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Effect of follicle age on conception rate in beef heifers[1]

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ABSTRACT: The objective of this study was to determine the effect of age of the ovulatory follicle on fertility in beef heifers. Ovulation was synchronized with the 5 d CO-Synch + controlled intravaginal drug release (CIDR) program in heifers in Montana (MT; $n = 162$, Hereford and Angus Crossbred) and Ohio (OH; $n = 170$, Angus Crossbred). All heifers received estradiol benzoate (EB; 1 mg/500 kg BW, [i.m.]) 6 d after the final GnRH of the synchronization program to induce follicular atresia and emergence of a new follicular wave (NFW) followed by prostaglandin F2α ($PGF_{2\alpha}$; 25 mg, i.m.) administration either 5 d ("young" follicle [YF]; $n = 158$) or 9 d ("mature" follicle [MF]; $n = 174$) after EB. Estrous detection was performed for 5 d after $PGF_{2\alpha}$ with AI approximately 12 h after onset of estrus. Ovarian ultrasonography (MT location only) was performed in YF and MF at EB, 5 d after EB, $PGF_{2\alpha}$, and AI. Heifers in MT ($n = 20$) and OH ($n = 18$) that were not presynchronized or did not initiate a NFW were excluded from further analyses, resulting in 142 and 152 heifers in MT and OH, respectively. Heifers from the MF treatment in MT that initiated a second NFW after EB but before $PGF_{2\alpha}$ (MF2; $n = 14$) were excluded from the primary analysis. In the secondary analysis, the MF2 group was compared to MF and YF treatments in MT. Estrous response was similar (90%; 252/280) between treatments and locations. Proestrus interval (from $PGF_{2\alpha}$ to estrus) and age of the ovulatory follicle at AI were similar for MF heifers between locations (54.6 ± 1.7 h and 8.3 ± 0.07 h) but were greater ($P < 0.01$) for YF heifers in OH (78.5 ± 1.4 h and 5.3 ± 0.06 h) than MT (67.4 ± 1.6 h and 4.8 ± 0.06 h; treatment × location, $P < 0.01$). However, conception rate did not differ for MF (63.8%; 74/116) and YF (67.0%; 91/136) treatments. In the MT heifers, follicle size and follicle age at AI in the YF treatment (10.4 ± 0.15 mm and 4.8 ± 0.06 d, respectively) was less ($P < 0.01$) than in the MF treatment (11.0 ± 0.18 mm and 8.3 ± 0.11 d, respectively), but conception rate to AI did not differ between treatments in MT. In the MF2 group proestrus interval was greater ($P < 0.01$); hence, diameter of the ovulatory follicle and age were similar to that for the YF treatment. Conception rate to AI did not differ between MF2, MF, and YF (61.5, 63.3, and 64.7%, respectively) in MT. In conclusion, manipulation of age of the nonpersistent ovulatory follicle at spontaneous ovulation did not influence conception rate.

Key words: conception rate, follicle age, heifers

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INTRODUCTION

It has been demonstrated that pregnancy rates were affected by follicular diameter following GnRH-induced ovulation in estrous synchronization programs (Vasconcelos et al., 1999; Lamb et al., 2001; Perry et al., 2005, 2007; Meneghetti et al., 2009; Sá Filho et al., 2010). Additionally, it has been demonstrated that proestrus length during follicular development affected fertility following AI (Bridges et al., 2008, 2010; Taponen et al., 1999; Peters and Pursley, 2003).

[1]Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA or the authors and does not imply its approval to the exclusion of other products that may also be suitable. USDA-ARS, is an equal opportunity/affirmative action employer. All agency services are available without discrimination. A portion of salaries and support provided by State and Federal funds appropriated to the Ohio Agricultural Resource Development Center (OARDC; Manuscript No. 017/13AS). Appreciation is extended to Gregg Fogle, Mike Woods, Doug Armstrong, Alan Mason, and Tyler Johnson for their assistance with this project and Pfizer Animal Health for their product support.

