Laparoscopic embryo transfer in rabbits

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A technique for endoscopic transfer of one-cell, one–two-cell, two-cell or morula stage embryos to the Fallopian tubes of rabbits was developed in two phases. Phase I of the experiments involved the transfer of 30–65 embryos to each of nine recipients and resulted in pregnancies in all animals. After 12 days six rabbits were killed and they showed an implantation rate of 27%. The remaining three rabbits continued pregnancy until birth. The embryo survival rate was 26%. In a second phase, 10–20 embryos were transferred to each of the 22 recipient animals, 19 of which went on to give birth (embryo survival rate in the animals that became pregnant was 47%).

Introduction

The rabbit has been an important animal in the study of reproductive biology since Walter Heape performed the first successful embryo transfer experiment in rabbits about 100 years ago (cited by Biggers, 1991). Numerous techniques have been developed involving surgical access into the ovaries, Fallopian tubes, uterus and vagina from the flanks or linea alba (Chang, 1950; Hafez, 1962; Adams, 1962). Such surgical techniques for gaining entry into the abdominal cavity for both observation of genital organs and embryo transfer is, however, stressful for the recipient animal. In addition, manipulation of the reproductive organs can lead to bleeding or lesion formation which reduces the efficiency of embryo transfer. Fujimoto et al. (1974) used the technique of endoscopy (Russel, 1988) to observe follicle maturation and ovulation in rabbits. This method was also used to measure the accuracy of the ovulation rate (Thea-Clement and Bolet, 1987; Santacreu et al., 1990) and to estimate the implantation rate on day 12 of gestation (Santacreu et al., 1990). Thea-Clement and Bolet (1987) and Santacreu et al. (1990) also noted that the use of laparoscopy does not have a negative effect on fetal survival. Viudes de Castro et al. (1991) used investigative surgery to examine the effect of varying the calorific content of food upon the rates of ovulation, implantation and the loss of fetuses and concluded that laparoscopy is invaluable in determining the effects of diets on oestrus and birth rate.

The aim of this study was to develop a new, simplified approach for the transfer of embryos to the Fallopian tubes of the rabbit. Here we report efficient pregnancy and implantation rates as a result of a reduction in handling and stress for the rabbit coupled with significantly less manipulation of the reproductive organs.

Materials and Methods

Recipients

Thirty-one female rabbits were used; they were caged individually and kept under conditions of 14 h light:10 h dark for 3 weeks before the beginning of the experiment. The rabbits were given pelleted rabbit feed (17.5% protein, 2.0% fat, 14% fibre, 8.0% ash, 20 000 iu vitamin A, 800 iu vitamin D3, 40 mg vitamin E and 25 mg copper kg−1) and water ad libitum. The trials were started in August 1991 and ended in April 1992.

Donors and embryos

Seventy-two hours before the induction of ovulation the donor animals received 20 iu pregnant mares’ serum gonadotrophin (PMSG; Intergonon: Vemie, Kempen) kg−1 body weight i.m. Ovulation was induced by i.v. administration of 180 iu hCG (Ekluton) per animal. The one–two-cell embryos were isolated from killed medium-sized cross-bred animals 19–21 h, and the morulae 60 h after induction of ovulation. Unfertilized ova were discarded. Embryo transfer was performed 22–24 h (one- or two-cell stage) or 48 h (morulae) after synchronization of recipient animals. Dulbecco’s phosphate-buffered saline containing 30 mg penicillin l−1, 100 mg streptomycin l−1 and 20% fetal calf serum was used for transfer medium and for washing purposes.

Phase 1

For this phase, nine medium-sized cross-bred rabbits (9–12 months old, 4-5 kg body weight) were used for developing the method for endoscopic transfer. Each recipient animal received 30–54 embryos (on average 45 embryos) at the one–two-cell, two-cell and morula stages. After 12 days, six of the animals were killed and the rate of successful implantation was determined. The remaining three animals continued through pregnancy to give birth.

Phase 2

Endoscopic transfer was used and 10–20 (average 12) embryos were implanted into each of 22 Zlka-hybrid rabbits...
Instruments

The following equipment (Storz) was used for endoscopy-mediated embryo transfer: small cold light fountain 150 watt, fibre optic light cable, inflation bulb, trocar with automatic valve and pyramidal tip, diameter 4.5 mm, Hopkins wide angle forward-oblique telescope 30°, diameter 4 mm.

