Non-surgical embryo collection in the mare and subsequent fertility of donor animals

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Summary. Observations were made on 14 mares which were non-surgically flushed on one or more occasions for recovery of embryos on Days 7–9 after ovulation. Flushing alone shortened the oestrous cycle by about 2·7 days but increased the number of mares failing to ovulate. Flushing followed by an injection of a prostaglandin analogue shortened the cycle by about 4·5 days and hence increased the frequency of ovulations. From a total of 70 flushings performed, 27 embryos were recovered and of 12 mares inseminated during the first oestrus after embryo collection, 6 conceived and foaled. The other 7 mares were used again as donors during the next season. They underwent a total of 21 non-surgical embryo collections and yielded 15 embryos. It is concluded that non-surgical embryo recovery and transfer provides a practical means of increasing the fecundity of valuable donor mares without depressing their fertility.

Introduction

Relatively few reports of embryo transfer in mares have appeared to date (Oguri & Tsutsumi, 1972, 1974; Allen & Rowson, 1972, 1975; Allen, Stewart, Trounson, Tischner & Bielański, 1976; Tischner, 1979; Vogelsang, Sorensen, Potter, Burns & Kraemer, 1979). Due largely to the difficulties involved in inducing superovulation in mares with exogenous gonadotrophins (see Allen, 1977; Douglas, 1979), the application of embryo transfer techniques to increase fecundity in equids must at present be limited to the repeated recovery of single embryos from desirable donor animals.

The purpose of the present study was to determine the fertility of donor mares after repeated non-surgical embryo collections.

Materials and Methods

The experiments were carried out during the spring and summer months of 1977 and 1978 on 14 mares and repeated in 1979 on 7 of them. The mares were of various breeds, aged 3–8 years and weighed 320–610 kg. All the mares were teased regularly for signs of oestrus and follicular development was determined by rectal palpation of the ovaries. Mares were mated naturally or inseminated with fresh or frozen semen (see Tischner, 1979), at the second phase of oestrus when a large and fluctuating Graafian follicle was present in the ovaries. Matings and inseminations were repeated daily until ovulation occurred.

Embryos were recovered non-surgically on Days 7, 8 or 9 after ovulation by a modification

of the method described by Allen & Rowson (1975). A plastic catheter with an inflatable cuff (decompression tube, 20 F.G. 1–9 75: G.F. Warne & Co., England) was passed through the cervix, and the uterine horn ipsilateral to the corpus luteum was flushed with 150 ml physiological saline (9 g NaCl/l) containing 0.1 g streptomycin. Embryos were located in the flushing fluid with the aid of a stereomicroscope. On 35 (50%) occasions the mares were injected with 250 µg of the prostaglandin analogue, fluprostenol (Equimate: ICI, Cheshire, U.K.), immediately after non-surgical flushing.

To determine the functional activity of the corpus luteum before and after embryo flushing, samples of peripheral blood were recovered daily from the 4th day after ovulation until the following ovulation during 14 oestrous cycles from mares receiving prostaglandin after flushing and during 10 oestrous cycles in the same mares when prostaglandin was not given. Daily samples were also recovered from one randomly chosen mare throughout a spontaneous oestrous cycle. The plasma was separated and later assayed for progesterone concentration by the radioimmunoassay method described by Furr (1973) using an antiserum (465/7) prepared against progesterone-11-succinyl-BSA conjugate. Plasma was extracted with 10 volumes of petroleum ether (40–60°) before assay and the within- and between-assay coefficients of variation were <10%. The sensitivity of the assay was 50 pg progesterone/ml.

After completion of the embryo recovery experiments in 1978, 12 of the mares were inseminated with deep-frozen semen during a single oestrous period which followed. Pregnancy diagnosis was made by rectal palpation on Days 20 and 40 after ovulation and re-checked during the second half of pregnancy. Supplementary observations were made on the mares that failed to conceive for the presence of uterine infection and the normality or otherwise of their subsequent oestrous cycles. In 1979 (April–June) foalings were checked and the non-pregnant mares were used again as donors.

Results

The mean length of a spontaneous oestrous cycle in the mares used in these experiments, calculated from 63 such cycles, averaged 22.1 ± 0.57 (s.e.m.) days. Ovulation occurred in 74% of these cycles and the mature Graafian follicle became atretic in the remaining 26% of cycles. Peripheral plasma progesterone concentrations in the spontaneous ovulatory cycles ranged from 4 to 9 ng/ml between Days 5 and 15 after ovulation (Text-fig. 1a).

