

EMBRYO TRANSFER IN THE COW

by

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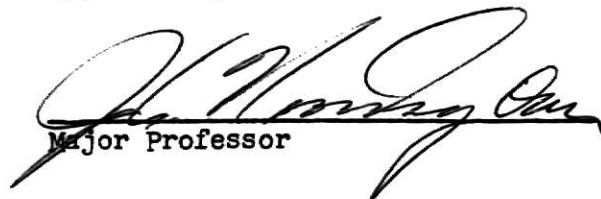
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SECTION J

INTRODUCTION

HISTORICAL INTRODUCTION

ADVANTAGES AND POSSIBLE APPLICATIONS OF EMBRYO TRANSPLANT

DISADVANTAGES AND RISKS OF EMBRYO TRANSPLANT

HISTORICAL INTRODUCTION

The first successful mammalian egg transfer was done in 1890, at Cambridge by Walter Heape, when he transferred fertilized ova from one rabbit to another. Since then embryo transplant has been widely studied in the rabbit by other investigators. Embryo transplant in farm animals was not vigorously studied until at the end of the second world war. There were however a few isolated successful efforts in sheep prior to this period (Warwick Berry and Horlacher 1934; Casida, Warwick and Meyer 1944). After the Second World War, artificial insemination was introduced as a cheap means of propagating the genetic qualities of a proven bull; and so embryo transplant was looked at, as a possible way of propagating similar qualities of a desired female counterpart. Hammond et al. (1950) contributed a lot in the investigation at this early stage of the study in farm animals.

The first conference on egg transfer was held in Texas in 1949, where several papers were presented on the problems, failures and successes in embryo transplant. This resulted in an increased interest in the field and publications of several more papers on embryo transplant in farm animals (Rowson et al. 1949, Dracy et al. 1950). As a result of the discussions at the Texas conference, it became obvious that embryo transplant was not as simple in the sheep or cow, as in the rabbit, and that surgery was necessary for both the recovery and transfer of the embryo in the cow and sheep.

Although currently there are several reports of successful embryo transplants in sheep, goats, cattle and swine (Dzuik, Hafez, Blandau et al.), even to the point of several commercial companies being formed, especially in U.S.A. and Canada (D. Dyrholm & T. Mitenko 1976), that deal in embryo

transplant, the aspirations of embryo transplant technique as the female counterpart of artificial insemination (AI) has not been fully realized.

ADVANTAGES AND APPLICATIONS OF EMBRYO TRANSPLANTS

(i) Perhaps the first advantage of the embryo transplant is that, one can increase the number of offspring per unit time from a valuable cow. George E. Seidel Jr. (1976) reported that fifteen or more calves can be realized from a surgical operation. Similar findings have been reported by several other workers (Sugie et al. 1970, Dzuik 1954).

D. Dyholm and T. Mitenbo pointed out that, mathematically, it is possible that one cow can, within the expected reproductive life of 8-9 years, produce 10,000 offsprings. While it is not possible to achieve this in practice, due to the logistics of large numbers involved, as well as the inconsistencies of biological factors involved, it serves to emphasize the potential of embryo transplant in propagating desired qualities of a cow speedily.

(ii) The second advantage is that, through this technique calves and infertile heifers and cows can have offsprings. Superovulation in calves has been experimentally done, and while there are several problems to be solved before it can be economically done, available evidence suggests that it could be done, in not a very distant future (George E. Seidel 1976).

While propagating infertile cow is not advocated, cows that have acquired infertility due to disease, injury or old age, can be made productive once again by making them carry "transferred embryo" to a full term.

(iii) Although not presently fully realized, embryo transfer could allow a reliable induction of twinning which would double the production

of a recipient cow.

(iv) Embryo transplant will also permit a cheap means of long distance transport of cattle, in form of eggs; once long-term frozen storage of cattle ova is economically possible.

(v) Another advantage of embryo transplant technique is that calves conceived from none native cows and bulls, can acquire immunity to local diseases from their foster-mothers (recipients) (B. T. Farrally 1975).

(vi) It is possible, experimentally, but not economical at present, to control sex of the calf produced, by determining in advance the sex of the blastomeres at the time of recovery of the fertilized egg (day 14 of pregnancy). A. C. Mills 1976 of Saskatoon Embryo Transplant reported that the charge of sex pregnancy is however, double that of unsexed pregnancy.

(vii) It is also possible to produce several calves of same parents at same time. This is particularly useful for research purposes.

DISADVANTAGES AND RISKS OF EMBRYO TRANSPLANT

Embryo transplant is however not without difficulties and risks.

(i) One of the greatest disadvantages of the technique in commercial operations, is the long time it takes to recognize the true genetic value of a cow; which for this purpose has been defined as the ability of the cow to transmit desirable traits, such as meat or milk production to her offsprings (Seidel Jr.). Even after these values have been discovered in a cow, it takes a longer time to get a calf with embryo transplant, than with normal mating. Seidel quotes an example of an operation in a commercial farm, which requires an animal to stay for at least 45 days, before it can be superovulated. This problem will be discussed in greater detail later when discussing syndronization of estrus.

(ii) Another problem encountered in embryo transplant, is the damage done to the reproductive tract of the donor. Several researchers have reported extensive adhesions in the uterus and the oviduct. This severely affects future reproductive capacity of the donor. Seidel (1976) estimated that in a commercial operation as much as 10% of the donors, are rendered infertile, at the first surgical recovery of the ova; This risk is doubled at the second surgical recovery. The exact cause of this extensive adhesions is not understood. Definately, this is more likely to occur where the surgeon is inexperienced or has broken asepsis; But the fact that this is seen even when strict asepsis is maintained, and that it seems to occur more commonly in embryo transfer than in other similar surgical procedures, will suggest that, the massive doses of hormones given prior to the surgery, might be a factor in the development of adhesions.

(iii) If hormones are injected too often, i.e., if the superovulation treatments are too close together, it interferes with the reproductive capacity of the donor. This risk can be minimized by allowing the donor to carry at least one pregnancy to full term for every two to three surgical ova recoveries. But if used in correct amounts and time intervals, hormones rarely interfere with the reproductive performances of the cow (Rowson et al. 1972).

(iv) Cecarean-sections are required to deliver almost all transplanted calves since the calves are usually big and recipients are of smaller breeds. This problem is important and must be taken into account when planning embryo transplant program.

(v) Perhaps more than anything else, the biggest disadvantage of embryo transplant is that it requires a lot of money. Embryo transplant as

done in cattle at present time is major operation and requires very well trained personnel, like reproductive physiologists as well as veterinary surgeons, to carry out a successful operation that is required on the farm. As pointed out earlier, in order for the operation to be economically viable it requires a large operation, since cost will outweigh the gain, if only a few calves are going to be produced. In practice the number of pregnancies resulting from one operation can vary from 0-20 and this will produce a large bill, which can be paid for, if fifteen or more calves are produced; But the owner will lose money, if only few calves are produced.

Whatever risks and disadvantages might be involved in embryo transplant in the cow, it is now known that, embryo transplant can be economically done in the cow. This is evidence by the number of commercial companies that are being formed in North America for this purpose. There are now more than ten such companies in U.S.A. and Canada. What impact this is going to have on the cattle industry remains to be seen.

