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FERTILITY FOLLOWING EGG TRANSFER IN THE COW; EFFECT OF METHOD, MEDIUM AND SYNCHRONIZATION OF OESTRUS

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Summary. A total of ninety-two heifers was used in a series of four experiments designed to investigate the effect of method of egg recovery, transfer, storage medium and degree of synchronization between donor and recipient compatible with fertility.

Experiments 1 and 2 involved the transfer of thirty-three eggs in homologous serum to eighteen recipients. In half of these, the eggs were transferred surgically and in the other half non-surgically. No pregnancies resulted from these transfers.

In Experiments 3 and 4, a total of seventy-six eggs was transferred to forty recipient heifers using TCM 199 as the transfer and storage medium. Twenty of these transfers were made surgically, the eggs also being recovered by surgical means in eleven cases and by slaughter in nine. The pregnancy rate obtained was 91% for surgically recovered and transferred eggs and 33% for eggs recovered at slaughter and then transferred surgically.

The remaining twenty animals received eggs, half of which were recovered surgically and half at slaughter, and all then transferred by non-surgical means.

It is concluded that homologous serum is an unsuitable medium for egg transfer but that TCM 199 is highly satisfactory. The recovery of eggs from slaughtered animals appears less satisfactory than if they are recovered *in vivo*. A degree of variation from exact synchronization of ±2 days can be tolerated.

INTRODUCTION

Despite extensive research, few successes have been recorded following egg transfer in the cow. The subject has been reviewed at some length by Dziuk, Donker, Nichols & Petersen (1958). There have been isolated instances of successful transfers such as those of Willett, Buckner & Larson (1953), Avery, Fahning, Pursel & Graham (1962) using surgical means, and of Mutter, Graden & Olds (1964), Sugie, Soma & Onuma (1965) and Rowson & Moor (1966b) following non-surgical transfers; but no consistent results have been obtained.

The findings of Rowson, Lamming & Fry (1953) indicated the extreme susceptibility of the uterus to infection during the luteal phase which may have accounted for some of the initial failures, yet despite aseptic precautions and the use of antibiotics, failures have continued. The further finding of Bennett & Rowson (1961) that stimulation, following the transfer of eggs by way of the cervix, could result in the ejection of eggs from the uterus added new light to the problem, but no completely satisfactory technique has yet been developed to overcome this effect.

The object of the present experiments was to investigate the different flushing and storage media for the purpose of improving egg-transfer in cattle and to compare the results by the surgical and non-surgical transfer procedures. In addition, the possible effect of recovering eggs from slaughterhouse material, as against those obtained surgically, was investigated together with the degree of synchronization compatible with fertility.

MATERIALS AND METHODS

A total of ninety-two maiden heifers was used in these experiments comprising thirty-four donors and fifty-eight recipients. The heifers were run with vasectomized bulls, the briskets of which were coated daily with coloured grease and all animals were examined at least once daily for service marks indicating oestrus.

Donor animals

These were injected on Day 16 of the cycle with pregnant mares serum gonadotrophin (1300 to 2000 i.u.) and observed for service marks over the next few days. At this oestrus, they were inseminated with semen obtained from the Cambridge A.I. Centre. The animals were either slaughtered or operated upon on the 4th or 5th day after the onset of oestrus.

In the case of the slaughtered animals, the uteri were brought back to the laboratory without any special precautions regarding temperature control. The oviducts and uterine horns were then flushed under aseptic conditions using either homologous serum or TCM 199 (Tissue Culture Medium 199, Glaxo Laboratories) according to the experimental group. Each horn was clamped near the body of the uterus, and after searing the oviduct with a hot iron, a sterile needle was passed through the seared area into the oviduct and about 20 ml of medium flushed through into the uterus. The tip of each horn was then seared and a glass tube inserted through an incision made in the seared area. An additional 20 ml of medium was then injected through a further seared area of the uterus close to the clamp placed at the base of the horn. The flushings then passed out of the glass tube and into special collecting cups which could be placed directly under the dissecting microscope for examination of the eggs.

