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EMBRYO TRANSFER IN DROMEDARY CAMELS

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ABSTRACT

Embryo recovery rates per attempt from naturally mated camels that superovulated following treatment with FSH or eCG were 261/68 (384%) and 193/84 (230%) respectively. The rate was affected by interval between ovulation and collection, male fertility and reproductive characteristics of the donor. Best recovery rates were achieved on Day 7.0 or 7.5 after natural mating from multiparous camels that had been superovulated with FSH. An overall pregnancy rate of 32% was achieved from the non-surgical transfer of 296 embryos. Factors affecting pregnancy rate included season, age and parity of recipient, number of recipient CL's, quality of embryo and donor/recipient/embryo synchrony.

Key words: Camel, Embryo, Superovulation, Pregnancy, Artificial Insemination, Embryo Transfer

INTRODUCTION

Dromedary camels used for milk, hair, hide and meat production, or for racing usually have only 1 calf every 2 years (1). Embryo transfer could provide more progeny from genetically superior and/or young valuable camels, subfertile animals and those calving late in the breeding season. It could also be used to test techniques such as AI with fresh, cooled, or frozen semen.

Providing camels have ovarian follicles that are sufficiently mature to ovulate in response to endogenous LH, they ovulate consistently following natural mating (4), insemination with whole semen or seminal plasma (14), or even seminal plasma administered im (10). However, management of camels for embryo transfer is complicated by the facts that (a) ovulation can occur without mating (especially after introduction of a male or following progesterone withdrawal) (5), and (b) on occasion, oestrus may be difficult to detect even in the presence of pre-ovulatory follicles. Furthermore, CL regression usually occurs 8 to 10 d after sterile mating, which in comparison with other domestic species, reduces the time available for transfer.

Ultrasonography greatly facilitates reproductive manipulations in the Dromedary camel (11,13) and live offspring have recently been produced by embryo transfer (6, 12). The pregnancy rates in these 2 studies were low: 1 pregnancy from transfer of 24 embryos into camels induced to ovulate (4%)(12), 5 pregnancies from transfer of 21 embryos into non-ovulating progesterone-treated recipients (24%) (12) and 40 pregnancies from transfer of 121 embryos (28%) (6).

This paper summarizes the development of embryo transfer in racing Dromedary camels in the United Arab Emirates between May 1989 and April 1993.

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MATERIALS AND METHODS

Management of Donors

All procedures were performed on standing camels, restrained in stocks with solid walls and doors. Plastic-covered chains were placed over and beneath the neck and under the caudal abdomen. Kicking bars were positioned behind the back legs and the tail was tied vertically. Most camels resented examination per vaginam or per rectum and some needed to be tranquilized with xylazine and ketamine iv (30 to 50 mg of each mixed together) for embryo flushing.

Ultrasonography was used to determine that donors were not pregnant and had no follicular cysts or intrauterine accumulation of fluid, and also to monitor follicular development and diagnose ovulation.

All donors were treated with progesterone-in-oil (100 mg Progestin, im) (Intervet (Aust) Pty. Ltd., Sydney, NSW, Australia) for 8 to 15 d. On the last day of progesterone therapy, camels were given either a single injection of 3,000 to 6,000 IU eCG (Pregnecol; Heriot Agrivet Pty. Ltd., VIC, Australia) or twice daily injections of 1 to 3 mg porcine FSH (Folltropin-V; Vetrepharm (A/ASIA) Pty. Ltd., VIC, Australia) in declining doses over 3, 5 or 7 d. Ovarian function was assessed from day 5 after commencing superovulation treatment, to determine which camels had responded and to assess the optimal time for breeding.

Follicles of 10 to 25 mm diameter were apparently most capable of ovulating in response to mating, but those measuring 16 to 18 mm diameter were preferred and were most common 8 to 12 days after gonadotrophin treatment. Ovulation was induced by mating once or twice, 12 h apart. For AI, ovulation was induced with either 3,000 IU hCG (Chorulon; Intervet (Aust) Pty. Ltd.) or 20 μg of the GnRH analogue, Buserelin (Bomac Laboratories, Sydney, NSW, Australia).

Selection and Management of Recipients

Wherever possible, nulliparous camels aged 5 to 8 yr were used as recipients. They were also treated with daily injections of 100 mg of progesterone-in-oil for 10 to 15 d, terminating on the day that gonadotrophin was first given to the donor. In later experiments (1993) recipients were treated with 1,500 to 3,000 IU eCG on the day of, or the day after, donor gonadotrophin treatment. The recipients were examined and grouped according to follicle size 5 d after ending the progesterone injections.

Ovulation in recipients was induced with either 3,000 or 5,000 IU, or with 20 μg Buserelin, administered according to the size of the follicle(s) present in the donor and the anticipated time of ovulation. Recipients were scanned to determine the size and numbers of CL(s), to eliminate camels with uterine pathology, and to diagnose pregnancy at day 14 after embryo transfer.

