Size of ovulatory follicles in cattle expressing multiple ovulations naturally and its influence on corpus luteum development and fertility

S. E. Echternkamp, R. A. Cushman and M. F. Allan

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Size of ovulatory follicles in cattle expressing multiple ovulations naturally and its influence on corpus luteum development and fertility[1,2]

S. E. Echternkamp,[3] R. A. Cushman, and M. F. Allan[4]

USDA-ARS, US Meat Animal Research Center, Clay Center, NE 68933-0166

ABSTRACT: Long-term genetic selection of cattle for fraternal twins has increased the frequency of twin and triplet ovulations. In contrast, the ratio of fetal numbers to ovulation sites in pregnant females with twin (0.83) or triplet (0.73) ovulations is <1.0 and the number of calves per parturition is 1.6 and 2.0, respectively. Failure of individual twin or triplet ovulations to yield a conceptus in fertile females indicates a significant contribution of ovulation or oocyte anomalies to increased fertilization failure or early embryonic mortality. The present objective was to identify physiological traits affecting conception in cyclic cattle expressing multiple ovulations naturally, including the effect of ovulation rate on follicle or corpus luteum (CL) size, and their relationship to conception. Diameter of the individual ovulatory follicles was measured by transrectal ultrasonography at AI and ranged from 8 to 30 mm, with a trend for diameter of the individual follicles, and associated CL, to decrease with increasing ovulation rate. Independent of ovulation rate, ovulatory follicles were smaller ($P < 0.05$) for nulliparous heifers (1.5 yr) compared with parous cows (≥2.5 yr). Pregnancy and fetal status were diagnosed by transrectal ultrasonography between 42 and 72 d after AI. Fertility was reduced ($P < 0.01$) for small twin or triplet ovulatory follicles (8 to 8.9 mm vs. 10 to 17.9 mm diam.), whereas fertility in monovular females was reduced ($P < 0.01$) for large ovulatory follicles (≥22 vs. 14 to 17.9 mm). Plasma progesterone concentrations increased with ovulation rate and were correlated positively with total CL or ovulatory follicle volume per female, indicating that CL size and function were influenced by the size of the follicle of origin. Progesterone was greater ($P < 0.05$) in the blood of nulliparous heifers compared with parous cows. The increased proportion of small ovulatory follicles associated with twin and triplet ovulations indicates that some ovulatory follicles were either selected to ovulate at a lesser stage of maturity or rescued while undergoing atresia, thus compromising oocyte competency or ovulation. Of greatest importance for reduced fertility was the greater incidence of pregnancy losses occurring in the middle of gestation in females gestating 2 or more fetuses as an apparent effect of uterine crowding, especially when 2 or more fetuses were contained within 1 uterine horn.

Key words: fertility, follicle size, ovulation rate, progesterone, twinning cattle

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INTRODUCTION

The most important factors contributing to reproductive inefficiency in beef cattle are failure to become pregnant (Bellows et al., 1979; Maurer and Echternkamp, 1985) and low prolificacy (Gregory et al., 1990). Failure to become pregnant has been attributed to fertilization failure, hormonal asynchrony or deficiencies, and embryonic anomalies; embryonic mortality within 16 d after fertilization is the most prevalent cause of fertility loss in cattle (Maurer and Chenault, 1983; Maurer and Echternkamp, 1985). Comparison of fertility between cows with twin vs. single ovulations (Echternkamp et al., 1990a) indicated that the per-service pregnancy rate was greater initially with twin ovulations but that a greater proportion of the twin and triplet pregnan-

[1]Mention of names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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[3]Corresponding author: Sherrill.Echternkamp@ars.usda.gov

[4]Current address: Pfizer Animal Genetics, 430 Terry Road, Aurora, NE 68818.

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cies were terminated prematurely, implicating maternal differences in uterine capacity to support a twin or triplet pregnancy (Echternkamp et al., 2007a). In addition, within pregnant females with twin or triplet ovulations, the ratio of calves born to the number of corpora lutea (**CL**) was 0.86 or 0.73, respectively; thus, 15 to 30% of the individual ovulations did not yield a conceptus, similar to losses in monovular females (Maurer and Chenault, 1983). Recent studies have shown that size of the ovulatory follicle affects the fertility of induced ovulations in estrus-synchronized cattle but that follicle size does not affect the fertility of spontaneous ovulations (Perry et al., 2005). Size of the ovulatory follicles and resulting CL are variable in cows with multiple ovulations (Echternkamp, 1992, 2000), and we hypothesize that differences in conception rate among oocytes from individual twin and triplet ovulations result from this variation in follicle size and its effects on CL development and embryo survival. The objective of this study was to identify physiological traits affecting conception in cattle expressing twin or triplet ovulations, including the effect of ovulation rate on follicle size and its relationship to conception.

MATERIALS AND METHODS

The experimental design and procedures used in this study were approved by the US Meat Animal Research Center (**USMARC**) Animal Care and Use Committee.

Animals and Experimental Design

A total of 438 individual animal records (156 nulliparous and 282 parous records) were collected from cyclic females in the fall-breeding herd of the Twinner population at the USMARC in 2004 (n = 148), 2005 (n = 143), and 2006 (n = 147); a total of 265 females contributed data in the study. The Twinner population is a composite of 9 cattle breeds and has been selected for 4 to 5 generations for the natural production of fraternal twin births, using repeated measurements of ovulation rate, individual animal EBV for twinning, and sires progeny proven for twinning (Echternkamp et al., 1990a; Gregory et al., 1990; Van Vleck et al., 1991). Selection protocols, management procedures, and breed composition have been reported (Gregory et al., 1990, 1996; Echternkamp and Gregory, 2002). The fall AI breeding season began in early November. Females were monitored for estrus twice daily for 1 natural estrous cycle (i.e., 21 d) and were inseminated 12 h after detection of estrus with semen from an assigned progeny-proven sire (Echternkamp et al., 2007a). Body weight of the females was measured at the beginning of the breeding season. Because of the repeated measurements of ovulation rate in all yearling heifers (Echternkamp et al., 1990a), heifers were first bred at 1.5 yr of age. The 2-yr-old nulliparous females failed to conceive during the previous spring breeding season. Because of age effects on ovulation rate, physical size, and biological maturity, the 2-yr-old females were evaluated separately from the 1.5-yr-old heifers. During the breeding season, all animals were provided ad libitum grass pasture supplemented with corn silage (70%) and alfalfa haylage (30%). Dams gestating, birthing, and nursing a single calf were fed a diet of 70% haylage and 30% corn silage supplemented with alfalfa hay ad libitum pre- and postpartum to the beginning of breeding. Females diagnosed with twins or triplets were fed a high-energy diet (80% corn silage, 17.5% high-moisture corn, and 2.5% protein supplement) starting an average of 50 d before the beginning of the calving season; dams nursing twins were fed this high-energy diet postpartum. Diets were fed to achieve a BCS of 5 to 6 on a scoring scale of 1 to 9 (NRC, 1996) during the pre- and postpartum periods.

Follicle, CL, and Ovulation Rate Measurements and Pregnancy Diagnosis

Diameters of both the ovulatory follicle(s) and its resulting CL were estimated by real-time ultrasonography with a 7.5-MHz linear-array probe (Aloka 500, Corometrics Medical Systems, Wallingford, CT). Both ovaries were scanned transrectally 12 h after detection of a natural estrus (i.e., concurrent with AI), and the diameter (average of 2 dimensions) and location of all antral ovarian follicles $\geq$5 mm within the ovaries were measured and recorded. Diameter and location of the resulting CL were measured 7 to 15 d after AI by the same procedure, and identity of the ovulatory follicles was reconfirmed based on location of the CL. An ultrasound image of each individual follicle and CL was photographed for confirmation of measurements. The volume of each follicle and CL was estimated from the measured diameter (volume = $4/3\pi r^3$), and total ovulatory follicle or CL volume was the summation of the individual ovulatory follicle or CL volumes within an animal. Some CL contained a small (2- to 5-mm diam.), fluid-filled cavity, but the frequency and size of the cavities were similar among ovulation rate groups, and the volume of the cavity was not subtracted. Data for 9 cows having a CL with a fluid-filled cavity >15 mm in diameter were excluded from analysis of CL traits.

