Introduction
Opportunities to improve reproductive efficiency in camels are limited by the long gestation period and the short breeding season, as well as the continuing use of traditional systems of reproductive management in most breeding herds. These age-old methods make it difficult to ensure an optimum number of females are pregnant at the end of the season and they can also lead to widespread venereal infections with consequent lowering of fertility.

The technique of embryo transfer can be used to overcome some of these problems as well as used to produce multiple progeny from desirable genetic combinations of sire and dam. However, there are two essential prerequisites of successful embryo transfer in large domestic species; firstly the induction of superovulation in donor animals by exogenous gonadotrophin therapy, and secondly simple methods for preparing groups of synchronised recipients.

Superovulation
Superovulation treatments, to stimulate the growth of multiple follicles, include the use of exogenous gonadotrophins such as equine chorionic gonadotrophin (eCG) or Follicle Stimulating Hormone (FSH) which may or may not, be given after a period of progesterone priming. This progesterone priming can be given either as a Progesterone Releasing Intravaginal Device (PRID) inserted into the vagina for a period of seven days, or as daily injections of 100-150mg progesterone - in - oil for up to 15 days [1-4]. However the best results, (i.e. the best stimulation of the ovaries) occur if the camel is treated with exogenous gonadotrophins when there is minimum follicular activity in the ovaries. If follicles are present at the time of treatment these tend to develop into overlarge follicles before the new stimulated wave of follicles have had a chance to develop [5].

i. Follicle stimulating hormone (FSH) - FSH of porcine or ovine origin has been used for superovulation in camels. In the dromedary a total dose of 18mg of ovine FSH (oFSH) or 400mg porcine FSH (pFSH) in 20ml is given over 4 days. Generally speaking two injections are given daily in gradually decreasing doses for example: Day 1: 2 x 4ml, Day 2: 2 x 3ml, Day 3: 2 x 2ml, Day 4: 2 x 1ml [1-3].

ii. Equine Chorionic Gonadotrophin (eCG) - equine Chorionic Gonadotrophin is well known for its FSH activity and has been used to promote follicular development and superovulation in camels. The dosage of eCG used varies from 1500 - 6000iu. It is generally injected in a single dose one day before, or on the day of, completion of a 5 - 15 day progesterone regime [2-4].

iii. Combined eCG and FSH - in my experience the best response is seen when a combination of both FSH and eCG are given. The eCG (2500iu) is given as a single injection on day 1 of treatment together with the first of the twice daily injections of FSH, followed by three more days of twice daily injections in decreasing doses of FSH as described above [6].

Problems With Superovulation in Camels
Superovulation treatments in the female camels are far from perfect as the ovulation response and embryo yield remain highly variable. The main problems are:
i. The high incidence of non-responsive females - approximately 20-30% of stimulated females do not develop follicles.

ii. The high incidence of follicle luteinization before breeding - this is particularly prevalent in eCG-treated females and could be due to the LH activity of this hormone.

iii. The high incidence of over stimulated ovaries - in some eCG or FSH stimulated females, the ovaries become very large and contain many generations of follicles of different sizes. This maybe due to an individual difference in response to the hormones.

iv. Dromedary camels can become refractory to superovulation with FSH and eCG - this is probably caused by immunization against these hormones. We have observed a complete arrest of ovarian activity in some females that have been superovulated with these hormones repeatedly over several years.

**Mating and Induction of Ovulation**

Some people may rely on behavioural oestrus to determine the best time of mating but this is not the best method for the management of superovulated donors because signs of oestrus do not correlate well with ovarian follicular status. In order to achieve a good ovulation rate, donors should be monitored by ultrasonography and palpation throughout the superovulation treatment and bred when the follicles reach a suitable size of between 1.3 - 1.8cm in diameter [7]. Follicles generally start to develop about 4 - 6 days after the start of treatment and reach 13 - 16mm in diameter approximately 8 - 12 days after the start of treatment (Fig. 1) [6,8]. The number of matings per donor can vary, but in our programmes we generally mate the donor twice at a 24h interval, and although ovulation occurs in response to mating, the donors are given a single intravenous (i.v.) injection of GnRH analogue (20µg Buserelin) at the time of the first mating in order to maximize ovulation response.

![Figure 1. Ultrasonograph of a camel ovary 8 days after treatment with exogenous gonadotrophins to stimulate follicle development.](https://www.ivis.org)

**Embryo Collection and Evaluation**

The methods of embryo collection from camels are similar to those described in other species. 

**Surgical embryo collection** - after exteriorization of the uterus via laparotomy surgical embryo collection is possible, however the use of this technique is only justified when collection of embryos at the tubal stage, i.e. at the morula stage of development, is desired.

