

Management of recipients for an embryo transfer program in dromedary camels (*Camelus dromedarius*)

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Summary

Embryo transfer can be used to improve the reproductive efficiency of camelids. Methods for the collection and transfer of Day 7 dromedary camel embryos are well established and the selection of good quality and well synchronised recipients has been crucial to the success of these studies. Previous results showed that the best pregnancy rates were achieved when embryos were transferred into recipients that were negatively synchronised to be one or two days behind the donor; however, as camels are induced ovulators and lack the cyclical corpus luteum (CL) of other domestic species, synchronisation of ovulation can be difficult. Consequently, methods to broaden the time window for embryos to be transferred into recipients are required. This review discusses the use of daily progesterone injections or oral treatment with meclofenamic-acid in ovulated, asynchronous recipients, as well as daily progesterone injections or treatment with a combination of progesterone and equine Chorionic Gonadotrophin (eCG) in non-ovulated recipients, to increase the number of available recipients in an embryo transfer program. Adequate pregnancy rates have been achieved in non-ovulated camels treated with progesterone and eCG (50%), ovulated, asynchronous, progesterone treated recipients (50–60%), and ovulated, asynchronous meclofenamic treated recipients (60–80%), thereby reducing the need for such tight synchrony between donors and recipients. The corpora lutea produced using these protocols can then be maintained by the ‘maternal recognition of pregnancy signal’ produced by the conceptus and thereby eliminate the need for exogenous progesterone administration throughout pregnancy.

Keywords: camel, embryo transfer, recipient

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Introduction

The reproductive efficiency of dromedary camels is generally regarded to be low due to the relatively short breeding season, long gestation period, long period of lactation-related anoestrus and the use of traditional methods for camel breeding (Nawito et al., 1967). However, with the use of embryo transfer, this efficiency can be improved by increasing the number of offspring from desirable genetic combinations. There are two

essential prerequisites for successful embryo transfer programs, namely inducing superovulation in the donor animals, and preparing groups of recipients. Early attempts to induce superovulation in camels used doses of 2000–6000 I.U. equine chorionic gonadotrophin (eCG; Folligon), or 20 I.U. ovine follicle-stimulating hormone (oFSH – Ovagen) and these studies resulted in the recovery of 2–12 embryos (McKinnon and Tinson, 1992; Skidmore et al., 1992). However, pregnancy rates were as low as 4 – 32% after transferring

these embryos non-surgically into recipients which had been induced to ovulate, and were not improved by surgical transfer techniques via left flank laparotomy (33%; McKinnon et al., 1994). Subsequent studies investigated the degree of synchrony required between donor and recipient and resulted in higher pregnancy rates, as much as 70%, following the transfer of embryos into recipients that had ovulated one day after the donor (McKinnon et al., 1994; Skidmore et al., 2002).

Synchronising the donor and recipient in camel embryo transfer is complicated by the fact that these species are induced ovulators and lack the cyclical CL of most domestic species. This means that the more conventional methods of oestrous synchronisation used in cattle either by giving two injections of prostaglandin (PG)F₂ α 11 days apart (Cooper et al., 1976) or by administration of a prostaglandin analogue with or without progesterone (Roche, 1976) are inappropriate in camels. However, the results of McKinnon et al., (1994) have indicated that a combination of progesterone and eCG could be used to synchronise camel recipients with donors. Alternatively, Nikjou, (2008) showed that two GnRH injections given 14 days apart could also be used to synchronise follicular wave cycles in camels. As these methods are time consuming and involve multiple injections and/or monitoring by ultrasound, there is a need to improve the efficacy of these procedures. This review gives a brief outline of methods used for camelid super-ovulation and embryo transfer,

and discusses the management of recipients for an embryo transfer program.

