Evaluating the Relationship Between Anogenital Distance, Reproductive Tract Score, and Fertility to Timed-artificial Insemination in Commercial *Bos taurus* Beef Heifers

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Abstract
Previous studies have found an inverse correlation between anogenital distance (AGD) and fertility in beef and dairy cattle, with greater fertility observed in females with shorter AGD. It has been previously demonstrated that heifers with mature reproductive tracts, as assessed by reproductive tract scores (RTS), have improved fertility to artificial insemination programs. The objective of this study was to explore the relationship between AGD, RTS, and conception rates to timed artificial insemination (TAI) in *Bos taurus* commercial yearling beef heifers (n = 884). All heifers were assigned an RTS on a scale from one to five at least two days before the beginning of the TAI protocol. Reproductive tract scores were determined based on uterine horn diameter and uterine tone: 1 = immature, <20 mm diameter and no tone; 2 = 20 - 25 mm diameter and no tone; 3 = 20 - 25 mm diameter with slight tone; 4 = 30 mm diameter with good tone; and 5 = >30 mm diameter with good tone. Heifers from eight different locations in Kansas and Tennessee were used during the 2023 and 2024 breeding seasons. All heifers were enrolled in a controlled internal drug release (CIDR)-based estrus synchronization protocol and received TAI. Pregnancy status was determined by transrectal ultrasonography approximately 45 days post-TAI. The AGD was defined as the distance from the middle of the anus to the base of the clitoris, and was assessed using digital calipers at CIDR insertion, withdrawal, TAI, or pregnancy diagnosis based on location. There was an effect of RTS ($P < 0.05$), but no effect of AGD ($P > 0.05$) on conception rates to TAI, and there was no difference in average AGD between heifers that were diagnosed as pregnant or open to TAI ($P = 0.15$; 83.09 mm, and 84.07 mm, respectively). We conclude that RTS is a more accurate assessment of beef heifer fertility to TAI than AGD.

Introduction
Research in Holstein cattle from Canada has found an inverse relationship between the anogenital distance (AGD) and the probability of pregnancy to timed-artificial insemination (TAI) protocols. While AGD has been studied in humans, rodents, and a

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few livestock species other than cattle, there is a lack of research in beef cattle. In other species, AGD has been found to be influenced by the concentration of androgens that the female fetus is exposed to during gestation. Research from Nebraska and Jordan has identified beef and dairy cattle with naturally high androgens and impaired folliculogenesis compared to their herd mates. Since little research has been conducted in beef cattle on AGD, our objective was to collect data on AGD and pregnancy outcomes in beef heifers enrolled in a fixed-time artificial insemination protocol. Another objective was to compare the use of AGD and reproductive tract scores (RTS) on the predictability of heifer fertility to TAI. We hypothesized that beef heifers with shorter AGD would have improved fertility to TAI when compared to heifers with longer AGD. The use of AGD in beef heifers may be able to help producers identify sub-fertile heifers, non-invasively identify cattle with high androgen concentrations, and improve management decisions for replacement heifers.

Experimental Procedure

Commercial *Bos taurus* yearling heifers (n = 888) from eight locations throughout Kansas and Tennessee were enrolled in a controlled internal drug release-based TAI estrous synchronization protocol. At least two days before the start of the TAI protocol, heifers were evaluated for RTS through transrectal palpation. The RTS scores were given on a scale of one through five based on uterine horn diameter, tone of the reproductive tract, and ovarian structures. The scoring system for RTS was: 1 = immature, <20 mm diameter and no tone, no palpable ovarian structures; 2 = 20 - 25 mm diameter and no tone, 8 mm follicles on ovary; 3 = 20 - 25 mm diameter with slight tone, 8 - 10 mm follicles on ovary; 4 = 30 mm diameter with good tone, 10 mm follicles or possible corpus luteum on ovary; and 5 = >30 mm diameter with good tone, greater than 10 mm follicles or corpus luteum present on ovary. The AGD was measured with digital calipers and was defined as the distance between the middle of the anus to the base of the clitoris. Heifers were retrospectively assigned to a quartile based on AGD for each location: Q1 heifers had the shortest 25% AGD, Q2 had an AGD of 26 - 50%, Q3 had an AGD of 51 - 75%, and Q4 had the longest AGD of 76% - 100%. Body weight (BW) was collected depending on the location during at least one day of the synchronization protocol. Pregnancy to TAI was determined through transrectal ultrasound approximately 45 days after TAI. Models of pregnant and nonpregnant heifers were analyzed with the GLIMMIX procedure in the 9.4 version of SAS.

Results and Discussion

Pregnant versus open heifers least square means are found in Table 1. Table 2 shows the percentage of pregnant heifers by AGD quartile and RTS. There was a difference ($P < 0.05$) for RTS between open heifers (3.72) compared to pregnant heifers (3.87). Trained technicians' ability to accurately score the reproductive tract prior to the breeding season allows for the culling of immature heifers before the breeding season. The average AGD of open heifers was 84.07 mm, while it was 83.09 mm for pregnant heifers, which was not significant ($P > 0.05$). Figure 1 shows the trendline for AGD and the probability of pregnancy based on the AGD length. Weights from six of the locations are included. Open heifers had an average weight of 806 lb while the pregnant heifers had an average weight of 813 lb. Although there was a numeric difference of 7 lb, there was no statistical difference ($P > 0.05$). A combined model of RTS and AGD was used to determine the probability of pregnancy when both variables were considered. Heifers that had an AGD over the average (84 mm) had a decreased probability of 1.2% of being pregnant for each mm over the average. The RTS was associated with pregnancy odds. Compared to RTS 1, the odds of pregnancy were generally higher for heifers with greater RTS, with differences ($P < 0.05$) observed between RTS 2 and 5, and RTS 3 and 5. Heifers that had a shorter AGD and higher RTS have a greater probability of becoming pregnant in the first round of TAI. Our results indicate that the average RTS is more related to TAI pregnancy rates than average AGD. However, when ranking females according to AGD within locations, the use of RTS and AGD ranking had a stronger prediction of pregnancy to TAI. We conclude that RTS is a better tool for identifying fertile heifers than AGD. However, AGD might be used as a second layer of selection to increase accuracy.

