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PRODUCTION OF TWINS IN CATTLE BY EGG TRANSFER

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Summary. A total of fifty-two heifers was used in an egg transfer experiment to study methods of inducing twinning in cattle.

Two groups of eleven recipients were made pregnant to determine the effect of either transferring two eggs to a single uterine horn or one egg to each. Seven animals in each group were slaughtered and the remaining four allowed to go to term.

Of the heifers into which two eggs were transferred to a single horn, 73% became pregnant and 45% had twins at slaughter or calving. In a single case, one of the eggs had undergone transuterine migration and developed in the opposite horn to that into which it was transferred. Of the eleven heifers receiving one egg in each uterine horn, 73% had twins and a pregnancy rate of 72% was obtained in this group. A much greater degree of embryonic loss was thus found in the slaughtered group which had received two eggs to a single uterine horn.

The number of cotyledons which developed was much greater in heifers with a bilateral twin pregnancy.

The experiments indicate that the development of twin embryos within a single uterine horn results in competition for nutrients and sometimes the loss of one or both embryos but, with bilateral transfers, a high percentage of normal twins is produced.

INTRODUCTION

It was demonstrated by Rowson, Moor & Lawson (1969) and Rowson, Lawson & Moor (1969) that, following the surgical transfer of two cow eggs to the uterine horn adjacent to the ovary containing the corpus luteum, the percentage of resulting pregnancies was high (91) but the percentage of twins actually born was low (12·5). It was postulated that the reason for the low percentage of twins might be due to: (1) failure of one of the eggs to migrate to the other uterine horn and consequent implantation of both embryos within the same uterine horn leading to competition for survival, or (2) occurrence of migration but failure of pregnancy to continue in the contralateral horn owing to the absence of a corpus luteum in the ovary adjoining that horn. Such a unilateral relationship is known to exist in both cattle and sheep if the embryo is actually confined to one horn. Gordon, Williams & Edwards (1962) in the cow, Baier

& Rüsse (1968) in the sheep, and Lyngset (1968) in the goat, had all reported that twins occur naturally at a higher proportion when a corpus luteum is present in each ovary than when two corpora are present unilaterally. Erdheim (1942) also reported that out of seventeen cases of twinning in dairy cattle, only two were unilaterally pregnant and out of nineteen beef cows, only three. In another report (Perkins, Olds & Seath, 1954), an examination of 255 cattle pregnancies revealed only four cases in which an egg had migrated to the contralateral horn.

MATERIAL AND METHODS

A total of fifty-two maiden heifers was used, nineteen as egg donors and thirty-three as egg recipients. The heifers were run with vasectomized bulls, whose briskets were painted daily with a coloured raddling grease. The heifers were examined twice daily for service marks which indicate oestrus.

Donor animals

Donor animals were injected on Day 16 or 17 of the oestrous cycle with pregnant mares' serum gonadotrophin (Gestyl, Organon Laboratories) at a dose level of 1500 to 2000 i.u. and closely checked for signs of oestrus over the next few days. They were then inseminated and, on the 4th to 7th day after the onset of oestrus, laparotomy was performed in order to recover the fertilized eggs; at the same time, ovulations were recorded. The eggs were recovered by flushing the uteri and oviducts of the donor animals with TCM 199 (Glaxo Laboratories) using the techniques described by Rowson, Moor & Lawson (1969). Before transfer, all eggs were stored in an incubator at 37° C and the time interval between recovery and transfer recorded to the nearest 5 min.

Recipient animals

No attempt was made to synchronize oestrus artificially, but the donor and recipient heifers were selected from the herd solely on the evidence of service marks or having been seen to be mated by the vasectomized bull and were always within ± 2 days of exact synchronization of oestrus.

Recipient animals were divided into two groups:

Group 1: those which received one egg to each uterine horn.

Group 2: those which received two eggs in the uterine horn adjacent to the corpus luteum.

After egg transfer, all recipients were run with a vasectomized bull and if they had not shown evidence of oestrus by 42 days, they were examined rectally for pregnancy. During examination, an attempt was made to determine whether the heifer was carrying a single embryo or twin embryos but, to avoid disturbing the pregnancy, no attempt was made to define the amniotic sacs and diagnosis of pregnancy was limited to establishing whether each uterine horn was distended to the appropriate size.

Seven out of a total of eleven pregnant recipients in Group 1 were slaughtered at 90 days of pregnancy while the remaining four were allowed to go to term. Similarly, seven out of eleven pregnant animals in Group 2 were also slaugh-

tered, but at 60 days of gestation, because it was felt that if prenatal losses were occurring due to overcrowding of a single uterine horn, then such losses would probably become evident at a stage of pregnancy much earlier than 90 days. The remaining four animals in Group 2 were allowed to go to term. Of the total of thirty-three recipients, twenty-two were used for the experiments, two were discarded (see Group 1 results) and nine failed to become pregnant.

At laparotomy, the uteri of all slaughtered recipients were removed, carefully opened and the membranes gently separated from the cotyledons so as to leave the conceptus intact. The number of cotyledons to which membranes were attached was recorded for individual uterine horns. The foetal membranes were then opened and the sex and crown–rump measurements of the embryos recorded.

RESULTS

Group 1

In order to provide the eleven pregnant experimental animals, eggs were transferred to a total of eighteen recipients. Thirteen became pregnant, giving a pregnancy rate of 72% but two of these pregnant animals were discarded; the first because of the abortion of twins early in pregnancy and the other because of the loss of an egg during transfer.

From Table 1, it will be seen that viable twin foetuses were found in five out of the seven recipients in the slaughtered group. A sixth heifer was diagnosed as carrying twins on Day 42 and, at slaughter, evidence of a degenerating embryo was found in the non-pregnant horn.

Three of the four heifers left to calve in Group 1 produced twins. The fourth animal was also carrying twins at examination on Day 42 but lost one of the foetuses between that time and term. Thus, in the eleven recipients that became pregnant after the transfer of one egg to each uterine horn, only two embryos failed to develop to Day 42 and thereafter only two further losses occurred, giving an overall embryonic survival rate on Day 90 of 82%.

Group 2

Following the transfer of two eggs to one uterine horn, eleven out of fifteen recipients became pregnant, giving a pregnancy rate of 73%. These results show that the transfer of one egg to each uterine horn or both eggs to one horn clearly does not influence the percentage of recipients that become pregnant following egg transfer.

At autopsy of the seven recipients on Day 60, it was found that only one set of twins had survived within the same uterine horn (Table 2). Twins were also found in a second heifer in Group 2 but, in this animal, transuterine migration of one embryo had occurred to give a bilateral twin pregnancy similar to those obtained in Group 1. Of the remaining five recipients slaughtered on Day 60, two had a normal conceptus together with a degenerate embryo present in the same horn, two had only one viable embryo present and one had two degenerate embryos in the one horn.

Three of the four heifers left to calve in Group 2 produced twins: in each case, foetal development occurred in the horn to which the two eggs had been transferred.

TABLE 1

RESULTS OF BILATERAL EGG TRANSFER TO HEIFERS WITH A UNILATERAL CORPUS LUTEUM: THE DEGREE OF SYNCHRONIZATION, STORAGE TIME OF EGGS AND NUMBERS OF FOETUSES DEVELOPING (GROUP 1)

Recipient no.	*Synchronization variation from donor (days)*	*Eggs transferred (cell stage)*	*Storage time (min)*	*Pregnant horn (left/right)*	*Corpus luteum present (left/right)*
124 (T)	0	2×12	115	+/+	−/+
190 (T)	−1	2×16	65	−/+	+/−
167 (T)	+2	2×32	105	+/+	−/+
208 (T)	−1	1×12, 1×32	65	+/+	+/−
149 (S)	−1	2×32	150	+/+	−/+
179 (S)	0	2×8	175	+/+	+/−
180 (S)	+1	2×8	140	+/d	+/−
199 (S)	0	1×12, 1×32	130	+/+	−/+
53 (S)	0	2×8	65	+/+	−/+
207 (S)	0	2×32	135	+/−	+/−
260 (S)	0	2×32	60	+/+	+/−
156 (NP)	−1	2×16	135	−/−	−/+
131 (NP)	−2	2×16	110	−/−	+/−
24 (NP)	+1	2×8	50	−/−	−/+
222 (NP)	0	2×32	105	−/−	−/+
162 (A)	−1	2×32	115	+/+	−/+
204 (P)	+2	2×32	185	−/+	−/+
214 (NP)	+1	2 blast.	70	−/−	−/+

(T) = Term animals.
(S) = Animals slaughtered at 90 days.
\+ = Normal embryos. Position of embryos determined by rectal examination in (T) group.
d = Degenerating embryo.
(NP) = Non-pregnant animals.
A = Aborted.
P = Pregnant but one egg lost.

The 55% embryonic loss in the eleven pregnant recipients with two eggs transferred to one uterine horn appears to take place during the first 60 days of pregnancy. No further foetal loss occurred after this stage, despite the presence of two foetuses within one uterine horn.