Where eggs were recovered by surgical means, the donor was anaesthetized with an initial intravenous injection of pentobarbitone sodium followed by closed circuit anaesthesia using Fluothane and oxygen. The animal was placed on its back on the operating table, the area immediately anterior to the pelvis

shaved and sterilized and a 15-cm incision made in the mid-line through which the uterus and ovaries were withdrawn. A similar flushing procedure was adopted as with the excised uteri from slaughtered animals except, that as aseptic conditions were observed, searing was not necessary. In a few cases, instead of flushing the eggs through a glass tube placed in the tip of the uterine horn, a flexible plastic tube was inserted into the ovarian end of the oviduct and the eggs flushed out in the standard manner used for the sheep (Hunter, Adams & Rowson, 1955). When the ovaries could readily be drawn into the wound, corpora lutea were counted. After flushing, the uterus was returned to the abdomen and the wound sutured, using two layers of No. 3 catgut followed by interrupted skin sutures of Ethicon tape.

Before transfer, all eggs recovered from the donor animals were stored in the special collecting cups in an incubator at 30° C. The time interval from recovery of the eggs to their transfer was recorded.

Recipient animals

All recipient animals were selected from the herd on the basis of either service marks or having been seen to be mounted by the vasectomized bulls; they were all within the range of ± 2 days of exact synchronization of oestrus with that of the donors. When the donor and recipient animals showed oestrus on the same day, the degree of synchronization is designated as '0' or exact. When the recipient showed oestrus 1 or 2 days before the donor, the symbol $+1$ or $+2$ days was used. Conversely, when the recipient showed oestrus 1 or 2 days after the donor animal then the symbol -1 or -2 days was used. To half of the recipients (twenty-nine) the eggs were transferred surgically and to the other half non-surgically. Surgical recipients were treated similarly to the donors so far as exposure of the uterus was concerned. The ovary containing the corpus luteum was identified and the eggs always transferred to the adjacent uterine horn. Most animals received two eggs. The transfer was carried out using a Pasteur pipette connected to a 1-ml syringe. All the animals recovered rapidly and were turned out within a few days with a raddled vasectomized bull to detect further oestrus. If oestrus was not exhibited by about 40 days, the recipients were examined and any pregnant animals dispatched to outlying pastures to await calvings.

Non-surgical egg transfers were carried out using the technique described by Rowson & Moor (1966b).

RESULTS

In Exp. 1, a total of sixteen eggs from seven donors was transferred to nine recipients by surgical means using homologous serum as the medium for both flushing and storage (Table 1). In six of these recipients, the stage of the cycle was 1 day less than that of the donor, in two recipients it was 2 days less, and in the remaining recipient it was synchronized exactly. Eggs were transferred after storage times of 45 to 95 min from their recovery, with an average interval of 60 min. No pregnancies were obtained in this experiment.

In Exp. 2, again using serum as the medium, a further seventeen eggs from

six donor animals were transferred non-surgically to uteri of nine recipients (Table 2). The cycles of five of these recipients were exactly synchronized with those of the donors, two were 2 days less, one, 1 day less, and one, 2 days in advance of the cycle of the donors. The average time of egg storage before transfer was 55 min with a range of 15 to 130 min. None of these animals became pregnant.

TABLE 1

RESULTS OBTAINED FOLLOWING SURGICAL TRANSFERS TO RECIPIENTS USING HOMOLOGOUS SERUM: THE STAGE OF DEVELOPMENT AND STORAGE TIME OF EGGS TOGETHER WITH THE DEGREE OF SYNCHRONIZATION

Donor	*Recipient*	*Synchronization variation from donor (days)*	*Eggs transferred (cell stage)*	*Storage time (min)*	*Pregnant*	*Post-operative cycle length (days)*
28 (O)	39	−1	2×8	55	–	22
75 (O)	53	−2	2×16	95	–	42
75 (O)	73	−1	3×16	50	–	43
79 (O)	69	0	1×8	60	–	39
68 (O)	59	−1	2×32	45	–	21
68 (O)	76	−1	1×32	80	–	24
39 (S)	70	−1	2×8	45	–	42
76 (S)	65	−1	2×32	80	–	49
77 (S)	57	−2	1×8	30	–	45

(O) = eggs recovered by operation; (S) = eggs recovered after slaughter.

