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# Ultrasonographic reproductive tract measures and pelvis measures as predictors of pregnancy failure and anestrus in restricted bred beef heifers

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## ABSTRACT

Previous reports have shown that reproductive tract score (RTS) can predict reproduction outcomes in seasonally bred beef heifers, although the accuracy can vary. Some ultrasonographic measures of the female reproductive tract and pelvis area have also been associated with reproductive outcome in young heifers. The objectives of this study were to determine which transrectal ultrasound or pelvis measures taken at a single examination are independent predictors of reproductive failure and whether the RTS system can be optimized with this information. In this observational study, year-old beef heifers (n = 488) in 2 birth cohorts were followed from just before the first breeding until confirmation of pregnancy. A single pre-breeding examination included body condition score, RTS, ultrasound measures of the reproductive tract (length and diameter of the left and right ovaries, presence and diameter of a CL, largest follicle diameter and left uterus horn diameter) and transverse and vertical diameters of the pelvis. Additional farm records including dam parity, sire, birth weight and birth date, weaning weight, weaning date, prebreeding body weight, AI dates, and semen used were available. Breeding consisted of 50 days of AI, followed 5 to 7 days later by a 42-day bull breeding period. Pregnancy failure was defined as the failure to become pregnant after the AI and bull breeding periods, while anestrus was defined as the failure to be detected in estrus during the 50-day AI period. From the prebreeding data and farm records, independent predictors of pregnancy failure and anestrus were identified using stepwise reduction in multiple logistic regression models. Age at the onset of breeding was the only consistent independent predictor of pregnancy failure and anestrus in both cohorts of this study (P < 0.05). Body condition score, uterus horn diameter, absence of a CL, largest follicle of less than 13 mm, and pelvis area (PA) were the prebreeding examination variables that remained in prognostic models (P < 0.1). Combining either the model based on the 3 remaining ultrasound measures or RTS with PA provided more accurate prognostic models for pregnancy failure and anestrus than using RTS alone (P < 0.05). It is concluded that ultrasound measures have prognostic value for pregnancy failure in restricted bred yearling heifers as a result of their association with anestrus, and that smaller PA has additional prognostic value for poorly performing heifers.

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## 1. Introduction

The ability to select young heifers that will reproduce effectively in a seasonal breeding system has advantages over the alternative approach of waiting until reproductive

failure occurs [1,2]. Reproductive tract score (RTS) predicts anestrus and pregnancy failure in heifers independently of age, body weight (BW), and body condition score (BCS) and is a valid selection tool to enhance reproductive performance of herds [3–7]. However, estrus cycle stage and proportion of heifers in anestrus affect the accuracy of RTS, the complexity of the RTS system affects its repeatability, and other tests with potential to improve RTS are available [5,7–9].

The ultrasonographic presence of a CL has been used to mark the onset of puberty, has substantial repeatability, is more accurate than blood progesterone determination, and is a predictor of reproductive outcome in seasonally bred cows and heifers [9–12]. Ovary size is associated with antral follicle count (AFC), which in turn is associated with follicular reserve and fertility, whereas AFC is not affected by estrus cycle stage [13,14]. Maximum follicle diameter is correlated with uterus, cervix, and vaginal diameter and increases in the 10 weeks before first ovulation in heifers because of increased LH pulse frequency [13,15–19]. Larger PA has been associated with early onset of puberty in heifers and improved libido in bulls [20–22].

The objective of this study was to determine which individual transrectal ultrasound or pelvis measures taken at one point in time before breeding are independent predictors of reproductive failure in seasonally bred beef heifers and whether this knowledge can be used to optimize RTS.

## 2. Materials and methods

This was an observational study of 488 uniquely identified Bovelder beef heifers born in either 2007 (n = 259) or 2008 (n = 229) (2007 and 2008 cohorts) that were followed from just before their first breeding season to confirmation of pregnancy. The farming system, breed, and location have been described previously [5,7,20,23–25]. Farm data collected included the following: birth weight and birth date, parity of dam, sire, bull allocated, and first to fourth AI day numbered from the mating start date (MSD).

