

# Developmental and reproductive characteristics of beef heifers classified by number of estrous cycles experienced by start of first breeding

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**ABSTRACT:** A retrospective analysis was conducted to evaluate the effect of number of estrous cycles exhibited before breeding on growth and reproductive performance of replacement beef heifers fed ad-libitum or restricted by 20% less than ad-libitum during postweaning development over a 9-yr period. Progesterone concentration in blood samples collected at 9- to 11-d intervals were used to assign heifers into groups by number of estrous cycles exhibited before the start of breeding: 0 (nonpubertal;  $n = 395$ ), 1 ( $n = 205$ ), 2 ( $n = 211$ ), 3 ( $n = 116$ ), or  $>3$  ( $n = 249$ ). Heifers ( $P < 0.01$ ) in the 0 cycle group were born 6 d later than the 1, 2, or 3 cycle groups, which were born 4 d later ( $P < 0.01$ ) than the  $>3$  cycle group. Weight of heifers at birth decreased ( $P < 0.05$ ) as the number of cycles increased. Weaning weight and ultrasound measures of loin area and fat thickness over the loin at 1 yr age increased as the number of cycles increased ( $P < 0.01$ ). Postwean weight gain, hip height at 1 yr age, and weights from the start of breeding through precalving increased with cycle numbers in a quadratic fashion ( $P < 0.02$ ) and were greater ( $P < 0.05$ ) in ad-libitum than restricted-fed heifers. Pregnancy rate in the 0 cycle group was lower (84%;  $P < 0.05$ )

than the 1 (90%) or  $>3$  (94%) estrous cycles groups and tended to differ ( $P < 0.1$ ) from the 2 (88%) and 3 (89%) estrous cycle groups. Interval from the start of breeding to calving was 3 to 5 d longer ( $P < 0.05$ ) for the 0 cycle group ( $300 \pm 1$  d) than other groups. Proportion of heifers calving in the first 21 d was less ( $P < 0.05$ ) in the 0 or 1 cycle groups than other groups. Pregnancy rates of 2-yr-old cows ( $n = 898$ ) were lowest ( $P < 0.05$ ) for the 0 (73%) and 2 (79%) estrous cycle groups than the 1 (85%), 3 (90%), or  $>3$  (92%) estrous cycle groups. Restricted level of feeding during postweaning development resulted in greater ( $P < 0.05$ ) proportion of heifers in 0 cycle group and lower ( $P < 0.05$ ) proportion in  $>3$  cycle group, but reproductive performance was not influenced ( $P > 0.1$ ) by level of feeding or interaction of feeding and estrous cycle grouping. In summary, date of birth and rate of physical maturation (weight, height, and fat deposition) were associated with timing of puberty. Pregnancy rate was greater in heifers that exhibited estrus before the start of breeding, but did not improve from having more than one estrous cycle. Proportion conceiving early was greater for heifers having two or more cycles before breeding.

**Key words:** beef heifers, heifer development, puberty, reproduction

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## INTRODUCTION

Obtaining high-pregnancy rates in replacement females is a common goal among beef cattle producers. One industry guideline recommends that heifers should be managed so they achieve puberty in sufficient time to experience

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multiple estrous cycles before the start of breeding. This is based on research that demonstrated a 21 percentage point increase in pregnancy rate in heifers inseminated on their third estrus compared with heifers inseminated on their first estrus (Byerley et al., 1987). It was concluded from these results that fertility of the first pubertal estrus was inferior to the third estrus. Additional studies confirming this conclusion are lacking and substantial genetic change in the cattle population has occurred since these data were collected over 25 yr ago (Endecott et al., 2013). Furthermore, Byerley et al. (1987) had potentially confounding effects of heifer age and weight at time of breeding between treatment groups. Heifers inseminated at first estrus were 53 d younger (322 vs. 375 d) and 31 kg lighter (295 vs. 326 kg) than heifers inseminated on their third estrus. The objective of this research was to provide a more thorough evaluation of the impact the number of estrous cycles exhibited before the start of breeding has on pregnancy rates of replacement beef heifers and to characterize growth and development associated with the number of estrous cycles exhibited before the start of breeding.

## MATERIALS AND METHODS

All research protocols used in this study were approved by the USDA, ARS, Fort Keogh Livestock and Range Research Institutional Animal Care and Use Committee. Heifers ( $n = 1,176$ ) used in this study were a stable composite population (CGC:  $\frac{1}{2}$  Red Angus,  $\frac{1}{4}$  Charolais,  $\frac{1}{4}$  Tarentaise; Newman et al., 1993) produced over a 9-yr period (average Julian birthdate  $96 \pm 15$ ). Date and weight were recorded for all heifers at birth and weaning. At weaning, heifers were placed in a feedlot and subsequently assigned to either control or restricted feeding levels for a 140-d period, beginning approximately 60 d after adaptation to the feedlot. In the first year of the study (2002), heifers were group fed in one of four pens (two pens/feeding treatment). In subsequent years, heifers were individually fed in an open shed equipped with electronic Calan gates (American Calan, Northwood, NH) as described previously (Roberts et al., 2007, 2009). Heifers averaged  $245 \pm 19$  d of age and  $220 \pm 25$  kg weight at initiation of the feeding period. Control heifers were fed to appetite and restricted heifers were fed at 80% of that consumed by controls adjusted to a common weight basis (weight measurements and feed adjustments made at 28-d intervals). The 140-d period ended  $40 \pm 11$  d before the start of breeding. At the end of the 140-d feeding trial,

body weight and hip height were measured on all heifers, and ultrasound carcass measures were collected on heifers from years 2 through 9 of the study, as described previously (Roberts et al., 2007). Following the 140-d trial, heifers were managed the same throughout the breeding season. A prebreeding weight was taken 0 to 16 d (varied over years) before the start of breeding. This population provided an opportunity to evaluate the objective using heifers developed at different rates of growth during the postweaning period.

Circulating concentrations of progesterone were used to estimate date of first estrus. Beginning at an average of 331 d of age and continuing through the start of breeding, 3 to 9 mL of blood were collected from each heifer by coccygeal venipuncture at 9- to 11-d intervals. After collection, blood was placed on ice and stored overnight at 4°C. Blood was then centrifuged at  $1,200 \times g$  for 30 min. Serum was harvested and stored at -20°C for subsequent progesterone analysis. Concentrations of progesterone in serum were determined directly without extraction by solid-phase radioimmunoassay (Coat-a-Count kit; Diagnostic Products Corp., Los Angeles, CA) as reported previously (Bellows et al., 1991). Intraassay and interassay CV were 8% and 16%, respectively, and assay sensitivity was 0.08 ng/mL. For the purpose of this study, date of first estrus was assumed to occur 6 d before the date of first progesterone sample measuring  $\geq 1$  ng/mL. Heifers were classified into one of five categories based on the estimated number of estrous cycles exhibited before the start of breeding, assuming a 21-d interval for each estrous cycle: category = 0 if no progesterone sample was  $\geq 1$  ng/mL (nonpubertal,  $n = 395$ ); category = 1 if number of days between first progesterone sample  $\geq 1$  ng/mL and the start of breeding was  $< 15$  d ( $n = 205$ ); category = 2 if number of days between first progesterone sample  $\geq 1$  ng/mL and the start of breeding was 15 to 35 d ( $n = 211$ ); category = 3 if number of days between first progesterone sample  $\geq 1$  ng/mL and the start of breeding was 36 to 56 d ( $n = 116$ ); category  $> 3$  if number of days between first progesterone sample  $\geq 1$  ng/mL and the start of breeding was  $> 56$  d ( $n = 249$ ). Beginning date of sampling did not allow for determination of the specific number of cycles in the latter group beyond greater than three estrous cycles.

Beginning approximately June 1 each year, at an average age of  $425 \pm 15$  d (youngest and oldest heifer over the 9-yr period was 370 and 465 d, respectively), heifers were inseminated followed by natural mating for a 48- to 55-d breeding season

( $n = 5$  yr) or natural mating only for a 46- or 62-d breeding season ( $n = 4$  yr). In the 5 yr estrous synchronization was employed, protocols varied across yr; with a CO-Synch protocol used in 1 yr, a CO-Synch + controlled internal drug-releasing device (CIDR; Zoetis Animal Health, Parsippany, NJ) protocol used for 2 yr, and protocols with either 1 or 2 PGF<sub>2 $\alpha$</sub>  injections used the other 2 yr. Depending on the estrous synchronization protocol used, breeding season duration was adjusted to provide heifers three opportunities to be bred if pubertal at the start of breeding (i.e., breeding season of 48 to 55 d). At 1 to 2 mo after bull removal, body weight and hip height were measured, and heifers were evaluated for pregnancy by transrectal ultrasonography using a 5-MHz transducer (Aloka, Wallingford, CT) and hip height was measured.

