

Using modern reproductive technologies such as embryo transfer and artificial insemination to improve the reproductive potential of dromedary camels

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Key words

Dromedary - Embryo transfer - Artificial insemination - Synchronization - United Arab Emirates.

Summary

Initial experiments evaluated the optimum extender for camel semen. Ejaculates collected from male camels were diluted 1:1 with green buffer, Laciphos or skim-milk glucose extender. Then a total of 300×10^6 live sperm were inseminated into each female camel that had been induced to ovulate with 20 µg of the GnRH analogue buserelin given 24 h previously. Pregnancy was confirmed in 47, 53 and 0% of females inseminated with semen diluted in green buffer, Laciphos and skim-milk extender, respectively. In experiment 2, donor camels were superovulated with a combination of 20 i.u. porcine FSH and 2500 i.u. equine chorionic gonadotrophin, and those that responded were mated to a chosen male when the majority of follicles had reached 1.3-1.8 cm in diameter. Their uteri were flushed non-surgically eight days after mating (day 7 after ovulation). The recovered embryos were either directly transferred singly into recipient camels at different levels of synchrony with respect to the day 7 donor (+1 to -3 days; n = 58), or cooled in embryo flushing media for 24 h in an Equitainer at 4°C before being transferred singly into recipient camels (n = 32) on day 6 after ovulation. The pregnancy rate increased to a maximum of 67% when the recipient was synchronized on one day behind the donor and it fell dramatically when the level of asynchrony increased to +1 (9%) or -3 (10%) days. Of the 32 recipients to which cooled embryos were transferred, 20 (63%) were confirmed pregnant at 18-20 days after ovulation to give a success rate similar to that attained with the control fresh embryos (67%).

■ INTRODUCTION

Artificial insemination and embryo transfer technology offer many advantages to commercial animal production and are used routinely nowadays in several species, including the cow, sheep, and horse (1). The method offers the chance to increase the productivity of a particular male or female and to shorten the generation interval thereby increasing the overall rate of progress in genetic improvement. In the camel, artificial insemination can be used to reduce the number of females the male has to mate and increase his number of offspring. Embryo transfer could be of

particular value to increase the number of progeny from desirable male and female genetic combinations, whether for racing or for the production of meat or milk (15). Furthermore, due to the camel's long gestation period of 13 months, and its restricted breeding season, judicious use of artificial insemination and embryo transfer could increase overall reproductive efficiency in the species. However, as all camelids are induced ovulators (8), there are some essential prerequisites required for such artificial insemination and embryo transfer programs. Firstly, induction of superovulation in donors and induction and synchronization of ovulation in donors and recipients are required for all camels undergoing embryo transfer. Ovulation also needs to be induced in each camel that is to be inseminated and the semen diluted in a suitable extender to maximize the use of each ejaculate.

This paper evaluates the optimum extender to dilute the camel semen and examines the degree of synchrony required between donors and recipients of fresh and cooled embryos.

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■ MATERIALS AND METHODS

Experiment 1: evaluation of the optimum extender for dilution of camel semen

Sixty adult female camels aged 6-14 years and three male dromedaries aged 5-8 years were maintained as part of the experimental herd at the Camel Reproduction Centre in Dubai. During the camel-breeding season in the Gulf region (November-April) the ovarian follicular wave patterns of the dromedaries were monitored regularly by serial transrectal ultrasound examinations as described by Skidmore *et al.* (12). When a dominant follicle reached 1.3-1.8 cm in diameter the camel was given an intravenous injection of 20 µg of the GnRH analogue buserelin (Receptal; Hoechst Animal Health, Beds., UK) to induce ovulation (13), and inseminated 24 h later.

Ejaculates (2-8 ml, motility 60-80%) were collected from the male camels, using a modified bull artificial vagina. They were diluted 1:1 (v:v) with green buffer (IMV, L'Aigle, France) containing 10% (v:v) added egg yolk, or Laciphos (IMV) also containing 10% (v:v) added egg yolk, or skim-milk glucose extender (4). Sperm concentration and motility were assessed before and after adding the diluent and a dose of 300×10^6 motile spermatozoa was deposited in the uterine body by means of a manually-guided bovine insemination catheter passed through the cervix.

Ovulation was diagnosed by ultrasound examination of the ovaries 48 h after the GnRH injection (13) and pregnancy was confirmed subsequently by ultrasound examination of the uterus from day 18 onwards (11).

