Effect of a unilateral or bilateral twin embryo distribution on twinning and embryo survival rate in the cow

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Summary. Uni- and bilateral twin embryo distributions were effected by the transfer of one embryo on Day 7 to the ipsi- or contralateral uterine horn of previously inseminated heifers (123, Exp. 1) or cows (95, Exp. 2). The embryo transfers were surgical in Exp. 1 and non-surgical in Exp. 2. Transferred and native embryos were distinguished by breed. Embryo survival rate was measured in a proportion ($N = 40$) of the heifers at Day 53 of gestation and in the remainder of the heifers and all of the cows at term. In the heifers (Exp. 1) overall pregnancy rates of 76% and 75% were recorded after uni- and bilateral twin embryo distributions respectively. Twinning rates of 55% and 60% at Day 53 of gestation and 60% and 60% at term were recorded for uni- and bilateral distributions respectively. In the cows (Exp. 2) calving rates of 61% and 63% and twinning rates of 33% and 38% were recorded following uni- and bilateral twin embryo distributions respectively. When the data from both experiments were combined, overall embryo survival rates were similar for both twin embryo distributions although the ipsilateral transfer of an embryo reduced the survival rate of the native embryo. It is concluded that the confinement of two embryos in one uterine horn on or after Day 7 does not depress pregnancy, twinning or overall survival rate to term.

Keywords: embryo distribution; twinning; survival rates; cow

Introduction

Most published estimates of the frequency of natural twin births in the cow fall within a range of 1–5% depending mainly on breed, parity and environmental conditions (see reviews by Gordon *et al.*, 1962; Scanlon *et al.*, 1974; Morris, 1984). Increasing this frequency would be expected to increase the biological efficiency of the cow (Dickerson, 1983; Diskin & Hickey, 1987).

Rowson *et al.* (1971) were first to show that ovulation rate and not uterine capacity is the main factor limiting twin-calving rate in the cow. This was later confirmed by many others (see review by Sreenan, 1977). Attempts to increase ovulation rate in the cow as a means of increasing twin-calving rate include mild superovulation (Mauleon *et al.*, 1978), genetic selection (Piper & Bindon, 1979; Morris & Day, 1986) and hormone immunization (Wise & Schanbacher, 1983; Sreenan, 1984; Webb *et al.*, 1984). These approaches, if successful in inducing a high proportion of twin ovulations, should result in the occurrence of unilateral twin ovulations in 50% of such cases. However, there are some reports showing a unilateral twin ovulation rate of 75% in twin-ovulating heifers (see review by Hanrahan, 1983). There are suggestions in the literature that unilateral twin ovulation results in a high rate of embryonic death and consequently a low twinning rate (Gordon *et al.*, 1962; Rowson *et al.*, 1971). Hanrahan (1983), reviewing the literature on embryo loss in cattle, calculated that the probability of embryo loss was increased by 22% for unilateral twin ovulations compared with bilateral or single ovulations, both of which had similar embryo survival rates. Unlike the sow or the ewe, transuterine migration of ova or embryos rarely occurs in the cow (Scanlon, 1972), and it has been postulated that the consequent overcrowding of two embryos

within one uterine horn results in the death of one of the embryos in a high proportion of cases (Rowson *et al.*, 1971). If this were the case, an increase in the proportion of cows with twin ovulations should not result in equal increases in twin-calving rates among animals with uni- and bilateral twin ovulation distributions.

In the studies reported here we have carried out either ipsilateral or contralateral embryo transfers to previously inseminated recipients to examine the effect of twin embryo distribution (unilateral *vs* bilateral) on embryo and fetal survival through to term in primiparous heifers (Exp. 1) and mature cows (Exp. 2).