In late November or early December of each year, heifers diagnosed as pregnant were divided into their postweaning treatment groups to allow for different levels of supplemental feeding through the winter as described previously (Roberts et al., 2016). For the majority of the study, pasture forage was readily available for winter grazing and the only additional supplemental protein provided was alfalfa cubes or hay, depending on the year. Supplement was fed either daily or every other day to achieve 1.8 or 1 kg/day offered for each control or restricted heifer, respectively. When pasture access was limited due to snow cover, heifers were provided 10.9- or 9.1-kg alfalfa hay/d for control and restricted treatments, respectively. At approximately 2 to 4 wk before the start of calving, heifers were recombined and weighed. Date of calving, calf birth weight, and interval from the start of breeding to calving were recorded for each animal. Pregnancy rates for the second breeding season were recorded for all cows nursing a calf ( $n = 898$ ).

### Statistical Methods

Data were analyzed with SAS (SAS Inst. Inc., Cary, NC). Differences in measurements taken on heifers and their calves due to different estrous cycle categories were evaluated by the GLIMMIX procedure with a model that included random effects of year ( $n = 9$ ), and fixed effects of postweaning feeding treatment ( $n = 2$ ), estrous cycle classification ( $n = 5$ ), and interaction of feeding treatment  $\times$  estrous cycle category. Interaction of feeding treatment  $\times$  estrous cycle category only approached significance ( $P < 0.06$ ) in the analysis for average daily gain during the postweaning feeding treatment and was therefore removed from the model

of all other variables. Estrous cycle classification was considered significant when  $P < 0.05$  for type 3 tests of fixed effects for estrous cycle classification. When significant, least square means for estrous cycle classifications were compared using the DIFF option. In addition, linear and quadratic contrast statements were used to provide insight on pattern of change as the number of estrous cycles increased from 0 to  $>3$ . Values presented represent least square means and SE. Effects of feeding treatment on many of the response variables analyzed have been reported previously and are not presented in this study (Roberts et al., 2017); except data on proportion of animals from each treatment within each estrous cycle category (analyzed by Chi Square), BW at the start of breeding, and pregnancy rate for treatment by estrous cycle grouping are presented.

## RESULTS

### Effect of Estrous Cycle Grouping

Heifers that were nonpubertal at the start of breeding (0 cycle group) were born 6 d later ( $P < 0.01$ ) than heifers in the 1, 2, or 3 cycle group, which were 4 d younger ( $P < 0.01$ ) than heifers in the  $>3$  cycle group (Table 1). Birth weight decreased as the number of cycles exhibited before breeding increased ( $P < 0.05$ , Table 2). Conversely, prewean ADG and weaning weight ( $P < 0.01$ ) increased as the number of cycles exhibited before breeding increased (Table 1). Postwean weight gain and hip height at 12.5 mo age were influenced by cycle numbers in a quadratic fashion ( $P < 0.03$ ) with increasing values from 0 to 3 cycles and a decrease or no change from 3 to  $>3$  cycles (Table 1).

Carcass ultrasound measurements of loin area, width-to-height ratio of loin, and fat thickness over the loin increased with increasing number of cycles exhibited before breeding ( $P < 0.01$ , Table 1). Intramuscular fat in the loin did not vary ( $P = 0.17$ ) by number of estrous cycles exhibited before breeding.

Heifer weight before breeding was influenced by cycle numbers in a quadratic fashion ( $P < 0.02$ ) with increasing weight from 0 to 3 cycles and a plateau from 3 to  $>3$  cycles (Table 1). Differences observed for day of birth among the estrous cycle groupings carried over to differences in age at the start of breeding, with 0 cycle heifers being youngest (421 d of age), followed by heifers that exhibited either 1, 2, or 3 estrous cycles before breeding (426 d of age), which were younger ( $P < 0.01$ ) than heifers exhibiting  $>3$

**Table 1.** Growth, carcass characteristics, and pregnancy rate of heifers classified by the number of estrous cycles exhibited before the start of breeding