Experiment 2: transfer of fresh and cooled embryos into recipient camels

Superovulation treatment

The ovaries of the donor camels ($n = 41$) were examined by transrectal ultrasonography as described above. When follicular activity declined to a minimum in the ovaries, each donor camel was injected with a combination of 2500 i.u. eCG (Folligon, Intervet Laboratories, Cambridge, UK) given as a single intramuscular injection on day 1 of the treatment program, and 400 mg of pFSH (Follitropin, Vetrepharm, Ireland) injected twice daily in declining doses of 2 x 80 mg, 2 x 60 mg, 2 x 40 mg, 2 x 20 mg over 4 days also beginning on day 1. A combination of both hormones was found to give better results, i.e. more follicles than if each hormone was used individually (9).

The ovaries of all donor camels were scanned at regular 1-2 day intervals until the majority of follicles had grown to 1.3-1.8 cm in diameter when they were mated twice 24 h apart.

Embryo recovery and transfer

The uteri of the mated donor camels were flushed non-surgically eight days after the first mating, i.e. day 7 after ovulation, as described by Skidmore *et al.* (10). Ovulation occurs 24-48 h after mating (5) so day 0 was taken as one day after mating. The uterus was filled with embryo flushing media (IMV), using an 18 French gauge two-way flexible gibbon balloon catheter (Benkat Instruments, Herts, UK). The medium was then collected by gravity flow into sterile beakers and passed through a sterile embryo filter (EmCon Filter, Immuno Systems, WI, USA). The residual filtrate was searched using a stereoscopic binocular microscope and all the recovered embryos were assessed morphologically and graded 1 to 5 (grade 1 = excellent, grade 5

= degenerate). Embryos of grade 2 or above were loaded, singly, into a 0.25 ml straw, which was then placed in a bovine/equine embryo transfer gun (IMV) and transferred into the anterior tip of the left uterine horn as described by Skidmore *et al.* (10). Pregnancy in the recipients was diagnosed as described in experiment 1.

Ovulation synchrony requirements between donor and recipient camels

The ovaries of the recipients were scanned regularly to monitor follicle development as described above. Those with a suitably mature follicle of 1.3-1.8 cm diameter in their ovaries from one day before ($n = 11$), on the same day ($n = 12$), to 1 ($n = 15$), 2 ($n = 10$) or 3 ($n = 10$) days after a donor camel was mated were injected (i.v.) with 20 µg buserelin. Ovulation was diagnosed by ultrasound examination of the ovaries 48 h later and confirmed by detecting the presence of a *corpus luteum* in the ovaries on the day of embryo transfer.

Embryo cooling

A total of 56 of the recovered embryos were washed in droplets of fresh flushing media before being transferred into small sterile bijou bottles containing 5 ml fresh flushing media. The tube was sealed and placed in an Equitainer (Hamilton Thorn, Massachusetts, USA), which is designed to cool the contents of the container to 4°C and hold this temperature for 36-48 h (3). The Equitainer was opened 24 h later and the embryos recovered from the holding tube and washed in fresh flushing media containing 10% (v:v) fetal calf serum (FCS, Sigma Chemical, Poole, Dorset, UK). Embryos considered to be of grade 2 or higher were then transferred into recipient camels ($n = 32$) on day 6 after ovulation as described previously, and pregnancy was diagnosed by ultrasonography of the uterus between days 18-20 after ovulation.

■ RESULTS

Experiment 1

The average volume of semen collected was 4 ml (range 2-8 ml) and the concentration of sperm was between $185-350 \times 10^6 \text{ ml}^{-1}$. However, approximately 70-80% of each ejaculate was composed of secondary sex gland secretions which made the semen very glutinous and difficult to mix with the various extenders straight after collection. After a period of approximately 20-30 min at room temperature, it had partially liquefied which aided slightly better mixing.

Pregnancy was diagnosed in 10/21 (47%), 7/13 (53%) and 0/6 (0%) female camels inseminated with semen diluted in green buffer, Laciphos or skim-milk extender, respectively. However, two of the camels inseminated with semen extended in Laciphos subsequently lost their pregnancies between days 30 and 40 after ovulation.

Experiment 2

Superovulation treatments

Thirty-seven of the 41 camels treated with 2500 i.u. eCG and 20 i.u. pFSH responded by developing between 4 and 35 follicles (mean \pm sem 19.7 ± 5.3 follicles), which took between 7-12 days (mean \pm sem 10 ± 1.0 days) after treatment to reach 1.3-1.8 cm in diameter when the camel was mated.

