3-3-2021

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PMCID: PMC9630398

Biology of Reproduction, 2021, 104(6), 1360–1372
doi:10.1093/biolre/ioab044
Research Article
Advance Access Publication Date: 11 March 2021

Research Article

Attainment and maintenance of pubertal cyclicity may predict reproductive longevity in beef heifers†

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†__Grant Support:__ This research was supported by the National Institute of Food and Agriculture 2013-67015-20965 to ASC, JRW, and JSD, the University of Nebraska Food for Health Competitive Grants to ASC, JSD, and JRW. United States Department of Agriculture Hatch grant NEB26-202/W3112 Accession #1011127 to ASC, Hatch–NEB ANHL Accession#1002234 to ASC and JRW. Competitive collaborative USMARC/UNL grants from UNL ARD and USMARC to ASC and JRW. We acknowledge and thank Dr Robert A. Cushman for helping us to develop the SAS program for classification of heifers and assistance with determining Antral Follicle Counts on this population of heifers.
‡These authors contributed equally to this manuscript and are co-first authors.
Conference Presentation: Portions of these data were presented at the 51st Annual Meeting of the Society for the Study of Reproduction, 10–13 July 2018, New Orleans, Louisiana.

Received 10 October 2020; Revised 2 March 2021; Editorial Decision 3 March 2021

Abstract

We hypothesized the manner that heifers achieve puberty may indicate their future reproductive longevity. Heifers with discontinued or delayed cyclicity during puberty attainment may have irregular reproductive cycles, anovulation, and infertility in their first breeding season contributing to a shorter reproductive lifespan. Therefore, plasma progesterone (P4) was measured from weaning to breeding on 611 heifers born 2012–2017 and four pubertal classifications were identified: (1) Early; P4 $\geq$ 1 ng/ml $<$ March 12 with continued cyclicity, (2) Typical; P4 $\geq$ 1 ng/ml $\geq$ March 12 with continued cyclicity, (3) Start-Stop; P4 $\geq$ 1 ng/ml but discontinued cyclicity, and (4) Non-Cycling; no P4 $\geq$ 1 ng/ml. Historical herd records indicated that 25% of heifers achieved puberty prior to March 12th in the 10 years prior to the study. Start-Stop and Non-Cycling yearling heifers were lighter indicating reduced growth and reproductive maturity traits compared with Early/Typical heifers. In addition, Non-Cycling/Start-Stop heifers were less responsive to prostaglandin F2 alpha (PGF2α) to initiate estrous behavior and ovulation to be artificially inseminated. Non-Cycling heifers had fewer reproductive tract score-5 and reduced numbers of calves born in the first 21-days-of-calving during their first breeding season. Within the Start-Stop classification, 50% of heifers reinitiated cyclicity with growth traits and reproductive parameters that were similar to heifers in

the Early/Typical classification while those that remained non-cyclic were more similar to heifers in the Non-Cycling group. Thus, heifers with discontinued cyclicity or no cyclicity during puberty attainment had delayed reproductive maturity resulting in subfertility and potentially a shorter reproductive lifespan.

Summary sentence

Puberty attainment of beef heifers as classified by circulating plasma progesterone profiles from weaning to breeding can be used to predict future reproductive fertility and longevity.

Key words: puberty, progesterone, ovary, domestic animal reproduction, female infertility, ruminants.

Introduction

Puberty is a dynamic and critical time point in the life of a female. At puberty, a female achieves regular cyclicity and is able to conceive and reproduce. Women who have altered or irregular cyclicity are often identified at puberty and diagnosed with reproductive disorders such as polycystic ovarian syndrome [1–3]. In beef herds, less is known about how attainment of puberty may affect cyclicity and reproductive lifespan. Heifers that achieve puberty early, calve earlier in subsequent years, allowing for them to be maintained in the herd with a longer reproductive lifespan. Furthermore, calves born earlier in the breeding season will be heavier and return more dollars back to the producer to replace investment costs of their dams' development [4]. Furthermore, in the US beef industry, it is crucial for heifers to reach puberty early enough to become pregnant and deliver a calf at 2 years of age [5, 6], and thus, they must become pregnant by 15 months of age. Previous studies suggest that a heifer's first few estrous cycles after puberty may be less fertile [7–9]; thus, it is ideal for heifers to attain puberty by 12–13 months of age in order to conceive at 15 months [4, 5].

While achieving puberty early is important and has been linked to a potentially longer reproductive lifespan [4, 6, 10–14], precocious puberty may be detrimental to a heifer remaining in the herd. Wehrman et al. [15] and others defined precocious puberty as initiating cyclicity prior to 300 days of age and indicated that females as early as 120 days of age [16–21] have initiated cyclicity when exposed to a bull calved at 12 months of age. These early pregnancies reduce the heifer's ability to reach her growth potential, often have problems with dystocia at calving, and utimately cause the heifer to be culled from the herd [15–21]. Precocious puberty has been induced by feeding a high-concentrate diet or increased quality forages to heifers after early weaning [22, 23].

Delayed puberty can also be a problem for beef producers since these females do not initiate cyclicity early in the breeding season. Often progesterone (P4) is used within a synchronization protocol on heifers to initiate their attainment of puberty [6, 13, 24]. However, very little is known if P4 in combination with synchronization regimes causes these females to remain in the herd long-term or if it allows females to be retained in the herd that should be culled. Heifers with delayed puberty also may have genetic, physiological, or hormonal problems that do not allow them to become cyclic early. Therefore, being able to identify heifers with precocious and delayed pubertal attainment would enhance the management tools of beef cattle producers.

In heifers, pubertal attainment is marked by behavioral estrus and is followed by ovulation and formation of a functional corpus luteum on the ovary [6, 13, 24]. Previous studies have used threshold levels of circulating progesterone from 1 to 2 ng/ml to identify puberty in beef heifers [4, 18, 25]. In the current experiment, 1-ng/ml progesterone was used as a threshold to evaluate puberty attainment but females also had to maintain continued cyclicity. Our hypothesis was the manner that heifers achieve puberty may be an indicator of their future reproductive longevity.

Adolescent girls with irregular cyclicity, precocious, or delayed puberty have been diagnosed with polycystic ovary syndrome [14, 26]. Similarly, heifers with discontinued or delayed cyclicity during pubertal attainment may have irregular reproductive cycles, anovulation, and infertility throughout their reproductive lifespan. Thus, pattern of pubertal attainment may indicate whether bovine females can be reproductively efficient. Also, linkages of pubertal attainment with physiological or hormonal problems may allow for better culling choices for the beef producer and may inform similar hormonal disorders in adolescent girls and women.

Materials and methods

Ethics

The University of Nebraska–Lincoln Institutional Animal Care and Use Committee approved all procedures and facilities used in this experiment.

Animals

A total of 611 beef heifers born in 2012–2017 were used in this experiment (Table 1). Heifers were from the physiology herd at the University of Nebraska–Lincoln and were kept at the Eastern Nebraska Research and Extension Center (ENREC). The physiology herd is made up of ~250 Red Angus composite cows. During 2012–2014, up to 10% of calves were sired by MARC III bulls, a composite breed of ¼ Red Angus, ¼ Hereford, ¼ Pinzgauer, and ¼ Red Poll. From 2015 to 2017, about 50% of cows were AI sired by Red Angus bulls, and about 50% were sired by Red Angus × Simmental composite herd bulls.

Dams of heifers were fed supplement during gestation (4.5-lb dried distillers' grains/head/day; which was above NRC requirements) for all years during this experiment, except dams of heifers born in 2016, which received no supplement. Heifers receiving no supplement still would have obtained a diet at or above NRC requirements. A drought occurred in 2012 which may have affected pregnant dams and offspring born in 2013. Heifers were born in the spring around March and grazed on pasture with dams until weaning in late October. At weaning (~6–7 months of age), heifers were separated from dams and male calves and retained as replacement heifers on pasture at ENREC.

