Examination of Female Camels for Reproductive Soundness

Restraint Techniques

i. Physical restraint - Physical restraint can be as simple as a halter and maintaining the animal in a sternal position and the use of ropes to restrain leg movements, or a more elaborate system such as placing the animal in stocks.

ii. Chemical restraint - Sedation is often necessary when the animal is too reluctant to stand in the stocks or when a more involved gynecological procedure is required. The drugs of choice are either detomidine hydrochloride, 30 - 35 mg/kg, i.v. for camels; xylazine, 0.25 - 0.5 mg/kg, im for C. dromedarius (sedation) and 1 - 2 mg/kg, im for C. dromedarius (immobilization)

Examination of Vulva and Perineum

Vaginal Discharge - Vaginal discharge in the female camelidae is almost always pathological in nature and signals the presence of an inflammatory or infectious process in the genital system. Thick mucopurulent discharge can be seen when the lips of the vulva are parted and vaginal discharge is to be suspected if dried material is observed on the ventral aspect of the tail in camels.

Ultrasonography of the Genital Tract - Ultrasonography of the uterus and ovaries should be carried out to identify follicular activity and any pathological changes in the ovaries and to see if there is any fluid in the uterus. The various conditions that may occur are:

i. Hypoplasia - The ovaries are sometimes so small that they cannot be found by rectal palpation or ultrasonography.

ii. Anovulatory Follicles and Cystic Conditions - In camelidae, development of large anovulatory haemorrhagic follicles is quite common in the absence of ovulation-inducing stimuli. Although these anovulatory follicles take the ultrasonographic appearance of follicular cysts they behave differently and have a limited impact on the ovarian activity.

iii. Ovario-bursitis with Encapsulation of the Ovary - Ovario-bursitis is a particular condition of the ovarian bursa characterized by the accumulation of variable amounts of fluid and encapsulation of the ovary. This condition has only been reported in the dromedary camel and can most easily be diagnosed by ultrasonography. The ovary most often appears floating inside the bursal sac and is easily identified when active.

Uterine Swabbing

Detection of uterine infection is very important in the prevention of venereal transmission to other animals. In addition, identification of the causative germ and determination of its sensitivity to different drugs allows the practitioner to choose the most efficient treatment. Bacteriological examination of the uterus can be done from either (i) direct uterine swabs or (ii) culture of discharge of uterine flushing medium.

Direct Uterine Swabbing - The uterine culture swab should be double guarded, i.e., the swab is within two protective coverings, in order to avoid contamination. The swab is inserted into the cervix manually with the hand of the examiner in the vagina. Once the swab is inside the uterine cavity it is pushed out of its second protective sheath and rotated gently against the endometrium several times, then retrieved into its protective sheath and the whole system is removed from the vagina. The organisms responsible for endometritis in the camel are those bacteria and protozoa that may be implicated in venereal transmission such as: *Pseudomonas aeruginosa*, *Campylobacter fetus*, and *Trichomonas fetus*.

Culture of Discharge of Uterine Flushing Medium - The uterus can be irrigated with 60 - 100 ml of physiological saline, without antibiotics. The recovered medium is collected into a clean beaker and the sterile swab dipped into the fluid. If the camel is infected the uterus should be flushed out 2 - 3 times or until the recovered medium is clear and then antibiotics
dissolved in approximately 20 ml of sterile water for injection can be infused.

Embryo Transfer
Embryo transfer can be used to produce multiple progeny from desirable genetic combinations of sire and dam. The induction of superovulation in donor animals by exogenous gonadotrophin therapy, and simple methods for preparing groups of synchronised recipients, are essential prerequisites of successful embryo transfer programmes in large domestic species.

Superovulation - Superovulation treatments, to stimulate the growth of multiple follicles, include the use of exogenous gonadotrophins such as equine chorionic gonadotrophin (eCG) or FSH which may, or may not, be given after a period of progesterone priming. This progesterone priming can be given either in PRID form (progesterone releasing intravaginal device) inserted into the vagina for a period of seven days, or as daily injections of 150 mg progesterone - in - oil for up to 15 days. 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.

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 20 i.u. of ovine FSH (oFSH) or porcine FSH (pFSH) in 20 ml is given over 4 days. Generally speaking two injections are given daily in gradually decreasing doses for example: Day 1: 2x4 ml; Day 2: 2x3 ml; Day 3: 2x2 ml and Day 4: 2x1 ml

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 - 6000 i.u. It is generally injected in a single dose one day before, or on the day of, completion of a 5 - 15 day progesterone regime.

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 (2500 i.u.) 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.

Problems with Superovulation in Camelidae
Superovulation treatments in the camelidae female 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 superovulated females do not develop follicles. This is probably due to immunization against eCG in some females.

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 super-ovulated females, the ovaries become very large and contain many generations of follicles with different sizes. This is probably 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 during several years.

Mating and Induction of Ovulation
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. Follicles generally start to develop about 4 - 6 days after the start of treatment and reach 13 - 16 mm in diameter approximately 8 - 12 days after the start of treatment. The number of matings per donor can vary, but in our programmes we generally mate the donor twice at a 24 h interval and although ovulation occurs in response to mating, we usually give donors a single intravenous injection of GnRH analogue (20 mg Buserelin) at the time of the first mating in order to maximize ovulation response.

Embryo Collection and Evaluation
The methods of embryo collection from camelidae are similar to those described in other species. The most widely used technique for the collection of embryos from camelidae remains the non-surgical technique.

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 donor can either be placed in stocks or restrained sitting on the ground after being sedated. 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 llamas, alpacas and young dromedaries because of the smallness of the pelvis. However, in larger females it is not usually necessary, especially if they are already sedated. Collection is made using a Gibbon Balloon (20 Gauge) or Foley catheter (18 - 20 Gauge for camels). Using a sterile gloved hand the catheter can be guided through the vagina; the cervix is then 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 - 120 ml of flushing medium, you can use commercially available bovine embryo flushing media or Dulbecco's phosphate buffered saline (DPBS) + 0.2% Bovine Serum Albumin (BSA), + kanamycin sulphate) using a total volume of approximately 500 ml. After each flush the medium is collected, by gravity flow, into sterile beakers and filtered through an embryo filter until only 20 - 30 ml 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 in size.

**Effect of Timing on Embryo Recovery Rate**

It is now well established that in camelidae the embryo does not reach the uterus until day 6 or 6.5 after ovulation. 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. The size of the embryo is highly variable at different stages post- ovulation. However, the majority of the embryos collected at day 6.5 or 7 are already hatched blastocysts. The embryos recovered from the dromedary camel 7 days after mating are very variable in size and have a diameter ranging from 0.18 - 0.50 mm. This variability of 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 embryo 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.

**Table 1. Classification of embryos**

<table>
<thead>
<tr>
<th>Grade of embryo</th>
<th>Characteristics</th>
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<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>
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<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>
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<tr>
<td>Grade IV</td>
<td>Collapsed embryos showing dark areas of degeneration and many extruded cells.</td>
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<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>
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**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:

**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. General examination of the recipient should focus on conformation and a good body condition and on symptoms of debilitating or contagious diseases. All potential recipients should be tested for brucellosis and trypanosomiasis. A complete breeding-soundness examination should be done on the potential recipient including palpation and ultrasonography of the reproductive tract, uterine culture, vaginal examination and examination of the udder.
Synchronization with Donors - 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 recipient should have ovulated 24 - 48 hours after the donor. Transfer of embryos into recipients that have ovulated one day before the donor, or three of more days afterwards, result in very low pregnancy rates.

Synchronization of Follicular Development and Ovulation - Synchronization of ovulation between the donor and recipient can be approached using two methods: (i) selection of recipients from a random group or (ii) preparation of recipients in such a manner that follicular development is synchronized with that of the donor.

In using the first technique a group of cycling recipients are examined 24 hours after the donor is bred and all females that have a mature follicle (1.3 - 1.7cm 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. Synchronization of follicular development in donors and recipients has been attempted using progestagen with variable degrees of success. Synchronization of recipients with Progesterone Releasing Intravaginal Devices (PRID's) has been attempted in the dromedary but was not successful. 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 (100 mg/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.

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

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.

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 0.25 ml or 0.5 ml 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 a second plastic sanitary sheath.

The recipient is prepared in the same manner described for embryo colletion. 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.

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