Materials and Methods

Experiment 1

Animals. A total of 123 sexually mature (20–24 months of age) Aberdeen Angus and Hereford cross heifers within a liveweight range of 370–440 kg were used as embryo recipients to determine the effect of twin embryo distribution on pregnancy, embryo survival, calving and twin-calving rates. The heifers were treated with short-term intravaginal progestagen-impregnated sponges to induce a synchronous oestrous response (Mulvehill & Sreenan, 1978) and were checked for the occurrence of standing oestrus with the aid of a vasectomized bull. All heifers were artificially inseminated once during an observed standing oestrus and all inseminations were carried out by the same technician at 6–12 h after the heifers were first observed to stand. Frozen–thawed semen (30×10^6 spermatozoa per straw) from one Charolais bull of known high fertility (90-day non-return rate of 78.0%) was used for each insemination.

Embryos. Embryos were collected following the induction of superovulation in donor heifers of a breed similar to that of the recipients. After the administration of PMSG (Folligon, Intervet Ltd, Milton Road, Cambridge, UK) and prostaglandin (Estrumate, Coopers Ltd, Berkhamsted, UK) during midcycle, the donor heifers were inseminated at the superovulation oestrus with frozen–thawed semen from one Simmental bull of known high fertility (90-day non-return rate of 83.2%). The use of different breed sires for the recipient and donor heifers allowed a distinction between the native and transfer calves at term. The superovulation and embryo recovery procedures were carried out as previously described (Sreenan & Diskin, 1982).

Twin-embryo distribution. Unilateral and bilateral twin-embryo distributions were effected as follows. After insemination recipient heifers were randomly allotted to receive an embryo in the ipsilateral or contralateral uterine horn. The side of the corpus luteum was first determined by rectal palpation before transfer and was confirmed by direct observation of the ovary at the time of transfer. The embryo transfers were carried out in each case directly into the lumen of the uterine horn that had been exteriorized through the flank region. All transfers were carried out to synchronous recipients (0 ± 1 day) on Day 7 of the donor's cycle (Day 0 = the day of oestrus) using freshly collected embryos. On a given day grade 1 embryos (morulae and blastocysts) collected from at least 3 donors were pooled and then randomly allotted to recipients. Embryo assessment was based on the criteria published by Elsden *et al.* (1978). Only heifers with a single corpus luteum were used as recipients. At the time of transfer about 15–18% of the recipients would not have a native embryo in the ipsilateral uterine horn because of a combination of fertilization failure and early embryonic deaths before Day 7 (see review by Sreenan & Diskin, 1986). However, random allocation would distribute these equally between the ipsilateral and contralateral transfer groups. During the course of the transfers several recipient heifers were excluded because of poorly formed corpora lutea or difficulty during transfer. Excluding these animals, a total of 66 recipients received an embryo in the uterine horn ipsilateral and 57 in the uterine horn contralateral to the corpus luteum.

Measurement of embryo survival. All recipient heifers were pregnancy tested by rectal palpation between 45 and 50 days from the insemination. A random sample of those diagnosed as pregnant was then slaughtered from each of the ipsilateral ($N = 20$) and contralateral ($N = 20$) transfer groups at Day 53 of gestation to provide a measure of embryo survival rate up to the time of completion of implantation. Confirmation of a viable pregnancy and embryo survival at this time was based on the expected weight for age of each fetus, its fetal fluids and membranes (Sreenan & Beehan, 1976). The remaining heifers diagnosed as pregnant ($N = 53$) were held to full term but were pregnancy tested by palpation again on Day 150 from insemination. At calving, the number and breed type of each calf born were recorded for each recipient heifer.

Experiment 2

Animals. A total of 95 Hereford $\times$ Friesian second and later parity beef cows were used in this experiment to determine the effect of twin-embryo distribution on pregnancy, embryo survival and twinning rates in mature cows. Cows that had calved a minimum of 50 days previously had their oestrous cycles synchronized as described for Exp. 1. Detection of standing oestrus, artificial insemination and embryo production were all carried out as described for Exp. 1. The embryo transfers in this experiment were carried out non-surgically using the procedure described by

Sreenan & Diskin (1982) but to ipsilateral or contralateral uterine horns as described for Exp. 1. Different breed sires were again used as in Exp. 1 to allow distinction between native and transfer calves at term. The cows were inseminated with semen from a Charolais bull (90-day non-return rate of 79.7%) and the donor heifers were inseminated with semen from a Simmental bull (90-day non-return rate, 75.3%).

