Relationship between size of the ovulatory follicle and pregnancy success in beef heifers[1]

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ABSTRACT: Previous research indicated that the size of the ovulatory follicle at the time of insemination significantly influenced pregnancy rates and embryonic/fetal mortality after fixed-timed AI in postpartum cows, but no effect on pregnancy rates was detected when cows ovulated spontaneously. Our objective was to evaluate relationships of fertility and embryonic/fetal mortality with preovulatory follicle size and circulating concentrations of estradiol after induced or spontaneous ovulation in beef heifers. Heifers were inseminated in 1 of 2 breeding groups: (1) timed insemination after an estrous synchronization and induced ovulation protocol (TAI n = 98); or (2) AI ~12 h after detection in standing estrus by electronic mount detectors during a 23-d breeding season (spontaneous ovulation; n = 110). Ovulatory follicle size at time of AI and pregnancy status 27, 41, 55, and 68 d after timed AI (d 0) were determined by transrectal ultrasonography. Only 6 heifers experienced late embryonic or early fetal mortality. Interactions between breeding groups and follicle size did not affect pregnancy rate (P = 0.13). Pooled across breeding groups, logistic regression of pregnancy rate on follicle size was curvilinear ($P < 0.01$) and indicated a predicted maximum pregnancy rate of 68.0 ± 4.9% at a follicle size of 12.8 mm. Ovulation of follicles <10.7 mm or >15.7 mm was less likely ($P < 0.05$) to support pregnancy than follicles that were 12.8 mm. Ovulatory follicles <10.7 mm were more prevalent (28% of heifers) than ovulatory follicles >15.7 mm (4%). Heifers exhibiting standing estrus within 24 h of timed AI had greater ($P < 0.01$) follicle diameter (12.2 ± 0.2 mm vs. 11.1 ± 0.3 mm) and concentrations of estradiol (9.9 ± 0.6 vs. 6.6 ± 0.7) and pregnancy rates (63% vs. 20%) than contemporaries that did not exhibit behavioral estrus. However, when differences in ovulatory follicle size were accounted for, pregnancy rates were independent of expression of behavioral estrus or circulating concentration of estradiol. Therefore, the effects of serum concentrations of estradiol and behavioral estrus on pregnancy rate appear to be mediated through ovulatory follicle size, and management practices that optimize ovulatory follicle size may improve fertility.

Key words: cattle, follicle size, pregnancy rate

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INTRODUCTION

Maternal prerequisites to production of a viable embryo include ovulation of a competent oocyte, adequate progesterone production by the CL, and an adequate uterine environment. In domestic cattle, luteal progesterone is required for maintaining pregnancy (McDonald et al., 1952) and for enhancing embryonic growth and development (Garrett et al., 1988; Mann et al., 1996). Bovine follicles achieve ovulatory capacity at a diameter of approximately 10 mm (Martinez et al., 1999; Sartori et al., 2001). Protocols that synchronize ovulation facilitate use of AI (Pursley et al., 1997; Martinez et al., 2001). An injection of GnRH to induce ovulation has improved success of timed insemination (**TAI**) protocols (Stevenson et al., 2003). However, use of GnRH results in a wide range of follicle sizes being induced to ovulate (Perry et al., 2005).

Cows induced to ovulate follicles <11.5 mm had smaller CL and secreted less progesterone than cows ovulating larger follicles (Vasconcelos et al., 2001). Decreased pregnancy rates (Lamb et al., 2001; Perry et

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al., 2005) and increased embryonic mortality (Perry et al., 2005) also occurred in postpartum cows induced to ovulate follicles <11.3 mm in diameter after a GnRH-induced ovulation and TAI protocol. However, ovulatory follicle size had no apparent effect on pregnancy rates or embryonic/fetal mortality of postpartum cows when ovulation occurred spontaneously after detection of standing estrus (Perry et al., 2005). Santos et al. (2004) suggested that induced ovulation of small, incompetent follicles might result in reduced embryo survival because of luteal inadequacy and short estrous cycles.

Therefore, the objective of this study was to evaluate relationships of fertility and embryonic/fetal mortality with preovulatory follicle size and circulating concentrations of estradiol after induced or spontaneous ovulation in beef heifers.

MATERIALS AND METHODS

All protocols involving animals used in this research were approved by the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee.