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SECTION II

EGG (EMBRYO) SOURCE

INTRODUCTION

A. SUPEROVULATION OF MATURE COWS

Superovulation Technique

Types of Gonadotrophin Used

PMSG

Effects of Gonadotrophin on the Cow

Factors Influencing Egg Yield in a Hormonally
Superovulated Cow.

B. PREPUBERAL COWS OR CALVES

C. TEST-TUBE FERTILIZATION OF OOCYTES

D. ISOLATED BLASTOMERES

EGG (EMBRYO) SOURCE

INTRODUCTION

This first consideration that one doing embryo transplant has to make is, the availability of the embryo; The source must be adequate and constant, if the person is going to make any profit, for the reasons mentioned earlier. There is not much in literature about the source of embryo for transplantation; but two approaches have been reported. The first involves the hormonal induction of superovulation of heifers, cows or calves, and artificially fertilizing the eggs, and recovering them usually by surgical means. The other source not commonly employed is, obtaining eggs from vesicular follicles of the cows at slaughter houses, maturing such egg and fertilizing them in the laboratory. These methods will now be discussed as they form a major step in embryo transplant technique.

A. SUPEROVULATION OF MATURE COWS

The first report of successful superovulation in the cow was in 1943 by Casida et al., when they used pituitary gonadotropin, to superovulate cows. But before this, hormonal superovulation had been performed in pigs, sheep and laboratory animals. Since then several other researchers, have reported varying degrees of success in hormonal superovulation in the cow (Hammon et al. 1944, Folley and Mapress 1944, Dowling 1949, Laming and Rowson 1942, Dzuik et al. 1958, Hafez et al. 1961, Avert et al. and Ballow et al. 1969). In all these trials a follicle stimulating hormone (FSH) was administered several days in advance of oestrus and ovulation, to grow 10-20 follicles to maturity.

Superovulation Technique

Regardless of whichever hormone is used, the technique requires the injection of a gonadotropin usually Pregnant Mare Serum (PMSG) or FSH, during the follicular phase of the estrus cycle, followed by Human Chronic Gonadotrophin (HCG) parentally.

Current gonadotrophin treatment involves a single intramuscular dose of 1,500-2,500 international units, preferably the higher dose, on the 16th day of observed estrus cycle.

On the 19th day of the cycle, and again on the 20th day of cycle, the donor cow receives 10 mg of estradiol 17B intramuscularly, to aid in the onset of estrus and regression of corpus luteum. If the cow shows estrus before day 19 it doesn't receive the estradiol treatments.

On the onset of estrus, the cow is given 2,000 international units of HCG intravenously to facilitate ovulation. If the cow does not come into heat despite the two treatments of estradiol it is given HCG on the 21st day of estrus cycle in the morning and inseminated in the evening and again 12 hours later (following morning).

The cow is artificially inseminated with freshly collected semen in milk diluent (200 million sperm/0.2 ml dose), within six hours after the start of estrus and again 12 hours later.

Good results have been obtained without the estradiol treatment.

Types of Gonadotrophin

Follicle Stimulating Hormone (F.S.H.), a crude anterior pituitary extract of the horse (HAP), was used by earlier researchers, to induce superovulation in laboratory animals with considerable success. Dowling was the first person to use HAP to induce superovulation in the cow in 1949.

FSH is a glycoprotein of variable mol. wt.; e.g. it has a M.W. of 29,000 in the pig and 67,000 in the sheep (McDonald 1975). Its exact structure has not been determined. It is produced by the pituitary gland under the influence of the hypothalamus. The physiological effect of FSH is to cause multiple follicle growth in the ovaries with estrogen production and ovulation in the female.

FSH from sources other than the horse including sheep or swine also have been used to induce superovulation in the cow (Gordon 1975). There is no available data to show which of them is superior, but it is now known that, at least in sheep, HAP is more effective in achieving high superovulation than Pregnant Mare Serum Gonadotropin (PMSG) (Moore and Shelton 1962 and 67, Dowling 1949 and Van Rensburg 1964). At present the most common source of FSH is pituitary gland of the swine (Foote and Onuma 1970) obtained from slaughter houses. Sheep are still also used.

The disadvantage of FSH is that it is rapidly inactivated in the body ($1\frac{1}{2}$ -4 hours), and so in order to maintain effective levels for long enough periods, as required in superovulation, it has to be injected every twelve hours. This requires on the average 10 injections intramuscularly between day fifteen and day twenty of the estrus cycle. For this reason FSH like hormones are more popular than the pituitary extracts.

Pregnant Mare Serum Gonadotropin (PMSG)

Pregnant Mare Serum Gonadotropin (PMSG) is a glycoprotein of larger molecular weight than FSH, M.W. being 68,000. It is produced in the endometrial cups of pregnant mare uterus and was first extracted by Cole and Hart in 1930. The hormone appears in the blood of a pregnant mare at about day forty of pregnancy and reaches its highest level at about day

60 to day 110 of the pregnancy. It then persists in measureable amounts to about 140 days after fertilization had taken place.

Although it has similar effects on the ovary as FSH, it also contains some Leutinising Hormone (LH). It therefore acts on the ovaries to bring about follicular development as well as ovulation.

It was first used to bring about superovulation in the cow in 1943 by Casida et al., and was also used by Hammond Jr. and Bhatta Chanya in 1944. Since then nearly all reports of superovulation in the bovine have been done with PMSG (Dowling 1949, Willet 1943, William, Gordon 1962, Dewy 1964, Rowson et al. 1966, 1972). It is also the hormone used to produce superovulation in many commercial firms (George E. Seidel Jr. 1976).

The advantage of PMSG over FSH is that it is readily available and at low cost, since it is produced commercially for other purposes. It persists in the body for a much longer time than FSH (up to 26 hours) (MacDonald 1975), so only a single intramuscular or subcutaneous injection is required to produce superovulation in the cow.

In reality, there is very little difference in effectiveness of producing superovulation between FSH and PMSG (Seidel). But the simpler scheme involving PMSG is preferred; However, where the animal does not respond to PMSG, FSH can be used. Both are used parently, during the follicular phase of estrous cycle (day 16-20) to bring about superovulation.

Effects of Gonadotropin Therapy on the Cow

The ovarian response to gonadotropin depends among other things on the stage of the estrus cycle at which treatment is given. If given during follicular phase, in general, it has consistantly resulted in multiple ovulation; But if given during luteal phase, it will cause cystic

follicles. Even when used during the follicular phase of estrus cycle it could produce some undesirable effects and must be watched closely.

(i) Dose-Response Relationship

There is a direct relationship between the dose of gonadotropin given and the number of ovulations in a given individual; But there is an enormous individual variation ranging from no ovulation to as many as 20-30 ovarian follicles for a given dose. This is consistently seen in data accumulated on the trials from different researchers. There is however no relationship between the dose and the size of the animal (Bellow 1969). Bellows et al. also found that gonadotropin at a dose of 12.50 mg and above gave excessive follicular response characterized by the development of abnormal follicles of more than twice the size of those produced with lower doses of gonadotropin.

