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Effects of administration of a growth promoting implant during the suckling phase or at weaning on growth, reproduction, and ovarian development in replacement heifers grazing native range

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Abstract

Management strategies utilized during pre-breeding development of replacement heifers can impact fertility and the ovarian reserve. Angus-Hereford crossbred heifers (n = 233) were utilized over a 3-yr period to determine the effects of administration of a growth promoting implant at either branding or weaning on growth, reproduction, and ovarian development. Heifer calves were randomly assigned to one of three treatments: 1) nonimplanted controls (CON; n = 79), 2) implanted at approximately 2 mo of age (average calf age = 58 d) with Synovex-C (BIMP, n = 82), or 3) implanted at approximately 7 mo of age (average calf age = 210 d) with Synovex-C (WIMP; n = 72). In years 2 and 3, a subset of heifers (year 2 n = 16; year 3 n = 14) were unilaterally ovariectomized. Heifers implanted at 2 mo of age were heavier at weaning, yearling (mid-February; average calf age = 332 d), and at the beginning of the breeding season ($P < 0.01$) compared to CON and WIMP heifers. Average daily gain (ADG) was similar among treatments from weaning to yearling and weaning to the start of the breeding season ($P \geq 0.61$); however, WIMP heifers had increased ($P = 0.05$) ADG from yearling to the start of the breeding season compared to BIMP heifers. Antral follicle count and reproductive tract scores were not influenced by implant treatment ($P \geq 0.18$). Response to synchronization of estrus was increased ($P = 0.02$) in WIMP compared to CON heifers, with BIMP heifers similar to all other treatments. First service conception rates tended to be increased ($P = 0.09$) in CON heifers compared to WIMP heifers, with BIMP heifers similar to CON and WIMP. Final pregnancy rates were similar ($P = 0.54$) among treatments. A treatment × yr interaction was detected ($P = 0.01$) for the number of primordial follicles/section with increased primordial follicles in WIMP heifers in year 3 compared to BIMP and WIMP heifers in year 2 and CON heifers in year 3, as well as in BIMP compared to WIMP heifers in year 2. Utilization of growth promoting implants did not negatively impact postweaning reproductive development or compromise pregnancy rates in beef heifers. Based on these results, administration of a growth promoting Synovex-C implant at 2 mo of age may allow for increased body weight at weaning, without hindering reproductive performance.

Lay Summary

Management of beef females during the first year of life can impact fertility and reproductive longevity. Cattle producers can improve calf weight gains by using growth promoting implants; however, to be applicable, they must not negatively impact heifer reproductive performance or development. Understanding the impact of growth promoting implants on growth, fertility, and reproductive development is important to determine if they can be utilized as an effective management strategy in heifers intended to be retained in the breeding herd. To determine if growth promoting implants influence fertility, 233 heifer calves either received no implant, a Synovex-C implant at 2 mo of age, or a Synovex-C implant at 7 mo of age. Implanting heifers at 2 mo of age increased body weight at weaning. Implanting heifers at 7 mo of age did not improve body weight gains. Implanting heifers at 2 or 7 mo of age resulted in similar pregnancy rates. By using a growth promoting implant at 2 mo of age in beef heifers, producers may be able to increase heifer weaning weight without negatively affecting reproductive development or pregnancy rates. Additional body weight at weaning may provide a profit advantage for heifers not retained as replacements.

Key words: growth promoting implants, heifer development, reproduction

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Abbreviations: ADG, average daily gain; AI, artificial insemination; BIMP, branding implant; BW, body weight; CON, control; CIDR, controlled internal drug release device; PGF2α, prostaglandin $F_{2\alpha}$; WIMP, weaning implant

Introduction

Management decisions made during the peri-pubertal development period play a key role in establishing heifer fertility and subsequent longevity (reviewed in Summers et al., 2019). Growth promoting implants have been utilized in the beef industry as an economical way to increase body weight gains. Due to inconsistencies in reproductive performance reported in the literature, growth promoting implants have not been recommended for use in replacements heifers.

Ralgro and Synovex-C increase body weight gains and yearling pelvic area (Staigmiller et al., 1983; Hancock et al., 1994) with no negative effect on puberty attainment (Hancock et al., 1994). Previous research, however, is inconsistent regarding the impact of growth promoting implants on fertility and subsequent pregnancy rates. Deutscher et al. (1986) and Hancock et al. (1994) report that percent pregnant did not differ between non-treated controls and heifers receiving a growth promoting implant, whereas others indicate a reduction in percent pregnant for heifers receiving an implant (Staigmiller et al., 1983). In addition to discrepancies in reproductive performance, the effect of growth promoting implants on ovarian dynamics and the ovarian reserve has not been evaluated. Management decisions made during the peripubertal period have been demonstrated to influence the size of the ovarian reserve (Freetly et al., 2014; Amundson et al., 2015). Evaluation of the influence of growth promoting implants on the ovarian reserve may allow for a more complete understanding of the influence of implants on fertility, ovarian development, and reproductive longevity in replacement beef heifers.

Therefore, the objective of this study was to investigate the effects of growth promoting implants on growth performance, reproductive efficiency, ovarian measurements, and the ovarian reserve. Our hypothesis was that heifers receiving a growth promoting implant at either branding or weaning would have increased growth performance, while maintaining similar overall reproductive performance, and utilization of growth promoting implants would not negatively impact ovarian dynamics.

Materials and Methods

All animal procedures and facilities used to conduct the present study were approved by the New Mexico State University Institutional Animal Care and Use Committee (IACUC approval # 2016-037).

