BIOLOGY OF REPRODUCTION 52, 676–682 (1995)

Transfer of Bovine Embryos Produced In Vivo or In Vitro: Survival and Fetal Development[1]

PETER W. FARIN[3] and CHARLOTTE E. FARIN[2,4]

Department of Food Animal and Equine Medicine,[3] Department of Animal Science[4]
North Carolina State University, Raleigh, North Carolina 27695–7621

ABSTRACT

The objectives of the present experiment were to compare survival after transfer of bovine embryos produced in vivo with those produced in vitro and to examine the physical characteristics of fetuses produced from these transfers. Embryos produced in vivo (Holstein × Angus) were recovered from uterine flushings of superovulated heifers 7 days after first artificial insemination, and embryos produced in vitro (Holstein × beef breeds) were collected 7 days after insemination. Embryos were paired by source (in vivo, in vitro), stage (compact morula, blastocyst), and quality grade (excellent = 1, good = 2), and transferred nonsurgically to recipient heifers on Day 7 (± 1 day) of the estrous cycle. Pregnancy status was monitored by determination of serum progesterone concentrations, ultrasonography, and palpation through 7 mo of gestation, at which time fetuses were recovered. In comparison with grade 1 embryos produced in vivo, the risk of embryonic death after transfer was similar for grade 2 embryos produced in vivo ($p = 0.56$) and for grade 1 embryos produced in vitro ($p = 0.88$). By contrast, grade 2 embryos produced in vitro were at greater ($p = 0.04$) risk of embryonic death. Embryo loss was associated ($p = 0.01$) with increased serum concentrations of progesterone in recipients at the time of transfer. At 7 mo of gestation, fetuses from embryos produced in vitro were heavier ($p = 0.02$) than fetuses from embryos produced in vivo and had skeletal measurements that were disproportionate ($p \leq 0.04$) to body weight.

INTRODUCTION

The production of cattle embryos in vitro is both a practical alternative to conventional in vivo techniques [1–3] and an important tool for the study of preimplantation embryo development [4]. Bovine embryos produced in vitro have resulted in embryos developing to the morula and blastocyst stage. Transfer of these embryos to recipient cattle has resulted in pregnancy rates comparable to those achieved after transfer of embryos produced in vivo from superovulated donor cattle [2, 3, 5–10], and in the birth of live calves [2, 5–7, 10, 11]. However, in most studies, embryos produced in vitro were transferred in the absence of a contemporary comparison group.

Reports concerning the growth characteristics of fetuses or calves from embryos produced in vitro are limited. Walker et al. [12] and Holm et al. [13] found that lambs from embryos cultured in vitro were heavier at birth and were associated with a longer gestation than lambs from embryos produced in vivo. In cattle, fetal development is influenced by many factors including breed, sire, and the sex and number of fetuses in utero [14, 15]. King et al. [16] found that birth weight and sex ratio of calves produced from transfer of in vivo-derived embryos were not significantly different from values for calves produced through artificial insemination.

The objectives of the present research were to compare survival of in vivo- and in vitro-produced embryos after transfer into recipient heifers and to examine the physical characteristics of fetuses resulting from these transfers. Specifically, we tested the hypothesis that bovine embryos produced either in vivo or in vitro do not differ in their ability to survive in utero or develop normally.

MATERIALS AND METHODS

Embryo Production

Thawed frozen semen from the same Holstein sire was used for the production of both in vivo and in vitro embryos. For production of in vivo embryos, Angus donors were superovulated by i.m. administration of 32–35 mg FSH (F.S.H.-P., Schering Corp., U.S.A., Kenilworth, NJ) given in a series of decreasing doses over a 4-day period beginning on Day 10, 11, 12, or 13 of the estrous cycle. Estrus was induced by the administration of two i.m. injections of 25 mg of prostaglandin $F_{2\alpha}$ THAM-salt solution ($PGF_{2\alpha}$, Lutalyse; Upjohn Co., Kalamazoo, MI) on the morning and evening of Day 3 of FSH treatment. Donors were artificially inseminated at 12 and 24 h after detection of standing estrus. Embryos were recovered nonsurgically using a modification of a previously described technique [17] on Day 7 (Day 0 = day of first detected estrus and first insemination).

