# Follicular Development of Beef Heifers Exposed to Bulls During an Estrus Synchronization Protocol that Included a 14-Day CIDR, $PGF_{2\alpha}$, and Timed Artificial Insemination (AI) and GNRH

**J.G. Berardinelli*, L.E. Ramos, J.R.C. Wilkinson, J.R. Olsen, T. Gibbs, L. Runnion, and S. A. Tauck[1]**

Montana State University, Bozeman, MT, 59717

## IMPACT STATEMENT

*Our results indicate that the continuous presence of mature bulls during an estrus synchronization protocol that included a CIDR for 14 days does not appear to influence ovarian follicular dynamics or the expression of estrus after PG injection in beef heifers. Thus, this may not be the mechanism whereby the presence of bulls increases fertility in the bovine. However, the relationship between the dominate follicle (ovulatory follicle) diameter and body condition score supports the concept that "more fit" females ovulate larger follicles which in turn improves fertility and reproductive efficiency in beef heifers.*

## SUMMARY

The objective was to evaluate the effect of presence of a bull on ovarian follicular development and its relationship to fertility in beef heifers using an estrus synchronization (ES) protocol that included a progesterone-containing, controlled internal drug release devices (CIDR) for 14 days, $PGF_{2\alpha}$, and, fixed timed AI (TAI) and GnRH. We found that ovulatory follicle (DF) diameters, at the time of CIDR removal and $PGF_{2\alpha}$ injection (d 0), did not differ between heifers exposed to bulls (BE) and heifers not exposed to bulls (NE). DF diameter of NE and BE heifers were 10.3 ± 0.3 mm (mean ± SE) and 10.9 ± 0.3 mm, respectively. Additionally, there was no difference in number of antral follicles between BE- and NE-treated heifers (1.7 ± 0.1 and 1.5 ± 0.1 follicles, respectively). Diameter of DF did not affect the proportion of heifers that showed estrus or time after $PGF_{2\alpha}$ to exhibition of estrus for heifers in either treatment. Thus, this may not be the mechanism whereby the presence of bulls increases fertility in the bovine. Interestingly, diameters of DF increased ($P < 0.05$) linearly as body condition score (BCS) increased at a rate of 3.4 mm per unit increase in BCS. This relationship supports the concept that "more fit" females ovulate larger follicles which in turn might improve fertility in beef heifers.

## INTRODUCTION

The occurrence at puberty has a significant effect on reproductive efficiency of beef cattle herds when heifers are bred to calve as 2-yr-olds, particularly in production systems that use restricted breeding seasons (Ferrell, 1982).

Tauck et al., (2007) reported that pregnancy rates increased in primiparous, suckled cows exposed to bulls before and during an ES protocol that included a CIDR for 7 d, $PGF_{2\alpha}$, and, fixed TAI and GnRH. Based on this and other observations, Berardinelli et al. (2007) suggested that there is the possibility that a pheromone(s) excreted by bulls might be responsible for this enhancement by altering follicular dynamics of ovulatory or dominant follicles (DF).

Previous research indicated that heifers that ovulated follicles >10.7mm and <15.7 mm in diameter at time of AI were more likely to become pregnant than cohorts that ovulated a follicle with a more extreme diameter (Perry et al., 2007). These authors suggested that the use of protocols that control follicular development and increase the likelihood of ovulating optimal sized follicles (10.8 to 15.6 mm) may result in positive benefits on

pregnancy rates in heifers. The biostimulatory effect of the bull might provide this "positive benefit".

Based on these observations, we postulated that exposing yearling beef heifers to bulls during and ES protocol that included a CIDR for 14 d, $PGF_{2\alpha}$, and, TAI and GnRH would increase DF diameters at time of CIDR removal and $PGF_{2\alpha}$ injection, and alter the relationship between DF diameters and proportion of heifers showing estrus.

## PROCEDURES

*Animals and Treatments*

Heifers were housed at the Montana State University, Bozeman Area Research and Teaching Facility. Animal care, handling, and protocols used in this experiment were approved by the Montana State University Agricultural Animal Care and Use Committee.