Animals, diets, and treatments

Over a 3-yr period, spring-born Angus × Hereford crossbred heifers (n = 233) were utilized in a completely randomized design to compare utilization of a growth promoting implant administered at either branding or weaning on developing heifers grazing native range. Research was conducted at the New Mexico State University Corona Range and Livestock Research Center located 13 km east of Corona, NM. Heifers were assigned to 1 of 3 treatments at branding: 1) non-implanted controls (CON); 2) heifers receiving a growth promoting implant (100 mg progesterone + 10 mg estradiol; Synovex-C; Zoetis Animal Health, Florham Park, NJ) at approximately 2 mo of age (average calf age = 58 d; branding; BIMP); or 3) heifers receiving a growth promoting implant (100 mg progesterone + 10 mg estradiol; Synovex-C) at approximately 7 mo of age (average calf age = 210 d; weaning; WIMP). Dams and suckling heifers were managed similarly from calving through weaning, with all dams located at the Corona Range and Livestock Research Center and supplemented to maintain similar body condition and calf performance. At branding heifers received a modified live vaccine (Bovi-Shield GOLD 5, Zoetis Animal Health) against infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza 3, and bovine respiratory syncytial virus, and a vaccination against blackleg caused by *Clostridium chauvoei*, malignant edema caused by *Clostridium septicum*, black disease caused by *Clostridium novyi*, gas-gangrene caused by *Clostridium sordellii*, and enterotoxemia and enteritis caused by *Clostridium perfringens* Types B, C, and D (ULTRABAC 7, Zoetis Animal Health). At weaning, heifers received a booster vaccination against infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza 3, and bovine respiratory syncytial virus, as well as vaccination against *Mannheimia haemolytica* (One Shot, Zoetis Animal Health) and were dewormed (Dectomax, Zoetis Animal Health). Following weaning, heifers were managed together grazing native rangeland. Rangeland pasture vegetation is described by Forbes and Allred (2001). Predominant grasses included blue grama (*Bouteloua gracilis*), sideoats grama (*Bouteloua curtipendula*), hairy grama (*Bouteloua hirsute*), sand dropseed (*Sporobolus cryptandrus*), common wolftail (*Lycurus phleoides*), threeawns (*Aristida* spp.), and black grama (*Bouteloua eriopoda*) with minor components of other grasses and annual forbs (Forbes and Allred, 2001). Heifers had *ad libitum* access to water and a loose salt-mineral mix formulated to complement available forage, composed of 10% Ca, 7% P, 2% Mg, 0.5% K, 2500 ppm Cu, 5000 PPM Zn, 2500 ppm Mn, 75 ppm I, 15 ppm Se, and 246 KIU/kg vitamin A (Hi-Pro Feed, Friona, TX). Heifers received supplementation (Rancher Pro 20% Cube, Hi-Pro Feed, Friona, TX) over the post-weaning development period to provide a minimum gain of 0.09 kg heifer^{-1} d^{-1} with supplementation rates adjusted based on forage availability and historic forage quality as needed. Historic forage values collected at the New Mexico State University Corona Range and Research Center are reported in Table 1. Minimum heifer gains were set at 0.09 kg heifer^{-1} d^{-1} to minimize supplementation costs over the post-weaning winter feeding period. Supplementation rates were adjusted each spring to target heifers to achieve approximately 55% of mature body weight (BW) by initiation of the breeding season. Supplementation started immediately post-weaning and continued through the pre-breeding development period.

Ultrasonographic evaluation of antral follicle count and reproductive tract development

Antral follicle counts were added in years 2 and 3 to further evaluate the impact of growth promoting implants on ovarian development, corresponding to the addition of ovariectomies in a subset of heifers in years 2 and 3. Heifers were submitted for ultrasonographic evaluation of antral

Table 1. Historical average nutrient analysis forage crude protein and total digestible nutrient collected at the New Mexico State University Corona Range and Livestock Research Center

Item	Crude protein, %	Total digestible nutrients, %
Month		
January	4.62	42.58
February	5.51	41.18
March	7.48	41.06
April	10.16	40.53
May	12.09	43.40
June	11.53	40.66
July	14.03	38.89
August	10.90	41.32
September	6.70	44.00
October	6.60	32.25
November	7.10	41.21
December	7.50	44.32

follicle count and reproductive tract development using an SSD-500v ultrasonic machine and a 7.5 MHz linear array transducer (Aloka, Wallingford, CT). Antral follicle count and reproductive tract measurements were performed by a single technician (McNeel and Cushman., 2015; Tenley et al. 2019). Transrectal ultrasonographic examination included a complete reproductive tract examination consisting of antral follicle count (all follicles ≥ 3 mm), corpus luteum count, ovarian length and height measurements, and uterine horn diameter. Reproductive tract score was based on uterine horn diameter, size of the ovaries, and ovarian structures as described previously (Martin et al., 1992).

Breeding

Estrus was synchronized utilizing the Select Synch + CIDR synchronization protocol with heifers receiving a controlled internal drug release device (CIDR, Eazi-Breed, Zoetis Animal Health) insert for 7 d after which the CIDR was removed and heifers were administered a single 5-mL intramuscular injection of prostaglandin $F_{2\alpha}$ (5 mg/mL; $PGF_{2\alpha}$, Lutalyse, Zoetis Animal Health). At the time of $PGF_{2\alpha}$ administration, an estrus detection aid (Estrotect, MAI Animal Health, Elmwood, WI) was applied to the tail head. Estrus detection was performed for 5 d following $PGF_{2\alpha}$ administration and heifers were artificially inseminated (AI) approximately 12 h after observed standing estrus. Approximately 10 d after the last day of AI, heifers were exposed to bulls for approximately 60 d. First service conception and overall pregnancy rates were determined 30 d after the last day of AI and at a minimum of 30 d after bull removal by analyzing whole blood for pregnancy specific protein-B (Biopyrn, Biotracking Inc. Moscow, ID; New Mexico Department of Agriculture, Veterinary Diagnostic Service, Albuquerque, NM).

Morphometric analysis of ovaries

At breeding in years 2 and 3, a subset of heifers (year 2 $n = 16$; year 3 $n = 14$) were unilaterally ovariectomized. Prior to ovariectomy, the Select Synch + CIDR protocol described above was administered to induce a follicular phase at the time of ovariectomy. Thirty-six hours after CIDR removal, unilateral ovariectomy occurred by right flank laparotomy (Youngquist et al., 1995; Summers et al., 2014). Immediately upon collection, weight, height, and length of each ovary were recorded. The height and length of the largest follicle were recorded, and visible antral follicles were counted on the ovary. Ovarian tissue was dissected, snap frozen in liquid nitrogen, and stored at −80 °C. A representative section (1.5 mm thick) from the center of the ovary was fixed in 4% paraformaldehyde overnight (Amundson et al, 2015; Tenley et al., 2019). The subsequent day, ovarian tissue was rinsed in phosphate buffered saline, followed by post-fixation in graded ethanol's to dehydrate the tissues. Ovarian tissue was then clarified in xylene and embedded in paraffin for subsequent sectioning and histological evaluation. Ovarian tissue was sectioned and 5 sections (6 µm thickness) were taken with a minimum of 10 sections between each collected section to ensure the same primordial, primary, and secondary follicles were not counted in consecutive sections. Ovarian sections were stained with eosin and counterstained with hematoxylin. Follicles were counted and classified as primordial (surrounded by a single layer of flattened pre-granulosa cells), primary (surrounded by a single layer of cuboidal granulosa cells), or secondary (surrounded by two of more layers of cuboidal granulosa cells), by the same trained individual each year according to previously utilized and established criteria (Cushman et al., 1999; Amundson et al., 2015; Tenley et al., 2019).

