

The effect of follicle age on pregnancy rate in beef cows¹

F. M. Abreu,*† T. W. Geary,† L. H. Cruppe,* C. A. Madsen,†
E. M. Jinks,‡ K. G. Pohler,‡ J. L. M. Vasconcelos,§ and M. L. Day*²

*Department of Animal Sciences, The Ohio State University, Columbus 43210; †USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT 59301; ‡Division of Animal Sciences, University of Missouri, Columbia 65211; and § Departamento de Produção Animal, Faculdade de Medicina Veterinária e Zootecnia-UNESP, Botucatu, SP, Brazil

ABSTRACT: The effect of the age of the ovulatory follicle on fertility in beef cows was investigated. Multiparous ($n = 171$) and primiparous ($n = 129$) postpartum beef cows in 2 groups (G1 and G2) received estradiol benzoate (EB; 1 mg/500 kg BW, intramuscular [i.m.]) 5.5 d (G1; $n = 162$) and 6.5 d (G2; $n = 138$) after the final GnRH of a synchronization program (5d CO-Synch + CIDR) to induce emergence of a new follicular wave (NFW), followed by prostaglandin F_{2α} (PGF_{2α}; 25 mg, i.m.) administration either 5.5 d (“young” follicle, YF; $n = 155$) or 9.5 d (“mature” follicle, MF; $n = 145$) after EB. Estrous detection coupled with AI 12 h later (estrus-AI) was performed for 60 h (MF) and 84 h (YF) after PGF_{2α}; cows not detected in estrus within this period received timed AI (TAI) coupled with GnRH at 72 and 96 h, respectively. Within the first 72 h after PGF_{2α}, more ($P < 0.01$) cows in the MF (76.3%) than YF treatment (47.7%) exhibited estrus, but through 96 h, the proportion detected in estrus ($P < 0.05$) and interval from PGF_{2α} to estrus ($P < 0.01$)

were greater in the YF than MF treatment (88.6% vs. 76.3%, 78.9 ± 0.8 vs. 57.5 ± 1.6 h, respectively). Age of the ovulatory follicle at AI was greater ($P < 0.01$) in the MF (9.32 ± 0.04 d) than YF (6.26 ± 0.02 d) treatment, but follicle diameter at AI and pregnancy rates did not differ between MF (13.1 ± 0.2 mm; 72.0%) and YF (12.9 ± 0.1 mm; 67.1%) treatments. Regardless of treatment, the diameter of the ovulatory follicle at AI and pregnancy rate were greater ($P < 0.01$) with estrus-AI (13.1 ± 0.1 mm; 75.0%) than TAI (12.6 ± 0.2 mm; 55.4%). Cows in the MF treatment that initiated a second NFW after EB but before PGF_{2α} (MF2; $n = 47$) were induced to ovulate with GnRH and TAI at 72h, when ovulatory follicles were 4 d old and 10.2 ± 0.2 mm in diameter. Pregnancy rate for TAI (51.1%) in MF2 did not differ from TAI pregnancy rate (55.4%) across the MF and YF treatments. In summary, the age of the ovulatory follicle affected interval to estrus and AI but did not influence pregnancy rate in suckled beef cows.

Key Words: cows, follicle age, pregnancy rate

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INTRODUCTION

In cattle, if progestins are used to extend the duration of follicle dominance beyond that which would oc-

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²Corresponding author: day.5@osu.edu

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cur spontaneously, fertility is reduced (Mihm et al., 1994; Ahmad et al., 1995) because of decreased oocyte quality (Revah and Butler, 1996) and embryo development (Ahmad et al., 1995). In spontaneously ovulating cows that have either 2 or 3 waves of follicular growth during an estrous cycle (Savio et al., 1988; Sirois and Fortune, 1988), the interval from follicle emergence to estrus (age of the follicle) is greater by approximately 3 d in cows with 2 follicular waves (Bleach et al., 2004), and pregnancy rate to AI is lower when compared to cows with 3 follicular waves during the estrous cycle (Townson et al., 2002). Synchronization programs that induce ovulation of follicles with GnRH at a shorter interval after emergence of a new follicular wave (NFW) have resulted in increased timed AI (TAI) pregnancy rates in postpartum