For production of in vitro embryos, chemicals and antimicrobials were from Sigma Chemical (St. Louis, MO) and were of tissue culture grade. Ovaries, primarily from beef cattle, were collected from a local abattoir, maintained at 30–33°C in saline with 0.75 μg/ml penicillin, and trans-

Accepted November 14, 1994.
Received May 31, 1994.
[1]This work was supported in part by grants from the North Carolina Biotechnology Center (9113-ARG-0903, C.E.F./P.W.F.), the National Science Foundation (DCB-9157841, C.E.F.), and the North Carolina Agricultural Research Service (C.E.F.).
[2]Correspondence. FAX: (919) 515-7780.

ported to the laboratory within 5 h of collection. After delivery to the laboratory, ovaries were washed three times with fresh saline/penicillin and held at 33°C. Cumulus-oocyte complexes (COC), aspirated from follicles 2–7 mm in diameter, were held in bovine follicular fluid (33°C). COC were allowed to settle for 5–10 min, and were collected and washed five times in modified Tyrode's medium (TL-HEPES; [18]) at 33°C. After washing, groups of 15–30 COC were transferred to 1 ml TCM-199 (Gibco BRL, Grand Island, NY) supplemented with 10 μg/ml LH, 5 μg/ml FSH, 1 μg/ml estradiol, 200 μM pyruvate, 50 μg/ml gentamicin, and 10% estrous cow serum, and cultured for 20 h at 39°C in an atmosphere of 5% CO_2 in air and 100% humidity [19]. After maturation, COC were inseminated for 18–20 h in 1.0 ml of fertilization medium containing 1×10^6 spermatozoa selected by a swim-up procedure [18]. Presumptive zygotes were transferred to TCM-199 with 10% estrous cow serum and 50 μg/ml gentamicin and cultured in the presence of oviductal epithelial cells [9] for 7 days (Day 0 = day of insemination). Culture medium was changed at 48–72-h intervals during the 7-day culture period.

Transfer of Embryos

Estrus was induced in recipient heifers by administration of two injections of 25 mg of $PGF_{2\alpha}$ separated by a 10-day interval. On the day of transfer (Day 7 of embryo development), embryos were evaluated [20] and paired by source (in vivo or in vitro), stage (compact morula or blastocyst), and quality grade (excellent = 1 or good = 2). Embryo grade was based on symmetry and compactness of blastomeres as well as the presence of extruded material within the perivitelline space [20]. Embryo evaluations were performed by the same person (CEF). On Day 7 (± 1 day) of the estrous cycle, individual pairs of embryos derived from in vivo or in vitro production were transferred nonsurgically [21] to the uterine horn ipsilateral to the ovary bearing the CL of Angus cross-bred heifers. Embryo pairs were washed in TL-HEPES, loaded into 0.25-ml straws, and transferred using a miniaturized embryo transfer syringe and sheath (I.M.V. International Corp., Minneapolis, MN). An attempt was made to synchronize stage of embryo development and day of recipient's estrous cycle such that morulae were transferred into recipients on Day 6 or 7, and blastocysts were transferred into recipients on Day 7 or 8 of the cycle. A total of 38 embryo pairs (19 pairs produced in vivo and 19 pairs produced in vitro) were transferred. Embryos were transferred by the same person (PWF) and were scheduled such that a group of in vivo embryos were transferred within 14 days of transfer of an equal-size group of in vitro embryos.

Monitoring Pregnancy

Pregnancy status in recipient heifers was monitored by determination of serum progesterone, palpation, and ultrasonography. Blood samples were collected by caudal venipuncture at weekly intervals from the day of transfer to 83 days after transfer for assay of serum progesterone concentration by RIA [22]. Intra- and interassay coefficients of variation were 9.4% and 18.2%, respectively (n = 7 assays). At approximately 53 days after embryo transfer, all recipient animals were examined for the presence or absence of viable fetuses using ultrasonography (Aloka 210 ultrasound with a 5.0-MHz probe; Corometrics Medical Systems Inc., North Wallingford, CT). Recipients were examined again at 93 days after transfer by ultrasonography and palpation per rectum to confirm pregnancy status. Additional examinations were conducted at monthly intervals until 7 mo of gestation. Conceptus loss was considered to have occurred when the concentration of serum progesterone was less than 1.0 ng/ml or when a fetus was not detected by palpation and ultrasound. Failure to detect one or more fetuses on Day 53 after transfer was consistent with at least one previous sample of serum progesterone being less than 1.0 ng/ml.

Assessment of Fetuses

Pregnant recipients were slaughtered on Day 222 (SE = 0.3 days) of gestation (Day 215 after transfer), and their reproductive tracts were recovered. Each fetus was weighed, necropsied, and measured. Internal organ weights (heart, liver, kidney, and adrenal glands) and skeletal measurements (crown-rump length, heart girth, biparietal diameter, length of long bones, and circumference of joints) were obtained for each fetus. The placentas were examined, and placentomes were counted.

Statistical Analyses

Proportional data for pregnancy status, twinning, and sex of fetus were analyzed by the Chi-square test [23]. Concentrations of serum progesterone on the day of transfer in recipient heifers that either remained pregnant for 7 mo or lost their pregnancies were analyzed by the Wilcoxon Rank Sum Test [23].