Measurement of embryo survival. All recipient cows were pregnancy tested by rectal palpation at 50 days after insemination and those diagnosed as pregnant were kept and again pregnancy tested at 150 days after insemination. All cows diagnosed as pregnant at this second test were kept to term. At calving the number of breed types of calves born were recorded for each recipient cow.

Statistical analysis

Within each experiment, differences between proportions were tested by χ^2 analysis and differences between means were tested by analysis of variance (Steel & Torrie, 1980).

Results

Experiment 1

In the heifers, pregnancy, twin-pregnancy and embryo survival rates were similar ($P > 0.10$) for unilateral and bilateral twin embryo distributions up to Day 53 of gestation (Table 1). Embryo survival rates through to term are presented in Table 2 for the remaining heifers that were diagnosed as pregnant at 45–50 days from insemination. Calving, twinning and embryo survival rates were similar ($P > 0.10$) for the unilateral and bilateral twin embryo distributions. The incidence of pregnancy loss between Day 45–50 and full term was 15.1% overall and was not affected by the twin-embryo distribution. Most of the pregnancy loss had occurred by Day 150 but with no indication of whether the losses were of singles or twins. Between Day 150 and term one heifer aborted twin fetuses in each group.

Table 1. Effect of a unilateral or bilateral twin-embryo distribution on pregnancy rate and embryonic survival up to Day 53 of gestation in heifers

	Embryo distribution	
	Unilateral	Bilateral
No. of recipients	66	57
No. pregnant at Days 45–50 (%)	50 (76)	43 (75)
No. pregnant, killed at Day 53	20	20
No. with twin embryos (%)	11 (55)	12 (60)
Embryo survival in pregnant recipients (%)*	31 (78)	32 (80)

*Assuming 100% fertilization in both groups.

Experiment 2

In the parous cows, pregnancy, calving, twinning and embryo survival rates to full term were similar ($P > 0.10$) for the unilateral and bilateral twin embryo distribution groups (see Table 3).

Table 2. Effect of a unilateral or bilateral twin-embryo distribution on fetal survival to term in heifers diagnosed pregnant at Days 45–50

	Embryo distribution	
	Unilateral	Bilateral
No. of recipients pregnant at Days 45–50	30	23
No. of recipients pregnant at 150 days (%)	26 (87)	21 (91)
No. of recipients calved (%)*	25 (83)	20 (87)
No. of sets of twins born (%)	15 (60)	12 (60)
No. of singles born (%)	10 (40)	8 (40)
Embryo survival in calved recipients (%)	40 (80)	32 (80)
Embryo survival overall†	50%	52%

*One set of twins aborted from each group.

†Calculated from the combined slaughter and calving data.

The overall incidence of pregnancy loss between Day 50 and term was 8% and was unaffected by the twin-embryo distribution.

Survival rate of native and transferred embryos

The use of different breed sires allowed distinction between native and transferred calves born and the data on embryo survival are summarized from both experiments in Table 4. The overall survival rates of native and transferred embryos were similar ($P > 0.10$). The survival rate of the native embryos and transferred embryos and both combined were similar ($P > 0.05$) for Exps 1 and 2. There was no interaction between embryo distribution and experiment for native, transferred or overall embryo survival rate ($P > 0.05$). Therefore, the data from both experiments were combined. In animals that calved there was a higher survival rate of the transferred embryos ($P < 0.05$) in the unilateral groups and of the native embryos ($P < 0.05$) in the bilateral group. More 'transfer' single calves ($P < 0.05$) were born from the unilateral than the bilateral group and more native singles ($P < 0.05$) from the bilateral group.

Discussion

The results presented show that the confinement of two embryos within the ipsilateral uterine horn did not increase the rate of embryonic or fetal mortality compared with a bilateral distribution. The transfer of the second embryo to an already inseminated recipient cow is therefore an effective means of inducing twin pregnancy, and the site of transfer (ipsilateral or contralateral) will not affect the twin-calving rate.