Eight-two, spring-born, Angus X Hereford heifers and two mature epididymectomized Angus X Hereford bulls were used in this experiment. Heifers were maintained in a single pasture and had no contact with bulls or their excretory products from the previous breeding season until the start of the experiment (D -32). Before the start of the experiment, ovarian functional status of each heifer was rated by two ultrasound examinations of each ovary for the presence or absence of a corpus luteum (cycling status). The first and second ultrasonic examinations were conducted 10 and 2 d, respectively, before the start of the experiment. Means for age, BW, and BCS of heifers were 12.8 ± 0.6 months (± SD), 337.3 ± 26.0 kg, and 4.9 ± 0.2, respectfully, at the start of the treatment.

Two d before the start of the experiment heifers were stratified by age, BW, BCS, and cycling status. Once stratified, heifers were assigned randomly within strata to one of two treatments: exposed to continuously to bulls EB; n = 41) or not exposed to bulls (NE; n = 41) for 32 d from D -32 to D 0.

*Facilities and Bull Exposure*

Two lots were used for this experiment, designated north and south by their geographic location. Lots were adjacent to each other and separated by a wooden, fixed fence and an additional barb-wire fence that separated EB and NE heifers by approximately 15 m. Lots were very similar in east-west configuration, bunk space, aspect, and slope.

The heifer to bull ratio was 10.5:1 throughout the exposure period. Heifers in one pen were prevented from seeing or direct, close contact with bulls by tarpaulins draped over the wooden fence.

*Nutrition*

Heifers were given 28 lb/hd/d of good quality, chopped mixed-grass alfalfa hay, 2.43 lb/hd/d cracked barley, 1 lb/hd/d supplement that contained 38% protein and 200 mg of Rumensin, water, and a trace mineral-salt supplement throughout the experiment. Heifers were fed one half of the ration in the morning (0800-1000 h) and one half late in the afternoon (1600-1700 h).

*Estrus Synchronization Protocol*

Each heifer received a progesterone-containing CIDR 32 d before administration of PGF2α (D 0). Fourteen d later (D -18) CIDRs were removed from heifers and each heifer received PGF2α (25 mg/heifer; i.m.). Each heifer was fitted with an estrus detection aid (Estrotect®; Rockway, Inc., Spring Valley, WI) and observed visually for signs of behavioral estrus. Heifers that showed behavioral estrus and heifers whose estrus-detection aid turned color from silver to red within 60 h after PGF2α were inseminated artificially 12 h later (AI 12). Heifers that did not show estrus and heifers whose estrus-detection aid did not change color within 60 h received GnRH (100 ug/cow; i.m.) and were fixed-time AI (TAI) 72 h after PGF2α (D 3). Heifers that did not exhibit estrus by 60 h after $PGF_{2\alpha}$ were assigned an interval to estrus of 72 h.

*Ultrasonography and Follicular Dynamics*

Right and left ovaries of each heifer were imaged ultrasonically with at Titan Ultrasound Imaging System (SonoSite, Walla Walla, WA) immediately before PG injection at time of CIDR removal. An image of the ovary containing the largest follicle was capture for morphometric

measurement using Sigma Scan Pro software (SysStat Software, Inc.). An example of the image and measurements to obtain an average diameter are shown in Figure 1.

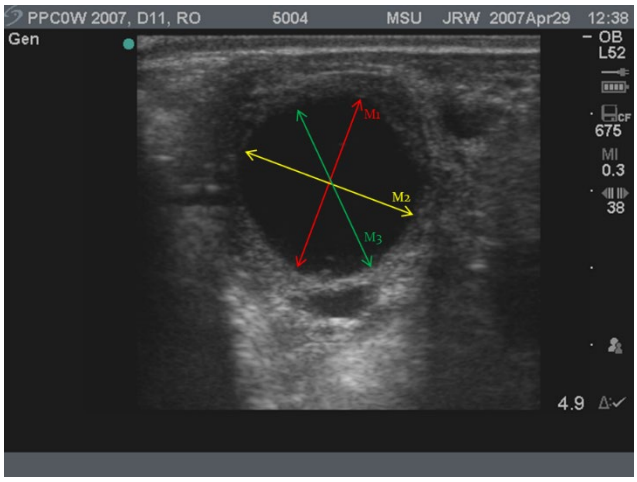


**Figure 1.** Method for determining measurements of dominant follicle (DF) diameter. Lengths (mm) of intersecting lines were averaged to yield the diameter of the largest follicle on either ovary of each heifer.