**Non-Surgical collection of embryos** - the most widely used technique for the collection of embryos from camelidae remains the non-surgical technique, but before the uteri of the camels can be flushed they have to be restrained and sedated. The drugs of choice are either

- detomidine hydrochloride:
  a) 30 - 35µg/kg i.v. for camels [9]

- xylazine:
  a) 0.25 - 0.5 mg/kg (im) for C.dromedarius (sedation)
  b) 1 - 2 mg/kg (im) for C. dromedarius (immobilization) [10]

The donor can either be placed in stocks or restrained sitting on the ground after being sedated. Then the rectum needs to be cleared of all faeces and the tail wrapped in a tail bandage before cleaning the perineal region thoroughly. Some people like to use epidural anaesthesia which can be advantageous in young dromedaries because of the smallness of the pelvis, however, in larger females it is not usually necessary, especially if they are already sedated.

The uterus is flushed using a Gibbon Balloon (20 Gauge) or Foley catheter (18 - 20 Gauge). Using a sterile gloved hand the catheter is guided through the vagina; then the cervix is dilated manually and the catheter inserted. Once the catheter is through the cervix the cuff is inflated with 30 - 40 ml of air or PBS medium and pulled back against the internal os of the cervix to seal it. The uterus is then flushed repeatedly with 60 - 120ml of flushing medium, which may be either commercially available bovine embryo flushing media or Dulbecco's phosphate buffered saline (DPBS) + 0.2% Bovine Serum Albumin (BSA), + 0.005% (w:v) kanamycin sulphate. The uterus is palpated whilst being flushed to monitor uterine filling and when it feels fully distended the medium is collected, by gravity flow, into sterile
beakers. The recovery of the medium has to be as complete as possible and this is aided by gentle massage of the uterine horns whilst collecting the fluid. The process is repeated at least 3 times or until a total volume of approximately 500ml has been used (Fig. 2) [2-4].

Figure 2. Female camel undergoing non-surgical embryo collection. Flushing medium is injected into the uterus via a 2-way Gibbon Balloon catheter passed through the cervix and collected subsequently by gravity flow into a sterile beaker. - To view this image in full size go to the IVIS website at www.ivis.org . -

Some authors prefer to flush each horn separately because the cervical canal can relax during flushing and the cuff may slip back into the vagina causing loss of some flushing media [8]. For flushing individual horns the catheter should be placed in the uterine horn so that the cuff is positioned in its lower third. This can be difficult to judge as the uterine horns in camelidae are separated internally by a septum that is not palpable. The cuff is inflated with air or flushing medium so that the catheter is well anchored and cannot move under the pressure of the medium. The horn is then flushed 4 to 5 times by injection of 30 - 120ml of flushing media. The operation is then repeated for the other horn. The collected medium is filtered through an embryo filter until only 20 - 30ml of medium remains. This is poured into a sterile petri dish and examined under a microscope for the presence of embryos. As many as 20 or more embryos have been recovered in a single flush but because not all the follicles will ovulate at the same time these embryos can vary greatly in size and development (Fig. 3).

Figure 3. A collection of embryos from one uterine flush of a dromedary camel. Note the difference in size of the blastocysts due to asynchronous ovulation of the follicles. - To view this image in full size go to the IVIS website at www.ivis.org . -

Effect of Timing on Embryo Recovery Rate

It is now well established that in camels the embryo does not reach the uterus until day 6 or 6.5 after ovulation (Day 0 = one day after mating). Therefore any attempt to collect embryos before day 6 post ovulation, results in low recovery rates. In practice the best recovery rates from dromedaries are achieved when the uterus is flushed on day 7 or 8 after ovulation.

Evaluation of Embryos

Embryos recovered from the uterus in camelidae are generally at the hatched blastocyst stage but the size of the embryo is highly variable at different stages post-ovulation. Those recovered from the dromedary camel 7 days after ovulation have a diameter ranging from 0.18 - 0.50 mm. This variability in the stage of development is probably due to the wide spread of ovulations in superovulated animals. Hatched embryos continue to grow rapidly and become easily visible to the naked eye as they expand. The evaluation system used by most authors classifies the embryos into 5 grades according to their morphological characteristics and stage of development (Table 1). The clinician should look for abnormalities such as:

i. extruded blastomeres (i.e. individual cells which have been extruded from the cell mass);
ii. signs of degeneration (dark areas), and
iii. obvious morphological anomalies such as folding or wrinkling.
### Classification of embryos