In Exp. 1, the pregnancy rates of 76% and 75%, for unilateral and bilateral twin embryo distributions respectively, at 45–50 days from insemination in the heifers are in agreement with the rates of 73% and 72% reported by Rowson *et al.* (1971) for similar distributions. Pregnancy rates within the range 71–76% at 40–50 days after bilateral embryo transfers have also been reported (Sreenan *et al.*, 1975; Anderson *et al.*, 1978). In Exp. 2 the calving rate of 63% in mature cows for

Table 3. Effect of a unilateral or bilateral twin-embryo distribution on embryo survival rate to term in parous cows

	Twin embryo distribution	
	Unilateral	Bilateral
No. of cows	49	46
No. pregnant at Day 50 (%)	31 (63)	33 (72)
No. pregnant at Day 150	30 (61)	31 (67)
No. calved (%)	30 (61)	29 (63)
No. of sets of twins born (%)	10 (33)	11 (38)
No. of singles born (%)	20 (67)	18 (62)
Embryo survival in calved recipients (%)	40 (67)	40 (69)
Embryo survival overall (%)	40 (41)	40 (43)

Table 4. The effect of a unilateral or bilateral twin-embryo distribution on the survival of native and transferred embryos

	Twin embryo distribution			
	Unilateral		Bilateral	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Total calves born	40	40	32	40
Native embryo survival (%)	19 (48)	25 (63)	19 (59)	27 (68)
Transferred embryo survival (%)	21 (52)	15 (40)	13 (41)	13 (33)
Single calves born	10	20	8	18
Native single calves surviving (%)	4 (40)	15 (75)	7 (88)	16 (89)
Transferred single calves surviving (%)	6 (60)	5 (25)	1 (12)	2 (10)

the bilateral embryo distribution is in agreement with a previously published rate of 58% from this laboratory (Sreenan *et al.*, 1981) and a rate of 57% published by Renard *et al.* (1979). There are no comparable reports of calving data for unilateral twin-embryo distributions.

The overall pregnancy loss between about Day 50 and term was higher for the gravid heifers (15.1%) than for the parous cows (8%) but was unaffected by the embryo distribution. The losses recorded for both parity groups are consistent with published estimates (see review by Sreenan & Diskin, 1986).

There was no difference in twin-pregnancy, twin-calving, or overall embryonic mortality rate between the unilateral and bilateral embryo distributions within Exps 1 and 2. This contrasts with the conclusions drawn by Rowson *et al.* (1971) who used embryo transfer to achieve similar embryo distributions. These authors recorded viable twinning rates of 40% (N = 10) and 73% (N = 11) and embryo survival rates of 65% (N = 20) and 86% (N = 22) in pregnant recipient heifers with

unilateral and bilateral embryo distributions respectively. While Rowson *et al.* (1971) concluded that embryo mortality was higher in the unilateral group, the number of observations was too limited to indicate a statistically significant difference. Following the surgical transfer of two embryos to the tip of the ipsilateral uterine horn, Newcomb *et al.* (1980) reported twin-pregnancy and embryo-survival rates of 56% and 78%, respectively, in a group of 16 pregnant recipient heifers. In a separate group of 18 pregnant recipients within the same study, these authors reported twin-pregnancy and embryo-survival rates of 61% and 73% respectively following a bilateral embryo distribution. These data are consistent with the results of the present Exps 1 and 2.