Table 1. P4 samples collected each year and heifers in each puberty group each year.

	Year						
	2012	2013	2014	2015	2016	2017	2012–2017
Number of samples/heifer	14	13	22	30	31	31	
Number of heifers per year	68	99	105	106	119	114	611
Number of heifers in each puberty group per year							
Typical	25	44	31	48	77	54	279
Early	24	6	38	30	8	37	143
Start-Stop	15	22	28	13	7	6	91
Non-Cycling	4	27	8	15	27	17	98

Weight and gain calculations

Birth weights, weaning weights, and yearling weights were collected for heifers each year. Adjusted birth weights, adjusted weaning weights, and adjusted yearling weights were calculated using Cow Sense herd management software (Midwest Microsystems L.L.C., Lincoln, NE). Calf and yearling average daily gain (ADG) were calculated as ADG from birth to weaning (calf ADG) and from weaning to yearling weight (yearling ADG). Weight per day of age (WDA) was calculated during the time from birth to yearling weight.

Blood samples

Blood samples were collected during the sampling period using coccygeal venipuncture into glass vacutainer blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) containing 12-mg EDTA and placed on ice. Within several hours, blood samples were centrifuged at ~700 g and 4 °C for 30 min, and plasma was removed and stored in polypropylene tubes (Globe Scientific, Inc., Paramus, NJ) at −20 °C. For the 2012- and 2013-born heifers, samples were collected monthly from October to December and twice monthly from January to May. In 2014, sample collection frequency was increased, and samples were collected monthly from October to December, twice monthly in January, and weekly from February to May. For the 2015-, 2016-, and 2017-born heifers, weekly blood samples were collected from October to May (Table 1).

Progesterone radioimmunoassays

Progesterone (P4) concentration for each plasma sample was detected using radioimmunoassay (RIA). Samples were run in duplicate, and the average of the duplicates was recorded as the P4 concentration for that sample. Duplicates with a coefficient of variation (CV) greater than 15% were re-analyzed. In years 2012 and 2013, the Coat-a-Count assay kit (Diagnostic Products Corporation, Los Angeles, CA) was used to determine P4 concentrations (intra-assay CV = 6.1%, inter-assay CV = 15.6%) [27–29]. In years 2014–2017, P4 concentrations were determined using the ImmuChem™ Coated Tube Progesterone ^{125}I RIA kit (ICN Pharmaceuticals, Inc., Costa Mesa, CA; intra-assay CV = 2.3%, inter-assay CV = 14.0%; due to DPC P4 kit no longer being available) [27–29].

Puberty group classifications

The first occurrence of P4 ≥ 1 ng/ml during the sampling period was considered puberty date. Continuous cyclicity was evaluated by percent of samples where P4 ≥ 1 ng/ml after puberty date during the sampling period; heifers with 50% or greater samples with P4 ≥ 1 ng/ml on and after puberty date were considered to have continuous cyclicity, and heifers with fewer than 50% of samples with P4 ≥ 1 ng/ml on and after puberty date were considered to have discontinuous cyclicity. Four distinct puberty groups were detected using an SAS analysis based on puberty date and whether cyclicity continued over the sampling period: (1) Early—reached P4 ≥ 1 ng/ml before March 12 and had continuous cyclicity from puberty until breeding; (2) Typical—reached P4 ≥ 1 ng/ml after March 12 and had continuous cyclicity from puberty until breeding; (3) Start-Stop—reached P4 ≥ 1 ng/ml during the sampling period but had discontinuous cyclicity from "puberty" to breeding; and (4) Non-Cycling—had no occurrence of P4 ≥ 1 ng/ml prior to breeding. Because elevated P4 concentrations at weaning could be attributed to weaning stress, P4 concentrations from samples collected on the date of weaning were not considered when determining puberty group.

March 12 was used to separate Early and Typical heifers because it marked the 25th percentile for puberty date in an analysis of puberty date distributions for heifers (previous 10 years) in the UNL physiology herd. A date was used to determine whether heifers were Early or Typical rather than separating groups by age at puberty because in a typical US beef cow-calf operation; heifers must reach puberty at a certain point in the season regardless of their age and birth date in order to achieve maximum reproductive efficiency [4, 12].

Start-Stop heifers born in 2012–2016 were further classified into two subgroups based on whether they showed evidence of reinitiating continuous cyclicity prior to breeding: (1) Start-Stop-Start (SSS) heifers (n = 40) showed evidence of continuous cyclicity prior to breeding, whereas (2) Start-Stop-Discontinuous (SSD) heifers did not regain continuous cyclicity prior to breeding (n = 34). The 2017 born heifers were not utilized since all Start-Stop heifers were SSD. An SAS code was developed to separate Start-Stop heifers into these two subgroups by considering P4 values for Start-Stop heifers in the month of May only (samples most immediately prior to breeding). Heifers with at least one occurrence of P4 ≥ 1 ng/ml in the month of May followed by 50% or greater samples where P4 ≥ 1 ng/ml until breeding were considered SSS heifers. Heifers with no occurrences of P4 ≥ 1 ng/ml in the month of May, or heifers with at least one occurrence of P4 ≥ 1 ng/ml in May followed by fewer than 50% of samples where P4 ≥ 1 ng/ml, were considered SSD heifers.

Prebreeding evaluation

Prior to breeding each year, heifers were evaluated for reproductive maturity using transrectal ovarian ultrasound. Uterine horn diameter, ovary size, number, and size of antral follicles on each ovary (any follicles greater than 5 mm), and presence or absence of corpora lutea were recorded for each heifer. Antral follicle count was considered the total number of antral follicles recorded for both left and right

ovaries. Uterine horn diameter was the diameter of the right uterine horn. A reproductive tract score was calculated for each heifer using the criteria outlined in Anderson et al. [30]. Briefly, heifers with an RTS-5 (presence of a corpus lutem and mature tract) would be optimal for breeding. However, heifers having RTS-4 (large antral follicle and mature reproductive tract) could also be viable to have large enough follicles to ovulate with eggs available for fertilization. Females with less than an RTS-4 would be immature and not optimal for breeding [30].

Breeding and pregnancy diagnosis

In May each year, heifers were synchronized with 2 injections of PGF2α 14 days apart and were fitted with estrus detection patches (Estrotect Breeding Indicator, WI). After the second injection of PGF2α, heifers were detected for visual signs of estrus and/or patch activation (60% of patch activated) for ~1 week; 12 h after heifers were observed in estrus they were artificially inseminated (AI). Ten days after the last heifer was detected in estrus, all heifers were placed with herd cleanup bulls for 45 days. Pregnancy was detected using transrectal ultrasound at ~6 weeks after AI for AI-pregnancy. Age at breeding was identified as age at time of AI for those heifers that conceived to AI and had a calf in the period of 285 days after AI. For heifers that were not AI, we used their calving date and subtracted 285 days from their age. Several heifers did not calve; thus we used the age of heifers at the date that bulls were removed from the heifers (45-day breeding season) and added two estrous cycles (42 days) to determine their age at breeding to penalize them for not producing a calf. Approximately 60 days after bull removal, heifers were pregnancy checked for overall pregnancy. Overall pregnancy diagnosis was performed using manual palpation for 2012–2015 born heifers. For 2016–2017 born heifers, overall pregnancy diagnosis was performed using blood samples analyzed by GeneSeek (Neogen Genomics, Lincoln, NE) through the Bovine Early Stage Pregnancy Detection (BioPRYN) test. The first 21 days of the calving season was determined by the first date calved and the 21 days on/after that date. Not all heifers in Table 1 were used for pregnancy and AI. A portion of heifers each year were utilized for intensive measures of follicle wave patterns and steroid hormone and gonadotrophin secretion and collection of ovaries with further analysis of ovarian tissue as follows: 2014-born: 6 Start-Stop, 4 Early; 2015-born: 3 from each of 4 pubertal classifications; 2016-born: 2 Early, 4 Typical, 2 Start-Stop, 4 Non-Cycling; and 2017-born: 3 Early, 3 Typical, 3 Start-Stop, 3 Non-Cycling. Thus, these heifers in each pubertal classification, as stated, were ovariectomized and not included in the data for synchronization and breeding/pregnancy.