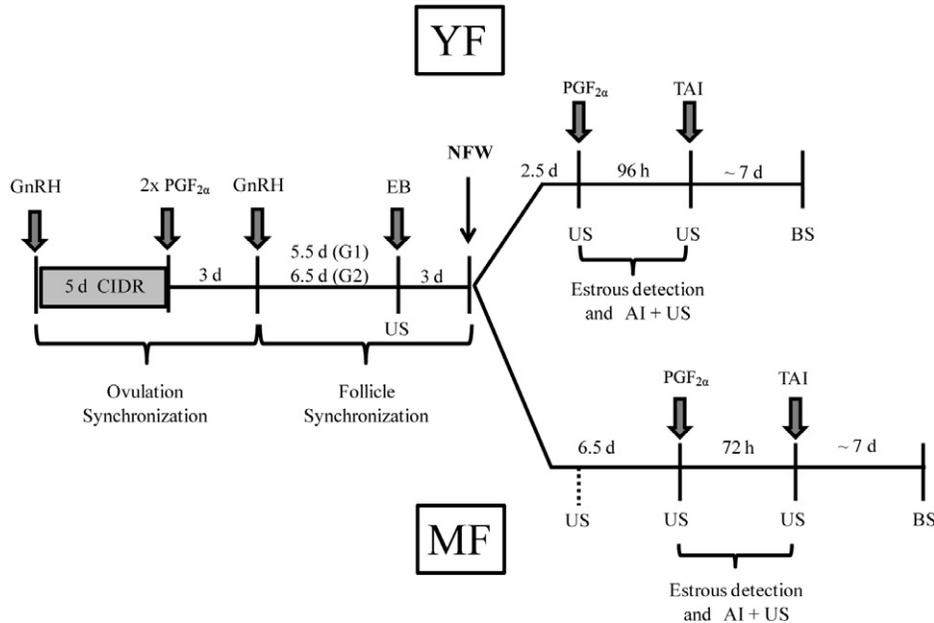


Figure 1. Diagram of experimental design of both treatments (MF: mature follicle; YF: young follicle). In group 1 (G1), the interval from GnRH to estradiol benzoate (EB) injection was 5.5 d, and in group 2 (G2), this interval was 6.5 d. For clarity, the diagram is lined up on the basis of the day of EB administration; however, treatments were offset so that timed AI (TAI) was performed on the same date for cows in both treatments. BS and US indicate the days that blood samples were collected and ultrasonography was performed in both treatments, respectively. In G1, BS was also collected 7 d after TAI in both treatments. Ultrasonography was also performed on d 34 and 88 (G1) and d 74 (G2) after AI for pregnancy diagnosis. New follicular wave is designated as NFW. $\text{PGF}_{2\alpha}$ = prostaglandin $\text{F}_{2\alpha}$.

beef (Bridges et al., 2008) and lactating dairy (Santos et al., 2010) cattle. Moreover, Cerri et al. (2009) demonstrated a greater proportion of good-quality embryos collected from lactating dairy cows that were induced to ovulate younger follicles when duration of dominance of the ovulatory follicle was manipulated, within the range normally observed in spontaneously ovulating females. The cumulative interpretation of reports in lactating dairy cows suggests that age of the follicle is a significant source of variation in fertility, but the direct effect of follicle age on pregnancy rate to AI in cattle has not been evaluated, and there is a paucity of data in this regard in beef cattle. The objective of the present study was to investigate the effect of the age of the ovulatory follicle on fertility in postpartum beef cows when AI was performed either on the basis of estrus or when ovulation was induced with GnRH. It was hypothesized that cows that ovulated younger follicles would be more likely to become pregnant to AI than cows that ovulated more mature follicles.

MATERIALS AND METHODS

Animals and Treatments

All procedures involving animals used in this research were approved by the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee. Crossbred (Angus \times Hereford) postpartum beef cows (multiparous, $n = 171$; primiparous, $n = 130$) at USDA-ARS's Fort Keogh Livestock and Range Research Laboratory