*Statistical Analyses*

Diameters of the largest follicle (DF) and number of follicles were analyzed by separate ANOVA for a completely randomized design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment. Means were separated by Bonferroni's procedure of SAS.

Proportions of heifers that exhibited proportions classified by DF diameters (small medium, or large) that showed estrus by 60 h after $PGF_{2\alpha}$ separate chi-square analyses using the PROC FREQ procedure of SAS.

Within treatment regressions of DF diameter and body condition score (BCS) were analyzed with PROC REGRESS of SAS.

## RESULTS AND DISCUSSION

Diameters of the DF at the time of CIDR removal and $PGF_{2\alpha}$ injection (d 0) did not differ between BE and NE heifers and averaged 10.3 ± 0.3 mm and 10.9 ± 0.3 mm, respectively (Table 1). Furthermore, there was no difference in number of antral follicles > 8.0 mm between BE- and NE-treated heifers (1.7 ± 0.1 and 1.5 ± 0.1, respectively; Table 1).

Table 1. Diameters of the dominant follicles (DF) and number of antral follicles >8.0 mm at the time of CIDR removal and $PGF_{2\alpha}$ injection in heifers exposed (BE) and not exposed (NE) to bulls during the ES protocol

| | Treatment | | |
|---|---|---|---|
| Item | BE | NE | *P*-value |
| n | 41 | 41 | |
| DF diam., mm | 10.3 ± 0.3 | 10.9 ± 0.3 | 0.70 |
| Number antral follicles >8.0mm | 1.7 ± 0.1 | 1.5 ± 0.1 | 0.50 |

Diameter of DF did not affect the proportion of heifers that showed estrus or time to estrus of heifers in either treatment (63% and 58% for BE and NE heifers, respectively) or intervals to estrus by 60 h after $PGF_{2\alpha}$ (49.5 ± 6 and 48.8 ± 9 h, respectively).

Interestingly, diameter of DF increased ($P < 0.05$) linearly as BSC increased in BE and NE heifers at an average rate of 3.4 ± 0.7 mm per unit increase in BCS.

In conclusion, the presence of mature bull during an estrus synchronization protocol that included a CIDR for 14 days does not appear to influence ovarian follicular dynamics or the expression of estrus after $PGF_{2\alpha}$ injection in beef heifer. Thus, this may not be the mechanism whereby the presence of bulls increases fertility in the bovine. However, the relationship between DF diameter and BCS supports the concept that "more fit" females ovulate larger follicles which in turn improve fertility.

## REFERENCES

Berardinelli, J. G., and S. A. Tauck. 2007. Conception rates to artificial insemination in primiparous, suckled cows exposed to the biostimulatory effect of bulls before and during a gonadotropin-releasing hormone-based estrus synchronization protocol. J. Anim Sci. 85:848-852.

Perry GA, Smith MF, Roberts AJ, MacNeil MD, Geary TW. 2007. Relationship between size of the ovulatory follicle and pregnancy success in

beef heifers. J. Anim. Sci. 85:684-689. https://doi.org/10.2527/jas.2006-519.

Tauck, S. A., and J. G. Berardinelli. 2007. Putative urinary pheromone of bulls involved with breeding performance of primiparous beef cows in a progestin-based estrous synchronization protocol. J. Anim. Sci. 85:1669-1674.

**ACKNOWLEDGEMENTS**

This work is/was supported by the USDA National Institute of Food and Agriculture, Hatch projects 185936 and 98058, and the Montana Agric. Exp. Sta.

*Corresponding author email: jgb@montana.edu