Implications

The RTS was a better predictor of pregnancy in virgin beef heifers than AGD. However, when both assessments were combined, there was greater prediction power for pregnancy to TAI.

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Table 1. Least square means of open and pregnant heifers

Variable	Open	Pregnant	*P*-value
RTS[1]	$3.72^{a} \pm 0.24$	$3.87^{b} \pm 0.24$	< 0.01
AGD,[2] mm	84.07 ± 1.93	83.09 ± 1.94	0.15
Weight, lb	806 ± 16	813 ± 16	0.37

[ab] Rows with uncommon superscripts differ ($P < 0.05$).

[1] Reproductive tract score: 1 = immature, <20 mm diameter and no tone, no palpable ovarian structures; 2 = 20 - 25 mm diameter and no tone, 8 mm follicles on ovary; 3 = 20 - 25 mm diameter with slight tone, 8 - 10 mm follicles on ovary; 4 = 30 mm diameter with good tone, 10 mm follicles or possible corpus luteum on ovary; and 5 = >30 mm diameter with good tone, greater than 10 mm follicles or corpus luteum present on ovary.

[2] Anogenital distance.

Table 2. Percent pregnant by reproductive tract score (RTS) and anogenital distance (AGD) quartile

	AGD Q1[1]		AGD Q2		AGD Q3		AGD Q4	
RTS[2]	Pregnancy, %	n[3]	Pregnancy, %	n	Pregnancy, %	n	Pregnancy, %	n
1	0.00	1	100.00	1	-	-	100.00	1
2	63.64	11	41.67	12	31.25	16	22.22	9
3	48.10	79	48.57	70	43.59	78	43.04	79
4	57.38	61	40.00	70	57.38	61	46.15	52
5	56.25	64	56.25	64	47.06	68	45.12	82

[1] Anogenital distance (AGD) quartile: Q1 heifers had the shortest 25% AGD, Q2 had an AGD of 26- 50%, Q3 had an AGD of 51 - 75%, and Q4 had the longest AGD of 76% - 100%.
[2] Reproductive tract score: 1 = immature, <20 mm diameter and no tone, no palpable ovarian structures;
2 = 20 - 25 mm diameter and no tone, 8 mm follicles on ovary; 3 = 20 - 25 mm diameter with slight tone, 8 - 10 mm follicles on ovary; 4 = 30 mm diameter with good tone, 10 mm follicles or possible corpus luteum on ovary; and
5 = >30 mm diameter with good tone, greater than 10 mm follicles or corpus luteum present on ovary.
[3] Number of heifers within RTS and AGD Quartile.

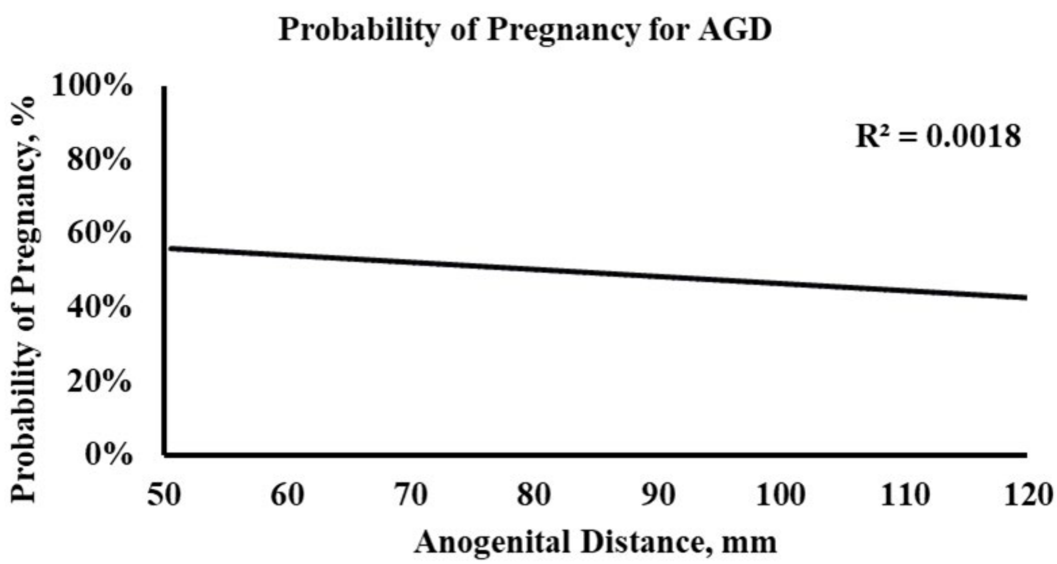

Figure 1. Predicted probability of pregnancy (%) as a function of anogenital distance (mm). The solid line represents the fitted regression model (R^2 = 0.0018).