

From the Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden.

RECIPROCAL EMBRYO TRANSFER BETWEEN REPEAT BREEDER AND VIRGIN HEIFERS — AN EXPERIMENTAL MODEL

By

H. Gustafsson and K. Larsson

GUSTAFSSON, H. and K. LARSSON: *Reciprocal embryo transfer between repeat breeder and virgin heifers — an experimental model.* Acta vet. scand. 1983, 24, 59—64. — The aim of the study was to evaluate the importance of mother-conceptus relationships for the elevated embryonic loss in repeat breeder heifers. Embryos were collected by non-surgical technique, classified and transferred surgically or non-surgically to synchronized, inseminated recipients. The embryos were transferred to the uterinehorn contralateral to the corpus luteum. The embryos were transferred from repeat breeder heifers (RBH) to virgin heifers (VH) or from VH to RBH. After slaughter 4 weeks after transfer there was no difference in embryonic survival between heifer categories following transfer or insemination. In some animals degenerated foetal membranes were found in the non-pregnant horn. The study indicates embryonic morphology rather than the category of donor or recipient as influencing the embryonic survival rate.

embryo; embryonic death; embryo transfer;
repeat breeder.

In repeat breeder heifers (RBH) characterized by normal oestrous cycles, inseminated in 3 or more cycles without conception and without clinical sign of disease or abnormality of the reproductive tract, the embryonic loss is elevated as compared with virgin heifers (VH) (*Linares* 1982). The ratio of normal vs. abnormal embryos, collected 7 days after insemination, was 1:3 in the RBH group while it was 3:1 in the VH group. The abnormal embryos will most likely undergo early embryonic death.

Reciprocal embryo transfer between RBH and apparently normal VH might be a useful experimental model to evaluate mother-

conceptus relationships. This assumption is based on the following statements:

- Repeated non-surgical collections of embryos can be done successfully in single-ovulating heifers without disturbing their reproductive functions (*Linares et al.* 1980);
- Satisfactory pregnancy rates are obtained after embryo transfer (*Rowe et al.* 1980, *Greve* 1981);
- Satisfactory survival rates are obtained after transfer of embryos to the uterine horn contralateral to the corpus luteum (*Tervit et al.* 1977, *Christie et al.* 1979);
- Contralateral transfer procedures do not affect the ability of the animal's own embryo to survive (*Sreenan & Mc Donagh* 1979).

The aim of this paper is to describe the reciprocal transfer model and to give some preliminary data concerning its efficiency.

MATERIAL AND METHODS

A total of 10 RBH and 10 VH were used in the experiment. The heifers were of the Swedish Red and White breed, Swedish Holstein and crosses between these 2 breeds. The RBH were investigated concerning chromosome aberrations and endometrial biopsies were taken for histological examination. Before being recipients for transfer all animals were inseminated at 2 spontaneous heats and non-surgical embryo collections were attempted 7 days after insemination. The insemination procedures and the collection technique have been described in detail by *Linares et al.* (1980). The embryos were classified as being normal (N), morphologically deviating (MD) or degenerated (D) (*Linares & King* 1980, *Linares & Plöen* 1981).

The oestrous cycles of the donor and the recipient were synchronized prior to transfer by injection of 500 µg of cloprostenol (Estrumate, ICI). Initially only one pair of animals (RBH + VH) was synchronized but during the course of the experiments it was found preferable to synchronize a larger group of animals. The donor and the recipient were inseminated 72 and 96 h after the injection. Only animals whose cycles were synchronous (0 ± 1 d) were used. Non-surgical collection of the embryo from the donor was done 7 days after the last insemination. The embryo was classified as previously described, photographed, transferred to clean medium and incubated at 35–37°C until transfer.

A total of 27 transfers were attempted and 15 reciprocal transfers were accomplished using a surgical method in 8 cases and a non-surgical in 7 cases. The surgical transfers were carried out on a standing animal in the flank on the opposite side to the C.I.-bearing ovary. Following epidural anaesthesia, paravertebral block and line infiltration with 2 % lidocain (Xylocain, ASTRA) a 20 cm incision was made and the horn of the uterus was pulled out into the incision by gentle traction. The embryo was transferred by means of a Pasteur pipette or a silicone tube with a 1 ml syringe attached to the lumen at the tip of the horn contralateral to the C.I. The non-surgical transfers were carried out using a Cassou artificial insemination gun (0.25 ml straw). The embryo and a small volume of medium were drawn into the straw and the straw was fitted in the a.i.-gun. The recipient received epidural analgesia and the instrument was guided through the cervical canal and led into the uterine horn contralateral to the ovary containing the C.I. The embryo was expelled 2—5 cm in front of the intercornual ligament. Recipients were observed for returns to oestrus and were slaughtered 4 weeks after transfer. The genital tract was removed as soon as possible after stunning and bleeding. The horns were carefully dissected to determine position and size of the embryo(s) and the appearance of foetal membranes. Data were statistically evaluated by Fischer's test.