Experimental Design

Crossbred beef heifers at USDA-ARS, Fort Keogh Livestock and Range Research Laboratory in Miles City, MT, were fitted with HeatWatch transmitters (DDx Inc., Denver, CO). Estrous cycles and ovulation were synchronized with the CO-Synch protocol. Briefly, heifers received an injection of GnRH (100 μg as 2 mL of Fertagyl i.m.; InterVet, Millsboro, DE) on d –9, and $PGF_{2\alpha}$ (25 mg as 5 mL of Lutalyse i.m., Pfizer Animal Health, NY) on d –2. Forty-eight hours after $PGF_{2\alpha}$, all heifers not yet detected in estrus and inseminated received GnRH (Fertagyl; 100 μg i.m.; d 0) and TAI by 1 of 6 inseminators with semen from 1 of 16 sires.

Heifers were assigned to the induced ovulation group (n = 98) if they (1) did not exhibit standing estrus more than 24 h before TAI; (2) ovulated in response to the second GnRH injection; and (3) did not exhibit a short luteal phase (returned to estrus <16 d after ovulation) after TAI. Heifers detected in standing estrus more than 24 h before or more than 48 h after TAI during a 23-d breeding season were inseminated approximately 12 h afterwards and are hereafter referred to as the spontaneous ovulation group (n = 110). Heifers were considered to be in standing estrus when 3 mounts of 2 s or longer in duration were recorded by the HeatWatch system within a 4-h period.

The contrast of induced and spontaneous ovulation groups may be challenged in that these groups were not randomly established before the experiment was initiated. Rather, heifers were assigned to the spontaneous ovulation group when they were detected in estrus before the second injection of GnRH or failed to become pregnant to TAI and returned to estrus during the 23-d breeding season. Because these criteria could be the result of factors that influenced ovarian follicular development or fertility of these heifers, it may be argued that the comparison of the induced vs. spontaneous ovulation groups is biased and the contrast of them was not interpreted herein. However, this potential bias does not compromise the determination of the effects of follicle size on fertility and embryonic/fetal mortality.

Blood Sampling

Blood samples were collected into 10-mL Vacutainer tubes (Fisher Scientific, Pittsburgh, PA) via puncture of a median caudal vessel on d –16, –9, –2, and 0 to determine circulating concentrations of progesterone (all samples) and estradiol (d 0 samples). Blood samples were also collected from heifers in the spontaneous ovulation group at the time of AI to determine circulating concentrations of estradiol. Blood was allowed to clot at room temperature, stored at 4°C for 24 h, and centrifuged at $1,200 \times g$ for 30 min. Serum was harvested and stored at –20°C until the concentrations of progesterone and estradiol-17β were determined by RIA. Heifers with serum concentrations of progesterone >1.0 ng/mL on d –16 or –9 were classified as being pubertal and having estrous cycles (Geary et al., 2006). Heifers (n = 18) with serum concentrations of progesterone <1.0 ng/mL on both d –16 or – were considered prepubertal and not used in this study.

RIA

Serum concentrations of progesterone were determined by RIA (Diagnostic Products Corporation, Los Angeles, CA; Bellows et al., 1991). Intra- and interassay CV were 7.6 and 16.1%, respectively, and assay sensitivity was 0.08 ng/mL. Serum concentrations of estradiol-17β were determined by RIA (Perry et al., 2004). Intra- and interassay CV for estradiol-17β were 3.8 and 11.8%, respectively, and assay sensitivity was 0.5 pg/mL.

Ultrasonography

Ovaries of all heifers were examined by transrectal ultrasonography using an Aloka 500V ultrasound with a 7.5 MHz linear probe (Aloka, Wallingford, CT) to characterize follicle size on d –2 and 0 (for induced ovulation heifers) and at insemination ~12 h after onset of spontaneous estrus (spontaneous ovulation heifers) and to confirm ovulation on d 2. All follicles ≥8 mm in diameter were recorded. Follicle size was determined by averaging follicular diameter at the widest point and perpendicular to the first measurement using the internal calipers on the Aloka 500V. Ovulation was defined as the disappearance of a large follicle from an ovary within 48 h after GnRH was administered (induced ovulation group). Pregnancy status and embryo viability (heartbeat) were determined on d 27, 41, 55, and 68 after TAI by transrectal ultrasonography using an Aloka 500V ultrasound with a 7.5 (d 27) or 5 MHz linear probe.

Statistical Analysis

Follicle size and circulating concentrations of estradiol-17β were analyzed by ANOVA in PROC MIXED (SAS Inst. Inc., Cary, NC). The model for follicle size included fixed effects for breeding group, whether or not the heifer expressed standing estrus nested within breeding group, and a linear effect of the concentration of progesterone on d –2. The model for circulating concentrations of estradiol-17β included fixed effects for breeding group, whether or not the heifer expressed standing estrus nested within breeding group, and linear and quadratic effects of follicle size.

In each breeding group, logistical regression models were fit by the method of maximum likelihood using PROC LOGISTIC of SAS to determine the effects of serum concentration of progesterone on d –2, change in follicular diameter from d –2 to 0, follicle size at time of insemination (d 0), and circulating concentrations of estradiol-17β on pregnancy rates at d 27 and 68, or first detectable pregnancy date and d 68 for heifers that ovulated spontaneously. First (linear) and second (quadratic) order continuous effects of follicle size were modeled because preliminary analyses indicated significant lack of fit to models that included only linear effects. For each group of heifers, the first derivative of pregnancy rate with respect to follicle size was solved for its root, in the range of observed follicle sizes, to obtain the follicle size resulting in the maximum predicted pregnancy rate. A 90% confidence interval for the predicted maximum was approximated by calculating the value at which the predicted pregnancy rate was significantly reduced ($P = 0.05$) relative to the predicted maximum, and the associated ordinal values of follicle size were determined. Follicle sizes for which pregnancy rate was less than the lower critical value of the confidence interval were thus concluded to be suboptimal. Effects of follicle size, breeding group, and serum concentration of progesterone at d –2 on whether ovulation occurred were also assessed by logistical regression.

RESULTS

At initiation of the synchronization protocol (i.e., d –9), 208 of the 226 heifers (92%) had initiated estrous cycles as established by serum progesterone concentrations ≥1.0 ng/mL on d –16 or –9. Data from the 18 heifers that were prepubertal were not used to produce the results reported hereafter. Of these 208 heifers, 157 (75%) had ovulated by 48 h after the second injection of GnRH. Of these heifers, 29 returned to estrus within 16 d of TAI. There were 51 heifers (25%) that did not ovulate by 48 h after the second injection of GnRH. Overall, 135 heifers became pregnant to AI, of which 60 resulted from TAI and 75 became pregnant to insemination after a detected estrus. Only 6 heifers experienced late embryonic or early fetal mortality, but this loss was independent of follicle size or day of gestation.

Small follicles were less likely to ovulate in response to exogenous GnRH than larger follicles ($P < 0.01$), with unovulated follicles having a diameter of 10.1 ± 0.7 mm compared with the 12.1 ± 0.2-mm diameter of ovulated follicles. Increased concentration of progesterone in serum at d –2 tended to increase follicle size ($P = 0.09$) at TAI but had no effect on whether or not ovulation occurred ($P = 0.75$). Heifers exhibiting standing estrus within 24 h of TAI had greater follicle diameter (12.2 ± 0.2 mm vs. 11.1 ± 0.3 mm), concentrations of estradiol (9.9 ± 0.6 vs. 6.6 ± 0.7, respectively), and pregnancy rates (63 vs. 20%, respectively) than contemporaries that did not exhibit behavioral estrus. Size of the ovulatory follicle and circulating concentrations of estradiol were linearly related and lowly correlated (r = 0.13; $P = 0.04$). When accounting for differences in ovulatory follicle size, pregnancy rates were independent of expression of behavioral estrus or circulating concentrations of estradiol-17β ($P = 0.28$ and 0.18, respectively). Therefore, the effects of serum concentrations of estradiol and behavioral estrus on pregnancy rate appear to be mediated through ovulatory follicle size.

Heifers ovulating spontaneously had 0.6 ± 0.3 mm larger ($P = 0.04$) ovulatory follicles at insemination than heifers induced to ovulate after GnRH administration. Interactions between breeding group (induced ovulation and spontaneous ovulation) and follicle size did not affect pregnancy rate ($P = 0.13$). Pooled across breeding groups, the relationship between pregnancy rate and follicle size was curvilinear ($P < 0.01$) and remained significant even if only those heifers that ovulated were considered. A maximum pregnancy rate of 68.0 ± 4.9% was predicted at a follicle size of 12.8 mm for the first pregnancy diagnosis after AI (i.e., 27 d). As a result of there being relatively few pregnancies lost between d 27 and 68 and those that were lost being independent of follicle size, the relationship between follicle size and pregnancy diagnosis was similar at d 68 to that observed on d 27. Based on the relationship portrayed in Figure 1, heifers with ovulatory follicles that were <10.7 mm or >15.7 mm in diameter at time of insemination were predicted to be less likely to be pregnant on d 68 compared with heifers that had 12.8 mm follicles at time of insemination. Twenty-eight percent of heifers ovulated follicles <10.7 mm, but only 4% of heifers ovulated follicles >15.7 mm (Figure 2) at time of insemination. Increased serum concentration of progesterone at d –2 also tended to increase pregnancy rate ($P = 0.10$). However, there was no effect of follicular growth from d –2 to 0 ($\Delta = 1.6 \pm 0.2$ mm) on pregnancy rate ($P = 0.77$).