Heifers were weighed either 22 days (2007 cohort) or 27 or 24 days (2008 cohort) before the MSD (prebreeding BW), and a single prebreeding examination was performed 7 days (2007 cohort) or 27 or 24 days (2008 cohort) before the MSD. During the prebreeding examination, heifers were restrained individually in a chute, and the following data were collected in the same order by one experienced veterinarian: First, BCS was determined using a 9-point scale [26]. This was followed by RTS by transrectal palpation using a 5-point scale [3], then followed by transrectal ultrasonographic measurements of the reproductive tract [27], using a real-time digital ultrasound imaging system set in B-mode with a variable frequency linear probe set at 5 MHz (SIUI CTS-900V; Shantou Institute of Ultrasonic Instruments, Shantou, China). The interpolar length of the left and right ovaries, the diameter of the left and right ovaries at the deepest point (2008 cohort only), the presence and diameter of a CL, the diameter of the largest follicle, and diameter of the left uterus horn near the base (UD) were recorded. Finally, internal vertical diameter (VD) and transverse diameter (TD) of the pelvis were measured by transrectal placement of a caliper type pelvimeter (Rice

pelvimeter; Lane Manufacturing, Denver, Colorado) [20,28,29]. Farm management and staff were blinded to all the measured prebreeding data throughout the trial, except for the prebreeding BW.

The MSD was October 15 of each year, and breeding consisted of 50 days of continuous estrus observation by visual inspection, and once daily AI of all heifers identified in estrus during the preceding 24 hours by the same inseminator. Five to 7 days after each 50-day AI season, all heifers were joined with bulls in a single multisire group at a heifer:bull ratio of 30-35:1 for 42 days. Pregnancy diagnoses were performed by transrectal palpation 138 or 165 days after MSD (2007 and 2008 cohorts, respectively).

For the purpose of regression models, BCS was categorized into 2 categories (<6 and ≥6) and RTS into 3 categories (1–2, 3, and 4–5) [30]. Diameter of the largest follicle was used either as a continuous variable or was dichotomized using various cutoffs (7, 8, 9, 12, 13, and 14 mm). Pelvis area (PA) was calculated as the product of the TD and VD, and rescaled values of PA (RPA) and uterus diameter (RUD) were calculated within birth cohort using the following formula:

$$X^* = (X - X_{minimum}) \div (X_{maximum} - X_{minimum})$$

where $X^*$ = RPA or RUD and X = PA or UD.

If a heifer was not detected in estrus, it was assumed that she remained prepubertal until the end of the 50-day AI season and was defined as anestrus, whereas pregnancy failure was defined as a negative pregnancy test at the end of the AI and bull breeding periods.

Correlations were estimated using Spearman correlation for non-normally distributed data (only age in this study) and Pearson correlation for normally distributed data. Independent proportions were compared using the Fisher exact test, and means and medians were compared using analysis of variance with the Tukey–Kramer multiple comparison test and the Kruskal–Wallis one-way analysis of variance, respectively.

Multiple linear regression models (for length of the longest ovary, diameter of the largest follicle, UD, and PA) and logistic regression models (for absence of a CL, absence of a follicle ≥13 mm, anestrus, and pregnancy failure) were constructed using a backward elimination process [31] with P < 0.20 for initial inclusion and $P_{Wald} < 0.10$ for retention in models. Predictors that were considered included year of birth, dam parity (1, 2, or ≥3), prebreeding BW (kg), growth rate (kg/day), age at onset of breeding (days) and BCS category at examination, presence or diameter (mm) of the CL, diameter (mm) of the largest follicle or presence of a follicle of at least 7, 8, 9, 12, 13, or 14 mm, RUD, RPA, length of the longest or shortest ovary (mm) or combined length of the 2 ovaries (mm), or ovary length difference (mm). Once only independent variables remained in each model ($P_{Wald} < 0.10$), each of the eliminated variables was included individually again to test for confounding. Confounding was considered if inclusion of a variable changed the coefficient of one of the independent predictors by more than 15%.