(ii) Refractoriness to Pregnant Mare Serum Gonadotropin (PMSG)

Refractoriness to hormones is uncommon in mammals (Rowlands et al. 1937), but several researchers have found that, there is a decline in the number of corpus lutea with successive superovulations with PMSG (Willet and Buckner, 1965, Hafez et al. 1962 and 1964, Jainudeen et al. 1966). Jainudeen et al. in their experiment on the effect of repeated therapeutic PMS injection on the cow, found among other things that, large doses, such as given in superovulation treatments, will continue to produce similar ovulatory response for 5-7 months; But after that it fails to stimulate the ovaries. This refractoriness to PMSG is not corrected by a period of withdraw from the hormone (Hafez), but increasing the dosage of the hormone will overcome it temporarily (Willet and Buckner 1965).

The refractoriness is believed to be caused by raise in the

formation of Antigonadotropins with successive PMSG injections. The level of the antigonadotropins in serum in cow has been measured by comparing its atrophying effects on the ovary of rats. (Cole et al. 1957).

Jainudeen et al. by measuring antigonadotropin levels using Cole's method were able to show that, the level of antigonadotropin rises with successive treatment of PMS and reaches its highest level on about the sixteenth day of PMS treatment. This activity of gonadotropin will then explain why repeated treatments with PMS, do fail to induce multiple ovulation in the cow, due to the increased levels of antigonadotropin in the blood.

(iii) Effect of Gonadotropin on Estrus Cycle

Hafez et al. 1964 reviewed the literature on the effect of PMS on estrus cycle of the cow. Dzuik et al. (1958) reported high incidence of silent heat in cows treated with PMS. Gordon et al. 1962 also reported that, as high as 14% of the animals treated with PMS showed silent heat (quiet ovulation). Similar high figures were also reported by Hafez himself in 1963. Hafez et al. in reviewing this data in 1964 observed that silent heat is not perculiar to gonadotropin treated cows alone. He pointed out that silent estrus were reported to be as high as 18-27% of all estrous cycles in cattle by Kidder et al. (1952) and Trimberger and Fincher (1956). So such estrus cycles seen in animals treated with gonadotropin may be from other causes like age, stage of lactation, interval from last calving, plane of nutrition and season of the year; None of which were adequately eliminated in the reported trials by Hafez, Dzuik and several others.

Split-estrous has been frequently reported following PMS treatment (Hafez and Sugue, 1961, Gordon et al. 1962, Hafez et al. 1964). In split heat, the animal allows to be mounted by a bull, and this is followed by

a period in which it won't allow to be mounted, and then another in which it could be mounted by a bull again. Hafez et al. (1966) reported that split-heat is related to the time interval between PMS injection and subsequent estrus and that it is more common if PMS is given subcutaneously than intramuscularly.

The most disturbing feature of PMS treatment is the great variability in the length of subsequent estrus. These tend to be erratic and of varying duration following PMS therapy. It appears at present no one is able to explain this abnormality.

Factors that Influence Egg Yield in a Superovulated Cow

It is too early to draw up a list of all the factors that do influence egg yield in a hormonally superovulate cow at this point; Because no one understands all this factors. But from the data accumulated so far few factors have been observed to influence the egg-yield in a cow. The list presented below is definitely incomplete because there is evidence to believe that there are other factors, which we do not understand at present, that do influence the number of eggs produced by a superovulated cow.

(i) Ovarian Follicle Population

It is very well established that the number of primordial follicles, as well as their size vary considerably, between one cow and another (Erickson 1966; Choudary et al. 1968). Summers and Campbell (1974), and Mariana et al. (1970) were able to show that superovulatory response bears a relationship with the number of ovarian follicles. But the strongest evidence was produced by Dublin workers in 1969 where they were able to show, in a study of vesicular follicle population and PMSG therapy response, in 258 cows, that the number of eggs produced with PMSG was influenced by

the vesicular content. It is difficult at present to see how this knowledge will help the breeder in practice as he can not access this on the farm.

(ii) Breed Differences

There is genuine evidence to show that there is variation in PMSG response in different breeds as well as strains in a breed. Gordon recorded in three different experiments, that the Friesian respond to PMSG appreciably less than Hereford/Angus and Charolais. In all the three experiments the average number of eggs recovered in Friesien were less than half of the other breed.

(iii) Seasonal and Nutritional Effects

The number of follicles produced seem to be higher in winter and spring than in summer and autumn (Rajakoski 1966; Denny 1964; Salcon 1969). Salcon suggested that this might be due to differences in the number of vesicular follicles capable of responding to PMSG at different periods of the year.

Lamond (1970) showed that, fasting a cow soon after PMSG treatment, can markedly reduce superovulatory response. Similar findings were reported by Hill et al. in the same year. He also showed that, the effect was most critical during the follicular phase of the cycle. Gordon (1976) however found in his experiment in cattle and sheep that, there is no such appreciable difference in PMSG response, unless there is radical difference in the planes of nutrition.

(iv) Effect of Hormonal Regimen

As pointed out earlier, crude horse pituitary extract of FSH (HAP)

is better in stimulating ovulatory response than PMSG at least in the sheep; but because PMSG is readily available and required less injection it is preferred for commercial use while HAP is more often used in research.

Also as pointed out, timing of the PMSG injection is most important. Most follicular response is achieved if it is given during the follicular phase of the oestrus cycle (about day 16 of the estrus cycle).

PMSG has been given at varying doses from 1,000 to 2,500 international units, to produce different levels of ovulation (Hammond Jr. and Bhattacharya 1944, Gordon et al. 1962, Denny 1964, Rowson 1971 and 1972). In all these data, there has been varying response based on the dose levels, but the degree of response vary widely. Gordon (1975) using 3,000 international units PMSG found ovulation response to vary from 1 to 112. But accumulated results suggests that, there is more variation in response at higher doses than lower doses, but at high doses all animals have been found to produce additional eggs. Some reports (Folley and Malpress 1944 and Hafez 1963) however, have suggested the use of dose 3,000 international units PMSG and above.

Variation in ovulatory response to PMSG have been noted to vary with preparation from different pharmaceutical companies (Salcon 1969 and Sreenan 1969) as well as the different batches of PMSG from the same company. (Baker 1973, Bowen and Rowson 1973).

Routes of administration of PMSG have also been demonstrated to cause variation ovulatory response to PMSG. Intramuscular route have consistently given better response than the subcutaneous route (Lyons worker 1975; Boshoff and Burgner 1973).

In considering the variation in ovulatory response to PMSG one has to bear in mind the "silent heat" that follows PMSG administration. As

pointed out earlier this will lead to different times in insemination and times at which ova recovery is attempted. This obviously will affect the number of eggs recovered; But as to what extent this affects the results present in the data can not be determined.

B. PREPUBERAL COWS AND CALVES AS SOURCE OF EMBRYO

While it is experimentally possible to induce superovulation in sexually immature cattle and fertilize the eggs for embryo transplant (Seidel et al. 1970), there appears to be much variation in the results of such trials.