DISCUSSION

Maternal requirements for production of a viable embryo include ovulation of a competent oocyte, adequate progesterone production by the CL, and an adequate uterine environment. Circulating concentrations of estradiol during the preovulatory period can influence the establishment and maintenance of pregnancy through altering the uterine environment (Miller and Moore, 1976; Miller et al., 1977). Luteal progesterone is also

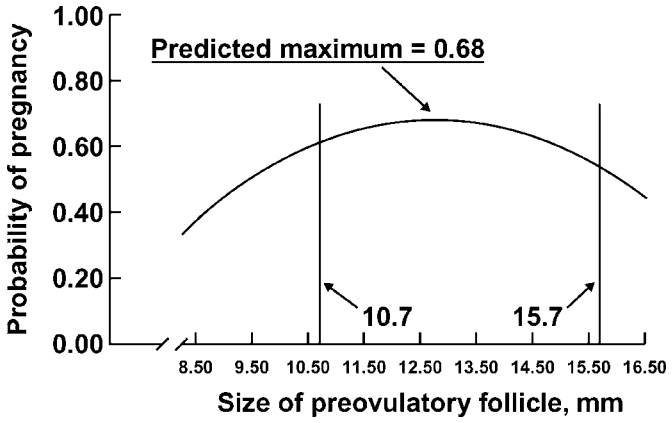

Figure 1. Effect of size of preovulatory follicle on the probability of pregnancy. Endpoints of the derived confidence interval (vertical lines, indicated by arrows) for the follicle size resulting in the maximum pregnancy rate indicate that follicles <10.7 or >15.7 mm in diameter at the time of AI were less likely ($P < 0.05$) to support pregnancy than an optimally sized follicle of 12.8 mm (at the predicted maximum probability of pregnancy of 0.68).

required for the maintenance of pregnancy (McDonald et al., 1952) and to stimulate endometrial secretions (Geisert et al., 1992) as well as embryonic growth and development (Garrett et al., 1988; Mann et al., 1996).

Size of the preovulatory follicle has been shown to be influenced by the number of follicular waves during the estrous cycle with larger follicles being produced by cows having 2 follicular waves as opposed to 3 (Ginther et al., 1989; Celik et al., 2005). In this situation, there is some indication of larger follicle size being associated with reduced fertility due to aging of the oocyte (Mihm et al., 1996; Revah and Butler, 1996). By applying the GnRH/$PGF_{2\alpha}$/GnRH protocol, synchronized ovarian follicular wave emergence is anticipated with a reduction in ovulation of a persistent follicle to the second GnRH injection (Britt et al., 1974; Zaied et al., 1980; Thatcher et al., 1989). Thus, some standardization of the duration of folliculogenesis can be achieved at TAI but not for subsequent spontaneous ovulations.

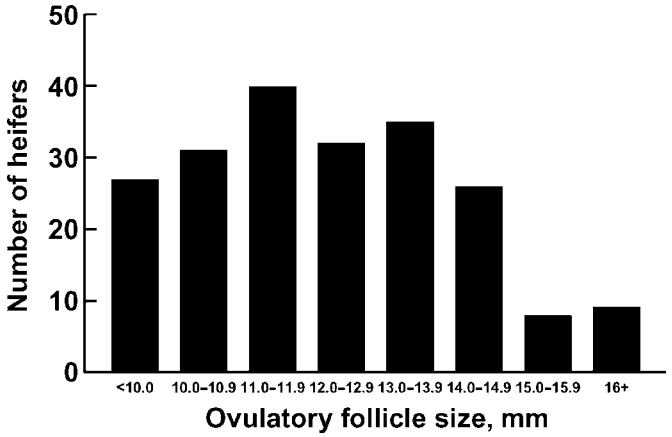

Figure 2. Distribution of ovulatory follicle size in crossbred beef heifers.

Follicles appear to acquire ovulatory capacity at a diameter of about 9 to 10 mm and several physiological changes, including increased circulating concentrations of estradiol, are coincident with this acquired capacity (Martinez et al., 1999; Sartori et al., 2001). In the current study, size of the ovulatory follicle and circulating concentrations of estradiol were lowly correlated. Greater serum concentrations of estradiol and increased pregnancy rates were observed in heifers exhibiting standing estrus within 24 h of TAI compared with heifers not exhibiting standing estrus, consistent with similar observations in postpartum cows (Perry et al., 2005).

