Anogenital distance reflects the sex ratio of a gilt's birth litter and predicts her reproductive success¹

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ABSTRACT: Anogenital distance (AGD) has been used to reflect masculinization in litter-bearing species. As masculinization affects behavior and reproduction, AGD could be measured to assist in selecting gilts with a temperament more suited to commercial production and greater reproductive potential. We hypothesized that gilts from a male-biased litter would have a longer AGD and poorer reproductive performance. In Exp. 1, AGD and weight were measured at day 1, day 21, and week 16 of age for gilts from male-biased litters ($\geq 60\%$ males; $n = 51$) and female-biased litters ($\geq 60\%$ females; $n = 51$). Sow AGD was measured 3 d after farrowing. In Exp. 2, AGD was measured at gilt selection at approximately 24 wk of age and gilts followed to second parity. Litter sex ratio affected AGD at 16 wk of age, with gilts from female-biased litters having longer AGD (mean $\pm$ SEM, 9.1 ± 0.7 mm vs. 11.0 ± 0.6 mm, $P = 0.013$). Anogenital distance was not different

on day 1 or day 21. There was no effect of sex ratio on weight at any time, and sow AGD was not associated with the sex ratio of her litter. Gilts with an AGD longer than the mean of 11.55 mm were heavier (mean $\pm$ SEM, 118.8 ± 0.4 kg vs. 117.7 ± 0.4 kg, $P = 0.023$), were achieved puberty earlier (179.6 ± 0.6 d vs. 182.2 ± 0.6 d, $P = 0.001$), were mated younger (200.6 ± 0.6 d vs. 203.2 ± 0.6 d, $P = 0.001$), and were more likely to be mated (91% vs. 83% , $P = 0.005$) than gilts with an AGD shorter than the mean. Gilts with an AGD greater than 11.55 mm had a greater born alive litter size (11.79 ± 0.20 vs. 11.20 ± 0.19 , $P = 0.018$) compared with gilts with an AGD shorter than 11.55 mm. At 16 wk, AGD was associated with sex bias and could be used as a selection tool to predict reproductive success of the first parity, with a longer AGD being associated with gilts that had been born into a female-biased litter and that had better reproductive performance.

Key words: gilts, litter sex bias, reproduction, selection

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INTRODUCTION

The proportion of males in a litter can affect the phenotype and physiology of gilts that develop in that litter. Gilts from male-biased litters were more likely to fail to conceive on their first mating, were more sensitive to gonadotropins, and had less teats than gilts from a female-biased litter (Drickamer et al., 1997, 1999; Seyfang

et al., 2017). These traits coincide with what has been recorded in masculinized female rodents. Masculinized female rodents had an altered phenotype that included nipple retention, increased anogenital distance (AGD), and impaired reproductive potential (Hotchkiss et al., 2007). The AGD is measured from the anus to the genitals and can be used as an indicator of female masculinization (Clark et al., 1993; vom Saal et al., 1999; Correa et al., 2013). Males have longer AGD than females, and there is variation within sex, with masculinized females having longer AGD than non-masculinized females (vom Saal et al., 1999). In rodents, masculinization can result from intrauterine position or litter sex ratio, with females developing between 2 males, or females from male-biased litters, becoming masculinized (Clark et al., 1993; vom Saal et al., 1999). Sex-biased litters are common in commercial pig production. Sex-biased litters ($\geq 60\%$ males or females) can result for a variety of reasons including a sex-biased conception, implantation, or fetal mortality (Clark et al., 1993; James, 2004; Grant et al., 2008; Rekiel et al., 2012). In the first of 2 experiments, we hypothesized that gilts from male-biased litters will have longer AGD than gilts from female-biased litters. The first experiment also aimed to determine an appropriate age to measure AGD to predict reproductive performance. In Exp. 2, we hypothesized that gilts with a longer AGD would have poorer reproductive performance than gilts with a shorter AGD. If AGD reflects the sex ratio of the birth litter and is reflective of future reproductive performance, then AGD could be a valuable selection tool.

MATERIALS AND METHODS

Experiment 1

This experiment was conducted at the Roseworthy Piggery, The University of Adelaide (South Australia) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). It was approved by the University of Adelaide Animal Ethics Committee (approval number S-2015-060B).

A total of 102 terminal line gilts from Large White $\times$ Landrace sows were selected from 40 litters consisting of $\geq 60\%$ male (male biased; $n = 51$) or $\geq 60\%$ female (female biased; $n = 51$) piglets, including stillborn piglets. Up to 3 gilts per litter were selected and were raised under standard Australian commercial conditions; born in farrowing crates in a batch farrow system; and weaned at approximately day 28 into a straw-based ecoshelter.