(Miles City, MT) were assigned by calving date and parity into 2 experimental groups (**G1**, $n = 162$, 493 ± 5.8 kg; **G2**, $n = 138$, 526 ± 5.3 kg). Ovulation was presynchronized in all cows before application of experimental treatments with the 5 d CO-Synch + CIDR program (Fig. 1; CIDR [Pfizer Animal Health, New York, NY] + GnRH [Fertagyl, 100 μg , intramuscular (i.m.), Intervet Inc., Millsboro, DE] followed 5 d later by CIDR removal and 2 injections of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) 12 h apart [Lutalyse, 25 mg each, i.m., Pfizer Animal Health] and GnRH [100 μg , i.m.] 3 d after $\text{PGF}_{2\alpha}$). Cows were assigned to receive estradiol benzoate (EB; 1 mg/500 kg BW, i.m. [β -estradiol 3-benzoate (Sigma-Aldrich Co. LLC, Milwaukee, WI) in 10% benzyl alcohol (Sigma-Aldrich) and 90% sesame oil]) either 5.5 d (G1) or 6.5 d (G2) after the final GnRH injection of the presynchronization program to induce atresia of the first-wave dominant follicle and result in emergence of a NFW approximately 3 d later (Burke et al., 2001). The day of the estrous cycle at EB was varied by 24 h between groups to accommodate scheduling. Within group, $\text{PGF}_{2\alpha}$ (25 mg, i.m.) was administered either 5.5 d ("young" follicle, **YF**; $n = 155$) or 9.5 d ("mature" follicle, **MF**; $n = 145$) after EB (Fig. 1) to induce regression of the corpus luteum (CL). Estrous detection was performed twice daily either for 72 h (MF) or 96 h (YF) after $\text{PGF}_{2\alpha}$. Based on estrous distribution of a preliminary study conducted with postpartum beef cows ($n = 32$), it was determined in the current study that after $\text{PGF}_{2\alpha}$, cows detected in estrus by 60 h (MF) or 84 h (YF) would be AI approximately 12 h later (**estrus-AI**), whereas cows not detected in estrus by this

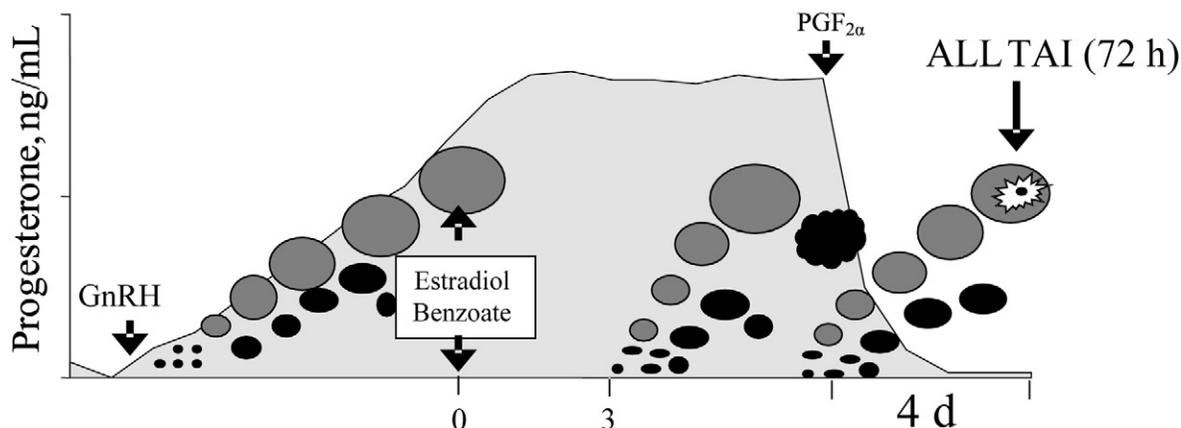


Figure 2. Diagram of cows identified from the mature follicle (MF) treatment that initiated a second new follicular wave after estradiol benzoate but 1 d before prostaglandin F_{2α} (PGF_{2α}) administration (MF2); TAI = timed AI

treatment-specific time received timed AI (TAI) coupled with GnRH at either 72 h (MF) or 96 h (YF) after PGF_{2α} (Fig. 1). Cows first detected in estrus at 72 h in the MF or 96 h in the YF treatment, although considered for the estrus detection data, were TAI (with GnRH) and were considered as being TAI in all analyses. To perform TAI in both treatments on the same date, initiation of the presynchronization program was offset by 3 d between treatments. Estrous detection was performed at least twice daily through visual observation with the aid of Estroject (Rockway Inc., Spring Valley, WI) estrous detection patches applied at the time of PGF_{2α} injection. Approximately 10 d after AI, cows were exposed to bulls for the remainder of a 55-d (G1) or 43-d (G2) breeding season.