Jainudeen, Hafez and Lineweaver (1965) conducted two sets of studies in which they tried to induce multiple ovulation in calves, using PMSG and LH. Superovulation was done by injecting different doses of PMSG in calves of ages 4-24 weeks intramuscularly. This was followed after 5 days of intravenous injection of human leutinising hormone LH or NIH-LH. The calves were then inseminated with frozen bull semen. Although they were able to recover 52 eggs from 10 calves giving an impressive average (5.2), only two calves yielded fertilized eggs. Similar findings were reported by Casida et al. (1943), Black et al. (1953), Avery and Graham (1952) and Jainudeen et al. (1966). From the result of their data we now know that superovulation response is highly variable in immature cows and calves, as is in the sexually mature cows; But insemination trial results have been disappointing.

Part of the problem is due to the immature reproductive tract in the calves which makes it difficult to inseminate the calves. In some cases the semen could not be deposited beyond the entrance to the cervix (Onuma et al. 1969) which might have contributed to the poor fertilization

rate. Also the calf's reproductive tract is harmful to the ova, and prolong stay in the tract reduces the survival rate of the ova (Seidel et al. 1970).

So although it has been possible to obtain calves, which have been produced by ova transplant, from sexually immature calves, calves as a source of ova for transplant is not reliable at present for the reasons given above.

C. TEST-TUBE FERTILIZATION OF OOCYTES

There are many reports in the literature of the isolation and recovery of oocytes from several mammalian species, (Umbaugh 1949, Noyes 1962, Chang 1955, Edwards et al. 1965 and 1966). Methods used in the recovery include extirpating and ovaries and mechanically rupturing the follicles in a laboratory. One can therefore envisage, the slaughter houses as a potential cheap source of cow oocytes for test-tube fertilization of cow oocytes.

Test-tube fertilization has been achieved in several animals including the cow (Edwards 1973). Hunter and Rowson (1972) also obtained encouraging results in culturing the fertilization of cow oocytes in the test tube using 50:50 mixture of Tyrode's solution and flesh follicular fluid. Similar results have also been reported by Leman and Dzuik (1971), Pope et al. (1974), Church et al. (1974), and several others, all within the last five years. But actual birth from such fertilization has been reported only in laboratory animals.

These findings have made some observers to feel that abattoir can be used as a source of oocytes for fertilization and subsequent inoovulation in recipient cows. While this may be possible in future, at present, utilization of the ovarian oocyte is in no way an acceptable

alternative to superovulation as source of cattle eggs for transfer. In any case the slaughter house will provide rather unreliable source of oocytes for transplantation, as proven cows are not commonly encountered at the abattoir.

D. ISOLATED BLASTOMERES

Moore (1973) showed that individual sheep egg blastomeres, from 2-8 cell eggs, can develop into normal young. Kanagawa and Basrur (1973) also reported similar findings in cattle egg blastomeres. Apparently cellular specialization has not occurred by eight-cell stage.

This suggests that even larger number of eggs can be obtained from genetically valuable cows by using individual blastomeres. It is however too early to speculate what impact this will be in cattle industry.

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SECTION III

RECOVERY MEDIA AND CULTURING

RECOVERY

SURGICAL RECOVERY

Paralumbar Laparotomy

Mid Ventral Laparotomy

Intravaginal Approach

Disadvantages of Surgical Recovery

SLAUGHTER AND HYSTERECTOMY

NON-SURGICAL RECOVERY

Factors that Might Affect Non-Surgical Recovery

MEDIA AND CULTURING

Homologous Serum

Follicular Fluid

Hang's TC 199

Laboratory Animals

RECOVERY OF FERTILIZED OVUM

The recovery of ova from uterus is the most exciting aspect of ova transplant. Great attention must be paid to prevent damage to the tract so that the donor could be used over and over again. Although this aspect of ova transplant has received a very serious attention, the recovery of fertilized ova from the uterus of the live cow without serious interference with the future reproductive capacity of the donor, remains the main obstacle in ova transplant industry. Over the years a number of different techniques have been employed to obtain ova from developed ovarian follicles.

SURGICAL RECOVERY OF EGGS

Cattle egg recovery has been most encouraging using surgical methods (Rowson et al. 1969). In most techniques described, a laparotomy is performed and uterus and ovaries exteriorised and ova flushed out of the uterus using any of the several media available.

(i) Para-Lumbar Laparotomy

Kraemer and Davies used this approach to recover ova from 24 donors. They used xylocaine to achieve paravertebral block. Several other workers including Dzuik et al. (1958), Lamond and Holmes (1965), Baker (1966) and Mariana (1969) have used this as method of recovering ova from the uterus.

(ii) Mid-Ventral Laparotomy

This is by far the incision most used. General anesthesia is used. The animal is placed on a soft rubber mat and sodium pentobarbitone is given intravenously, just enough to induce light general anesthesia. The

cow is premedicated with atropine to reduce saliva secretions. Anesthesia is maintained by gas machine using halothane and oxygen mixture. Halothane anesthesia has been found to be particularly suitable since it relaxes the uterine muscles making it easier to manipulate.

The area anterior to the udder is shaved, scrubbed and sterilized. A mid-ventral laparotomy is performed and uterus exteriorised.

Whether the ova are recovered from oviduct or uterus will depend on the time of collection. If the recovery is attempted about 5 days after insemination, the ova will be in the tip of uterine horn (Scanlon and Sreenan 1969). Ova will be found higher up or lower depending on the time of surgery. In general at day 9 after insemination the zona pellucida has been shed off and the blastocyst is very delicate and liable to damage. Therefore recovery must be done before 9 days after last insemination.

To collect the ova, a fine canula is introduced into the ovarian end of the Fallopian tube and flushing the warm fluid gently through the top part of the uterus using a syringe and pipette. A better technique using simultaneous flushing with a 14 French gauge two-way female urological catheter 23, canula and syringe has been reported by Newcomb and Rowson (1975).

(iii) Intravaginal Recovery

Ovariectomy by the vagina method is a common surgical procedure in veterinary practice (O'Connor, 1965, and Baker 1968). In this surgery an incision is made in the vulva and the ovaries and the horns of the uterus are brought into vagina and ovaries removed.

Bedirian and Baker (1972) used this approach to recover 14 oocytes

in 18 trials. Using a vaginospeculum an incision was made into the dorsal wall of the vulva and horns of the uterus brought into the vagina with a curved piece of plaxiglass and Allis tissue forceps. The surgery was done with only sedation and caudal epidural anesthesia. The surgery itself is simple compared with mid-ventral laparotomy where general anesthesia must be employed.

There is however no other report of this technique available and so it is not possible to evaluate this technique properly. The poor egg yield in this trial will tend to discourage the use of this technique. Obviously the lack of sufficient cervical relaxation compared with halothane anesthesia will be responsible impart to the very low recovery yield. They also reported extensive adhesions post surgically in all cases. From all indications this method is not very promising; but might be useful in viewing the ovaries in short term studies, such as follicular development and ovulation.