Survival of embryos after transfer was examined using survival analysis techniques [24, 25]. The Kaplan-Meier method was used to estimate survival functions for embryos through Day 215 after transfer, and plots of these functions, known as survival curves, are presented. Cox's proportional hazards regression model was then used to examine factors that could contribute to the time of embryo or fetal loss after transfer. Factors examined were embryo source (in vivo, in vitro), stage of embryo development (compact morula, blastocyst), embryo quality grade (excellent = 1, good = 2), synchrony of embryo age and recipient's day of the estrous cycle (−1, 0, or 1 day), side of transfer (left or right uterine horn), serum progesterone concentration (ng/ml) of recipient on the day of transfer, and potential two-way interactions. Inclusion of factors into

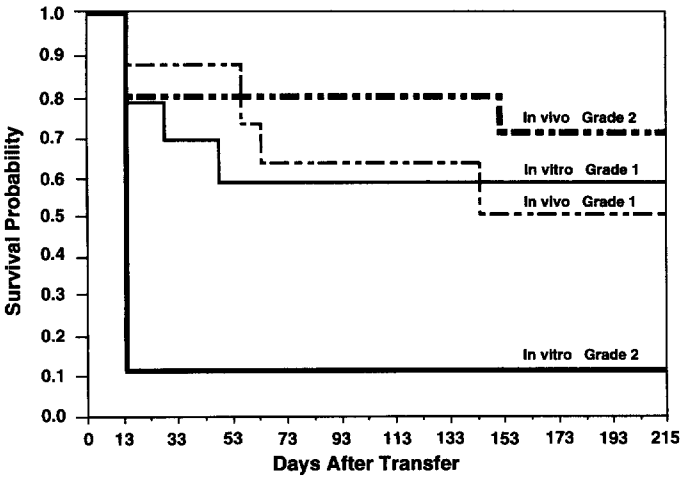

FIG. 1. Survival curves for embryo pairs produced either in vivo or in vitro of quality grade 1 (excellent) or 2 (good) from day of transfer to recipient heifers through Day 215 after transfer. The proportion of heifers that remained pregnant until Day 215 after transfer was 4 of 8 for in vivo grade 1, 8 of 11 for in vivo grade 2, 6 of 10 for in vitro grade 1, and 1 of 9 for in vitro grade 2.

the final model was based on significance at $p < 0.15$. The final Cox regression model included the interaction of embryo source × embryo quality grade, and the effect of serum progesterone concentration of recipient cattle on the day of embryo transfer. Hazard ratios, analogous to risk ratios [24], were determined for embryo loss relative to a baseline hazard of 1.0. The baseline for the interaction of embryo source × embryo quality grade was that for grade 1 embryos produced in vivo.

Fetal and placental measurements were analyzed by linear model procedures [23]. The final model included the main effects of embryo source (in vivo, in vitro), number of fetuses in utero (1, 2), and phenotypic sex of fetus (male, female). For all measurements, two-way interactions were found to be nonsignificant and were removed from the model.

RESULTS

Embryonic and Fetal Survival

The proportion of recipients pregnant at Day 53 after transfer was greater for those that received in vivo embryos than for those that received in vitro embryos (15 of 19, 79% vs. 7 of 19, 37%; $p = 0.01$). Plots of survival probability through Day 215 after transfer revealed that embryonic loss was similar ($p = 0.44$) for embryos of grade 1 and grade 2 produced in vivo and embryos of grade 1 produced in vitro, but survival rate was extremely low for embryos of grade 2 produced in vitro (Fig. 1). Compared with other embryo source and grade combinations, grade 2 embryos from in vitro production underwent substantial ($p = 0.002$) early embryonic loss within 14 days of transfer (i.e., prior to Day 21 of gestation). This resulted in only 1 of the 9 recipients remaining pregnant until 7 mo of gestation (Fig. 1).

There was no significant disparity ($p \geq 0.66$) in the survival of embryos based on stage of development, synchrony between embryo age and day of the recipient's estrous cycle, or side of transfer.

By regression analysis, embryo survival was described by the interaction of embryo source and grade, and the continuous effect of serum progesterone concentration in recipient heifers on the day of transfer (Table 1). Embryo source × grade was significant ($p = 0.01$); however, no other interaction examined was significant ($p > 0.30$). Grade 1 embryos produced in vitro were at no greater risk ($p = 0.88$) of embryonic or fetal mortality than grade 1 embryos produced in vivo. By contrast, in vitro-derived embryos of grade 2 were 4 times more likely ($p = 0.04$) to die after transfer than in vivo-derived embryos of grade 1. Embryonic mortality increased slightly ($\beta = 0.34$; $p = 0.01$) as serum concentrations of progesterone increased beyond 1.0 ng/ml on the day of transfer.

The concentration of serum progesterone for recipients ranged from 1.0 to 8.6 ng/ml on the day of transfer. Serum

TABLE 1. Cox proportional hazards regression model of embryo loss in cattle after transfer of embryos produced either in vivo or in vitro.

				Hazard ratio[a]		
					95% CI	
Variable	β[b]	SE β	*p* value	Ratio	Lower	Upper
Embryo source × grade[c]						
In vivo, grade 1	0			1.00		
In vivo, grade 2	−0.45	0.78	0.56	0.64	0.14	2.86
In vitro, grade 1	−0.10	0.71	0.88	0.90	0.22	3.60
In vitro, grade 2	1.41	0.68	0.04	4.11	1.07	15.77
Progesterone[d]	0.34	0.13	0.01	1.40	1.08	1.81

[a]The hazard or risk of embryo loss in recipient heifers after embryo transfer compared with in vivo grade 1 embryos. For example, the risk of embryo loss for recipients that received in vitro grade 2 embryos was greater (4.11 times) than for those that received in vivo grade 1 embryos.
[b]Regression coefficient.
[c]Embryos were graded as 1 (excellent) or 2 (good) [20].
[d]Serum progesterone concentration (ng/ml) in recipient cattle on the day of embryo transfer.

TABLE 2. Concentrations of serum progesterone (ng/ml) on the day of transfer for recipient heifers that either remained pregnant for seven months or lost their pregnancy.*

Recipient group	Maintained pregnancy	Lost pregnancy	Overall
In vivo	3.8 ± 0.5 (12)[a]	6.2 ± 0.6 (7)[b]	4.7 ± 0.5 (19)
In vitro	4.2 ± 0.7 (7)[a]	5.6 ± 0.5 (12)[b]	5.1 ± 0.4 (19)
Overall	4.0 ± 0.5 (19)[a]	5.8 ± 0.4 (19)[b]	4.9 ± 0.3 (38)

*Mean ± SE (number in parentheses = number of recipients).
[a,b]$p \leq 0.04$, Wilcoxon's rank sum test.

concentrations of progesterone did not differ ($p > 0.10$; Table 2) between recipients that received in vivo embryos and those that received in vitro embryos. However, the levels of progesterone on the day of transfer were lower ($p \leq 0.04$) for recipient cattle that remained pregnant for 7 mo when compared with those that lost their pregnancies.

Fetal Development

Thirty fetuses were recovered in utero on Day 222 (SE = 0.3 days) of gestation. The proportion of twin fetuses that resulted from transfer of in vivo and in vitro embryos was identical (12 of 20 and 6 of 10, respectively; $p = 1.00$). In addition, there was no significant difference ($p = 0.59$) in the proportion of male fetuses from the in vivo embryo transfers (12 of 20) compared with the in vitro embryo transfers (7 of 10). The analysis of variance model for body weight is presented in Table 3, and in Tables 4 and 5 are the least-squares means for measurements of fetuses in both transfer groups. There were significant increases in body weight (3.2 kg), heart girth (4.1 cm), long-bone lengths (metatarsus length, 1.8 cm), and heart weight (23.5 g), for fetuses derived from embryos produced in vitro compared with fetuses from embryos produced in vivo (Table 4). When fetal measurements were expressed on a per kilogram body weight basis, fetuses from in vitro embryos had consistently smaller ($p \leq 0.04$) skeletal measurements than fetuses from in vivo embryos (Table 5). By contrast, the weights of the visceral organs expressed on a body weight basis were not significantly different between the two groups. There were fewer ($p = 0.02$) placentomes on a body weight basis for fetuses from in vitro embryos (Table 5).

TABLE 3. Least-squares linear model for body weight of seven-month-old bovine fetuses resulting from embryos produced either in vivo or in vitro.

Source	df	Mean square	*p* value
Model	3	53.59	0.01
Error	26	11.01	
Corrected total	29		
$R^2 = .36$			
		Type III	
Embryo source (in vivo, in vitro)	1	70.36	0.02
No. of fetuses (1, 2)	1	60.81	0.03
Sex of fetus (M, F)	1	17.75	0.22

DISCUSSION

Pregnancy rate on Day 53 after transfer was 42% lower for recipients that received embryos produced in vitro than for those that received embryos produced in vivo. The pregnancy rate for each group of recipients was within the range of those reported by others [2, 3, 8, 10, 26]. In our study, pregnancy rates were comparable after transfer of grade 1 in vivo and in vitro embryos and grade 2 in vivo embryos. However, significantly fewer pregnancies were obtained after transfer of grade 2 embryos produced in vitro. Grade 2 embryos produced in vitro were also at higher risk of dying after transfer than were grade 1 embryos produced in vivo. These data indicate that only grade 1 embryos produced in vitro were as capable of establishing pregnancy as high-quality (grades 1 or 2) embryos produced in vivo. A similar conclusion was reached by Hasler [2], who examined pregnancy data from a large number of recipient heifers.