TABLE 2

RESULTS OBTAINED FOLLOWING NON-SURGICAL TRANSFERS TO RECIPIENTS USING HOMOLOGOUS SERUM: THE STAGE OF DEVELOPMENT AND STORAGE TIME OF EGGS TOGETHER WITH THE DEGREE OF SYNCHRONIZATION

Donor	*Recipient*	*Synchronization variation from donor (days)*	*Eggs transferred (cell stage)*	*Storage time (min)*	*Pregnant*	*Post-operative cycle length (days)*
26 (S)	35	0	1×8	40	–	20
34 (S)	40	0	1×8	15	–	16
33 (S)	27	−1	1×32	20	–	Animal ill
29 (S)	37	0	2×8	20	–	21
29 (S)	32	+2	3×8	30	–	10
31 (S)	26	0	2×8	30	–	21
25 (S)	39	0	3×8	100	–	39
25 (S)	38	−2	2×8	110	–	21
25 (S)	27	−2	2×8	130	–	21

(S) = eggs recovered after slaughter.

In Exp. 3, a total of thirty-nine eggs from sixteen donors was transferred to twenty recipients using surgical means, but TCM 199 instead of serum as the flushing and storage medium (Table 3). The average time interval of egg storage before transfer was 81 min with a range of 25 to 170 min; for eleven of the recipients, the eggs were recovered surgically and for nine, following slaughter. Of the twenty recipients, thirteen became pregnant (65%). The proportion of recipients that became pregnant to eggs recovered by surgical means was 91%,

TABLE 3

RESULTS OBTAINED OF SURGICAL TRANSFERS USING TCM 199: THE STAGE OF DEVELOPMENT, STORAGE TIME OF EGGS AND THE DEGREE OF SYNCHRONIZATION

Donor	*Recipient*	*Synchronization variation from donor (days)*	*Eggs transfered (cell stage)*	*Storage time (min)*	*Pregnant*	*Post-operative cycle length (days)*
52 (O)	67	0	1×8, 2×16	35	−	18
88 (O)	85	0	2×8	140	+	—
88 (O)	71	0	2×8	90	+	—
70 (O)	112	−2	2×32	125	+	—
87 (O)	108	0	2×32	80	+	—
121 (O)	119	−2	2×12	85	+	—
102 (O)	64	+1	2×8	75	+	—
81 (O)	95	−1	2×16	30	+	—
81 (O)	103	−1	2×16	145	+	—
113 (O)	51	−1	2×16	122	+	—
113 (O)	82	0	2×16	55	+	—
38 (S)	72	−1	2×32	40	+	—
40 (S)	93	−2	1×16	25	−	21
73 (S)	124	0	2×8	150	+	—
57 (S)	119	0	2×8	40	−	37
122 (S)	54	0	2×8	30	−	15
122 (S)	106	−1	2×8	170	−	17
89 (S)	123	−1	2×16	25	−	17
111 (S)	125	+1	2×8	55	+	—
40 (S)	65	0	1×16	85		20

(O) = eggs recovered by operation; (S) = eggs recovered after slaughter.

TABLE 4

RESULTS OBTAINED OF NON-SURGICAL TRANSFERS USING TCM 199: THE STAGE OF DEVELOPMENT, STORAGE TIME OF EGGS AND THE DEGREE OF SYNCHRONIZATION