Blood collection and radioimmunoassay

Blood samples were collected every 2 wk starting in February of each year and continuing through the start of the breeding season to determine attainment of puberty. Blood samples were collected by coccygeal venipuncture into serum separator vacuum tubes (Corvac, Kendall Healthcare, St. Louis, MO). Samples were subjected to centrifugation (1200 × *g* for 20 min at 4 °C) and serum was decanted and stored at −20 °C until hormone assays were conducted. Serum progesterone concentrations were quantified by radioimmunoassay utilizing components of a solid phase kit (MP Biomedicals, LLC, Santa Ana, CA) and modified for use in ruminant serum as reported by Schneider and Hallford (1996). Intra-assay coefficients of variation were 7.0%, 9.3%, and 11% in years 1, 2, and 3, respectively. Inter-assay coefficients of variation were 18.6%, 5.9%, and 9.1% in years 1, 2, and 3, respectively. Progesterone concentrations greater than 1.0 ng/mL in a single sample were interpreted to indicate attainment of puberty (Henricks et al., 1971).

Follicular fluid progesterone and estradiol concentrations were quantified by radioimmunoassay using components of a solid phase kit (MP Biomedicals, LLC, Santa Ana, CA) as reported by Castañon et al. (2012). Follicular fluid was diluted 1:100 for both progesterone and estradiol. Intra-assay coefficients of variation for progesterone were 4.5% and 3.7% in years 2 and 3, and 13.4% and 7.5% for estradiol in years 2 and 3, respectively.

Statistical analysis

Data were analyzed utilizing the MIXED and GLIMMIX procedures of SAS (SAS Inst. Inc., Cary, NC). Heifer body weight, average daily gain, antral follicle count, uterine horn diameter, reproductive tract score, and ovarian measurements were analyzed using the MIXED procedure. The model included implant treatment, year, and the interaction of implant treatment × year. One heifer was removed from analysis of the preovulatory follicle diameter and follicular fluid hormone concentrations due to a follicle diameter of 5.0 mm and undetectable concentrations of estradiol in the follicular fluid indicating the follicle measured was not a dominant follicle. Puberty attainment, estrus response, and pregnancy rates were analyzed using the GLIMMIX procedure of SAS with a binomial distribution and a logit link to examine the fixed effect of implant treatment. No significant ($P > 0.05$) treatment × year interactions were detected for binomial data; thus, the treatment × year interaction was removed from the model, and the data are presented as the main effect of treatment and year. The influence of implant treatment on microscopic follicle number was analyzed using the MIXED procedure of SAS. A statistical power analysis was completed to determine a minimum of 6 animals is needed per treatment to detect a difference in ovarian histology of 40 vs. 95 primordial follicles with a standard deviation of 33 (Freetly et al., 2014; Amundson et al., 2015; Tenley et al., 2019) at a power of 0.80. Data are presented as the least-squares means and SE. Significance was determined at $P \leq 0.05$ and a tendency was reported if $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

Heifer performance

Heifer growth performance over the post-weaning development period is reported in Table 2. Heifers receiving a Synovex-C growth promoting implant at 2 mo of age were heavier ($P = 0.001$) at weaning compared to CON and WIMP heifers. Synovex-C has an active payout period of 100 to 140 d and is designed to increase growth performance and BW in suckling calves under 182 kg. Therefore, increased BW at weaning was anticipated in heifers receiving a Synovex-C implant at branding (approximately 2 mo of age). Yearling BW was greater ($P = 0.001$) in BIMP heifers compared to CON and WIMP heifers. This BW advantage was maintained through the beginning of the breeding season, with an increased BW ($P = 0.009$) in BIMP heifers compared to CON and WIMP heifers. Similar to results in the current study, Hancock et al. (1994) reported heifers receiving a growth-promoting implant at 2 mo of age were heavier at weaning compared to non-implanted heifers. This BW advantage was maintained through 1 yr of age in heifers receiving a Synovex C implant at 2 mo of age. Furthermore, authors reported heifers implanted at 6 mo of age did not display additional growth as a result of administration of a growth promoting implant (Hancock et al., 1994). Additional BW at weaning in heifers receiving a Synovex C implant at 2 mo of age is advantageous for producers making marketing and selection decisions at weaning. The BW advantage gained from use of a growth promoting implant provides additional marketing options and profit advantage for heifers not retained as replacements. In a review, Duckett and Andrae (2001) evaluated the effect of implanting at each production phase on ADG, BW, and value. Administration of a growth promoting implant during the suckling phase in steer calves provided an 8-kg increase in BW and $16.32 increase in value. Heifers in the current study had a 15-kg increase in BW compared to control heifers suggesting a potential increase in value if heifers had been marketed at weaning.

Average daily gain from weaning to yearling did not differ ($P = 0.93$) between treatments. From the yearling time point to the start of the breeding season ADG was increased ($P = 0.05$) in heifers receiving a growth promoting implant at 7 mo of age compared to heifers implanted at branding (2 mo of age), with non-implanted control heifers similar to all other treatments. Average daily gain over the entire postweaning development period, weaning to the start of the breeding season, did not differ ($P = 0.61$) between CON, BIMP, and WIMP heifers. Heifers receiving a Synovex-C implant at 7 mo of age did not demonstrate an increase in BW gain from weaning through the yearling timepoint as anticipated. The lack of increased growth performance reported in WIMP heifers may be partially attributed to the lower dose of hormones in Synovex-C (100 mg progesterone + 10 mg estradiol) compared to growth promoting implants intended for use in older and heavier heifers containing greater concentrations of hormones. Additionally, postweaning management of heifers consisted of grazing low-quality dormant native forage and supplementation to achieve a minimum ADG of 0.09 kg of gain per day. Nonetheless, BW gains each year were not