In the current study, measurements of ovulatory follicle size were collected at the time of insemination for heifers in each group. Induced ovulation of heifers following the GnRH/$PGF_{2\alpha}$/GnRH protocol would be anticipated approximately 28 h after the second GnRH injection (Pursley et al., 1995), whereas spontaneous ovulation of heifers exhibiting estrus would be anticipated approximately 31 h after the onset of estrus (Christenson et al., 1975) or approximately 19 h after insemination. Thus, groups differed by approximately 9 h relative to time of ovulation when follicles were measured. The observation that follicle size of heifers at the time of insemination was 0.6 ± 0.3 mm greater for heifers ovulating spontaneously compared with those induced to ovulate is consistent with our observed 1.6 ± 0.2 mm increase in diameter of the ovulatory follicle from d –2 to 0 for heifers receiving TAI.

Heifers that ovulated follicles <10.7 mm in diameter had decreased pregnancy rates compared with heifers that ovulated follicles ≥12.8 mm in the current study. Competence of the oocyte ovulated, postovulation progesterone production by the CL, and the uterine environment may all be involved in this decrease in pregnancy rates. Vasconcelos et al. (2001) reported that when follicles of dairy cows were aspirated to reduce their size (11.5 ± 0.2 mm vs. 14.5 ± 0.4 mm for nonaspirated control), circulating concentration of estradiol at the time of the second GnRH injection, serum concentrations of progesterone 7 and 14 d afterwards, and pregnancy rates per AI at d 27 were reduced. However, by controlling the interval between the injection of $PGF_{2\alpha}$ and the second injection of GnRH, Peters and Pursley (2003) linearly increased size of the ovulatory follicle and pregnancy rates per AI without affecting subsequent concentration of serum progesterone. The average follicle size of each treatment group reported by Peters and Pursley (2003) was greater than the follicle size coincident with the maximal predicted pregnancy rate in the current study.

Work of Santos et al. (2004) can be interpreted to indicate that induced ovulation of small and incompetent follicles, resulting in increased embryonic mortality is more severe than fertilization failure in heifers.

Moore et al. (2005) observed lower concentrations of serum progesterone coincident with embryo loss between d 24 and 28. Competence of bovine oocytes increased as follicular diameter increased (Arlotto et al., 1996) and RNA synthesis was still observed in large oocytes (Fair et al., 1995). Perry et al. (2005) reported induced ovulation of follicles <11.3 mm in diam. also resulted in increased late embryonic, early fetal mortality, or both compared with cows induced to ovulate optimal sized follicles (14.6 mm). The inability to detect effects of follicle size on embryonic mortality in the current study likely results from the low overall rate of embryonic mortality and sample size.

In the current study, ovulatory follicle size influenced pregnancy success when heifers spontaneously ovulated after detection in standing estrus or were induced to ovulate with GnRH. Ovulation of follicles >10.7 mm and <15.7 mm in diam. at time of AI resulted in increased pregnancy rates of heifers. In contrast, ovulatory follicle size had no influence on pregnancy success in postpartum cows that ovulated spontaneously following behavioral estrus (Perry et al., 2005). The reason for the difference between heifers and cows when follicles spontaneously ovulate is not known. However, Byerley et al. (1987) reported a 19% lower pregnancy rate in heifers inseminated at their pubertal estrus vs. contemporaries inseminated at their third estrus and speculated that the greater fertility of the third estrus may be related to maturational changes associated with cycling activity. Based on the observation of 8.4% of heifers that were initially allocated to this study being prepubertal, an estrous cycle duration that is approximately two-thirds of the phenotypic standard deviation of age at puberty length (s ≈ 31d, Splan et al., 1998; Bennett and Gregory, 2001), and assumed normality of the distribution of age at puberty; the first injection of GnRH would have been coincident with the inherently less fertile pubertal estrus in approximately 16% of the heifers. The relationship of follicle size and number of estrous cycles postpuberty with fertility remains to be elucidated.

In summary, heifers that ovulated follicles >10.7 mm 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. Heifers detected in standing estrus within 24 h of TAI had greater follicle size, greater circulating concentrations of estradiol, and increased pregnancy rates compared with heifers not observed in estrus. However, when accounting for differences in ovulatory follicle size, pregnancy rates were independent of expression of behavioral estrus or circulating concentrations of estradiol-17β. Therefore, ovulatory follicle size appears to be a strong indicator of follicle maturity and perhaps a better indicator of fertility than serum concentration of estradiol at time of AI or expression of estrus. 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.

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