Survival analysis of data in the present study had several distinct advantages over the use of more conventional statistical methods [24]. Survival analysis was selected because survival times of embryos vary, and typically the survival distribution is skewed. By using survival analysis, we could plot the estimated survival functions for embryos from different sources and quality grades, examine factors that could contribute to embryonic and fetal loss during the first 7 mo of gestation in a regression format, and quantify *risk* associated with each of these factors.

On the basis of survival curves, loss of grade 2 embryos produced in vitro occurred prior to Day 21 of gestation. This observation is consistent with other data on early embryonic loss in cattle [8, 27, 28] and suggests that these embryos were not successful in signalling maternal recognition of pregnancy [29]. Our estimates of day of embryonic loss are conservative because they are based on detection of a low concentration of serum progesterone (< 1.0 ng/ml) in weekly samples and data on returns to estrus after transfer were not available. In addition, levels of serum progesterone are typically greater than 1.0 ng/ml during the luteal phase of the estrous cycle, which would prevent accurate detection of embryo loss prior to Day 21. Thus, grade 2 embryos produced in vitro may have been lost at anytime within 14 days of transfer.

Although all recipient heifers had at least 1.0 ng/ml of serum progesterone at the time of transfer, risk of embry-

TABLE 4. Least-squares means (± SE) for measurements of seven-month-old bovine fetuses that developed from embryos produced either in vivo or in vitro.[a]

Measurement	In vivo (n = 20)	In vitro (n = 10)	p value
Body weight (kg)	15.4 ± 0.8	18.6 ± 1.1	0.02
Skeletal (cm)			
Heart girth	52.4 ± 0.9	56.5 ± 1.2	0.01
Crown-rump	65.1 ± 1.3	67.2 ± 1.9	NS
Biparietal diameter	10.6 ± 0.2	11.2 ± 0.2	0.05
Right metacarpus	13.1 ± 0.3	14.0 ± 0.4	0.07
Right metatarsus	21.3 ± 0.4	23.1 ± 0.6	0.02
Right hip-fetlock	42.7 ± 0.8	46.1 ± 1.1	0.02
Right fetlock circumference	12.8 ± 0.2	13.7 ± 0.3	0.06
Right pastern circumference	11.3 ± 0.2	12.1 ± 0.3	0.04
Viscera (gm)			
Heart	116.2 ± 5.8	139.7 ± 8.3	0.02
Liver	477.5 ± 28.4	566.2 ± 40.4	0.08
Paired kidneys	161.0 ± 12.1	184.3 ± 17.2	NS
Right adrenal gland	0.5 ± 0.1	0.8 ± 0.2	0.07
Number of placentomes	113 ± 7	110 ± 11	NS

[a]Values are adjusted for sex of fetus and number of fetuses in utero.

onic death increased 1.4 times for each additional 1.0 ng/ml increase in serum progesterone (Table 1). The negative impact of relatively high circulating concentrations of progesterone on pregnancy rates was similar for recipients that received in vivo or in vitro embryos, suggesting that the influence of progesterone on pregnancy maintenance is independent of embryo type present in the uterus. We are not aware of any other reports concerning the relationship between the levels of progesterone in recipients at the time of transfer of in vitro-produced embryos and subsequent pregnancy rates. However, this relationship has been studied after transfer of in vivo-derived embryos with equivocal results [30–35]. Our findings are consistent with several reports [30, 31, 35] indicating that pregnancy rates were suppressed in recipients with relatively high circulating progesterone concentrations (> 5.0 or 6.0 ng/ml) at the time of transfer, but are at variance with others [32–34]. Additional research with a larger number of recipients is needed to clarify this relationship.

When fetuses and placentas were examined at 7 mo of gestation, abnormalities were detected only in fetuses from in vivo embryos. One male fetus that was co-twin to a female (freemartin) fetus in the in vivo transfer group had marked hydrocephalus and bilateral microphthalmia. Placentas from both transfer groups were similar in color and texture; however, the number of placentomes was significantly less on a per body weight basis for fetuses in the in vitro transfer group. These findings suggest that nutrient availability or gas exchange may be limited late in gestation for fetuses derived from embryos produced in vitro. If this

TABLE 5. Least-squares means (± SE) for measurements expressed as a ratio of body weight for seven-month-old bovine fetuses that developed from embryos produced either in vivo or in vitro.[a]

