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Twin Production by Embryo Transfer in Japanese Black-Holstein Crossbred Cows

Osamu SUZUKI*, Mari AOKI and Koji KIMURA

Department of Grazing Animal Production, National Grassland Research Institute (NGRI)
(Nishinasuno, Nasu, Tochigi, 329-2793 Japan)

Abstract

Calving traits, postpartum reproduction and growth of calves in 28 Japanese Black-Holstein crossbred cows pregnant with twins including 3 cows with triplets by embryo transfer were compared with the data of 60 crossbred cows pregnant with single calves. After calving, all the cow-calf pairs were fed mainly roughage, grazed on pasture during summer and fed with grass silage during the winter season. No concentrate supplement for calves was given. Gestation length of cows giving birth to twins and calf birth weight were shorter by 8 days and lighter by 6 kg compared with cows giving birth to single calves, respectively. Significantly ($P < 0.01$) more cows giving birth to twins required assistance and retained placenta compared with cows giving birth to single calves. Twenty-five percent of all the cows that produced twins still gave birth to one calf or twin calves. In cows nursing twins, postpartum days to first ovulation and first estrus were significantly ($P < 0.01$) longer compared with the cows nursing single calves. However, the interval between postpartum and conception and the number of embryo transfers (inseminations) required for conception were not different between the 2 groups. Daily gain (DG) of twin calves from birth to 90 days of age was significantly ($P < 0.01$) lower than that of single calves, but DG from 90 to 180 days of age did not differ. At weaning after 180 days of age, body measurements of twin calves were 92-97% of those of single calves except for the body weight which was 82% of that of single calves. After all the 48 calves were produced (171%) and raised to weaning from 28 cows giving birth to twins, cows raising twins produced 136 kg more total weaning weight than did cows raising single calves. The results obtained in this study indicate that twin production by embryo transfer to the crossbred cows is efficient for beef production, although a relatively higher incidence of neonatal calf death was recorded.

Discipline: Animal industry
Additional key words: calving, reproductive performance, growth of calf

Introduction

The primary objective of the beef cattle industry should be to increase the efficiency of beef production. The maintenance of reproductive cows throughout a year accounts for more than 70% of the production cost per Japanese Black calf. Therefore, increased frequency of twinning should markedly increase the reproductive efficiency and reduce the production cost of beef calf. Recently several investigators have demonstrated that twinning could enhance the biological and economical efficiency of beef production[4,5,9,12].

As the embryo transfer technique has been developed for practical use in farms, attempts to produce twins by embryo transfer have been increasing in Japan. However, twinning is often associated with management problems such as abortion[10,16,17], increased dystocia[11], retention of placenta[8,9,15] and longer breeding intervals[8,19,20]. In addition, twinning in pure beef breed (Japanese Black) may result in calves with lower weaning weights due to their dam's insufficient milk yield to nurse twin calves[13]. On the other hand, although about 300 thousands of Japanese Black-Holstein crossbred cattle are kept for fattening in Japan, almost all the females are fattened and slaughtered without reproducing. It

Present address:
* Department of Research Planning and Coordination, NGRI (Nishinasuno, Nasu, Tochigi, 329-2793 Japan)

was confirmed that the Japanese Black-Holstein crossbred cows can produce more than 12 kg of milk per day which is sufficient to nurse 2 calves[2]. Therefore profitability should increase in twin production by the transfer of embryos to Japanese Black-Holstein crossbred cows.

The objectives of the present study were to investigate the various traits associated with twinning and to examine the feasibility of twin production by embryo transfer to Japanese Black-Holstein crossbred cows.