Statistical analyses

Data analyses were conducted using SAS v9.4. All data are presented as mean ± standard error of the mean. For all analyses, differences were considered significant when $P \leq 0.05$ and a tendency when $P \leq 0.10$ and > 0.05. Age at puberty, age at weaning, weight data (adjusted birth weight, adjusted weaning weight, adjusted yearling weight, ADG, and WDA), antral follicle count, uterine horn diameter, and age at breeding were analyzed using the MIXED procedure of SAS with year, group, and group*year in the model statements. For comparisons between the four puberty groups, if there was no significant group*year interaction, this term was dropped from the model. For weight data, for comparisons between the Start-Stop subgroups since there were a low number of heifers for each subgroup, group*year interactions were included in the model. Percent of samples where P4 ≥ 1 ng/ml was evaluated using the GLIMMIX procedure of SAS with a binomial distribution and logit link and year and group*year interaction included in the model. Reproductive tract score data were analyzed as a percent of heifers with a score of 5; data were distributed binomially and analyzed using the GLIMMIX procedure. If no significant group*year interactions were observed, this interaction term was dropped from the model. Pregnancy and calving data (percent showed estrus in response to PGF2α, percent pregnancy to AI, percent overall pregnancy, and percent calved in the first 21 days) were analyzed using the GLIMMIX procedure with a binomial distribution and logit link. For comparisons between puberty groups, model included year, group, and group*year interaction. If there was no significant group*year interaction, this term was dropped from the model. Year was not included in the model for comparisons between subgroups for pregnancy and calving data.

Results

Puberty groups

The total 611 heifers over the 6 years of data is shown in Figure 1A with the total number of heifers per pubertal classification (Typical and Early) that achieved puberty (Figure 1B and C) or the first P4 ≥ 1 ng/ml (Figure 1D; Start-Stop Group). No data are presented for the Non-Cycling Group since they did not have any rise in P4 and no dates could be calculated for them Puberty dates or first P4 ≥ 1-ng/ml date distributions differed for each year (Figure 2A–F). Representative P4 profiles for puberty groups are shown in Figure 3A–D with variation in Start-Stop heifers (discussed later). Of the 611 heifers evaluated over the 6 years, 45.7% of heifers were Typical (Figure 3A), 23.4% of heifers were Early (Figure 3B), 14.9% were Start-Stop (Figure 3C), and 16.0% were Non-Cycling (no rise in P4).

Average age at first P4 ≥ 1 ng/ml was lowest in Start-Stop heifers (265 ± 4 d), followed by Early (317 ± 4 d), and Typical (378 ± 2 d) heifers (Tables 1 and 2). Since Non-Cycling heifers did not have P4 ≥ 1 ng/ml, we could not calculate this measure for them. Typical (82.7 ± 2.4%) and Early (74.2 ± 4.7%) heifers had a greater percent of samples from weaning to breeding where P4 ≥ 1 ng/ml compared with Start-Stop (27.6 ± 5.6%) heifers (Table 2). Early (208 ± 2 d) heifers were the oldest puberty group at weaning, followed by Typical (203 ± 1 d) heifers which were not significantly older than Start-Stop (200 ± 2 d) heifers but were older than Non-Cycling (198 ± 2 d) heifers (Table 2).

Weights, ADG, and WDA

Adjusted birth weight was not different between puberty groups (Figure 4A). However, adjusted weaning weight was significantly greater in Typical (246 ± 1.41 kg) and Early (246 ± 1.95 kg) compared with Start-Stop (240 ± 2.56 kg) heifers but was not different from Non-Cycling (242± 2.37 kg/d) heifers (Figure 4B). Average adjusted yearling weight was higher in Typical (335 ± 1.72 kg) and Early (334 ± 2.40 kg) compared with Start-Stop (326 ± 3.13 kg) and Non-Cycling (326 ± 2.92 kg) heifers (Figure 4C). Birth to weaning ADG was greater in Typical (0.98 ± 0.01 kg/d) and Early (0.98 ± 0.01 kg/d) than in Start-Stop (0.94 ± 0.01 kg/d) heifers but was not different from Non-Cycling (0.96 ± 0.01 kg/d) heifers (Figure 4D). Weaning to yearling ADG was not different between groups (Figure 4E). Overall WDA from birth to yearling weight was

