Effects of Twinning on Postpartum Reproductive Performance in Cattle Selected for Twin Births[1,2,3]

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ABSTRACT: The effects of twinning, dystocia, retained placenta, and body weight on postpartum reproduction were evaluated for 3,370 single and 1,014 twin births. Females were bred by AI for 40 d followed by 20 or 30 d of natural service with equal numbers bred and calved in spring and fall. Percentage of dams cyclic by the end of the AI period was lower (*P* < .05) for dams birthing and nursing a single calf (92.4%) than for dams birthing twins and nursing zero (98.7%) or two (94.7%) calves. Whereas the interval from parturition to first estrus was shorter (*P* < .01) for dams birthing and nursing a single (56.9 d) than for dams birthing twins and nursing one (68.5 d) or two (69.6 d) calves, length of the interval was further reduced by dystocia in nonlactating dams of either twins or singles (type of birth × dystocia, *P* < .05). Ensuing pregnancy rates were also affected by type of birth and dystocia. Without dystocia, dams birthing and nursing a single calf had a higher pregnancy rate (79.2%) than dams birthing twins and nursing one (61.7%) or two (66.3%) calves, whereas the lower ensuing pregnancy rates associated with dystocia in dams of singles (71.9%) resulted in similar rates among dams of singles and twins with dystocia (type of birth × dystocia; *P* < .01). Having a retained placenta resulted in a lower incidence of (93.5 vs 96.4%, with vs without; *P* < .05) and a longer interval to (64.7 vs 59.2 d; *P* < .01) estrus while reducing subsequent pregnancy rates ($\overline{X}$ = 9.6%) in 3 of the 7 yr evaluated (retained placenta × year, *P* < .01). Because all parous females were bred during the same calendrical period, the shorter gestation length for twin calves (275.6 vs 281.3 d) resulted in a longer interval from parturition to conception for twin births, whereas means for conception date differed by only 2 d between dams of twins and singles. Furthermore, a reduction (*P* < .01) in the interval to conception occurred with dystocia in dams of singles (89.3 vs 85.0 d, without vs with dystocia) and of twins nursed by zero (116.9 vs 83.5 d), one (100.2 vs 92.8 d), or two (96.1 vs 97.2 d) calves. Another detriment to fertility was the higher incidence of fetal mortality or abortions associated with twin vs single pregnancies (12.4 vs 3.5%; *P* < .01). However, despite the lower conception rates for dams of twins, the increased prolificacy provides an opportunity to increase total beef production with a twinning technology.

Key Words: Twinning, Reproductive Performance, Postpartum Interval, Fetal Death, Beef Cattle

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Introduction

Annual frequency of natural twin births in cattle selected primarily for twin ovulations at the Roman L. Hruska Meat Animal Research Center (MARC) now exceeds 35%. Thus, the production of twin calves presents a new paradigm in beef cattle management and production and affords an opportunity to increase both reproductive and economic efficiency. However, part of the potential economic gain from twinning in cattle is compromised by reduced calf survival (Gregory et al., 1996), by increased incidence of dystocia (Cady and Van Vleck, 1978; Gregory et al., 1990b, 1996) and retained placenta (Turman et al., 1971; Bellows et al., 1974; Anderson et al., 1982;

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[3]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 same by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Guerra-Martinez et al., 1990), and by reduced and(or) delayed conception after twinning (Erb and Morrison, 1959; Turman et al., 1971; Gregory et al., 1990b). The objective of this study was to evaluate the effects of twinning on postpartum reproduction and fetal survival in a population of cattle with a high frequency of natural twinning.

Materials and Methods

Reported data are a summary of results for a 7-yr period (1988 through 1994) of a comprehensive study at MARC to evaluate the feasibility of increasing twinning in cattle by selecting for ovulation rate in puberal heifers and twinning rate in parous cows (Gregory et al., 1990a, 1997). Management procedures, selection protocols, and historical data on herd formation and breed composition have been reported previously (Echternkamp et al., 1990; Gregory et al., 1990a,b, 1996). Females were bred to calve first at 2.5 yr of age. Breeding seasons were 70 or 60 d long (i.e., 40 d of AI followed by 30 [spring] or 20 [fall] d of natural service). Spring breeding was from late May until early August and fall breeding was from late October until late December. Breeding season for the nulliparous heifers began approximately 2 wk earlier than for the parous cows. Approximately equal numbers of females were bred to calve in the spring and fall. Cows were monitored twice daily for signs of estrus starting 40 d after the first parturition (i.e., full-term pregnancy) and continuing through the AI period.

Beginning with spring breeding 1990, all females were examined by ultrasonography between 40 and 70 d of gestation to determine number of fetuses in utero. Examinations were performed transrectally by scanning the dorsal surface of the uterine body and horns using a real-time ultrasound scanner (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) equipped with a 3.5-MHz convex array rectal probe. Pregnancy was reconfirmed by rectal palpation at an average of 100 d of gestation. Cows diagnosed with twins (i.e., 1991 through 1994) were fed a high-energy diet starting an average of 70 d before the beginning of the calving season. Cows birthing twins were always provided a higher plane of nutrition postpartum than were dams of singles. Cross-fostering was used for one of the calves in a set of triplets and for twins if there was a high probability of calf death if both remained with their dam.

Throughout the calving season, cows were monitored frequently, especially cows gestating twins, for symptoms of parturition. Obstetrical assistance was provided to dams of twins within .5 to 1 h after detection of approaching parturition and sooner than for singles. Criteria for classification of dystocia were the same as described previously (Echternkamp and Gregory, 1998).

Fetal membranes not sloughed within 48 h after parturition were classified as retained placenta. Treatment protocol for a retained placenta included an initial treatment of penicillin/streptomycin (200,000 units penicillin G and 250 mg dihydrostreptomycin/mL; 4.5 mL/100 kg BW) i.m., 6 mg of estradiol cypionate (ECP, Upjohn Co., Kalamazoo, MI) i.m., and 50 mL of oxytetracycline hydrochloride (100 mg/mL) intrauterine on d 3 postpartum; nonlactating dams also received 10 USP units of oxytocin i.m. Dosage of penicillin/streptomycin was increased to 7.7 mL/100 kg BW in 1993. The antibiotic-oxytocin treatment was continued daily until the placenta was sloughed.

Body weight was measured at 1) an average of 70 d before beginning of the calving season, 2) the beginning of the breeding season, and 3) the end of the breeding season.