Embryo collection performed without subsequent injection of prostaglandin significantly reduced mean oestrous cycle length to 19.4 ± 1.51 (s.e.m.) days ($P < 0.05$; 20 cycles). Plasma progesterone concentrations tended to fall from around 9.0 to about 2.0 ng/ml within 3–4 days after flushing (Text-fig. 1b) and the interval between embryo collection and the following ovulation averaged 11.4 days. Ovulation occurred normally in 57% of these cycles, atresia of the Graafian follicle in 37% and in the remaining 6% neither behavioural oestrus nor follicular development was detected.

Embryo collections followed immediately by an injection of fluprostenol further shortened mean oestrous cycle length to 17.6 ± 0.74 (s.e.m.) days ($P < 0.01$; 27 cycles). It caused a rapid drop in blood progesterone concentrations, from 7 to around 2 ng/ml within 24 h after collection, and decreased the interval between embryo collection and the following ovulation to 9.6 days (Text-fig. 1c). Ovulation occurred normally in 77% of these cycles, the Graafian follicle underwent atresia in 17% (statistically significant improvement, $P < 0.05$) and neither behavioural oestrus nor follicular growth occurred during the following 2–3 weeks in the remaining 6%.

During the course of the experiment, the 14 mares underwent a total of 70 non-surgical embryo collections (average 5 per mare; range 2–12) and yielded a total of 27 embryos (average 2 per mare; range 0–5). The fertility of the mares, calculated on the basis of the number of

Text-fig. 1. Mean times of onset of oestrus and ovulation (OV) and mean $\pm$ s.e.m. peripheral plasma progesterone concentrations in: (a) one mare during a spontaneous oestrous cycle; (b) donor mares during 14 cycles when embryo collection alone (EC) was performed during dioestrus; and (c) donor mares during 10 cycles when embryo collection combined with prostaglandin treatment (EC + Equimate) was performed during dioestrus.

embryos recovered, was similar for the 41% of mares flushed after a spontaneous ovulation, 38% of mares flushed after an ovulation which followed an embryo collection during the preceding dioestrus and 43% of mares flushed after an ovulation which followed embryo collection and prostaglandin treatment in the preceding dioestrus.

Six of the 12 mares inseminated with frozen semen at the conclusion of the experiment during an oestrus which followed a previous embryo collection conceived and gave birth to 6 healthy foals. One of the mares which failed to conceive suffered from a bacteriological infection of the uterus. The other 7 mares which did not show any abnormalities were used again as donors. During the season of 1979, they underwent a total of 21 non-surgical embryo collections (average 3 per mare; range 2–5) and yielded a total of 15 embryos (average 2.1 per mare; range 1–3). After completion of the experiments the mares continued to cycle regularly.

Discussion

The procedure for non-surgical collection of embryos, using a flexible plastic catheter, did not appear to have any detrimental effect on the fertility of the donor mares. Conception rates after embryo collection were relatively high and the only uterine infection which occurred was probably induced by insemination rather than embryo collection.

Only 150 ml physiological saline was used as the flushing medium and this was removed from the uterus almost immediately. Nevertheless, this brief uterine infusion, together with the necessary manipulation of the uterine horn ipsilateral to the corpus luteum, was sufficient stimulus to hasten luteolysis and the onset of the next oestrous period. As expected, luteolysis was greatly hastened when embryo recovery was accompanied by administration of prostaglandin and this treatment resulted in an overall increase in the frequency and number of ovulations.

Concentrations of plasma progesterone measured in one mare during a spontaneous cycle were typical, confirming previous results (Palmer, 1978). Similarly detailed observations on other mares were therefore considered unnecessary.

The mares were mostly inseminated with frozen rather than fresh semen which, as shown by Tischner (1979), could be expected to have significantly reduced conception rate and hence embryo recovery rate. Nevertheless, the study demonstrated clearly that repeated insemination with fresh or frozen semen, combined with repeated attempts to recover embryos between 7 and 9 days after ovulation, does not affect the fertility of donor animals. It may therefore be concluded that repeated collection of embryos and their transfer to recipient animals provides a practical method for increasing the fecundity of genetically desirable mares.

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