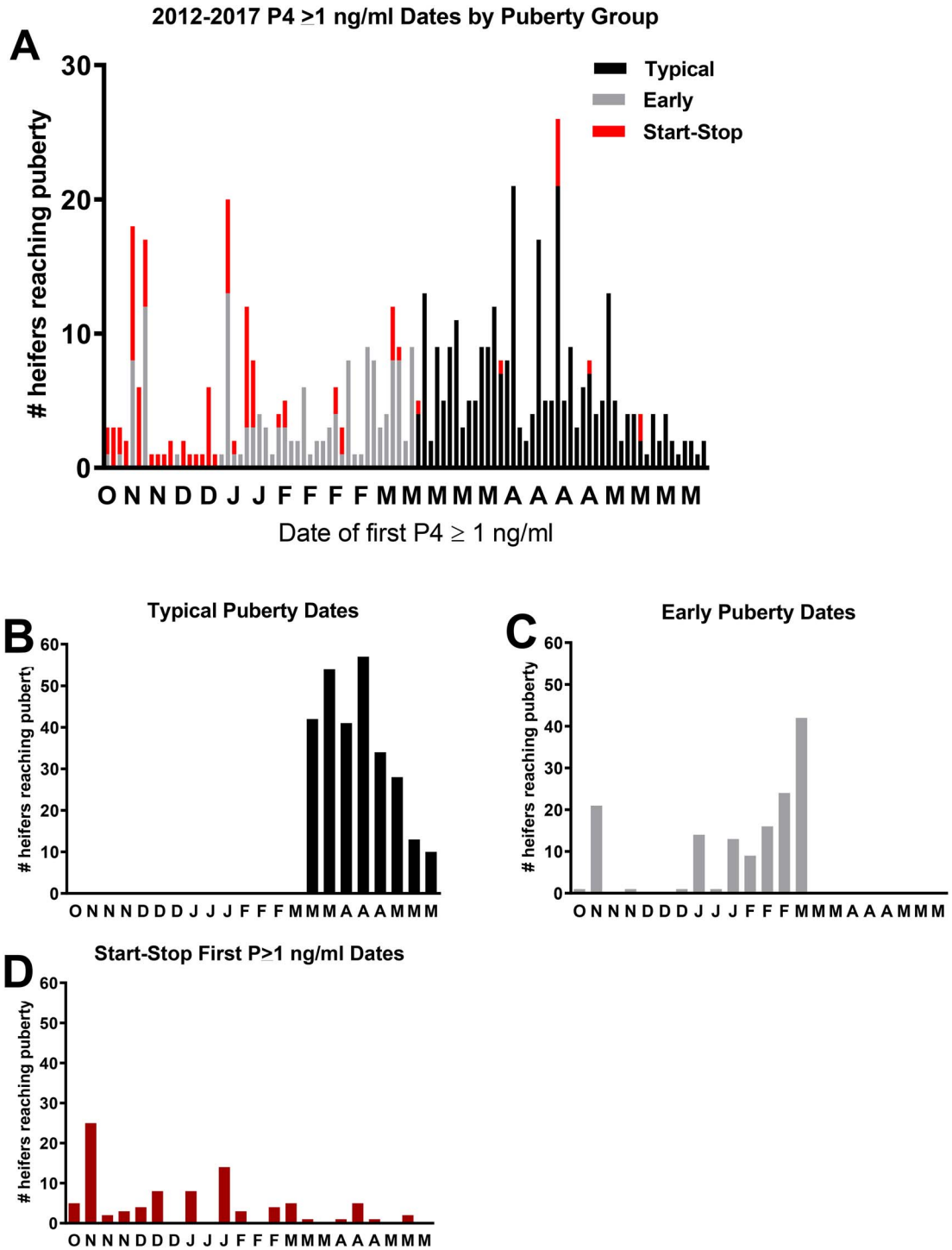

Figure 1. (A) Distribution of total number of heifers by puberty group for the first P4 ≥ 1 ng/ml rise for all heifers in the experiment that displayed them over all years: 2012–2017; (B) distribution of total number of heifers classified as Typical for the initial P4 ≥ 1 ng/ml (which also indicates their puberty dates, $n = 279$); (C) total number of heifers classified as Early for the initial P4 ≥ 1 ng/ml (which indicates their puberty dates, $n = 143$); (D) total number of Start-Stop heifers initial P4 ≥ 1 ng/ml from October to May ($n = 91$). Months are represented on *x*-axis (October–May with singular letter; October = O, November = N, December = D, January = J, February = F, March = M, April = A, and May = 2nd set of M's). A number of heifers at that blood collection with P4 ≥ 1 are on the *y*-axis. Since 2012–2014 did not have weekly blood collections, all individual puberty classifications were represented by looking at those reaching P4 ≥ 1 ng/ml at every one-third of the month.

higher in Typical (1.07 ± 0.01 kg/d), Early (1.05 ± 0.01 kg/d), and Non-Cycling (1.05 ± 0.01 kg/d) compared with Start-Stop (1.01± 0.02 kg/d) heifers (Figure 4F).

Prebreeding AFC, UHD, and RTS

There was a group∗year interaction for prebreeding antral follicle count, where most years antral follicle count tended to be higher in

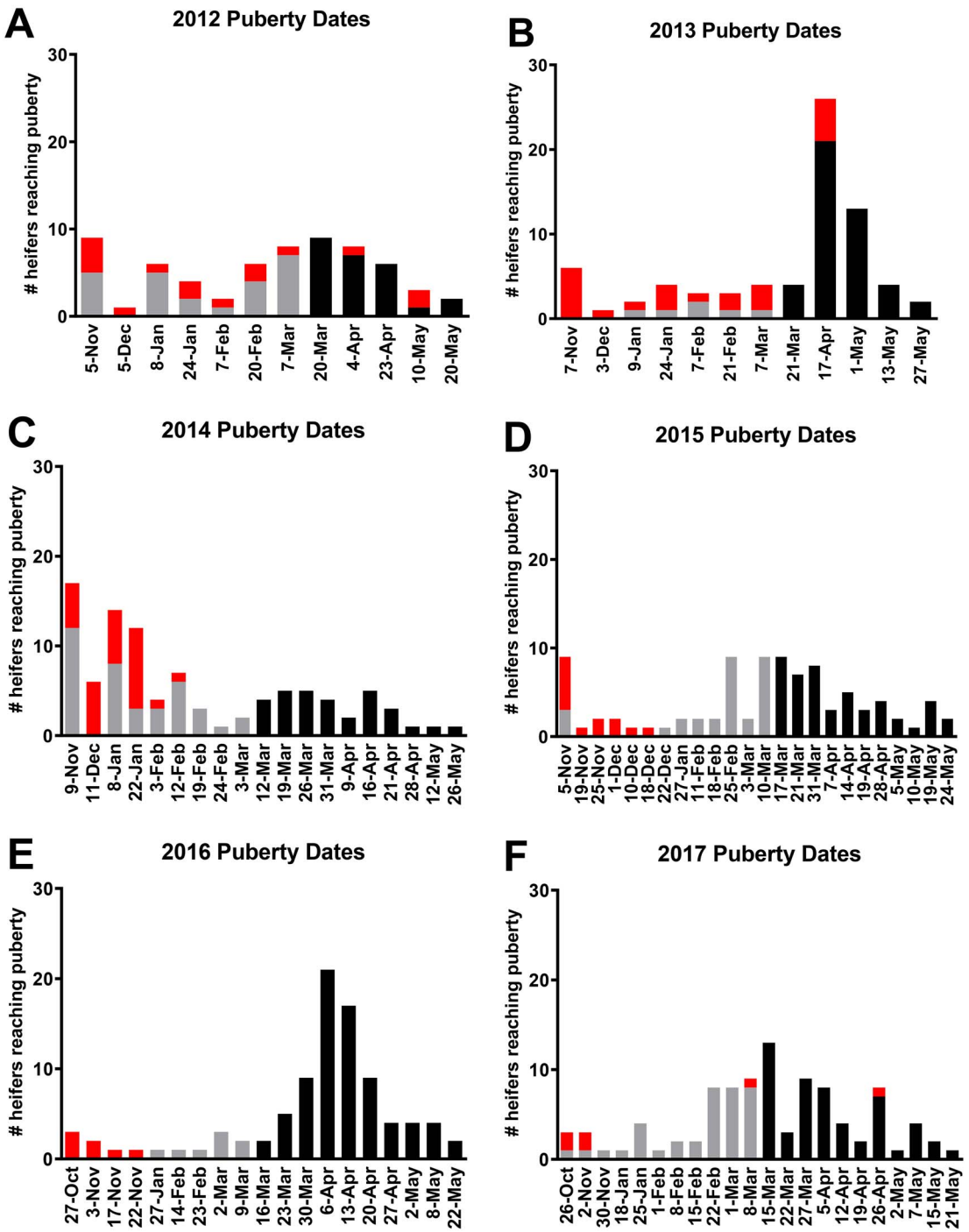

Figure 2. Distribution of dates of first P4 ≥ 1 ng/ml in Typical, Early, and Start-Stop heifers for (A) 2012, (B) 2013, (C) 2014, (D) 2015, (E) 2016, and (F) 2017. Abbreviated dates are represented on *x*-axis. Note that this would not be a "true puberty" for Start-Stop since they do not maintain cyclicity.

Non-Cycling heifers than in Typical or Start-Stop heifers ($P = 0.07$; Figure 5A). Prebreeding uterine horn diameter was larger in Typical (10.02 ± 0.11 mm) than in Non-Cycling (9.29 ± 0.19 mm) heifers and not different from Early (10.00 ± 0.15 mm) or Start-Stop (9.77 ± 0.19 mm) heifers (Figure 5B). At prebreeding, the highest percent of Early (92.7 ± 2.29%) heifers had a reproductive tract score of 5 (presence of corpora lutea), followed by Typical (76.2 ± 2.81%), Start-Stop (48.6 ± 5.70%), and Non-Cycling (1.27 ± 1.27%) heifers (Figure 5C). Average reproductive tract scores were 4.92 in Early heifers, 4.71 in Typical heifers, 4.46 in Start-Stop heifers, and 3.98 in Non-Cycling heifers.