Data were analyzed by least squares fixed-model procedures (Harvey, 1985). Data for triplet births (28 sets) were excluded from the statistical analyses. The following traits were evaluated: percentage detected and days to first estrus prior to the breeding period; percentage detected and days to first estrus through the AI period; percentage pregnant at calving; days to conception; calving date; conception date; postpartum BW (i.e., beginning and end of breeding season); and postpartum BW change. Fixed effects in the model were type of birth-lactational status (**TOB-N**), dystocia, retained placenta, age of dam, year of birth, season of birth (spring vs fall), and all possible two-way interactions. Nonsignificant ($P > .10$) two-way interactions were deleted from the final analyses for each trait, and three-way interactions were assumed to be nonsignificant. Lactation variables were the consequence of calf death or management decision. Statistical models for analysis of calving date, conception date, and the intervening interval were modified to evaluate only main effects and to evaluate data for spring- and fall-calving dams separately. Comparisons of means were conducted with a *t*-test. Differences in fetal mortality between diagnosed single and twin pregnancies were assessed for each time period with chi-square analysis.

Results and Discussion

Postpartum Estrus

Estrus was detected in 94.4% of the dams by the end of the AI season. Although the percentage of dams expressing estrus by the end of the AI season was similar ($P > .10$) for both seasons, a significantly higher percentage of fall- vs spring-calving dams expressed estrus prebreeding (73.8 vs 50.6%, respectively; $P < .01$). Therefore, data for estrus were evaluated for two time periods: 1) prebreeding alone and 2) prebreeding and AI season combined.

Prebreeding Estrus. The F-values and least squares means for significant effects of twinning, age of dam, season, year, and their significant interactions on the reinitiation of estrous cycles before the breeding season are reported in Tables 1 and 2, respectively. The general trend was for fall-calving dams to have a higher ($P < .01$) incidence of detected estrus prebreeding than spring-calving dams; the exception was nonlactating dams of twins, which had a high incidence of estrus in both seasons (TOB-N × season; $P < .01$). In addition, estrus detection did not differ between dams birthing and nursing twins vs singles in the fall (Table 2), but dams birthing and nursing twins had a lower incidence of estrus detection in the spring (TOB-N × season; $P < .01$).

The negative effect of lactation on return to estrus was reflected in lactating dams (i.e., either single or twin birth) having a lower ($P < .01$) incidence of estrus than nonlactating dams in both seasons. The increased estrus detection in fall-calving dams coincided with a shorter ($P < .01$) interval (~1 wk) from parturition to first estrus for fall- vs spring-calving dams, except in nonlactating dams of singles, in which the interval did not differ between seasons (TOB-N × season; $P < .05$). The interval from parturition to first estrus prebreeding (Figure 1) was consistently longer for dams of twins nursing one or two calves than for dams birthing and nursing a single, whereas the interval varied significantly among years for nonlactating dams of twins (TOB-N × year; $P < .01$).

Despite the variation among TOB-N groups both within and between years (TOB-N × year; $P < .05$), there was a general trend for prebreeding estrus detection to increase ($P < .01$) between 1989 and 1994 with the increase being of greater magnitude for dams of twins nursing one or two calves than for dams birthing and nursing a single (Figure 2). In contrast, the interval from parturition to first estrus did not change with years (Figure 1), except in nonlactating dams of twins in which the interval increased between 1988 and 1994 (TOB-N × year; $P < .01$). In addition to providing increased prepartum dietary energy for females diagnosed with twins, there was a general improvement in the nutritional state of the herd across years, which apparently enhanced the return to estrus for dams of singles, also. Similarly, fall-calving dams had greater ($P < .01$) BW gains during the breeding season (Table 3) and higher ($P > .01$) incidences of detected estrus prebreeding than spring-calving dams, and nonlactating dams of twins were the heaviest ($P < .01$) postpartum (Table 3) and had the highest ($P < .01$) estrus detection prebreeding. Collectively, these observations suggest that plane of nutrition and(or) BW had a positive effect on the reinitiation of estrous cycles in dams of both twins and singles.

Table 1. Summary of F-statistics from analysis of variance for postpartum estrus and conception

Variable	df	Estrus (prebreeding) %	Estrus (prebreeding) Days	Estrus (total season) %	Estrus (total season) Days	Conception %	Conception Days
		F-value					
Status (TOB-N[a])	4	17.54**	6.24**	2.52*	52.88**	8.61	20.94**
Dystocia (D)	1	10.99**	21.53**	2.54	15.25**	2.77	19.99**
Retained placenta (RP)	1	1.56	13.91**	3.94*	18.59**	12.79**	25.69**
Age (A)	3	12.32**	12.69**	3.79**	15.23**	4.32**	24.29**
Year (Y)	6	8.11**	.80	3.40**	4.04**	2.96**	1.41
Season (S)	1	44.10**	4.63*	4.92*	377.56**	22.52**	.004
TOB-N × D	4	—	9.83**	—	6.35**	3.74**	6.54**
TOB-N × RP	4	—	3.68**	—	—	—	—
TOB-N × Y	24	1.65*	2.29**	—	3.15**	2.46**	2.01**
TOB-N × S	4	4.07**	4.29**	—	—	—	3.64**
D × RP	1	—	—	—	—	—	4.84*
D × A	3	—	—	—	—	3.13*	—
D × S	1	—	6.08*	—	—	—	4.36*
RP × Y	6	—	—	—	—	3.59**	—
RP × S	1	—	21.16**	—	—	—	8.91**
A × Y	18	1.84*	1.67*	—	2.81**	—	2.10**
A × S	3	4.45**	—	—	—	—	—
Y × S	6	7.79**	2.61*	—	6.44**	—	6.77*
Residual mean square	—	2,074.13	207.35	523.78	360.26	1,575.48	408.61
(df)	—	(4,262)	(2,296)	(4,317)	(4,023)	(4,280)	(3,046)

[a]Type of birth by number of calves nursed.
*$P \le .05$.
**$P \le .01$.

Two-year-old dams had the highest percentage returning to estrus but the longest ($P < .01$) postpartum anestrous period (Figure 3); however, the effects of age were also influenced by season (age × season; $P < .01$) and year (age × year; $P < .05$). For example, the higher incidence of estrus (Table 2) for 2-yr-old dams differed from dams ≥ 5 yr of age in both seasons but from 3- and 4-yr-olds in the spring only; 3- and 4-yr-old dams also differed from dams ≤ 5 yr of age in the spring (age of dam × season; $P < .01$). In addition, the decline in estrus detection with increasing age was significantly greater in spring- than in fall-calving dams (age of dam × season; $P < .01$). Estrus detection also varied among ages within and between years (age of dam × year; $P < .05$), but the increase in estrus detection from 1989 to 1994 was evident for all age groups (Figure 3). The higher incidence of estrus and the longer postpartum anestrous period for first-parity dams likely resulted from nulliparous heifers being exposed to bulls 2 wk earlier

Table 2. Least squares means for percentage of dams returning to estrus prebreeding and interval from parturition to estrus