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In lactating dairy cows that experienced 3 follicular waves during the estrous cycle, it was demonstrated that age of the ovulatory follicle was approximately 3 d less than in cows with 2 follicular waves (Bleach et al., 2004). Moreover, cows with 3 follicular waves during the estrous cycle preceding AI had a greater pregnancy rate than cows with 2 follicular waves (Townson et al., 2002). In both postpartum beef (Bridges et al., 2008) and lactating dairy (Santos et al., 2010) cattle, GnRH induced ovulation earlier after emergence of a new follicular wave (**NFW**) increased timed-AI (**TAI**) pregnancy rate but was confounded by proestrus interval. Furthermore, when duration of dominance of the ovulatory follicle was manipulated, induced ovulation of younger follicles increased the proportion of excellent and good quality embryos (Cerri et al., 2009). However, the effect of follicle age on fertility has not been directly evaluated in cattle. The objective of the present study was to investigate the effect of age of the ovulatory follicle on fertility in beef heifers. It was hypothesized that spontaneous ovulation of younger follicles would result in greater fertility (conception rate to AI) in beef heifers.

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 or The Ohio State University Agricultural Animal Care and Use Committee. Heifers in Montana (**MT**) were Hereford ($n = 46$) and Angus Crossbred ($n = 116$), and heifers in Ohio (**OH**) were Angus Crossbred ($n = 170$). Heifers were randomly assigned to treatment by breed (MT only) and weight (both locations). Ovulation was presynchronized in all heifers with the 5 d CO-Synch + controlled intravaginal drug release (**CIDR**) program (Fig. 1), in which an intravaginal progesterone insert (CIDR; Pfizer Animal Health, New York, NY) and GnRH injection (100 μg, i.m.; Cystorelin [Merial, Inselin, NJ] used in OH and Factrel [Pfizer Animal Health] used in MT) were administered at the same time followed 5 d later with CIDR removal and prostaglandin F2α ($PGF_{2\alpha}$; Lutalyse, 25 mg; Pfizer Animal Health) administration, and 3 d after $PGF_{2\alpha}$ with GnRH (100 μg, i.m.; as described above per location). Heifers received estradiol benzoate (**EB**; 1 mg/500 kg BW, i.m.) 6 d after the final GnRH injection of the presynchronization program to reset follicular growth and initiate a NFW approximately 3 d later (Burke et al., 2001). To induce regression of the corpus luteum (**CL**), $PGF_{2\alpha}$ (25 mg, i.m.) was administered either 5 d ("young" follicle [**YF**]; $n = 158$] or 9 d ("mature" follicle [**MF**]; $n = 174$) after EB (Fig. 1). Following $PGF_{2\alpha}$, estrous detection was performed at least twice daily for 5 d with AI approximately 12 h after estrus. To perform AI in both treatments randomly during the same period in time, treatments were offset by 4 d at the beginning of the presynchronization program so that all heifers received the final $PGF_{2\alpha}$ on the same date.

Ultrasonography

Transrectal ultrasonography (**US**; Fig. 1) was performed (MT only) using a 7.5 MHz linear array transducer (Aloka 500V; Aloka, Wallingford, CT) to characterize ovarian structures in all heifers at the time of EB, $PGF_{2\alpha}$, and AI and 5 d after EB (MF only, which corresponded to the same day that heifers in the YF treatment received $PGF_{2\alpha}$). Follicle size was determined 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 $PGF_{2\alpha}$ to AI for all heifers and from d 11 to 15 for MF heifers. Pregnancy diagnoses were determined using a 5.0 MHz transrectal linear array transducer (Aloka 500V) approximately 30 to 40 d after AI in both locations.

Blood Collection and Radioimmunoassay

Montana. Blood samples were collected (Fig. 1) via tail venipuncture into 10 mL vacutainer tubes (Fisher Scientific, Pittsburgh, PA) on d 11 in all heifers (day of $PGF_{2\alpha}$ for YF heifers) and also on d 15 in the MF treatment only (day of $PGF_{2\alpha}$ for MF heifers). After collection, blood was incubated for 24 h at 4°C followed by

