How to Collect and Transfer Oocytes  (21-Nov-2003)

E. M. Carnevale, M. A. Coutinho da Silva and E. L. Squires

Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO, USA.

Abstract
Oocyte transfer provides a method to obtain pregnancies from many mares that are unsuccessful embryo donors. The procedure requires more expertise than embryo transfer. However, oocyte transfer provides mare owners with a viable method to obtain pregnancies from mares that would otherwise be considered infertile.

1. Introduction
For some valuable mares, standard breeding methods or embryo transfer fail to produce offsprings. Failure to collect embryos from a mare can be associated with reproductive pathologies that affect fertilization or early embryo development. These pathologies include ovulation failure, oviductal blockages, uterine infections, and cervical tears or scarring. For these mares, oocyte transfer provides a potential method to obtain additional offspring. During oocyte transfer, the oocyte is collected from a donor's preovulatory follicle and transferred into the oviduct of an inseminated recipient. Therefore, fertilization, embryo development, and fetal development occur within the healthy reproductive tract of the recipient. The only requirement of the donor is to produce a preovulatory follicle with a viable oocyte.

2. Materials and Methods
Procedures for oocyte transfer involve: 1) collection of a preovulatory oocyte between 0 and 16 h before the anticipated time of ovulation; 2) selection and insemination of a recipient; and 3) transfer of the oocyte.

In the donor, the reproductive tract is scanned daily during estrus to determine the proper timing of administration of human chorionic gonadotropin (hCG, 1,500 - 2500 IU, IV) or a GnRH analog (2.1 mg implant of deslorelin acetate). The compounds are administered when a combination of the following criteria are observed: 1) follicle >35 mm in diameter; 2) relaxed uterine and cervical tone; 3) uterine edema indicative of estrus; and 4) estrous behavior. Follicular maturation and ovulation is anticipated to occur approximately 36 h after hCG and 40 h after deslorelin.

The oocyte is usually collected from the follicle between 0 and 16 h before ovulation. Attempting to collect oocytes from follicles that have not begun the maturation process is usually unsuccessful because the cumulus oocyte complex is tightly adhered to the follicular wall. Two primary methods of oocyte collections have been used. Oocytes can be collected by using a needle placed through a trocar in the flank area. This requires transrectal manipulations of the ovary, so the ovary is firmly positioned against the abdominal wall. A trocar is placed through the abdominal wall, and a needle is inserted through the trocar and into the follicular lumen. In our laboratory, most oocytes are collected by using transvaginal, ultrasound-guided follicular aspirations. This procedure requires an ultrasound probe that is housed in a plastic casing with a needle guide. Prior to aspirations, the donor mare is given mild sedation and a compound is administered to reduce rectal contractions (propantheline bromide, 0.04 mg/kg, IV). The vulvar area is thoroughly washed, and the ultrasound transducer, encased in a plastic housing, is inserted to the anterior vagina. A double-lumen, 12-gauge needle is inserted through the needle guide. The ovary containing the preovulatory follicle is positioned by transrectal manipulations so the antrum of the follicle is adjacent to the needle port. The needle is then advanced through the vaginal and follicular walls and into the follicular antrum. As the follicular fluid is gently suctioned from the follicle by using a pump set at 150 mmHg, the antrum is lavaged with 50 - 100 ml of a flush medium (bases of phosphate-buffered saline or synthetic oviduct fluid with additions of a fetal calf serum or bovine serum albumin, antibiotic, and heparin at 10 U/ml). Upon recovery, fluid is immediately searched to determine if the oocyte is present. Because oocytes are sensitive to temperature fluctuations, media and plastic ware are warmed to approximately body
temperature (38 - 38.5°C). Upon identification, the oocyte is either immediately transferred into the oviduct of a recipient or is cultured for the completion of maturation. Success of oocyte transfer has been similar when oocytes were cultured or collected just before ovulation and immediately transferred. In our laboratory, oocytes are cultured in Tissue Culture Medium 199, with additions of 10% fetal calf serum, 0.2 mM pyruvate, and 25 mg/ml gentamicin. Oocytes are cultured at 38.5°C in an atmosphere of 6% CO2 and air. If oocytes are collected 24 h after administration of hCG, then they are cultured for an additional 12 - 16 h before transfer. If oocytes are collected ≥ 30 h after hCG, then they are immediately transferred into the oviduct.