While the overall embryo survival rate was unaffected by the embryo distribution, there was a tendency within each of the two experiments for the survival rate of the native embryo to be reduced in the unilateral twin embryo distribution. When the data from both experiments are combined for all recipients the estimated embryo survival rate of native embryos in a bilateral distribution is 59% (46/78) compared with 49% (44/89) in a unilateral distribution. This trend is also evident in pregnant recipients. When the data from both experiments are combined for pregnant recipients, the survival rate of native embryos in pregnant recipients is reduced from 94% (46/49) for a bilateral distribution to 80% (44/55) for a unilateral distribution ($P < 0.05$). This would suggest that the transferred embryo adversely affected the survival rate of the native embryo. Because of the local unilateral relationship between the corpus luteum, its adjacent uterine horn and the embryo (Del Campo *et al.*, 1977), the presence of two embryos within the ipsilateral uterine horn should significantly increase the chances of establishing a pregnancy compared with one embryo located in each uterine horn, provided that each embryo acts independently of the other. Based on an expected embryo-survival rate to term of about 60% following spontaneous ovulation and insemination (Sreenan & Diskin, 1986) and on an expected embryo-survival rate to term also of about 60% following single embryo transfer (Greve & Del Campo, 1986), the calving rate for a unilateral twin embryo distribution derived in this manner should be 84%. However, the observed calving rate for the unilateral group in Exp. 2 is only 61% and the estimated calving rate for the unilateral group in Exp. 1 is 64%. Therefore, while the confinement of two embryos within the ipsilateral uterine horn resulted in similar calving, twinning and overall embryo-survival rates as for a bilateral distribution, these rates are clearly lower than would be expected if each embryo acted independently of the other. It is unlikely that the transfer procedure adversely affected the survival rate of the native embryos as it has been shown that the fertility of mated heifers was unaffected by sham embryo-transfer procedures carried out to the contralateral (Brand & Drost, 1977) or ipsilateral (Seidel *et al.*, 1975) uterine horns.

The higher survival rate of native embryos in this report with bilateral distribution is in agreement with previous published reports from this laboratory. After bilateral surgical transfers, embryo survival rates of 60% and 31% were reported for the ipsilateral and contralateral uterine horns, respectively (Sreenan *et al.*, 1975). Following contralateral embryo transfers to previously inseminated recipients, embryo survival rates of 60% and 44% and again 50% and 32% were reported for the native and transferred embryos, respectively (Sreenan & McDonagh, 1979; Sreenan *et al.*, 1981). Of the single calves born in this study a higher proportion was from the transferred embryos in the unilateral distribution groups compared with the bilateral groups. The very low proportion of single calves that derived from the transferred embryos in the bilateral distribution groups is consistent with the published literature. Single embryo transfers to the contralateral horn, without a viable embryo in the ipsilateral horn, have consistently resulted in a high rate of embryonic mortality because of failure of the luteotrophic and antiluteolytic signals from the embryo to reach the corpus luteum (see review by Seidel, 1980).

Several abattoir surveys (Erdheim, 1942; Scanlon *et al.*, 1974; Al-Dahash & David, 1977) on the distribution of twin ovulations in twin-pregnant cows have indicated a higher proportion of bilateral than unilateral twin pregnancies. Reviewing these data, Hanrahan (1983) estimated that the probability of embryo loss was increased by 22% for unilateral twin ovulations compared with bilateral or single ovulations. Data from Morris *et al.* (1988) similarly suggest a lower embryo

survival rate after unilateral twin ovulations than after bilateral twin ovulations in spontaneously ovulating cows. In that study twin-calving rates of 11% and 38% were recorded following unilateral and bilateral double ovulations, respectively. These data contrast with the present results and also with those of Echternkamp & Gregory (1987), who found that pregnancy and embryo-survival rates were similar for cows with single and multiple ovulations irrespective of whether these occurred unilaterally or bilaterally. They recorded pregnancy rates of 81.9% and 75.8% and twinning rates of 45.5% and 58.6% following bilateral and unilateral multiple ovulation, respectively.

While the published data on embryo-survival rates in spontaneously twin-ovulating cows are confounded with fertilization failure, the results of our experiments demonstrate that confinement of two embryos in one uterine horn on or after Day 7 does not depress pregnancy, twinning or overall embryo-survival rates to term. Further studies are required to determine fertilization rate and embryo survival accurately in spontaneously uni- and bilateral twin-ovulating cows.

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