Variable	n	% or d	n	% or d
Estrus detection, %				
	No		Yes	
Dystocia	3,111	62.7	1,223	57.3
	Spring		Fall	
TOB-N × season**				
1, 0[a]	57	57.7	60	79.8
1, 1	1,670	37.8	1,569	65.9
2, 0	41	93.4	28	86.3
2, 1	99	31.5	134	63.5
2, 2	320	23.0	356	60.9
Age × season**				
2 yr	595	58.4	540	73.4
3 yr	513	50.2	481	72.1
4 yr	408	46.2	353	71.1
> 5 yr	671	39.9	773	68.4
Days to estrus				
	No		Yes	
TOB-N × dystocia**				
1, 0	46	54.4	38	49.9
1, 1	1,405	47.1	361	47.8
2, 0	28	69.1	33	45.2
2, 1	62	56.5	67	54.8
2, 2	194	55.6	141	56.5
TOB-N × retained placenta**				
1, 0	76	41.4	8	62.9
1, 1	1,740	45.7	26	49.2
2, 0	30	56.2	31	58.1
2, 1	102	53.1	27	58.2
2, 2	262	56.4	73	55.6
	Spring		Fall	
TOB-N × season**				
1, 0	34	48.5	50	55.7
1, 1	694	48.2	1,072	46.8
2, 0	37	64.2	24	50.1
2, 1	38	57.6	91	57.3
2, 2	97	58.2	238	53.8
Dystocia × season*				
No	639	57.3	1,096	55.8
Yes	261	53.4	379	48.3
Retained placenta × season**				
No	816	55.3	1,394	45.7
Yes	84	55.4	81	58.3

[a]Type of birth (1 = single and 2 = twins) followed by number of calves nursed postpartum.
*$P \leq .05$.
**$P \leq .01$.

in the previous breeding season and calving 8 d earlier (Table 6) than parous females, providing first-parity dams additional time to reinitiate estrous cycles.

Interval to estrus in nonlactating dams was also influenced by occurrence of a retained placenta (TOB-N × retained placenta; $P < .05$) and dystocia (TOB-N × dystocia; $P < .01$). Having a retained placenta delayed the return to estrus in nonlactating dams of singles and in dams of twins nursing one calf (TOB-N × retained placenta; $P < .05$), which resulted in the overall increase ($P < .01$) in the anestrous interval associated with retained placenta. The TOB-N × dystocia interaction ($P < .01$) resulted from an extended interval to estrus in nonlactating dams of twins without dystocia, whereas dystocia had no effect on the return to estrus for dams in the other TOB-N groups.

Total Season. The cumulative percentage of dams detected in estrus through AI (94.4%), or artificially inseminated (91.6%, unpublished data), was high (Table 4). In general, dams birthing twins had a

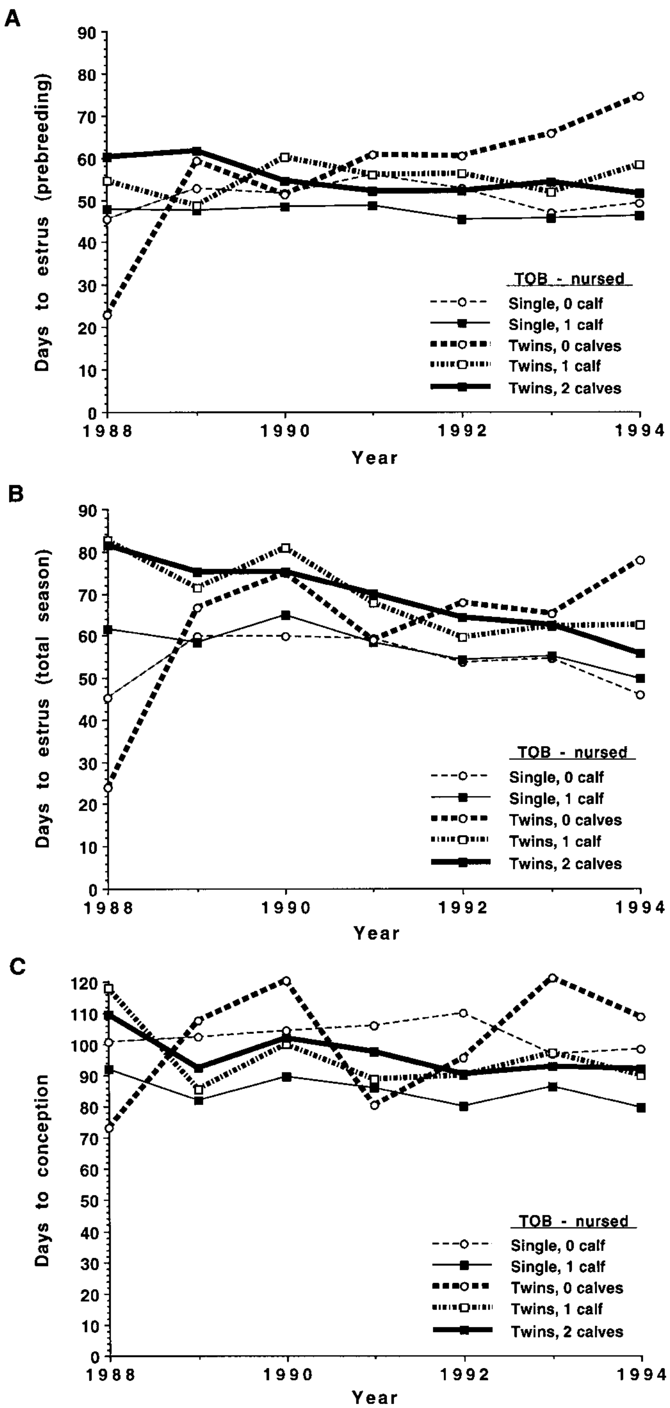

Figure 1. Time trend for effect of type of birth (1 = single and 2 = twin) and number of calves nursed postpartum (TOB-nursed) on interval to first estrus prebreeding (panel A), interval to first estrus (total season, panel B), and interval to conception (panel C). TOB-nursed × yr, $P \leq .01$.