Donor	*Recipient*	*Synchronization variation from donor (days)*	*Eggs transferred (cell stage)*	*Storage time (min)*	*Pregnant*	*Post-operative cycle length (days)*
87 (O)	104	+2	2×32	95	−	29
87 (O)	102	−1	2×32	40	−	20
70 (O)	35	0	2×32	35	−	39
88 (O)	76	+2	2×8	60	+	—
88 (O)	69	+1	2×8	45	−	25
52 (O)	69	0	1×16	90	−	12
70 (O)	84	0	2×32	100	−	59
121 (O)	54	−2	2×12	90	+	—
121 (O)	95	+1	2×12	105	−	23
81 (O)	107	−2	1×16	115	−	19
73 (S)	92	+1	2×8	170	−	37
73 (S)	80	0	2×8	75	−	20
57 (S)	48	0	2×8	110	−	23
122 (S)	59	0	2×8	65	+	—
122 (S)	87	+1	3×8	135	+	—
89 (S)	92	−1	1×16	75	−	26
89 (S)	12	−1	2×16	55	−	23
111 (S)	101	+1	1×8	68	−	39
104 (S)	106	0	2×16	55	−	40
104 (S)	123	+2	2×16	75	−	18

(O) = eggs recovered by operation; (S) = eggs recovered after slaughter.

but in those that received eggs recovered after slaughter it was 33%. It can be seen from Table 3 that pregnancies were obtained when the degree of synchronization varied between −2 to +1 days.

In Exp. 4, a further group of thirteen donor animals was used from which a total of thirty-seven eggs was transferred to twenty recipients non-surgically, i.e. by way of the cervix (Table 4). Ten received eggs recovered from donors by operation and another ten were given eggs recovered at slaughter. A total of four recipients became pregnant in this group. The average interval from egg recovery to transfer was 83 min. There was, therefore, no difference between the degree of success obtained by egg recovery at slaughter or operation in this experiment. A feature of this experiment, and to some extent of Exp. 1, was the rather irregular return to oestrus of the non-pregnant animals (Tables 1 and 4).

DISCUSSION

The results of these experiments show quite clearly that blood serum, even though homologous, is not a satisfactory medium for the flushing and storage of cow eggs before transfer. On the other hand, TCM 199 appeared to be highly satisfactory. Using this medium and a combination of surgical methods for both recovery and transfer of the eggs, a very high rate of pregnancy has been obtained, namely 91%. The reasons for the lower pregnancy rates obtained after transfer of eggs recovered from slaughtered animals are not obvious at the moment. Perhaps some changes deleterious to the eggs have taken place in the uterus during the interval between slaughter of the donor and recovery of the eggs.

It is difficult to draw any concrete conclusions as to the degree of synchronization of oestrus which is compatible with normal fertility. However, of the seventeen animals which became pregnant in Exps. 3 and 4, the range of successes was ±2 days. The relationship of these seventeen animals to that of the donor's oestrous cycle was as follows: three animals −2 days, four animals −1 day, six animals (synchronized) Day 0, three animals +1 day and one animal +2 days. The groups are too small to make any comparison of success or failure at the various times of synchronization but, out of a total of eight recipients which were either ±2 days, four became pregnant indicating that the degree of synchronization necessary for successful transfer is probably no closer than that of the sheep (Rowson & Moor, 1966a).

The longest period of storage of eggs which, after transfer, resulted in pregnancy was 135 min in the non-surgical Group 4 and 150 min in the surgical Group 3. Both of these were at or near the extremes of storage times for their particular group and it is possible that the period of successful storage of eggs could be greatly extended.

In most of the experiments in this study two eggs were transferred to each recipient. This was done to ensure that the recipient had every chance of becoming pregnant should one egg be non-viable, and, also, to determine the ability of the recipient to produce twins. So far, we have failed to induce twins and up to the present, each recipient has borne only a single offspring. The question arises as to whether twinning in cattle is merely a question of double ovulation

or whether a uterine carrying factor is also involved. The calving data from these experiments will be presented separately at a later date.

The most interesting and encouraging result of the present study is that, using surgical means for both the recovery and transfer of cow eggs, in combination with TCM 199, a high rate of pregnancy can be obtained in cattle. Moreover, the whole experimental procedure, including full anaesthesia, requires only about 40 min per animal.

The method for transfer of cattle eggs, now shown to be fully successful, should be of great value not only for the purpose of genetic improvement but also as a useful tool for investigations into various fundamental aspects of reproductive physiology in the cow.

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