Measurement	In vivo (n = 20)	In vitro (n = 10)	p value
Skeletal:body wt (cm/kg)			
Heart girth	3.59 ± 0.15	3.04 ± 0.22	0.04
Crown-rump	4.45 ± 0.18	3.61 ± 0.26	0.01
Biparietal diameter	0.73 ± 0.04	0.60 ± 0.05	0.04
Right metacarpus	0.90 ± 0.03	0.76 ± 0.05	0.02
Right metatarsus	1.45 ± 0.06	1.25 ± 0.08	0.04
Right hip-fetlock	2.93 ± 0.12	2.49 ± 0.17	0.04
Right fetlock circumference	0.88 ± 0.04	0.74 ± 0.05	0.03
Right pastern circumference	0.78 ± 0.03	0.65 ± 0.05	0.03
Viscera:body wt (gm/kg)			
Heart	7.70 ± 0.17	7.45 ± 0.25	NS
Liver	31.34 ± 0.92	30.41 ± 0.31	NS
Paired kidneys	10.53 ± 0.58	10.15 ± 0.83	NS
Right adrenal gland	0.03 ± 0.01	0.04 ± 0.01	NS
Placentomes:body wt (number/kg)	7.7 ± 0.5	6.0 ± 0.7	0.04

[a]Values are adjusted for sex of fetus and number of fetuses in utero.

is true, it may help explain the increase in morbidity and mortality observed in newborn lambs derived from embryos cultured in vitro [12]. However, it does not explain increased birth weight of lambs [12] or the increase in body weight of fetuses observed in the present study.

Fetal and placental measurements were all within ranges reported by others [14, 15, 36]. However, when fetuses from in vivo- and in vitro-derived embryos were compared, fetuses in the in vitro group were 17% larger than their in vivo contemporaries. Interestingly, when fetal measurements were examined on a per kilogram body weight basis, fetuses that resulted from in vitro embryos had smaller skeletal, but not visceral, measurements than fetuses from in vivo embryos. These data imply that heavier fetuses resulting from in vitro-produced embryos developed disproportionately to their body weight. To our knowledge this is the first study to demonstrate that bovine fetuses from embryos produced in vitro are actually larger in the third trimester of pregnancy and that the increase in weight of offspring derived from in vitro-produced embryos reported by others [12, 13, 37] is not simply associated with extended gestation length. Sinclair et al. [37] recently reported that, compared with calves produced through artificial insemination, calves from in vitro-produced embryos were significantly heavier at birth, had a higher growth rate, and had increased carcass yield at 7 mo of age. In addition, Walker et al. [12] and Holm et al. [13] found that lambs from embryos exposed to in vitro culture were also heavier at birth than lambs from embryos not exposed to an in vitro culture system. In contrast, an increased incidence in the birth of large calves following the commercial transfer of in vitro-produced embryos has not been observed [2].

The source of the difference in body weight between the two groups of fetuses in the present study is unclear. Increased body weight in fetuses from embryos produced in vitro may have resulted from increased bone density, muscle mass, fat deposition, fluid volume, or some combination of these factors. Fetal or calf body weight is known to be influenced by sex of fetus, number of fetuses in utero, breed of sire and dam of fetus, and nutrition of the dam [14, 15, 36, 38, 39]. The effect of genotype of fetus on birth weight of calves from conventional embryo transfers is especially evident when the breeds of embryo donor and recipient are different [39]. In the present study, we used the same Holstein bull for production of both in vivo and in vitro embryos; however, we could not control for potential differences in breed of dam. In vivo embryos were produced with Angus heifers as donors, and in vitro embryos were produced with ovaries collected primarily from beef cows and heifers. Because oocytes for production of embryos in vitro were from a variety of breeds, we could not examine the effect of breed of dam on body weight of fetuses. The final model, including the effects of embryo source, sex of fetus, and number of fetuses in utero, accounted for 36% of the variation in fetal body weight.

Development of the mammalian embryo as well as growth of the fetus has been shown to be modulated by growth factors [40–43]. It is tempting to speculate that alterations in the concentration of growth factors in the culture medium, or in expression of growth factor ligands and receptors in embryos produced in vitro, may have contributed to the observed difference in fetal size. Messenger RNAs for several growth factors and their receptors including insulin-like growth factor (IGF)-I and -II are present in bovine embryos cultured in an oviductal epithelium coculture system [44]. Furthermore, evidence in mice suggests that alterations in expression of genes for IGF-II or its receptor have been associated with alterations in body weight of newborn pups [41, 43].

In summary, embryo survival after transfer was similar for grade 1 in vitro-produced embryos and grade 1 and 2 in vivo-produced embryos. By contrast, embryo survival was lowest for in vitro-produced embryos of grade 2. In addition, pregnancy rates were lower in recipient heifers with elevated serum concentrations of progesterone at the time of transfer. Survival analysis was found to be a valuable statistical technique for examining embryonic and fetal loss. At 7 mo of gestation, fetuses from embryos produced in vitro were normal in appearance, but were disproportionately larger than fetuses from embryos produced in vivo.