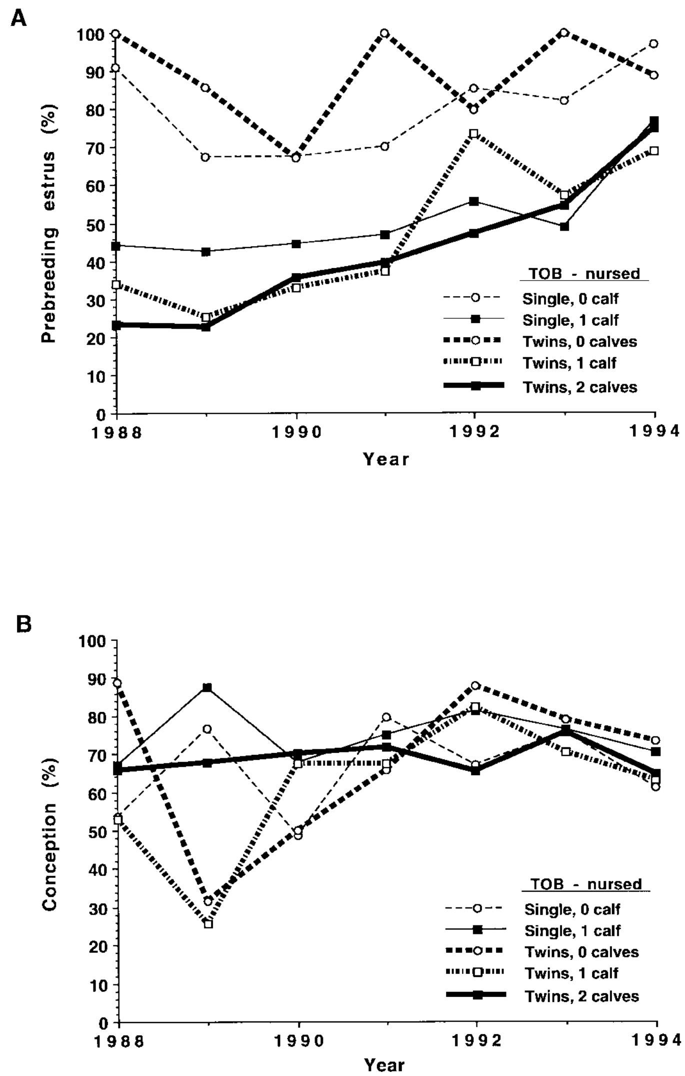

Figure 2. Time trend for effect of type of birth (1 = single and 2 = twin) and number of calves nursed postpartum (TOB-nursed) on percentage of dams expressing estrus before the breeding season (panel A, TOB-nursed × yr, $P \leq .05$) and on percentage of dams conceiving (panel B, TOB-nursed × yr; $P \leq .01$).

higher incidence of estrus than dams of singles; the difference was statistical significant ($P < .05$) between dams birthing and nursing a single and dams birthing twins and nursing zero or two calves. Thus, it is unlikely that the 5.9% lower pregnancy rate for dams of twins vs singles, or the low pregnancy rates in general, resulted from prolonged postpartum anestrus or failure to reinitiate estrous cycles within the breeding season. However, the higher incidence of estrus detection prebreeding in dams of singles vs twins implies that dams of singles had more estrous cycles prior to the breeding season, and this may have enhanced fertility. Incidence of estrus detection was reduced ($P < .05$) in dams with a retained placenta and in dams ≥ 5 yr of age vs 2 or 3 yr of age, which concurred with observations for prebreeding estrus detection. Despite the higher incidence of estrus prebreeding in fall-calving dams, the overall incidence of estrus detection was slightly lower ($P < .05$) in fall-calving dams. Also, more ($P < .05$) females were detected in estrus in 1989, 1990, and 1994 than in 1988, 1991, 1992, and 1993.

The interval from parturition to first estrus (total season) was affected by several variables (Tables 1 and 4). Collectively, the postpartum anestrous period was 11.9 d longer ($P < .01$) for dams of twins than for

Table 3. Factors affecting prebreeding and postbreeding BW and BW change during the breeding season

Variable	df		Prebreeding BW		Postbreeding BW		BW Change	
Analysis of variance			*F*-value					
Status (TOB-N[a])	4		5.5**		16.4**		27.5**	
Dystocia (D)	1		.001		.000		.01	
Age (A)	3		628.5**		674.2**		2.2	
Year (Y)	6		1.4		3.7**		21.6**	
Season (S)	1		5.9*		5.0*		89.7**	
TOB-N × D	4		2.4*		2.9*		.5	
TOB-N × Y	24		1.9**		1.9**		6.6**	
TOB-N × S	4		3.7**		2.1		4.5**	
A × Y	18		7.1**		3.8**		8.1**	
A × S	3		22.4**		13.2**		9.6**	
Y × S	6		21.4**		66.9**		138.2**	
Residual mean square	4,164		3,773.7		3,576.0		896.9	
Least squares mean	n		kg		kg		kg	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
TOB-N[a]								
1, 0	55	60	634.8	637.9	649.8	682.6	15.1	44.7
1, 1	1,629	1,552	609.5	612.0	609.0	623.6	–.6	11.6
2, 0	38	28	671.5	625.2	694.8	678.6	23.3	53.5
2, 1	95	131	645.6	625.4	635.3	638.0	–10.3	12.7
2, 2	304	355	625.1	628.7	612.5	630.2	–12.6	1.6
Age of dam								
2 yr	581	537	582.2	544.6	579.7	572.2	–2.5	27.7
3 yr	505	477	624.1	612.7	628.7	635.3	4.5	22.6
4 yr	396	350	652.2	657.0	660.5	682.0	8.4	25.0
> 5 yr	639	762	690.7	689.0	692.3	713.0	1.6	24.0
Year								
1988	166	219	625.8	627.3	658.2	671.4	32.5	44.2
1989	254	334	626.7	640.0	577.8	657.8	–49.0	17.9
1990	317	321	664.3	615.4	656.6	639.6	–7.8	24.0
1991	355	310	617.6	629.7	647.0	663.7	29.4	34.1
1992	381	293	654.0	633.3	673.2	626.7	19.0	–6.7
1993	335	323	646.0	612.3	642.8	631.1	–3.2	18.8
1994	313	326	626.6	622.7	626.5	664.0	–.2	41.4
			Dystocia		Dystocia			
TOB-N[a]			No	Yes	No	Yes		
1, 0	62	53	623.1	649.6	651.8	680.6		
1, 1	2,481	700	607.2	614.4	612.6	620.0		
2, 0	27	39	664.3	632.4	701.0	672.5		
2, 1	109	117	637.6	633.3	640.6	632.7		
2, 2	370	289	625.2	628.5	621.1	621.6		

[a]Type of birth (1 = single and 2 = twin) followed by number of calves nursed postpartum.
*$P \le .05$.
**$P \le .01$.