Table 2. Effect of growth-promoting implants administered at either branding or weaning on heifer body weight, average daily gain, and reproductive performance

Item					*P*-value		
	CON[1]	BIMP[2]	WIMP[3]	SEM	Trt	Yr	Trt x Yr
No. of heifers	79	82	72				
BW							
Weaning BW, kg	219^{a}	234^{b}	219^{a}	3.54	<0.01	0.51	0.26
Yearling BW[4], kg	215^{a}	229^{b}	215^{a}	3.30	<0.01	0.53	0.21
Breeding BW, kg	241^{a}	252^{b}	241^{a}	3.19	<0.01	<0.01	0.15
ADG, kg/d							
Weaning to Yearling	-0.01	-0.02	-0.01	0.03	0.93	<0.01	0.99
Yearling to Breeding	0.33^{ab}	0.31^{a}	0.36^{b}	0.01	0.05	<0.01	0.78
Total[5]	0.13	0.11	0.13	0.02	0.61	<0.01	0.95

^{a,b}Means within a row without a common superscript differ ($P < 0.05$).

[1] CON, heifers received no growth-promoting implant.

[2] BIMP, heifers received a single Synovex-C implant (100 mg progesterone + 10 mg estradiol; Zoetis Animal Health) at 2 mo of age.

[3] WIMP, heifers received a single Synovex-C implant (100 mg progesterone + 10 mg estradiol; Zoetis Animal Health) at 7 mo of age.

[4] Yearling body weight was collected in mid-February of each year. Heifers were an average of 332 d of age.

[5] Heifer average daily gain from weaning to the start of the breeding season.

observed from weaning to yearling resulting in WIMP heifers lacking the protein and energy intake necessary for appropriate response to the Synovex-C implant. Furthermore, heifers in all treatments in the current study had a negative ADG over the winter grazing period, indicating inadequate nutrient availability. Paisley et al. (1999) conducted research with steers receiving either no implant, a Synovex-C implant, a Synovex S implant, or a Revalor G implant and developed on dormant tallgrass prairie with a protein supplement. Utilization of a growth promoting implant resulted in increased overall weight gains and average daily gains for implanted steers. Specifically, implants improved winter gains compared to non-implanted controls with daily gains in all steers below 0.47 kg/d in period 1 and below 0.22 kg/d in period 2 (Paisley et al., 1999). Differences in the growth promoting implants utilized and supplementation of steers by Paisley et al. (1999) compared to heifers in the current study likely resulted in differences in BW gains and ADG between the two studies. These results suggest with adequate nutrient availability growth promoting implants can improve gains during winter grazing of low-quality dormant native range. Results from the current trial indicate that adequate nutrient availability is necessary over the winter grazing period for Synovex-C implants administered at weaning to be effective and result in increased BW gains (Duckett and Andrae, 2001).

Reproductive performance

Heifer antral follicle count, reproductive tract score, and uterine horn diameter are presented in Table 3. Antral follicle count was similar ($P = 0.17$) among CON, BIMP, and WIMP heifers. Antral follicles are visualized by ultrasonography and utilized as a prediction tool for characterizing fertility as well as the size of the ovarian reserve in beef heifers (Ireland et al., 2008; Ireland et al., 2011). The number of primordial follicles has been positively correlated with the number of antral follicles (Cushman et al., 1999; Ireland et al., 2008; Tenley et al., 2019), allowing antral follicle count to be utilized as a tool to potentially assess and predict fertility in beef heifers. To the best of our knowledge, previous literature has not investigated the influence of growth promoting implants administered to beef heifers at approximately 2 or 7 mo of age on antral follicle counts. In heifers fed to achieve 55% or 65% mature BW prior to the breeding season there was no effect of nutritional treatment on total antral follicle count (Eborn et al., 2013). These results suggest that development of heifers on a low rate of gain during the post-weaning development period in the current study likely did not influence the total number of antral follicles. No differences were observed between treatments for reproductive tract score ($P = 0.80$). Reproductive tract score is an indicator of reproductive tract maturity as well as an estimate of pubertal status, taking into consideration ovarian size, structures present on the ovary, and uterine horn diameter, assigning heifers a score ranging from 1 to 5. A reproductive tract score of 1 is indicative of an immature, underdeveloped reproductive tract and the heifer is prepubertal, whereas a reproductive tract score of 5 is indicative of a mature reproductive tract and the heifer is considered pubertal. The average reproductive tract score of all treatments was greater than 4.29, suggesting that heifers in all treatments were at a similar stage of reproductive tract maturity and development, as well as puberty attainment. Furthermore, similar antral follicle count and reproductive tract scores among heifers despite utilization of a growth promoting implant provide evidence that administration of a Synovex-C implant at either 2 or 7 mo of age did not deleteriously impact reproductive development prior to the onset of the first breeding season.

Uterine horn diameter tended to be increased ($P = 0.08$) in CON heifers compared to BIMP heifers, with WIMP heifers similar to all other treatments. In addition, uterine horn diameter was increased ($P < 0.01$) in year 3 compared to yr 2 (10.1 ± 0.17 vs. 8.5 ± 0.21, respectively). Bartol et al. (1995) reported heifers administered a Synovex-C implant at days 0, 21, or 45 after birth had decreased uterocervical weight compared to non-implanted controls. In contrast, Prichard et al. (1989) reported heifers implanted at 56 and 146 d of age had a greater uterine horn diameter and heavier uterine weight when slaughtered at 210 d of age compared to non-implanted controls. Uterine horn diameter increases from 2 wk of age through 60 wk of age (Honaramooz et al., 2004); therefore, heifers may have been at slightly different stages of maturity among years and between BIMP and CON heifers. McNeel et al. (2017) reported slight discrepancies may exist between uterine horn diameter measurements taken by ultrasound compared to physical measurements taken after harvest. Discrepancies can result from small alterations in placement of the ultrasound probe during examination. In the current study, similarities in overall pregnancy rates imply that uterine development was not hindered by administration of a Synovex-C implant at 2 or 7 mo of age. Further research is necessary, however, to determine the influence of Synovex-C implants administered at branding (2 mo of age) or weaning (7 mo of age) on uterine development and function.