The study began in May 2015 and was completed in December 2015 over 4 farrowing batches. Pigs were allowed free access to feed and water at all times.

Using digital calipers, the AGD (mm) was measured from the anus to the start of vulva opening (AGD1), to the end of the vulva opening (AGD2), and to the ventral tip of the vulva (AGD3) of 40 sows 3 days after farrowing at which time they were also weighed. Four sows were excluded from the analysis due to injury to the vulva. The AGD of 3 female offspring from each litter were also measured at day 1 and day 21 after birth and at week 16. The same animals were used at each time point with a maximum sample size at day 1. Due to management practices, not all animals could be used at each of the subsequent time points. Mortalities were recorded throughout; however, no animals that died prior to week 16 were included in this analysis. The piglets were weighed at each time. Day 21 was used as a measure of weaning AGD. The AGD was measured at week 16 in Exp. 1 as this is when gilt selection occurs at the Roseworthy piggery, and we wanted to determine whether AGD measurement at the time of selection could be included in the selection criteria. The birth sow AGD was used to determine whether there was a relationship between sow AGD and the sex ratio of her litter.

Experiment 2

From November to December 2016 on a commercial SunPork facility in South Australia, 747 Landrace, and Landrace $\times$ Large White gilts were selected at approximately 24 wk of age. All procedures complied with the farm's internal animal welfare standards. Gilts weighed from 105 to 130 kg, had at least 14 teats and had good leg conformation. The AGD from the anus to the start of the vulva opening was measured at selection using digital calipers. Boar exposure for 20 min/d began the day after selection in a detection-mating area. A boar was housed with approximately 20 gilts and with fenceline contact with a further 3 boars until the gilts exhibited their pubertal estrus. Gilts were considered estrous if they stood to backpressure in the presence of a boar. If estrus was not detected within 6 wk of exposure, the gilts were culled. After estrus detection, gilts were housed in pens with other gilts achieving estrus and checked daily for second estrus from 18 d in the detection mating area. Gilts were inseminated with 3×10^9 sperm in 80 mL extender at detection of their second estrus and again 24 h later if still in estrus. After mating gilts were transferred to a group-housed straw-based system with

50 to 100 gilts per group. Animals were fed a standard commercial diet and had free access to water at all times. Pregnancy status was confirmed at 4 wk by ultrasound, and nonpregnant gilts were removed. Gilts were rehoused into conventional farrowing crates 3 d before due date. Immediately after farrowing the litter size born alive, stillborn, and mummified fetuses was recorded, as was total number of females (born alive and stillborns). The total litter size was calculated as born alive, stillborns, and mummified fetuses combined. The percentage of females was calculated as [total number of females (born alive + stillborn)/litter size (born alive + stillborn)]. Retrospective data mining of Metafarms was used to collect remating and second parity data. Culled gilts were recorded throughout the experimental period from selection, with reasons for culling being anestrus, negative pregnancy test, not in pig, lameness, illness, injury, death, or unknown reason. Culling results were then separated into first and second parity, with first parity being from selection until weaning of their first litter and second parity from weaning of their first litter until weaning of the second litter.

Statistical Methods

To determine whether there was a difference in AGD in gilts from male-biased litter compared with gilts from a female-biased litter in Exp. 1, AGD 1, 2, and 3 were analyzed using a linear mixed model (IBM SPSS Statistics 22) for each time point. The fixed factors included in the model were weight, parity (0 to 5), and skew ($\geq 60\%$ male = male biased or $\geq 60\%$ female = female biased), with litter ID as a random factor and weight included as a covariate. Gilt body weight at each time was also run as the dependent variable with fixed factors sow parity and skew, with litter ID as a random factor. The same method was employed to analyze sow AGD 1, 2, and 3, with sow weight as a covariate, to determine whether there was any relationship with the sex ratio of their offspring litter. To determine if there was a difference in reproductive performance between gilts with short AGDs compared with longer AGDs, Exp. 2 data were used. The AGD measurements were divided into short and long AGDs based on the mean AGD (11.55 ± 2.68 mm). Short AGDs were defined as those that were shorter than the mean (5.81 to 11.55 mm) and long AGDs as those longer than the mean (11.56 to 17.36 mm). A general linear model was used for weight at selection, age at puberty, age at first mating, born alive litter size, total born litter size, proportion of females

in the litter, and the wean to estrus interval with weight at selection included as a covariate and breed (Landrace, Landrace $\times$ Large White), date selected, and AGD category as fixed factors. Due to the distribution of the data, a generalized linear model with a Poisson distribution was used for the still-born and mummified litter size. A generalized linear model with a binomial distribution was used for the percentage of gilts mated, positive pregnancy check, percentage that did not farrow after a positive pregnancy check (not in pigs [NIPs]), farrowing rate, and culled percentage. Again, these tests included weight as a covariate and breed, date selected, and AGD category as fixed factors. A chi-square test was used for the cull reason classifications. The threshold for significance was $P < 0.05$. All AGD data were analyzed and is presented in millimeters.