Pregnancy was diagnosed within the herd by real-time ultrasonography (3.5-MHz convex-array probe, Corometrics Medical Systems; Echternkamp and Gregory, 1999) at approximately 60 d after onset of the AI breeding season. Fetal age, fetal number, and distribution of fetuses within the uterus were also determined; gestation length for females pregnant to AI ranged from 42 to 72 d. Pregnancy was reconfirmed by palpation per rectum of the uterus and its contents between 155 and 179 d after AI. At ultrasound and palpation pregnancy diagnosis and at calving, females were assigned a numerical diagnostic value of 1 for pregnant or 0 for nonpregnant.

The fertility of individual twin and triplet ovulations was confirmed when the number of fetuses within a uterine horn equaled the number of CL on the adjacent ovary or no fetuses were present within the horn. Conversely, individual fetuses could not be linked to individual twin or triplet ovulations when the number of fetuses within a uterine horn was less or greater than the number of CL on the adjacent ovary; fertility of those follicles was not assessed.

Plasma Progesterone and IGF-I Concentrations

A single blood sample (10 mL) was collected from each female at CL measurement 7 to 15 d after AI and at ultrasound pregnancy diagnosis. Blood was collected from the tail by venipuncture into a heparinized tube (15 IU of lithium-heparin, Sarstedt Inc., Newton, NC) and stored immediately on ice until processed. Plasma was recovered by centrifugation (1,250 × *g* for 20 min at 4°C) and stored at −20°C until assayed for progesterone and IGF-I. Progesterone was measured directly in plasma by using a commercial solid-phase RIA procedure (Siemens Medical Diagnostics Solutions, Los Angeles, CA). The intraassay CV was 2.4%, and the interassay CV was 1.8%. The minimum detectable concentration of progesterone in plasma was 0.05 ng/mL.

Concentrations of total IGF-I were measured in plasma by acid-ethanol extraction and the RIA protocols reported by Echternkamp et al. (1990b). The primary antibody was antiserum to human IGF-I (AFP4892898, National Hormone and Pituitary Program, Torrance, CA). The standard was bovine recombinant IGF-I (4045676, Monsanto, St. Louis, MO), and ^{125}I-labeled human recombinant IGF-I (H-5555, Bachem Bioscience Inc., King of Prussia, PA) was the tracer. The intraassay CV was 4.8%, and the interassay CV was 9.1%. The minimum detectable concentration of IGF-I in plasma was 0.20 ng/mL.

Data Analyses

Data were analyzed by ANOVA using the Mixed procedure (SAS Inst. Inc., Cary, NC), and results are reported as least squares means ± SEM. Initial ANOVA models included all possible 2-way interactions; nonsignificant ($P > 0.05$) interactions were subsequently excluded from the model. Main effects of animal age and year (2004 to 2006) on ovulation rate (number of CL present on both ovaries 7 to 15 d after AI) were determined by ANOVA; animal was included in the model as a random effect. Data for cows 6 yr of age and older were combined (≥6 yr).

The initial statistical model to determine the effect of ovulation rate on diameter of the individual ovulatory follicles and CL (Figure 1) included animal age and year as fixed effects and animal as a random effect; days after AI were included as a covariate for CL diameter. Because of the small number of cattle with 4 (n = 11), 5 (n = 2), or 6 (n = 1) CL, subsequent ANOVA analyses used data from only those females with an ovulation rate of 1, 2, or 3. Analyses of the effects of ovulation rate on diameter of the individual ovulatory follicles or CL, total ovulatory follicle or CL volume, total number of follicles ≥5 mm, plasma progesterone or IGF-I concentrations, conception, fetal numbers in utero, and prebreeding BW included animal age and year as fixed effects and animal as a random effect. In determining the effects of ovulation rate on individual CL diameter, total CL volume, and plasma progesterone concentration, the statistical model also included the fixed effects of pregnancy status (pregnant vs. nonpregnant) and days after AI. At ultrasound pregnancy diagnosis, days after AI were consolidated into 6 time groups; ≤45, 46 to 50, 51 to 55, 56 to 60, 61 to 65, and >65 d.

To further assess the effect of ovulation rate on size of the individual ovulatory follicle(s), individual ovulatory follicles were categorized based on the ovulation rate (1 to 3 CL) of the animal plus the size ranking (largest to smallest) of the ovulatory follicle within the animal, resulting in 6 follicle status categories: ovulation rate = 1 and follicle ranking = 1, ovulation rate = 2 and follicle ranking = 1 or 2, or ovulation rate = 3 and follicle ranking = 1, 2, or 3. Follicle status was substituted for ovulation rate in the preceding MIXED procedure ANOVA model to determine the effects of follicle status on diameter of the individual ovulatory follicles or their associated CL and on conception linked to these individual ovulations.

To assess the effect of diameter of the individual ovulatory follicles on fertility, ovulatory follicles were categorized by 1-mm increments from 8 to >22 mm. Data were analyzed by ANOVA using the MIXED procedure, with follicle diameter as the independent variable, animal age and year as fixed effects, and animal as a random effect. Because of major differences in the size range between single and twin or triplet ovulatory follicles, data for single ovulatory follicles were analyzed separately.

Effects of postpartum interval (number of days from parturition to AI) and previous type of birth (none, single, twin, or triplet) on ovulatory follicle or CL diameter, plasma progesterone or IGF-I concentrations, fetal number, fetus:CL ratio, and pregnancy rate at ultrasound or palpation pregnancy diagnoses were evaluated in parous cows by ANOVA. Analysis of variance using the Mixed procedure included postpartum interval or previous type of birth as an independent variable; ovulation rate, animal age, and year as fixed effects; and cow as a random effect. Postpartum interval was consolidated into 6 time intervals: ≤50, 51 to 60, 61 to 70, 71 to 80, 81 to 90, and >90 d postpartum. Data for plasma IGF-I concentration and BW were reanalyzed for parous cows without ovulation rate in the ANOVA model.

Effect of type of ovulation on pregnancy rate (0 = nonpregnant vs. 1 = pregnant) at ultrasound and pal-