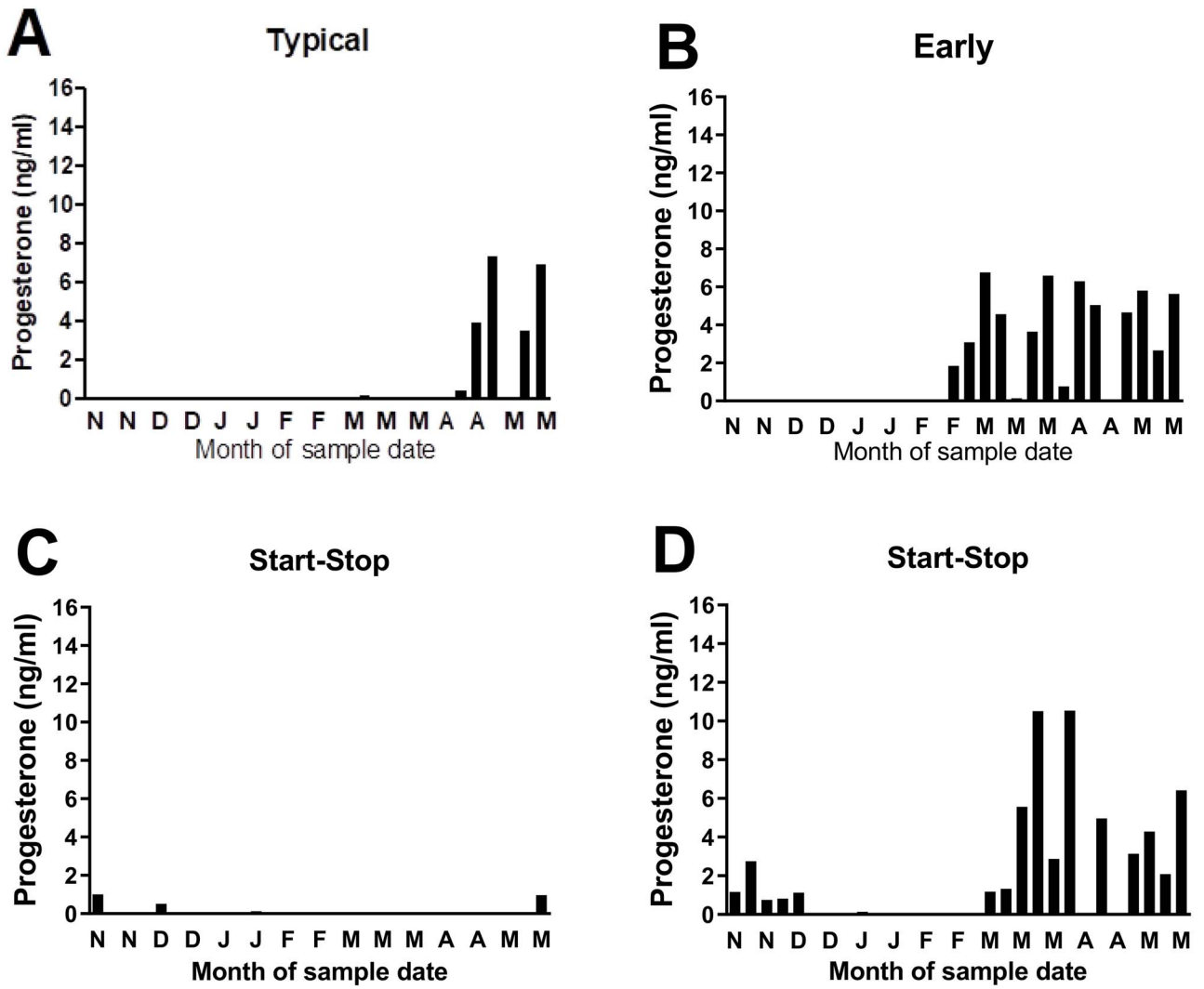

Figure 3. Progesterone profiles of representative (A) Typical, (B) Early, and (C, D) Start-Stop heifers. Month of sample date is on *x*-axis represented by single letter as stated in Figure 1 and progesterone (ng/ml) on *y*-axis. March 12 date separated Early and Typical heifers.

Table 2. Average age at first P4 rise, average age at weaning, and percent of samples where P4 ≥ 1 ng/ml after first P4 rise for each puberty classification.

	Puberty group				*P*-value		
	Typical	Early	Start-stop	Non-cycling	Group × year	Group	Year
Average age at first P4 ≥ 1 ng/ml, d	378 ± 2[a]	317 ± 4[b]	265 ± 4[c]	N/A	<0.0001	<0.0001	<0.0001
Avg age at Weaning, d	203 ± 1[b]	208 ± 2[a]	200 ± 2[b,c]	198 ± 2[c]	0.0005	0.0014	<0.0001
Percent of samples where P4 ≥ 1 ng/ml after initial P4 ≥ 1 ng/ml	82.7 ± 2.4[a]	74.2 ± 4.7[a]	27.6 ± 5.6[b]	N/A	0.999	<0.0001	0.9858

[a,b,c,d]Means in a row with different superscripts are different ($P \leq 0.05$).

Estrous response to PGF2α, age at breeding, pregnancy to AI, overall pregnancy rates, and calved in the first 21 days

A greater percentage of Typical (80.3 ± 2.82%) and Early (79.9 ± 4.26%) heifers showed estrus in response to PGF2α, followed by Start-Stop (49.7 ± 6.01%) and Non-Cycling (9.71 ± 3.81%) heifers (Figure 5D). Following AI, all heifers were placed with a herd bull. Age at breeding was calculated from time of AI which was conducted on heifers that showed estrus—Typical (429.66 ± 1.51), Early (437.47 ± 2.20), Start-Stop (430.16 ± 2.73), and Non-Cycling (439.40 ± 2.90; Figure 5E). For those heifers that were not AI, their calving date was obtained and 285 days

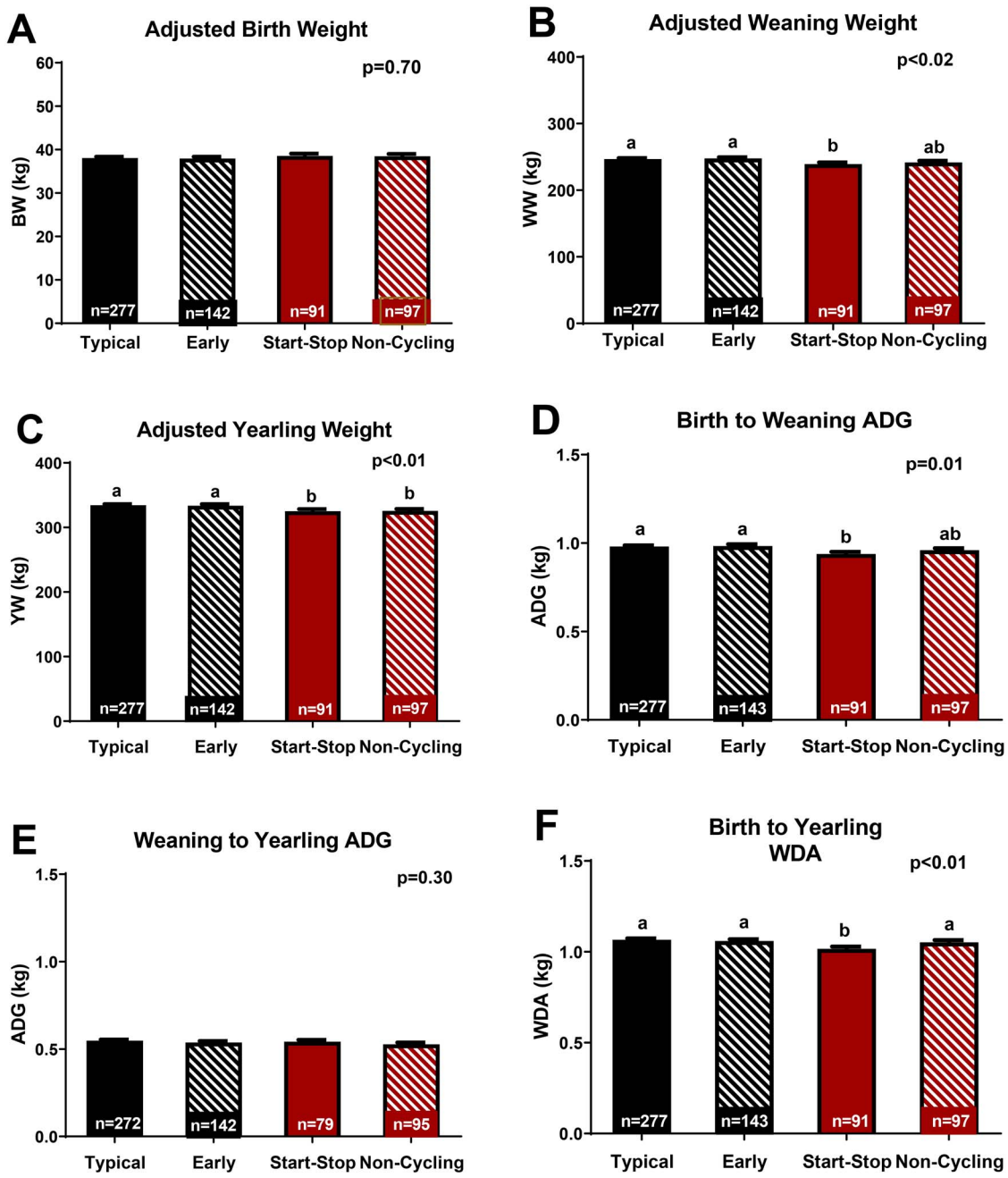

Figure 4. Effect of puberty group on (A) adjusted birth weight, (B) adjusted weaning weight, (C) adjusted yearling weight, (D) birth to weaning ADG, (E) weaning to yearling ADG, and (F) birth to yearling WDA. Numbers of heifers per group are indicated on bars. Bars with different letters represent differences at the *P*-value indicated in the right corner of the graph.

