Embryo-initiated oviductal transport in mares

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Summary. The hypothesis that equine embryos initiate oviductal transport in mares was tested by placing day 6 uterine embryos in the oviducts of day 2 ($n = 10$) or day 5 ($n = 10$) recipient mares and attempting to collect the embryos from the uterus 48 h later. To determine whether the surgical transfer procedure initiated oviductal transport, medium alone was placed in the oviducts of day 2 ($n = 10$) inseminated mares (sham transfer), and uterine embryo collections were attempted 48 h later.

Embryos were transported through the oviduct of day 2 recipients by day 4 (instead of day 5 to 6) in six of ten mares, which was not significantly less ($P > 0.1$) than in day 5 recipients (9 of 10). Oviductal transport was not primarily initiated by the surgical transfer procedure, since oviductal transport occurred in only one sham transfer.

There was no significant difference ($P > 0.1$) in the diameter of embryos placed in the oviducts of day 2 and day 5 recipient mares (180 ± 13.8 versus 187 ± 11.3 μm , respectively). However, embryos collected from the uterus were significantly smaller ($P < 0.05$) in day 2 than in day 5 recipients (375 ± 85.4 versus 659 ± 43.6 μm , respectively). One uterine embryo had shed its zona pellucida before being placed in, and transported through, the oviduct of the recipient mare.

Keywords: horse; embryo; oviductal transport

Introduction

Most mammalian embryos and unfertilized oocytes enter the uterus at similar times after ovulation (Harper, 1988). However, in hamsters (Ortiz *et al.*, 1986), rats (Villalon *et al.*, 1982) and ferrets (Mead *et al.*, 1988), embryos and unfertilized oocytes enter the uterus at different times after ovulation. In horses (Van Niekerk & Gerneke, 1966; Steffenhagen *et al.*, 1972; Betteridge & Mitchell, 1974; Betteridge & Mitchell, 1975; David, 1975; Onuma & Ohnami, 1975; Flood *et al.*, 1979) and long-tongued bats (Rasweiler, 1979) embryos enter the uterus but unfertilized oocytes are retained in the oviduct. Horse embryos therefore bypass unfertilized oocytes within the oviduct of the mare (Betteridge & Mitchell, 1975; Onuma & Ohnami, 1975). Retained, unfertilized oocytes apparently move distally in the oviducts (Steffenhagen *et al.*, 1972) or occasionally enter the uterus (Wilson *et al.*, 1985) with the passing embryos.

Most mammalian embryos enter the uterus 24–86 h after ovulation at the four- to 16-cell stage of development. Pig embryos enter the uterus at the four-cell stage 24–54 h after ovulation (Pomeroy, 1955; Oxenreider & Day, 1965), cow embryos at the eight- to 16-cell stage 57–86 h after ovulation (Hamilton & Laing, 1946; Crisman *et al.*, 1980), and sheep embryos at the eight-cell stage 60–66 h after ovulation (Holst, 1974). In contrast, horse embryos enter the uterus at the late morula to early blastocyst stage 120–144 h after ovulation (Oguri & Tsutsumi, 1972; Betteridge *et al.*, 1982; Boyle *et al.*, 1989; Hinrichs & Riera, 1990; Freeman *et al.*, 1991). Oviductal transport may occur in mares when embryos have developed to a critical stage when they can initiate

transport. The objective of this experiment was to test the hypothesis that horse embryos initiate oviductal transport in mares.

Materials and Methods

Animals and treatments

Day 6 uterine embryos were placed in the oviductal ampullae of day 2 or day 5 recipient mares (one embryo per recipient mare, $n = 10$ mares). Initiated oviductal transport was estimated by the number of embryos that were recovered from the uterus 48 h after transfer (4 days after ovulation).

The experiment was conducted in July, 1990. Mares aged 3–15 years and weighing 300–500 kg were housed in a dry lot and fed approximately 7 kg of alfalfa hay and 1–2 kg of corn per day, as well as free-choice trace mineral salt mix. Stage of oestrous cycle and ovulation were detected by rectal palpation and transrectal ultrasonography (Ginther, 1986). Mares were estimated to be in oestrus when they had endometrial oedema and an ovarian follicle greater than 30 mm in diameter. Mares in oestrus were examined between 04:00 and 07:00 h daily to detect ovulation. Embryo donor and sham transfer mares in oestrus were artificially inseminated with pooled semen from three stallions every other day until ovulation was detected. The day of ovulation was defined as day 0.

Collection of uterine embryos

Day 6 embryos were collected by transcervical uterine lavage (McKinnon *et al.*, 1988). Embryos were immediately placed into a defined medium consisting of a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's medium (Sigma, St Louis, MO, USA) supplemented with insulin ($5 \mu\text{g ml}^{-1}$), transferrin ($5 \mu\text{g ml}^{-1}$), sodium selenite (5 ng ml^{-1}), penicillin (50 iu ml^{-1}) and streptomycin ($50 \mu\text{g ml}^{-1}$).

Embryo transfer

Mares were premedicated with 0.04–0.08 mg acepromazine kg^{-1} body weight. General anaesthesia was induced by intravenous infusion of 5% guaifenesin with 2 g sodium thiamylal l^{-1} and maintained by inhalation of a halothane–oxygen mixture. The ovary and oviduct ipsilateral to ovulation were exposed through a flank laparotomy. Uterine embryos (one per mare) were surgically placed in the mid-ampullary region of the oviducts of the recipient mares (Peyrot *et al.*, 1987). Medium alone was placed into the oviducts of inseminated sham-transfer mares.

Statistical analysis

The post-transfer proportions of uterine embryo collection were compared using Fisher's Exact Test. The diameters of pre-transfer and post-transfer embryos were compared using Student's *t*-test.

Results

Six of ten uterine embryos placed in the oviducts of day 2 recipient mares were transported to the uterus by day 4. The number of embryos transported through the oviducts of the mares was not significantly less ($P > 0.1$) for day 2 than for day 5 recipient mares (6 of 10 versus 9 of 10, respectively; Table 1). The number of embryos transported through oviducts of mares was significantly greater ($P < 0.05$) for day 2 recipient mares than for sham transfer mares (6 of 10 versus 1 of 10, respectively, Table 1).

There was no significant difference ($P > 0.1$) in the pre-transfer diameter of embryos transferred to the oviducts of day 2 and day 5 recipient mares ($180 \pm 13.8 \mu\text{m}$ versus $187 \pm 11.3 \mu\text{m}$, respectively). The post-transfer diameter was significantly smaller ($P < 0.05$) for embryos collected from the uteri of day 2 than for day 5 recipient mares ($375 \pm 85.4 \mu\text{m}$ versus $659 \pm 43.6 \mu\text{m}$, respectively; Table 1).

Table 1. Oviductal transport after surgical transfer of day 6 embryos into the mid-ampullary region of recipient mares

	Day 5 recipient mares	Day 2 recipient mares	Day 2 sham transfer mares
Number of mares	10	10	10
Oviductal transport*	9/10 ^a	6/10 ^a	1/10 ^b
Embryo diameter† (µm)			
Before transfer	187 ± 11.3	180 ± 13.8	—
After transfer	659 ± 43.6 ^a	375 ± 85.4 ^b	—

*Recovery of embryos from uterus 48 h after transfer.

†Mean ± SEM.

^{a,b}within rows ($P < 0.05$).

Discussion

The hypothesis that the horse embryo initiates oviductal transport was supported. Six of ten embryos placed in the oviducts of day 2 recipients were subsequently recovered from the uterus at day 4, which is before the expected time of uterine entry at days 5–6. Oviductal transport in day 2 recipients was not significantly less than that found in day 5 recipients.

Oviductal transport did not appear to be initiated by the surgical transfer procedure, since oviductal transport occurred in only one sham transfer. A surgery-related inflammatory response may have initiated oviductal transport in this one mare.

Oviductal transport does not appear to be initiated by increased embryonic size since embryos do not increase in diameter before transport (Freeman *et al.*, 1991). Oviductal transport does not appear to be initiated by a change in embryo surface morphology since the surface morphologies of oocytes and embryos are similar (Betteridge *et al.*, 1976). In addition, embryos with markedly different surface morphologies (zona-enclosed compared to zona-free) initiated oviductal transport. The surface of embryos during normal oviductal transport is the zona pellucida (Betteridge *et al.*, 1982). One embryo in this study and two embryos in a previous study (Freeman *et al.*, 1989b) had shed their zonae pellucidae before they were placed in and transported through oviducts of recipient mares. The surface of these zona-free embryos during oviductal transport was the capsule (Betteridge *et al.*, 1979).

It is suggested that horse embryos initiate oviductal transport by secreting a chemical. As horse embryos in the uterus initiate oviductal transport and uterine embryos develop *in vitro* (Freeman *et al.*, 1989a), it would be logical to culture uterine embryos and identify embryonic secretory chemicals that potentially initiate oviductal transport.

Embryos increased in size at a slower rate after transfer to the oviduct of day 2 compared with day 5 recipient mares. The slower rate of growth may have been caused by asynchrony between embryos and recipient mares, as a decrease in embryonic growth rate occurs when cow and sheep uterine embryos are transferred to the uteri of less advanced recipients (Lawson *et al.*, 1983; Albiñ *et al.*, 1991). Embryos were in oviducts of day 2 and day 5 recipient mares for undetermined times; the relative effect of oviductal or uterine environments could therefore not be evaluated.

The authors thank D. C. Kem, Twin Willows, D. W. Jacklin, T. Scheumann, D. Agnew and the Advisory Board of the Northwest Equine Reproduction Laboratory for support. The authors are grateful to D. Bingaman, T. Byrd, B. Hamilton, R. Haws, L. Lindsley, J. Lyon and L. Mitten for excellent technical assistance. Idaho Agricultural Experiment Station Publication Number 91A05. University of Idaho Research Council Seed Grant Number 681-Y112.

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Received 9 April 1991