Before transfer, a suitable recipient must be obtained. Young mares, between 3 and 10 years old, are preferred. Because the ovary must be exposed for transfer into the oviduct, mares with long, broad ligaments and thin flanks are preferred. Cyclic recipients are synchronized with donors, with hCG administered to donors and recipients at the same time. If an oocyte is collected from the donor, then the recipient's oocyte is removed by follicular aspiration. If an oocyte is not collected from the recipient or if a secondary follicle (e.g., ≥ 27 mm) is present, then the recipient is determined to be unsuitable. This is based on the potential for the recipient's own oocyte to be fertilized from the aspirated follicle or from a second ovulation. We also use noncyclic mares as oocyte recipients. These include mares in anestrus or in transition, or mares that have been given large doses of deslorelin acetate (> 4.2 mg) to induce a period of follicular suppression. Prostaglandin should be administered to mares with a functional corpus luteum before treatment with estradiol. Estradiol (3 mg daily, IM) is administered to noncyclic recipients for 3 - 6 d before transfer. To be a suitable recipient, the cervix should be open and uterine edema should be present. The recipient can be inseminated before, after, or before and after transfer. In our experience, if high-quality semen is available, then recipients can be inseminated approximately 12 h before transfer with good success. Sufficient numbers of high-quality sperm are necessary to maximize the success of oocyte transfer.

Transfer of the oocyte is performed through a standing flank laparotomy. Before surgery, the flank area is shaved and scrubbed for surgery. Recipients are tranquilized with xylazine HCl and butorphanol tartrate (0.3 mg/kg and 0.01 mg/kg, respectively, IV) before a line block at the incision site. Before the start of surgery, the recipient will receive injections of detomidine HCl (9 µg/kg, IV) and butorphanol tartrate (0.01 mg/kg, IV). A skin incision, approximately 10 cm in length, is made approximately midway between the last rib and tuber coxae. The muscle layers are bluntly dissected, and the peritoneum is punctured. The ovary is manually located and gently moved to the exterior of the incision. The oviduct is easily visible along the surface of the ovary. To transfer the oocyte, the oocyte is pulled into a fire-polished glass pipette with a minimal volume of media (< 0.05 ml). The external os of the oviduct is located within the infundibulum, and the glass pipette is gently advanced between 2 and 3 cm into the oviduct. The oocyte can then be released into the oviduct. Antibiotics (penicillin G procaine, 23,000 IU/kg, IM, daily) and phenylbutazone (2 g) are administered for 6 and 3 d, respectively, after surgery. After transfers, non-cyclic recipients receive progesterone (150 - 200 mg in oil, daily, IM) or altrenogest (0.44 mg/kg, PO) for the maintenance of pregnancy. Pregnancy exams are performed at 12, 14, and 16 d after transfer.

3. Results
In previous experiments, oocyte transfers have resulted in high pregnancy rates (50 - 90%) when oocytes were collected from young recipients and when semen was of good quality. However, commercial oocytes are typically transferred from older, subfertile mares, and semen is obtained from different stallions with varying fertility. During the 2000 - 2002 breeding seasons, we collected oocytes during 339 cycles and from 434 follicles, with collection rates of 98% (331/339) per cycle and 76% (331/434) per follicle. The pregnancy rate per transfer at 16 d was 40% (111/281).

4. Conclusions
Oocyte transfer involves the transfer of a donor's oocyte into an inseminated recipient's oviduct. By using this procedure, the process of ovulation and the tubular genitalia of the donor are avoided. Therefore, many valuable broodmares that are infertile with standard breeding techniques or embryo transfer can be successful donors for oocyte transfer.

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