Materials and methods

1) Animals and embryo transfer

Twenty-eight Japanese Black-Holstein crossbred cows pregnant with twins including 3 cows pregnant with triplets and 60 Japanese Black-Holstein crossbred cows pregnant with single calves were used. These cows became pregnant after the transfer of 2 or 3 embryos bilaterally or after the transfer of 1 or 2 embryos to the uterine horn opposite to the corpus luteum 7 days after insemination. *In vivo* fertilized embryos transferred were collected from superovulated Japanese Black cows 7 days after estrus and frozen in a medium containing 20% of calf serum and 10% of ethylene glycol as described by Dohchi et al.[6]. *In vitro* fertilized embryos transferred were produced by *in vitro* maturation and *in vitro* fertilization and then frozen in the above medium. These embryos were transferred nonsurgically 6-8 (mostly 7) days after estrus without any dilution of the medium after thawing.

These cows gave birth in March-August, 1992 through 1995. Calving traits such as calving difficulty, retention of placenta, birth weight were recorded. All the cows were fed about 8-10 kg of grass hay with 1-2 kg of concentrates in a calving pen 10 to 20 days before and 10 days after calving. Thereafter cow-calf pairs were mainly fed roughage, grazed on pasture during summer and group-fed with grass silage during the winter season. Concentrates were given at the rate of 0-2 kg/day for cows nursing single calves and 0-4 kg/day for cows nursing twin calves, respectively, but no concentrate supplements were given to the calves. Of 21 sets of twins, 14 sets including one set of triplets were weaned at 180 days of age and the remainder at 90 days of age. Of 60 calves born as single calves, 57 were weaned at 180 days of age.

2) Resumption of breeding

Postpartum ovarian activity was examined by rectal palpation and/or with a linear-array ultrasound scanner 2 times a week and cows were checked for estrus daily from 15 days after calving. Cows showing estrus received 2 embryos bilaterally 7 days after estrus or received 1 embryo contralaterally 7 days after insemination at estrus. Resumption of breeding was not consistent because of estrous synchronization. Pregnancy was diagnosed with an ultrasound scanner.

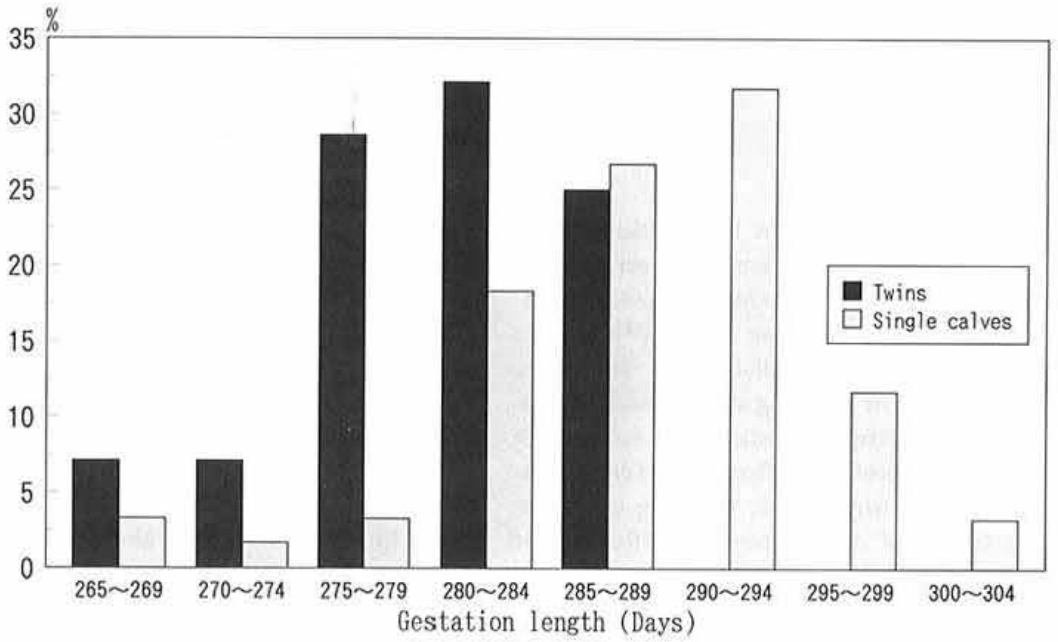

Fig. 1. Gestation length of crossbred cows which produced twin or single calves

3) Measurement of body condition score and milk yield

Body condition score (BCS) of dams was measured at calving and thereafter monthly until 6 months after calving by the method described by Bellows et al.[3]. At the same time ultrasonic back fat thickness (BFT) of dams was measured. Daily milk yield was measured by the calf-suckling method 2 times a day for 2 days at 2, 4, 8, 12, 16, 21, and 26 weeks postpartum. Body measurement was carried out at birth as well as at 90 and 180 days of age.