Ultrasonography

Transrectal ultrasonography (Fig. 1) was performed using a 7.5-MHz linear array transducer (Aloka 500V; Aloka, Wallingford, CT) to characterize ovarian structures in all cows at the time of EB, PGF_{2α}, and AI/TAI (Fig. 1). In addition, ultrasonography was also performed 5.5 d after EB in the MF treatment, which corresponded to the same day after EB that YF cows received PGF_{2α}. Follicle size was measured by averaging follicular diameter at the widest point and perpendicular to the first measurement. Follicles and CL were recorded on ovarian maps during each examination. Growth rate of the ovulatory follicle was determined from PGF_{2α} to AI for all cows and from 5.5 d after EB to PGF_{2α} for cows in the MF treatment. Pregnancy diagnoses were determined using a 5.0-MHz transrectal linear array transducer (Aloka 500V) approximately 34 d and 88 d (G1) or 74 d (G2) after TAI.

Blood Collection and Radioimmunoassay

Blood samples were collected (Fig. 1) via tail venipuncture into 10-mL Vacutainer tubes (Fisher Scientific, Pittsburgh, PA) 5.5 d after EB (before PGF_{2α} in the YF treatment) and in G1 cows approximately 7 d after AI to compare progesterone concentration after ovulation of different-aged follicles. After collection, blood was incubated for 24 h at 4°C followed by centrifugation at 1200 × g for 25 min at 4°C. Serum was collected and stored at -20°C until RIA. Serum concentrations of progesterone were measured using a Coat-a-Count RIA kit (Diagnostic Products Corporation, Los Angeles, CA; Bellow et al., 1991). Intra-assay and interassay CV were 1.4% and 2.5%, respectively. The average sensitivity of the assay was 0.08 ng/mL.

Data and Statistical Analyses

Cows that failed to initiate a NFW after EB (G1, *n* = 6; G2, *n* = 5) were excluded from statistical analyses. Also, in the MF treatment, cows in which the new EB-induced follicular wave became atretic and a second-wave dominant follicle emerged before PGF_{2α} (G1, *n* = 25; G2, *n* = 22) were excluded from the primary analysis and were analyzed separately. This response created a unique (and unplanned) group of cows (MF2) from the original MF treatment that were estimated to have emergence of a NFW 1 d before PGF_{2α} administration (Fig. 2). Therefore, the MF2 cows ovulated a follicle that was very young (~4 d after emergence) at the time of GnRH given at 72 h after PGF_{2α}.

Estrous response within the first 72 h after PGF_{2α}, overall estrous response, and pregnancy rate were analyzed with a model that included group, treatment (MF, *n* = 93; YF, *n* = 149), cow age, and their interaction using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC; version 9.2). Interval from PGF_{2α} to estrus within the first 72 h, interval from PGF_{2α} to estrus, follicle size

and age at AI, progesterone concentrations, and follicular growth rate (between PGF_{2α} and AI) were analyzed with a model that included group, treatment, cow age, and their interaction using the MIXED procedure of SAS. The correlation between follicular growth rate and pregnancy rate was analyzed with the CORR procedure of SAS.

In a secondary analysis, pregnancy rate and progesterone concentrations approximately 7 d after AI were investigated between cows in the MF2 and TAI (MF and YF combined) groups. In addition, estrus response at TAI (i.e., at 72 h for MF and MF2 and at 96 h for YF treatment) among MF, YF, and MF2 was also analyzed as previously described. The means for follicle age, follicle diameter at TAI, and follicular growth rate were analyzed using the MEANS procedure of SAS. To calculate follicle age in the MF2 group, emergence of the second NWF after EB was estimated to have occurred 1 d before PGF_{2α} administration. Data are expressed as the mean ± SEM.

RESULTS

All animals included in the analyses had a functional CL and progesterone concentrations greater than 1.0 ng/mL 5.5 d after EB. A greater ($P < 0.01$) percentage of cows in the MF treatment exhibited estrus within the first 72 h after PGF_{2α} than YF cows (Table 1). However, throughout the estrous detection period (between PGF_{2α} and TAI; Fig. 3), the proportion of cows detected in estrus was greater ($P < 0.01$) in the YF than in the MF cows (Table 1). The interval from PGF_{2α} to estrus was 22.3 h greater ($P < 0.01$) among YF than MF cows, and a group by treatment interaction ($P < 0.05$) was detected. Cows in the MF treatment in G2 were detected in estrus more rapidly ($P < 0.05$; 52.7 ± 3.2 h) than cows in the MF treatment in G1 (60.6 ± 1.4 h); the interval from PGF_{2α} to estrus was similar between groups in the YF treatment (79.1 ± 1.0 and 78.6 ± 1.4 h, respec-