prior to that date were considered their breeding date. Several heifers did not calve; therefore, we used the date the herd bull was removed (bulls were in for 45 days) plus two estrus cycles to determine their breeding date (Figure 5E). Overall pregnancy rate did not differ between puberty groups (Figure 5F). More Typical (57.9 ± 3.85%), Early (51.0 ± 6.01%), and Start-Stop (45.2 ± 6.60%) heifers calved in the first 21 days of the calving season compared with Non-Cycling (20.9 ± 5.92%) heifers (Figure 5G).

Start-Stop subgroup differences

In the Start-Stop Group, there were females that did not return to cyclicity (SSD) and others that initiated cyclicity prior to breeding (SSS). Between SSS and SSD, there were no differences in adjusted birth weight, but SSS (246 ± 4.98 kg) heifers tended to have greater adjusted weaning weights than SSD (231 ± 5.65 kg) heifers (Figure 6A). There were no significant differences in adjusted yearling weight, ADG, or WDA between the subgroups. Age at weaning differed significantly between subgroups

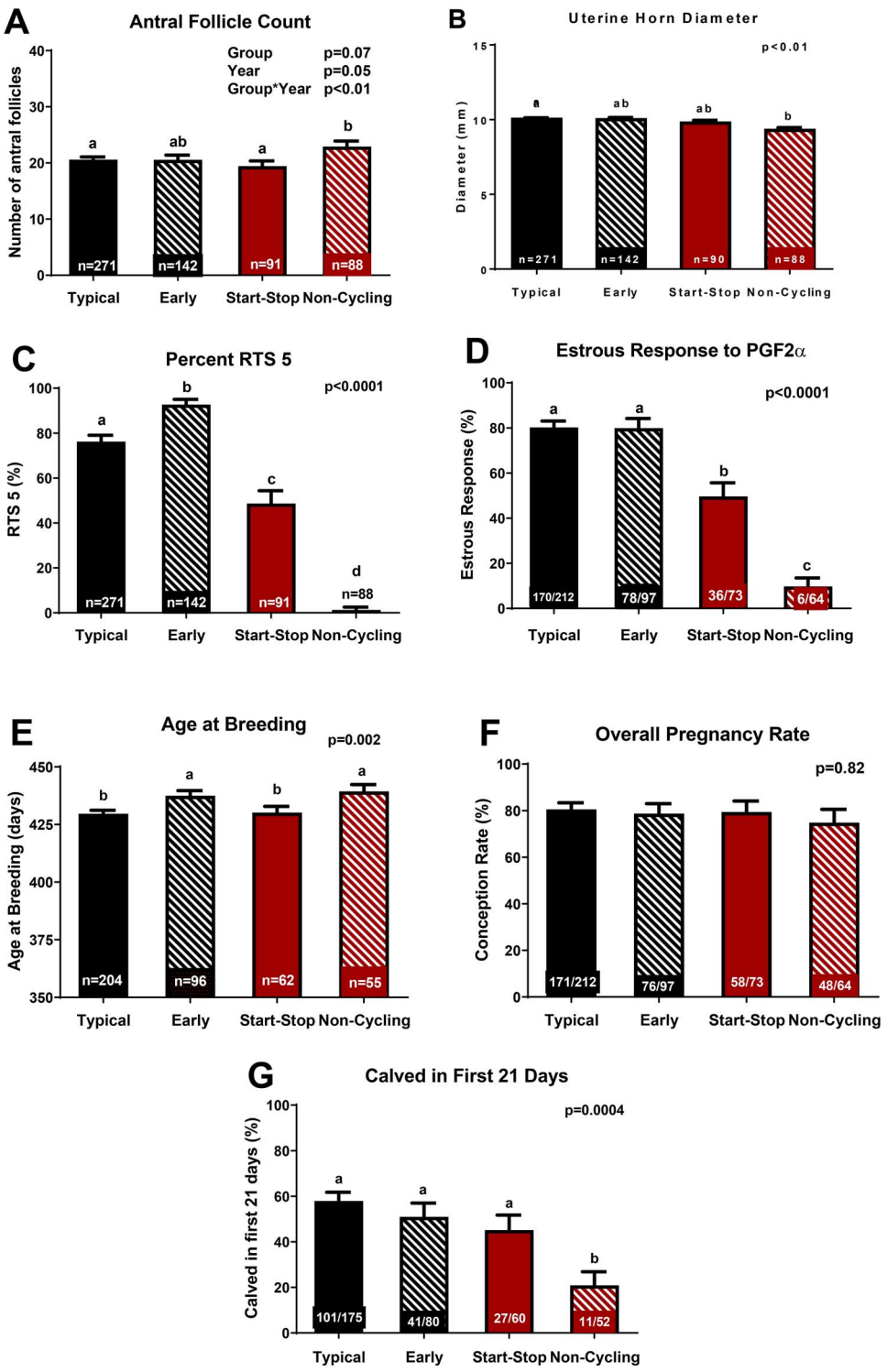

Figure 5. Differences in reproductive parameters by puberty group: on (A) antral follicle count, (B) uterine horn diameter, (C) percent RTS 5, (D) percent displayed estrus after PGF2α, (E) age at breeding, (F) overall pregnancy rate, and (G) percent heifers calved in the first 21 days of the calving season. Numbers of heifers per group are indicated on bars. Bars with different letters represent differences at the *P*-value indicated in the right corner of the graph.

(SSS—203 ± 3.39 d, SSD—190 ± 3.80 d, $P < 0.02$). At prebreeding, no differences in antral follicle count or uterine horn diameter were seen between subgroups, but a greater percent of SSS (71.3 ± 8.14%) heifers had a reproductive tract score of 5 compared with SSD (24.0 ± 8.34%) heifers (Figure 6B). Average reproductive tract scores were 4.7 in SSS heifers and 4.2 in SSD heifers. At breeding, a greater percent of SSS (79.5 ± 6.47%) heifers showed estrus in response to PGF2α than SSD (14.7 ± 6.07%) heifers (Figure 6C). Demonstrating that the SSS heifers were more similar to Typical and Early and SSD were more similar to Non-Cycling. All heifers