Heifer puberty attainment, response to synchronization of estrus, first service conception rates, and overall pregnancy rates are reported in Table 4. The percentage of heifers

Table 3. Effect of growth-promoting implants administered at either branding or weaning on heifer antral follicle count, reproductive tract score, and uterine horn diameter in years 2 and 3

Item	CON[1]	BIMP[2]	WIMP[3]	SEM	*P*-value		
					Trt	Yr	Trt x Yr
Antral follicle count	23	23	25	1.14	0.18	0.28	0.46
Uterine horn diameter, mm	9.68	8.97	9.14	0.24	0.08	<0.001	0.65
Reproductive tract score[4]	4.41	4.29	4.37	0.13	0.80	0.06	0.40

[1] CON, heifers received no growth-promoting implant.
[2] BIMP, heifers received a single Synovex-C implant (100 mg progesterone + 10 mg estradiol; Zoetis Animal Health) at 2 mo of age.
[3] WIMP, heifers received a single Synovex-C implant (100 mg progesterone + 10 mg estradiol; Zoetis Animal Health) at 7 mo of age.
[4] Reproductive tract score (Martin et al., 1992).

Table 4. Effect of growth-promoting implants administered at either branding or weaning on heifer puberty attainment, estrus response, first service conception rates, and overall pregnancy rates

Item	CON[1]	BIMP[2]	WIMP[3]	SEM	*P*-value	
					Trt	Yr
Puberty, %						
Yearling[4]	58	46	44	6.11	0.22	<0.001
Prebreeding	70	73	65	6.12	0.58	<0.001
Estrus response,%	48^{a}	58^{ab}	71^{b}	5.87	0.03	0.73
First service conception rate,%	77	63	52	7.68	0.09	0.72
Overall pregnancy rate,%	88	81	85	4.74	0.54	0.13

[a,b]Means within a row without a common superscript differ ($P < 0.05$).
[1]CON, heifers received no growth-promoting implant.
[2]BIMP, heifers received a single Synovex-C implant (100 mg progesterone + 10 mg estradiol; Zoetis Animal Health) at 2 mo of age.
[3]WIMP, heifers received a single Synovex-C implant (100 mg progesterone + 10 mg estradiol; Zoetis Animal Health) at 7 mo of age.
[4]Yearling samples were collected in mid-February of each year. Heifers were an average of 332 d of age at sample collection.

attaining puberty by approximately a year of age was similar ($P = 0.22$) regardless of implant treatment. There was no difference ($P = 0.58$) in the proportion of heifers attaining puberty before the start of the breeding season among treatments. Similarly, Hancock et al. (1994) reported no significant difference in the percentage of heifers attaining puberty before the start of the breeding season. McCraw et al. (1991) reported that similar proportions of heifers achieved puberty by 14 mo of age regardless of growth promoting implant treatment. Previously, heifers implanted at 2 to 3 mo of age exhibited a 10% increase in the proportion of heifers attaining puberty before the start of the breeding season compared to non-implanted control heifers (67.5% vs. 57.8%, respectively; Whittier et al., 1991). Whittier et al. (1991) reported an increase in yearling BW in implanted heifers, potentially resulting in the increased proportion of heifers attaining puberty at the start of the breeding season. Rusk et al. (1992) reported age at puberty was reduced in heifers implanted at 3 mo of age compared to controls. Differences in puberty attainment between studies may potentially be explained by differences in nutritional management and growth rate of heifers among trials.

Response to synchronization of estrus was increased ($P = 0.02$) in heifers implanted at 7 mo of age compared to CON heifers, with heifers implanted at 2 mo of age similar to all other treatments. Conversely, Hancock et al. (1994) reported a similar proportion of heifers in estrus the first 21 d of the breeding season between non-implanted controls, heifers receiving an implant at 2 mo of age, and heifers receiving an implant at 6 mo of age. In the current study, estrus response was recorded in response to a 7-d CIDR synchronization protocol over a 5-d heat detection period. First service conception rates to AI tended to be increased in non-implanted controls ($P = 0.09$) compared to WIMP heifers, with BIMP heifers similar to all other treatments. Rusk et al. (1992) reported no difference in pregnancy rates the first 10 d of the breeding season between non-implanted controls and heifers receiving a Synovex-C implant at 3 mo of age (57% vs. 41%, respectively); however, heifers implanted at both 3 and 8 mo of age had reduced pregnancy rates compared to controls (35% vs. 57%, respectively). In heifers receiving a Synovex-C implant at 2 or 6 mo of age, first service conception rates were similar among heifers receiving a growth promoting implant and non-implanted controls (Hancock et al., 1994). Furthermore, McCraw et al. (1991) also reported similar first service conception rates for heifers implanted at 3 mo of age with an estradiol benzoate and progesterone implant compared with controls.

The percentage of heifers pregnant at the end of the 60-d breeding season was similar among treatments ($P = 0.54$). Whittier et al. (1991) also reported no differences in overall pregnancy rates between heifers implanted at approximately 2 to 3 mo of age and control heifers (76.8% vs. 80.7%, respectively). Additionally, Hancock et al. (1994) reported implanting heifers with Synovex-C at 2 or 6 mo of age resulted in a similar proportion of heifers becoming pregnant in the first 21 d of the breeding season, as well as over the entire 63 d breeding season in Trial 1. In Trial 2, however, the percentage of heifers pregnant in the first 21 d was reduced in heifers receiving a Synovex-C implant a 6 mo of age (Hancock et al., 1994). In contrast, at d 60 of the breeding season, Rusk et al. (1992) reported a decrease in pregnancy rates in heifers implanted at 3 mo of age compared with control heifers (74% vs. 94%, respectively). Over the entire 95-d breeding season, however, pregnancy rates of heifers receiving a growth promoting implant increased from 74% to 90%, resulting in overall pregnancy rates similar to control heifers (99%; Rusk et al., 1992). Results reported by Rusk et al. (1992) suggest heifers receiving a growth promoting implant may require a longer breeding season to attain pregnancy rates similar to nonimplanted heifers. Decreased first service conception rate to AI in WIMP heifers may suggest that fertility was decreased entering the breeding season for heifers administered a growth promoting implant at 7 mo of age. Comparable overall pregnancy rates between WIMP, BIMP, and CON heifers, however, suggest that WIMP heifers were able to attain acceptable pregnancy rates over a 60-d breeding season. Similar first service conception rate and overall pregnancy rate between non-implanted controls and BIMP heifers suggests that administration of a Synovex C implant at 2 mo of age did not negatively impact fertility.