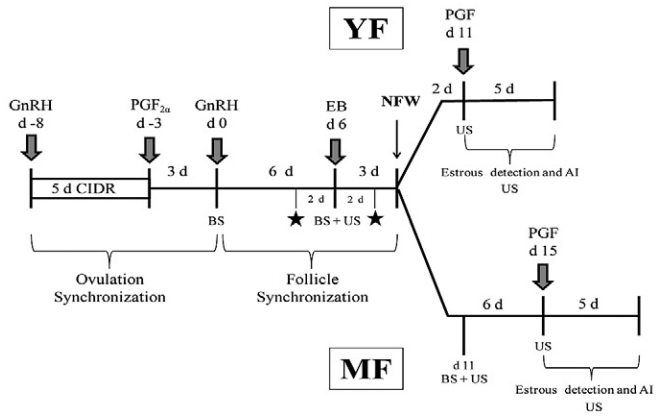

Figure 1. Diagram of treatments (young follicle [YF] and mature follicle [MF]), blood samples (BS), and ultrasonography (US; Montana only). Day 0 and 6 blood samples were taken from heifers in Ohio only, and BS on d 11 and d 15 (MF only) of the experiment were taken from heifers in Montana only. For clarity, the diagram is standardized to the second GnRH injection (d 0) of the presynchronization treatment; however, the initiation of treatments was offset by 4 d so that prostaglandin F2α ($PGF_{2\alpha}$) was administered on the same day for heifers in both treatments. New follicular wave (NFW) formation was created in all heifers by administration of estradiol benzoate (EB) on d 6 of the estrous cycle. ★ indicate the additional US performed in 28 MF and 27 YF heifers in Montana. CIDR = controlled intravaginal drug release.

centrifugation at 1,200 × *g* for 25 min. Serum was collected and stored at –20°C until serum concentrations of progesterone were analyzed using a Coat-a-Count RIA kit (Siemens, Los Angeles, CA) as described previously (Bellows et al., 1991). Average intra-assay CV was 3.0%, and interassay CVs (2 assays) for pooled serum samples at different stages of the estrous cycle (anestrus, d 4, d 5, and d 14 of the luteal phase, and estrus) were 6.6, 3.3, 2.9, 2.8, and 3.0%, respectively. The average sensitivity of the assay was 0.13 ng/mL.

Ohio. Blood samples were collected (Fig. 1) via jugular venipuncture into 10 mL EDTA Vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ) on the day of the final GnRH of the 5 d CO-Synch program and at EB administration. Samples were stored on ice immediately after collection and then centrifuged at 1,500 × *g* for 20 min. Plasma was decanted and stored at –20°C until plasma concentrations of progesterone were determined using a Coat-a-Count RIA kit (Siemens) as described previously (Burke et al., 2001). Average intra-assay CV was 3.1%, interassay CVs (5 assays) for pooled plasma samples containing 0.14, 1.16, and 3.31 ng/mL of progesterone were 2.4, 4.1, and 3.0%, respectively, and the average sensitivity of the assays was 0.07 ng/mL.

Data and Statistical Analyses

Animals in OH were excluded from analyses if progesterone concentrations were either greater than 1 ng/mL at the final GnRH of the presynchronization program ($n = 9$) or lower than 1 ng/mL at EB ($n = 9$). In MT, animals were excluded if a CL was not visible via ultrasound at EB ($n = 2$) or if progesterone concentrations were less than 1 ng/mL on d 5 after EB ($n = 7$). Furthermore, heifers that did not initiate a NFW that was detectable by US 5 d after EB in MT ($n = 11$) were also excluded from further analyses. In MT, 14 heifers in the MF treatment were determined by US to have the dominant follicle from the new, EB-induced follicular wave undergo atresia and a second wave emerge before $PGF_{2\alpha}$ (**MF2**). Only heifers that were conclusively identified by ultrasound as undergoing this second follicular wave were included in this group. This group of animals was excluded from the primary analysis. Exclusion of animals that did not respond to the animal model resulted in a total of 150 heifers in the YF treatment (OH, $n = 75$; MT, $n = 75$), 130 heifers in the MF treatment (OH, $n = 77$; MT, $n = 53$), and 14 heifers designated as MF2 in MT.