<table>
<thead>
<tr>
<th>Grade of embryo</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>Excellent quality embryo. Size corresponds to the stage of collection in relation to ovulation. Before day 8 it should be perfectly spherical with a smooth surface.</td>
</tr>
<tr>
<td>Grade II</td>
<td>Good embryo, same as above with some irregularities of the contour and very few protruded cells.</td>
</tr>
<tr>
<td>Grade III</td>
<td>Medium quality, small embryo with dark patches, irregular contour and some protruded cells.</td>
</tr>
<tr>
<td>Grade IV</td>
<td>Collapsed embryos showing dark areas of degeneration and many extruded cells.</td>
</tr>
<tr>
<td>Grade V</td>
<td>Non-transferable. Collapsed very dark embryos or embryos that are retarded, dark morulae, and all stages that are younger than morula or unfertilized ova.</td>
</tr>
</tbody>
</table>

### Management of Recipients

The quality of the recipient is the most important factor in the success of any embryo transfer program. The two main aspects of selection of recipients for embryo transfer are:

(i) the screening of reproductive and health problems and (ii) the preparation and synchronization with the donor.

#### Criteria for selection of recipients - the screening programme can be summarized as follows:

- **a. History:** The potential recipient should be young (less than 12 years of age), have had at least one normal pregnancy with a normal delivery and be either currently pregnant or recently weaned.
- **b. General examination:** The recipient should focus on conformation, a good body condition and on symptoms of debilitating or contagious diseases. All potential recipients should be tested for brucellosis and trypanosomiasis.
- **c. Breeding-soundness examination:** A complete breeding-soundness examination should be done on the potential recipient including firstly, palpation and ultrasonography of the reproductive tract to check the ovaries are active (i.e. some follicular activity present) and the uterus free of uterine fluid and secondly, uterine swabs should be taken and cultured to check for *Pseudomonas aeruginosa*, *Campylobacter fetus* and *Trichomonas fetus*, and (iii) vaginal examination and examination of the udder.

#### Synchronization with donors

- **Synchronization of the cycle in female camels** has met with many difficulties due to the peculiar nature of follicular activity in these species. Techniques used in other domestic animals, for the synchronization of oestrus and ovulation in a group of females such as treatment with progesterone or prostaglandins or a combination of both are not practical, or have only limited success, in these animals. However, synchronization of the reproductive cycle between the donor and the recipient is very critical, and embryo transfer results in the dromedary suggest that the best recipients should ovulate 24 - 48 hours after the donors. Transfer of embryos into recipients that have ovulated one day before the donor, or three or more days afterwards, result in very low pregnancy rates [3,6].

- **Synchronization of ovulation between the donor and recipient** can be approached using one of the following methods:
  
  - **a. Selection of recipients from a random group.** If using this technique a group of recipients at known stages of their reproductive cycles are examined 24 hours after the donor is bred and all females that have a mature follicle (1.3 - 1.8cm in diameter) are treated with GnRH or hCG. This method of selection is time consuming and can only be used if the number of donors are limited [6,11].
  
  - **b. Preparation of recipients in such a manner that follicular development is synchronized with that of the donor.** Synchronization of follicular development in donors and recipients has been attempted
using progestagen treatments such as PRIDs or subcutaneous implants (Norgestomet). However these methods had only limited success as they do not seem to completely arrest follicular development and therefore have very limited efficacy in synchronizing follicular development [1].

c. Better results can be obtained when recipients are induced to ovulate with hCG or GnRH following a treatment combining progesterone and eCG. The recipients are treated daily with progesterone-in-oil (100mg/day) for 10 to 15 days, to try and dampen the development of more follicles, and on the last day of progesterone treatment, 1500 - 2500 i.u. eCG is injected to induce follicular development. Progesterone treatment is scheduled to end on the day of injection of gonadotrophin in the donor in an attempt to synchronize the recipient and donor. The eCG treatment guarantees the presence of mature follicles in the recipient at the same time or 24 - 48 hours after the donor [3].

d. Preparation of recipients with progesterone. Synchronization between the embryo and the uterus can be obtained by progesterone therapy, without induction of ovulation. Progesterone (100mg) is given daily starting 2 days after mating of the donor. However, because there is no corpus luteum (CL), progesterone treatment has to be continued throughout pregnancy [2].

e. Bilaterally ovariectomized females can also be used as recipients. The females are treated for two days with oestradiol 17-β (40mg/day) followed by daily injections of progesterone (100mg/day). This has resulted in 30% pregnancy rates but again has the disadvantage of daily progesterone injections having to be continued throughout pregnancy [8].

Screening of recipients - all recipients should be screened on the day of transfer to ascertain that ovulation has occurred and that a mature CL is present. This can be done either by determination of progesterone concentration in the blood or by ultrasonographic visualization of the CL. Ultrasonographic detection remains the most accurate method and it also allows the screening of the recipients for the presence of fluid in their uteri.

Transfer of Embryos
Embryos can be transferred surgically or non-surgically.