dams of singles; however, length of the interval did vary among years of birth (TOB-N × year; $P < .01$, age of dam × year; $P < .01$, and year × season; $P < .01$). Comparison of postpartum intervals among years (Figure 1) revealed that dams of twins nursing one or two calves consistently had a longer ($P < .01$) interval from parturition to first estrus compared to dams of singles, and that the postpartum anestrous period decreased ($P < .01$) between 1988 and 1994, except in nonlactating dams of twins, in which the interval increased (TOB-N × year; $P < .01$). Again, the TOB-N × dystocia interaction ($P < .01$) resulted from an extended interval to estrus in nonlactating dams of twins without dystocia, whereas dystocia had no effect on the return to estrus for dams in the other TOB-N groups (Table 4). Having a retained placenta increased ($P < .01$) the interval about 1 wk (Table 4); however, 92.8% of these cows did express estrus by the end of the AI season. Retained placentae were associated primarily (i.e., 81.6%) with twin births, and with shorter gestation lengths (Echternkamp and Gregory, 1998) and correspondingly earlier calving dates (Table 6). Thus, dams with retained placenta had a longer interval from parturition to breeding, and most of the spring-calving dams were first detected in estrus during the breeding season. Although the effect of age of dam on return to estrus (Figure 4) varied among years (age of dam × year, $P < .01$), 2-yr-old dams took longer ($P < .01$) to return to estrus than 3-, 4-, or ≥ 5-yr-old dams. Fall-calving dams consistently returned to estrus sooner ($P < .01$) than spring-calving dams, but the magnitude of the difference varied among years (year × season; $P < .01$). Because the earlier onset of estrus in the fall or in recent years (Figure 1) was not accompanied by an increase in pregnancy rates, it is suggested that factors other than folliculogenesis or return to estrus contribute to the lower pregnancy rate in dams of twins.

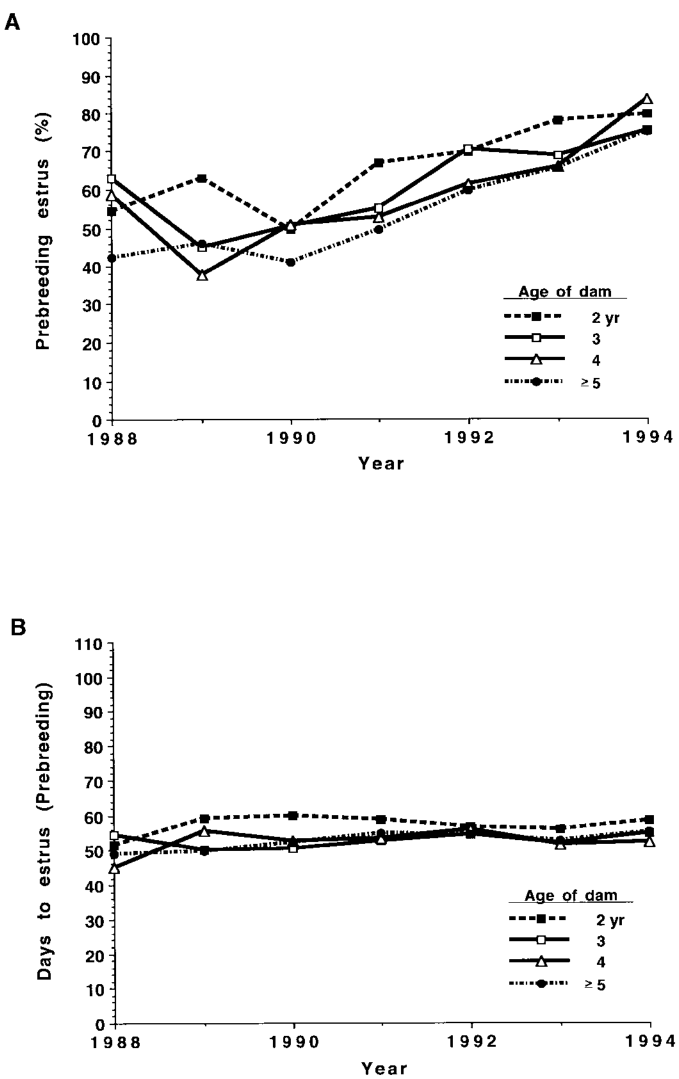

Figure 3. Time trend for effect of age of dam on expression of (panel A) and interval to first estrus prebreeding (panel B). Age × yr, $P \leq .05$.

A longer interval from parturition to first estrus, ovulation, or conception in dams of twins vs singles is well documented (Turman et al., 1971; Cady and Van Vleck, 1978; Wheeler et al., 1982; Guerra-Martinez et al., 1990). Such delays may result from additional lactational stress with twins; weaning the calves soon after birth removed the negative effect of twins on postpartum estrus (Bellows et al., 1974), and weaning enhanced conception (Turman et al., 1971). Likewise, the interval from parturition to first estrus and ovulation was lengthened in beef cows birthing one calf but nursing two calves (i.e., fostered second calf) independent of nutritional influences (Wyatt et al., 1977; Wetteman et al., 1978). The additional calf increased milk production 39%, and their dams required 72% more supplemental feed compared to dams nursing one calf, as well as increasing the frequency and total time the cow was nursed (Wyatt et al., 1977). In the present study, reinitiation of estrous cycles was also enhanced in nonlactating dams of twins, which were also heavier and fatter postpartum than nursed dams of twins; return to estrus did not differ between dams of twins nursing one vs two calves. In contrast to the preceding studies, present lactational treatments were a consequence of pre- or perinatal calf mortality associated with dystocia and(or) environmental and management consequences rather than randomized assignment to lactational treatments; thus, the return of estrous cycles was likely influenced by a combination of factors in the present study.

Pregnancy Rate

Pregnancy rates (measured at calving) for dams exposed in 1988 through 1994 are illustrated in Figure 2. Pregnancy rate was highly variable among TOB-N groups both within and among years (TOB-N × year;

$P < .01$), but dams birthing and nursing a single had a higher ($P < .01$) ensuing pregnancy rate (Figure 2) than dams birthing and nursing twins in 1989 (88.4 vs 68.6%, single vs twin) and in 1992 (82.3 vs 66.4%, respectively) and than dams birthing twins and nursing one calf overall (75.6 vs 59.6%, respectively). The overall effect of twinning was a 5.9% ($P = .06$) reduction in conception at the subsequent breeding season. The cause(s) for the lower pregnancy rate in dams of twins was not apparent but may have been associated with the increased incidence of dystocia and(or) retained placenta. As noted before, nursing differences were primarily the result of calf mortality within 72 h after birth, associated with premature birth or with dystocia (Gregory et al., 1996). However, a reduction in conception after dystocia (Table 5) was found only in dams of singles, whereas dystocia had no effect on subsequent pregnancy rate in dams of twins (TOB-N × dystocia; $P < .01$).

The 9.6% decrease in pregnancy rate associated with retained placentae resulted from significant ($P < .01$) reductions in pregnancy rate in 1988 (53.2 vs 77.1%, with vs without), 1990 (50.9 vs 74.1%), and 1994 (55.7 vs 77.9%, respectively; retained placenta × year; $P < .01$). Because the majority of the retained placentae were associated with twin births (Echternkamp and Gregory, 1998), the reduction in fertility occurred primarily in dams of twins.