Embryo transfer

Superovulation

Several treatments have been used to stimulate super-ovulation in camels; McKinnon and Tinson (1992) injected donor animals with 100 mg of progesterone-in-oil (i.m.) daily for 10–15 days prior to super-ovulation treatment to limit follicular growth and provide an environment suitable for recrudescence of follicular activity. Then, starting on the last day of progesterone treatment, they administered a single injection of 3000–6000 I.U. eCG or twice daily injections of 1–3 mg ovine FSH over 3–5 days. The results showed that more follicles developed, and therefore more embryos were recovered, from those treated with FSH (75 embryos /18 donors; 417%) than from those treated with eCG (118/52; 227%). However, these methods are both time consuming and involve an excessive handling of the camels to give daily injections. Follicle stimulation has also been achieved by inducing ovulation in all donor animals with GnRH first, then starting on Day 4 after ovulation (Day 0 = the day after GnRH injection as ovulation occurs 24–48 h after ovulation stimulus; Marie and Anouassi, 1986), each donor received a combination of 2500 I.U. eCG (Day 1) and 400 mg porcine FSH (pFSH: Follitrophin), given in declining doses over a period of four days (days 1–4). The results in this study showed that 36 of the 42 treated camels responded by

developing between 4–35 follicles, (mean $\pm$ SEM 19.7 $\pm$ 5.3). Donors were then mated approximately 10 days later when the majority of follicles had reached a mature size of between 1.3–1.7 cm in diameter, and the mean $\pm$ SEM number of embryos per flushing attempt was 7.8 $\pm$ 1.4 (Skidmore et al., 2002).

Subsequently Vyas et al., (2004) used an alternative method whereby they induced ovulation in the donor animal with human chorionic gonadotrophin (hCG; Day 0) and then administered either 50 mg Armour units (FSH-P) in 10 diminishing doses at 12 h intervals starting from Day 6 after hCG administration; or 75 NIH-FSH-SI units (SUPEROV) in eight equal doses at 12 h intervals starting from Day 8 after hCG administration. They found considerable individual variation in response between animals, although no significant difference between the two groups in terms of the superovulatory response and number of embryos recovered. However, only a total of 47 CL were

recorded; nine embryos were recovered from the 10 animals treated with FSH-P and 56 CL, and 21 embryos from the seven animals treated with SUPEROV.

Embryo recovery and transfer

The uteri of the mated donor camels were flushed non-surgically on Day 7 after ovulation (eight days after the first mating) by trans-cervical uterine lavage (McKinnon et al., 1994; Skidmore et al., 2002). In brief, a 22-gauge catheter was passed through the cervix and the uterus filled with 70–120 ml aliquots of embryo flushing media, which was recovered by gravity flow. The recovered medium was filtered through an embryo filter and searched for embryos which were subsequently individually transferred, trans-cervically, into the uterus of the recipient camel using a sheathed bovine/equine embryo transfer pipette guided per rectum into the left horn before the embryo was deposited (Skidmore et al., 2002; see Figure 1).



Figure 1. Transcervical embryo transfer using a bovine/equine embryo transfer gun

Management of recipients

The most important factor in the success of an embryo transfer program is the quality and selection of the recipients. Ideally all potential recipients should be 5–12 years old, in good condition, free of parasites and diseases and have had at least one normal pregnancy and parturition. A complete breeding soundness examination should be undertaken. This would include ultrasonography of the reproductive tract to check the ovaries are active and the uterus free of fluid, a uterine culture to check for any infection and a vaginal examination to check it is free of adhesions (Tibary and Anouassi, 1997).

Synchronisation of recipients with donors

It is now well established that the chances of survival of the embryo are greatly influenced by the endocrinological environment of the recipient, which is why synchronisation with the donors can be critical. Previous results in camels indicated that the highest pregnancy rates (70%) were achieved if the recipient had ovulated 1–2 days after the donor, i.e. embryos transferred to recipients on Day 5 or 6 after ovulation (McKinnon et al., 1994; Skidmore et al., 2002). However, pregnancy rates dramatically decreased if the level of asynchrony increased to +1 (9%; recipient

ovulated one day ahead of the donor) or -3 days (10%; recipient ovulated 3 days behind the donor) (Skidmore et al., 2002). This is not surprising because camels have a relatively short luteal lifespan of only 8–10 days (Marie and Anouassi, 1987; Skidmore et al., 1996), which means that the time window for transferring embryos into the uterus, before luteolysis occurs, is short.