4) Statistical analysis

Significant differences between the data of twin- and single-birth groups were tested by χ^2 test and Student t-test.

Results and discussion

1) Parturition

As shown in Fig. 1, the gestation length of 28 cows giving birth to twins, including 3 cows giving birth to triplets mostly ranged for 275 to 289 days, while the gestation length of cows giving birth to single calves was widely distributed with a peak at 290–294 days. The average gestation length was significantly ($P<0.01$) shorter by 8 days for cows giving birth to twins than for cows giving birth to single calves. Gestation length in this study was

Table 1. Calving traits of crossbred cows producing twin or single calves

Group	No. of cows	No. of gestations	Body weight at calving (kg)			Gestation length (days)	Birth weight (kg)	No. of cows which required assistance (%)	No. of cows which retained placenta (%)
			Before	After	Difference				
Twins*	28	5.8 (2.6)	598.9 (60.2)	519.3[a] (59.6)	79.6[c] (23.2)	279.9[c] (6.0)	28.6[c] (8.2)	7[c] (25.0%)	6[c] (21.4%)
Single calves	60	5.5 (2.6)	598.9 (72.6)	561.9[b] (74.8)	37.0[d] (22.5)	287.8[d] (7.6)	34.5[d] (5.4)	1[d] (1.7%)	0[d] (0.0%)

Values in parenthesis indicate standard deviations.
[a, b, c, d] Means with different superscripts within a same column are significantly different at 5 and 1%, respectively.
* Includes 3 cows which produced triplets.