Item	Number of estrous cycles					SE*	P value <sup>†</sup>
	0	1	2	3	> 3		
Number of heifers	395	205	211	116	249		
Julian day of birth	101 <sup>a</sup>	95 <sup>b</sup>	95 <sup>b</sup>	95 <sup>b</sup>	91 <sup>c</sup>	2.3	<0.001
Weight at birth, kg	35.7 <sup>a</sup>	34.9 <sup>ab</sup>	34.4 <sup>bc</sup>	34.5 <sup>bc</sup>	33.7 <sup>c</sup>	0.6	<0.001
Weight at wean, kg	193 <sup>a</sup>	202 <sup>b</sup>	204 <sup>b</sup>	206 <sup>bc</sup>	210 <sup>c</sup>	4.0	<0.001
Gain birth to wean, kg/d	0.88 <sup>a</sup>	0.90 <sup>b</sup>	0.91 <sup>bc</sup>	0.92 <sup>bc</sup>	0.93 <sup>c</sup>	0.02	<0.001
Gain 8 to 12.6 mo, kg/d	0.55 <sup>a</sup>	0.57 <sup>b</sup>	0.58 <sup>bc</sup>	0.61 <sup>c</sup>	0.58 <sup>bc</sup>	0.04	<0.002
Hip height <sup>‡</sup> , cm	117.0 <sup>a</sup>	117.7 <sup>b</sup>	118.2 <sup>b</sup>	118.1 <sup>b</sup>	117.8 <sup>b</sup>	0.6	<0.004
Loin muscle area <sup>‡</sup> , cm <sup>2</sup>	52.2 <sup>a</sup>	53.7 <sup>b</sup>	56.0 <sup>c</sup>	57.2 <sup>cd</sup>	58.0 <sup>d</sup>	1.3	<0.001
Loin width:height <sup>‡</sup>	0.45	0.46 <sup>b</sup>	0.47 <sup>c</sup>	0.47 <sup>cd</sup>	0.48 <sup>d</sup>	0.04	<0.001
Fat over loin <sup>‡</sup> ,mm	3.13 <sup>a</sup>	3.52 <sup>b</sup>	3.81 <sup>c</sup>	3.81 <sup>cd</sup>	4.07 <sup>d</sup>	0.21	<0.001
Weight, start of breeding, kg <sup>  </sup>	307 <sup>a</sup>	321 <sup>b</sup>	321 <sup>b</sup>	327 <sup>bc</sup>	329 <sup>c</sup>	7.5	<0.001
420-d adjusted weight, kg	311 <sup>a</sup>	322 <sup>bx</sup>	321 <sup>bx</sup>	328 <sup>by</sup>	326 <sup>b</sup>	8.2	<0.001
Weight at pregnancy test, kg	391 <sup>a</sup>	397 <sup>b</sup>	398 <sup>b</sup>	403 <sup>b</sup>	400 <sup>b</sup>	10.4	0.002
Gain 14 to 19 mo, kg/d	0.54 <sup>a</sup>	0.50 <sup>b</sup>	0.50 <sup>b</sup>	0.50 <sup>b</sup>	0.46 <sup>c</sup>	0.05	<0.001
Heifer first season pregnancy rate, %	84 <sup>ax</sup>	90 <sup>b</sup>	88 <sup>ay</sup>	89 <sup>aby</sup>	94 <sup>b</sup>	3.5	<0.008

\*Largest SE of mean.

<sup>†</sup>P value for the effect of estrous cycle category.

<sup>‡</sup>Measured at 12.6 mo of age (range 330 to 432 d of age).

<sup>||</sup>Weight was adjusted to 420 d of age. Trend for interaction ( $P = 0.058$ ) of estrous cycle classification and postweaning feeding treatment (shown in Figure 1).

<sup>a-c</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).

<sup>xy</sup>Means within a row without a common superscript tend to differ ( $P \leq 0.11$ ).

**Table 2.** Body weight, calving characteristics, and rebreeding of pregnant heifers classified by the number of estrous cycles exhibited before the start of first breeding

Item	Number of estrous cycles					SE*	P value <sup>†</sup>
	0	1	2	3	>3		
Number pregnant heifers	322	176	184	103	225		
Weight at start of calving, kg	418 <sup>a</sup>	425 <sup>b</sup>	425 <sup>b</sup>	430 <sup>b</sup>	424 <sup>ab</sup>	12	0.032
Age at calving, d	720 <sup>a</sup>	723 <sup>abx</sup>	722 <sup>a</sup>	722 <sup>a</sup>	727 <sup>by</sup>	3.3	0.019
Days start breeding to calving	300 <sup>a</sup>	296 <sup>b</sup>	295 <sup>b</sup>	295 <sup>b</sup>	296 <sup>b</sup>	1.4	<0.001
Percent calving in 21 d	49 <sup>a</sup>	54 <sup>a</sup>	60 <sup>b</sup>	63 <sup>b</sup>	60 <sup>b</sup>	6.0	0.033
Weight, calves at weaning, kg	180 <sup>a</sup>	187 <sup>b</sup>	188 <sup>b</sup>	189 <sup>b</sup>	184 <sup>b</sup>	6.3	0.001
Second pregnancy rate, %	73 <sup>a</sup>	85 <sup>bx</sup>	79 <sup>a</sup>	90 <sup>b</sup>	92 <sup>by</sup>	4.7	<0.001

\*Largest SE of mean.

<sup>†</sup>P value for the effect of estrous cycle category.

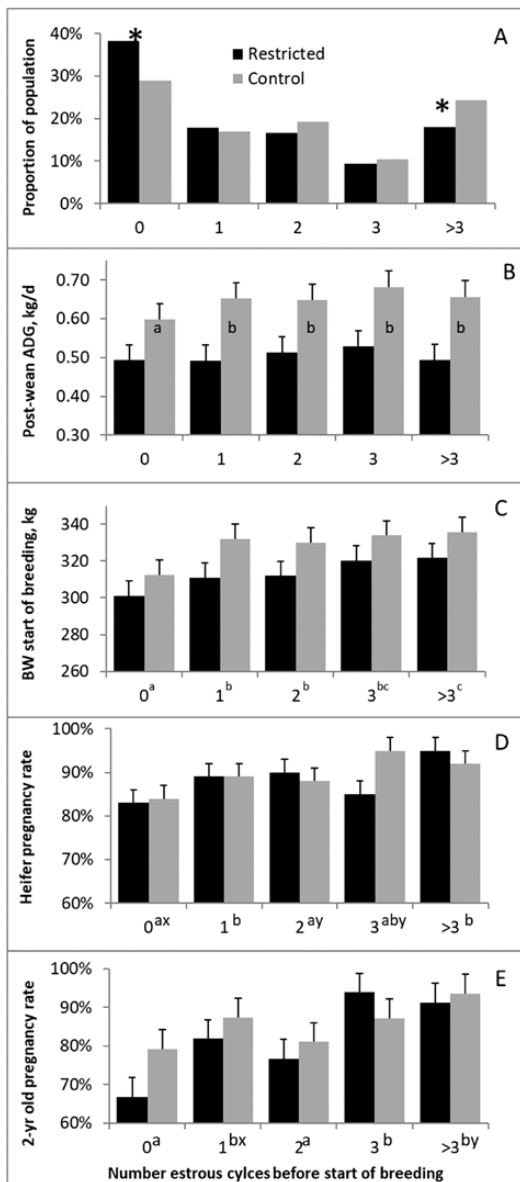
<sup>ab</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).

<sup>xy</sup>Means within a row without a common superscript tend to differ ( $P < 0.07$ ).

estrous cycles before breeding (430 d). Adjustment of weight to a common age at the start of breeding (420 d of age, Table 1) reduced numeric differences among groups and changed numeric ranking of heifers in the >3 cycle group from being heaviest to intermediate in weight. These adjusted weights followed a quadratic response ( $P < 0.02$ ) to estrous cycle number as was observed for nonadjusted weights.

Heifer weight at pregnancy diagnosis at approximately 19 mo of age remained lighter ( $P < 0.05$ ) for the 0 cycle group than other estrous cycle groups,

which did not differ among each other (Table 1). However, average weight gain from breeding to pregnancy diagnosis differed ( $P < 0.001$ ) due to estrous cycle grouping, being greatest in the 0 cycle group, intermediate in the 1, 2, and 3 estrous cycle groups, and least in the >3 cycle group (Table 1). Because of the greater weight gain in the 0 cycle group, magnitude of differences in body weight between this and the other estrus cycle groups decreased compared with differences observed at earlier measurements. Hip height at pregnancy