Finally, independent prebreeding examination predictors of anestrus and pregnancy failure were combined

**Table 1**
Prebreeding measures and reproductive outcomes per year of birth and per sampling day

| Variable | Sampling day | | |
| --- | --- | --- | --- |
| | Day 7 (n = 259) | Day 27 (n = 134) | Day 24 (n = 95) |
| | Born 2007 | Born 2008 (n = 229) | |
| Age at examination (d)[d] | 401 ± 31[a] (311–449) | 383 ± 27[b] (308–453) | |
| Age at the onset of breeding (d)[d] | 407 ± 31[a] (317–455) | 408 ± 27[a] (331–479) | |
| Prebreeding body weight (kg)[d] | 292 ± 36[a] (195–392) | 272 ± 34[b] (184–349) | |
| Vertical pelvis diameter (cm)[d] | 12.9 ± 0.9[a] (11–16) | 12.6 ± 1.1[b] (8–15) | |
| Transverse pelvis diameter (cm)[d] | 11.0 ± 0.9[a] (8.5–13) | 10.6 ± 1.0[b] (8–13) | |
| Largest follicle diameter (mm)[d] | 10.7 ± 2.8[a] (4–18) | 11.1 ± 2.4[a] (4–17) | |
| Proportion with CL | 101/259 (39%)[a] | 56/229 (24%)[b] | |
| CL diameter (mm)[d] | 21.9 ± 4.5[a] (11–30) | 20.7 ± 4.4[a] (11–30) | |
| Left ovary interpolar length (mm)[d] | 24.3 ± 5.7[a] (13–43) | 23.1 ± 4.7[b] (12–36) | |
| Left ovary diameter (mm)[d] | N/D[e] | 14.0 ± 4.2 (5–40) | |
| Right ovary interpolar length (mm)[d] | 25.8 ± 5.6[a] (13–42) | 24.1 ± 5.1[b] (14–43) | 26.5 ± 6.0[a] (11–42) |
| Right ovary diameter (mm)[d] | N/D | 14.0 ± 4.2[a] (8–30) | 15.1 ± 4.1[b] (8–29) |
| Left uterus horn diameter (mm)[d] | 15.3 ± 2.6[a] (10–24) | 12.2 ± 1.9[b] (7–17) | 11.7 ± 1.5[c] (8–15) |
| Proportion with pregnancy failure | 56/258 (22%)[a] | 38/219 (17%)[a] | |
| Proportion with anestrus | 51/259 (20%)[a] | 50/229 (22%)[a] | |

[a,b,c]Means or proportions in rows with differing superscripts differ significantly (P < 0.05).
[d] Mean ± standard deviation (minimum and maximum).
[e] Not done.

into different prognostic models to estimate which models provided the best predictions of the outcomes. Areas under the receiver operating characteristic (ROC) curves (ROC-AUC) of prognostic models for anestrus and pregnancy failure were compared using the algorithm of DeLong et al. [32].

Data analysis was done using NCSS 2007 (NCSS, Kaysville, UT, USA) and STATA 11.1 (StataCorp, TX, USA).

## 3. Results

The age (mean ± standard deviation) of heifers at prebreeding weighing (384 ± 28.8 days) and at the onset of breeding (407 ± 28.7 days) were similar for the 2 birth cohorts (P = 0.74 and 0.27, respectively), but heifers born in 2007 were examined at an older age than those born in 2008 (P < 0.01, Table 1). Heifers born in 2007 were significantly heavier prebreeding than those born in 2008 (P < 0.01, Table 1) and BCS (median, interquartile range [IQR]) was also higher in the 2007 cohort (6 [5–6] and 5 [5–6], respectively, P < 0.01). More heifers were in RTS categories 4 or 5 (247 of 488) compared to categories 1 or 2 (102 of 488) and RTS 3 (139 of 488; P < 0.01), and the

proportions were similar between birth cohorts (P = 0.87). Pelvis and ultrasound measures of the reproductive tract are reported in Table 1. The UD differed between the 2 birth cohorts (Table 1). Furthermore, in the 2008 cohort, sampling day was associated with right ovary length and diameter and with UD (P < 0.05, Table 1). The left ovaries had shorter mean interpolar length than that of the right (23.7 and 25.5 mm, respectively, P < 0.01), but the mean diameter of the left and right ovaries for heifers born in 2008 did not differ (14.0 and 14.4 mm, respectively, P = 0.26).