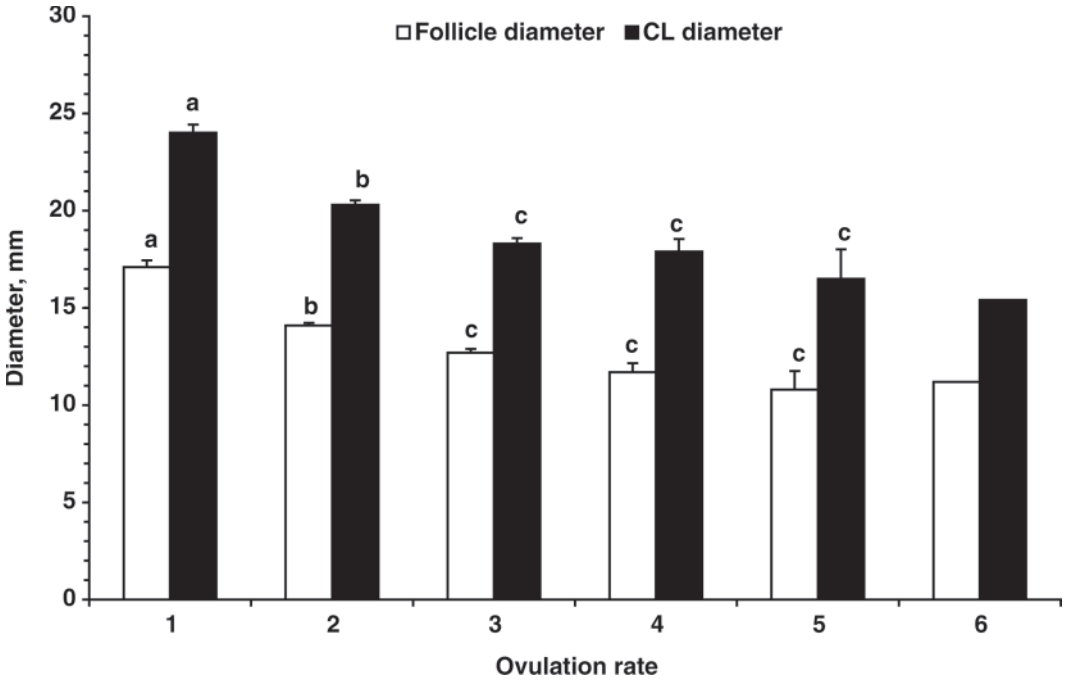

Figure 1. Effects of ovulation rate (number of CL present on both ovaries) on diameter of individual ovulatory follicles at AI and on diameter of their associated corpora lutea (CL) 7 to 15 d after AI (least squares means ± SEM). An ovulation site was confirmed by the subsequent formation of a CL. Number of ovulations per female per estrous cycle ranged from 1 to 6. Diameters of the individual follicles and associated CL were correlated positively (r = 0.054; $P < 0.001$). Means without a common letter (a to c) differ, $P < 0.01$.

pation pregnancy diagnosis and at calving, and on fetal number and the fetus:CL ratio in females conceiving to AI was assessed by ANOVA and included animal age and year as fixed effects and animal as a random effect in the model. Type of ovulation was classified by number (1, 2, or 3) and distribution (either, bilateral, or unilateral) of the ovulations between the 2 ovaries. The effect of ovulation rate on the distribution of animals by type of birth (single, twin, or triplet) was evaluated within type of ovulation classification by chi-square analysis.

Analysis of factors affecting maintenance of pregnancy was conducted by ANOVA and included fetal status as an independent variable, animal age and year as fixed effects, and animal as a random effect; effect of previous type of birth (none, single, twin, or triplet) was assessed also in a separate model for parous cows. Fetal status was classified by the number and distribution of fetuses between the left and right uterine horns: single left, single right, unilateral twins left, unilateral twins right, bilateral twins, unilateral triplets, and bilateral triplets; data for the small number of unilateral triplets in the left or right horn were combined. Records having a discrepancy between number of diagnosed fetuses and number of calves born were deleted from this analysis. Previous type of birth, dam age, and year did not affect maintenance of pregnancy and were deleted from the final model.

Pearson or residual correlations were calculated among CL number, follicle diameter, total ovulatory follicle volume, total number of antral follicles ≥5 mm, CL diameter, total CL volume, plasma progesterone or IGF-I concentrations, BW, animal age, and postpartum interval by using the CORR procedure analysis of the Mixed procedures of SAS; residual correlations were calculated by using the GLM procedures of SAS to adjust for the effects of ovulation rate, animal age, and year.

RESULTS

Ovarian Traits

Ovulation Rate. The overall mean for ovulation rate for the 3 yr evaluated was 2.1 ± 0.03 CL; however, ovulation rate was greater ($P < 0.05$) in 2006 (2.2 ± 0.06) compared with 2004 (2.0 ± 0.06), but not 2005 (2.1 ± 0.06). In 2006, one cow had an ovulation rate of 6 CL, 2 cows had a rate of 5 CL, and a greater proportion of cows had 4 CL in 2006 (n = 6) compared with 2004 (n = 4) or 2005 (n = 1).

Follicle Diameter and Numbers. Diameters of the individual ovulatory follicles ranged from 8 to 30 mm; the magnitude of the range decreased with increasing ovulation rate. Ranges in diameter by ovulation rate were as follows: 1 = 12 to 30 mm; 2 = 8 to 27 mm; 3 = 8 to 20 mm; 4 = 8 to 21 mm; 5 = 8.5 to 12.5 mm; and 6 = 8 to 13 mm. Means for diameters of the individual ovulatory follicles (Figure 1) decreased ($P < 0.01$) with each incremental increase in ovulation rate from 1 to 3; means were similar among ovulatory follicles in females with ovulation rates of 3, 4, 5, or 6. Means for diameters of the individual CL originating from these ovulatory follicles (Figure 1) also decreased ($P < 0.01$) with increasing ovulation rate. Diameters

Table 1. Effects of ovulation rate (1 to 3), animal age, and year on the size of ovulatory follicles and number of antral follicles ≥5 mm

Item	n	Individual follicle diameter, mm	n	Total follicle volume,[1] cm^3	Number of antral follicles[2]
Ovulation rate					
1	75	17.1 ± 0.4[a]	75	3.0 ± 0.2[a]	2.9 ± 0.1[a]
2	520	14.1 ± 0.1[b]	260	3.3 ± 0.1[a]	3.5 ± 0.1[b]
3	267	12.7 ± 0.2[c]	89	3.8 ± 0.2[b]	4.5 ± 0.1[c]
Age, yr					
1.5	249	14.0 ± 0.3[a]	126	3.0 ± 0.2	3.4 ± 0.1
2	41	13.9 ± 0.5[ab]	25	3.0 ± 0.3	3.7 ± 0.2
2.5	172	14.7 ± 0.3[bc]	77	3.4 ± 0.2	3.5 ± 0.1
3	157	14.8 ± 0.3[bc]	73	3.5 ± 0.2	3.6 ± 0.1
4	106	14.5 ± 0.3[ac]	51	3.5 ± 0.2	3.9 ± 0.2
5	65	15.2 ± 0.4[c]	33	3.6 ± 0.3	3.7 ± 0.2
≥6	72	15.1 ± 0.4[c]	39	3.6 ± 0.3	3.5 ± 0.2
Year					
2004	284	15.1 ± 0.2[a]	145	3.7 ± 0.1[a]	3.3 ± 0.1[a]
2005	287	14.8 ± 0.2[a]	141	3.6 ± 0.1[a]	3.6 ± 0.1[b]
2006	291	13.9 ± 0.2[b]	138	2.8 ± 0.1[b]	3.9 ± 0.1[c]

[a–c]Means ± SEM within a column and category without a common superscript differ, $P < 0.05$.
[1]Total ovulatory follicle volume (cm^3) is the summation of the estimated volume ($4/3\pi r^3$) of the individual ovulatory follicles within a female.
[2]Number of antral follicles ≥5 mm on both ovaries.

of the CL and of the follicle of origin were correlated positively (Pearson correlation coefficient, r = 0.54; residual correlation coefficient, r = 0.45; $P < 0.001$).

Subsequent assessments of ovarian traits were limited to females having ovulation rates ranging from 1 to 3 (Table 1). Again, means for individual ovulatory follicle diameters decreased ($P < 0.05$) with incremental increases in ovulation rate. In addition, ovulatory follicles of nulliparous heifers were smaller ($P < 0.05$) in diameter compared with follicles of parous cows, and ovulatory follicles were smaller ($P < 0.05$) in 2006 compared with 2004 or 2005. In contrast, total ovulatory follicle volume per female (Table 1) increased ($P < 0.05$) with increasing ovulation rate from 1 to 3 and was smaller ($P < 0.05$) in 2006 compared with 2004 or 2005.

Total number of antral follicles ≥5 mm on both ovaries (Table 1) increased ($P < 0.05$) with incremental increases in ovulation rate and differed ($P < 0.05$) among years. Total number of antral follicles ≥5 mm was correlated positively (r = 0.46; $P < 0.01$) with ovulation rate. Numbers of antral follicles were not influenced by animal age and the 2 variables were weakly correlated (r = 0.04), but follicle numbers were somewhat influenced by BW (r = 0.16; $P < 0.01$).