Data were analyzed using a model that included treatment, location, and the treatment × location interaction. Estrous response (proportion of heifers detected in estrus during the 5 d period after $PGF_{2\alpha}$) and conception rate were analyzed with the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). The MIXED procedure of SAS was used to analyze length of proestrus (interval from PGF administration to detection of estrus), follicle age (calculated as the interval between predicted emergence 3 d after EB and estrus), ovulatory follicle diameter at AI, and follicular growth rate (determined by the increase in diameter between $PGF_{2\alpha}$ and AI divided by the number of days between these events). In the secondary analysis, the effect of treatment on estrous response, conception rate, proestrus interval, and follicle age and diameter at AI were compared as described above between the MF2 group and the MF and YF treatments in MT. For calculation of follicle age in the MF2 group, emergence of the second NFW after EB was estimated to have occurred 1 d before $PGF_{2\alpha}$ administration. Data are expressed as the mean ± SEM.

RESULTS

Estrous response was similar (90%; 252/280) between treatments and locations (Table 1). The interval from $PGF_{2\alpha}$ to estrus (proestrus interval) was greater in the YF than MF treatment ($P < 0.05$; Table 1; Fig. 2 and 3); however, a treatment × location interaction ($P < 0.01$) was detected for proestrus interval. In the MF treatment, proestrus interval did not differ by location whereas interval to estrus was greater ($P < 0.01$) in YF heifers in OH than MT. As a result of differences in the timing of $PGF_{2\alpha}$ relative to emergence of the ovulatory follicle and

Table 1. Effect of treatment (Trt) on estrous response, proestrus interval, follicle age and size at AI (mean ± SE), and conception rate in both locations (heifers in Montana classified as mature follicle 2 are not included)

	Trt[1]	*n*	Estrous response, %	Proestrus interval, h[2]	Follicle age at AI, d[3]	Follicle size at AI, mm	Conception rate, %
Montana	MF	53	92.5	55.8 ± 2.7[a]	8.8 ± 0.11[a]	11.0 ± 0.18[a]	63.3
	YF	75	90.7	67.4 ± 1.6[b]	5.3 ± 0.06[b]	10.4 ± 0.15[b]	64.7
Ohio	MF	77	87.0	53.7 ± 2.2[a]	8.9 ± 0.10[a]	–	64.2
	YF	75	90.7	78.5 ± 1.4[c]	5.8 ± 0.06[c]	–	69.1

[a–c]Values with different superscripts in the same column differ ($P < 0.01$).

[1]MF = mature follicle; YF = young follicle.

[2]Proestrus interval was defined as the interval from prostaglandin F2α ($PGF_{2\alpha}$) administration to estrus.

[3]Follicle age was defined as the interval from estradiol benzoate (EB) administration to 12 h after estrus minus 3 d for new follicle wave formation to occur for heifers that received $PGF_{2\alpha}$ either 5 (YF) or 9 d (MF) after EB.

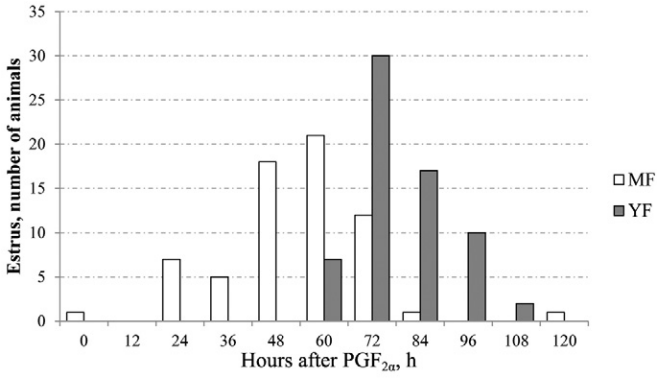

Figure 2. Distribution of estrus for Ohio heifers receiving prostaglandin F2α ($PGF_{2\alpha}$) either 5 (young follicle [YF]) or 9 (mature follicle [MF]) d after estradiol benzoate was used to create a new follicular wave. Mean interval to estrus was greater in YF than MF heifers ($P < 0.01$).