Embryo Recovery

The day of natural mating or injection of hCG or GnRH was termed Day 0 and embryo recovery was attempted from Day 6.0 to 9.0. Embryo recovery techniques have previously been described (6) and were similar to cattle (3).

Embryo recovery was attempted from 152 females that had been mated naturally and from 58 females that had been inseminated artificially. The number of flushes per camel varied from 1 to 3 per cycle because some animals were re-flushed 12 or 24 h later. The total number of flushes was 256.

Embryo Transfer

Non-surgical embryo transfer. The technique has previously been described (6). Briefly, the transfer gun was advanced through the vagina within the palm of the operator's hand and, after the index finger had penetrated the cervix, the tip of the instrument was guided to the middle of the annular cervical rings. The sanitary sleeve was then retracted and the operator's hand was placed in the rectum to direct the instrument tip into the left horn. On occasions, the right horn was entered inadvertently and, because the junction of the horns was so caudal, no attempt was made to reposition the instrument.

Surgical embryo transfer. Embryos were transferred with. a glass pipette, through a standing left flank laparotomy incision in tranquilized camels, as in horses (7).

Statistical Analysis

Proportional data (pregnancy) were analyzed by Chi Square analysis. Continuous data (embryo recovery) were not analyzed due to the huge variability in responses.

RESULTS AND DISCUSSION

Factors Affecting Embryo Recovery Rates

Age of embryo. Embryo recovery rates in superovulated donors flushed at various times after natural mating were less on Day 6.0 (8/7, 114%) compared to Day 6.5 (79/44, 179%) and Day 7 (286/107, 267%). This suggested that the embryo enters the uterus at about Day 6.0 to 6.5.

Delayed oviductal transport, or asynchronous ovulations, may have prevented some embryos being in the uterus at the time of collection attempts. In 117 cases in which donors were not given $PGF_{2\alpha}$ after the embryo collection attempt, 30 animals became pregnant. Of the 43 donors that had given no embryos, only 8 (17%) became pregnant, whereas of the 74 that gave 1 or more embryos, 22 (30%) became pregnant ($p<0.18$). A few donors became pregnant despite being flushed twice, 24 h apart (i.e. on Days 6.5 and 7.5).

Male effect. On one occasion that no embryos were recovered from 10 females mated to one male and 31 embryos were recovered from 11 females mated to another male. Electroejaculation and semen analysis demonstrated that the first male had a low (<10%) proportion of progressively motile spermatozoa, a low sperm concentration ($< 20 \times 10^6$/mL) and a low total sperm count in his ejaculate (80×10^6), despite having testicles of normal size and consistency.

Artificial insemination. Donors that were mated naturally gave more embryos per collection attempt (454/152, 299%) than those inseminated artificially (71/58, 122%)(the number of flushes varied from 1 to 3 per cycle). The embryo recovery rate from camels inseminated with raw semen (19/16, 199%) or extended semen (54/42, 124%) were similar. Similar rates of embryo collection were obtained whether camels were treated with hCG (48/42, 114%) or GnRH (23/16, 144%) to

induce ovulation. The lower overall recovery following AI may have resulted from inadequate stimulation of ovulation.

Age and reproductive characteristics of the donor. The numbers of embryos per cycle were similar in donors aged 12 yr or older (226/86, 263%) and younger females (299/124, 241%). More embryos were obtained from parous females (322/84, 383%) than from nulliparous females (203/126, 161%). Large, unovulated follicles in the ovaries (13) may have affected embryo recovery in naturally mated females as fewer embryos were recovered when these large follicles were present (59/35, 169%) than when they were not present (466/175, 266%) at the beginning of superovulatory treatment. The follicles occurred in 43% that were superovulated randomly, but in only 4% that were pre-treated with progesterone for 15 d ($p<0.001$).

Superovulatory treatments. More embryos were recovered per attempt from naturally mated females treated with FSH (261/68, 384%) than from those treated with eCG (193/84, 230%). AI resulted in embryo recovery rate of 49/26 (188%) for FSH-treated camels and 22/32 (69%) for eCG treated camels.

Repeated embryo recovery attempt. There was no adverse effect of repeated embryo recovery attempts. Two camels were flushed 5 times and 14 were flushed 3 times. High producers at the first flush tended to remain high producers at subsequent flushes. Nevertheless, embryo recovery rate tended to decline in donors treated repeatedly with gonadotrophin.

Embryo Morphology and Development

All donors in this study were superovulated and thus ovulated asynchronously. It was not possible to assess accurately the stages of embryo development in relation to mating or ovulation. On Day 6.5 the embryos collected were usually morulae and early blastocysts still within the zona pellucida. On Day 7.0 embryos ranged from late morulae to small hatched blastocysts but by Day 7.5 most embryos were hatched expanding blastocysts. Thus, the transition from early blastocyst within an intact zona pellucida to hatched blastocyst was apparently rapid. After Day 7.5, the embryo continued to expand rapidly but did not elongate as in cattle (3) or remain spherical as in horses (8). Rather, it increased in size while remaining collapsed, folded or crinkled.