Age, BCS, length of the longest ovary, diameter of the CL and UD at the time of examination, and prebreeding BW were all positively correlated with each other (P < 0.05). The diameter of the largest follicle was positively correlated with the length of the longest ovary and the length of the shortest ovary (P < 0.05). Reproductive tract score was most markedly associated with the length of the longest ovary, the length of the shortest ovary, and the absence of a CL (Table 2). It was also associated with the diameter of the CL and the diameter of the largest follicle, less so with UD and the absence of a follicle ≥8 mm but not associated with the absence of a follicle ≥13 mm (Table 2).

**Table 2**
Different ultrasonographic measures of the reproductive tract per reproductive tract score (RTS) category.

| Ultrasound variable | RTS 1 (n = 15) | RTS 2 (n = 87) | RTS 3 (n = 139) | RTS 4 (n = 120) | RTS 5 (n = 127) |
| --- | --- | --- | --- | --- | --- |
| Longest ovary length (mm)[f] | 20.7 ± 4.3[a] | 23.0 ± 3.0[b] | 25.6 ± 3.2[c] | 28.4 ± 3.5[d] | 33.0 ± 4.5[e] |
| Shortest ovary length (mm)[f] | 16.8 ± 2.9[a] | 19.1 ± 2.6[b] | 21.3 ± 3.2[c] | 22.8 ± 3.6[d] | 23.0 ± 4.0[d] |
| Absence of a CL[g] | 15/15 (100%)[a,b] | 87/87 (100%)[a] | 127/138 (92%)[b] | 82/120 (68%)[c] | 19/127 (15%)[d] |
| CL diameter (mm)[f] | — | — | 18.0 ± 4.7[a] | 18.9 ± 3.9[a] | 22.7 ± 4.1[b] |
| Largest follicle diameter <8 mm[g] | 5/15 (33%)[a] | 12/87 (14%)[a,b] | 16/138 (12%)[b] | 8/120 (7%)[b] | 12/127 (9%)[b] |
| Largest follicle diameter <13 mm[g] | 14/15 (93%)[a] | 69/87 (79%)[a] | 105/138 (76%)[a] | 82/120 (68%)[a] | 93/127 (73%)[a] |
| Largest follicle diameter (mm)[f] | 8.7 ± 3.3[a] | 10.4 ± 2.5[b] | 10.7 ± 2.5[b] | 11.4 ± 2.5[c] | 11.1 ± 2.7[b,c] |
| Uterus horn diameter (mm)[f] | 12.7 ± 3.0[a] | 13.4 ± 2.6[a] | 13.6 ± 2.9[a] | 14.0 ± 2.9[a,b] | 14.2 ± 2.6[b] |

[a,b,c,d,e]Values in rows with different superscripts differ significantly (P < 0.05).
[f] Mean ± standard deviation.
[g] Proportion of the total number of heifers in each RTS category (%).