When individual ovulatory follicles were ranked by size within an animal and compared among ovulation rates (Table 2), the largest ovulatory follicle (F1 follicle) was of greater ($P < 0.01$) diameter in females with an ovulation rate of 1 vs. 2 or 3, whereas the diameter of neither the largest (F1) nor the second largest (F2) ovulatory follicles differed between females with 2 vs. 3 ovulations. Within females having an ovulation rate of 3, diameters differed ($P < 0.01$) among F1 vs. F2 vs. F3 (third largest) ovulatory follicles. For comparison, the mean diameter of the subordinate follicle in females with a single ovulation was 12.5 ± 0.4 mm.

Number of days postpartum (range = 44 to 114 d) did not affect diameters of individual ovulatory follicles

Table 2. Effects of ovulation rate (1 to 3) and order of size ranking on diameter of ovulatory follicles and associated corpora lutea (CL) and on fertility

Ovulation rate	Order ranking[1]	n	Individual follicle diameter, mm	Individual CL diameter, mm	Conception,[2] %
1	F1	72	17.1 ± 0.3[a]	24.3 ± 0.4[a]	53.7 ± 5.7
2	F1	233	15.7 ± 0.2[b]	21.8 ± 0.2[b]	56.1 ± 3.4
2	F2	233	12.4 ± 0.2[c]	19.3 ± 0.2[c]	52.9 ± 3.4
3	F1	78	15.4 ± 0.3[b]	20.3 ± 0.3[d]	56.1 ± 5.6
3	F2	78	12.4 ± 0.3[c]	18.1 ± 0.3[e]	55.3 ± 5.6
3	F3	78	10.3 ± 0.3[d]	16.6 ± 0.3[f]	44.1 ± 5.6

[a–f]Means ± SEM within a column without a common superscript differ; $P < 0.01$.
[1]Individual ovulatory follicles were categorized based on the ovulation rate (1 to 3 ovulations) of the animal and the size ranking of the ovulatory follicle within the animal from largest (F1) to second (F2) or third (F3) largest ovulatory follicle. Animals with incomplete ovarian and fertility data were omitted from the analysis.
[2]Conception rate linked to individual ovulatory follicles. Data for twin and triplet ovulations required the number of fetuses within a uterine horn to equal the number of CL on the adjacent ovary, except when no fetuses were present within the horn.

Table 3. Effects of ovulation rate (1 to 3), reproductive stage, animal age, and year on corpus luteum (CL) diameter and plasma progesterone concentrations

Item	n	Individual CL diameter,[1] mm	n	Total CL volume,[2] cm^3	Postbreeding progesterone,[3] ng/mL	n	Gestational progesterone,[4] ng/mL
Ovulation rate							
1	72	24.1 ± 0.4[a]	72	7.9 ± 0.5[a]	6.5 ± 0.3[a]	35	10.6 ± 0.3[a]
2	512	20.3 ± 0.2[b]	254	9.5 ± 0.3[b]	8.5 ± 0.2[b]	167	11.9 ± 0.3[b]
3	267	18.1 ± 0.3[c]	89	10.5 ± 0.4[c]	9.6 ± 0.3[c]	65	12.7 ± 0.3[b]
Days after AI							
7	29	19.6 ± 0.7[a]	18	8.7 ± 1.0	6.5 ± 0.6[a]		
8	101	21.2 ± 0.4[bcd]	45	9.9 ± 0.6	6.2 ± 0.4[a]		
9	96	20.8 ± 0.4[ac]	44	9.5 ± 0.6	7.0 ± 0.4[a]		
10	128	21.8 ± 0.4[b]	65	10.3 ± 0.5	7.9 ± 0.3[b]		
11	115	21.4 ± 0.4[bc]	56	9.4 ± 0.6	8.8 ± 0.3[bc]		
12	120	20.8 ± 0.4[ac]	61	9.3 ± 0.5	9.0 ± 0.3[c]		
13	109	21.1 ± 0.4[bcd]	55	9.3 ± 0.6	9.4 ± 0.3[cd]		
14	94	20.6 ± 0.4[a]	48	8.9 ± 0.6	10.2 ± 0.4[d]		
15	57	20.2 ± 0.6[a]	25	8.3 ± 0.9	9.0 ± 0.5[c]		
Age, yr							
1.5	244	19.9 ± 0.3[a]	121	7.9 ± 0.6[a]	9.0 ± 0.2[a]	79	12.2 ± 0.3[a]
2	41	20.7 ± 0.6[ab]	25	9.0 ± 0.8[ab]	8.6 ± 0.5[ab]	19	12.5 ± 0.3[a]
2.5	167	20.9 ± 0.3[b]	76	9.5 ± 0.5[b]	8.2 ± 0.3[b]	51	12.8 ± 0.3[a]
3	156	21.0 ± 0.3[b]	73	9.3 ± 0.5[b]	8.1 ± 0.3[b]	50	12.5 ± 0.3[a]
4	106	20.7 ± 0.4[b]	51	9.3 ± 0.6[b]	7.9 ± 0.3[b]	29	11.6 ± 0.3[a]
5	63	21.3 ± 0.5[b]	32	10.1 ± 0.7[b]	8.2 ± 0.4[b]	20	11.0 ± 0.3[ab]
≥6	72	21.4 ± 0.5[b]	39	10.0 ± 0.7[b]	7.4 ± 0.4[b]	19	9.6 ± 0.3[b]
Year							
2004	281	21.3 ± 0.2	142	9.5 ± 0.4[a]	9.5 ± 0.2[a]	85	12.1 ± 0.3
2005	279	21.2 ± 0.3	138	9.8 ± 0.4[a]	7.5 ± 0.2[b]	86	11.4 ± 0.3
2006	289	20.7 ± 0.2	137	8.5 ± 0.4[b]	7.6 ± 0.2[b]	96	11.7 ± 0.3

[a–d]Means ± SEM within a column and category without a common superscript differ, $P < 0.05$.

[1]Corpora lutea with a fluid-filled cavity >15 mm in diameter were excluded from the data set, which excluded data for 9 animals.

[2]Total CL volume (cm^3) is the summation of the estimated volume ($4/3\pi r^3$) of the individual CL within an animal. A few CL contained a small fluid-filled cavity but the volume of the cavity was not estimated.

[3]Plasma progesterone concentrations between 7 and 15 d after AI.

[4]Plasma progesterone concentrations in pregnant females between 42 and 72 d of gestation.

in suckled females (data not shown), and there was no interaction between days postpartum and ovulation rate. In addition, the residual correlation coefficient for number of days postpartum and follicle diameter was small (r = 0.04). Effects of ovulation rate, year, and animal age on follicle diameter in parous cows were the same as for nulliparous and parous females combined.

CL Diameter. In comparisons of CL among females having ovulation rates of 1 to 3 (Table 3), means for individual CL diameter decreased ($P < 0.05$), whereas total CL volume per female increased ($P < 0.05$) with each additional ovulation. Both individual CL diameter and total CL volume were smaller ($P < 0.05$) for heifers 1.5 yr of age compared with cows ≥2.5 yr of age. Measured between 7 and 15 d after AI (Table 3), individual CL diameter was greatest ($P < 0.05$) on d 10, differing ($P < 0.05$) from d 7, 9, 12, 14, or 15, whereas total CL volume did not differ among days. There was no interaction between ovulation rate and days after AI. Neither individual diameter nor total CL volume differed between females conceiving or not conceiving to AI (data not shown). Total CL volume was less ($P < 0.05$) in 2006 vs. 2005 or 2004, but CL diameter did not differ among years.

