MORPHOMETRIC CHARACTERISTICS OF FEMALE PIGLETS FROM DIFFERENT SEX RATIOS

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ABSTRACT

In a male-biased litter, there is a greater chance that a female is positioned in between two male siblings while in utero. This may lead to masculinized females with different phenotype, behavior and reproductive performance. To determine the effects of sex ratio on morphometric characteristics of female piglets, litters were classified as having ≥60% (H group) or <60% (L group) male siblings. Crown-rump length (CRL), body weight (BW), and anogenital distance (AGD1, AGD2, AGD3) of one hundred seventeen three-day-old female piglets from thirty-one litters were measured. Weight by length and adjusted anogenital distance were calculated, and the number of teats was recorded. H group had significantly lower CRL (*P*<0.05) and BW (*P*<0.05) compared to L group. AGD and teat number were not different between groups. Female AGD3 is correlated with BW (r=0.635, *P*<0.001), weight by length (r=0.581, *P*<0.001), and CRL (r=0.714, *P*<0.001) while AGD2 has similar positive relationships with weight by length (r=0.370, *P*<0.05) and CRL (r=0.508, *P*<0.001). Results suggest that BW and CRL are sensitive to the effects of sex ratio.

Key words: anogenital distance, sex ratio

INTRODUCTION

Prenatal exposure of the female fetus to high levels of testosterone has long-term effects on the reproductive performance in some polytocous species (vom Saal, 1989; Monclus *et al*., 2014). Testosterone influence the development of various tissues in utero, including the brain, and can mediate masculinization or defeminization of the individual (vom Saal, 1989). Anogenital distance (AGD), the distance between the anus and genitalia, is considered as a biomarker of prenatal androgen exposure (Hotchkiss *et al*., 2007). A masculinized female is distinguishable by its longer AGD compared to females positioned near female sibs *in utero* (vom Saal, 1989; Hotchkiss and Vandenbergh, 2005). This indicates that intrauterine position enhances testosterone exposure of females positioned between two males.

Because the intrauterine position of fetuses is hard to determine in litter-bearing animals, the sex ratio at birth acts as a proxy. It is based on the premise that the probability of a male fetus to be positioned adjacent to another fetus is the same as the sex ratio of the

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litter (Lamberson *et al*., 1988). Thus, in a male-biased litter, a female fetus growing and developing between two males is more likely to happen (Seyfang *et al*., 2017). Similar to the masculinization of female mice, the anogenital distance of female piglets is associated with a male-biased litter (Drickamer *et al*., 1997). Apart from AGD, there is no study on the effect of sex ratio on morphometric characteristics of female suckling piglets.

We, therefore, hypothesize that female piglets coming from litters with more males have different morphological characteristics compared with female piglets from litters with less than 60% males. The objectives of the study were to describe morphometric characteristics in 3-day old female piglets, to determine if these characteristics are different between females from litters with a high and low percentage of male siblings, and to examine relationships between morphometric parameters with AGD.

MATERIALS AND METHODS

The study was conducted at the University Animal Farm (Swine Section). One hundred seventeen female piglets from thirty-one litters of primiparous sows (Large White x Landrace) were used in the present study. Body weight (BW) and AGD were determined after three days. The female piglets' legs were outstretched when AGD was measured using a caliper. The anogenital distance was measured in three different ways:(1) distal end to proximal end measurement of the anus and vulva, (2) the center of the anus up to the middle of the vulva, and (3) the distal end of the anus to the distal end of the vulva (Figures 1a-c). Crown-rump length (CRL) was measured from the base of the head of the animal down to the base of the tail (Figure 1d). Weight by length was estimated by dividing BW by CRL, and the number of teats was counted. The adjusted anogenital distance was computed by dividing AGD with crown-rump length.

Overall average and standard error of the mean of the different morphometric characteristics were calculated to provide general information on the female piglets used in the study. Thirty-one litters were then classified into either the H group (male-biased, ≥60% males) and L group (non-male-biased, <60% males). A litter is considered male-biased if there are >60% males (Seyfang *et al*., 2017). In the present study, litters in the L group had 12.5% to 57% males in the litter. Average morphometric measurements of the female piglets per litter were calculated. To determine the effect of sex ratio and to adjust for the month the animal was bred, Proc GLM of SAS (SAS University Edition) was used. Further, the average body weight and weight by length of the females in the litter were adjusted to litter size in the model. If the effect of sex ratio is significant ($P<0.05$), least square means were compared using Student's *t*-test. Pearson product moment correlation was used to determine significant relationships between morphometric parameters.

RESULTS AND DISCUSSION

The present study (1) provides baseline information on morphometric characteristics including AGD, (2) reveals sex ratio effects on body weight and crown-rump length, and (3) establishes relationships between morphometric parameters taken from offspring of first parity sows.