**Table 3**

Summary of the multiple logistic or linear regression models of selected prebreeding measures.

| Predictor | Multivariable model outcome | | | | |
|---|---|---|---|---|---|
| | Presence of a CL[a] | Largest follicle ≥13 mm[a] | Uterus diameter (mm)[b] | Longest ovary length (mm)[b] | Pelvis area (cm²)[b] |
| Dam parity >1 (vs. 1) | # | # | # | # | −4.15 (−7.63, −0.66) |
| Wean weight (10 kg units) | 1.16 (1.08, 1.25) | # | # | # | 0.89 (0.05, 1.74) |
| Prebreeding body weight (10 kg units) | # | 1.08 (1.02, 1.14) | 0.06 (−0.01, 0.13) | # | 2.35 (1.61, 3.09) |
| Age at examination (wk) | 1.13 (1.06, 1.20) | # | 0.07 (0.01, 0.12) | 0.09 (0.00, 0.18) | 0.58 (0.16, 1.00) |
| BCS at examination ≥6 | # | # | # | # | −3.25 (−5.92, −0.59) |
| Presence of a CL | N/a | N/a | 0.58 (0.14, 1.01) | 6.38 (5.56, 7.21) | 7.64 (5.00, 10.30) |
| Largest follicle diameter (mm) | N/a | N/a | # | 0.31 (0.16, 0.45) | # |
| Year of birth 2007 | 1.55 (1.00, 2.40) | # | 3.13 (2.72, 3.55) | # | 3.68 (0.78, 6.59) |

Abbreviations: BCS, body condition score; N/a, not analyzed; #, not an independent predictor (P > 0.10).
[a] Odds ratios (95% confidence interval [CI]) of independent predictors (P < 0.10) in logistic regression models.
[b] Regression coefficients (95% CI) of independent predictors (P < 0.10) in multiple regression models.

In the multivariate models, weaning weight and age at examination were independently associated with presence of a CL, whereas only prebreeding BW was independently associated with presence of a follicle ≥13 mm (Table 3). Prebreeding BW, age at examination, and presence of a CL were independently associated with UD, and age at examination, presence of a CL, and largest follicle diameter were independently associated with the length of the longest ovary (Table 3). Dam parity 1 (vs. > 1), weaning weight, prebreeding BW, age at examination, BCS, and presence of a CL were all independently associated with PA (Table 3).

Body condition score, absence of a CL, largest follicle less than 13 mm, RUD, and RPA were the prebreeding examination variables that remained in multivariable models of pregnancy failure and anestrus (P < 0.1, Table 4). The prognostic model for anestrus using the 3 remaining prebreeding ultrasonographic measures of the reproductive tract in combination with RPA (US + RPA model) yielded an ROC-AUC of 0.81 (Table 5, Fig. 1). The US + RPA model and the model combining RTS with RPA (RTS + RPA model) provided more accurate predictions of pregnancy failure and anestrus than using RTS alone (P < 0.05, Table 6). The RPA model and the model using ultrasonographic absence of a CL, absence of a follicle ≥13 mm, and RUD (US model) tended to predict anestrus better than RTS (P = 0.09 and 0.06, respectively). The RPA model tended to predict pregnancy failure better than RTS (P = 0.09).

## 4. Discussion

In this study, ultrasonographic measures of the reproductive tract and pelvis measures were compared as prebreeding predictors of pregnancy failure and anestrus in seasonally bred beef heifers, and this information was used to determine if the current RTS system can be optimized.

Despite good heritability of age at puberty [11,33] ($h^2$ = 0.52 and 0.43, respectively), environmental factors from fetal development to puberty can influence the phenotypic expression of reproductive potential [5,11,19], which may have led to different levels of pubertal development achieved by the MSD in the 2 birth cohorts in this study. However, the anestrus and pregnancy failure proportions were not different, and we assumed that considering year of birth as a potential covariate in models would adequately adjust for any differences between the years.