When CL were classified by both ovulation rate and follicle size ranking (Table 2), CL diameter decreased ($P < 0.01$) within a size ranking with each increment of increase in ovulation rate and decreased ($P < 0.01$) within an ovulation rate group by decreasing rank. Thus, diameter of CL originating from F1 ovulatory follicles differed ($P < 0.01$) among females with ovulation rates of 1, 2, or 3, and CL originating from F1 ovulatory follicles were larger in diameter than CL from F2 follicles ($P < 0.01$) and CL from F2 vs. F3 follicles ($P < 0.01$).

Plasma Progesterone Concentrations

Postbreeding. In blood samples collected 7 to 15 d after AI, means for plasma progesterone concentrations (Table 3) increased ($P < 0.01$) with each incremental increase in ovulation rate from 1 to 3 and with progression (days) after AI. Progesterone concentrations were greater ($P < 0.05$) in heifers 1.5 yr of age than in cows ≥2.5 yr of age and in 2004 compared with 2005 or 2006, but concentrations did not differ between females conceiving or not conceiving to AI (8.3 vs. 8.4 ± 0.2 ng/mL, respectively). Plasma concentrations were

Table 4. Effects of number and distribution of ovulations on pregnancy rates to AI measured at ultrasound or palpation pregnancy diagnosis or at calving

			Pregnancy rate, %		
Ovulation rate	Distribution[1]	n	Ultrasound[2]	Palpation[3]	Calving[4]
1	Either	75	50.4 ± 2.6[a]	48.1 ± 2.8[a]	47.6 ± 2.8[a]
2	Bilateral	100	66.6 ± 2.3[b]	62.3 ± 2.4[b]	62.0 ± 5.2[b]
2	Unilateral	160	64.5 ± 1.8[b]	57.1 ± 1.9[ab]	54.7 ± 4.3[ab]
3	Bilateral	68	74.4 ± 2.8[b]	66.1 ± 2.9[b]	64.1 ± 2.9[b]
3	Unilateral	21	57.1 ± 5.0[ab]	37.4 ± 5.2[a]	32.3 ± 5.2[a]

[a,b]Means ± SEM within a column without a common superscript differ, $P < 0.05$.
[1]Ovulation occurred on either the left or right ovary or both ovaries (bilateral).
[2]Percentage of females pregnant to AI at 42 to 72 d after AI.
[3]Percentage of females pregnant to AI at 155 to 175 d after AI.
[4]Percentage of females pregnant to AI at calving.

correlated positively with total CL volume (r = 0.26; $P < 0.01$) as well as total ovulatory follicle volume (r = 0.29; $P < 0.01$). Adjusted for the effects of ovulation rate, CL age, and year, residual correlation coefficients were negative for progesterone concentration vs. animal age (r = −0.20; $P < 0.01$) or BW (r = −0.21; $P < 0.01$).

Early Pregnancy. Plasma progesterone concentrations (Table 3) for females pregnant at ultrasound pregnancy diagnosis (i.e., 42 to 72 d after AI) were greater ($P < 0.05$) in females with 2 or 3 CL vs. 1 CL. Again, progesterone concentrations were affected by animal age, being greater ($P < 0.01$) for females 1.5 to 4 yr of age vs. ≥6 yr of age. Concentrations did not differ among days from AI to pregnancy diagnosis or among years.

Conception

Ovulation Rate. Percentages of females pregnant (Table 4) at the initial ultrasound pregnancy diagnosis (42 to 72 d after AI) were greater ($P < 0.05$) for females with twin (bilateral or unilateral) ovulations or bilateral triplet ovulations compared with a single ovulation. Neither animal age nor year affected pregnancy rates. Although not included in the analysis, 6 of 11 females with 4 ovulations, 0 of 2 females with 5 ovulations, and 1 of 1 female with 6 ovulations were pregnant at ultrasound pregnancy diagnosis.

Among females pregnant to AI (Table 5), conception rate (fetus:CL ratio) for individual ovulations decreased ($P < 0.01$) as ovulation rate increased from 1 (1.00 ± 0.03) to 2 (0.83 ± 0.02) to 3 (0.72 ± 0.03). The fetus:CL ratio was not affected by whether twin or triplet ovulations occurred on one or both ovaries or on the left vs. right ovary or by animal age or year.

Follicle Diameter. Ovulation rate, through its effect on follicle size, had an effect on fertility (Figure 2) of individual ovulations when ovulatory follicles were categorized by diameter in 1-mm increments. Among monovular females (Figure 2A), ovulatory follicles ranging in diameter from 14.0 to 17.9 mm (1-mm increments) yielded the greatest conception rates, which differed ($P < 0.01$) from the 6 infertile ovulatory follicles ≥22 mm in diameter. In contrast, analysis of follicular data pooled for females with an ovulation rate of 2 or 3 (Figure 2B) revealed that individual ovulatory follicles ranging in diameter from 8.0 to 8.9 mm yielded fewer ($P < 0.01$) conceptuses than ovulatory follicles ranging in diameter from 10.0 to 17.9 mm, 19 to 19.9 mm, or 20.0 to 20.9 mm. Ovulatory follicles ranging in diameter from 9.0 to 9.9 mm yielded fewer ($P < 0.01$) conceptuses than follicles 10 to 11.9 mm, 13.0 to 13.9 mm, or 19 to 19.9 mm in diameter.

Fetal Number

Evaluated between 42 and 72 d after AI, number of fetuses (Table 5) per pregnant female increased ($P <$

Table 5. Effects of number and distribution of ovulations (corpora lutea, CL) between ovaries on number of fetuses and fetus:CL ratio at ultrasound pregnancy diagnosis (42 to 72 d after AI) for females pregnant to AI

Ovulation rate	Location[1]	n	Number of fetuses	Fetus:CL
1	Either	38	1.0 ± 0.05[a]	1.00 ± 0.01[a]
2	Bilateral	67	1.7 ± 0.04[b]	0.83 ± 0.01[b]
2	Unilateral	103	1.7 ± 0.03[b]	0.83 ± 0.01[b]
3	Bilateral	51	2.1 ± 0.04[c]	0.72 ± 0.01[c]
3	Unilateral	12	2.2 ± 0.08[c]	0.73 ± 0.01[bc]

[a–c]Means ± SEM within a column without a common superscript differ, $P < 0.01$.
[1]Ovulation occurred on either the left or right ovary or on both ovaries (bilateral).

0.01) proportionally with ovulation rate from 1 to 3 CL. Number of fetuses per pregnant female was not affected by whether twin or triplet ovulations occurred on one or both ovaries; however, subsequent fetal survival was reduced when twin or triplet fetuses were located within the same uterine horn because of an increased incidence of pregnancy failure (Table 6). Fetal number was not affected by animal age or year (data not shown).

Maintenance of Pregnancy

Pregnancy rates (Table 4) decreased between ultrasound and palpation pregnancy diagnoses (42 to 72 d vs. 155 to 179 d after AI, respectively), a period of approximately 110 d. Females with unilateral triplet ovulations had the greatest incidence of pregnancy failure, and pregnancy rates at palpation diagnosis (Table 4)