Table 1. Effect of treatments on response variables (mean ± SE) in beef cows

Item ¹	Mature follicle	Young follicle	P-value
Estrous response within 72 h, %	76.3	47.7	<0.01
Estrous response from PGF _{2α} to TAI (72 vs. 96 h), %	76.3	88.6	<0.01
Interval from PGF _{2α} to estrus, h	57.5 ± 1.6	78.9 ± 0.8	<0.01
Follicle age at AI, d	9.32 ± 0.04	6.26 ± 0.02	<0.01
Follicle diameter at AI, mm	13.1 ± 0.2	12.9 ± 0.1	>0.10
Follicle growth rate (PGF _{2α} to AI), mm/d	0.95 ± 0.07	1.14 ± 0.04	<0.05
Follicle growth rate 5.5 d after EB to PGF _{2α} , mm/d	0.77 ± 0.06	n/a	—
Pregnancy rate, %	72.0	67.1	>0.10
Progesterone concentration (~7 d) after AI, ng/mL (G1 only)	3.56 ± 0.21	3.85 ± 0.13	>0.10

¹TAI = timed AI; EB = estradiol benzoate; G1 = group 1; PGF_{2α} = prostaglandin F_{2α}.

tively). The experimental design created 3.04 d difference ($P < 0.01$) in age of the ovulatory follicle between MF and YF treatments (Table 1). The diameter of the dominant follicle at 5.5 d after EB was 8.26 ± 0.12 for the YF treatment (coinciding with the day of PGF_{2α} administration) and 7.96 ± 0.13 for the MF treatment (4 d preceding PGF_{2α} treatment; $P < 0.05$). Dominant follicles grew at a relatively slow rate from 5.5 d after EB until PGF_{2α} administration in the MF treatment, and the rate of growth from PGF_{2α} administration to AI was greater ($P < 0.05$) in the YF than in the MF treatment (Table 1). The diameter of the ovulatory follicle at AI did not differ ($P > 0.10$) between treatments. Pregnancy rates and serum progesterone concentrations at 7.40 ± 0.05 d (MF) and 7.7 ± 0.04 d (YF) after AI were similar ($P > 0.10$) between MF and YF treatments (Table 1). Regardless of treatment, the diameter of the ovulatory follicle at AI and pregnancy rate were greater ($P < 0.01$) with estrus-AI (13.1 ± 0.1 mm; 75.0%) than TAI (12.6 ± 0.2 mm; 55.4%), however, the diameter of the

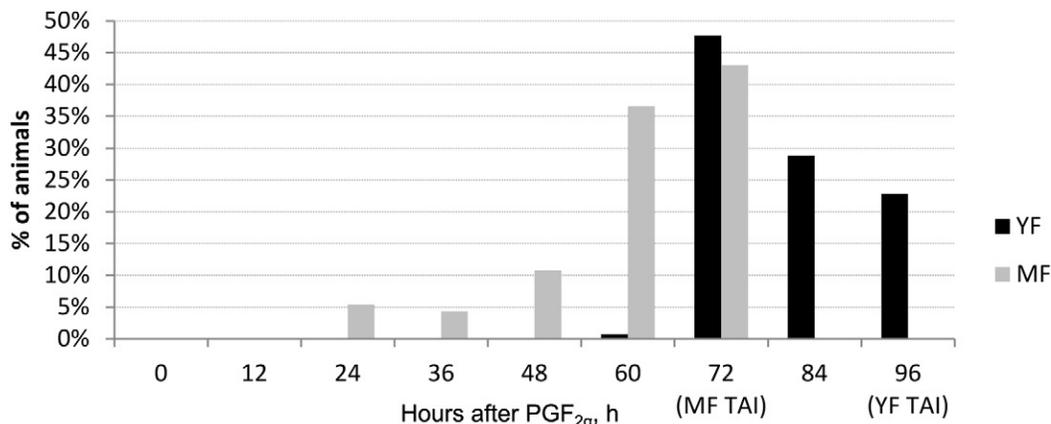


Figure 3. Distribution of estrus after prostaglandin F_{2α} (PGF_{2α}) injection (0 h) across treatments. Animals detected in estrus within 60 h (mature follicle, MF) or 84 h (young follicle, YF) were AI approximately 12 h after detection of estrus. Cows that were not detected in estrus in this interval were timed AI (TAI) at 72 h (MF) or 96 h (YF).

ovulatory follicle was not related ($P > 0.10$) to pregnancy rate within type of AI. Across treatments, follicular growth rate between PGF_{2 α} and AI was positively correlated ($r = 0.16$; $P < 0.05$) with pregnancy rate.