The difference in UD between the 2 birth cohorts in this study may have occurred as a result of a true biological difference in the younger group of heifers in 2008 [19] or may have been a systematic measuring error that occurred during sampling [34]. The relatively small UD reported in the 2008 cohort of the present study compared to the 2007 cohort and previous reports [3,19] indicates that a systematic error was more likely to have occurred in the 2008 cohort than in the 2007 cohort. The possibility of such an error to occur justifies the use of a rescaled value of the raw data in analyses, and we assumed that the relative uterus diameter within birth cohort in the present study, rather than the absolute diameter, provided a better indication of

**Table 4**

Summary of logistic regression models for pregnancy failure and anestrus in the 2 birth cohorts combined.

| Independent predictor | Outcome: pregnancy failure[a] | Outcome: anestrus[a] |
|---|---|---|
| CL absent | 1.69 (0.94–3.05) | 6.13 (2.32–16.21) |
| Largest follicle <13 mm | 2.07 (1.10–3.90) | 2.13 (1.09–4.16) |
| Rescaled uterus diameter | 0.43 (0.18–1.02) | 0.39 (0.15–1.01) |
| Rescaled pelvis area | # | 0.15 (0.05–0.48) |
| Age at onset of breeding (wk) | 0.88 (0.83–0.93) | 0.90 (0.84–0.96) |
| BCS <6 | # | 2.90 (1.66–5.08) |

Abbreviations: BCS, body condition score; #, not an independent predictor (P > 0.10).
[a] Odds ratios (95% confidence interval) of independent predictors (P < 0.10).

**Table 5**

Logistic regression model for prediction of anestrus using independent transrectal ultrasound measures of the reproductive tract in combination with pelvis area (n = 488).[a]

| Predictor | Coefficient | OR | 95% CI | | P |
|---|---|---|---|---|---|
| Constant | −2.13 | 0.12 | 0.03 | 0.41 | <0.01 |
| CL absent | 1.97 | 7.15 | 2.75 | 18.57 | <0.01 |
| Largest follicle < 13 mm | 0.81 | 2.25 | 1.19 | 4.27 | 0.01 |
| Rescaled uterus diameter | −0.96 | 0.38 | 0.16 | 0.94 | 0.04 |
| Rescaled pelvis area | −2.77 | 0.06 | 0.02 | 0.18 | <0.01 |

Abbreviations: CI, confidence interval; OR, odds ratio.
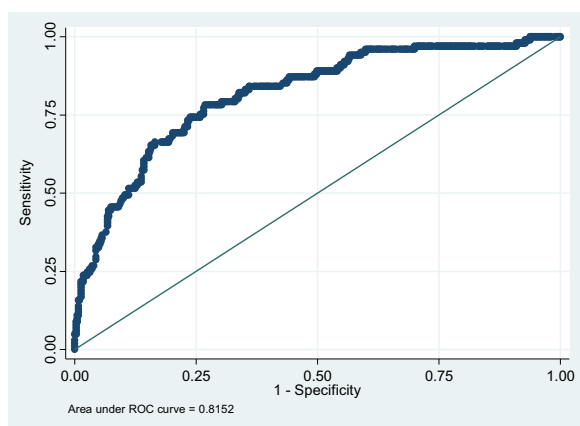[a] Area under the receiver operating characteristic curve = 0.81.

**Fig. 1.** Receiver operating characteristic (ROC) curve for anestrus using the model based on the ultrasonographic absence of a CL, absence of a follicle ≥13 mm, rescaled uterus diameter, and rescaled pelvis area (Table 5).

the relative pubertal development stage of an animal within a group. We assumed the same for PA data.

The difference in length between the left and right ovaries could either have been a true biological difference [35] or it could also have been a systematic measuring error due to the operator using the same hand and the alignment of the ultrasound probe being different on the left and right ovaries [27]. Honaramooz et al. [19] could not demonstrate a difference in ultrasonographic size between the left and right ovaries; however, the largest follicle on the right side was 1 mm larger than on the left side in their data. The fact that there was a numerical difference in ovary diameter between the 2 sides in the 2008 cohort, that was not significant, is not useful to support either of the 2 hypotheses. It may be that an adjustment for the side of the largest ovary may improve the ability of the length of the longest ovary to predict reproductive outcomes, but because of obvious confounding by the size of the largest follicle and the presence of a CL on the predictive ability of ovary length, this was not investigated any further.

