Nonsurgical Embryo Recovery and Transfer in Cattle

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Embryo transfer is a technique which makes it possible for a cow to produce more than one or two calves per year. The value of embryo transfer in cattle is two-fold. In its practical application, a considerable contribution has already been made in the multiplication of the exotic breeds and the transfer of eggs from the better animals of a herd into the poorer ones. As a research tool, it has contributed significantly toward the understanding of reproductive physiology, particularly in the areas of endocrine relationships during the estrous cycle, ovulation, fertilization and early development of the fertilized egg.

Embryo Recovery

The technique of embryo *recovery* in cattle has evolved from slaughter of the donor animal to surgical recovery in the anesthetized living animal and, more recently, nonsurgical recovery from the standing animal via the cervix. Development of nonsurgical recovery techniques has been exceedingly slow. Several workers over the years have described devices for the recovery of bovine embryos via the cervix from superovulated cows (Rowson and Dowling, 1949; Dowling, 1949; Dracy and Petersen, 1950; Dziuk, Donker, Nichols and Petersen, 1958). Success was variable and limited to an average of one ovum per attempt (range 0-20). Sugie, Soma, Fukumitsu and Otsuki (1972) succeeded in recovering an average of 6.2 ova (range 1-18) from 45 out of 60 superovulated cows using a two- or three-cannula apparatus along with 2-3,000 ml of flushing medium. More recently, Elsden, Hasler and Seidel (1976) succeeded in recovering an average of 6.9 ova (range 0-25) nonsurgically from 26 superovulated cows while a recovery rate of 71% was obtained in 51 unsuperovulated cows. Using similar techniques, Rowe, et al., 1976, achieved comparable results of an average of 6.3 ova (range 1-14) from 15 superovulated heifers and 65% recovery from single ovulating heifers.

Recovery Technique: A two-way Foley catheter (O.D. 5.4 mm) with or without a 10-cm extended tip (Drost, Brand and Aarts, 1976) may be used. A stylet is inserted the full length of the device to provide sufficient rigidity to enable placement into the uterine horn. Epidural anesthesia (5 ml 2% Xylocaine) is given to prevent defecation and straining. The catheter is introduced through a sterile vaginal tube speculum (O.D. 4 cm) and threaded through the cervical canal under rectal guidance. As soon as the tip enters the uterus, the speculum is removed and the catheter manipulated into the appropriate horn until the balloon resides in the body of the uterus. The balloon is inflated with 10 to 20 ml of air and the stylet is removed.

The distal end of the catheter is connected by siliconized Teflon tubing by means of a Y connector to a bottle containing the flushing medium and to a conical receptacle covered with tinfoil. Each uterine horn is flushed with 500 ml of Dulbecco's phosphate-buffered saline, enriched with 4 mg of bovine serum albumin and 100 I.U. sodium penicillin G and 0.05 mg dihydrostreptomycin sulphate per ml.

Initially, 75 to 150 ml of medium is allowed to distend the horn while the outflow tube is blocked. Next, the inlet tubing is clamped off and the outflow released. This process is repeated 5-7 times. During the flush the uterus is manipulated per rectum by alternately lifting and extending the tip of the horn or by retraction of the entire uterus. The horn is also gently massaged or pressed against the pelvic wall. In superovulated cows the procedure is repeated in the opposite horn using a second sterile speculum and device. After a settling period of 10-20 minutes, 5-ml aliquots are aspirated from the bottom of the conical glasses with a sterile pipette and placed in a concave dish and examined for the presence of embryos under a stereomicroscope.

Discussion

Compared to surgical embryo recovery procedures, nonsurgical methods offer greater potential primarily by virtue of their repeatability and the fact that they can be carried out under field conditions. Additional relative advantages of

nonsurgical versus surgical embryo recovery techniques are listed in Table 1.

Table 1

Relative Advantages and Disadvantages of Nonsurgical Versus Surgical Embryo Recovery Techniques in Cattle

Nonsurgical	Surgical
Advantages	Advantages
repeatable (no adhesions)	patient immobilized
can be performed on farm	sterile technique possible
no fasting required	direct manipulation of uterus
time saving	accurate evaluation of ovarian response
no recovery period	20-50 ml recovery medium needed
no surgical risks	
Disadvantages	Disadvantages
manipulative skills required	formation of adhesions
500 ml recovery medium required per cow	surgical facilities needed
sterile procedure difficult to maintain	risk of general anesthesia
can only be performed in animals in which the cervical canal can be passed	surgical risk
	difficult in lactating animals
	fasting required
	recovery period required

A serious constraint on success with either recovery approach is the tremendous variation which occurs in the individual cow or heifer in response to gonadotropin treatment. Scanlon (1972) reported a response in 124 out of 152 cows (81.6%), Betteridge and Mitchell (1974) an even lower percentage of 56.4 (22/39 cows). Such differences in response may be due to a wide variety of factors including such things as age, breed, season, genetic makeup, lactational status, physical condition, stage of the estrous cycle and dose level employed. The average number of ovulations, eggs recovered and eggs fertilized are reported in the literature for 791 cows responding to PMSG treatment as 15.9, 8.9 and 4.8 respectively (Table 2). This illustrated that the entire process of embryo transfer is a factorial one, whereby the end result, the number of calves born, is the product of the various factors (steps) involved, a process of diminishing returns.

Table 2

Yield of Fertilized Bovine Ova After Superovulation

Reference	Donors n	Average Number of Ova ovulated	recovered	fertilized
Scanlon, 1972	124	12.1	7.5	4.1
Betteridge & Mitchell, 1974	44	10.3	4.8	3.5
McGaugh, Olds & Kratzer, 1974	40	10.3	5.3	2.0
Gordon, 1975	436	18.1	9.8	4.3
Gordon, 1976	147	15.7	9.6	8.4
Total	791	15.9	8.9	4.8
(%)			(56)	(54)

Transfer to Recipients

Early attempts at nonsurgical *transfer* via the cervix failed to meet with success (Dowling, 1949; Lamming and Rowson, 1952). The first successful cervical transfer was reported by Mutter, Graden and Olds (1964). Workers in Cambridge, England, believed uterine infection (Lamming and Rowson, 1953; Rowson, Lamming and Fry, 1953) and ejection of the embryos (Bennett and Rowson, 1961; Harper, Bennett and Rowson, 1961; Rowson, Bennett and Harper, 1964) to be major contributing factors to the lack of success after transfer via the cervix. It was further believed that inflation of the uterus with carbon dioxide gas just after deposition of the embryo reduced the loss of eggs from the uterus due to its relaxing effect or some unknown mechanism. Conception rates with this technique have been low (Sugie, 1965; Rowson and Moor, 1966; Rowson, Moore and Lawson, 1969; Vincent, Mills and Rundell, 1969; Lawson, Rowson, Moor and Tervit, 1975). The last workers tentatively concluded that nonsurgical transfer of fertilized eggs to heifers may best be done during midcycle, after Day 6. Brand, et al. (1976a), in a study of the electrical activity of the myometrium of the cow, demonstrated that myometrial activity started on Day −3, culminated on Days 0 (= estrus) and 1 and disappeared on Day 4 or 5 of the next cycle. Manipulations of the genital tract, such as those performed during nonsurgical embryo transfer procedures or irrigation of the uterus by gravity flow, did not induce myometrial contractions. Only nonspecific electrical activity of the myometrium was noted during manipulation, both before and after Day 5, which ceased immediately upon removal of the instruments. They postulated that spontaneous myometrial activity before Day 5 is responsible for low pregnancy rates when nonsurgical embryo transfers are performed early (Days 3-5) in the cycle. Brand, et al. (1976b), further demonstrated a significant reduction in the number of bacterial isolates obtained from the vulva to the external os of the cervix by using three concentric cannulas. They concluded that contamination of the uterus by introduction of pipettes through the cervix is not a major contributing factor in the low pregnancy rates after nonsurgical transfer in the cow if adequate precautions have been taken. This conclusion is supported by the finding that the fertility of bred heifers was not adversely affected by sham transfer to the contralateral horn on Days 6-10. This is in agreement with the work of Seidel, Bowen, Homan and Okun (1975) who carried out sham transfers to the ipsilateral horn of bred heifers on Day 6.

Transfer Technique: The nonsurgical transfer device consists of two concentric, stainless steel cannulas, 6 and 3 mm in diameter, and a round tip inner flexible ureteral catheter, 1.65 or 1.98 mm in diameter, with two laterally placed holes. The three catheters are 35, 45 and 70 cm long, respectively (Brand, Drost, Aarts and Gunnink, 1976). The outer cannula is covered at one end with a piece of nonabsorbent paper prior to autoclaving. An epidural injection of 4-6ml 2% Xylocaine is given to suspend defecation and prevent straining. The vulva of the recipient is cleaned with 70% alcohol. The labia are parted and the outer two sterile cannulas, one inside the other, are introduced into the vagina and 1-2 cm into the external os of the cervix. The second cannula is then pushed through the protective paper cover and extended 3-4 cm into the horn ipsilateral to the corpus luteum per rectal guidance. Next, the flexible catheter is introduced, using sterile technique, into the middle cannula and advanced as far as possible into the uterine horn. Its passage is facilitated by gentle undulating movements of the horn as it is manipulated per rectum. The embryo contained in 0.5 ml medium is deposited in the middle of the uterine horn via the flexible catheter. After this, the catheter is flushed with an additional 0.5 ml of medium. The content of the catheter is 0.5 ml.

Discussion

Results of nonsurgical embryo transfer in cattle are presented in Table 3. Of the authors listed in Table 3 only Sugie, et al. (1972), transferred embryos which were collected nonsurgically. Considerable variation exists in the pregnancy rate after nonsurgical transfer. Generally, the results are below the 65% obtained after surgical transfer (Gordon, 1975). It is possible that technical failures, such as lack of experience, is a factor in the reduced pregnancy rate. Trauma of the endometrium with a resulting leucocytosis, or a disturbance of the endocrinological and biochemical environment of the uterus may also play a role.

Table 3

Results of Nonsurgical Embryo Transfer in Cattle

Recipients n	Day of Transfer	Pregnant n (%)	Reference
2	4-6	2 (100)	Sugie, 1965 (a,d)
8	2-6	3 (38)	Rowson & Moor, 1966 (a)
20	3-5	4 (25)	Rowson, et al., 1969
68	3-7	17 (25)	Sugie, et al., 1972 (a,d)
24 (c)	5-7	6 (25)	Boland, et al., 1975
15	6-7	6 (40)	Brand, 1975 (e)
42	4-7	10 (24)	Hahn, et al., 1975
20	6-9	7 (35)	Lawson, et al., 1975
10	3-5	1 (10)	Lawson, et al., 1975
10	6-9	1 (10)	Lawson, et al., 1975 (a)
10	3-5	0 (0)	Lawson, et al., 1975 (a)
20	6-9	8 (40)	Lawson, et al., 1975 (b)
10	3-5	1 (10)	Lawson, et al., 1975 (b)
8	6	4 (50)	Sreenan, 1975

(a) CO_2 insufflation used; (b) under fluothane anesthesia; (c) bred recipients; (d) cervix bypassed; (e) unpublished data.

Summary and Conclusions

The feasibility of the embryo transfer technique in cattle is well established. There is little question regarding its role in reproductive research, while its practical application depends entirely upon economic factors. Nonsurgical embryo recovery and transfer methods offer greater potential than surgical techniques by virtue of their repeatability and adaptability to field conditions. Development of nonsurgical procedures has been slow. It has been shown that with adequate precautions the hazard of introduction of infection via the cervix can be minimized. Ejection of embryos after cervical transfer can be avoided when transfers are made after Day 5 when spontaneous electrical activity of the myometrium is very low. The results of nonsurgical embryo transfer, as well as the yield of bovine ova after superovulation, in the literature are reviewed. It is clear that a more reliable superovulatory treatment, objective criteria for evaluation of embryo quality, and long-term storage methods of embryos need to be developed before embryo transfer will find large-scale application in either practice or research.

References

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Discussion

Question: The breed of the cow that was featured there was an MRI cow, which is a red and white production breed just coming into Canada and the United States. It is named after three big rivers: the Muse, Rhine and Iso Rivers, so they call it an MRI cow.

Question: Dr. Drost, on your discussion of long-term storage of these embryos, have you done any work on the conception rate of long-time storage embryos over and above the fresh ones?

Answer: Yes, we have done only limited work on that. When I say we, I am speaking for the embryo-transfer group on the Davis campus in conjunction with the Animal Science Department. Dr. Anderson, in particular, who is our embryo man, has been particularly interested in developing culture techniques for short-term storage, cold storage, and is currently working on freezing techniques. I am not prepared to give you any specific results because I think any percentage would be misleading. But, this is a technique which is being tried in a number of laboratories and, as you perhaps know, calves have resulted from embryos that have been frozen, subsequently thawed and transferred. I am not prepared to give you any percentages. The numbers simply are not large enough.

Question: Dr. Kendrick, would you recommend that we stay on our bulls (rest of question inaudible)?

Answer: When this vaccine is used, annual boosters have been given.