ACKNOWLEDGMENTS

The authors thank L. Yang, B. Collins, N. Martus, C. Wolf, and C. McDonell for providing excellent technical assistance, and Drs. J.H. Britt, B.D. Slenning, J.F. Levine, and J.D. Armstrong for critical review of this manuscript.

REFERENCES

1. Gordon I, Lu KH. Production of embryos in vitro and its impact on livestock production. Theriogenology 1990; 33:77–87.
2. Hasler JF. Applications of in vitro fertilization technology to infertile dairy cows. In: Proceedings of 12th Annual Convention of American Embryo Transfer Assoc; 1993; Portland, ME: 43–52.
3. Looney CR, Lindsey BR, Gonseth CL, Johnson DL. Commercial aspects of oocyte retrieval and in vitro fertilization (IVF) for embryo production in problem cows. Theriogenology 1994; 41:67–72.
4. Gandolfi F. Autocrine, paracrine and environmental factors influencing embryonic development from zygote to blastocyst. Theriogenology 1994; 41:95–100.
5. Sirard MA, Parrish JJ, Ware CB, Leibfried-Rutledge ML, First NL. The culture of bovine oocytes to obtain developmentally competent embryos. Biol Reprod 1988; 39:546–552.
6. Goto K, Kajihara Y, Kosaka S, Koba M, Nakanishi Y, Ogawa K. Pregnancies after co-culture of cumulus cells with bovine embryos derived from in-vitro fertilization of in-vitro matured follicular oocytes. J Reprod Fertil 1988; 83:753–758.
7. Fukuda Y, Ichikawa M, Naito K, Toyoda Y. Birth of normal calves resulting from bovine oocytes matured, fertilized, and cultured with cumulus cells in vitro up to the blastocyst stage. Biol Reprod 1990; 42:114–119.
8. Reichenbach HD, Liebrich J, Berg U, Brem G. Pregnancy rates and births after unilateral or bilateral transfer of bovine embryos produced *in vitro*. J Reprod Fertil 1992; 95:363–370.
9. Eyestone WH, First NL. Co-culture of early cattle embryos to the blastocyst stage with oviductal tissue or in conditioned medium. J Reprod Fertil 1989; 85:715–720.
10. Monson R, Northey DL, Gottfredson R, Peschel DR, Rutledge JJ, Schaefer DM. Pregnancy rates of in vitro produced bovine embryos following nonsurgical transfer. Theriogenology 1992; 37:261 (abstract).