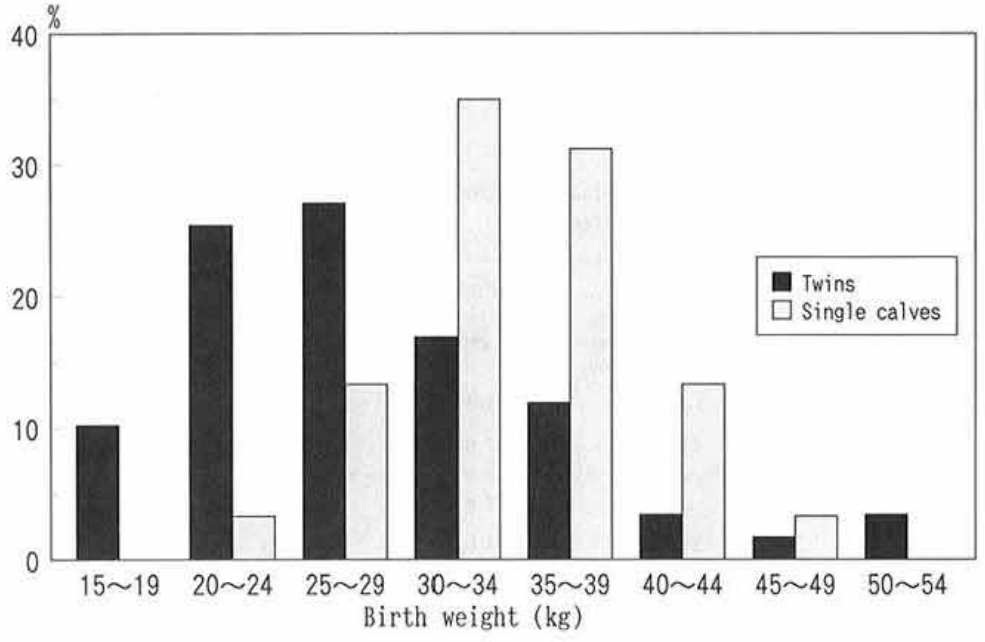

Fig. 2. Birth weight of twin or single calves

similar to the results reported in Japanese Black[13] and other breeds[8,16]. Cows giving birth to twins required significantly ($P<0.01$) more assistance (25.0% vs 1.7%) and had retained placentas (21.4% vs 0.0%) compared with cows giving birth to single calves (Table 1). The percentage of cows requiring assistance and retaining placentas was within the range reported by other workers[1,7,9]. It has been reported that the retention of placentas increased in multiple births or premature calving and could be reduced by increasing the nutritional levels of cows[7,15]. Assistance at calving was mainly required in the case of breech presentation for the second calf.

Distribution of birth weight of 59 twins (including 9 triplets) and 60 single calves is shown in Fig. 2. Although the birth weight of twin calves showed a wide range of distribution, a large proportion was within the range of 20–34 kg. Birth weight of triplets was less than 20 kg and twin calves weighing more than 40 kg were derived from *in vitro* fertilized embryos. On the other hand birth weight of single calves was normally distributed with a peak at 30–39 kg. The average birth weight was significantly ($P<0.01$) smaller by 6 kg for twin calves than for single calves.

Most of the twin births occurred under supervision. In most of the cases the second calf was born 1 h after the first calf when the delivery was not assisted. Of 28 sets of twins born, both calves from 2 sets (7.1%) were born dead and 1 calf from 5 sets (17.9%) including 2 sets of triplets was born dead (Table 2). Only one stillbirth was observed in single births.

Table 2. Survival of twin calves

	No. of cows	Percentage (%)
Total no. of cows which produced twins	28	100.0
Both twins alive*	21	75.0
One of twins dead**	5	17.9
Both twins dead	2	7.1

* Includes 1 cow which produced triplets.
** Includes 2 cows which produced triplets.

2) Postpartum reproductive performance

The mean postpartum days to the first ovulation and first estrus were 49 and 65 days for 21 cows nursing twins, respectively. These intervals were significantly ($P<0.01$) longer by 13 days and 18 days than those of cows nursing single calves, respectively (Table 3). However, there were no significant differences in the postpartum days to first embryo transfer (insemination) and conception and the number of transfers required for conception between the 2 groups. Yonai et al.[20,21] reported that in dams bearing twins fed with 100% TDN levels of Japanese Feeding Standard (JFS) 2 months before and 3 months after calving, the interval between postpartum and the first estrus was longer due to repeated quiet ovulations before the first estrus. They also reported that delayed postpartum estrus was improved by increase of the nutritional levels to 120% of JFS. Wheeler et al.[18] observed that postpartum intervals did not differ between twin- and single-bearing cows under the management scheme where dams were maintained to allow for body weight gain during the early postpartum period. In this study postpartum days to conception exceeded 100 days since resumption of breeding was delayed due to cattle management. However, the results of our study

Table 3. Postpartum reproductive performance of crossbred cows which produced twin or single calves

Group	No. of cows	Postpartum days to				No. of transfers required for conception**
		1st ovulation	1st estrus	1st transfer**	Conception	
Twins*	21	48.8[a]	65.3[a]	103.9	110.4	1.2
		(13.1)	(17.9)	(40.6)	(38.6)	(0.4)
Single calves	57	36.1[b]	47.4[b]	88.5	110.7	1.5
		(11.2)	(13.6)	(39.1)	(56.9)	(0.8)

Values in parenthesis indicate standard deviations.
[a, b] Means with different superscripts within a same column are significantly different at 1%.
* Includes 1 cow which produced and nursed triplets and 2 cows which produced triplets and nursed twins.
** Embryo transfer or insemination.

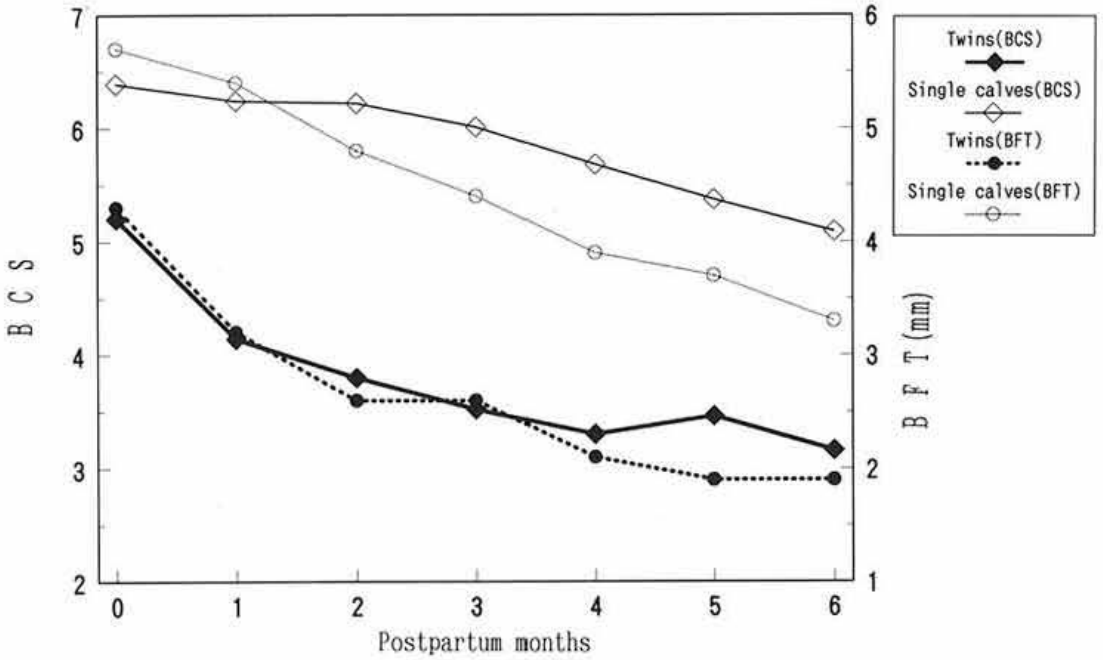

Fig. 3. Changes in body condition score (BCS) and back fat thickness (BFT) of crossbred cows which produced twin or single calves

showed the possibility of a year-calving interval even in cows nursing twins.

As shown in Fig. 3, postpartum body condition score and back fat thickness gradually declined in cows that produced twins or single calves. There were, however, marked decreases in the BCS and BFT of dams nursing twins during the early postpartum period, especially in the twin-nursing cows which did not receive concentrate supplement. These cows tended to display longer intervals to the first estrus among the cows nursing twins. Wyatt et al.[19] also reported that cows rearing simulated twins produced 39% more milk and required 72% more winter supplement to sustain a winter weight loss comparable to that of cows rearing single calves, and showed extended postpartum anestrous periods.

Daily milk yield of cows nursing twins reached a peak of 13.2 kg at 4 weeks and gradually decreased to 7.2 kg at 26 weeks postpartum. On the other hand, daily milk yield of cows nursing single calves

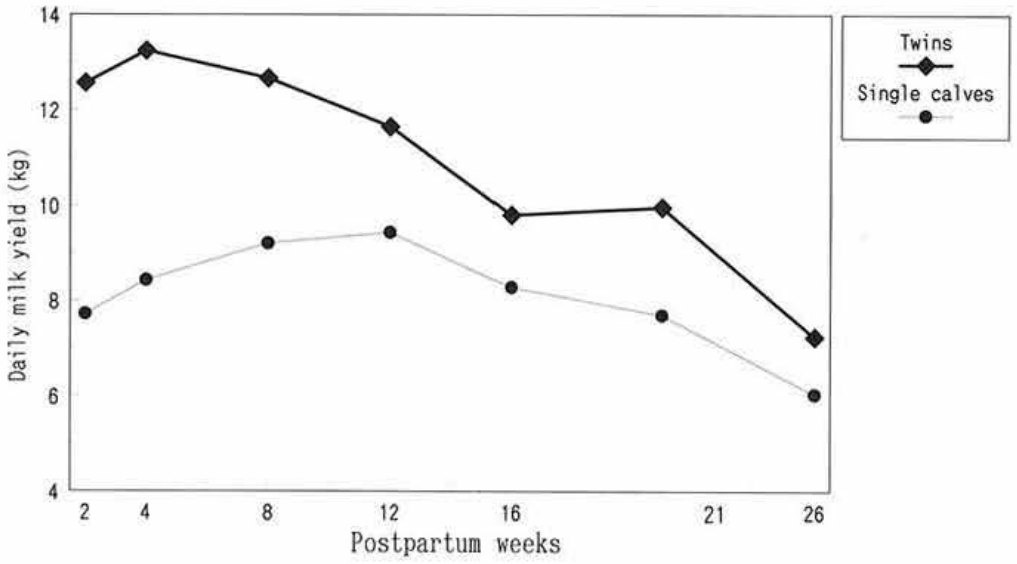

Fig. 4. Changes in daily milk yield of crossbred cows which produced twin or single calves

gradually rose from 7.7 kg at 2 weeks to 9.4 kg at 12 weeks and thereafter gradually fell to 6.0 kg at 26 weeks postpartum (Fig. 4). Cows nursing twins produced a daily milk yield about 5 kg higher than cows nursing single calves during the early postpartum period. Changes in BCS and milk yield recorded in this study revealed that concentrate supplement to cows nursing twins was important especially during the first 3 months postpartum.

3) Growth of calves

Birth weight, body weight at 90 days of age and daily gain from birth to 90 days of age were significantly ($P<0.05$) lower for twin calves than for single calves. However, no significant difference was observed in the daily gain from 90 to 180 days

Table 4. Growth of twin or single calves

Group	Sex	No. of calves	Body weight (kg) at			Daily gain (kg) during		
			Birth	90 days	180 days	Birth to 90 days	90 to 180 days	Birth to 180 days
Twins*	Male	15	28.5[a] (5.7)	95.4[a] (16.3)	171.5[ab] (33.4)	0.74[a] (0.15)	0.84 (0.23)	0.79[a] (0.16)
	Female	14	28.8[a] (5.1)	99.9[a] (14.9)	169.1[a] (31.2)	0.79[a] (0.13)	0.77 (0.20)	0.78[a] (0.15)
Single calves	Male	29	35.7[b] (5.5)	130.8[b] (15.8)	212.0[c] (25.1)	1.05[b] (0.13)	0.90 (0.15)	0.98[b] (0.12)
	Female	28	33.9[b] (4.5)	119.9[b] (12.1)	195.1[bc] (19.5)	0.95[b] (0.11)	0.82 (0.12)	0.89[ab] (0.09)

Values in parenthesis indicate standard deviations.
[a, b, c] Means with different superscripts within a same column are significantly different at 5%.
* Includes a set of triplet calves.
Breeds of twin calves: 8 Japanese Black, 21 crossbred.
Breeds of single calves: 14 Japanese Black, 43 crossbred.