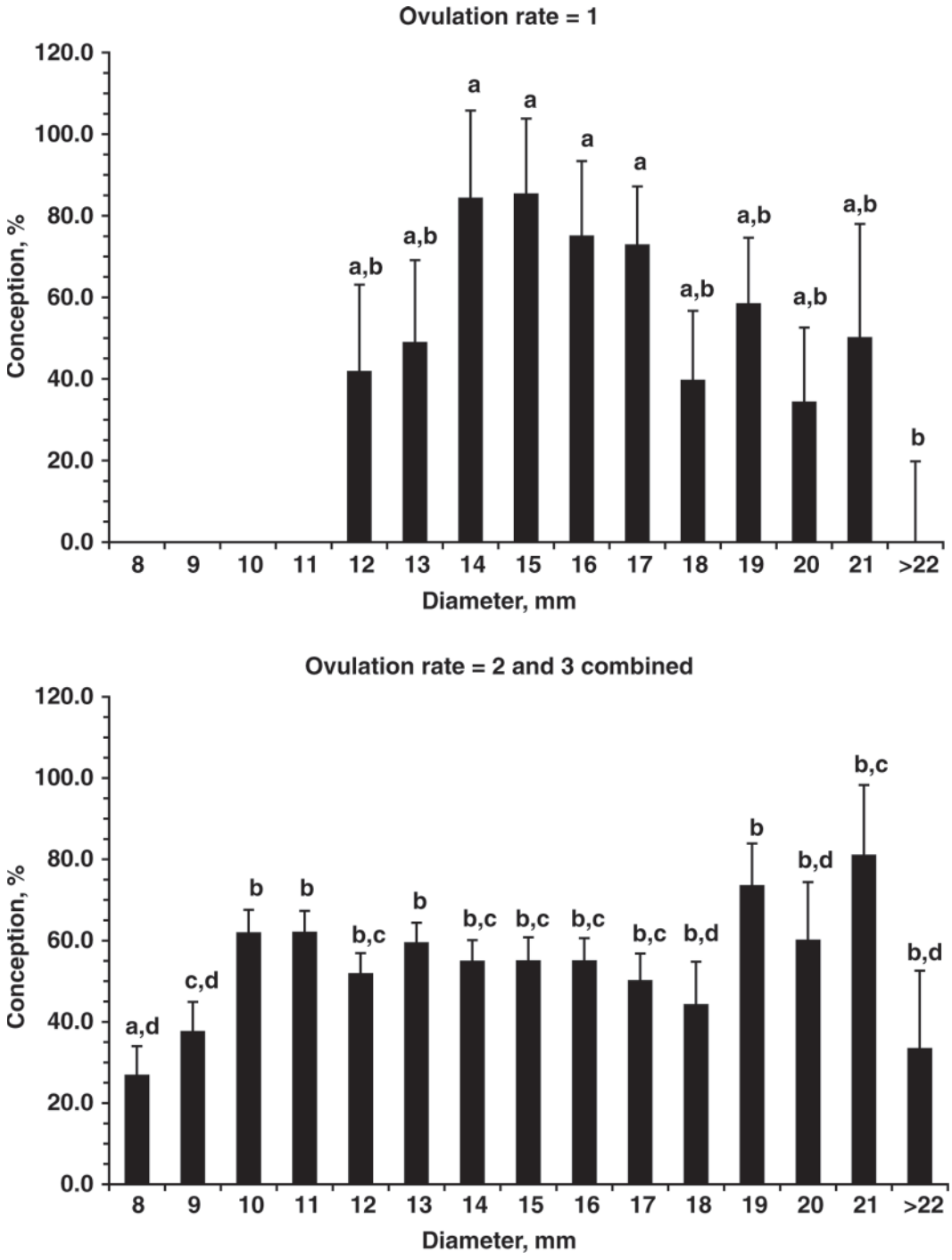

Figure 2. Effect of follicle size on conception rate of individual ovulatory follicle(s); data were analyzed separately for (top panel) females expressing a single ovulation vs. (bottom panel) combined data for females with twin or triplet ovulations (least squares means ± SEM). Individual ovulatory follicles were categorized by 1-mm increments from 8 to >22 mm. Means without a common letter (a to d) differ, $P < 0.01$.

Table 6. Effects of fetal number and distribution in utero on maintenance of pregnancy from ultrasound pregnancy diagnosis to palpation pregnancy diagnosis or calving[1]

Number of fetuses	Location	n	Palpation,[2] %	Pregnancy loss,[2] %	Calving,[3] %	Total loss,[3] %
1	Either	106	96.6 ± 2.8[a]	3.4 ± 2.8[a]	96.4 ± 2.9[a]	3.6 ± 2.9[a]
2	Bilateral	57	93.0 ± 3.8[ab]	7.0 ± 3.8[ab]	91.5 ± 4.0[ab]	8.5 ± 4.0[ab]
2	Unilateral	80	84.8 ± 3.2[bc]	15.2 ± 3.2[bc]	83.1 ± 3.3[bc]	16.9 ± 3.3[bc]
3	Bilateral	15	73.7 ± 7.3[c]	26.3 ± 7.3[c]	73.7 ± 7.7[c]	26.3 ± 7.7[c]
3	Unilateral	6	33.7 ± 11.6[d]	66.3 ± 11.6[d]	33.1 ± 12.2[d]	66.9 ± 12.2[d]

[a–d]Means ± SEM within a column without a common superscript differ, $P < 0.05$.

[1]Number of fetuses and their location within either the left or right uterine horn or both uterine horns at ultrasound pregnancy diagnosis (42 to 72 d of gestation).

[2]Percentage of females maintaining pregnancy from ultrasound pregnancy diagnosis (42 to 72 d of gestation) to palpation pregnancy diagnosis (155 to 175 d of gestation) vs. the inverse relationship.

[3]Percentage of females maintaining pregnancy from ultrasound pregnancy diagnosis to term (calving) vs. the inverse relationship.

were less ($P < 0.05$) for females with unilateral triplet ovulations compared with bilateral twin or bilateral triplet ovulations. Pregnancy rates at palpation did not differ between females with twin vs. single ovulations but were greater ($P < 0.05$) for females with bilateral triplet vs. single ovulations.

The incidence of pregnancy failure between palpation and term was small within the ovulation rate groups; thus, percentages of females calving (Table 4) were similar to pregnancy rates at palpation diagnosis. A greater ($P < 0.05$) percentage of females with bilateral twin or triplet ovulations calved compared with females with single ovulations. Percentage of females pregnant at palpation diagnosis or calving did not differ among animal ages or years (data not shown).

Maintenance of pregnancy during the second and third trimester of gestation was influenced primarily by number and distribution of the fetuses within the uterus (Table 6). Of the females gestating 1, 2, or 3 fetuses at 42 to 72 d after AI, pregnancy rates at palpation diagnosis were 96.7, 88.8, and 62.2%, respectively, differing ($P < 0.01$) among females with 1 or 2 fetuses vs. 3 fetuses. The reduction in pregnancy rates between ultrasound and palpation pregnancy diagnosis, or calving, was small for single-fetus pregnancies, whereas two-thirds of the females gestating triplets in one uterine horn and one-fourth of the females with triplets in 2 uterine horns aborted between ultrasound and palpation pregnancy diagnosis. The resulting smaller pregnancy rate at palpation for unilateral triplet fetuses differed ($P < 0.05$) from all other fetal groups. In addition, pregnancy rates were less ($P < 0.05$) for bilateral triplets compared with bilateral twins or singles at both palpation diagnosis and calving. Pregnancy rates did not differ between unilateral and bilateral twin pregnancies, but rates were reduced ($P < 0.05$) for unilateral twins compared with singles. Survival of single or unilateral twin fetuses was not affected by whether they were gestated in the left or right uterine horn. Pregnancy losses between palpation pregnancy diagnosis and term were nominal for all of the fetal groups; thus, pregnancy rates at palpation pregnancy diagnoses and calving were similar.

Plasma IGF-I Concentrations

Plasma IGF-I concentrations (Table 7) were greatest for heifers 1.5 yr of age and decreased ($P < 0.05$) with increasing animal age; thus, IGF-I concentrations were correlated negatively ($r = -0.51$; $P < 0.01$) with animal age and with the increase in BW linked with age ($r = -0.48$; $P < 0.01$). Plasma IGF-I concentrations did not differ among ovulation rate groups or by number of antral follicles ≥5 mm per female (data not shown), but IGF-I, ovulation rate, and BW were all greater ($P < 0.05$) in 2006 vs. 2004 or 2005. Plasma IGF-I concentrations did not differ between females conceiving or not conceiving to AI (156.3 ± 3.4 vs. 165.6 ± 4.5 ng/mL, respectively; $P = 0.11$).

Data for plasma IGF-I concentrations in parous cows were analyzed for the effects of number of days postpartum (range = 44 to 114 d), type of birth at the preceding calving, ovulation rate, animal age, and year. Concentrations (Table 7) were greatest ($P < 0.05$) for parous females not pregnant in the preceding calving period and were greater ($P < 0.05$) after a single vs. twin birth; IGF-I concentrations were not affected by number of days postpartum. Among the parous cows, IGF-I concentrations decreased ($P < 0.05$) with animal age and were greater ($P < 0.05$) in 2006 vs. 2004 or 2005.

DISCUSSION

Selection of cattle for spontaneous twin ovulations increased the frequency of twin and triplet ovulations (Echternkamp et al., 1990a, 2007a) and the total number of preantral (Cushman et al., 2000) and antral (Echternkamp et al., 2004) follicles within the ovaries. Although diameter of the individual ovulatory follicles varied both within and among ovulation rate groups, the trend was for the median size of the individual ovulatory follicles to decrease with increasing ovulation rate and for the minimal diameter of the twin and triplet ovulatory follicles to be 4 mm smaller than for monovular follicles. A similar relationship between ovulation rate and follicle size has been observed in ewes from

Table 7. Influence of animal age and year on the relationship between plasma IGF-I concentrations and BW

Item	n	IGF-I,[1] ng/mL	BW,[2] kg
Age, yr			
1.5	127	199.6 ± 4.8[a]	502.9 ± 4.8[a]
2	26	172.5 ± 10.8[b]	583.5 ± 10.6[a]
2.5	76	153.0 ± 6.2[b]	584.9 ± 6.2[a]
3	71	148.0 ± 6.4[bc]	645.6 ± 6.4[b]
4	54	143.8 ± 7.4[bc]	697.5 ± 7.4[c]
5	36	129.5 ± 9.0[cd]	712.7 ± 9.0[cd]
≥6	40	111.5 ± 8.6[d]	734.3 ± 8.5[d]
Year			
2004	148	134.6 ± 4.5[a]	666.3 ± 4.5[a]
2005	143	133.5 ± 4.6[a]	668.5 ± 4.5[a]
2006	146	197.7 ± 4.5[b]	692.0 ± 4.5[b]
Type of birth[3]			
0	47	171.9 ± 7.0[a]	668.3 ± 8.7
1	108	129.4 ± 4.6[b]	648.8 ± 5.8
2	110	113.0 ± 4.6[c]	667.5 ± 5.7
3	12	133.5 ± 13.9[bc]	700.6 ± 17.4

[a–d]Least squares means ± SEM within a column and category without a common superscript differ, $P < 0.05$.

[1]IGF-I concentrations were measured in blood samples collected 7 to 15 d after AI.

[2]Females were weighed at the beginning of the breeding period.

[3]Type of birth at the preceding parturition in parous cows only; 0 = nonpregnant, 1 = single, 2 = twin, and 3 = triplet birth.

prolific breeds of sheep (Webb et al., 1989; Driancourt et al., 1990). This decrease in follicle size indicates that some of the twin and triplet ovulatory follicles (e.g., F2 or F3 follicles) were developing follicles selected and ovulated at a smaller size, and possibly lesser physiological maturity, or were subordinate follicles rescued from atresia. The presence of smaller ovulatory follicles in Twinner cows concurs with previous comparisons (Echternkamp, 2000; Echternkamp et al., 2004) of follicular fluid estradiol concentrations between medium (8 to 11.9 mm) and large (≥12.0 mm) antral follicles collected at 44 h after $PGF_{2\alpha}$ from cows selected vs. not selected for twinning. Estradiol concentrations for large ovulatory follicles were similar between the 2 cattle populations, but concentrations were greater and more variable in medium follicles from selected cattle. In addition, preovulatory estradiol concentrations were greater in the blood of cows with twin ovulatory follicles, which may enhance the onset of estrus and preovulatory gonadotropin release (Echternkamp et al., 2004). Because the smaller ovulatory follicles were already present in the Twinner cows before the preovulatory increase in gonadotropin secretion, the smaller ovulatory follicles are likely the consequence of selection at a smaller size rather than rescue of a subordinate follicle(s).

Either scenario for the smaller ovulatory follicles may have compromised ovulation or oocyte competence and contributed to the reduced fertility of twin or triplet ovulatory follicles 8 to 9.9 mm in diameter because developmental competence of bovine (Arlotto et al., 1996) and human oocytes (Bergh et al., 1998) increases with increasing follicle diameter. Bovine follicles reportedly achieve ovulatory capacity at approximately 10 mm in diameter (Sartori et al., 2001). Size of the ovulatory follicle did not affect fertility in cyclic cows having a spontaneous ovulation (Perry et al., 2005). In contrast, in estrus-synchronized beef heifers, optimal follicle size to achieve pregnancy after induction of ovulation with GnRH was reported to be between 12.5 and 13.5 mm in diameter; ovulatory follicles <11.5 and >16 mm were less likely to yield a pregnancy at 27 d after breeding (Perry et al., 2007), which concurs with the reduced fertility of the 8- to 9.9-mm twin and triplet ovulatory follicles. Conversely, the reduced fertility for single ovulatory follicles ≥22 mm in diameter agrees with reports that large, persistent ovulatory follicles resulting from suboptimal progesterone dosages frequently yield a poorer quality oocyte, leading to reduced embryonic survival (Ahmad et al., 1995; Vasconcelos et al., 1999).

Ovulatory follicles being of smaller diameter in nulliparous heifers compared with parous cows concurs with previous observations in heifers that size of the dominant follicle progressively increased with animal age from 2 to 34 wk (Evans et al., 1994). Follicle diameter was not affected by number of days postpartum in the current study; however, these cows were between 44 and 114 d postpartum, in positive BW gain, and cyclic, so an effect of postpartum interval on follicle diameter was unlikely.

Within pregnant females, conception rates (i.e., fetus:CL ratio) for the individual twin (0.83) or triplet (0.73) ovulations were less than 1.00. Likewise, twin and triplet ovulations increased the number of calves per parturition only 1.6- and 2-fold, respectively, rather than 2- or 3-fold. Some of the infertile triplet ovulations were linked with the reduced fertility of the 8- to 9.9-mm ovulatory follicles; 19% of the triplet ovulations

were within that size range, whereas only 6% of the twin ovulations were 8 to 9.9 mm in diameter. Failure of 15% of the individual twin ovulations and 27% of the triplet ovulations to yield a conceptus in conceiving females indicates that a significant proportion of the multiple ovulations were infertile. These losses likely resulted from ovulation or oocyte anomalies causing fertilization failure or early embryonic mortality, as opposed to uterine or placental anomalies or insufficiencies compromising conceptus development and maintenance of pregnancy. Similar reproductive losses have been reported for monovular cattle; 20 to 30% of the embryos were either degenerate or absent from the reproductive tract by 12 d after breeding (Maurer and Chenault, 1983; Maurer and Echternkamp, 1985). Thus, regardless of cause, it appears that 15 to 20% of the ovulations in cattle are inherently infertile. In addition, pregnancy rates are generally 10 to 15% less for cyclic parous cows in the Twinner population compared with contemporary cows in other research populations at USMARC (unpublished data), indicating that additional causes of infertility are unique to the Twinner population.

Diameter of the individual CL decreased and total CL volume and plasma progesterone concentrations increased with increasing ovulation rate in patterns similar to those for ovulatory follicles. Thus, diameters of the ovulatory follicles and their resulting CL were correlated positively, and total ovulatory follicle volume was correlated positively with plasma progesterone concentrations 7 to 15 d after AI, indicating that size and functionality of the CL were influenced by size of the ovulatory follicle of origin for both spontaneous and induced (Vasconcelos et al., 2001) ovulations. However, the progressive increase in plasma progesterone concentrations between 7 and 15 d after AI was not accompanied by a significant increase in total CL volume; thus, plasma progesterone concentrations are contingent on factors in addition to total size of the luteal tissue mass or previous follicle size. In addition, plasma progesterone concentrations were greater in nulliparous heifers (1.5 yr of age) compared with parous cows ($\geq$2.5 yr of age), whereas follicle and CL diameters were smaller for the nulliparous heifers. The greater progesterone concentrations in nulliparous heifers may have been a consequence of their smaller body size and blood distribution volume or of decreased feed intake and metabolism of progesterone (Sangsritavong et al., 2002) rather than increased luteal steroidogenesis in the heifers compared with the larger, lactating parous cows. Progesterone concentrations were correlated negatively with animal BW.

Although progesterone concentrations and pregnancy rates were greater for females with twin or triplet ovulations vs. a single ovulation, progesterone concentrations and total CL volume 7 to 15 d after AI did not differ within ovulation rate categories between females conceiving or not conceiving to AI. Some investigators found greater blood progesterone concentrations in those females conceiving and attributed the greater fertility to the increased progesterone concentrations (Mann and Lamming, 2001; Inskeep, 2004); however, the increased pregnancy rates for twin and triplet ovulations are likely the consequence of the additional oocytes rather than greater progesterone concentrations. Alternatively, the additional embryos with twin or triplet ovulations may have increased the production of embryonic or luteotropic factors to facilitate the establishment and maintenance of pregnancy within the uterus and to increase progesterone synthesis by the CL. Plasma progesterone concentrations also were greater in pregnant females with 2 or 3 CL or fetuses at 42 to 72 d after AI. Because the number of fetuses was sometimes less than the number of CL within females with 2 or more CL, plasma progesterone concentrations during this stage of gestation more closely reflected the number of CL rather than the number of fetuses when evaluated separately; thus, progesterone concentrations in the blood were more likely influenced by the total amount of luteal tissue rather than the total amount of placental tissue. The effect of animal age on plasma progesterone concentration was still detectable in the pregnant females at d 42 to 72 of gestation, decreasing with increasing age.

Comparisons of IGF-I concentrations between cattle selected vs. not selected for twin births indicated greater IGF-I in the blood and ovarian follicular fluid of Twinner cattle (Echternkamp et al., 1990b, 2004; Echternkamp, 2000). Similarly, both ovulation rate and IGF-I were greater in 2006 compared with 2004 or 2005; however, IGF-I concentrations were not linked to differences in ovulation rate or total number of antral follicles $\geq$5 mm among the Twinner females, negating the potential use of blood IGF-I measurements as a subphenotype to estimate propensity for multiple ovulations or twinning.

A decline in GH synthesis and secretion concomitant with a decline in IGF-I secretion during normal aging is well documented in mammalian species (Müller et al., 1993) and concurs with IGF-I concentrations being greater in nulliparous heifers vs. parous cows and the continued decline in IGF-I concentrations with increasing age in the present study. Elevations in blood IGF-I concentrations are generally associated with positive energy status, especially in postpartum beef cows (Spicer et al., 2002), which concurs with IGF-I concentrations being greater in parous cows without a parturition vs. lactating cows and after a single vs. twin parturition. Plasma IGF-I concentrations were not influenced by days postpartum in the present study. Again, these Twinner cattle were between 44 and 114 d postpartum, cyclic, and in positive BW gain, whereas previously reported associations between postpartum infertility and IGF-I were primarily early in the postpartum period and associated with restricted dietary energy intake (Roberts et al., 1997). In addition, plasma IGF-I concentrations did not differ between females conceiving or not conceiving to AI, indicating that es-

tablishment of pregnancy was not influenced by plasma IGF-I concentrations in females with adequate nutritional supplementation.

Development of the USMARC cattle population with an annual twinning rate of 50 to 60% (Gregory et al., 1990; Echternkamp et al., 1990a, 2007b) affords an opportunity to increase both reproductive and economic efficiency in beef cattle through the production of additional calves per dam. Females conceiving to twin or triplet ovulations produced 60 and 100% more calves, respectively, per parturition than females with a single ovulation (1.6 vs. 2.0 vs. 1.0 calves/parturition, respectively). The primary selection response has resulted in increased ovarian folliculogenesis and a greater frequency of twin, triplet, and quadruplet ovulations (Gregory et al., 1990; Echternkamp et al., 1990a, 2007a), the first prerequisite for fraternal twin, triplet, and quadruplet births. In the current study, ovulation rate increased from 2.0 in 2004 to 2.3 in 2006. The greater ovulation rate in 2006 coincided with 1 female having an ovulation rate of 6 CL, 2 females having an ovulation rate of 5 CL, and a greater proportion of females having quadruplet ovulations. Concurrent with previous findings (Echternkamp et al., 2004), females with an increase in ovulation rate had increased numbers of antral follicles.

Increases in ovulation and twinning rate in cattle can impose some constraints to improving productivity, such as calving rates being 10 to 15% less in the Twinner population relative to other research populations at USMARC (Echternkamp et al., 2007b). The reduced fertility for Twinners is likely the consequence of both current and previous reproductive performance. For example, the increased ovulation rate in 2006 was not accompanied by an increase in fetal numbers because the 2 females with 5 CL were not pregnant, and the female with 6 CL conceived triplets. In addition, females with 4 CL (n = 11) had reduced fertility and failed to birth quadruplet calves; however, a set of live quadruplet calves was born in the spring of 2004. As observed previously (Echternkamp et al., 1990a), pregnancy rates were greater early in gestation for cattle with twin or triplet ovulations vs. a single ovulation, but these differences had narrowed by palpation pregnancy diagnosis (155 to 179 d after AI) because of a greater incidence of pregnancy failure in females with twin or triplet ovulations, especially when the twin or triplet fetuses were localized to one uterine horn. These observed increases in pregnancy loss in some, but not all, females gestating twin or triplet fetuses may reflect maternal differences in uterine capacity and fetal support. Some evidence exists for Twinner females having a superior maternal uterine environment for fetal development and for the existence of a QTL linked to twinning rate separate from ovulation rate (Thallman et al., 2000). In cattle, chorionic blood vessels begin to anastomose between placentas of twin fetuses at approximately 40 d of gestation, and death of a fetus after anastomosis of the blood supplies generally terminates the pregnancy (Echternkamp, 1992); thus, cattle gestating twins or triplets have a greater abortion rate than the 2 to 3% mortality found in cattle with a single fetus (Hawk, 1979; Echternkamp et al., 2007a). Only approximately 50% of the twin ovulations at breeding actually yield a twin birth. In contrast to a previous study (Echternkamp et al., 2007b), there was no decreased maintenance of unilateral triplet pregnancies in nulliparous females or decreased fertility in cows ≥6 yr of age in the present study.

In summary, selection for increased ovulation rate in this population of cattle increased variability in the size of the ovulatory follicles, but the general trend was for the diameter of the ovulatory follicles to decrease with increasing ovulation rate. An apparent effect of follicle size on fertility was a reduction in fertility for ovulatory follicles 8 to 9.9 mm in diameter in females with twin or triplet ovulations vs. a reduction in fertility for large monovular ovulatory follicles (≥22 mm). The increased proportion of small ovulatory follicles associated with twin and triplet ovulations indicates that some of the ovulatory follicles were either selected at a lesser stage of maturity or rescued while undergoing atresia, thus compromising oocyte competency. Size and functionality of the CL were also influenced positively by the size of the follicle of origin; however, the greater blood progesterone concentrations with twin and triplet ovulations did not increase calving rates. Of greatest importance for reducing fertility in cattle expressing twin or triplet ovulations was the greater incidence of pregnancy losses occurring during the last two-thirds of gestation in females gestating 2 or more fetuses as an apparent effect of uterine crowding, especially when 2 or more fetuses were gestating in one uterine horn.

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