Pregnancies attained after collection and transfer of oocytes from ovaries of five euthanized mares

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- Oocyte transfer involves the collection of an oocyte from a donor mare and transfer of the oocyte into the oviduct of an inseminated recipient mare.
- Upon death of a valuable mare, oocytes can be collected from the excised ovaries for transfer into recipients.

Five mares of light-horse, performance breeds were euthanatized. Horses (referred to as donors) 1 through 5 were 23, 14, 9, 10, and 16 years of age, respectively. Reasons for euthanasia included neoplastic (donor 1), musculoskeletal (donors 2, 4, and 5), and gastrointestinal (donor 3) conditions with poor prognoses. Available histories included medical treatments and surgical procedures (donors 3 and 5) before euthanasia. Two donors (1 and 5) were euthanatized with an overdose of barbiturates; methods of euthanasia for the other donors were not known.

After euthanasia, ovaries were recovered by attending veterinarians. Intervals between euthanasia and ovary collection ranged from 30 minutes to 5 hours. Intervals between ovary collection and delivery to the Equine Reproduction Laboratory at Colorado State University ranged from 30 minutes to 8 to 10 hours (**Table 1**). Ovaries from the 5 donors were maintained at various temperatures during transportation. Ovaries from donors 4 and 5 were collected at different locations within the country and transported to Colorado State University by airplane; both sets of ovaries were transported within shipping containers designed for the transportation of equine semen.[a]

Upon arrival at Colorado State University, ovaries were rinsed with saline (0.9% NaCl) solution or lactated Ringer's solution. Forty-six oocytes were collected via aspiration of follicles, scraping the follicular wall,[1] or slicing the ovary.[2] The collected cells were placed in medium[b] that was immediately searched for oocytes. Recovered oocytes were placed in **synthetic oviduct fluid (SOF)** or tissue culture medium-199 at 38.5°C (101.3°F) in 6% CO_2 and air. Most oocytes were cultured between 24 and 30 hours, as previously described.[3,c] Donors 1 and 2 had large follicles and were in estrus; these donors received human chorionic gonadotropin (2,500 U, IV) the day before euthanasia. For donor 1, an oocyte collected from a preovulatory follicle and 1 additional oocyte were transferred 8 hours after collection; oocytes collected from small follicles were cultured for 24 to 30 hours. For donor 2, oocyte collections were only attempted from 2 preovulatory follicles. One oocyte was collected and transferred 6.5 hours after collection. Oocytes collected from the preovulatory follicles should have started the maturation process and therefore required shorter culture intervals than oocytes collected from small follicles.

Thirty-six oocytes were transferred to 10 recipient mares via flank laparotomies performed with the horses standing. Recipients were noncyclic mares (for donors 1, 2, 4, and 5) that had been treated with estradiol[d] in cottonseed oil (0.007 mg/kg [0.003 mg/lb], IM, q 24 h) for at least 3 days before transfer and mares in early to mid-estrus (for donor 3) in which the dominant follicle was aspirated during surgery. For transfers, recipients were restrained in stocks. Mares were sedated with xylaxine HCl (0.33 mg/kg [0.15 mg/lb], IV) and butorphanol tartrate (0.01 mg/kg [0.005 mg/lb], IV), and a surgical area was clipped and scrubbed. A line block (100 mL of 2% lidocaine) was used to anesthetize the incision site. Immediately before surgery, butorphanol (0.01 mg/kg [0.005 mg/lb]) and detomidine hydrochloride[e] (0.0088 mg/kg [0.004 mg/lb], IV) were administered. The dermis was incised, and muscle layers were opened bluntly to allow exposure of the ovary and oviduct through the incision. Multiple oocytes (2 to 6) from the culture medium were aspirated into a fire-polished glass pipette with < 0.1 mL of a transfer medium (buffered SOF). Oocytes were deposited into the oviduct after threading the pipette through the infundibular os and approximately 3 cm into the oviduct.

Recipients were inseminated within the uterus. Semen for most transfers was cooled, transported semen from various stallions, and recipients were inseminated before or after transfer, or both. Frozen semen was used to inseminate 1 of the recipients 3 hours after the transfer of oocytes (recipient 3-3 in Table 1).