Follicles in the MF2 group ($n = 47/141$) were estimated to have emerged 1 d before PGF_{2 α} administration. All MF2 cows received TAI at 72 h after PGF_{2 α} and although only 15% (7/47) of these cows were detected in estrus at TAI, this proportion did not differ ($P > 0.05$) from cows detected in estrus at TAI in the MF (19%; 18/93) and YF (11%; 17/149) treatments. The dominant follicle growth rate between PGF_{2 α} and AI in the MF2 treatment was 0.97 ± 0.10 mm/d. At GnRH-induced ovulation MF2 follicles were estimated to be approximately 4 d of age and were 10.2 ± 0.2 mm in diameter. The pregnancy rate in the MF2 group was 51.1% and did not differ ($P > 0.10$) from the pregnancy rate to TAI (55.4%) across the MF and YF treatments. Serum progesterone concentration 7 d after AI was lower ($P < 0.01$) in MF2 (1.8 ± 0.2 ng/mL) compared with the TAI group (3.4 ± 0.3 ng/mL) across the MF and YF treatments.

DISCUSSION

In the present experiment, administration of PGF_{2 α} earlier following the emergence of the dominant follicle resulted in spontaneous or induced ovulation of younger follicles. Follicle turnover and emergence of a NFW was successfully induced approximately 3 d after EB administration, as previously described (Burke et al., 2001). In cows ovulating younger follicles, proestrus interval and follicle growth rate were greater. However, no differences between treatments were detected in follicle diameter at AI (estrus-AI and TAI), progesterone concentrations after AI (G1 only; estrus-AI and TAI), or pregnancy rate. Regardless of treatment, pregnancy rates were greater in cows that received AI after estrous detection than TAI.

An unexpected and interesting group of cows (MF2; $n = 47/141$) in the MF treatment was detected through ultrasonography. In this MF2 group, the dominant follicle from the EB-induced NFW underwent atresia, and a second wave of follicle development emerged before PGF_{2 α} administration. Cows classified as MF2 experienced a shorter follicular wave than anticipated following EB treatment. However, this follicle emerged and regressed in the presence of a fully functional CL (approximately d 9 to 16 of the estrous cycle), much like a second-wave follicle in female cattle that experience 3 waves during their estrous cycle. Savio et al. (1990) reported that in postpartum dairy cows, the interval between detection of the second-wave follicle (d 14 of the estrous cycle) and detection of the third-wave dominant follicle (d 19 of the estrous cycle) was 5 d in cows with 3 follicular waves. Since there were 6.5 d between expected emergence of

the EB-induced follicle wave and PGF_{2 α} , the endocrine environment established with the animal model used in this study may have contributed to the prevalence of MF2 cows. An additional factor that could contribute to the observation of the emergence of a second dominant follicle after EB was that some cows may have responded to EB with NFW emergence more quickly than other cows, resulting in cows classified as MF2. Regardless, the MF2 follicles were estimated to emerge 1 d before PGF_{2 α} and this response was detected in 33% of the cows in this treatment, suggesting that the animal model used nearly maximized follicle lifespan in MF cows.

In the present study proestrus interval was limited to 72 and 96 h in MF and YF treatments, respectively, with a GnRH injection coupled with TAI used in cows not in estrus by 60 and 84 h, respectively. This 24 h difference in proestrus was afforded on the basis of data from a preliminary study conducted with 32 beef cows receiving the same treatments as the present experiment that were allowed to exhibit estrus and spontaneously ovulate. With these preliminary data, along with the data from a similar heifer study (Abreu et al., 2013), we were able to predict the most appropriate time to perform TAI. Moreover, Burke et al. (2001) demonstrated that females that received PGF_{2 α} 1 d vs. 4 d after follicle emergence would require a greater proestrus interval. The interval from PGF_{2 α} to estrus (proestrus) was 21.4 h longer in the YF than in the MF treatment; however, pregnancy rates did not differ between treatments. This finding is inconsistent with recent reports that a longer proestrus would result in a greater pregnancy rate to AI after spontaneous ovulation (Geary et al., 2010) or with TAI in all cows (Bridges et al., 2010).