Disadvantages of Surgical Technique

Since most of the surgical recoveries are done under general anesthesia, there is a risk of the animal dying from anesthetic over dose or complication. This is relatively a minor risk but the surgeon has to consider how many times an animal could continue undergoing general anesthesia without any effect on its production.

Also as pointed out in the introductory paragraph, for unknown reasons, surgical techniques produce adhesions even where strict asepsis is maintained. Damage of this kind is even more extensive where inexperienced or careless personnel are involved (Seide Jr., 1976). This makes the donor unproductive in further trials.

Regardless of the disadvantages of surgical technique in the recovery of ova, at present time, embryos from valuable cows are recovered surgically (Seidel 1976). Many researchers are working on non-surgical means of recovery of the embryo but none of these methods have been sufficiently developed to approach the success of surgical recovery.

SLAUGHTER OR HYSTERECTOMY

Reproductive organs of the cows are removed intact and horns of the uterus clamped off and aseptically flushed in the laboratory to collect the ova, in much the same way as was described in surgical recovery technique. Where the animal is not yet ready for slaughter, hysterectomy is performed and ova recovered as for abattoir source (Forte 1972).

NON-SURGICAL RECOVERY

If ova transplant is to make the desired impact in the propagation of offspring from the genitically superior females, then a way other than surgical method currently used must be replaced by techniques that will be less damaging to the reproductive capacity of the donor female cow. Seidel (1976) estimated that with the best techniques and experience the reproductive capacity of several donors are completely destroyed while additional 10% are rendered "sub-fertile". This figures are doubled with each subsequent surgery. Over the years several researchers have tried to overcome this by employing non-surgical techniques so as to preserve better the reproductive capacity of the donor cow. One must however add that the attempts have been far too few and result equally discouraging.

The first attempt to collect fertilized ova non-surgically in the cow was made by Lowson and Dracy (1949). They employed an apparatus

which was made of three concentric rubber tubes, through which they passed fluid in one and collect the injected fluid in another. The out tube had a cuff into which air was injected once the apparatus was passed into the uterus to hold it in place. There is however no data on the performance of the apparatus and why it seems not to be in use at present. But Dowling (1949) in the same year reported data on an apparatus with much the same specifications as the one described above, in which two out of six attempts were successful. There is no information as to how many ova were recovered in each of the successful trials.

Dracy and Patersen (1950) reported two non-surgical attempts to recover fertilized ova from the uterus of a cow. In one they used a catheter to flush the oviduct and aspirate back the injected fluid. None of the seven attempts were successful. They however had 12 successes out of 23 attempts with as many as 20 eggs in one cow using a canula and an inner tube. The instruments were made of stainless steel.

Dzuik et al. (1958) made three attempts to recover fertilized ova non-surgically in the cow. In the first attempt, he used a canula with inner tubing and a retained balloon, and he was unable to recover any ova in 26 trials. In the second trial they used a tube with a retaining balloon and they were successful in 2 out of 20 trials. The third trial involved a use of a tubural device, with holes and retaining balloon, with which they collected 10 ova out of 39 trials. Dzuik also in 1954 reported a much better result when he used a catheter with 10 holes and self retaining cuffing. He collected a total of 15 ova in 5 successful recoveries out of 13 attempts.

Yamcuchu (1961) in Japan reported woeful failure with a uterine douche unit. No ova was collected in any of his 3 attempts.

The most successful attempt in non-surgical recovery of ova in the cow was carried out by Sugie, Soma, Fukumitsu and Otsuki in 1970. Their result has so far been the most impressive and rekindled the hope of non-surgical ova transplant in the bovine specie.

Two types of apparatus were used in the study. A two-way device was first employed which had no cuffing and a total of 123 ova out of an estimated 334 were recovered non-surgically. The result with the three-way apparatus was slightly better. A total of 158 ova were collected out of 374 estimated to have ovulated. This result includes all the trials done over a period of 4-7 days after insemination. Since all other trials were performed at the best time of recovery, i.e. 5 to 6 days after insemination, a more valid analysis would be 154 ova out of 216 ova in the three-way apparatus and 116 recovered out of 272 with the two-way apparatus.

The results of the surgical method of recovery is compared with the non-surgical method in Table 1 and that of the different non-surgical methods are compared in Tables 2 and 3.

Some Factors That Might Affect Non-Surgical Recovery of Ova

From the Uterus

(i) Factors Related to the Cow and Superovulation

All the factors that will affect the number of ova available in the uterus will have direct effect on the number of ova recovered regardless of which method is used. These factors include breed and strain of cattle used, ovarian follicle population, seasonal and nutritional effects, and regimen adopted in the hormone therapy (i.e. dose level, source and route of administration). How these factors affect the number of ova

available at the time recovery is attempted was discussed in Section II. These factors are not limited to non-surgical recovery and thus one cannot explain the low number of ova recovered by non-surgical means compared with other means on this basis.

(ii) Time at Which Recovery is Attempted

The number of days, post-ovulation, after which the recovery is attempted will influence the location of the ova in the reproductive tract, which is critical to the success of the operation. Sreenan (1969) reported that at four days after ovulation the ova are found mainly in the oviduct; But five days after ovulation they can be expected to be at the tip of the oviduct. This may account for, to some degree, the failures which have been reported with attempts carried out four days after insemination. Sugie et al. (1970) found remarkable difference in the number of days. Trials on the 4th and 7th day yielded significantly fewer eggs than the ones on the 5th and 6th day regardless if a 3-way or 2-way apparatus is used. By the 9th day post insemination, the zona pellucida has been hatched and one is dealing with very fragile cells which will produce considerably lower yield than when dealing with normal eggs (Boland et al.).

(iii) Amount of Media Used

In all attempts reported, the basic principle is to infuse some media into the uterine horn and oviduct so as to cause the ova to float in the media and flow out through a tube into a waiting container. One is struck by the variation in the amount media used in attempt to recover the ova non-surgically. Dzuik et al. (1958) in a study of 58 uterine specimen found that, non-pregnant uterus of a cow can hold from 193-325 mls.

They subsequently used 200 mls of the media to recover 15 ova in 13 attempts (1954). In another study Dzuik (1958) used 112 mls. The lack of knowledge as to how much fluid is to be used might very well be responsible for some failures, since there is such great variation in the volume capacity of the cow uterus.

(iv) Cuffing

It is not clear from the data accumulated whether the presence or absence of the cuffing makes any difference in the success of the recovery attempt. But since there is much variation in the size of the cuffing used with the different devices, it is possible that in some cases there is no closure of the uterus. Dzuik et al. (1958) used 10 mls to achieve the same. The data collected from several trials in which cuffing was used is compared with one in which cuffing was not used and is presented in Table 4.

MEDIA FOR COLLECTION AND CULTURING OF EMBRYO

Introduction

Very little is known about the requirement of the media for recovery or culturing of mammalian embryo, although appreciable work has been done in the rabbit, some of which have been applied in the bovine. But if ova transplant is to achieve the desired impact, a media that will be optimal for embryo survival in vitro and would facilitate storage and transport of the ova must be available. Several solutions have been tried.

Homologous Serum

This was the first media tried. Chang in 1948 was able to store

rabbit embryo in the homologous serum for up to seven days without losing their viability. He worked with serum from different mammals and found that most serum, except pig serum, contained an ovicidal factor, and that this factor is destroyed if the serum is heated (Sreenan et al. 1968) to about 55°C for 30 mins. (Chang 1949). Attempts to transfer this finding in the rabbit to the cow was disappointing. Rowson et al. (1968) found that homologous serum is an unsuitable medium for egg transfer or culturing in the cow. Onuma and Foote (1968) however achieved encouraging results with bovine or rabbit serum.

Follicular Fluid

This media is obtained from the follicle of the ovary of sheep. Earlier trials with this media were disappointing (Brock and Rowson 1952). Sreenan et al. (1968) however found this media superior to homologous serum.

Hang's Tissue Culture (TC 199)

This media has yielded more encouraging results than any other and currently is the one used by most breeders. Rowson, Moor and Lowson (1968) found TC 199 to be superior to follicular fluid. They obtained no pregnancy with follicular fluid but when they used TC 199, 9 out of 13 animals were pregnant. TC 199 is usually warmed to body temperature (37°C) before use.

Laboratory Animals

There are some reports of attempts to store cow embryo in the oviduct of laboratory animals, especially rabbit and mouse (Brinster 1968, Graham 1953, Onuma and Foote 1969).

In general embryo development in laboratory animals is superior to that obtained in other media. Rowson et al. (1972) obtained successful pregnancies from ova that had been stored in the rabbit oviduct for five days.

Miscellaneous

Several other solutions have been used to recover or culture ova from the uterus. Among them are Tyrode's supplemented with albumin, Dulbeccos media (Gordon 1975) and physiological saline. Physiological Saline Solution (PSS) is used in some commercial ova transplant houses in U.S.A. and Canada. Most of these media contain some antibiotic to prevent bacteria infection of the reproductive tract.

Table 1. Surgical methods versus non-surgical methods of recovery of ova in the cow.

	Surgical	Non-Surgical
Ave. No. ova attempted	18.1	6.2
Ave. No. ova recovered	9.3	1.3
% Recovery	51%	20%
Highest % recovery recorded	90%*	33%**

References:

- (i) I. Gordon (1975), - a Review.
- (ii) R. H. Foote and H. Onuma (1970), - a Review.
- *(iii) L. E. A. Rowson (1971).
- ** (iv) T. Sugie et al. (1970).

Table 2. Non-Surgical Ova Recovery

Device	Attempts	Success	Time	No. Egg Recovery	Range/ Ave.	%	Source
3-WAY	24	17	5	112	1-18	-	Sugie et al. (1970)
2-WAY	9	7	5	55	1-16	-	Sugie et al. (1970)
1-WAY (Douche)	3	0	-	-	-	-	Yamuchu (1961)
1-WAY (Tubing c cuff)	39	10	-	-	1-6(0.7)	25%	Dzuik et al. (1958)
1-WAY (Cather c 10 holes & cuffing)	13	5	-	15	1-5(3)	31%	Dzuik (1954)
Canula or tubing c cuffing	10	2	-	-	1-7	20%	Dzuik et al. (1958)
Canula, inner- tube & cuffing	26	0	0	0	-	-	Dzuik et al. (1958)
Canula & innertube	37	12	-	-	* -20(1.1)	33%	Dracy & Petersen (1950)
Catheter in oviduct 1-WAY	7	0	-	-	-	-	Dracy & Petersen (1950)
3-Concentric Rubber tubes	6	2	-	-	-	33%	Dowling (1949)
3-Concentric stainless steel c cuff (3-WAY)	**	**	-	-	-	-	Dowling

* Lower limit not given.

** Successful but data not published.

Table 3. Comparison of Different Devices Used in Non-Surgical Recovery

	1-WAY	2-WAY	3-WAY
Attempts	72	78	24
Success	17	29	19
%	25%	35%	71%
Egg Recovered	15	55	112
Maximum	5	16	18

Table 4. Effects of Cuffing

	No Cuffing	Cuffing
Attempts	62	112
Success	21	34
%	33%	30.3%
No. Egg Recovered	55	127
Maximum	16	18

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SECTION IV

SYNCHRONIZATION OF ESTRUS

I INTRODUCTION

II INDICATIONS OF ESTRUS SYNCHRONIZATION

Artificial Insemination

Management Tool

Ova Transplant

Research

III METHODS OF SYNCHRONIZATION

PHYSIOLOGICAL BASIS OF SYNCHRONIZATION

PROGESTERONE

Oral Progestagens

Subcutaneous Implants

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PROSTAGLANDINS (PGF 2 a) AND ANALOGUES

Parenteral Use

Uterine Use

OTHER METHODS

Natural

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Oxytocin

Uterine Irritants

SYNCHRONIZATION OF ESTRUS

INTRODUCTION

Estrus synchronization is a method by which cattle in the herd are brought to heat at the same time. The survival of the transferred egg depends on how closely their development stage corresponds to the developmental stage of the reproductive system of the recipient. It is now known without doubt that the rate of pregnancy in the recipient depends on the closeness of the estrus cycles of the donor and recipients (Sugie et al. 1970). Chances of pregnancy are higher if there is not more than 24 hours difference in their time of estrous (Rowson et al. 1969) as was pointed out earlier. This has made synchronization of estrus of the donor and recipient a very important phase in ova transplant.

INDICATIONS OF SYNCHRONIZATION OF ESTRUS

(a) Artificial Insemination

Synchronization of estrus is now employed widely in artificial insemination programmes (Schrutz 1976) in controlling the time of calving. The breeder selects the desired time of calving and synchronizes the estrus time of his herd so that all insemination can be done within a relatively short time.

(b) As Management Tool

Estrus synchronization is also used as an effective management tool in planning the calving season. This does produce a herd which are virtually of the same age; They can then be vaccinated together, dehorned at the same time and ready for sale at same time. Animals that do not come into heat at the desired time are culled at this time. This is

now a common practice among German and British breeders (Schultz 1976).

(c) Ova Transplant

With the current interest in ova transplant in cattle, estrus synchronizaton is now employed as a very important tool in ova transplant; Although in several established farm, the breeder uses natural synchronization. He maintains a large herd of cows and when he recovers fertilized ova from the donor cow, he just transfers it into all the cows that were in heat at about the same time as the donor. However several workers now employ other methods, usually hormonal synchronization.

(d) Research

It is also employed by many researchers who are involved in embryo transplant studies or other aspects of reproductive studies.

METHODS OF ESTRUS SYNCHRONIZATION

Physiological Basis of Estrus Synchronization

The physiological basis for estrus synchronization is that, progesterone, which is produced by the corpus luteum, supresses the release of L. H. by the anterior pituitary (Roberts 1971). This then inhibits the maturation of Graafian Follicles in the ovary and prevents ovulation. Estrus synchronization can also be achieved by inducing ovulation at a desired time or by induction or delay of corpus luteum regression (Lauderdale and Zimbelman 1974).

Regardless of which method or methods are used, the method must be effective, easy to administer and above all safe for the animal.

CORPUS LUTEAL ENUCLEATION

This is the oldest method of estrus synchronization. The corpus

luteum is mechanically "enucleated" by finger pressure applied through the rectum. The success of the operation depends on how completely the C. L. has been expressed (Hafez et al. 1963). The method is however crude and sometimes fatal. Hemorrhage has been reported in association with this method. Adhesions also commonly follow this method of synchronization. It is definitely not the method of choice in valuable cows.

PROGESTERONE

Progesterone was first used by Ulberg Christian and Casida about 25 years ago. This drug was used vigorously in the 1960 as an agent for estrus synchronization in the cow.

(i) Oral Progestagens

Upjohn's MAP (medroxy-progesterone acetate) and MGA (malengestrol acetate) have been used orally along with Syntax's CAP (chlormadinone acetate) to bring about estrus synchronization in the cow. They are fed orally for a period of about 18 days. Wiltbank and Kasson (1968) reported success with combination of oral progestagen, incorporated in the feed for 9 days, and estrogen for two days to synchronize estrus. MAP is used at the dose level of 180-200 mg/day/animal, CAP 10 mg/day/animal and MGA at 1 mg/day per animal. The animals treated with progestogens will develop normal Corpus Luteum, but follicle formation and ovulation is suppressed. The conception rates at the estrus following progestogen treatment is appreciably lower than in non-synchronized cows, but the conception rate in subsequent estrus is the same as untreated animals (Roberts 1971).

(ii) Sub-Cutaneous Implants

In 1974 French worker (Maulen) reported synchronization of estrus in cattle with subcutaneous ear-implants of 6 mg SC 2009 (alpha-acetoxy-11 beta-methyl 19 nor. preg. 4 ene 3, 2 diona) for 9 days followed by 3 mg of SC 21009 and estradiol intramuscularly.

(iii) Vaginal Sponges

Use of intravaginal sponges containing progesterone in cattle has been reported by Scalnon (1969) and Sreenan (1969). This method was useful in beef cattle, but the lack of retention of the sponges in the vagina limits its use at present.

PROSTAGLANDINS (PGF_2a) AND ANALOGUES

This is the newest addition to the compounds that can be used to achieve synchronization in cattle. It has been used by several researchers in the past five years (Rowson et al. 1972, Tervit et al. 1973, Cooper 1974, Cooper and Furr 1974, Smith 1974).

Prostaglandin F_2a will destroy the functional corpus luteum in the cow, mare and ewe. Prostaglandin is naturally secreted by the prostate. It is now known that prostaglandins is a mixture of several hormones (V. K. Ganiam 1976). Two analogues of prostaglandins are available commercially for use 1C1 80996 and 1C1 81008. These are structurally similar to the "F" series of prostaglandins and also have luteolytic property of prostaglandin F_2a .

(i) Parenteral Use

1C1 80996 given intramuscularly at dose of 500 micrograms will bring about luteolysis in the cow. Natural prostaglandins PGF_2a will

produce the same effect if given at a dose level of 20-30 mg. In order for a single injection to be effective it must be given at between day 5 and 16 of estrus cycle (Cooper and Fum 1974) as prostaglandins has no effect on the CL during the first 4 days estrus cycle nor on natural luteal regression.

Where the cycle period of the animal is not known, two injections of about 10-12 days apart, have to be employed to achieve complete synchronization in a herd; Although Cooper and Fum have reported only 60% synchronization in a herd. As far as is known at present fertility after prostaglandins synchronization is the same as naturally synchronized animals. 1C1 80996 has a wide margin of safety. Even at the dose 200 times the effective dose the only signs described are mild diarrhea.

1C1 81008 is used to synchronize estrus in the mare. For unknown reason the result obtained from prostaglandin trials in the gilt has been very disappointing.

(ii) Uterine Use

Prostaglandins given at the dose of 0.5 to 1.0 mg intrauterine in the horn containing the CL will induce luteal regression and estrus in 48 to 96 hours. This method is not practical in natural herd condition as it requires time and skill and carries some risk of uterine infection.

Preliminary investigation by Susan Lewis, Cumming and Lawson have suggested that, the use of PMSG together with PGF_2a is superior to PMSG alone in terms of the proportion of animals ovulating and higher ovulation achieved in the animals which respond. Other embryo transplant firms use this combination in cows whose length of cycle is uncertain. PGF_2a is given two days after PMSG or FSH administration (G. E. Seidel Jr.

1976).

OTHER METHODS

Natural synchronization seems very popular among commercial herds in North America (Gordon 1975 and Seidel 1976). Such farms maintain a large herd from which they selected all recipient who happen to be on heat the same time as the donor and use for their embryo transplant. Other methods of estrus synchronization includes estrogen in combination with other means and infussion of the uterus with irritants e.g. alcohol or iodine to shorten the estrus cycle (Schurtz 1976). Oxytocin also has been employed to synchronize estrus in the cow.

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SECTION V

INOVULATION

INTRODUCTION

VAGINAL TRANSFER

SURGICAL TRANSFER

TECHNIQUE OF INOVULATION IN THE COW

Transport and Storing

Anesthesia

Surgery

SPECIAL INSTRUMENTS

SOME FACTORS THAT MIGHT AFFECT PREGNANCY OF RECIPIENT

Degree of Synchronization

Method of Transfer

Age of Egg at Transfer

Others

INOVULATION

INTRODUCTION

The first successful ova transfer in farm animals was performed by Hunter along with others in 1955 in sheep in Britain. This was followed by several others including Avenill (1956), Avenill and Rowson (1958) and Moor et al. (1960). Much the same success has been reported in pigs and other farm animals including cattle since then. The term inovulation is used here to describe the process of placing the recovered ovum in the uterus of the donor, to avoid confusion with the whole process of ova transfer.

VAGINAL TRANSFER

Earlier efforts in the transfer of ova in the cow were concentrated on non-surgical transfer via the cervix of the cow (Dracy 1953, Dowling 1949, Rowson 1951). No successful pregnancy was reported by this method. This failure was due to two main reasons.

First the uterus of the cow is very highly susceptible to infection during estrus and few days following it, (Rowson 1953). Therefore the passing of instrument through the cervix introduce infection into the uterus which results in pregnancy failure. But to what extent this is responsible to the pregnancy failure is not known, since addition of antibiotic to the media which would prevent possible uterine infection by Dzuik (1968) failed to result in pregnancy.

The second and perhaps the most important factor is that, the fertilized ova injected into the uterus is quickly rejected out of the uterus by uterine contractions (Bennett and Rowson 1953) presumably due to irritation. This phenomenon is not prevented by drugs which are known

to prevent uterine contractions (Rowson et al. 1964).

Non-surgical transfer is now receiving much attention from researchers and at least one transfer unit is transferring its ova non-surgically (P. Elsdein personal communication Sept. 1976).

SURGICAL TRANSFER

This method of transfer has given the encouraging results. Pregnancy results as high as over 90% have been reported by Rowson (1972). Other workers have reported pregnancy rates much lower than Rowson (Graham 1974, Sreenan 1974) using the same surgical procedure as he did. This would suggest that there are some other factors that influence the transfer, some of which will be discussed later. The results of flank and midventral laparotomies are compared in Table V(a).

TECHNIQUE OF INOVULATION IN THE COW

Transport and Storing

If eggs are to be transferred the same day they are collected, it is sufficient to store it at the temperature of 37°C until the recipient is ready for the transfer. Success with ova stored for longer periods have been reported with ova stored at 8°C , but as pointed out earlier, the degree of success goes down rapidly with length of time. In general the ova should be transferred to the recipient as soon as possible to achieve maximum result.

Anesthesia

Some embryo transfer units prefer local anesthesia (paravertebral block) and flank laparotomy, but the result obtained by this method of transfer is poor compared with general anesthesia.

Most commonly, anesthesia is induced by thiopentone sodium followed by close circuit anesthesia with fluothane and oxygen as in surgical recovery.

Surgery

The mid ventral abdominal area is shaved and scrubbed and a lower ventral laparotomy is performed and both uterine horns and ovaries are exteriorised. Both ovaries are checked for the presence of a C.L.

One egg is transferred into each of the horns using a sterile Pasteur pipette through a stab wound made with a sterile needle about 2-5 cm proximal to the uterotubal junction (Sreenam et al. 1974, Gordon 1975, Seidel 1976). There is no mention of "free-martinism" in association with inovulation by this method. Only ova certified to be normal by the microscopic examination is transferred into the recipient.

The uterus and the ovaries are returned to the pelvic canal and the incision closed in routine manner.

Post-surgical care is routine. Animals are watched for return to heat usually by the use of vasectomized marker bull and pregnancy is confirmed by rectal examination after two months.

SPECIAL INSTRUMENT

Sugie, Soma and Fukumitsu (1972) reported considerable success with a special instrument, they designed to transfer ova without general anesthesia. Twelve out of seventeen animals became pregnant and 13 calves including a set of twins were delivered from the 12 animals which were able to carry the pregnancies to full term.

The procedure involved an incision into the dorsal wall of the vulva and on into the horn of the uterus into which the media containing

the ova is injected. One hand is passed into the rectum to help maneuver the uterus. It is not known whether he used epidural anesthesia.

SOME FACTORS THAT MIGHT AFFECT PREGNANCY

It must be emphasized right from the start that there is no conclusive evidence that any of these factors do affect the rate of pregnancy resulting from ova transfer, but in some cases the little evidence accumulated would suggest that they influence the rate of pregnancy.

Degree of Estrus Synchronization

The importance of this in the success of ova transplant was discussed previously. The effect of synchronization is summarized in Table V(b). Best results are obtained when synchronization is zero.

Method of Transfer

Best results are obtained from ventral laparotomy under flouthane-oxygen anesthesia. This method of transfer is compared with the best reported non-surgical method in Table V(b).

Age of Egg at Transfer

There is no critical data about the optimal time to perform the transfer; It is however clear that, transfer must be carried out within 3-6 days of ovulation. The general consensus among commercial units seems to be 5-6 days. This might be because it corresponds to the earliest time the ova reaches the horn of the uterus, the site of transfer; and because identical location in the uterus of donor and recipient is required to obtain best result. By day 9 the zona pellucida would have been shed and the embryo is very fragile and pregnancy could not be expected.

Others

Other factors believed to affect pregnancy rate in the recipient are the status of the recipient, its health and plane of nutrition. The handling of the ova before transfer is also believed to affect the rate of pregnancy in the recipient. There are however no critical data on these factors in the cow.

Table V(a). Surgical Methods of Involuation

	Flank Laparotomy	Mid-Ventral Laparotomy
Number of Attempts	7	37
No. Pregnancies	1	20
No. Calves	1	17
No. Abortions	0	3

Sources:

Avery et al. (1962)

Umbaugh, R. E. (1949)

Willet et al. (1952 and 1953)

Rowson et al. (1966 and 1969)

Table V(b). Effects of Method of Transfer

Synchronization	Non-Surgical Transfer	Surgical Transfer
-3	0	0
-2	25	30
-1	30	52
0	29.4	91.1
+1	36	56
+2	0	40
+3	0	20

SOURCE - Summary of

Rowson et al. (1972)

Screen et al. (1975)

Sugie et al. (1972)

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EMBRYO TRANSPLANT IN THE COW

by

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EMBRYO TRANSPLANT IN THE COW

INTRODUCTION

The first embryo transplant, in the cow was performed in 1943 by Berry et al. Since then embryo transplant has been studied extensively in the cow, with the aim of increasing beef production.

I. SOURCE OF EMBRYO

Four methods have been used to obtain fertilized ova for transplanting.

(i) Superovulation of Adult Cows

This is the commonest source employed. A cycling cow is given gonadotropin during the follicular phase of the estrus cycle to stimulate the production of 10-20 ova. FSH of crude pituitary extract, and PMSG have been used for this purpose; Of the two, PMSG is preferred. Gondotropin is used along with estradiol and HCG.

The response to gonadotropin is influenced by the health and nutritional status of the animal, season of the year, source, batch and route of administration of PMSG as well as breed and individual differences. Refractoriness to repeated doses of PMSG due to antigonadotropin production has been reported by some workers.

(ii) Immature Cows and Calves

Homonal superovulation has been achieved in prepuberal cows and calves. Results reported in these trials have been very varied. This method is not used as source of ova except for research purposes.

(iii) Test-Tube Fertilization

Test-tube fertilization has been done in the cow, however, no

actual calving has been reported from test-tube fertilization in the bovine species.

(iv) Blastomeres

Individual blastomeres, from 2-8 cell eggs, have been transferred with resultant pregnancy and parturition in the cow. Much more research has to be done before this can be used in the beef industry.

II. RECOVERY

Three methods are employed in ova recovery:

(i) Surgical Method

This is the most used method. The uterus is exteriorised by laparotomy and oviduct flushed with a recovery media and ova collected in the media. Extensive damage to the uterus very often follows surgical ova recovery.

(ii) Slaughter and Hysterectomy

The animal is either killed or hysterectomised and ova recovered as in the surgical method.

(iii) Non-Surgical Method

This method is currently being extensively investigated. Results obtained by this method have been discouraging, compared with the surgical method.

III. ESTRUS SYNCHRONIZATION

The degree to which the estrus cycles of the donor and recipient are synchronized greatly influences the rate of pregnancy. Best results

are obtained when synchronization is not more than $\pm$ 24 hours.

Progesterone, prostaglandins and their analogues are used.

IV. TRANSFER

(i) Vaginal

This was the first method attempted to transfer ova. No pregnancy has been reported by this method.

(ii) Surgical Transfer

This is the best method available; Pregnancy rates up to 90% have been reported by this method. The ova is introduced into the uterus by a pipette through a stab incision at tubro-uterine junction of the uterus.

(iii) Special Instrument

Sugie, et al. reported high pregnancy rates with an instrument they designed for the purpose.

CONCLUSION

From the data accumulated, it can be seen that embryo transfer can make a great impact on the cattle industry by increasing the out-put of a genetically good cow in much the same way as artificial insemination has done to the male counterpart. More research will have to be done to solve the different problems, especially in regard to the recovery of ova from the donor, before such a goal can be fully realized.