Pregnancy rates (Table 5) were consistently lower ($P < .01$) in fall- vs spring-calving dams. Conversely, fall-calving dams had a 40% lower ($P < .01$) incidence of retained placenta, a positive weight gain postpartum, and a higher ($P < .01$) incidence of and a shorter ($P < .01$) interval to estrus prebreeding, all factors that are reported to increase pregnancy rate. However, dietary differences did exist between the spring- and fall-breeding seasons; dams were managed on improved pasture during the spring breeding season vs a

Table 4. Least squares means from analysis of variance for postpartum estrus (total season)

	Estrus			
Variable	n	%	n	Days
TOB-N[a]		*		**
1, 0	117	93.5	111	52.7
1, 1	3,239	92.4	3,046	56.9
2, 0	69	98.7	68	62.0
2, 1	233	95.4	223	68.5
2, 2	676	94.7	644	69.9
Retained placenta		*		**
No	4,001	96.4	3,781	59.2
Yes	333	93.5	311	64.7
Age of dam		**		**
2 yr	1,135	95.2	1,072	65.5
3 yr	994	96.4	955	60.3
4 yr	761	94.8	721	59.9
≥ 5 yr	1,444	93.3	1,344	61.9
Year		**		**
1988	392	93.9	366	59.3
1989	593	97.0	570	65.0
1990	648	97.0	624	68.2
1991	691	93.6	643	63.5
1992	678	93.0	628	60.0
1993	670	94.0	627	59.7
1994	662	96.0	634	57.9
Season		*		**
Spring	2,187	95.7	2,079	68.2
Fall	2,147	94.2	2,013	55.7
TOB-N[a] × dystocia				**
			No	Yes
1, 0			60 56.0	51 49.3
1, 1			2,394 57.2	652 56.6
2, 0			30 73.2	38 50.8
2, 1			105 68.2	118 68.9
2, 2			354 68.7	290 70.5

[a]Type of birth (1 = single and 2 = twins) followed by number of calves nursed postpartum.
*$P \leq .05$.
**$P \leq .01$.

dietary change from improved pasture to a corn silage-alfalfa haylage based diet during the fall breeding season. This dietary change may account for the higher estrus activity early in the breeding season but lower conception rates and lighter calf weaning weights (150 d of age; Gregory et al., 1996) for fall-calving dams. Also, the shorter breeding season in the fall vs spring (i.e., 60 vs 70 d) likely contributed to the lower pregnancy rate in fall-calving dams.

Pregnancy rates were significantly lower in dams ≥ 5 yr of age than in 2- ($P < .01$) and 3- ($P < .05$) yr-old dams (Table 5), which may have been associated with the lower ($P < .01$) percentage of dams ≥ 5 yr of age expressing estrus before the AI season (Table 2). However, the percentage inseminated was similar among age groups (unpublished data). Other causes for the lower fertility in older dams were not apparent but may include a combination of factors such as repeated incidences of dystocia or retained placenta,

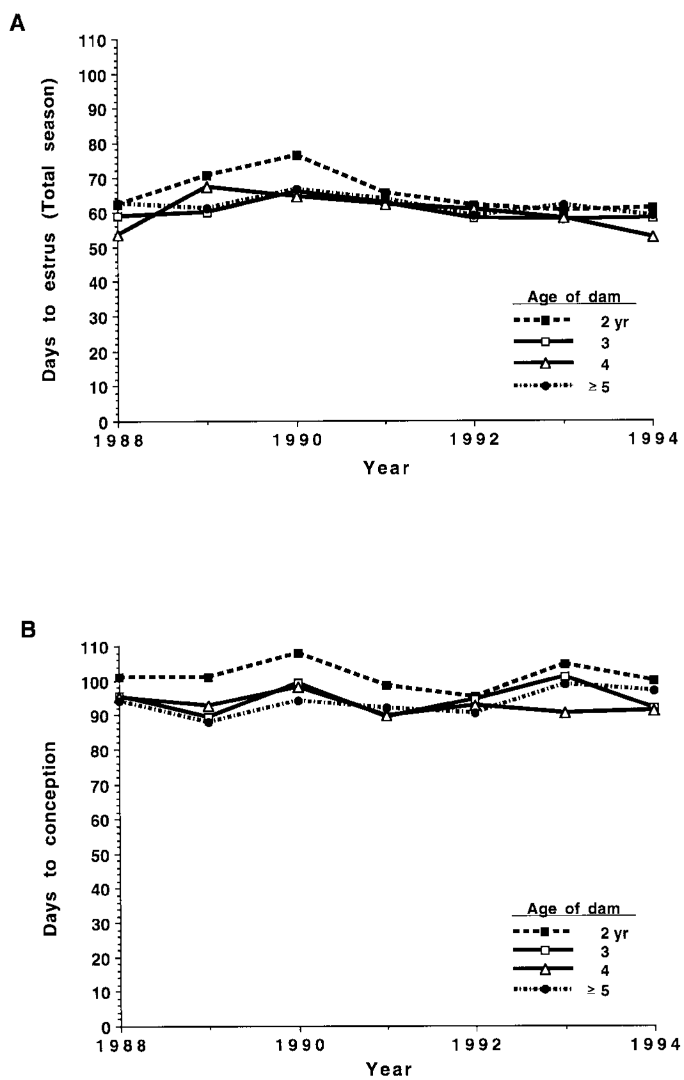

Figure 4. Time trend for effect of age of dam on interval to first estrus (panel A) and interval to conception (panel B). Age × yr, $P \leq .01$.

chronic infection from dystocia or retained placenta, and enlarged physical size of the uterus. The ≥ 5 yr age group was composed primarily of dams retained for their high twinning rate, and the uterus of such dams is very large, which may impede gamete transport. However, the effect of uterine involution or size on subsequent fertility was not evaluated.

Days to Conception

Because all of the cows were bred during the same fixed time period, the interval from parturition to conception (Table 5) was the consequence of both calving and conception date (Table 6). Calving date (Table 6) was significantly earlier ($P < .01$) for dams of twins than for dams birthing and nursing a single in both the spring- and fall-calving season due to the shorter gestation length for twins (Echternkamp and Gregory, 1998). Whereas only dams birthing and nursing twins in the spring had a later ($P < .05$) conception date than dams birthing and nursing a single, conception dates did not differ ($P > .1$) between dams of twins nursing zero or one calf vs dams of singles. Conception date was not affected by TOB-N in the fall. Similarly, dams with a retained placenta calved earlier ($P < .01$) in both the spring and fall, but conception was delayed ($P < .05$) only in the fall.