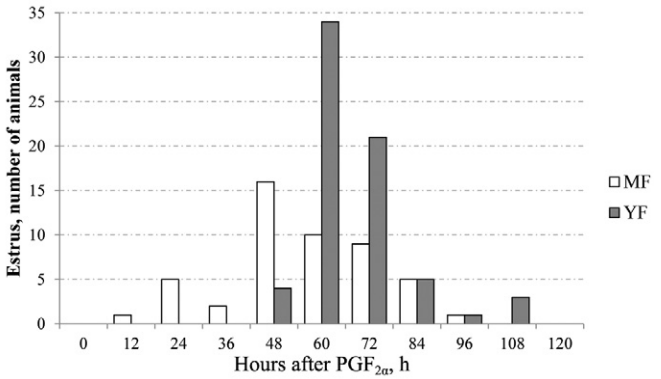

Figure 3. Distribution of estrus for Montana heifers receiving prostaglandin F2α ($PGF_{2\alpha}$) either 5 (young follicle [YF]) or 9 (mature follicle [MF]) d after estradiol benzoate was used to create a new follicular wave. Mean interval to estrus was greater in YF than MF heifers ($P < 0.01$).

the proestrus interval, a divergence in ovulatory follicle age between the YF and MF treatments of 3.5 ± 0.17 d in MT and 2.9 ± 0.16 d in OH was detected (treatment × location for follicle age, $P < 0.01$; Table 1). Additionally, follicle diameter at AI was greater ($P < 0.01$) in the MF (11.0 ± 0.18 mm) than YF (10.4 ± 0.15 mm) heifers in MT. However, regardless of location and impacts of treatment on proestrus interval, ovulatory follicle age, and diameter of the ovulatory follicle, conception rates did not differ between MF and YF heifers (Table 1).

When the MF2 group was compared to the MF and YF treatments in MT, no differences were detected in estrous response (Table 2), but proestrus interval was greater ($P < 0.01$) in MF2 heifers than both MF and YF heifers (Table 2; Fig. 4). Diameter and estimated age of ovulatory follicles in MF2 heifers at AI were similar to YF heifers but, like the YF treatment, of lesser diameter and age than the MF heifers. However, follicle growth rate from $PGF_{2\alpha}$ to AI and conception rate in the MF2 group did not differ from the YF and MF treatments (Table 2).

DISCUSSION

In the present study, the experimental design created a difference of approximately 3 d in age of the ovulatory follicle between treatments. Interval from $PGF_{2\alpha}$ to estrus (proestrus) was greater and follicle size at AI was less in heifers in the YF treatment; however, conception rate did not differ from the MF treatment.

Administration of $PGF_{2\alpha}$ to induce luteolysis at different stages during follicular growth, estimated at 2 d and 6 d after ovulatory follicle emergence in YF and MF, respectively, enabled different environments for follicle development across treatments, resulting in follicles that spontaneously ovulated at different ages. Through the US performed in heifers in MT, the new dominant follicle induced with EB underwent atresia earlier than was expected (before $PGF_{2\alpha}$ administration) in MF2 heifers, resulting in emergence of a second follicular wave after EB. Since it appeared that this second new follicle emerged 1 d before $PGF_{2\alpha}$ and that this response was detected in 21% ($n = 14/67$) of MF heifers at MT, it is suggested that the model used effectively extended follicles to almost their maximum age in the heifers that remained in the MF treatment in MT. Heifers classified as MF2 experienced a shorter follicular wave than anticipated following EB treatment. This short follicular wave surprised us, but it is possible that a contributing factor to this short wave of follicular growth was that these heifers responded to EB faster than other heifers or were experiencing an estrous cycle with 3 waves of follicular growth. Others (Ginther et al., 1989) have reported the second wave of 3-wave

Table 2. Effect of treatments or groups (Grp) on response variables (mean ± SE) for heifers in Montana

Grp	*n*	Estrous response, %	Proestrus interval, h[1]	Follicle age at AI, d[2]	Follicle size at AI, mm	Follicle growth rate from d 11 to 15, mm/d	Follicle growth rate from $PGF_{2\alpha}$ to AI, mm/d	Conception rate, %
MF	53	92.5	55.8 ± 2.7[a]	8.8 ± 0.11[a]	11.0 ± 0.18[a]	0.57 ± 0.07	1.07 ± 0.08	63.3
YF	75	90.7	67.4 ± 1.6[b]	5.3 ± 0.06[b]	10.4 ± 0.15[b]	N/A[3]	0.97 ± 0.06	64.7
MF2	14	92.9	92.3 ± 3.7[c]	5.3 ± 0.15[b]	10.0 ± 0.3[b]	N/A	0.97 ± 0.07	61.5

[a–c]Values with different superscripts in the same column differ ($P < 0.01$).