Synchronisation of the donor and recipient can be achieved by either synchronising ovulation or by hormonal preparation. Synchronisation of ovulation can be achieved by random selection of animals from a large recipient herd of 15–20+ animals, or by preparing recipients so that their follicular development is synchronised with the donor. Using the random selection technique the group of recipient animals is checked 24–48 h after the donor is mated and those with a mature follicle in their ovaries are given GnRH to induce ovulation (McKinnon et al., 1994; Skidmore et al., 2002). This means they are negatively synchronised to -1 or -2 days behind the donor. This method can be very time consuming and can only be used if there is a large recipient herd or only a small number of donors.

Synchronisation of follicular development has been attempted using Progesterone Releasing Intravaginal Devices (PRIDs), but as many animals developed a vaginal discharge or ovulated while the PRID was *in situ*, this was not very successful (Cooper et al., 1992). Better results were obtained if the recipients were treated with

progesterone (100 mg/day) for 10–15 days, followed 24 h later by a single injection of 1500 I.U. eCG, with the progesterone treatment programmed to end the day the donor received gonadotrophin (McKinnon et al., 1994; Tinson et al., 2000). The eCG treatment of the recipient should therefore guarantee the presence of follicles in their ovaries 24–48 h after the donor. However, it has been reported that pregnancy rates are lower in recipients with 1, 2 or more than 6 corpora lutea (CLs) compared with recipients with 3–6 CLs, so a high response to eCG in the recipients is not desirable (McKinnon et al., 1994).

Other studies by Skidmore et al., (2009) have shown that if camels are injected with GnRH when follicles of between 1.0 and 2.0 cm are present, then these follicles will ovulate or luteinise. If prostaglandin (PG) is then injected on Day 7 after ovulation to induce luteolysis, another mature follicle capable of ovulating should be present 6–7 days later, i.e. 14 days after ovulation. Therefore, if recipient camels receive GnRH at the same time or 24 h after the donors receive their GnRH prior to gonadotrophin treatment, the recipients should have a mature follicle present in their ovaries 24 h after the donor is ready for mating.

The use of asynchronous recipients

It can be particularly difficult when trying to synchronise a small group of recipients with a particular donor, as even with careful planning of progesterone, eCG and GnRH injections, follicles may develop either too early or too late for the recipient to be of use. Consequently,

protocols have been developed to establish pregnancies in ovulated, asynchronous, progesterone or meclofenamic-acid treated recipients.

*Ovulated, asynchronous,
progesterone-treated recipients*

In a recent study (Skidmore and Billah, 2011) recipients with a mature follicle in their ovaries three or four days after the donor was mated were given GnRH to induce ovulation, and embryos transferred to them on Day 3 or 4 after ovulation. As serum progesterone concentrations were still below 1 ng/ml in the recipients at this stage, each recipient received daily injections of 75 mg of progesterone-in-oil from two days before embryo transfer until Day 6 after ovulation. Thereafter the dose was reduced to 50mg on Day 7 and 25 mg on Days 8 and 9, after which treatment was discontinued. Pregnancy rates of 50% (4/8) and 62.5% (5/8) were achieved for the ov + 3 and ov + 4 recipients, respectively, compared with 0% (0/8) for the controls, where the embryos were transferred into Day 4 recipients that did not receive any progesterone. These results show that recipients that ovulate three to four days after the donor can be maintained on progesterone until the embryo is established and secretes sufficient 'maternal recognition of pregnancy signal' to maintain the CL itself.

*Ovulated, asynchronous
meclofenamic acid – treated
recipients*

If recipients ovulate three or more days before the donor then they would undergo luteolysis before the embryo could be transferred. Studies