Table 1 shows the characteristics of female offspring of primiparous sows. On average, the number of male and female piglets in a litter size of 9 from first parity sows is

a. Method 1 of measuring AGD (AGD1).

b. Method 2 of measuring AGD (AGD2).

c. Method 3 of measuring of female AGD (AGD3).

d. Measurement of CRL.

Figure 1. Body measurements taken on female pigs.

equal, leading to a male-to-female ratio of 1.01. In the present study, only eight sows had a male-biased litter (>60% males born in a litter). Using the three different methods mentioned above, AGD ranges from 0.24 to 1.60 cm. This is larger than the AGD measured within three days as reported by Drickramer *et al*. (1997) ranging from 0.54 to 0.76 cm. The difference may be due to the instrument and method used. As described by Drickramer *et al*. (1997), the anogenital distance was measured from the nearest point of the anus and the genital opening. In the present study, method 1 of measuring AGD in females had the highest (28.8%) coefficient of variation, while Methods 2 and 3 AGD values were the same (13%). Method 1 of measuring AGD in females gave the shortest distance because of the closeness of the tip

Table 1. Characteristics of litters of primiparous sows.

Parameters	Mean	SEM
General		
Male to female ratio	1.01	0.10
Proportion of male, %	49.85	2.87
Female		
BW, kg	1.77	0.05
CRL, cm	29.81	0.28
AGD1, cm	0.239	0.013
AGD2, cm	1.037	0.025
AGD3, cm	1.599	0.038
Adjusted AGD1	0.008	0.001
Adjusted AGD2	0.035	0.001
Adjusted AGD3	0.054	0.001
Teat number	16.30	1.96

of the vulva with the anus, thus, this method is most susceptible to human error.

Sex ratio pertains to the percentage or proportion of males in a litter. Results show that while AGD measurements (including adjusted to CRL) were not significantly different between groups, females in male-biased litters had lower body weight and shorter body length (Table 2). Previous report showed that the AGD of female piglets was not affected by intrauterine position (Rohde Parfet *et al*., 1990). Recently, AGD of females at 16 weeks of age but not at day old, was seen to be associated with sex ratio (Seyfang *et al*., 2018). Interestingly, females from male-biased litters had lower body weight and shorter length than females from litters with a lower percentage of males. At 104d of gestation in pigs, a fetus surrounded by the opposite sex on each side (ex., female in between two males) was shown to be lighter in weight than a fetus surrounded by the same sex (Wise and Christenson, 1992). The results of the present study suggest that CRL and BW, but not AGD at day 3 of age, are sensitive to the effects of sex ratio.

Teat number of female piglets did not differ between H and L groups. Contrary to the previous report in pigs, the proportion of male siblings is one of the predictors of teat number in gilts (Drickamer *et al*., 1999). Though testosterone was not measured in the study, it is speculated that its concentration in utero may not be sufficient to elicit changes in mammary development.

Anogenital distance (Methods 2 and 3) was positively correlated with estimated weight by length and CRL. However, AGD Method 3 is correlated with BW but not AGD Method 2 (Table 3). It is likely that the same mechanisms are involved in regulating these characteristics, thus these relationships are established.

In conclusion, body weights were lower and body length was shorter in females from male-biased litter, indicating that these characteristics are sensitive to the effects of sex ratio in females.

Table 2. Effect of sex ratio on female characteristics.

Parameters	High	Low	P-value
BW, kg	1.52 ± 0.10	1.82 ± 0.05	0.017*
CRL, cm	28.28 ± 0.67	29.99 ± 0.31	0.028*
Weight by Length	0.058 ± 0.004	0.060 ± 0.002	0.643
AGD1, cm	0.226 ± 0.030	0.254 ± 0.014	0.414
AGD2, cm	0.937 ± 0.067	1.052 ± 0.029	0.128
AGD3, cm	1.508 ± 0.100	1.614 ± 0.047	0.343
Adjusted AGD1	0.008 ± 0.001	0.009 ± 0.001	0.381
Adjusted AGD2	0.036 ± 0.002	0.034 ± 0.001	0.590
Adjusted AGD3	0.053 ± 0.003	0.054 ± 0.001	0.887
Teat number	17.01 ± 4.82	17.44 ± 2.25	0.936

Data are presented as Least square means + SEM, *Significant at *P*<0.05.

Table 3. Significant relationships between AGD and morphometric measurements.

	AGD2 Female	AGD3 Female
Weight by length	0.370	0.581
	0.044	0.001
Crown-rump length	0.508	0.714
	0.005	<0.0001
Body weight	0.303	0.635
	0.132	0.0003

Values in the first and second rows are the correlation coefficient and *P*-value, respectively.

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