RESULTS

Experiment 1

At day 1 of age, the AGD (AGD1, AGD2, or AGD3; mean $\pm$ SEM) of gilts from female-biased litters was not different to the AGD of gilts from male-biased litters. At day 21, AGD2 was longer for gilts from female-biased litters than gilts from male-biased litters ($P = 0.036$, Table 1). At week 16, AGD1 and AGD2 were longer for gilts from female-biased litters than gilts from male-biased litters ($P = 0.013$ and $P = 0.019$, respectively, Table 1). The body weight of gilts from female-biased litters was not different to the body weight of gilts from male-biased litters at any time. The AGD of the birth sow (AGD1, AGD2, or AGD3) was not associated with the sex ratio of her litter (Table 2).

Experiment 2

Gilts with long AGDs were heavier at selection at 24 wk of age ($P = 0.023$), and with weight as a covariate, they achieved puberty earlier and were mated earlier than those having shorter than average AGD ($P = 0.001$, Table 3) and were more likely to be mated ($P = 0.005$, Table 3). Gilts with a short AGD were predominantly culled for anestrus (40% of culls), whereas those with a long AGD were more likely to be culled for a negative pregnancy check (35% of culls). There was an increase in total born alive for gilts with long AGDs compared with short AGDs in their first parity ($P = 0.018$, Table 3). There were no other differences in reproductive performance in first or second parity.

Table 1. Mean $\pm$ SEM weight (kg) of gilts and mean $\pm$ SEM anogenital distance (mm) as measured from the anus to the start of vulval opening (AGD1), to the end of the vulval opening (AGD2), and to the ventral tip of the vulva (AGD3) at day 1 after birth, day 21, and week 16 for gilts from male-biased litters and gilts from female-biased litters

	Male biased	Female biased	P-value
Day 1	<i>n</i> = 51	<i>n</i> = 51	
Weight (kg)	1.77 $\pm$ 0.07	1.70 $\pm$ 0.08	0.406
AGD1 (mm)	4.62 $\pm$ 0.25	5.26 $\pm$ 0.25	0.051
AGD2 (mm)	11.26 $\pm$ 0.55	11.73 $\pm$ 0.57	0.514
AGD3 (mm)	17.09 $\pm$ 0.72	17.14 $\pm$ 0.76	0.963
Day 21	<i>n</i> = 26	<i>n</i> = 33	
Weight (kg)	7.01 $\pm$ 0.32	7.59 $\pm$ 0.27	0.107
AGD1 (mm)	5.73 $\pm$ 0.41	6.48 $\pm$ 0.34	0.105
AGD2 (mm)	10.33 $\pm$ 2.60	11.63 $\pm$ 2.58	0.036
AGD3 (mm)	16.46 $\pm$ 0.65	17.27 $\pm$ 0.54	0.265
Week 16	<i>n</i> = 26	<i>n</i> = 35	
Weight (kg)	70.40 $\pm$ 2.24	71.20 $\pm$ 1.71	0.735
AGD1 (mm)	9.09 $\pm$ 0.72	11.01 $\pm$ 0.55	0.013
AGD2 (mm)	21.87 $\pm$ 1.22	25.17 $\pm$ 0.93	0.019
AGD3 (mm)	32.73 $\pm$ 1.61	34.94 $\pm$ 1.22	0.199

The maximum sample size was reached at day 1, and a subset of those animals were recorded at the subsequent time points due to management restraints.

DISCUSSION

The current research provides evidence that gilts with an AGD greater than 11.55 mm at 24 wk of age performed more effectively in a commercial breeding herd than gilts with an AGD less than 11.55 mm. In addition, our research indicates that gilts with a greater AGD at 16 wk of age were probably from a female-biased litter. Females from female-biased litters are generally better suited as potential replacement females as they are reproductively more efficient and productive than females from male-biased litters and our results align with these findings (Edgerton and Cromwell, 1987). Our result that gilts from a female-biased litter had a longer AGD than gilts from a male-biased litter is inconsistent with previous research conducted in rodents and pigs (McDermott et al., 1978; Drickamer et al., 1997; vom Saal et al., 1999; Nagao et al., 2004; Correa et al., 2013). Nonetheless, we provide evidence that AGD can be used to predict reproductive success in pigs and that the AGD of pigs from female-biased litters was greater than the AGD of pigs from male-biased litters.