Due to the range in the proportion of heifers attaining puberty, first service conception rates, and overall pregnancy rates between treatments, a statistical power test was conducted. To achieve a statistical difference in puberty attainment, first service conception rate, or overall pregnancy rate among treatments an additional 200 to 340 heifers would be needed per treatment, depending on the trait of interest. While approximately 80 heifers per treatment lacks sufficient statistical power to draw conclusions, these data provide the basis for the number of heifers that would be needed to observe detectable differences. Bartol et al. (1995) reported administration of a Synovex C implant in newborn calves negatively impacted uterine morphology and the uterine environment, likely resulting in detrimental effects on reproductive performance. While pregnancy results in the current study lack sufficient statistical power, negative impacts on uterine morphology and uterine endometrial gland development similar to results reported by Bartol et al. (1995) would potentially result in much larger impacts on fertility and pregnancy rates. Nonetheless, additional research utilizing a larger number of heifers would allow for a more complete understanding of the impact of utilization of growth promoting implants on pregnancy rates.

Ovarian measurements

Ovarian measurements, follicular fluid hormone concentrations, and ovarian histology results are reported in Table 5. Ovarian weight did not differ ($P = 0.89$) between CON, BIMP, and WIMP heifers. Hancock et al. (1994) reported implanting heifers at 2 mo of age resulted in an increase in total ovarian weight. A treatment × yr interaction ($P < 0.03$) was found for ovarian length and surface antral follicle count at time of ovariectomy. Ovarian length was decreased in CON heifers in year 3 compared to all other treatments. Ovarian height had a tendency for a treatment × yr interaction ($P = 0.09$) being driven by a slight decrease in ovarian height in year 3. In contrast, Prichard et al. (1989) reported implanting heifers at 56 and 146 d of age with 36 mg zeranol had no effect on ovarian weight or size. Furthermore, Eborn et al. (2013) reported development of the female reproductive system in beef heifers was not influenced by lower energy intake in heifers on a low rate of gain over the post-weaning development period. Heifers in the current study, however, had a negative ADG between weaning and the yearling time point, which could potentially impact ovarian development. Ovarian measurements in the current study are similar to those reported by Freetly et al. (2014) and Rosasco et al. (2020), suggesting that while ADG was decreased over the winter feeding period, ovarian development was not negatively impacted. The number of surface antral follicles were counted for each ovary at time of ovariectomy. The number of follicles were decreased ($P = 0.03$) in CON heifers in year 3 compared to CON heifers in year 2 and WIMP heifers in year 3, with heifers in all other treatments and years being similar. It has been suggested that an inherently high variation in ovary size and the ovarian reserve exists and mechanisms contributing to this variation are not well understood (Cushman et al., 2009; Ireland et al., 2011). Follicle numbers can be influenced by many factors including birthweight, maternal environment, breed, and maternal age (Cushman et al., 2009; Ireland et al., 2011; Cushman et al., 2019; Tenley et al., 2019). Therefore, differences in the number of surface antral follicles could potentially be the result of inherent variation among animals, suggesting animals that had naturally high or low surface antral follicle numbers were randomly assigned in different frequencies among the two years. This is demonstrated by the decrease in surface antral follicle numbers in non-implanted control heifers from year 2 to year 3 (35.9 vs. 18.8, respectively). Furthermore, developing heifers to 55% mature BW did not influence antral follicle counts, a predictor of the ovarian reserve, compared with heifers developed to 64% mature BW (Eborn et al., 2013), suggesting that the lack of nutrients during the winter in the current study did not impact antral follicle counts.

Administration of a growth promoting implant at weaning resulted in a decrease in preovulatory follicle diameter ($P = 0.02$) in WIMP heifers compared to BIMP heifers (10.37 vs. 13.46 mm, respectively), with control heifers (11.88 mm) similar to BIMP and WIMP heifers. Concentrations of estradiol in the follicular fluid of the dominant follicle were similar ($P = 0.27$) among treatments regardless of implant treatment. Follicular fluid estradiol concentrations were increased in year 3 compared to year 2 ($P = 0.05$). Follicular fluid progesterone concentrations were similar ($P > 0.47$) among all treatments and years. Estradiol:progesterone ratio was similar ($P = 0.51$) between CON, BIMP, and WIMP heifers; however, there was an increase in estradiol:progesterone ratio ($P = 0.04$) in heifers in year 3 compared to heifers in year 2.

Table 5. Effect of growth-promoting implants administered at either branding or weaning on heifer ovarian measurements and follicular fluid hormone concentrations in years 2 and 3

Item	Year 2			Year 3				*P*-value		
	CON[1]	BIMP[2]	WIMP[3]	CON[1]	BIMP[2]	WIMP[3]	SEM	Trt	Yr	Trt x Yr
No of heifers	7	5	4	4	5	5				
Ovarian weight, g	4.63	4.13	3.27	4.48	5.26	6.98	1.34	0.89	0.13	0.29
Ovarian length, mm	30.2^{a}	30.2^{a}	24.8^{a}	17.0^{b}	24.5^{a}	25.2^{a}	1.6	0.05	<0.01	<0.01
Ovarian height, mm	19.8	21.6	17.2	15.4	13.5	19.4	2.4	0.94	0.07	0.09
Surface antral follicles[4]	35.9^{a}	32.8^{ab}	23.7^{ab}	18.8^{b}	22.0^{ab}	36.6^{a}	6.60	0.85	0.27	0.03
Preovulatory follicle, mm	11.4^{ab}	13.6^{a}	11.1^{ab}	12.3^{ab}	13.3^{a}	9.7^{b}	1.2	0.02	0.76	0.53
Estradiol, ng/mL	319.6	145.4	141.1	654.1	605.9	250.2	194.3	0.27	0.05	0.63
Progesterone, ng/mL	55.1	46.6	51.4	66.8	53.0	40.3	14.2	0.47	0.83	0.66
Estradiol:progesterone	5.92	2.98	2.97	8.68	12.67	7.14	3.42	0.66	0.04	0.51

[a,b] Means within a row without a common superscript differ ($P < 0.05$).

[1] CON, heifers received no growth-promoting implant.

[2] BIMP, heifers received a single Synovex-C implant (100 mg progesterone + 10 mg estradiol; Zoetis Animal Health) at 2 mo of age.

[3] WIMP, heifers received a single Synovex-C implant (100 mg progesterone + 10 mg estradiol; Zoetis Animal Health) at 7 mo of age.

