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Characterization of anogenital distance and its relationship to fertility in lactating Holstein cows

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ABSTRACT

Anogenital distance (AGD) serves as a marker for prenatal androgenization, reproductive development, and fertility in humans and rodents. The primary objectives of this observational study in lactating dairy cows were to (1) characterize the distribution and variability of AGD, (2) determine the relationship among AGD and potential postnatal AGD determinants of age and height, and (3) evaluate the associations between AGD and pregnancy to first artificial insemination (P/AI) and cumulative pregnancy by 250 d in milk (DIM) within parity groups (first, second, and third+ parities). The secondary objective was to evaluate the association between AGD and testosterone concentrations. The AGD (mm), age (yr), and height at hip (cm) at the time of AGD determination, and aforesaid reproductive outcomes were determined in 921 Holstein cows (first, second, and third+ parity; n = 360, 256, and 305, respectively). Plasma concentrations of testosterone were determined in a subset of 93 cows. Overall, AGD had a normal distribution and high variability [mean (±standard deviation); 131.0 ± 12.2 mm], was weakly associated with cow age and height (coefficient of determination = 0.09 and 0.04, respectively), and had an inverse relationship with P/AI in first- and second-parity cows, but not in third+ parity cows. For every 1 mm increase in AGD, the odds of P/AI decreased by 3.4 and 2.4% for first- and second-parity cows, respectively. The optimal AGD threshold to predict probability of P/AI was 127.1 mm for both first- (sensitivity: 66.4; specificity: 56.6%) and second-parity cows (sensitivity: 46.0; specificity: 70.4%). Accordingly, first- and second-parity cows were categorized into either short or long AGD (≤ or >127.1 mm), and associations with reproductive outcomes were evaluated. First-parity cows with long AGD had lower P/AI (30.9 vs. 53.6%) and decreased likelihood (hazard ratio: 0.68) of pregnancy by 250 DIM than those with short AGD. Similarly, second-parity cows with long AGD had reduced P/AI (28.3 vs. 44.4%) and a tendency for decreased likelihood (hazard ratio: 0.76) of pregnancy by 250 DIM than in cows with short AGD. The association between AGD and testosterone was weak and nonsignificant. In summary, AGD in Holstein cows was normally distributed, highly variable, and weakly associated with age and height. Besides, AGD had an inverse relationship with P/AI and cumulative pregnancy by 250 DIM in first- and second-parity cows; however, such a relationship was not evident in older (third+ parity) cows.
Key words: anogenital distance, age, height, fertility

INTRODUCTION

Anogenital distance (**AGD**) has been defined as the distance from the center of the anus to either the posterior fourchette (Salazar-Martinez et al., 2004) or the clitoris (Sathyanarayana et al., 2010) in females. The in utero development of the perineum and caudal migration of genital tubercle, relative to the anus, are androgen dependent in humans and rodents (Langman, 1975; Bowman et al., 2003). Therefore, the variation in AGD is a reflection of fetal androgen exposure during its reproductive programming window in those species (Macleod et al., 2010; Dean at al., 2012). In this regard, Mendiola et al. (2012) reported that AGD was normally distributed in a population of young women with high variability. Several other studies demonstrated that the AGD was approximately twice as long in males as in females (Salazar-Martinez et al., 2004; Swan, 2008; Thankamony et al., 2009; Macleod et al., 2010; Sathyanarayana et al., 2010). Hence, AGD is not only a biological indicator of prenatal androgenization, but also a sexually dimorphic trait that may be used to determine fetal sex during early pregnancy.