Embryo transfer

The recipient rabbits were anaesthetized by i.v. injection of xylazine–ketamine (1.8 mg xylazine, 2%, kg⁻¹ body weight Rompun: Bayer, Leverkusen and 15 mg ketamine hydrochloride 10%, kg⁻¹ body weight WDT, Garbsen). The navel region of the rabbit was shaved, and the rabbit was then held on a movable operating table in a vertical position (head down). After making a small incision (<1 cm), the endoscope trocar was introduced through the abdominal wall. After removal of the trocar, the abdomen was inflated with air (by the help of an inflation bulb). The endoscope was then inserted (see Fig. 1) and the number of visible corpora lutea counted and the status and positioning of the infundibulum and ampulla were determined without any manipulation. An entry point for the insertion of a transfer capillary was then made at a site 2–3 cm from the infundibulum using a vene catheter (Abbocath-T: Sligo, Ireland, 51 × 2.5/2.9 mm). The vertical positioning of the animal ensures that the stomach and intestines are cranially located so that the Fallopian tubes form a downwardly pointing loop between the ovaries and uterus. The proximal end of the ampulla runs in a straight line (2–3 cm) over the infundibulum. The medium containing the embryos can be separated by air bubbles and transferred, using a glass capillary (5 µl Caps: Brand, Wertheim), into the ampulla via the infundibulum (Fig. 2). Afterwards, the air is removed from the abdominal cavity and the incision is closed using a Michel clamp. The recipient animal was then examined by palpation on day 12 after ovulation to determine whether it was pregnant.

The implantation rate was defined as the percentage of embryos that were implanted and the embryo survival rate as
the percentage of transferred embryos into pregnant recipients that resulted in live offspring.

**Results**

In the first phase experiment (Table 1), 403 embryos at the one-two-cell, two-cell and morula stages were transferred into nine animals. All nine recipient animals became pregnant. An implantation rate of 27% (74 implantations) was observed for the six animals that were killed and examined. The remaining three animals that came to term showed an embryo survival rate of 26% (33 offspring). On average each recipient animal showed ten corpora lutea. In the second phase (Table 2) 260 one-cell, one-two-cell or two-cell embryos were transferred into 22 recipient animals and 19 (86%) of these rabbits became pregnant. These animals gave birth to 107 offspring, which represents an embryo survival rate of 47%. On average, six corpora lutea were counted from each animal.

No pregnancy resulted after the transfer of one-cell embryos in two recipients and for two-cell embryos in one recipient. Nevertheless, the embryo survival rate for the transfer of one-cell and two-cell embryos in the second phase was 47 and 65%, respectively, compared with the embryo survival rate of 26% in phase 1 when more embryos were transferred. Taking the results of both phases of the experiment together, the 31 recipient animals received on average 21 embryos. Of these 31 animals, 28 became pregnant (90%).

**Discussion**

The aim of the work reported here was to develop a new procedure for the transfer of embryos into the Fallopian tubes of recipient rabbits using endoscopy. In the first phase of this study, a large number of embryos were transferred to each animal to increase the chance of pregnancy and the absolute number of implanted embryos (Adams, 1962), thereby facilitating the development and the establishment of this embryo transfer procedure. As all of nine recipient animals from the first phase of the experiment became pregnant and had up to 26 implantations, the number of embryos transferred in the second phase was reduced to 10–20 per recipient, to avoid overcrowding effects (Hafez, 1964). This resulted in an improved rate of survival from 26 to 47%.

Garcia-Ximenez *et al.* (1991) described a method for transferring embryos into the uterus by laparoscopy. They transferred on average 10.8 embryos to twelve lactating recipients. All

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<th>Table 1. Trial 1: transfer of rabbit embryos of different stages</th>
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Total: 9 recipients; 403 embryos transferred; 74 implantations; 33 pups.

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<th>Table 2. Trial 2: transfer of rabbit embryos of different stages</th>
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Values shown are means ± SD.
Total: 32 recipients; 260 transferred embryos; 19 recipients pregnant; 228 transferred embryos; 107 pups.
et lutea only transferred possibility navel each after endoscopy-mediated of and procedure rabbits could therefore be reused for multiple, endoscopy-mediated embryo transfer during or immediately after lactation. Another advantage of this technique is the considerable reduction in the time required for transfer, each procedure taking 5 min instead of the usual 15 min for laparotomy-mediated transfer (Besenfelder, 1990). Furthermore, this procedure necessitates that only the area around the navel needs to be shaved, thus saving time and reducing the possibility of injury to the skin and nipples.

The advantage of the first phase of this experiment was that it did not depend on planning a programme and thus permitted the transfer of embryos that were at the one–two-cell, two-cell and morula stages. This phase demonstrated that embryos from all of these stages could be successfully transferred into the Fallopian tube using this procedure. In the second phase, embryos could be used that were isolated at the pronuclear stage and, after a short period in culture, could be transferred at the one-, one–two- and two-cell stages of development. Two of the three recipients that did not become pregnant received one-cell embryos. It should be noted, however, that owing to the developmental stage of the embryos, it would appear that one-cell embryos give less guarantee for further development than did embryos that already showed division.

The visual control of the surface of the ovaries was permitted only to determine the number and status of corpora lutea that represented a successful synchronization of the recipients. The ovary was not manipulated during the counting of the corpora lutea and thus it is likely that more corpora lutea were present than were estimated.

The method described in this paper for the transfer of rabbit embryos into the Fallopian tubes of recipient animals is less time consuming and easier to perform than existing techniques. The procedure requires minimal operative procedure as well as manipulation of the reproductive organs. This procedure represents the method of choice for efficient routine embryo transfer.

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Chang MC (1950) Development and fate of transferred rabbit ova or blastocysts in relation to the ovulation time of recipients Journal of Experimental Zoology 114 351–381