**Figure 1.** Sample distribution (A), postwean average daily gain (ADG; B), body weight at the start of breeding (BW; C), heifer pregnancy rate (D), and rebreeding pregnancy rate at 2 yr of age (E) for heifers classified by postweaning development treatment and number of estrous cycles exhibited before the start of breeding. Heifers were fed to appetite (Control) or were fed at 80% of that consumed by controls adjusted to a common weight basis (Restricted) for a 140-d period from approximately 60 d after weaning to 40 ± 11 d before the start of breeding. Heifers were classified into five groups based on the number of estrous cycles exhibited before breeding (0, 1, 2, 3, or >3). Restricted feeding resulted in a smaller ( $P = 0.008$ ) proportion of heifers in the >3 estrous cycle group and greater ( $P < 0.001$ ) proportion in the 0 cycle group (A). Average daily gain during the postweaning feeding treatment differed due to interaction ( $P = 0.058$ ) of feeding treatment × estrous cycle classification, where the 0 estrous cycle group differed from other estrous cycle groups in control fed heifers but not restricted heifers (B). Heifer weight at the start of breeding (C) was decreased by restricted feeding ( $P < 0.001$ ) and varied by estrous cycle classification ( $P < 0.001$ ). Postweaning development did not affect pregnancy rate (D,  $P = 0.66$ ) or pregnancy rate at 2 yr age (E,  $P = 0.19$ ), so differences across estrous cycle groupings are same as shown in Tables 1 and 2, respectively. Positive Y error bars extend from top of each bar in (B) through (E). \*Denotes differences ( $P < 0.05$ ) due to nutritional treatment. <sup>abc</sup>Bars or estrous cycle classifications without common superscripts differ ( $P < 0.05$ ). <sup>xy</sup>Bars or estrous cycle classifications without common superscripts differ ( $P < 0.11$ ).

diagnosis did not differ ( $P = 0.48$ , 125.7 cm) due to estrous cycle grouping.

Pregnancy rates differed ( $P < 0.007$ ) by number of estrous cycles exhibited before the start of breeding (Table 1). Heifers that had 0 estrous cycles before breeding had lower pregnancy rates ( $P < 0.05$ ) than heifers exhibiting 1 or >3 estrous cycles before breeding and tended to differ ( $P < 0.1$ ) from the 2 and 3 estrous cycle groups.

For heifers that became pregnant, precalfing body weights were influenced by cycle numbers in a quadratic fashion ( $P = 0.032$ ), where heifers in the 0 estrous cycle group weighed less than heifers exhibiting 1 to 3 estrous cycles before breeding, and weights of heifers in the >3 cycle group were not different from other groups (Table 2). Heifers in the 0 estrous cycle group had 3- to 5-d longer ( $P < 0.05$ ) interval ( $300 \pm 1$  day) from the start of breeding to calving than other estrous cycle groups (Table 2). Proportion of heifers calving in the first 21 d of the calving season was greater ( $P < 0.05$ ) in heifers that had two or more cycles than heifers with 0 or 1 cycle (Table 2). Birth weight of calves of heifers calving was not influenced by estrous cycle grouping ( $P = 0.6$ , 32 kg). However, calf weight at weaning was influenced by estrous cycle grouping ( $P = 0.001$ ), with calves born to heifers in the 0 estrous cycle group being lighter than calves from heifers in the other groups (Table 2). Rebreeding pregnancy rates at 2 yr of age were lowest ( $P < 0.05$ ) for heifers from the 0 and 2 estrous cycle groups than heifers that had 1, 3, or >3 estrous cycles before first breeding (Table 2).

### Effect of Feeding Level During Postweaning Development

Proportion of heifers in each estrous cycle classification was influenced ( $P < 0.006$ ) by feeding treatment during the postweaning period, with the restricted feeding resulting in a smaller proportion in the >3 estrous cycle group and greater proportion in the 0 cycle group (Figure 1A). Average daily gain during the postweaning feeding treatment tended to differ due to interaction ( $P = 0.058$ ) of feeding treatment and estrous cycle classification, where the pattern of numerical differences across estrous cycle groupings was similar for both treatments, but statistical differences between estrous cycle groupings only existed in control fed heifers and not restricted heifers (Figure 1B). Differences in BW resulting from the postweaning treatments remained evident at the start of breeding (Figure 1C,  $P < 0.001$ ) and patterns of change

across the estrous cycle groupings were similar for both feeding treatment ( $P = 0.4$  for interaction of feeding treatment by estrous cycle classification). Neither heifer pregnancy rate (Figure 1D,  $P = 0.66$ ) nor rebreeding rate (Figure 1E,  $P = 0.19$ ) was influenced by postweaning feeding or interaction of feeding treatment by estrous cycle grouping ( $P > 0.26$ ). Thus, differences in pregnancy rates due to the main effect of estrous cycle classification are as indicated in Tables 1 and 2.