[4] Surface antral follicles ≥ 1mm were counted following ovary removal.

Ovulatory capacity is achieved in bovine follicles at approximately 9 to 10 mm (Sartori et al., 2001). Perry et al. (2007) reported ovulation of follicles > 10.7 mm and < 15.7 mm in diameter resulted in increased pregnancy rates in heifers, regardless of whether heifers spontaneously ovulated or were induced to ovulate with gonadotropin releasing hormone. While all treatments had a mean preovulatory follicle diameter over 9 mm, indicating follicles likely achieved ovulatory capacity, decreased dominant follicle diameter in WIMP heifers below 10.7 mm suggests that heifers implanted at 7 mo of age potentially had a slight decrease in follicle maturity and oocyte competency. While ovulatory follicle diameter can be an indicator of the stage of maturation of the follicle, the follicular microenvironment plays a crucial role in determining oocyte competency and reproductive success. Estradiol concentrations in the follicular fluid of the dominant follicle can be an indicator of the ability of the oocyte to become successfully fertilized. In cattle, oocytes from preovulatory follicles with greater concentrations of estradiol had an increased likelihood of developing to the blastocyst stage during in vitro maturation (reviewed in Pohler et al., 2012). Preovulatory follicles with an estradiol:progesterone ratio > 1 are considered estrogen active (Sunderland et al., 1994). Estradiol:progesterone ratios > 1 in all treatments indicate that a similar number of dominant follicles were estrogen active among treatments, suggesting that oocytes collected from heifers in all treatments had the potential to become successfully fertilized. Increased estradiol in year 3 compared to year 2 may suggest that follicles in year 3 would have had a microenvironment that would have allowed for a slight increase in fertilization rate and success if the oocyte had been ovulated. Similar overall pregnancy rates, follicular fluid estradiol concentrations, and a ratio of estradiol:progesterone > 1 suggest that reproductive performance was not negatively impacted by administration of the Synovex-C implant at 2 mo of age or 7 mo of age in beef heifers.

Histological evaluation

There was a significant treatment × yr interaction detected ($P < 0.03$) for primordial, primary, and secondary follicles per section. Primordial follicles per histological section were increased ($P = 0.01$) in WIMP heifers in year 3 compared to BIMP and WIMP heifers in year 2 and CON heifers in year 3. Moreover, primordial follicles per section were increased ($P = 0.01$; Figure 1) in CON heifers in year 2 compared to WIMP heifers in year 2. Heifers administered a Synovex-C implant at 7 mo of age (WIMP) in year 2 had a decreased number of primary follicles per histological section ($P = 0.03$; Figure 2) compared to CON heifers in years 2 and 3, BIMP heifers in year 3, and WIMP heifers in year 3, with all other treatments being similar. Secondary follicles per histological section were increased ($P = 0.01$; Figure 3) in BIMP heifers in year 2 compared to WIMP heifers in year 2, as well as in WIMP heifers in year 3 compared to CON heifers in year 3 and WIMP heifers in year 2. Primordial follicles within the ovary represent the ovarian reserve, from which follicles are recruited for development. Primordial follicles form at approximately day 90 of gestation in cattle and gain the capacity to activate after day 140 of gestation resulting in formation of the growing pool of follicles (Fortune et al., 2013). Primordial follicles are activated and continue to grow until the follicle is either selected to become a dominant follicle and proceed through ovulation or undergoes atresia. Therefore, heifers are born with a finite number of follicles in their ovaries. Recent research, however, has indicated that management strategies may be able to influence the number of primordial follicles in the ovaries during the first year of life (Freetly et al., 2014; Amundson et al., 2015). Furthermore, previous research has provided evidence that the size of the ovarian reserve may positively influence fertility in cattle (Cushman et al., 2009; McNeel and Cushman, 2015). To the best of our knowledge, previous research investigating the effects of growth promoting implants administered to heifers at either branding or weaning has failed to evaluate the impact on the ovarian reserve. Differences in primordial follicle numbers were driven by a significant increase in primordial follicles in WIMP heifers between years 2 and 3 (26 vs. 95 primordial follicles/section, respectively). There is limited evidence suggesting alteration of primordial follicle numbers within the WIMP treatment are a result of the Synovex-C implant. The significant increase within the WIMP treatment between years 2 and 3 could be a result of natural variation in the ovarian reserve among animals as primordial, primary, and secondary follicles were all increased in WIMP heifers in years 3 vs. 2. Primordial follicle numbers were positively associated with surface antral follicle counts in year 2 ($r = 0.754$, $P = 0.001$) and year 3 ($r = 0.53$, $P = 0.05$), suggesting that deviations in the ovarian reserve between years in WIMP heifers may be attributed to naturally occurring variation in the ovarian reserve among animals. Additional research may be warranted to confirm the impact of administration of a Synovex-C implant at 7 mo of age on the ovarian reserve. In the current study, there was no difference in primordial follicle numbers between non-implanted control

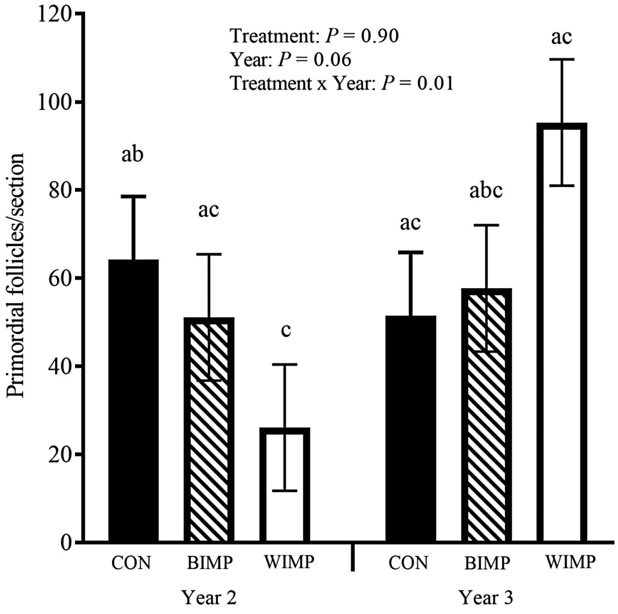

Figure 1. Number of primordial follicles per histological section of heifers administered a growth-promoting implant at branding, weaning, or nonimplanted control. Primordial follicles per histological section were increased ($P = 0.01$) in heifers receiving a Synovex C implant at 7 mo of age (WIMP) in year 3 compared to heifers receiving a Synovex C implant at 2 mo of age (BIMP) and WIIMP heifers in year 2 and nonimplanted control heifers (CON) in year 3. Moreover, primordial follicles per section were increased ($P = 0.01$) in CON heifers in year 2 compared to WIMP heifers in year 2. ^{a,b,c}Bars with different superscripts are different ($P < 0.05$).