Evaluation of Embryos

Embryos were graded morphologically from 1 to 5 (8). More pregnancies were obtained from the transfer of Grade 1 and 1.5 embryos (52/139, 37%), than from embryos of Grade 2 to 5 morphology (44/158, 28%) ($p<0.08$). The most common structural abnormalities detected were extruded blastomeres, dark or degenerate-looking blastomeres and retarded development in relation to embryo age (e.g. 4-cell embryos collected within a group of hatched blastocysts).

Factors Affecting Pregnancy Rates

Season. No pregnancies were obtained from 24 fresh or 45 frozen embryos collected and transferred to recipients out of the breeding season. Further, embryos collected at this time were remarkably uniform in stage of development (morulae) compared to the spread of developmental stages encountered during the breeding season. Camels mated naturally or inseminated with raw semen during the same period (June to August) failed to become pregnant even though they showed

normal follicular development and ovulation, and semen characteristics of the males were normal. Pregnancy rates did not differ when transferring embryos at different times within the breeding season (November to April).

Recipients. Initially, only around 75% of recipients showed normal follicular development and ovulated. This was improved to 90% by gonadotrophin treatment at time of progesterone withdrawal. Recipient quality/fertility influenced the results of embryo transfer. Recipients graded 1 showed better pregnancy rates (77/209, 37%) than those graded 1.5 (15/53, 28%) ($p<0.25$), 2 (4/24, 17%) ($p<0.05$) or 3 to 5 (0/7) ($p<0.05$). More recipients aged up to 11 yr became pregnant (92/272, 34%) than those aged over 11 yr (4/24, 17%) ($p<0.005$). Further, a higher pregnancy rate was achieved in recipients that were nulli- or uniparous (72/203, 36%) than in multiparous recipients (24/93, 26%) ($p<0.09$).

Embryo age. Age of the embryo at the time of transfer may have influenced pregnancy. The rates achieved with morulae or early blastocysts (10/39, 26%) were lower than with hatched blastocysts (86/249, 35%) ($p<0.27$). This was surprising in view of the delicate nature of hatched blastocysts. Transfer of larger collapsed blastocysts resulted in no pregnancies from 8 transferred.

Embryo number and quality. A lower pregnancy rate was achieved with embryos recovered in uterine flushes that contained < 5 embryos (27/102, 26%) compared to flushes of ≥ 5 embryos (69/194, 36%) ($p<0.01$). Higher pregnancy rates were achieved from the transfer of Grade 1.0 and 1.5 embryos (52/139, 37%) than from embryos graded 2.0 and above (44/158, 28%) ($p<0.08$).

Synchrony of donor, recipient and embryos. If the donor camel ovulated 1 d before or 1 d after the recipient, the degree of synchrony was termed -1.0 or +1.0 respectively. Likewise if the embryo appeared, developmentally, to be 1 d ahead the recipient, the synchrony between embryo and recipient was +1.0. For this purpose, morulae were classed arbitrarily as being 6.0 d old, early blastocysts 6.5 d, hatched blastocysts 7.0 d and expanded blastocysts 7.5 d. Pregnancy rates ranged from a peak of 48% for a donor/recipient synchrony of -1.5 d to a low of 11% for a synchrony of +1.0 d. Similarly, pregnancy rates with regard to embryo/recipient synchrony ranged form 44% with +1.0 d synchrony to 0 for a synchrony of-1.0 d. These results showed clearly that the chance of achieving pregnancy was much higher when embryos were transferred to recipients that had ovulated after, rather than before, the donor.

Method of transfer. Non-surgical transfer resulted in a pregnancy rate of 32% (92/285) compared to 33% (4/12) for surgical transfer. Surgical transfer gave higher pregnancy rates than non-surgical methods in cattle (3) and horses (7). In the camel, non-surgical transfer involved penetration of the 4 or 5 annular folds of the cervix with a finger prior to introduction of the transfer gun, probably increasing the risk of infection. Surgical transfer was also difficult (standing flank) due to tension associated with retraction of the left uterine horn, so only multiparous camels were suitable candidates. The pregnancy rates from surgical transfer (4/12, 33%) compared favourably with non-surgical transfer into multiparous recipients (24/91, 26%).

Side of transfer. Nineteen of 58 (33%) transferred to the right uterine horn resulted in pregnancies compared to 73/232 (32%) embryos transferred to the left horn. Because all the pregnancies developed initially in the left horn, this confirms that a high rate of transuterine migration occurs (4,1). Two pregnancies were obtained from 6 transfers of fresh embryos into the uterine body.

Number of CL's. In the recipients that were stimulated by eCG, more became pregnant with 3 to 6 CL's (32/67, 48%) than amongst those with 1 or 2 CL's (26/98, 27%) ($p<0.005$) or >6 CL's (2/10, 20%) ($p<0.09$).

Transfer technician. Technician did not appear to affect pregnancy rates as results were similar for the experienced technician (69/199, 35%) compared to the inexperienced technician (27/97, 28%) ($p<0.24$).

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