## DISCUSSION

Results from this study demonstrate the number of estrous cycles heifers exhibit before the start of breeding correspond to biological differences evident at time of birth, and throughout preweaning and postweaning development. Although classification by 21-d intervals may not result in the most distinct distinction between adjacent groups (animals at the beginning of one 21-d period are very close to animals at the end of the adjacent period), a general conclusion from the data may be the differences observed are consistent with a continuum in maturation rate across the population that also reflects biological differences in size at maturity. Heifers that reached puberty earlier in life, and thus exhibited a greater number of estrous cycles before breeding, were born earlier and exhibited greater growth rates from birth to weaning. Statistical separation of the measurements analyzed provides support of three distinct groupings: animals that did not exhibit estrus before the start of breeding, animals exhibiting 1 to 3 estrous cycles, and those exhibiting  $>3$  estrous cycles. As would be expected, the group exhibiting the greatest number of estrous cycles before breeding exhibited characteristics indicative of earlier maturation of skeletal (hip height), muscle (loin development), fat deposition, and an earlier plateau in growth rate compared with heifers exhibiting fewer or no estrous cycles before the start of breeding. Heifers that did not exhibit estrus before breeding were heavier at birth, with slower growth rates up to breeding, but greater growth rate at later stages of development, indicative of slower growing, later maturing animals.

In the present study, heifers that had not exhibited estrus before breeding would have their first opportunity to conceive on their pubertal estrus. Heifers expressing 2 estrous cycles before breeding would have their first opportunity to conceive on their third estrus. These groups of heifers are comparable to the treatment groups of Byerley et al. (1987). Although both studies provide evidence

for lower pregnancy rates at first estrus compared with third estrus, magnitude of difference was much greater in the study by Byerley et al. (1987) than in the present study (20 vs. 5 percentage point reduction for the respective studies). In addition, results from the present study do not support the theory that pregnancy rate was improved in heifers expressing  $>1$  estrous cycle before the start of breeding. The discrepancies in the results obtained by Byerley et al. (1987) and the present study are likely due to differences in experimental design and may also reflect genetic changes in age of puberty in heifers over time, as suggested in the reviews by Funston et al. (2012a) and Endecott et al. (2013). In the study by Byerley et al. (1987), insemination date corresponded to when heifers first expressed estrus, whereas initiation date of breeding remained constant in the present study. Heifers inseminated at first estrus were approximately 11 mo old at the time of breeding in the study by Byerley et al. (1987), whereas breeding was not initiated until approximately 14 mo of age in the present study, which is more representative of current industry practices. The importance of differences in the age of breeding between these two studies was alluded to by Byerley et al. (1987) where increased age resulted in increased pregnancy rates for heifers inseminated at first estrus, but not in heifers inseminated on their third estrus. Another difference between the two studies is that heifers were only provided one opportunity to conceive in Byerley et al. (1987), whereas multiple opportunities for conception were possible in the present study.

The observation from the present study that heifers exhibiting only 1 estrous cycle before breeding had a lower proportion calving in the first 21 d than groups exhibiting  $>1$  cycle could be indicative of improved performance in heifers exhibiting more than one estrous cycle before breeding. However, average interval between the start of breeding and calving, and weight of calves at weaning (unadjusted for age) did not differ among groups that exhibited one or more cycles before the start of breeding.

Results of the present study indicate that second breeding season pregnancy rates were influenced by estrous cycle category. However, results were inconsistent with a hypothesis of a linear relationship between rebreeding pregnancy rate and number of cycles exhibited prior to first breeding. Results support a qualitative response, where greater rebreeding performance was observed in animals that had expressed three or more estrous cycles before the start of first breeding.