Effect of degree of synchronization

All recipient heifers exhibited oestrus within the range of ±2 days of the date of oestrus of the donor animals. There was evidence of an effect of variation from exact synchronization which could have slightly influenced the pregnancy rate in favour of Group-2 recipients since, in nine out of eleven heifers in this group, synchronization was exact, whereas the numbers of exactly synchronized heifers in Group 1 were six out of eleven. Data on the influence of synchronization on the pregnancy rate in cattle will be published elsewhere.

Birth weight of calves

The birth weights of calves born as singles or twins in Groups 1 and 2 are shown in Tables 3 and 4 respectively. No special feeding regimen was adopted for those animals known to be carrying twins. All recipients were young maiden heifers, one of which (No. 208) weighed only 672 lb at calving and although her twin calves were underweight at birth, they developed rapidly and thrived normally.

TABLE 2

RESULTS OF UNILATERAL EGG TRANSFERS TO HEIFERS WITH A UNILATERAL CORPUS LUTEUM: THE DEGREE OF SYNCHRONIZATION, STORAGE TIME OF EGGS AND NUMBER OF FOETUSES DEVELOPING (GROUP 2)

Recipient no.	*Synchronization variation from donor (days)*	*Eggs transferred (cell stage)*	*Storage time (min)*	*Pregnant horn (left/right)*	*Corpus luteum (left/right)*
202 (T)	0	2×16	190	−/+	−/+
212 (T)	0	2×blast.	70	++/−	+/−
165 (T)	0	2×32	65	++/−	+/−
172 (T)	0	2×32	70	++/−	+/−
238 (S)	0	2×32	75	d+/−	+/−
193 (S)	0	2×8	110	+/−	+/−
206 (S)	0	2×8	155	−/+	−/+
192 (S)	0	2×32	195	−/++	−/+
64 (S)	+1	2×32	265	d+/−	+/−
209 (S)	+1	2×32	145	+/+	+/−
203 (S)	0	2×16	55	dd/−	+/−
175[a] (NP)	+1	2×16	75	−/−	+/−
175[b] (NP)	−1	2×32	110	−/−	+/−
160 (NP)	+1	2×32	85	−/−	−/+
216 (NP)	+1	2×32	60	−/−	−/+

(T) = Term animals.
(S) = Animals slaughtered at 60 days.
d = Degenerating embryos.
+ = Normal embryos.
a and b = This animal used twice.
(NP) = Non-pregnant.

Effect of unilateral pregnancy on cotyledon numbers

The number of cotyledons per horn in relation to bilateral or unilateral pregnancy is shown in Table 5. In many cases where the pregnancy was unilateral, the number of cotyledons in the contralateral horn was very small. The average number of cotyledons where animals were carrying twins was much greater than with a single pregnancy. There was some indication of a

TABLE 3

THE EMBRYONIC DEVELOPMENT AND SEX OF CALVES FROM GROUP-1 HEIFERS

Heifer no.	*Heifer wt at calving (lb)*	*Sex and wt of calves (lb)*	*Crown–rump length embryos (cm)*
124 (T)	1126	♂ 70, ♀ 61	
190 (T)	1084	♂ 78	
167 (T)	1124	♂ 62, ♀ 59	
208 (T)	672	♂ 50, ♀ 44	
149 (S)			♂ 18·0, ♀ 17·1
179 (S)			♂ 16·3, ♂ 16·2
180 (S)			♂ 15·5
199 (S)			♂ 18·0, ♀ 17·5
53 (S)			♂ 14·5, ♀ 13·5
207 (S)			♀ 17·5
260 (S)			♂ 16·8, ♀ 14·2

TABLE 4

THE EMBRYONIC DEVELOPMENT AND SEX OF CALVES FROM GROUP-2 HEIFERS

Heifer no.	*Heifer wt at calving (lb)*	*Sex and wt of calves (lb)*	*Crown–rump length embryos (cm)*
202 (T)	884	♂ 78	
212 (T)	964	♂ 65, ♀ 62	
165 (T)	992	♂ 68, ♂ 59	
172 (T)		♀ 52, ♀ 52	
193 (S)			♂ 8·2
206 (S)			♂ 8·3
192 (S)			♀ 8·4, ♀ 8·0
64 (S)			♂ 7·4, d? 0·3
209 (S)			♀ 8·5, ♀ 8·8
203 (S)			d? 1·4, d? 1·3
238 (S)			♂ 6·2, d♂ 5·4

(T) = Term animals.
(S) = Animal slaughtered at 60 days.
d = Degenerating embryos.
? = Unable to determine sex.

TABLE 5

NUMBER OF COTYLEDONS IN SLAUGHTERED ANIMALS TO WHICH MEMBRANES WERE ATTACHED IN RELATION TO PREGNANT OR NON-PREGNANT HORNS

Heifer no.	*Pregnant horn (left/right)*	*Cotyledons/ horn*	*Total cotyledons*
GROUP 1			
149	+/+	55/59	114
179	+/+	53/56	109
180	+/d	45/ 2	47
199	+/+	66/62	128
53	+/+	57/36	93
207	+/−	71/44	115
260	+/+	56/48	104
GROUP 2			
193	+/−	54/31	85
206	−/+	6/39	45
192	−/++	45/60	105
64	d+/−	42/22	64
209	+/+	45/46	91
203	dd/−	32/ 0	32
238	d+/−	54/ 4	58

d = Degenerating embryos.
+ = Normal embryos.

direct relationship between cotyledon numbers and crown–rump length of embryos.

Effect of storage of eggs before transfer

There was no obvious falling off in pregnancy rate where eggs were stored at 37° C for up to 4½ hr, but investigations into more prolonged periods of storage are now in progress.

DISCUSSION

The results of this study show that a very much higher percentage of viable twins is obtained (73) in heifers that conceive following the transfer of a single egg to each uterine horn, than if two eggs are transferred to a single horn (45). They provide evidence that failure to obtain a satisfactory twinning rate when two eggs are transferred to a single uterine horn is due to the fact that in cattle, transuterine migration of one of the two eggs rarely occurs and the resulting overcrowding of one horn sometimes may result in embryonic death.

It is interesting to note in this connection that the number of cotyledons to which conceptuses were attached was usually much greater where the pregnancies were bilateral. A similar observation has been made by Testart & Du Mesnil Du Buisson (1970).

The development of two embryos, one in each uterine horn would, therefore, appear to provide a much better physiological environment for normal growth. This also follows from the finding on the Group-2 animals that at 60 days, a high proportion of degenerate embryos resulted from transfer of two eggs to one horn.

In this study, there was no evidence for the existence of a clear-cut unilateral utero-ovarian-embryo relationship when the embryo was not confined. Presumably, progesterone secreted from a single corpus luteum is capable of maintaining a twin pregnancy in a high proportion of cases.

An effective way to apply these findings would be to transfer a second egg to an animal which has already become pregnant following routine artificial insemination; the practicability of such a procedure is now the subject of further experiments. The importance of that kind of approach would be particularly far-reaching in the beef industry and the next obvious steps to take are further research into methods of non-surgical egg transfer and the provision of large numbers of fertilized eggs which could be stored and called upon as required.

There is a belief among farmers that twinning is uneconomical because the calves are small, the dam often retains the placenta, and her milk and butterfat production are reduced. In addition, it could be argued that freemartins resulting from mixed sex twins constitute a distinct disadvantage in dairy breeds. It has been shown (Gordon *et al.*, 1962), however, that provided the cows that carry twins are well fed during the last 2 months of gestation, then the birth weight of each twin is not materially below that of single calves. The same workers also demonstrated that if the animals were well fed, then the incidence of retained placentae was very low indeed. The possible fall in butterfat content is another point for concern, since it has been reported (Ward, 1950) that a fall of 11 to 12 lb of fat per lactation occurred in animals which carried twins; Cloninger & Thoele (1957) also reported a slight reduction in milk yields, but the findings were in animals which had not been suspected of carrying twins and which had received no additional feeding during the last few weeks of pregnancy.

It would appear from the experiments described here that the failure of the superovulation techniques (by injecting gonadotrophins) as a means of producing a twinning rate of economic importance (Gordon *et al.*, 1962) is probably

due to variation in individual response to injected gonadotrophin or to unilateral superovulation. If more than one ovulation occurs in one or both ovaries, then it is inevitable that there will be prenatal loss, which in some cases may be total.

In conclusion, it can be said that bilateral transfer of eggs to cows provides an effective method whereby a very high percentage of twins can be safely produced but application of this technique on a practical scale must wait until general technical difficulties have been overcome. In the bovine, the foetal membranes fuse in the vast majority of twin pregnancies and the foetuses develop as chimaeras. This provides an opportunity of producing, in the same cow, different breed twins with intermixed blood types. Work is now in progress to see whether breeds of widely differing types, such as the Jersey and Friesian, can be used to study the effects of chimaerism on production and development.

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