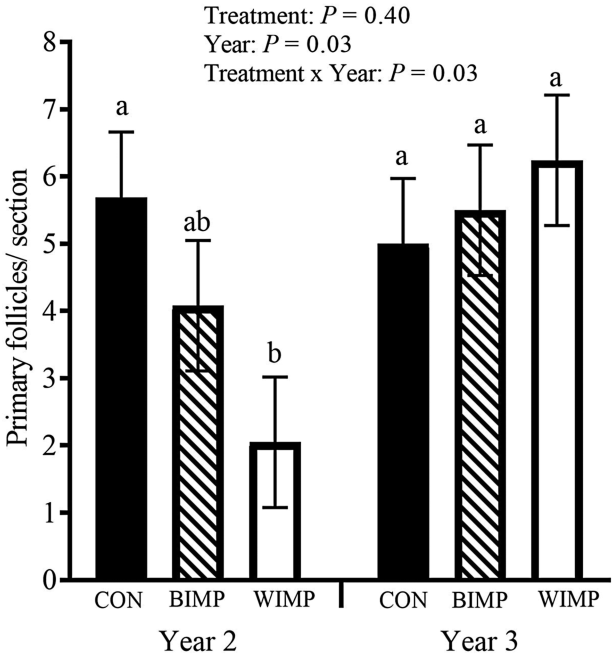

Figure 2. Number of primary follicles per histological section of heifers administered a growth promoting implant at branding, weaning, or nonimplanted control. Heifers administered a Synovex-C implant at 7 mo of age (WIMP) in year 2 had a decreased number of primary follicles per histological section ($P = 0.03$) compared to nonimplanted control heifers (CON) in years 2 and 3, heifers receiving a Synovex C implant at 2 mo of age (BIMP) in year 3, and WIMP heifers in year 3, with all other treatments being similar. ^{a,b}Bars with different superscripts are different ($P < 0.05$).

heifers and BIMP heifers. Therefore, it does not appear that administration of a Synovex-C implant at 2 mo of age negatively impacts the ovarian reserve.

Longevity is a critically important trait to producers as costs associated with development of replacement heifers are recovered through subsequent calf crops. There are limited studies investigating the long-term impact of growth promoting implants on longevity and reproductive performance past the first breeding season. Hancock et al. (1994) reported similar second season overall pregnancy rates between cows implanted with Synovex-C at 3 mo of age and non-implanted controls. Similarly, Deutscher et al. (1986) reported that second season pregnancy rates were not influenced by treatment with a zeranol growth promoting implant. Survival analysis in a previous study demonstrated that a similar proportion of heifers receiving a Synovex-C growth promoting implant at branding (3 mo of age) and non-implanted heifers remained in the herd to produce a fourth calf (Rosasco et al., 2018). Further research investigating the influence of growth promoting implants on heifer retention rates and lifetime reproductive performance, specifically evaluating timing of administration and comparison of different growth promoting implants, would allow for a more complete understanding of the long-term impact of implants on reproductive efficiency.

Utilization of a growth promoting implant during the suckling phase in beef heifers resulted in an increase in BW at weaning without negatively affecting overall pregnancy rates, ovarian development, and the ovarian reserve of heifers intended to be retained as replacement animals.

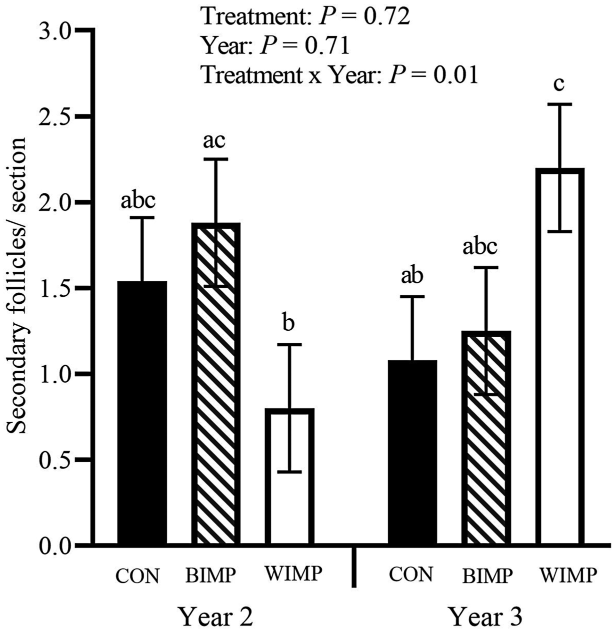

Figure 3. Number of secondary follicles per histological section of heifers administered a growth-promoting implant at branding, weaning, or nonimplanted control. Secondary follicles per histological section were increased ($P = 0.01$) in heifers receiving a Synovex C implant at 2 mo of age (BIMP) in year 2 compared to heifers receiving a Synovex C implant at 7 mo of age (WIMP) in year 2, as well as in WIMP heifers in year 3 compared to nonimplanted control heifers (CON) in year 3 and WIMP heifers in year 2. ^{a,b,c}Bars with different superscripts are different ($P < 0.05$).

Administration of a Synovex-C implant at weaning did not influence overall pregnancy rates; however, implanting heifers at weaning did not increase growth performance and tended to decrease first service conception rates. The variation in the number of primordial follicles in heifers implanted at weaning among years suggests that additional research is necessary to confirm the impact of implanting heifers at 7 mo of age on the ovarian reserve. Implanting heifers at 2 mo of age did not have a detrimental effect on the ovarian reserve. Administration of a growth promoting implant at 2 mo of age can potentially be integrated into production systems by producers to allow for increased BW at weaning without hindering reproductive performance, ovarian development, and the ovarian reserve. Additional body weight at weaning gained from administration of a growth promoting implant at 2 mo of age is advantageous for producers making replacement heifer selection decisions at weaning, providing a potential profit advantage for heifers not retained as replacements.

Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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