Rebreeding performance can be affected by age. Impact of age appears to be a function of when an animal was born (i.e., early vs. later in the calving season), which will translate into differences in age at the start of breeding and may also carry over to age at first calving. Age differences at calving will also be influenced by differences in when an animal conceives (Lesmeister et al., 1973; Funston et al., 2012b; Cushman et al., 2013). In the present study, heifers in the >3 estrous cycle group had the numerically highest second season pregnancy rate. These animals were born earlier and thus older at the start of breeding than other heifers. These differences carried over to this group being oldest at first calving.

The nutritional treatments in this study resulted in differences in weight at the start of breeding and puberty attainment, as reflected by the altered proportion of heifers in the 0 and >3 estrous cycle groupings (Figure 1A). However, a definitive impact of nutritional treatment on heifer pregnancy rate was not evident. These observations are consistent with previous analyses on this population with different statistical models (Roberts et al., 2009, 2016, 2017). Although studies from several decades ago found that postweaning nutritional alterations in puberty rates at the start of breeding were associated with altered heifer pregnancy rate, association between proportion pubertal at the start of breeding and pregnancy rate has become less evident over time (Funston et al., 2012a; Endecott et al., 2013). Differences in measurements up to weaning observed in the present study support previous findings that early developmental differences can have greater impact on subsequent reproductive performance than postweaning growth rate, as discussed previously (Roberts et al., 2009).

With the statistical model used in the present study, rebreeding performance did not differ significantly due to nutritional treatments. These results differ from previous results obtained using a statistical model that did not account for pubertal status, where rebreeding pregnancy rates were reduced by the restricted level of feeding (Roberts et al., 2016). Although interaction of nutritional treatment and estrous cycle classification did not ( $P = 0.26$ ) affect rebreeding performance in the present study, visual appraisal of data in Figure 1D indicates numeric decreases for restricted animals that exhibited <3 estrous cycles before first breeding. An important consideration for results on rebreeding performance is that nutritional treatments were imposed at two time points: postweaning to prebreeding and the last trimester of pregnancy. Thus, it is not

clear if the lower rebreeding pregnancy rates were associated with altered timing of puberty through management strategies in the postweaning period, or from providing less winter supplementation, or the combination of both. Funston and Deutscher (2004) found no difference in rebreeding rates of heifers developed at two rates of postweaning gain very similar to those in the present study. However, all heifers were treated similarly subsequent to the postweaning phase in the Funston and Deutscher study. Thus, it was speculated less winter supplement prior to first calving contributed to the decreased rebreeding in the population evaluated in the present study, and not the dietary differences prior to first breeding (Roberts et al., 2016).

In the present study, different breeding protocols were implemented across years. Differences in breeding protocols, such as breeding season length and inclusion of the CIDR in the estrous synchronization protocol, could have influenced results concerning breeding performance of animals classified into the different estrous cycle categories. However, insights into impacts of differences in breeding protocol on the results were not evaluated because breeding protocols were confounded by year.

With exception to information discussed in the three preceding paragraphs, variation in the measurements observed across estrous cycle categories would be expected to result from inherent genetic variation in traits influencing maturation rate and size at maturity. For example, differences in day of birth are likely the culmination of traits associated with time of conception and gestation length. Differences in weight at birth would also be influenced by gestation length, in addition to variation in intrauterine growth rate, which will be correlated with subsequent growth rate and mature size. Current management strategies for heifer development during the postweaning period are to provide sufficient nutritional input to promote earlier puberty with the intent to improve heifer pregnancy rate. This approach may be counter-productive over the long term by masking genetic differences that could contribute to improved efficiency through greater retention over time with less nutritional inputs. Although selecting replacement heifers based on the number of estrous cycles exhibited before breeding may not be practical, results from this study provide precedent to select heifers based on differences in birthdate, birth weight, and preweaning growth rate. Initial application of this approach may be best implemented by selection against animals with characteristics exhibited by the 0 cycle group, and exposing more females to

first breeding than needed to maintain static female inventory. Additional selection could be imposed by implementing a relatively short breeding season or fetal aging during pregnancy diagnosis to ensure retention of heifers that conceived early.

Results of the present study indicate that second breeding season pregnancy rates were influenced by estrous cycle category. However, results were inconsistent with a hypothesis of a linear relationship between rebreeding pregnancy rate and number of cycles exhibited prior to first breeding. Results support a qualitative response, where greater rebreeding performance was observed in animals that had expressed three or more estrous cycles before the start of first breeding.

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