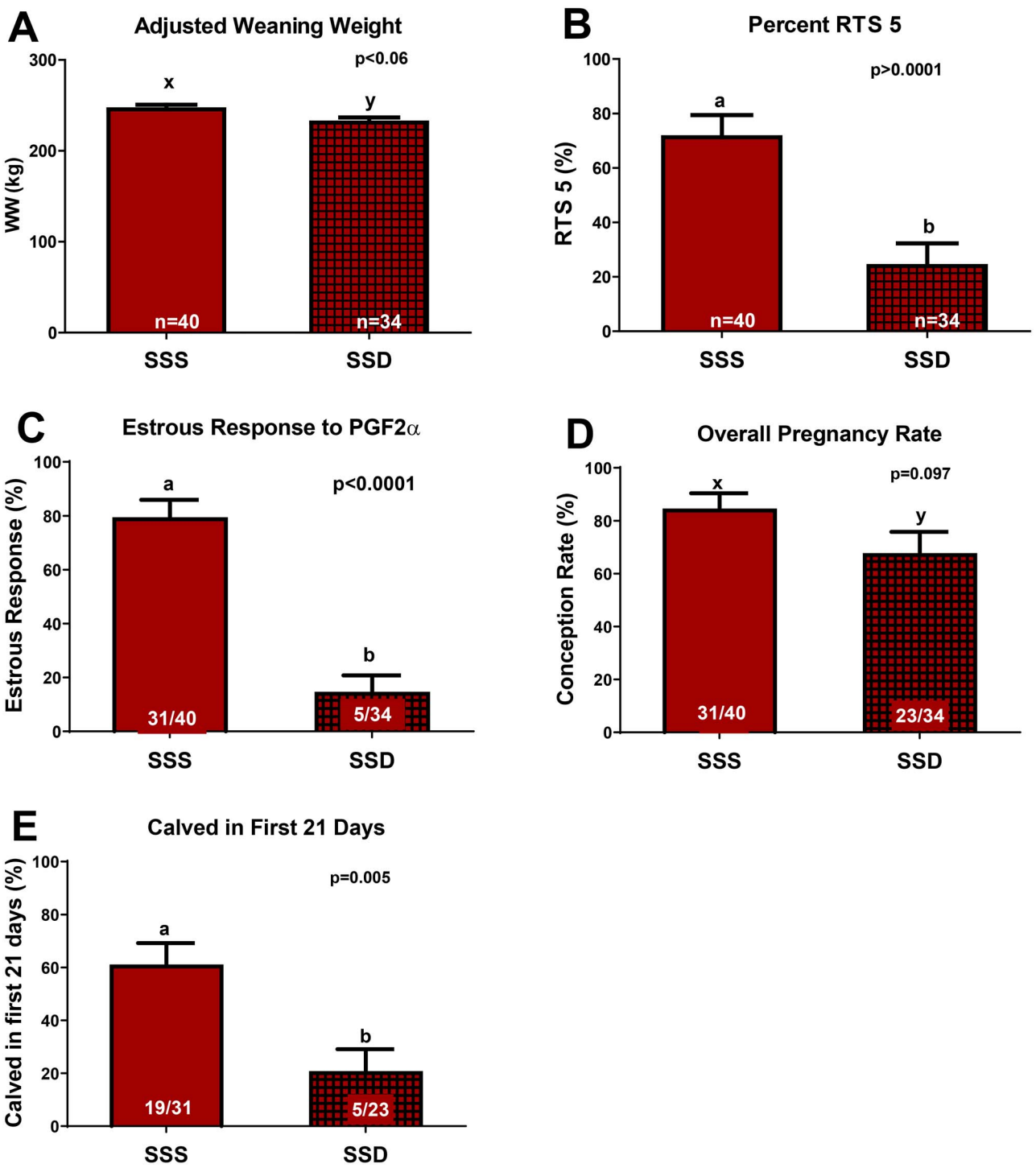

Figure 6. Differences of Start-Stop subgroup on (A) adjusted weaning weight, (B) percent reproductive tract score 5, (C) percent showed estrus after PGF2α, (D) overall pregnancy rate, and (E) percent heifers calved in the first 21 days of the calving season. Numbers of heifers in each group are shown on bars. Bars with different letters represent differences at the *P*-value indicated in the right corner of the graph.

that showed estrus were AI. Following AI, all heifers were placed with a herd bull. There was no differences in age at breeding for SSS and SSD heifers [SSS (n = 35) 429.14 ± 3.64 vs. SSD (n = 27) 431.48 ± 4.15]. However, overall pregnancy rate tended (P = 0.097) to be greater in SSS (84.6 ± 5.78%) heifers than SSD (67.8 ± 8.02%) heifers (Figure 6D). More SSS (61.1 ± 8.13%) heifers calved in the first 21 days of the calving season than SSD (20.8 ± 8.29%) heifers (Figure 6E). Again both of these parameters, response to PGF2α and percentage calved in the first 21 days, demonstrated that the SSS heifers were more similar to Early and Typical heifers, while the SSD heifers were more similar to Non-Cycling.

Discussion

In young girls, precocious, delayed, or irregular establishment of cyclicity at puberty may suggest a metabolic or reproductive disorder [14, 26]. It is not known if heifers who display precocious or irregular establishment of cyclicity are less likely to have longer reproductive lifespans. In the current study, four initial pubertal classifications were identified—Typical, Early, Start-Stop, and Non-Cycling. The Start-Stop and Non-Cycling groups had irregular, precocious, or delayed puberty, which affected growth traits, reproductive characteristics, and performance in their first breeding season. In the beef herd, females that achieve and maintain puberty earlier are more likely to have calves at an earlier time frame during calving, which increases the number of pounds at weaning for the beef producer [7, 8, 10, 15]. The Early and Typical pubertal classification heifers had continuous cyclicity after pubertal attainment, increased number of reproductive tract score 5, and a greater response to synchronization with PGF2α indicating that they had more corpora lutea. Furthermore, these phenotypic measures indicated that Typical and Early females had greater reproductive maturity than Start-Stop or Non-Cycling females. Thus, the manner in which heifers achieved puberty did affect their ability to be successful in their first breeding season and may impair reproductive performance in successive breeding seasons.

In this study, 1-ng/ml circulating plasma P4 was used as a threshold to indicate puberty date. This level has been commonly used in previous research studies when identifying puberty date in beef cattle [15, 29]. For Early and Typical heifers, this threshold appeared to accurately identify approximate date of puberty attainment as indicated by continuous cyclicity following first occurrence of P4 $\geq$ 1 ng/ml. This threshold also appeared to accurately identify those heifers that were not cycling prior to puberty (Non-Cycling heifers). In contrast, the Start-Stop heifers did not acquire puberty on the date that they first achieved P4 $\geq$ 1 ng/ml, instead this time was a "false start" since continued cyclicity was not ensured afterwards. Using the SAS analysis, we were able to identify the group of Start-Stop heifers, which differed from their contemporaries and deviated from typical puberty attainment. For Start-Stop heifers, the first occurrence of P4 $\geq$ 1 ng/ml was a questionable indicator of reproductive competency due to their discontinued cyclicity after initial "puberty date."

Start-Stop heifers showed the most variation in P4 profiles prior to breeding. While many of the Start-Stop females had P4 $\geq$ 1 ng/ml earlier than even the Early heifers, they did not maintain their cyclicity and did not attain puberty until later. Most Start-Stop heifers had the first occurrence of 1 ng/ml P4 during the months of November–January; 34 of 77 Start-Stop heifers showed 1–3 incidences of 1 ng/ml P4 but no evidence of cyclicity prior to breeding; they were categorized as SSD heifers (Tables 1 and 2). The other 40 of 77 Start-Stop heifers had at least one occurrence of P4 $\geq$ 1 ng/ml, discontinued cycling, and then began cycling continuously prior to breeding; these were categorized as SSS heifers.

Interestingly, there seem to be similarities between the SSS heifers in this study and the precocious puberty heifers in the study discussed by Wehrman et al. [15]. These precocious puberty heifers showed evidence of temporary luteal function at an early age, resumed anestrus, and then resumed cyclicity again later. Wehrman et al. [15] hypothesized that precocious puberty occurs when the inhibitory effects of estradiol are delayed, and estradiol from follicles on the ovary could induce an LH surge, leading to ovulation and cyclicity without the negative feedback of estradiol which accounts for the transient occurrence of circulating P4 at an early age [6, 24, 31, 32]. The authors suggested that when estradiol began to have an inhibitory effect on the Hypothalamic–Pituitary–Gonadal (HPG) axis, this would cease cyclicity leading to temporary anestrus until at a normal weight and age signaling would be appropriate for cyclicity to resume [20, 33]. This mechanism could explain one possibility for the irregularity in puberty attainment demonstrated in P4 profiles of Start-Stop heifers.