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Prenatal exposure to excess androgen in female fetuses leads to poor reproductive system development in utero, subsequently resulting in long AGD and poor postnatal fertility outcomes in rodents, rabbits, and humans (Zehr et al., 2001; Banszegi et al., 2012; Mendiola et al., 2012; Mira-Escolano et al., 2014a; Wu et al., 2017, respectively). The onset of puberty was delayed in female mice with long AGD (Zehr et al., 2001). Rabbit does with long AGD delivered fewer and lighter offspring, and had male-biased litters (Banszegi et al., 2012). Women with long AGD, presumably exposed prenatally to high androgen concentrations in utero, had increased numbers of ovarian follicles (Mendiola et al., 2012) and greater testosterone concentrations during the early follicular phase (Mira-Escolano et al., 2014a) compared with women with short AGD. Recently, Wu et al., (2017) reported that women with longer AGD were approximately 18 times more likely to develop polycystic ovarian syndrome, which is characterized by hyperandrogenism and anovulation, than those with shorter AGD. The placenta is the primary source of androgens in dams bearing female fetuses in dairy cows (Mongkonpunya et al., 1975). Maternal concentrations of testosterone (110 to 166 pg/mL) and androstenedione (936 to 1,400 pg/mL) during gestation were highly variable among individual cows bearing female fetuses (Gaiani et al., 1984). Thus, it is plausible that the high variability in in utero exposure of female bovine fetuses to androgens affects AGD and postnatal reproductive functions as reported in humans and rodents. In women, AGD was not associated with the postnatal determinants of age, height, and weight, but it was associated with body mass index (Mira-Escolano et al., 2014b; Wu et al., 2017). Moreover, in one study (Mira-Escolano et al., 2014a), for each millimeter increase in AGD in women, testosterone concentration increased by 0.006 ng/mL. Similar studies characterizing AGD and its associations with age, height, reproductive outcomes, and testosterone concentrations have not been conducted in dairy cows.

If an association exists between the simple morphologic measure of AGD and reproductive performance in dairy cows, AGD could become a new reproductive phenotype with potential for use in future genetic selection to augment fertility. Therefore, the primary objectives of this observational study were to (1) characterize the distribution and variability of AGD, (2) determine the relationship among AGD and potential postnatal AGD determinants of age and height, and (3) evaluate the associations between AGD and pregnancy to first AI (**P/AI**) and cumulative pregnancy by 250 DIM within parity groups (first, second, and third+). The secondary objective was to evaluate the association between AGD and testosterone concentrations.

MATERIALS AND METHODS

Animals and Management

This study was conducted at the Dairy Research and Technology Centre of the University of Alberta and 3 commercial dairy herds located in Alberta, Canada. Animals were housed and cared for in accordance with the requirements of Canadian Council on Animal Care (2009). Cows were fed a TMR (primary ingredients were barley or corn silage, alfalfa silage, alfalfa hay, and concentrates) formulated according to NRC (2001) to meet the requirements of a 650-kg lactating cow producing 45.0 kg of milk/d, and had ad libitum access to water. Whereas cows were subjected to presynchronization followed by Ovsynch (first AI) and Ovsynch only (second+ AI) in the university research herd and one of the commercial herds (timed AI; herds A and B, respectively), cows were predominantly inseminated based on estrus detection in 2 of the commercial herds (insemination at detected estrus; herds C and D, respectively).

Determination of Anogenital Distance, Age, Height, Milk Yield, and Reproductive Measures

Anogenital distance was defined as the distance from the center of the anus to the base of the clitoris (Figure 1a), and was measured using a stainless-steel digital calipers (Procise, The Innovak Group, Montreal, QC, Canada). The age of the cow (yr) at the time of AGD measurement was calculated by subtracting the date of birth from the date of AGD determination. The height at hip (hereafter referred to as "height") was determined using a livestock measuring stick (Jeffers, Dothan, AL) from the ground to the top of the cow's back (above hook bones). The AGD and height were measured by 2 individuals, with one person always measuring AGD and the other person measuring height. Data on AGD were collected during a single visit to each herd. Anogenital distance and height measurements were obtained from 921 cows (mean $\pm$ SD: 171 $\pm$ 93 DIM) that had no apparent perineal abnormalities such as inflamed or lacerated vulva as indicators of trauma at parturition, and that were later than 14 DIM at the time of AGD determination. Data on 305-d mature-equivalent milk yield and reproductive measures (P/AI and pregnancy by 250 DIM) were retrieved for all cows using DairyComp 305 herd management software (CanWest DHI, Guelph, ON, Canada).