The gilts from male-biased litters had shorter AGD than gilts from female-biased litters at 16 wk of age suggest species differences accounting in AGD determinations. Rodents have a different

Table 2. Mean $\pm$ SEM weight (kg) of sows and mean $\pm$ SEM anogenital distance (mm) as measured from the anus to the start of vulva opening (AGD1), to the end of the vulva opening (AGD2), and to the tip of the vulva (AGD3) 3 d after farrowing for male-biased and female-biased offspring

	Male biased	Female biased	P-value
Sow	<i>n</i> = 21	<i>n</i> = 15	
Weight (kg)	224.10 $\pm$ 8.03	218.75 $\pm$ 9.80	0.687
AGD1 (mm)	25.53 $\pm$ 2.01	26.96 $\pm$ 2.44	0.596
AGD2 (mm)	50.13 $\pm$ 3.78	51.55 $\pm$ 4.60	0.779
AGD3 (mm)	68.25 $\pm$ 4.26	66.92 $\pm$ 5.17	0.816

endocrine profile during pregnancy than sows, and this difference may result in greater aromatization of testosterone to estrogen at the time when the external genitalia are being formed. The progesterone levels in the maternal environment of rabbits, rats, and mice are low during sexual differentiation and external genitalia development, which occurs in early gestation (Garland et al., 1987). In the pig, however, progesterone reaches its peak concentration by early pregnancy (Robertson and King, 1974). When the concentration of progesterone is high aromatase activity is high, therefore, testosterone is more likely to be aromatized to estrogen. This means that although testosterone concentrations may be increased in male-biased litters around the time of external genitalia development, it is more likely to be aromatized to estrogen in pigs as they have higher maternal levels of progesterone at this time than in other species. This would then lead to an increase in estrogen exposure during the development of external genitalia. In rodents, increased AGD is usually reflective of exposure to excessive concentrations of androgens in utero (McDermott et al., 1978; vom Saal et al., 1999; Nagao et al., 2004), whereas excessive levels of estrogen would result in a decreased AGD (Mitchell et al., 2015). This may mean that in our study the AGDs were reduced in male-biased litters and that AGDs from female-biased litters were normal, not increased. Mack et al. (2014) found that males from a female-biased litter had an increased AGD compared with those from a male-biased litter, highlighting that the sex ratio of a litter can alter the AGD in pigs, and that a female-biased litter may result in phenotypic masculinization. In pigs, this may be due to increasing the number of females in a litter decreasing the amount of estrogen, which may result in masculinization of the offspring in female-biased litters (Mack et al., 2014). Mitchell et al. (2015) also highlighted that AGD can change over time depending

Table 3. The anogenital distance (AGD) as measured from the anus to the opening of the vulva in millimeters separated into 2 AGDs; short and long (short = 5.81 to 11.55 mm and long = 11.56 to 17.36 mm) with (mean $\pm$ SEM) reproductive data from selection until second parity as well as weight to assess the effect of the AGD on reproductive performance

	Short AGD	Long AGD	
	<i>n</i> = 398	<i>n</i> = 349	<i>P</i> -value
Weight at selection (kg)	117.68 $\pm$ 0.36	118.78 $\pm$ 0.38	0.023
Age at puberty (days)	182.17 $\pm$ 0.61	179.56 $\pm$ 0.62	0.001
Age at first mating (days)	203.17 $\pm$ 0.61	200.56 $\pm$ 0.62	0.001
Mated (%)	83	91	0.005
Positive pregnancy check (%)	91	91	0.871
Not in pigs (%)	9	8	0.761
Parity 1			
Farrowing rate (%)	79	80	0.732
Born alive	11.20 $\pm$ 0.19	11.79 $\pm$ 0.20	0.018
Stillborn	0.77 $\pm$ 0.06	0.76 $\pm$ 0.06	0.948
Mummified	0.18 $\pm$ 0.03	0.14 $\pm$ 0.03	0.244
Total litter size	12.21 $\pm$ 0.20	12.70 $\pm$ 0.21	0.060
Females in offspring litter (%)	45.84 $\pm$ 1.13	46.76 $\pm$ 1.15	0.513
Culled (%)	33	27	0.096
Top cull reason	Anestrus (40%)	Negative pregnancy check (35%)	0.011
Remated (%)	90	92	0.602
Wean to estrus interval (days)	5.92 $\pm$ 0.38	6.24 $\pm$ 0.39	0.509
Positive pregnancy check (%)	84	88	0.137
Parity 2			
Subsequent farrowing rate (%)	84	88	0.205
Born alive	11.99 $\pm$ 0.25	11.99 $\pm$ 0.26	0.996
Stillborn	0.50 $\pm$ 0.06	0.64 $\pm$ 0.07	0.054
Mummified	0.13 $\pm$ 0.03	0.13 $\pm$ 0.03	0.930
Total litter size	12.70 $\pm$ 0.26	12.85 $\pm$ 0.27	0.651
Culled (%)	23	19	0.284
Top cull reason	Negative pregnancy check (36%)	Negative pregnancy check (27%)	0.433