As with other traits, the interval from parturition to conception varied among TOB-N groups both within and among years (Figure 1). Collectively, the interval from parturition to conception did not differ among dams birthing twins but was increased 5.9 d ($P < .01$) by twinning. Separately, dams birthing and nursing a single had a shorter ($P < .01$) interval to conception compared to dams birthing and nursing twins or nonlactating dams of singles, or compared to ($P < .01$) dams birthing twins but nursing one calf in 1989 and 1991 (TOB-N × year; $P < .01$). As a consequence of the breeding season for heifers beginning 2 wk earlier than for parous females, calving date for 2-yr-old dams (i.e., first parity) was approximately 1 wk earlier ($P < .01$) than for parous dams, and the subsequent interval to conception was increased ($P < .01$) proportionately. However, magnitude of the differences in interval length among age groups varied among years (age of dam × yr; $P < .01$), including no significant ($P > .10$) difference in 1992 (Figure 4).

Dystocia (Table 5) was associated with a decrease in the interval to conception for dams birthing and nursing a single ($P < .01$) and for dams of twins nursing no ($P < .01$) or one ($P < .05$) calf (TOB-N × dystocia, $P < .01$). Because dams of singles with dystocia had a lower pregnancy rate but a shorter interval to conception, dystocia apparently had no effect on conception in some females but delayed fertility beyond the breeding season in others. Also, females with dystocia had a later calving date (Table 6) in the fall ($P < .01$), but not spring, which shortened ($P < .01$) the interval to breeding and, subsequently, conception. In addition, the increased ($P < .01$) interval to conception in dams having a retained placenta was shortened ($P < .01$) by dystocia (Table 5). Seasonal differences in interval to conception occurred among TOB-N groups and years (Table 5). For example, nursed dams had a shorter interval to conception in the spring than in the fall (dams birthing and nursing a single, $P < .01$; dams birthing and nursing twins, $P < .05$), whereas nonsuckled dams of twins had a shorter ($P < .01$) interval in the fall (TOB-N × season, $P < .05$). The year × season interaction ($P < .01$) resulted from the interval being longer in the spring than in the fall in 1989 and in 1992, shorter in 1990, and not differing between seasons in the other years.

Fetal Mortality

Females diagnosed by ultrasonography to be gestating twin fetuses had a higher ($P < .01$) incidence of fetal mortality during the first trimester (i.e., pregnancy rate at ultrasound diagnosis vs rectal palpa-

Table 5. Least squares means for conception rate (percentage of dams calving) and interval from parturition to conception

Variable	n	% or d	n	% or d
Conception, %	No		Yes	
TOB-N × dystocia**				
1, 0	64	75.8	53	61.0
1, 1	2,531	79.2	708	71.9
2, 0	30	71.3	39	67.8
2, 1	111	61.7	122	57.5
2, 2	375	66.3	301	72.2
Age × dystocia*				
2 yr	653	75.0	482	67.4
3 yr	718	71.4	276	66.8
4 yr	584	67.3	177	70.2
> 5 yr	1,156	69.7	288	59.8
Season**	Spring		Fall	
Days to conception	2,187	71.4	2,147	65.5
	No		Yes	
TOB-N × dystocia**				
1, 0	39	99.1	30	94.3
1, 1	1,899	89.3	500	85.0
2, 0	20	116.9	24	83.5
2, 1	71	100.2	78	92.8
2, 2	245	96.1	216	97.2
Retained placenta × dystocia*				
No	2,170	94.2	743	88.0
Yes	104	106.5	105	93.1
	Spring		Fall	
TOB-N × season**				
1, 0	34	95.9	35	97.5
1, 1	1,242	84.0	1,157	90.3
2, 0	28	110.4	16	89.9
2, 1	74	93.3	75	99.7
2, 2	226	93.2	235	100.1
Dystocia × season*				
No	1,144	99.3	1,130	101.3
Yes	460	91.4	388	89.7
Retained placenta × season**				
No	1,465	93.5	1,448	88.6
Yes	139	97.2	70	102.4

[a]Type of birth (1 = single and 2 = twins) followed by number of calves nursed postpartum.
*$P \le .05$.
**$P \le .01$.

tion) and throughout pregnancy (ultrasound diagnosis vs parturition) compared to females gestating a single fetus (Table 7). The 3.5% pregnancy loss in females gestating a single fetus is consistent with previously reported fetal losses of 5 to 10% after d 16 of gestation (Ayalon, 1978; Hawk, 1979; Maurer and Chenault, 1983). Similarly, a higher (P > .05) incidence of embryonic and fetal mortality was previously observed in this population between females with multiple vs single ovulations (Echternkamp et al., 1990). Echternkamp (1992) reported that when the fetal placentae were anastomosed, death of one twin fetus generally resulted in death of the other twin fetus and termination of the pregnancy rather than a reduction from twins to a single birth. Furthermore, < 1% of the single-born females in this population exhibit the freemartin syndrome, compared to 96% freemartin among females born co-twin to a male (Gregory et al., 1996), further suggesting that seldom does one of the twin fetuses survive alone after fusion of the placentae. Most of the mortality associated with multiple bovine fetuses/calves results from fetal mortality occurring at about d 35 of gestation when the placentomes are developing, and from increased incidence of premature births or late-term abortions (Anderson et al., 1982).

Effects of Type of Birth and Lactation on Postpartum Body Weight

Pre- and postbreeding BW and resulting BW changes during the breeding seasons are presented in Table 3. Pre- and postbreeding BW, and consequently BW changes, were highly variable among years and

Table 6. Factors affecting calving date (Julian), conception date (Julian), and the intervening interval for spring- and fall-calving dams analyzed separately

Variable		Spring season				Fall season		
		Calving date	Conception date	Interval		Calving date	Conception date	Interval
Analysis of variance	df		*F*-value		df		*F*-value	
Status (TOB-N)[a]	4	11.3**	2.9*	19.9**	4	15.4**	1.4	10.3**
Dystocia	1	.02	1.4	.4	1	20.4**	2.8	4.3*
Retained placenta	1	11.3**	.01	3.4	1	20.9**	4.2*	24.5**
Age of dam	3	19.7**	1.5	9.2**	3	26.2**	.2	16.8**
Year	6	9.7**	24.9**	3.3**	6	9.8**	8.1**	18.5**
Residual	1,588	301.3	271.0	413.1	1,502	234.4	246.7	424.2
Least squares means	n	Date	Date	d	n	Date	Date	d
μ	1,604	78.0	171.4	95.5	1,518	224.8	320.4	95.7
TOB-N[a]								
1, 0	34	78.7	174.4	94.6	35	222.7	320.2	97.5
1, 1	1,242	85.5	170.6	84.0	1,157	231.7	321.8	90.0
2, 0	28	65.9	166.5	113.8	16	221.7	314.8	93.1
2, 1	74	78.5	171.4	92.5	75	223.6	322.1	98.5
2, 2	226	81.2	174.2	92.6	235	224.2	323.3	99.1
Dystocia								
No	1,144	78.0	170.8	95.1	1,130	222.6	319.6	97.0
Yes	460	77.9	172.0	95.9	388	226.9	321.2	94.3
Retained placenta								
No	1,465	80.9	171.5	93.6	1,448	229.4	318.3	88.9
Yes	139	75.0	171.3	97.4	70	220.2	322.5	102.4
Age of dam								
2 yr	476	72.3	170.2	100.0	408	218.8	320.9	102.0
3 yr	377	79.2	171.3	94.8	329	226.4	320.4	94.0
4 yr	303	80.3	171.5	93.0	236	226.2	320.2	94.0
≥ 5 yr	448	80.1	172.6	94.1	545	227.6	320.2	92.6
Year								
1988	136	83.7	183.0	101.2	171	222.2	325.2	103.1
1989	203	83.1	175.8	94.7	262	229.1	317.1	88.0
1990	275	78.5	171.1	94.6	237	220.8	322.4	101.5
1991	292	77.1	172.0	96.7	237	225.1	321.9	96.9
1992	234	75.0	167.0	93.7	200	228.9	316.8	87.9
1993	235	73.9	166.2	95.6	219	222.9	321.5	98.6
1994	229	74.5	164.9	92.1	192	224.4	318.0	93.6

