Anim. Reprod., v.2, n.3, p.161-171, Jul./Sept. 2005

Reproduction in dromedary camels: an update

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Abstract

This review summarizes recent developments in camel reproduction, and it describes characteristics of the ovarian follicular wave cycle and exogenous hormonal control of ovulation and luteolysis. In addition, an account is given of the developments in assisted reproductive technologies in camels such as methods for collection, transfer, and deep-freezing of embryos and semen. Details of recent advances in *in vitro* maturation and fertilization of camel oocytes are also discussed.

Keywords: reproduction, dromedary, camels.

Introduction

The family Camelidae contains two subfamilies: Camelinae (Old World Camelids) and Laminae (New World Camelids). The genus Camelus comprises two species: *Camelus dromedarius*, the dromedary or one-humped camel and *Camelus bactrianus*, the Bactrian or two-humped camel. The New World camelids include the llama (*Lama glama*), the alpaca (*Lama pacos*), the guanaco (*Lama guanacoe*), and vicuana (*Vicugna vicugna*).

For centuries, the camel has been a very important animal in the desert regions because of its ability to provide milk, meat, and transport in harsh, dry conditions whereas llamas and alpacas play an important role for the people of the Andean region providing hair, meat, pelts, transport, and dung for fertilizer and fuel (Fernandez-Baca, 1993). However, as camels are generally used in less well-developed countries, research into improving characteristics such as fertility and milk and meat production have been lacking. However, the development of camel racing in the Middle East has led to an increase in value of the racing dromedary and thus increased interest in improving reproductive efficiency.

The reproductive efficiency of camels under natural conditions is generally regarded to be low. For example, Djellouli and Saint-Martin (1992) reported an overall calving rate of approximately 40% for 30 herds in Tunsia and a mortality rate of 17% between birth and 1 yr of age. This is probably due to the relatively short breeding season, a longer prepubertal period, a long gestation period of 13 months, a prolonged (8–10 months) period of lactation-related anestrus leading to a long inter-calving interval, and the lack of use of assisted reproductive techniques such as embryo transfer and artificial insemination. This review looks at recent developments in the field of camel reproduction.

Ovarian follicular kinetics

All camelids are induced ovulators; therefore, if mating does not occur, follicles tend to regress after initial periods of growth and maturity (Musa and Abusineina, 1978; Adams *et al*., 1990; Skidmore *et al*., 1995). Studies, using real-time ultrasonography to monitor ovarian follicular changes, have shown that the follicular wave pattern varies considerable between camels and can be divided into three phases: the growth phase of 10.5 ± 0.5 d, a mature phase of 7.6 ± 0.8 d, and a regression phase of 11.9 ± 0.8 d (Skidmore *et al*., 1996). In approximately 50% of the cycles studied, the follicle reached a mature size of between 1.5–2.0 cm in diameter whereas in the other 50% of the cycles, the follicle continued to grow until it reached a mean diameter of 4.1 ± 0.2 cm (range = 3.0–6.5 cm). These overgrown, anovulatory follicles took approximately 18.4 ± 0.8 d (range = 11–33 d) to reach maximum diameter, remained at the same size for 4.6 ± 0.5 days, and took approximately 15.3 ± 1.1 d to regress (Skidmore *et al*., 1996). Nevertheless these follicles did not inhibit the growth of other follicles in the same or contralateral ovary, which would grow, mature, and ovulate if the appropriate stimulus was applied. However, various methods have been applied to try and hasten the regression of these large follicles. For example, camels that had a mature follicle of > 3.0 cm in diameter in their ovaries were treated with either a single injection of 20 μg buserelin (Receptal) or received daily injections of 150 mg of progesterone-in-oil for a total of 14 d. The results indicated that whereas it took approximately 22 ± 1.5 d for the overly large follicle to regress in the control group that received no treatment, these follicles regressed in approximately 14.6 ± 1.3 d in animals injected with Buserelin and 12.7 ± 1.5 d in those animals that received daily progesterone injections. It may be speculated that the progesterone therapy suppressed the basal secretion rate of LH from the pituitary gland, thereby preventing any further growth or maintenance of the follicle (Skidmore, 1994).

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Received: August 24, 2005
Accepted: January 6, 2006

Control of ovulation

In dromedaries, ovulation can be induced by mating to an intact or vasectomized male camel. However, manual stimulation of the cervix or intrauterine injection of water or the prostaglandin F analogue, cloprostenol, does not induce ovulation perhaps because these do not stimulate the release of sufficient LH from the pituitary to cause ovulation (Musa and Abusineina, 1978; Sheldrick *et al*., 1992). In Bactrian camels, it has been shown that ovulation can also be induced by deep intravaginal deposition of whole semen or sperm-free seminal plasma (Chen *et al*., 1985) as well as intramuscular injection of seminal plasma (Pan *et al*., 1992). Similarly, llama and alpaca seminal plasma will induce ovulation in both llamas and alpacas if given intramuscularly (Adams *et al*., 2005). This ovulation-inducing factor in seminal plasma was retained after mild heating and treatment with acid or alkali but was destroyed by trypsin digestion, indicating that there is an active protein or polypeptide in camel semen that can express GnRH–like activity (Zhao *et al*., 1992; Pan *et al*., 2001).

When preparing animals for embryo transfer and artificial insemination, ovulation has to be controlled and synchronized. However, mating to a vasectomized male or inseminating or injecting seminal plasma is impractical due to the difficulty of collecting camel semen and also because of the risk of spreading veneral diseases. This leaves treatment with an LH-like, gonadotropic hormone at the optimal time in the follicular growth cycle as the most practical alternative. