THE INFLUENCE OF A CANNULA IN THE RABBIT OVIDUCT

II. EFFECT ON EMBRYO SURVIVAL

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Sloan & Johnson (1974) have shown that a cannula in the infundibular end of the oviduct causes no significant changes in the lipid and proteins of the oviduct fluids. This suggests that embryos might survive and develop within the cannulated oviduct by embryo transfer.

The normal development of mammalian eggs transferred from one individual to another was first reported in the rabbit by Heape in 1890. Since that time successful embryo transfer has been accomplished in the rabbit by many workers.

At the present time, there are no satisfactory means of transferring ova in rabbits without laparotomy of the recipient at the time of transfer. This study was undertaken in an attempt to develop a practical technique for transferring ova to the oviducts of recipient does through an indwelling cannula. This procedure would allow any number of transfers at any time after cannulation.

The twenty-eight does used in this experiment were sexually mature virgins weighing between 3.5 and 5.5 kg. All were kept in individual cages environmentally controlled near 21°C.

The operation to install the cannula and extra-abdominal flask, which is shown in Pl. 1, Fig. 1 and Text-fig. 1, was performed with aseptic precautions as follows: anaesthesia was induced by a slow injection of sodium pentobarbitone into the ear vein. An incision was made in the left flank approximately 3.5 cm from the loin mid-way between the ribs and hipbone. The ovary and oviduct were exposed through the incision. A silastic tube (1.57 mm i.d., 2.42 mm o.d.) was inserted into the fimbriated end of the oviduct and sutured in place (Pl. 1, Fig. 2). The cannula was carried out through the incision and the extra-abdominal flask, which housed the external end of the cannula, was sutured in place in the flank (Pl. 1, Fig. 3).

The cannula was measured from the mouth of the flask to the fimbriated end before each experiment. The pick-up tube was marked so that it could be passed to the end of the cannula but not into the oviduct.

Varying numbers of days after cannulation, donors and recipients were mated and injected with HCG (800 i.u.) to ensure ovulation. The donors were anaesthetized 48 to 52 hr after mating and mid-ventral laparotomy was per-

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formed. The oviducts were flushed with 5 ml Krebs-Ringer bicarbonate (KRB) (Lardy & Phillips, 1943) and collected in a watch-glass.

The ova were aspirated directly from the watch-glass and transferred immediately to the recipient. The maximum length of time from collection to transfer was less than 15 min.

The transfer of ova was accomplished by passing the ova pick-up tube (0.58 mm i.d., 0.965 mm o.d.) down the cannula to the fimbria and flushing the ova into the oviduct with 0.5 ml KRB as shown in Text-fig. 1. The external end of the cannula was secured so that no fluid escaped and was then housed in the extra-abdominal flask.

On Day 12 after transfer, the recipients were subjected to mid-ventral laparotomy and normal developing embryos were counted. They were then allowed to go to term.

TEXT-FIG. 1. Schematic drawing of the technique of rabbit embryo transfer with transfer tube in place within the cannula. C, cannula; E-AF, extra-abdominal flask; M, muscle layer; S, skin; T, transfer tube; V, velour cuff.

Twenty-eight does were unilaterally cannulated and designated as recipients. Due to infection and constriction of the cannula inside the body cavity, only twenty-three recipients received embryos. At laparotomy, it was found that five of the twenty-three recipients receiving ova had developed genital infection leaving only eighteen actual recipients. In these eighteen does, 44.4% of the transfers resulted in implantation. Thirty-five ova were transplanted to the eight recipients that showed implantation sites on Day 12, and twenty-one (60%) of these ova implanted.

Three animals into which embryos had previously been transferred by this method had embryos transferred a second time. In the first transfer, two had

PLATE I

FIG. 1. Cannula and extra-abdominal flask.
FIG. 2. Cannulation of a rabbit oviduct.
FIG. 3. Extra-abdominal flask sutured in the flank of a rabbit.

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successful pregnancies and one did not. In the second transfer, several months after the first, the same two became pregnant and bore young to term for the second time.

Although antibiotics were administered immediately after surgery and several days thereafter and the operation was performed as aseptically as possible, oviducal infection did occur and continued to be a problem.

Some cannulae were being constricted inside the body cavity. This resulted in failure of the transfer tube to pass to the fimbriated end of the cannula and thus failure of the embryos to reach the oviduct. Laparotomy showed that constriction was caused by pressure of the abdominal fat pad against the extra-abdominal flask housing the cannula. This was eventually overcome by passing the cannula directly through the fat tissue instead of around it.

Several modifications in the procedure were made in successive trials. Various cannula lengths and times between cannulation and transfer were tested, and the cannula was flushed with physiological saline after its suture into the oviduct to clear the cannula of any blood or other debris that might hinder the transfer.

Since normal fetuses were obtained from some of the does, this experiment indicates that the presence of an oviducal cannula does not significantly affect the normal function and secretion of the rabbit oviduct. This verifies the 'normality' of previous oviduct work in which cannulation has been used (Bishop, 1957; Clewe & Mastroianni, 1960; Mastroianni, Beer, Shah & Clewe, 1961; Mastroianni & Wallach, 1961; Hamner & Williams, 1965; Edgerton, Martin, Troutt & Foley, 1966; Huang, Coley, Kraft & Johnson, 1972; Sloan & Johnson, 1974) and supports the validity of collection of oviducal fluids with proper cannulation of the fimbriated end of the oviduct. While conditions may not be perfectly 'normal' within the cannulated oviduct, regular cleavage and subsequent uterine implantation can still take place.

The technique described could allow ovum transfer to be performed many times post-operatively without any apparent stress to the recipient, and might provide a means for studying the nutrient requirements for fetuses by the addition of substrates and labelled isotopes. The technique could also be used to study maternal influences and the effects of uterine environment on the survival of embryos.

Control of genital infection and improvement of the technique could greatly improve implantation percentages.

REFERENCES

- BISHOP, D. W. (1957) Metabolic conditions within the oviduct of the rabbit. *Int. J. Fert.* **2**, 11.
CLEWE, T. H. & MASTROIANNI, L., JR (1960) A method for continuous volumetric collection of oviduct secretions. *J. Reprod. Fert.* **1**, 146.
EDGERTON, L. A., MARTIN, C. E., TROUTT, H. F. & FOLEY, C. W. (1966) Collection of fluid from the uterus and oviduct. *J. Anim. Sci.* **25**, 1265.
HAMNER, C. E. & WILLIAMS, W. L. (1965) Composition of rabbit oviduct secretions. *Fert. Steril.* **16**, 170.
HEAPE, W. (1890) Preliminary notes on the transplantation and growth of mammalian ova within a uterine foster mother. *Proc. R. Soc. B*, **28**, 457.
HUANG, F. S., COLEY, S. L., KRAFT, L. A. & JOHNSON, A. D. (1972) Oviduct fluid organic acids of synchronized heifers. *J. Anim. Sci.* **34**, 353.
LARDY, H. A. & PHILLIPS, P. H. (1943) Effects of pH and certain electrolytes on the metabolism of ejaculated spermatozoa. *Am. J. Physiol.* **138**, 741.

- MASTROIANNI, L., JR, BEER, F., SHAH, U. & CLEWE, T. H. (1961) Endocrine regulation of oviduct secretions in the rabbit. *Endocrinology*, **68**, 92.
- MASTROIANNI, L., JR & WALLACH, R. E. (1961) Effects of ovulation and early gestation on oviduct secretions in the rabbit. *Am. J. Physiol.* **200**, 815.
- SLOAN, M. H. & JOHNSON, A. D. (1974) The influence of a cannula in the oviduct. I. Oviduct fluid lipids and proteins. *J. Reprod. Fert.* **37**, 149.