on the balance of estrogen and testosterone in the animal. This may be why our results at 16 wk of age differ from those of [Drickamer et al. \(1997\)](#) who found that day old gilts from male-biased litters did exhibit a masculine phenotype with an increased AGD. The timing of AGD development may occur during a short window of time, as indicated by [Petric et al. \(2004\)](#), who found that female pigs from sows treated with testosterone at day 30 of gestation would exhibit masculinized external genitalia but the same treatment at day 40 resulted in no change. Female-biased litters may have lower levels of estrogens in utero at day 30 of gestation compared with male-biased litters as female fetuses produce estrogens later in development than do males ([Parma et al., 1999](#); [McCoard et al., 2002](#)). Furthermore, testes also release estrogens and in pigs the testes produce more estrogen than many other species ([Raeside et al., 1993](#); [Booth, 1983](#)), again suggesting a species difference in the hormone profile of the uterine environment at the time of

genitalia development, which could impact AGD. Boars also have higher levels of plasma estrogens than gilts both pre- and postnatally ([Choong and Raeside, 1974](#); [Raeside and Sigman, 1975](#); [Booth, 1983](#); [Tarrar and Knight, 1995](#)). This contributes to the theory that AGD are feminized in male-biased litters, but that these females are masculinized in other aspects such as their reproduction due to the difference in timing of development relative to the AGD. The effects of a male-biased litter on reproduction were consistent with the literature, suggesting that gilts from a male-biased litter have been masculinized in utero ([Clark et al., 1993](#); [vom Saal et al., 1999](#); [Bánszegi et al., 2012](#)). Being able to determine the birth litter sex ratio using AGD would be beneficial in a gilt selection scheme as the sex ratio of the birth litter can affect behavior and elements of reproduction in pigs ([Seyfang et al., 2017, 2018](#)).

Experiment 2 showed that gilts that had a longer AGD achieved puberty earlier and had larger litter

sizes than gilts with shorter AGD, which, based on data from Exp. 1, suggests these females were from female-biased litters and presumably not masculinized. It is interesting that the effect on litter size was limited to the first parity. The reason for the parity effect is not known but may reflect additional management factors masking potential improvements in reproduction, particularly lactation management. Previous studies in pigs have found that gilts from female-biased litters were less aggressive and less likely to ovulate at 18 wk of age in response to exogenous gonadotrophins (Seyfang et al., 2017, 2018), and Drickamer et al. (1997) found that gilts from male-biased litters were less likely to become pregnant after their first mating than gilts from female-biased litters. This again highlights that the sex ratio of the birth litter can affect masculinization and reproductive performance.

The AGD of female pigs was associated with birth litter sex ratio, with the current work showing that at 16 wk of age, AGD could reflect the sex bias of the birth litter. Although we have not tested the mechanism controlling the AGD in pigs, it is possible that the physiological mechanism in pigs is different to that in rodents. In pigs, the steroids produced by males in utero do not influence the concentration of testosterone in the neighboring females (Framstad et al., 1990; Wise and Christenson, 1992). However, rodents have hemochorial placentation, whereas pigs have an epitheliochorial placenta. This may change the mode of transfer of androgens or other male hormones that could influence the AGD, therefore resulting in a species difference in AGD. This also supports the theory that the effects of males on their littermates is litter-wide in pigs, rather than an effect of the intrauterine position, as the male-biased uterine environment does not affect testosterone levels between gilts within a litter, but it could still affect the testosterone levels of the litter compared with a litter with a different bias. Alternatively, it could be a different effect of the male-biased uterine environment that affects the AGD in pigs. Measurement of AGD when gilts are selected for the breeding herd could enable producers to determine whether the gilt was from a male- or female-biased birth litter, with a longer AGD being associated with better reproductive performance as shown by a decreased age at puberty, increased mating rate, and increased born alive in parity 1. Importantly, because the AGD at 16 wk of age reflected the birth sex ratio and the AGD was also positively associated with reproductive performance of gilts, it has the potential to be included in the selection

procedure to increase reproductive uniformity and production.

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