A second possibility is that androgens are elevated in these females and affecting establishment of the HPG axis. Adolescent girls with greater than normal androgen levels during adolescence have been associated with irregular cyclicity and anovulation. Often, young girls with these elevated androgens continue to have problems throughout their reproductive lifespan and are diagnosed with polycystic ovary syndrome (PCOS) [3]. Our physiology herd has a population of females that have excess A4 in follicular fluid and have a PCOS-like phenotype so it is possible that heifers with irregular cyclicity or lack of cyclicity may be females that will become our High A4 population of cows [27].

A third explanation could be that P4 may be produced in the adrenal gland in response to stress which has been observed in rodents [34], women [35], and in the cow [36]. No ultrasound was conducted during the early rises in P4 seen mostly in Start-Stop heifers in October, November, and December. Thus, the source of P4 could be the adrenal gland and not luteal tissue on the ovary due to ovulation.

When comparing heifers within the Start-Stop puberty group, heifers that began cycling regularly prior to breeding (SSS heifers) tended to have higher adjusted weaning weights than those that did not cycle regularly prior to breeding (SSD heifers; Figure 6A), making SSS heifers similar in weight gain to Typical or Early heifers. Maternal undernutrition can reduce weight at weaning in offspring and affect their metabolic status to delay time of puberty [37]. SSS heifers were also significantly older than SSD heifers and similar in age to Typical or Early heifers. No differences were seen in calf ADG due to low experimental numbers, but SSS heifers tended to be heavier than SSD heifers at prebreeding, and significantly more SSS heifers had a reproductive tract score of 5 than SSD heifers at prebreeding (Figure 6B). Following the pattern of Typical or Early heifers, a greater percent of SSS heifers responded to PGF2α by showing estrus, were AI, and calved within the first 21 days of the calving period. Although all heifers categorized as Start-Stop reached a threshold of 1 ng/ml of P4 in the time between weaning and breeding, those that had evidence of continuous cyclicity especially in the month prior to breeding, followed the patterns of Typical and Early heifers, and are more likely to become cows with greater lifetime fertility and reproductive longevity. Both age and weight of SSS heifers may have allowed for resumption of cyclicity later as age and weight both contribute to earlier age at puberty [6].

Average puberty date as well as the distribution of puberty dates differed each year in this study. Factors such as dam supplementation or nutrient restriction impacted average puberty date, as seen previously in other studies [30, 38]. Heifers born in 2013 were gestating during the 2012 drought and had a later average puberty date. Dams of 2016-born heifers received no supplement during pregnancy, and 2016-born heifers had a later average puberty date. Corresponding to this later puberty date distribution and later average puberty date, a smaller percentage of 2013-born and 2016-born heifers were Early, and more 2013-born and 2016-born heifers were Non-Cycling compared with the overall trend of puberty group distributions.

Weight, plane of nutrition, and body fat are important regulators of puberty attainment [39]. There were no differences in adjusted birth weights between puberty groups, but Early and Typical had higher adjusted weaning weights than Start-Stop heifers, and ADG from birth to weaning was higher in Typical and Early heifers than in Start-Stop heifers. There were no differences in ADG between groups during the period after weaning until yearling weight, but Typical and Early heifers remained significantly heavier than both Start-Stop and Non-Cycling heifers at their adjusted yearling weights. These data show that the difference in adjusted yearling weights between Typical and Early vs. Start-Stop and Non-Cycling heifers was achieved primarily during the preweaning period, which corresponds to previous research showing that preweaning body weight gain has a larger impact on reducing age at puberty than postweaning body weight gain [4, 24]. This period of accelerated preweaning rate of gain also coincides with the window from 4 to 8 months of age when leptin has the largest effect on regulating age at puberty in heifers [5, 25].

Uterine horn diameter is correlated to reproductive tract development in heifers [38]. In the current study, we observed that the most immature females, Non-Cycling, had reduced uterine horn diameter when compared with Typical heifers. Furthermore, the Early puberty group had the highest percentage of reproductive tract score of 5 suggesting that this group had the most reproductively mature females. Since Early females initiate cyclicity earlier, it is logical that these females also have greater maturity with reproductive tract and uterine horn diameter measures. These Early females would be more easily identified and retained in the herd after annual palpation. A group less easily identified is the heifers that are in the Start-Stop pubertal classification, specifically the SSD heifers might be identified as cyclic and retained in the herd while decreasing overall herd reproductive efficiency.

After PGF2α injection at breeding, a greater percentage of Typical and Early heifers displayed estrus followed by Start-Stop and Non-Cycling heifers. Because all heifers that showed estrus were AI, a greater percent of Typical and Early heifers were AI, followed by Start-Stop and Non-Cycling heifers. There were no differences in conception rate to AI between puberty groups. However, a greater number of Typical, Early, and Start-Stop heifers calved in the first 21 days of the calving season compared with Non-Cycling heifers. Females that calve earlier in the calving season are likely to have increased fertility over their lifespan and last a longer time in the herd [38, 40]. In addition, heifers born earlier in the calving season make better replacement heifers because they are more likely to reach puberty before breeding, calve earlier as heifers, and produce calves with heavier weaning weights [40]. Early heifers were the oldest puberty group at weaning, followed by Typical heifers, with Start-Stop heifers not different than Typical heifers, and Non-Cycling the youngest on average at weaning.

Following artificial insemination, all heifers were placed with a herd bull. Overall pregnancy rate did not differ between puberty groups, demonstrating that Start-Stop heifers and Non-Cycling heifers were able to become pregnant by a bull even without evidence of previous cyclicity during the sampling period. This could be due to an extended opportunity for attainment of puberty/regular cyclicity within a larger time window or through bull exposure. Bull exposure has been known to influence puberty attainment in heifers [24, 31, 41], and heifers at a certain weight range and between 12 and 14 months are most influenced by bull exposure prior to puberty [24, 31, 41]. This age range corresponds to the time of bull exposure for the heifers in this study.

Bull exposure impacts the female HPG axis through pheromones, which are air-borne chemicals released from the urine or feces of the male and taken up by the olfactory system of females. Pheromones can increase GnRH signaling in the hypothalamus, which may induce puberty in heifers or cause re-induction of cyclicity in cows after anestrus periods such as pregnancy [24, 31, 41] Thus, exposure to the bull may have greater effects on inducing and maintaining cyclicity in the SSD and Non-Cycling heifers.

In the present study, we have identified two novel heifer pubertal classifications: SSD and Non-Cycling. The SSD heifers appear to attain "early puberty" but puberty is not maintained, and they display irregular cyclicity early after weaning. The Non-Cycling heifers have delayed puberty attainment and may have genetic or environmental factors that may contribute to their delayed puberty. Both classifications of puberty are reproductively immature and have less response to synchronization with PGF2α. These data support the notion that Non-Cycling and SSD females may be the majority of females that respond to synchronization regimes using a progestin (CIDR) or bull exposure which appears to overcome their genetic and/or metabolic inhibition of cyclicity. Also, the current use of progestins to induce cyclicity in heifers may allow for the retention of heifers in the herd that need these increased management practices [24, 31, 41]. Identifying the SSD and Non-Cycling heifers and selecting against them or understanding the added inputs that are needed to manage them differently would increase the ability of cow/calf producers to maintain females that fit their production system. Further analysis of these pubertal classifications, changes in hormonal concentrations and ovarian characteristics are being conducted to understand the biological implications of how each pubertal classification initiates cyclicity. Specifically, the SSD and Non-Cycling puberty classifications may increase our understanding of the genetic and biological processes involved in pubertal attainment of adolescent girls with irregular cyclicity or delayed cyclicity and how that may impact diagnosis of future reproductive disorders.

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