[a]Type of birth (1 = single and 2 = twins) followed by number of calves nursed postpartum.
*P ≤ .05.
**P ≤ .01.

Table 7. Effect of fetal numbers on pregnancy losses (%) in first trimester and to term as determined with chi-square analysis

		Stage of gestation		
Diagnosis[a]	n	US to 100 d[b]	100 d to term[c]	Total
Single	2,314	2.7[d]	.8[d]	3.5[d]
Twins	877	6.3[e]	6.1[e]	12.4[e]

[a]Number of fetuses in utero was determined by ultrasonography (US) between 40 and 70 d of gestation.

[b]Pregnancy was reconfirmed by rectal palpation at an average of 100 d (range, 70 to 130 d) of gestation.

[c]Differentiation between short gestation length (> 250 d) and premature births or abortions was based on hair, dental, and hoof development.

[d,e]Means within a column without a common superscript differ ($P < .01$).

seasons (year × season; $P < .01$) both within and among TOB-N groups. Prebreeding BW was heavier in the spring than in the fall for dams birthing twins and nursing no or one calf but did not differ between seasons for the other three TOB-N groups (TOB-N × season; $P < .01$), whereas postbreeding BW was consistently heavier ($P < .01$) in the fall than in the spring. Dams birthing and nursing a single were the lightest ($P < .01$) prebreeding in both seasons but similar ($P > .10$) to dams birthing and nursing twins postbreeding. Also, dams birthing and nursing twins were lighter ($P < .01$) postbreeding than dams birthing twins and nursing one calf; lactating dams were lighter ($P < .01$) than nonlactating dams in both birth groups. All of the TOB-N groups gained BW during the fall breeding season. Whereas in the spring only nonlactating dams gained BW during the breeding season, dams birthing and nursing a single maintained their BW, and lactating dams of twins lost BW (TOB-N × season; $P < .01$).

Two- and 3-yr-old dams were lighter prebreeding in the fall than in the spring, but dams 4 or ≥ 5 yr of age did not differ between seasons (age × season; $P < .01$). In contrast, postbreeding BW was similar between seasons for 2- and 3-yr-old dams, but dams 4 or ≥ 5 yr of age were significantly heavier in the fall than in the spring (age × season; $P < .01$). Consequently, BW gains during the breeding season were significantly greater, but similar among age groups, in the fall than in the spring; only 3- and 4-yr-old dams had measurable BW gains in the spring.

Dams of singles with dystocia were heavier both pre- and postbreeding than dams of singles without dystocia, whereas pre- and postbreeding BW did not differ between dams of twins with and without dystocia (TOB-N × dystocia; $P < .05$). Dams of singles with dystocia were also heavier prepartum than dams of singles without dystocia (Echternkamp and Gregory, 1998), suggesting that dystocia with single births was associated with dams of larger physical size and(or) body condition.

Discussion

Implementation of a twinning technology requires development of beef production and husbandry procedures to reduce the negative impact of twinning and its associated complications on calf survival and on fertility. Although early diagnosis of twin pregnancies by ultrasonography and increased dietary energy intake did not improve calf survival and rebreeding performance for dams of twins in the present study, timely obstetrical assistance and increased pre- and postpartum nutrition for dams with twins are essential to the implementation of a twinning technology. Without them, previous experience indicates that calf survival and pregnancy rates would have been significantly lower than those reported here. Increased pre- and postpartum nutrition did hasten onset of estrus and increase prebreeding estrus detection in dams of twins between 1988 and 1994 (Figures 1 and 2), which is a prerequisite for achieving conception. Unfortunately, the increased estrus activity between 1988 and 1994, and in fall- vs spring-calving females did not increase pregnancy rate; for example, pregnancy rate was 5.9% lower ($P < .01$) in fall-calving dams (Tables 1 and 5). Regardless, it is unlikely that the 5.9% lower pregnancy rate for dams of twins vs singles, or the generally lower pregnancy rates in this population, resulted from a failure to reinitiate estrous cycles or to maintain ovarian folliculogenesis within the breeding season. Some of the reduction in pregnancy rate after twinning was associated with the increased incidence of retained placenta with twin births; having a retained placenta reduced subsequent pregnancy rate approximately 10%.

Also, contributing to the lower pregnancy rate for dams with twins was the increased incidence of fetal mortality and premature births with twin fetuses, which were included as conception losses in the analysis of the pregnancy data. To date, there are no treatments to prevent these premature births in cattle. However, regardless of the inability to alleviate the constraints of increased dystocia and retained placentae and of reduced fertility and calf survival associated with twinning in cattle, dams of twins produce 53.1% more total calf birth weight (live), produce 58.4% more total weaning weight, and wean 65.2% more calves at 200 d of age (Gregory et al., 1996).

Implications

Increased productivity from twinning in beef cattle is compromised by the negative effects of increased dystocia and retained placenta on conception (10% lower), and fetal mortality and(or) premature births are increased 9% in dams conceiving twins. A further reduction in pregnancy rate for fall-calving dams of twins advocates spring calving, provided spring breeding occurs in months with increased ovulation rate. Although the interval from parturition to conception was increased by twin births, > 90% of the dams with twins and(or) a retained placenta expressed estrus and were inseminated during the subsequent 40-d artificial insemination season. Thus, their lower pregnancy rate did not result from failure to reinitiate estrous cycles. Increasing postpartum body weight has a positive effect on reinitiating estrous cycles but has no relationship to the lower fertility after twin births when body condition was adequate.

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