Embryo recovery and transfer

A total of 242 embryos were recovered from 32 of the 37 camels flushed on day 7 after ovulation, the remaining 5 donors did not produce any embryos. The mean (\pm sem) number of embryos per flush was 6.5 ± 1.07 (range 1-22) and they ranged from 300 to 500 μ m in diameter. A total of 90 embryos (58 fresh and 32 cooled) were transferred to synchronized recipients and the remaining 152 were either used in other experiments ($n = 118$) or were regarded as degenerate ($n = 34$) and were discarded.

Ovulation synchrony between donor and recipient camels

The pregnancy rate after transfer of fresh embryos increased to a maximum of 67% (10/15) when the recipient was negatively synchronized to have ovulated one day behind the donor camel (-1). But the rate fell dramatically when the degree of asynchrony increased to +1 (1/11; 9%), 0 (6/12; 50%), -2 (5/10; 50%) or -3 days (1/10; 10%).

Transfer of cooled embryos

A total of 56 embryos were placed in the Equitainer and 54 (96%) were considered to be of grade 1 or 2 morphologically after 24 h cooling. However, due to only a limited number of recipients being available, only 32 embryos were transferred to recipient camels, on day 6 after ovulation. Of these 20 (63%) were diagnosed pregnant by transrectal ultrasonography between days 18 and 20 after ovulation to give a success rate similar to the pregnancy rate of 67% achieved when transferring fresh embryos into day 6 recipients.

All the pregnant recipients calved spontaneously between days 379 and 392 after ovulation and all produced healthy calves.

DISCUSSION

The results of this study support those of Anouassi *et al.*, (2) in that acceptable conception rates can be achieved following insemination of the camel 24 h after induction of ovulation. However, whereas these authors used mating to a vasectomized male to induce ovulation, we were able to show similar results using a single intravenous injection of 20 μ g busserelin to induce ovulation. This is a much more practical method as a vasectomized male cannot mate that many females at any one time and it also reduces the risk of transmission of venereal and other diseases.

The results in this study also indicate that extenders containing egg yolk, i.e. Laciphos and green buffer, were better suited to liquid preservation of camel semen at room temperature for short periods of time. The results confirm those of Musa *et al.* (7) as they also obtained better preservation of camel semen in extenders containing egg yolk and lactose (e.g. Laciphos, sodium citrate/egg yolk and Dimiropolous II). However, the fertility of the extended semen was not tested in this particular study as no pregnancy results were given.

Earlier studies have shown that it was possible to produce live offspring by embryo transfer, but initial pregnancy rates after non-surgical transfer into ovulated recipients were only between 4 and 32%, and this was not significantly improved by surgical transfer via left flank laparotomy (33%) (6). In this study the pregnancy rate after embryo transfer was improved when the recipient was negatively synchronized at one day behind the donor but then fell dramatically when the level of asynchrony increased to +1 or -3 days. These results agree with the findings of McKinnon *et al.* (6) who reported pregnancy rates of 44% when embryo:recipient synchrony was +1.0 day. This is not altogether surprising as good evidence exists in cattle, sheep, horses and other species that the shock and trauma of recovery and transfer processes will induce a temporary slowdown in development of most embryos (1). If this occurs in the camel, transfer of expanding blastocysts recovered from the donor on day 7 after ovulation to synchronous or day 8 recipients may not allow the required interval for the embryo to recover and be sufficiently advanced developmentally to release enough maternal recognition of pregnancy message to suppress release of the uterine luteolysin that is poised to commence by as early as day 9 (14).

These results also showed that there did not seem to be any reduction in viability of the embryo due to storage at 4°C (in embryo flushing media) in an Equitainer, as pregnancy rates of 63% were achieved when embryos were transferred into day 6 recipients.

To summarize, these results show that semen is best diluted in extenders containing egg yolk, and satisfactory results can be obtained by inseminating the camel 24 h after induction of ovulation. In addition, better pregnancy rates were obtained if embryos were transferred to recipients that were negatively synchronized at one day behind the donor, and cooling of embryos for 24 h at 4°C did not seem to have any detrimental effect. This provides tremendous encouragement for the transporting of camel embryos within the UAE and possibly internationally as well.