[1]Proestrus interval was defined as the interval from prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administration to estrus.

[2]Follicle age was defined as the interval from estradiol benzoate (EB) administration to 12 h after estrus minus 3 d for new follicle wave formation to occur for heifers that received $PGF_{2\alpha}$ either 5 (YF) or 9 d (MF) after EB. Heifers designated as MF2 were a subset of MF heifers that experienced new follicular wave formation approximately 1 d before $PGF_{2\alpha}$.

[3]N/A = not applicable.

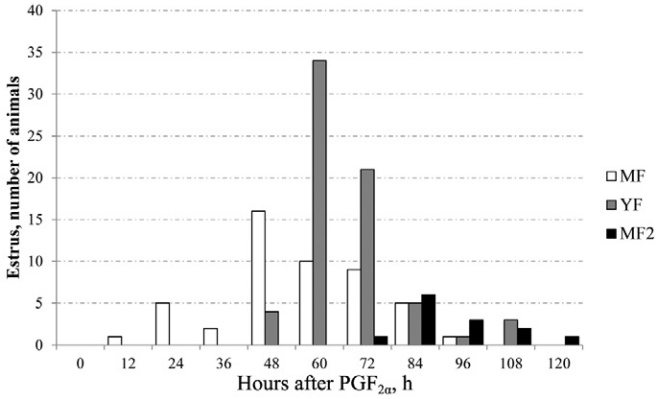

Figure 4. Distribution of estrus for Montana heifers receiving prostaglandin F2α ($PGF_{2\alpha}$) either 5 (young follicle [YF]) or 9 (mature follicle [MF]) d after estradiol benzoate was used to create a new follicular wave. A subset of heifers from the MF group experienced follicular turnover approximately 1 d before PGF administration (MF2). Mean interval to estrus was greater in MF2 heifers than in both the MF and YF heifers ($P < 0.01$).

heifers to be about 7 d, but the growth phase of this second wave to be 5 d. In OH, US was not performed, but comparison of the distribution of estrus between locations suggests that the subgroup of heifers designated as "MF2" in MT included very few heifers in OH. While differences in genotype and/or environment could explain the variation in response between the MF treatments as well as the difference in timing of estrus in the YF treatments between locations, a definitive explanation for these deviations in response between locations remains unclear.

Since only heifers detected in estrus received AI in the present study, all heifers inseminated attained sufficient threshold concentrations of estradiol during proestrus to induce behavioral estrus. The greater duration of proestrus observed in the YF treatment is in agreement with previous work from our lab (Burke et al., 2001) in which the interval to estrus was increased when luteolysis was induced 1 d instead of 4 d after emergence of the ovulatory follicle. In the present study, induction of luteolysis on d 2 as compared to 6 d after emergence increased interval to estrus by approximately 12 h in MT and 25 h in OH. This predicted response was one component of the basis of our hypothesis that heifers induced to ovulate younger follicles would have a greater conception rate. However, unlike a recent report (Geary et al., 2010), in which conception rate increased as interval from $PGF_{2\alpha}$ to estrus increased in estrus synchronized heifers, the interval from $PGF_{2\alpha}$ to estrus did not influence conception rate to AI in the present experiment. Using a controlled model that modified duration of proestrus in animals that were induced to ovulate follicles of similar age and diameter, Bridges et al. (2010) demonstrated that increased duration of proestrus resulted in increased TAI pregnancy rate. Furthermore, extending proestrus through modification of the CO-Synch + CIDR synchronization program substantially increased TAI pregnancy rate (Bridges et al., 2008). An important distinction between the work of Bridges et al. (2008, 2010) and the present study is that in the present experiment all females were inseminated based on estrus detection whereas in previous reports, TAI was used. Hence, while increased duration of proestrus may be beneficial with fixed time AI programs, no relationship was detected between length of proestrus and conception rate when females were inseminated based on estrus detection and ovulation in the present study.