Another aspect supporting the hypothesis that younger follicles would result in greater fertility was based on the findings that maximum peripheral estradiol concentrations are achieved approximately 3 d after follicle emergence (Rhodes et al., 1995). On the basis of this report, the negative effects of progesterone on LH (Beck et al., 1976) were removed at the time of peak estradiol concentrations during follicular growth in the YF treatment, potentially prolonging gonadotropic stimulation of a highly estrogenic ovulatory follicle. Indeed, a greater percentage of cows in the YF treatment was detected in estrus than in the MF treatment during the 96 and 72 h of proestrus afforded, respectively, suggesting that more cows in the YF treatment attained the threshold concentrations of estradiol to induce estrous behavior. The importance of a greater estradiol concentration before ovulation for pregnancy success in synchronization programs followed by TAI has been clearly demonstrated in beef (Perry et al., 2005; Bridges et al., 2008) and dairy (Lopes et al., 2007) cattle.

The hypothesis that the age of the ovulatory follicle could be a source of variation in fertility was based primarily on findings from a variety of studies in which the

ovulatory follicle age was either directly or indirectly investigated in beef and dairy cattle. In dairy cows, Cerri et al. (2009) identified embryos of greater quality 6 d after AI in cows induced to ovulate follicles approximately 1.5 d earlier during follicular dominance. Additionally, it has been demonstrated that lactating dairy cows with 3 follicular waves spontaneously ovulate younger follicles and have a greater conception rate than cows experiencing 2 follicular waves during the estrous cycle (Townson et al., 2002). In beef cattle, the age of the ovulatory follicle was investigated indirectly by Bridges et al. (2008). In that study, induced ovulation of follicles that were estimated to be approximately 1.5 d younger in cows resulted in greater pregnancy rates when cows with younger follicles were afforded an extended proestrus. A similar finding was reported by Santos et al. (2010), in which lactating dairy cows induced to ovulate younger follicles had a 7% increase in TAI pregnancy rate compared with cows induced to ovulate follicles approximately 2 d older. The present study and the companion paper on heifers (Abreu et al., 2013) are the first to directly test the impact of follicle age on fertility to AI. On the basis of the findings of the present study and the accompanying report (Abreu et al., 2013), we reject the hypothesis that pregnancy rate to AI is influenced by age of the ovulatory follicle in cattle. On the basis of the numerous experiments cited above that suggest follicle age is an important source of variation in the fertility of lactating dairy cattle, the potential exists that the impact of follicle age on fertility is inconsistent between lactating dairy cows and postpartum beef cows and heifers. If this inconsistency between cattle type exists, one possibility is that the heightened liver metabolism of steroids in lactating cows (Wiltbank et al., 2006) and the impact of this on follicular growth (Sartori et al., 2004; Wiltbank et al., 2006; Cerri et al., 2011a,b) contribute to the differential responses between beef cattle and dairy cows.

Ovulatory follicle diameter has also been investigated as a factor influencing pregnancy rates in beef (Lamb et al., 2001; Perry et al., 2005, 2007; Mussard et al., 2007) and dairy cattle (Vasconcelos et al., 2001), particularly in TAI programs. In the present study, although the age of the ovulatory follicle differed between treatments, follicle diameter at AI did not differ. Perry et al. (2005) concluded that cows that were induced with GnRH to ovulate small follicles (≤ 11 mm in diameter) had a decreased pregnancy rate; however, follicle diameter did not affect fertility after spontaneous ovulation. Similar findings were reported in dairy cows induced to ovulate smaller follicles (Vasconcelos et al., 1999). In the present study, differences in the age of the ovulatory follicle did not affect the follicle diameter at AI or pregnancy rates between treatments. The limited number of MF2 cows provides for an interesting comparison in this regard; the MF2 cows were induced to ovulate very young

follicles that were apparently younger and smaller and had decreased circulating concentrations of progesterone 7 d after AI, yet they had similar pregnancy rates to cows that were TAI in the MF and YF treatments.

In conclusion, manipulation of the age of the ovulatory follicle in postpartum beef cows resulted in a longer interval to estrus and to AI in cows ovulating younger follicles but did not influence pregnancy rate or follicle size at AI. These data suggest that age of the ovulatory follicle is not a major contributor to variation in fertility among beef cows.

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