**Table 6**
Areas under the receiver operating characteristic curves (ROC-AUC) of different predictive models for pregnancy failure and anestrus in the two birth cohorts separately and combined.

| Model | Pregnancy failure | | | Anestrus | | |
|---|---|---|---|---|---|---|
| | 2007 Cohort | 2008 Cohort | Combined data | 2007 Cohort | 2008 Cohort | Combined data |
| RTS[d] | 0.59[a] | 0.63[a] | 0.60[a] | 0.72[a] | 0.69[a] | 0.71[a] |
| RPA[e] | 0.62[a,b] | 0.63[a] | 0.62[a] | 0.74[a,b] | 0.78[b] | 0.76[a] |
| Ultrasound[f] | 0.70[b,c] | 0.64[a] | 0.65[a,b] | 0.77[a,b,c] | 0.76[a,b] | 0.76[a,b] |
| RTS + RPA | 0.63[a,b] | 0.65[a] | 0.64[a,b] | 0.79[b,c] | 0.80[b,c] | 0.79[b,c] |
| Ultrasound + RPA | 0.71[c] | 0.67[a] | 0.68[b] | 0.81[c] | 0.83[c] | 0.81[c] |

[a,b,c]ROC-AUC values in columns with differing superscripts differ significantly (P < 0.05).
[d] Reproductive tract score (RTS) categorized as 1 to 2, 3, and 4 to 5, with 4 to 5 as reference value.
[e] Rescaled pelvis area.
[f] Ultrasonographic absence of a CL, absence of a follicle ≥13 mm, and rescaled uterus diameter.

Age at examination was associated with UD and PA independent of BW, confirming that the development of the reproductive system is a function of both age and BW and that age and BW when puberty is reached vary between animals, even in a uniform group such as the study population [5]. The age range of the study population fell in or just after the second phase of rapid development of the reproductive tract [15,19], and as such, a lot of variance could be expected because of the proximity to puberty. The results of this study may therefore not necessarily be extrapolated to heifers in other age ranges.

None of the ovary length variables were independently associated with reproductive outcomes in this study. However, 2 significantly independent predictors of reproductive outcomes, largest follicle ≥13 mm and the presence of a CL at the time of examination, were both also independent predictors of ovary length, and we conclude that the effect of ovary length on reproductive outcome is confounded by the presence of ovarian structures. Cushman et al. [13] and Ireland et al. [14] suggested that the size of the ovaries may give a reflection of antral follicle count, which is associated with fertility in young adult cattle. Antral follicle count was not considered as an input variable in the present study; however, we assumed that either the longest, the shortest, or the combined ovary length would provide a reflection of antral follicle count after adjustment for the size of the largest follicle and the size or presence of a CL.

The diameter of the largest follicle was not correlated with UD as was the case in the study of Honaramooz et al. [19] and was only independently associated with the length of the longest ovary, both which appear in this study to be confounded in their prognostic value for reproductive outcomes. After testing several cutoff points to dichotomize the diameter of the largest follicle, less than 13 mm was the only predictor of anestrus and pregnancy failure in this study, which is in agreement with the observation by Honaramooz et al. [19] that the maximum follicle size increases before puberty from 10 to 12 mm. We conclude that heifers in the current trial that did not have a CL and also had a largest follicle diameter less than 13 mm were at risk of being too far from puberty at the time of examination to show estrus during the 50-day AI season or to become pregnant during the breeding season. None of the other follicle size cutoffs tested had any significant associations in this study, indicating that whether dominance of a follicle has occurred (using a cutoff between 7 and 9 mm [36]) did not have prognostic value for reproductive outcome in our study.

Previous findings indicating the superior ability of ultrasonography to detect the presence of a CL [10] are supported by this study because of the fact that significant proportions of heifers with ultrasonographically visible CLs were assigned RTS 3 or 4. These CLs were smaller than those of heifers with RTS 5 and were most likely not easily palpable; however, the tendency of the ultrasound model to have a better predictive value for anestrus and pregnancy failure compared to RTS is likely partly as a result of the better sensitivity of ultrasound to detect a CL. In the present study, the absence of a CL predicted not only anestrus but also pregnancy failure. Keeping in mind that the total breeding season length was 90 days, this can be partly explained by the fact that the first few ovulations

after puberty have lower fertility than later ovulations [37,38], which will further decrease the ability of heifers that reach puberty after the MSD to become pregnant during a restricted breeding season.