have therefore been carried out to determine means of prolonging the lifespan of CL. Skidmore et al., (1998) showed that there was evidence for the involvement of prostaglandins in luteolysis in camels, as the oral administration of the prostaglandin synthetase inhibitor meclofenamic acid prevented both the luteolytic action of exogenous PGF2 α and the normal increase in peripheral plasma PGFM concentrations in late dioestrus, thereby prolonging the luteal phase. This led to a further study that investigated whether camels treated with meclofenamic acid during the luteal phase to prolong the lifespan of the CL could be used as asynchronous recipients for embryo transfer (Skidmore and Billah, 2005). In this experiment, recipients that had a mature follicle in their ovaries one, three or five days before the donor was mated were given GnRH to induce ovulation. Meclofenamic acid was then administered orally to all recipient camels from Day 7 after ovulation until seven days after embryo transfer, and embryos transferred into these treated recipients on Days 8, 10, or 12 after ovulation. Pregnancy rates of 80%, 60% or 70% respectively were achieved as compared to 10% in the control animals, where embryos were transferred into non-treated recipients on Day 8 after ovulation (Skidmore and Billah, 2005). These results indicated that recipients that ovulate four to five days before the donor can be maintained on meclofenamic acid until the donor is flushed and the transferred embryo established enough to be able to maintain the CL itself.

Non-ovulated, progesterone treated recipients

Skidmore et al., (1992; 2002) showed that it was possible to achieve and maintain pregnancies in non-ovulated progesterone-treated recipients by giving them daily i.m. injections of 150 mg of progesterone-in-oil starting two days before embryo transfer. This suggested that the degree of synchrony between embryo age and that of the recipient's uterus may not be so important as long as serum progesterone concentrations remain elevated. However, since no CL would be present in the ovaries of these recipients they would need daily injections of progesterone for the entire 13-month gestation period, which would not be practical for large numbers of recipients. This is because the placenta does not contribute to progesterone secretion and all camelids depend entirely on progesterone from the CL to maintain their pregnancy to term (El Wishy et al., 1981). In an attempt to reduce the need for daily injections of progesterone, Norgestamet (progestagen) implants have been injected subcutaneously into pregnant recipient camels at 10-day intervals. However, for some unknown reason, two of four such treated recipients aborted 10–12 days after the start of treatment with the implants, so their use is not recommended (Skidmore et al., 1992). Camels that are maintained on progesterone should be checked regularly for abnormalities and signs of pyometra. It is often difficult to know exactly when to stop progesterone treatment prior to parturition, so it is possible that

recipients will experience dystocia due to insufficient relaxation of the cervix (Tibrary and Anouassi, 1997).

Non-ovulated progesterone eCG treated recipients

As daily injections are impractical, a subsequent study examined the possibility of stimulating the development of follicles in non-ovulated, progesterone-treated pregnant camels by injecting them with eCG and inducing the follicles that mature to ovulate with GnRH. For this purpose, recipient camels were treated with 75 mg of progesterone from three days before to 12 days after embryo transfer, at which time pregnancy was diagnosed (embryo age 19 days). All pregnant recipients continued to receive progesterone until Day 25 (embryo age), when each camel received 2000 I.U. eCG (i.m.) to induce follicular development and progesterone treatment was reduced to 50 mg /day. Approximately 10 days later, when follicle(s) of at least 1.3 cm in diameter were present, the recipients received 20 µg GnRH to induce ovulation. Once the CL(s) had fully developed (by Day 7 after ovulation), progesterone treatment was reduced to 25 mg for a final three days. Fourteen of 18 (77%) recipients that had received daily progesterone injections became pregnant and 7 (50%) remained pregnant after injections of eCG and GnRH. These results show that follicles can develop and subsequently ovulate in progesterone-treated animals and that the foetal maternal recognition of pregnancy signal produced by the conceptus can maintain the CL(s)

that develop for the remainder of gestation (Skidmore and Billah, 2011).

Conclusions

The increasing need to improve camel production has led to a more scientific approach to management of these animals, especially in embryo transfer programs. Hormonal methods using GnRH with or without PG or progesterone and eCG can be used to synchronise follicular waves of donor and recipient animals, which is a necessary prerequisite for embryo transfer. Ovulation can then be induced in those recipients that have a mature follicle in their ovaries 24–48 h after the donor is mated. Alternatively, reasonable pregnancy rates can also be achieved using daily progesterone injections or oral treatment with meclofenamic-acid in ovulated, asynchronous recipients, and daily progesterone injections or treatment with a combination of progesterone and equine Chorionic Gonadotrophin in non-ovulated recipients. These methods reduce the need for such tight synchronisation between donors and recipients and have the added advantage that the CL produced can be maintained by the conceptus once it is established, and there is no further need for exogenous progesterone throughout pregnancy.

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