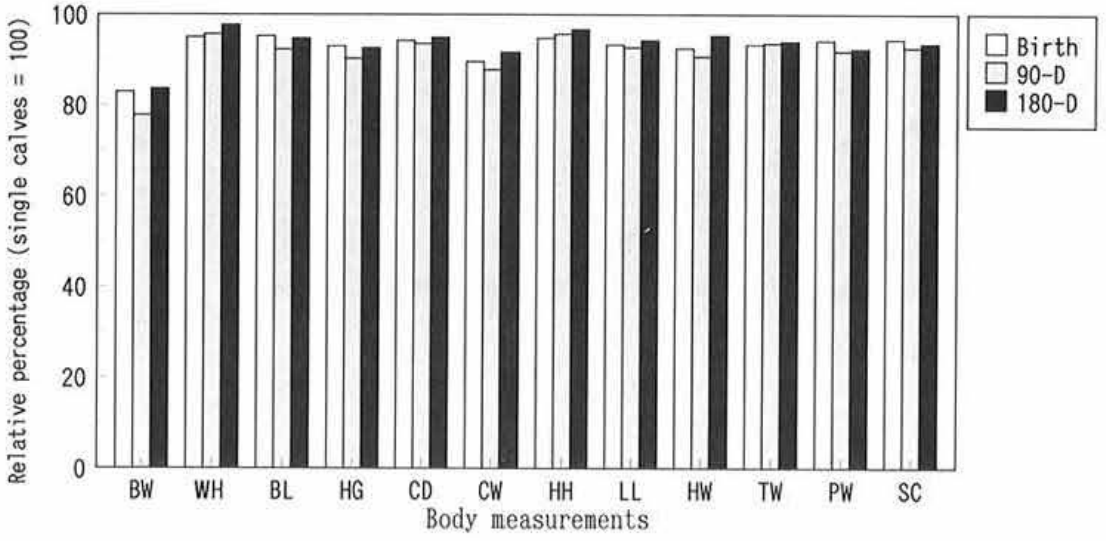

Fig. 5. Body measurements of twin calves at birth, 90 and 180 days of age
BW: Body weight, WH: Withers' height, BL: Body length, HG: Heart girth, CD: Chest depth, CW: Chest width, HH: Height at hip cross, LL: Lump length, HW: Hip width, TW: Thurl width, PW: Pin bone width, SC: Shank circumference.

between the twin and single calves. Although 180-day weaning weight of twin calves was smaller by 40 kg for a male and 26 kg for a female than in the case of single calves, twin calves showed relatively higher daily gains of 0.79 and 0.78 kg for a male and female, respectively (Table 4). Cows nursing twins produced 136 kg more of total 180-day weaning weight than did cows nursing single calves. De Rose & Wilton[5] reported that twin-bearing cows produced an additional 186 kg of total 200-day weaning weight in an experiment in which calves were given *ad libitum* access to creep feed, while dams with twins produced 91–97 kg more of 170-day weight when no additional supplement was given to the calves[14].

As shown in Fig. 5, body weight of twin calves at 180 days of age was about 82% of that of single calves. However, in twin calves, withers height and height at hip cross were 97% and other body measurements were 92–95% of those of single calves at 180 days, respectively. In the experiment[13] in which calves were fed a pelleted creep ration up to a maximum of 2.5 kg per day, it has been reported that the 26-week weaning weight of Japanese Black male calves was 207 and 177 kg, and that of female calves was 185 and 152 kg for single calves and twins, respectively. In spite of the absence of creep ration for calves, twin calves in this study showed almost the same body weight gain as that recorded in a previous report[13].

Conclusion

In the production of twins by embryo transfer to Japanese Black–Holstein crossbred cows, the percentage of calf accidents at calving increased. However, 48 calves were alive and raised until weaning from 28 cows. The final calf productivity for cows giving birth to twins was 171%. In addition, twin calves grew well even though no creep feed was given, and cows bearing twins produced 136 kg more weaning weight compared with cows bearing single calves. Results presented here revealed that twin production in Japanese Black–Holstein crossbred cows could be efficient and may enable to reduce the cost of beef calf production.

Most cases of calf accidents at parturition occurred when cows gave birth without supervision during the middle of the night. It was therefore necessary to observe pregnant cows closely to provide assistance for delivery and to the newborn calves. In an other experiment we confirmed that no calf accidents occurred in 10 twin births in which parturition was induced during the daytime by feeding at 18:00 for about 3 weeks before calving.

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