. In litter bearing species, female fetuses can be exposed to androgens from male fetuses secreting testicular androgens during gonad differentiation (Raeside and Sigman, 1975). Although litter bearing species such as pigs may be expected to produce litters of 50:50 male:female, sows often produce sex-biased litters (male-biased litters ≥60% males or female-biased litters ≥60% females) and there are a variety of reasons for this including sex-biased conception, and implantation and fetal mortality favouring one sex over the other (Clark et al., 1993; James, 2004; Grant et al., 2008; Rekiel et al., 2012). In rodents, the effects of androgen exposure from male litter mates can be a result of their intrauterine position, with a female developing between two males exhibiting a masculine phenotype, or it can be related to the total proportion of males in the litter (Clark et al., 1993; vom Saal et al., 1999). Female sheep exposed to abnormally high exogenous testosterone during their fetal development variably exhibit an advanced onset of puberty, irregular ovulatory cycles, anovulation, or polycystic ovaries (Padmanabhan and Veiga-Lopez, 2013). These effects could also be seen in viviparous lizard females exposed to endogenous testosterone in litters with a high proportion of males (Uller et al., 2005). Having a high proportion of males in the litter increases the likelihood that a female will develop between two males. For this reason, a male-biased litter is more likely to produce masculinized females. We hypothesize that due to increased androgen exposure in utero, neonatal gilts from male-

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http://dx.doi.org/10.1016/j.anireprosci.2017.05.012
Received 14 November 2016; Received in revised form 12 May 2017; Accepted 23 May 2017
Available online 25 May 2017
0378-4320/

biased litters will have increased basal testosterone and subsequent impaired ovarian function compared to gilts from female-biased litters.

2. Materials and methods

This experiment was conducted at the University of Adelaide Roseworthy Piggery (South Australia) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC 2013) and approved by the University of Adelaide Animal Ethics Committee (approval number S-2015-060B). A total of 49 terminal line gilts from Large White x Landrace sows were selected from 20 litters consisting of > 60% male (male-biased; n = 22) or female (female-biased; n = 27) piglets, including stillborn piglets. Up to three gilts per litter were selected and tagged as focus pigs and were monitored from birth until slaughter at 20 wks of age. They were raised under standard Australian commercial conditions with pigs being weaned at 28 d into a straw-based ecoshelter. The study began in May 2015 and was completed in November 2015 over two farrowing batches. All pigs were weighed at 24 h and 21 d, and focus pigs also at 16 wks. Pigs were allowed free access to feed and water at all times.

2.1. Testosterone

At 24 h of age a 3 mL blood sample was taken from all focus pigs via jugular venepuncture into heparinised tubes and the plasma harvested after centrifugation at 1500 x *g* for 10 min at 4 °C and then stored at −20 °C until required for assay. Plasma was assayed for testosterone using a testosterone enzyme immunoassay kit (Arbor Assays, catalogue number K032-H5) as described by Graham et al. (2015), following the manufactures instructions and using a double extraction with diethyl ether. All samples were assayed in triplicate and the intra- and inter-assay coefficients variation were 12% and 0.77%, respectively, and assay sensitivity was 9.92 pg/mL.

2.2. Ovarian response to stimulation

At 18 wks of age, all gilts were injected with 400 IU equine chorionic gonadotrophin plus 200 IU human chorionic gonadotrophin (PG600®, Intervet Australia, Bendigo, Victoria). Gilts were then exposed to fenceline contact with boars in a detection-mating area for 20 min/d for 6 d for oestrus detection. Gilts were deemed to be oestrous if they exhibited a standing response to back pressure in the presence of a boar. At 20 wks of age the gilts were slaughtered and their ovaries collected for determination of numbers of corpora lutea (CL). Gilts without CL were classified as anovular.

2.3. Statistical analysis

Testosterone concentrations were analysed using a one way ANOVA (IBM SPSS Statistics 22) with testosterone concentration as the dependent variable and the sex-bias (male- vs female-bias) as the independent variable. Oestrus and ovulation data were analysed using a general linear model for the proportion of gilts that exhibited oestrus and ovulated, and numbers of CL from each sex-bias with birth weight and weaning weight as covariates. Regression analysis was conducted to examine the relationship between the sex ratio of the birth litter and the number of CL. A one-way ANOVA was used to analyse differences in farrowing data, rearing data, and weights for each bias.

3. Results

3.1. Testosterone

There was no detectible effect of birth litter sex ratio on plasma testosterone concentrations in female piglets 24 h after birth. The mean (± SEM) testosterone concentrations for female piglets from female-biased litters was 0.52 ± 0.06 ng/mL compared to 0.66 ± 0.08 ng/mL for female piglets from male-biased litters (P = 0.228).

3.2. Ovarian response

Although there were differences in total born litter size (P = 0.003) and pigs weaned (P = 0.003) in the birth litters, there no treatment differences in piglet weights at any time (Table 1). By 20 wks of age fewer gilts from female-biased litters had exhibited oestrus than did gilts from male-biased litters (59.5% vs 86.0%, P = 0.047). All gilts that exhibited a standing response during oestrus detection had ovulated and all gilts that did not stand were anovular, as determined by presence or absence of CL on the ovaries. There was no difference in the PG600 injection to oestrus detection interval. For gilts that did ovulate, those from female-biased litters had fewer CL than did those from the male-biased litters (7.2 ± 1.7 vs 13.1 ± 1.5, P = 0.015).

The gilts from male-biased litters had a greater range of CL counts (3–31) compared to gilts from female-biased litters (3–14). There was a negative linear relationship between the sex ratio of the litter and CL number, with CL number decreasing with an increase in proportion of females (R^2 = 0.242; P = 0.003; Fig. 1).