11. Brackett BG, Bousquet D, Boice ML, Donawick WJ, Evans JF, Dressel MA. Normal development following in vitro fertilization in the cow. Biol Reprod 1982; 27:147–158.
12. Walker SK, Heard TM, Seamark RF. In vitro culture of sheep embryos without co-culture: successes and perspectives. Theriogenology 1992; 37:111–126.
13. Holm P, Walker SK, Petersen BA, Ashman RJ, Seamark RF. *In vitro* vs *in vivo* culture of ovine IVM-IVF ova: effect on lambing. Theriogenology 1994; 41:217 (abstract).
14. Eley RM, Thatcher WW, Bazer FW, Wilcox CJ, Becker RB, Head HH, Adkinson RW. Development of the conceptus in the bovine. J Dairy Sci 1978; 61:467–473.
15. Anthony RV, Bellows RA, Short RE, Staigmiller RB, Kaltenbach CC, Dunn TG. Fetal growth of beef calves. II. Effect of sire on prenatal development of the calf and related placental characteristics. J Anim Sci 1986; 62:1375–1387.
16. King KK, Seidel GE Jr, Elsden RP. Bovine embryo transfer pregnancies. I. Abortion rates and characteristics of calves. J Anim Sci 1985; 61:747–757.
17. Elsden RP, Hasler JF, Seidel GE Jr. Non-surgical recovery of bovine eggs. Theriogenology 1976; 6:523–528.
18. Parrish JJ, Susko-Parrish JL, Leibfried-Rutledge ML, Critser ES, Eyestone WH, First NL. Bovine in vitro fertilization with frozen-thawed semen. Theriogenology 1986; 25:591–600.
19. Ball GD, Leibfried ML, Lenz RW, Ax RL, Bavister BD, First NL. Factors affecting successful in vitro fertilization of bovine follicular oocytes. Biol Reprod 1983; 28:717–725.
20. Lindner GM, Wright RW. Bovine embryo morphology and evaluation. Theriogenology 1983; 20:407–416.
21. Elsden RP. Bovine embryo transfer. In: Proceedings of Annual Meeting, Society for Theriogenology; 1980; Omaha, NE: 101–133.
22. Villa-Godoy A, Hughes TL, Emery RS, Chapin LT, Fogwell RL. Association between energy balance and luteal function in lactating dairy cows. J Dairy Sci 1988; 71:1063–1072.
23. SAS. SAS User's Guide, Statistics, Release 6.03 ed. Cary, NC: Statistical Analysis System Institute, Inc.; 1988.
24. Lee ET. Statistical Methods for Survival Data Analysis, 2nd ed. New York: John Wiley & Sons, Inc.; 1992.
25. EGRET Statistical Software, Seattle: Statistics and Epidemiol. Res. Corp.; 1991.
26. Hasler JF, McCauley AD, Lathrop WF, Foote RH. Effect of donor-embryo-recipient interactions on pregnancy rate in a large-scale bovine embryo transfer program. Theriogenology 1987; 27:139–168.
27. Diskin MG, Sreenan JM. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. J Reprod Fertil 1980; 59:463–468.
28. Markette KL, Seidel GE Jr, Elsden RP. Estimation of embryonic losses in bovine embryo transfer recipients from progesterone profiles and returns to estrus. Theriogenology 1985; 23:45–62.
29. Thatcher WW, Bazer FW, Sharp DC, Roberts RM. Interrelationships between uterus and conceptus to maintain corpus luteum function in early pregnancy: sheep, cattle, pigs and horses. J Anim Sci 1986; 62(suppl II):25–46.
30. Niemann H, Sacher B, Elsaesser F. Pregnancy rates relative to recipient plasma progesterone levels on the day of nonsurgical transfer of frozen/thawed bovine embryos. Theriogenology 1985; 23:631–639.
31. Northey DL, Barnes FL, Eyestone WH, First NL. Relationship of serum progesterone, luteinizing hormone and the incidence of pregnancy in bovine embryo transfer recipients. Theriogenology 1985; 23:214 (abstract).
32. Hasler JF, Bowen RA, Nelson LD, Seidel GE Jr. Serum progesterone concentrations in cows receiving embryo transfers. J Reprod Fertil 1980; 58:71–77.
33. Payas AJ, Broadbent PJ, Dolman DF, Christie WB. Factors affecting pregnancy rate in embryo transfer recipients with reference to plasma progesterone. Theriogenology 1989; 31:238 (abstract).
34. Stubbings RB, Walton JS. Relationship between plasma progesterone concentrations and pregnancy rates in cattle receiving either fresh or previously frozen embryos. Theriogenology 1986; 26:145–155.
35. Remsen LG, Roussel JD, Karihaloo AK. Pregnancy rates relating to plasma progesterone levels in recipient heifers at day of transfer. Theriogenology 1982; 18:365–372.
36. Hubbert WT, Stalheim OHV, Booth GD. Changes in organ weights and fluid volumes during growth of the bovine fetus. Growth 1972; 36:217–233.
37. Sinclair KD, Broadbent PJ, Dolman DF, Taylor AG, Reaper JF. In vitro produced embryos as a means of achieving pregnancy and improving productivity in beef cows. Theriogenology 1994; 41:294 (abstract).
38. Anthony RV, Bellows RA, Short RE, Staigmiller RB, Kaltenbach CC, Dunn TG. Fetal growth of beef calves. I. Effect of prepartum dietary crude protein on birth weight, blood metabolites and steroid hormone concentrations. J Anim Sci 1986; 62:1363–1374.
39. Guilbault LA, Roy GL, Beckers JF, Dufour JJ. Influence of breed of fetus on periparturient endocrine responses and subsequent milk production of Ayrshire dams. J Dairy Sci 1990; 73:2766–2773.
40. Gluckman PD, Barry TN. Relationships between plasma concentrations of placental lactogen, insulin-like growth factors, metabolites and lamb size in late gestation ewes subject to nutritional supplementation and in their lambs at birth. Domest Anim Endocrinol 1988; 5:209–217.
41. DeChiara TM, Efstratiadis A, Robertson EJ. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature 1990; 345:78–80.
42. Rappolee DA, Sturm KS, Behrendtsen O, Schultz GA, Pedersen RA, Werb Z. Insulin-like growth factor II acts through an endogenous growth pathway regulated by imprinting in early mouse embryos. Genes & Dev 1992; 6:939–952.
43. Forejt J, Gregorová S. Genetic analysis of genomic imprinting: an *Imprintor-1* gene controls inactivation of the paternal copy of the mouse *Tme* locus. Cell 1992; 70:443–450.
44. Watson AJ, Hogan A, Hahnel A, Wiemer KE, Schultz GA. Expression of growth factor ligand and receptor genes in the preimplantation bovine embryo. Mol Reprod Dev 1992; 31:87–95.