Surgical embryo transfer - surgical embryo transfer in the dromedary and Bactrian camels is done via the left flank laparotomy. The embryo is transferred into the uterine cavity through a puncture made in the exteriorized horn by a Pasteur pipette. However, this technique cannot be used in young and primiparous animals because the uterine horn is too short and difficult to exteriorize.

Non-surgical technique - the non-surgical technique for embryo transfer consists of placing the embryo directly into the uterine lumen through the cervix using a regular bovine insemination gun. The embryo is loaded into a 0.25ml or 0.5ml sterile plastic straw and placed in the gun for transfer. The inseminating gun is first covered by a sterile sheath with a side opening, so that the embryos can escape even if the pipette is up against the wall of the uterus, then this is covered with a second plastic sanitary sheath.

The recipient is prepared in the same manner described for embryo collection. Then the embryo is transferred as follows:

i. The inseminating gun is introduced into the vagina and guided towards the cervix using a sterile gloved hand.

ii. The sanitary sheath is perforated after passage of the first cervical ring, by pulling the plastic sheath backwards towards the technician, and the gun is further guided into one of the uterine horns with a hand in the rectum.

iii. The plunger of the transfer pipette is pushed home and the embryo deposited into the uterus.

The passage through the cervix and uterine deposition of the embryo should be done as quickly as possible to avoid excessive irritation of the cervix and uterine mucosa which may cause prostaglandin F2α release and CL demise.

Factors Affecting Pregnancy Rates

i. Effect of quality of recipient. The fertility, age and parity of the recipients have a significant effect on pregnancy rates following embryo transfer in dromedary camels [3-4]. Recipients that are under 12 have better pregnancy rates than older camels and the pregnancy rate in maiden or primiparous females is almost double that of multiparous females. This is no doubt due to the increased
reproductive problems with advancing age and number of parturitions.

ii. Effect of synchronization. As mentioned earlier, pregnancy rates are higher if the embryos are transferred into recipients that have ovulated one or two days after the donor than those that have ovulated beforehand. This is probably due to the early demise of the corpus luteum because the tiny embryo has not had sufficient time to secrete enough of the very important ‘maternal recognition of pregnancy signal’ to the mother to prevent luteolysis occurring [12].

iii. Effect of method of transfer. In several species higher pregnancy rates are achieved following surgical transfer rather than non-surgical, however this does not seem to be the case with the dromedary where comparable rates have been achieved with both methods [3,4].

iv. Effect of the side of transfer. Many people have suggested that transfer of the embryos into the left horn is an advantage over the right because if pregnancies are to be carried to term in camelidæ they have to implant in the left horn. However in the dromedary, our work and that of others does not show any significant effect of the side of transfer [3,4,8] probably because at this stage the embryo is highly mobile and can easily migrate into the left horn. It seems to be more important to carry out the transfer as smoothly and quickly as possible causing minimum trauma to the endometrium.

v. Effect of season. Although female camelidæ can show ovarian activity throughout the year, pregnancy rates are zero if the embryo transfers are carried out during the hottest months of the year due to an increase in early embryonic death [3,4]. Breeding dates during this time should be avoided anyway, not only due to the poor fertility but also due to poor growth and survival of calves born during this time.

Conclusions

A combination of eCG and ovine or porcine FSH can stimulate multiple follicular development in donor camels for the purposes of embryo transfer. The donors are best treated when there is little or no follicular development in the ovaries as this leads to more follicles developing and maturing to between 1.3 - 1.9 cm in diameter in the same time interval. For best pregnancy results recipients should be synchronized so that they ovulate 24 - 48 hours after the donor. This can be achieved by random selection of the recipients from a group of camels and injecting them with hCG or GnRH 24 - 48 h after the donor, or by treating them daily with progesterone for 10 - 15 days and injecting eCG (1500 - 2500i.u.) on the last day of progesterone treatment. This usually ensures the presence of mature follicles in the recipient 24 - 48 h after the donor. They can then be injected with hCG or GnRH to induce ovulation. Embryos can be flushed and non-surgically transferred on day 7 after ovulation and pregnancy diagnosed by ultrasonographic examination of the uterus on day 17 - 20 of gestation.

References


7. Skidmore JA., Billah M and Allen WR. The ovarian follicular wave pattern and induction of ovulation
in the mated and non-mated one-humped camel (*Camelus dromedarius*). J. Reprod. Fert. 1996; 106: 185 - 192. -PubMed-


10. Custor R, Krame, L, Kennedy S and Bush M. Hematologic effects of Xylazine when used for restraint of Bactrian camels. JAVMA. 1977; 171: 899 - 901. -PubMed-


All rights reserved. This document is available on-line at www.ivis.org. Document No. A1011.1000 .