Although only heifers that were detected in estrus received AI in the present study, another underlying facet in support of the hypothesis for this experiment was that heifers in YF may have an extended duration of exposure to elevated estradiol during their lengthened proestrus. Indeed, it has been demonstrated that peripheral estradiol concentrations from a follicular wave are greatest approximately 3 d after emergence of the first follicular wave of the estrous cycle in cattle and decline thereafter (Rhodes et al., 1995). Therefore, in the YF treatment, luteal regression was induced at the anticipated apex of estradiol secretion whereas in the MF treatment, this occurred after the anticipated peak secretion of estradiol. The importance of preovulatory estradiol concentrations in determination of fertility has been well established when coupled with GnRH-induced ovulation and in TAI programs for beef cattle (Perry et al., 2005; Bridges et al., 2008, 2009). It was speculated (Bridges et al., 2008) that inducing luteolysis earlier during follicular development decreased circulating progesterone concentrations and its negative feedback on LH release (Echternkamp et al., 1976; Kinder et al., 1996) resulting in earlier and perhaps greater duration of gonadotropic stimulation of the ovulatory follicle and thus may have increased circulating and/or intrafollicular estradiol concentration before induced ovulation.

The hypothesis that follicle age in itself would influence conception rate was based on considerable data derived from estrous synchronization and TAI programs and findings in lactating dairy cattle that suggested cows with 3 waves of follicular growth were more fertile. In a comparison of estrous synchronization programs (Bridges et al., 2008), follicles were induced to ovulate 8 or 9.5 d after GnRH was given to induce emergence of a NFW, which likely occurred 1 to 2 d after GnRH (Macmillan and Thatcher, 1991; Twagiramungu et al., 1994, 1995). Treatment with GnRH at the initiation of synchronization has been shown to initiate a new wave of follicles in 66% of beef cows (Geary et al., 2000). In this ovulation synchronization experiment, TAI pregnancy rates were increased by 10.5 percentage points. Subsequently, similar findings were reported in dairy cows induced to ovulate 8 or 10 d after the GnRH treatment to induce emergence of a NFW (Santos et al., 2010). Hence, in ovulation synchronization programs, herds in which females

were induced to ovulate younger follicles had increased pregnancy rate to TAI. Beef cows in the aforementioned studies ovulating supposedly younger follicles also were allowed a longer proestrus interval. Using a controlled animal model, greater embryo quality was detected in cows induced to ovulate younger follicles (Cerri et al., 2009). In spontaneously ovulating lactating dairy cows, decreased age of the ovulatory follicle, primarily as a result of whether cows experienced 2 or 3 follicular waves during the estrous cycle, has been associated with increased fertility (Bleach et al., 2004; Townson et al., 2002). Alternatively, the design of synchronization programs that result in induced ovulation of younger follicles may benefit fertility through mechanisms unrelated to follicle age, as discussed previously. It is important to note that in the present experiment, all heifers had a functional midcycle CL during growth of the ovulatory follicle; hence, variability that exists in whole herd synchronization programs, relative to stage of the estrous cycle, or whether cows are cyclic or anestrus, did not exist.

Ovulatory follicle diameter at AI was measured in MT heifers and was increased by advanced follicle age in the present study. The impact of follicle diameter on fertility has been extensively investigated, particularly in TAI programs, and has been reported to positively influence pregnancy in beef (Lamb et al., 2001; Perry et al., 2005; Mussard et al., 2007) or dairy cows (Vasconcelos et al., 2001) and beef heifers (Perry et al., 2007). However, in the present study, greater ovulatory follicle diameter at estrus in MF heifers (11.0 ± 0.18 mm) did not result in greater pregnancy rates than YF heifers (10.4 ± 0.10 mm). Results of the present study suggest that if heifers are permitted to progress to a spontaneous estrus after regression of a functional midcycle CL, putative impacts of extended proestrus, increased ovulatory follicle size, and/or estrogenic capacity of the ovulatory follicle at the time of luteal regression are not accurate determinants of fertility.

In summary, manipulation of age of the ovulatory follicle also altered diameter of the ovulatory follicle and length of proestrus in heifers that exhibited estrus. Neither follicular age nor the associated changes in other reproductive characteristics (follicle diameter or length of proestrus) were a significant source of variation in fertility in beef heifers that exhibited estrus.

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