Although not validated against estrus or pregnancy outcomes during the breeding period after examination, Archbold et al. [6] estimated the sensitivity of ultrasonography to determine pubertal status to be reduced during proestrus and metestrus, because of the relatively poor ability to visualize the regressing corpus albicans and the corpus hemoragicum, respectively. We therefore assume that the reason why the absence of a follicle ≥13 mm remained an independent predictor in our models was either that some heifers that were pubertal at the time of examination had their first estrus in the few days after the examination or that in postpubertal heifers, a CL was not detected because of stage of the estrus cycle. Because of the fact that some heifers may have been at stages of the follicular wave before divergence of the dominant follicle at the time of examination [36], the absence of a follicle ≥13 mm cannot completely rule out cyclicity but improves the predictive ability when a CL is not present.

Because of the inaccuracy of transrectal palpation relative to ultrasonography to detect a CL, to distinguish between follicles less than 13 mm and 13 mm or greater, and to estimate the uterus horn diameter, transrectal ultrasonography tended to provide better prognostic models for reproductive failure than the current palpation model of Andersen et al. [3]. However, the accuracy of RTS by palpation may be improved by putting more emphasis on the presence of a CL, the size of the largest follicle, and the diameter of the uterus horn and less emphasis on the absolute size of the 2 ovaries. Our data confirm that the operator assigning the RTS scores weighed the size of the ovaries relatively heavily in the scoring system, but this study further indicated that the size of the ovaries after adjusting for ultrasonographically visible structures on the ovaries was not an independent predictor of reproductive outcome and should preferably not be emphasized.

Pelvis area, a measure previously used only to predict dystocia in heifers [28,29], had a strong and independent association with reproductive outcome in this study and added significant prognostic value to models based on palpation or ultrasonography of the reproductive tract. This is in agreement with previous reports of associations between PA and reproductive outcomes [21,22]. Similar to uterus diameter, PA has the potential to overcome the inaccuracy to predict pubertal stage at a single point in time caused by different stages of the estrus cycle. We assume that the reason why PA predicted reproductive outcome independent of other measures and why it added significant prognostic value to models predicting reproductive failure is because it develops gradually over time and is probably not significantly associated with the daily estrus cycle stage.

The absence of a CL, being the best predictor of reproductive failure, was a particularly good predictor in the 2007 birth cohort, in which case, heifers were examined closer to the MSD and the proportion of heifers with a CL was also higher. In the 2008 cohort, when heifers were examined more than 3 weeks before the MSD, BCS, uterus diameter, and PA were more important predictors of anestrus and pregnancy failure. We suggest that emphasis should be placed on different predictors depending on the age of heifers at the time of examination or depending on the proportion of heifers with CLs at the time of examination. When heifers are examined long before the MSD or when only a small proportion of heifers have CLs, more emphasis should be placed on the relative diameter of the uterus horn and the relative PA, whereas when examination is done shortly before the MSD or when a larger proportion of heifers have CLs, more emphasis should be placed on the absence of a CL and the absence of a follicle ≥13 mm diameter. Further research is needed, possibly using Bayesian modeling, to establish if different prognostic models should be applied based on herd status.

### 4.1. Conclusions

Transrectal ultrasonography of the reproductive tracts of beef heifers can provide prognostic models of pregnancy failure because of its association with anestrus during a restricted breeding season. The ultrasonographic measures that remained independent predictors of pregnancy failure and anestrus were the absence of a CL, absence of a follicle ≥13 mm, and relatively smaller uterus horn diameter.

Relatively smaller PA can either replace or add value to reproductive tract scoring by transrectal palpation or ultrasonography as predictor of poor reproductive performance in restricted bred beef heifers.

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