Recipients were treated with procaine penicillin G (23,000 U/kg [10,500 U/lb], IM, q 24 h) and phenylbutazone (4.4 mg/kg [2 mg/lb], IV or PO, q 24 h) for 6

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Table 1—Transport conditions, oocyte recovery, and pregnancy outcome after oocyte transfer from 5 euthanatized horses into 10 recipient horses

Horse (donor) No.	Transport interval	Temperature during shipment	No. of oocytes recovered	Recipient No.	No. of oocytes transferred	Pregnancy outcome
1	< 30 min	30–38°C	13	1-1	2*	NP
				1-2	5	NP
				1-3	5	EED
2	< 30 min	30–38°C	1	2-1	1*	NP
3	< 30 min	Ice for short duration	7	3-1	2	NP
				3-2	2	NP
				3-3	2	EED
4	8–10 h	5°C	15	4-1	6	EED
				4-2	6	NP
5	8–10 h	Ambient	10	5-1	5	Foal

*One oocyte for each transfer was recovered from a preovulatory follicle.
NP = No pregnancy. EED = Early embryonic death. To convert °C to °F, multiply by 9/5 and add 32.

and 3 days, respectively. Beginning on the day of surgery or the day after surgery, recipients received daily injections of progesterone[f] in cottonseed oil (0.3 mg/kg [0.14 mg/lb], IM, q 24 h). After a diagnosis of pregnancy, progesterone administration was stopped, and recipients were given a synthetic progestin[g] (altrenogest, 0.05 mg/kg [0.02 mg/lb], PO, q 12 h) for maintenance of pregnancy.[4]

From the 36 transferred oocytes, 6 (17%) embryonic vesicles were detected via ultrasound (Table 1). Sixteen days after transfer, 4 of the 10 recipients were pregnant, with at least 1 embryonic vesicle each. One recipient per donor was pregnant for 4 of the 5 donors. The recipient for donor 5 received 5 oocytes, and 3 embryonic vesicles were detected on day 13 after transfer. The 2 smallest embryonic vesicles were manually reduced on days 13 and 14 after transfer. Pregnancy development in the recipient appeared normal, and a healthy foal was born in 2002. Embryonic vesicles from donors 1 and 4 appeared normal when viewed on days 27 and 29, respectively; however, on days 45 and 38, respectively, early embryonic death was diagnosed because of loss of the embryonic vesicle (donor 1) or loss of the embryonic heartbeat (donor 4). Donor 3 provided 2 oocytes for recipient 3-3, and pregnancy resulted from the use of frozen semen. The conceptus appeared viable at 50 days of gestation; however, on day 62, the fetal heartbeat was not detected, and the vesicle was subsequently lost.

Euthanasia or death of valuable mares results in loss of genetic potential and can be devastating to the client. However, development of new reproductive techniques allows clinicians to offer clients a method to potentially obtain offspring after a mare's death.

Oocyte transfer in mares was first reported in 1988.[5] With recent advances in procedures for oocyte transfers, pregnancies are consistently being obtained in valuable mares with reproductive abnormalities that cause infertility that is unresponsive to other techniques, including embryo transfer.[4] The success of oocyte transfer varies with the quality of oocytes and semen used in the procedure.[6] Typically, older mares are entered into commercial oocyte transfer programs, and semen is obtained from different stallions with variable fertility. Pregnancy rates were 23,[4] 37,[4] and 40%[6] per transfer for 3 consecutive years in a commercial program. However, when oocytes from younger mares and fertile stallions were used in experiments, pregnancy rates of 54 to 83% per transfer were obtained.[6]

Oocytes collected for commercial transfers usually are obtained from preovulatory follicles within 16 hours of expected ovulation. In the mares reported here, 2 oocytes were collected from preovulatory follicles of euthanatized mares; however, no pregnancies resulted. Most oocytes collected from the ovaries probably had not started the maturation process and were immature or in various stages of atresia; therefore, their developmental potential varied. In a previous study,[3] oocytes were collected at euthanasia from ovaries and matured in vitro; the embryo development rate after transfer of the oocytes into recipients was 10% (4/40). After in vitro maturation and transfer of oocytes in the horses reported here, 6 of 34 (18%) oocytes developed into embryonic vesicles. Factors such as illness, medical treatments, and method of euthanasia could have affected oocyte viability. However, the embryo development rate indicated that some oocytes remained viable despite illness of donor horses and prolonged transport of the ovaries.

Pregnancies were obtained with oocytes from 4 of the 5 donors. Developing embryos and embryonic heartbeats were detected in all pregnancies. Embryo losses did not appear to be caused by problems with recipient mares, and all recipients were administered a progesterone replacement. A similar regimen was successfully used to maintain pregnancies in a commercial program for oocyte transfer.[4] Recipients had good uterine and cervical tones when embryo losses were detected. Potentially, embryo losses were caused by abnormal embryo development. One of the 4 recipients carried the pregnancy to term, resulting in birth of a healthy foal.

Optimal conditions for the shipment of equine ovaries have not been determined, although some research has been conducted with bovine ovaries. When bovine ovaries were stored at temperatures from 15 to 25°C (59 to 77°F) for ≥ 11 hours, blastocyst formation after in vitro fertilization was not reduced, compared with controls.[h,i] However, storage of ovaries at 4 versus 25°C resulted in substantially reduced cleavage rates and blastocyst formation.[i] In another experiment,[7] bovine oocytes stored at temperatures ≥

24°C (75°F) had high cleavage rates; however, exposure of oocytes to ≤ 4°C (39°F), even for a short period, resulted in approximately 50% reduction in cleavage. Cooling to low temperatures appears to mainly affect the ability of oocytes to form a meiotic spindle.[7] In the horses reported here, 2 pregnancies were obtained with oocytes from ovaries that had been exposed to low temperatures (5°C [41°F] and ice). However, both pregnancies ended with embryonic death. The effect that exposure to low temperatures could have had on embryo viability is not known. In our laboratory, we recommend that ovaries be removed from mares as soon as possible after death. The ovaries should be kept clean and rinsed with warm saline solution to remove excess blood or debris. Ovaries should be packaged in a clean plastic bag that contains enough saline solution to keep the tissue moist and placed in an insulated shipping container at ambient temperature. Fluctuations in temperature should be avoided. The interval between ovary and oocyte collections should be minimized. A sample of DNA, usually mane hair including roots, should be collected from the donor as required by the respective breed association for parentage identification. Semen must be obtained from the desired stallion for insemination of the recipient.

A single foal resulted from the oocyte transfers reported here. Oocytes for the successful transfer were collected from donor 5, although the interval from death to ovarian removal was longer for that mare (by approx 5 hours) than for other donors, and the ovaries were transported across the country at ambient temperature. For that mare, 3 embryonic vesicles were detected after the transfer of 5 oocytes, and 2 vesicles were manually crushed. The reasons why oocytes from donor 5 appeared to be more viable than those from other donors were unknown. Potentially, more oocytes from this mare's ovaries were in a viable stage of development. In addition, oocytes transferred from donor 5 were more highly selected; only 50% of oocytes collected from donor 5 were transferred versus approximately 90% of oocytes collected from the other donors.

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[a]Equitainer, Hamilton Thorn, South Hamilton, Mass.
[b]EmCare embryo flush solution, ICP, Auckland, New Zealand.
[c]Maclellan LJ, Lane M, Sims MM, et al. Effect of sucrose or trehalose on vitrification of equine oocytes (abstr). *Theriogenology* 2001;55:310.
[d]Sigma Chemical Co, St Louis, Mo.
[e]Dormosedan, Pfizer Animal Health, Exton, Pa.
[f]Sigma Chemical Co, St Louis, Mo.
[g]Regumate, Intervet, Millsboro, Del.
[h]Schernthaner W, Schmoll F, Brem G, et al. Storing bovine ovaries for 24 hours between 5 and 21°C does not influence in vitro production of blastocysts (abstr). *Theriogenology* 1997;47:297.
[i]Yang NS, Lu KH, Gordon I. In vitro fertilization (IVF) and culture (IVC) of bovine oocytes from stored ovaries (abstr). *Theriogenology* 1990;33:352.

References

1. Hinrichs K, DiGiorgio LM. Embryonic development after intrafollicular transfer of horse oocytes. *J Reprod Fertil Suppl* 1991;44:369–374.

2. Choi YH, Hochi S, Braun J, et al. In vitro maturation of equine oocytes collected by aspiration and additional slicing of ovaries. *Theriogenology* 2000;54:981–987.

3. Scott TJ, Carnevale EM, Maclellan LJ, et al. Embryo development rates after transfer of oocytes matured in vivo, in vitro, or within oviducts of mares. *Theriogenology* 2001;55;705–715.

4. Carnevale EM, Squires EL, Maclellan LJ, et al. Use of oocyte transfer in a commercial breeding program for mares with reproductive abnormalities. *J Am Vet Med Assoc* 2001;218:87–91.

5. McKinnon AO, Carnevale EM, Squires EL, et al. Heterogenous and xenogenous fertilization of in vivo matured equine oocytes. *J Equine Vet Sci* 1988;8:143–147.

6. Carnevale EM, Maclellan LJ, Coutinho da Silva MA, et al. Equine sperm-oocyte interaction: results after intraoviductal and intrauterine inseminations of recipients for oocyte transfer. *Anim Reprod Sci* 2001;68:505–314.

7. Wu B, Tong J, Leibo SP. Effects of cooling germinal vesicle-stage bovine oocytes on meiotic spindle formation following in vitro maturation. *Mol Reprod Dev* 1999;54:388–395.