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Résumé

Skidmore J.A., Billah M., Allen W.R. Emploi de technologies de reproduction modernes telles que le transfert d'embryons et l'insémination artificielle afin d'améliorer le potentiel reproducteur du dromadaire

Les premiers essais ont évalué le dilueur optimum pour le sperme du dromadaire. Les éjaculats de dromadaires mâles recueillis ont été dilués à 1:1 avec un tampon vert, du Laci-phos ou du glucose de lait écrémé. Puis, un total de 300 x 10⁶ spermatozoïdes vivants ont été inséminés dans chaque femelle dont l'ovulation avait été provoquée à l'aide de 20 µg de bussereline (analogue du GnRH) administrés 24 h plus tôt. La gestation a été confirmée chez 47, 53 et 0 p. 100 des femelles inséminées avec le sperme dilué dans des dilueurs respectivement à base de tampon vert, de Laci-phos et de lait écrémé. Dans la seconde expérimentation, les femelles donneuses ont subi une superovulation avec un mélange de 20 UI de FSH porcine et 2 500 UI de gonadotrophine chorionique équine. Les femelles répondant à ce traitement ont été accouplées à des mâles sélectionnés dès que la majorité des follicules avaient atteint 1,3 à 1,8 cm de diamètre. L'utérus était lavé sans intervention chirurgicale huit jours après l'accouplement (sept jours après l'ovulation). Les embryons collectés étaient soit directement transférés, un à la fois, chez des receveuses à différents stades de synchronisme par rapport au 7^e jour de la donneuse (+1 à -3 jours, n = 58), soit réfrigérés dans un milieu de lavage spécifique pendant 24 h dans un Equitainer à 4 °C avant d'être transférés, un à la fois, chez des receveuses (n = 32) au 6^e jour après l'ovulation. Le taux de gestation a augmenté jusqu'au maximum de 67 p. 100 quand la receveuse était synchronisée à 1 jour avant la donneuse. Ce taux a décru fortement quand le niveau d'asynchronie a augmenté à +1 (9 p. 100) ou à -3 (10 p. 100). L'état de gravidité de 20 receveuses (63 p. 100) sur les 32 qui avaient reçu les embryons réfrigérés a été confirmé entre 18 et 20 jours après l'ovulation, entraînant un taux de succès similaire à celui atteint dans le groupe de contrôle qui avait reçu des embryons frais (67 p. 100).

Mots-clés : Dromadaire - Transfert embryonnaire - Insémination artificielle - Synchronisation - Emirats arabes unis.

Resumen

Skidmore J.A., Billah M., Allen W.R. Uso de técnicas reproductivas modernas, como la transferencia de embriones y la inseminación artificial para mejorar el potencial reproductivo de los dromedarios

Experimentos iniciales evaluaron el extensor óptimo para el semen de camello. Se diluyeron eyaculados recolectados de camellos, con buffer verde 1:1, Laci-phos o extensor de glucosa de leche descremada. Luego, se inseminó un total de 300 x 10⁶ de esperma vivo en cada hembra camello, inducida a la ovulación con 20 µg de GnRH análoga, busserelina, administrada 24 h antes. La preñez se confirmó en 47, 53 y 0% de las hembras inseminadas con semen diluido con buffer verde, Laci-phos y extensor de leche descremada respectivamente. En el experimento 2, camellas donadoras fueron súper ovuladas con una combinación de 20 u.i. de FSH porcina y 2500 u.i. de gonadotropina coriónica equina y aquellas que respondieron fueron montadas por un macho escogido, esto una vez que la mayoría de los folículos alcanzó 1,3-1,8 cm de diámetro. Se hizo un lavado uterino no quirúrgico ocho días después de la copulación (día 7 post ovulación). Los embriones recuperados fueron transferidos directamente, uno por uno en camellas recipientes en distintos niveles de sincronización con respecto a la donadora en el día 7 (+1 a -3 días, n = 58), o bien enfriados en medio de lavado embrionario durante 24 h en un «Equitainer» a 4°C antes de ser transferidos uno por uno a camellas recipientes (n = 32) en el día 6 post ovulación. La tasa de gestación aumentó a un máximo de 67% cuando la recipiente estuvo sincronizada negativamente un día antes del donador y cayó dramáticamente cuando el nivel de asincronía aumentó a +1 (9%) o -3 (10%) días. De las 32 recipientes a las que se transfirieron embriones enfriados, 20 (63%) fueron confirmadas gestantes 18-20 días post ovulación, dando una tasa de éxito similar a la alcanzada con los embriones control frescos (67%).

Palabras clave: Dromedario - Transferencia de embrión - Inseminación artificial - Sincronización - Emiratos arabes unidos.