RESULTS AND DISCUSSION

A total of 15 transfers were performed out of 27 attempted transfers. On 2 occasions transfer was omitted due to failing oestrus synchronization between donor and recipient. In 1 case the collector could not possibly be introduced through the donor's cervical canal. The major cause of transfer failure was, however, unsuccessful embryo collection. This problem was met by synchronizing 2 potential donor heifers with 1 recipient. In 2 cases unfertilized ova were recovered and also the problem of unwanted embryonic morphology could be partly overcome by utilizing more than 1 potential donor. The total recovery rate of 71 % is in agreement with results previously reported (*Linares 1980*).

During the course of the study the transfers technique was changed from surgical to non-surgical. At the beginning of the experiments data from the literature indicated better results with surgical than with non-surgical transfer. Simultaneously with

the experiment, improved results with non-surgical transfer were reported (Rowe *et al.* 1980, Greve 1981). In a pilot study (Gustafsson & Larsson 1982) acceptable results were obtained and it was decided to use the non-surgical transfer technique also in this experiment.

The embryonic survival following insemination and contralateral transfer, respectively, is shown in Table 1. In 5 cases morphologically N embryos were transferred from VH to RBH

Table 1. Embryonic survival in relation to category of heifer as recipient.

| Recipient | Embryonic survival 4 weeks after transfer | | |
|-----------|---|-----------------|-------|
| | Own embryo | Received embryo | Total |
| VH | 5/7 | 1/7 | 6/14 |
| RBH | 4/8 | 3/8 | 7/16 |
| Total | 9/15 | 4/15 | |

VH: virgin heifers.

RBH: repeat breeder heifers.

and in 3 cases from RBH to VH. There was no difference in embryonic survival between the heifer categories following transfer or insemination; however, there was a numerically but insignificantly lower survival rate following contralateral transfer than following insemination in the total material. The survival rate of the recipient's own embryo seems not to be affected by the contralateral transfer procedure.

The survival of transferred embryos in relation to embryonic morphology is shown in Table 2. Four out of 9 embryos with a morphology classified as N survived, but no embryo classified as

Table 2. Survival of transferred embryos in relation to morphology.

| Morphology of transferred embryo | Findings 4 weeks after transfer | | |
|----------------------------------|---------------------------------|--------------------------|------------|
| | Normal foetus | Signs of embryonic death | Empty horn |
| N | 4/9 | 3/9 | 2/9 |
| MD | 0/5 | 2/5 | 3/5 |
| D | 0/1 | 1/1 | 0/1 |

N: normal.

MD: morphologically deviating

D: degenerated.

MD or D. It is well known that the pregnancy rate is very much influenced by the quality of the transferred embryo (Shea 1981). The classification under the microscope is, however, a subjective procedure and there are different criteria for the evaluation of embryos. In this investigation there seems to be good agreement between the morphological evaluation before transfer and the embryonic survival rate.

In 7 animals degenerated foetal membranes were found in the non-pregnant horn, indicating a certain degree of development after transfer followed by embryonic death. Six of these had a normal embryo of their own, while in 1 RBH the transferred foetus was normal. "Signs of death" requires the presence of a viable foetus in the contralateral horn to prevent the membranes from being expelled or resorbed during the following oestrus. Thus in the animals that had a normal oestrus between transfer and slaughter the degree of embryonic development could not be evaluated.

In conclusion, our preliminary data show that the transfer model is useful provided excess animals are available. The results from this study indicate embryonic morphology rather than category of donor or recipient as influencing the embryonic survival rate.

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SAMMANFATTNING

Korsvis överföring av embryon mellan symtomlösa omlöparkvigor och förut oinseminerade kvigor — en försöksmodell.

Syftet med försöksmodellen var att studera livmodermiljöns och det befruktade äggets betydelse för uppkomsten av den förhöjda frekvensen tidig fosterdöd hos symtomlösa omlöparkvigor.

Embryon uppsamlades med oblodig teknik från givardjur 7 dagar efter insemination. Embryona klassificerades såsom tillhörande någon av grupperna normala, morfologiskt avvikande eller degenererade embryon. Embryona överfördes kirurgiskt (8 fall) eller icke-kirurgiskt (7 fall) till synkroniserade mottagardjur som seminerats samtidigt med givardjuret. I 7 fall överfördes embryon från symtomlösa omlöpare (RBH) till förut oinseminerade kvigor (VH) och i 8 fall från VH till RBH. Embryot överfördes till mottagerdjurets livmoderhorn kontralateralt till gula kroppen. Vid slakt av mottagerdjuren 4 veckor efter transfer påvisades ingen signifikant skillnad i det överförda eller det egna (efter insemination) embryots överlevnad i relation till djurkategori. Endast embryon som klassificerats som normala överlevde efter transfer. I några fall påvisades degenererade fosterhinnor i det icke dräktiga hornet. Resultatet tyder på att för embryots överlevnad tycks embryots morfologi ha större betydelse än vilken djurkategori som varit givare eller mottagare.

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Reprints may be requested from: H. Gustafsson, the Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden.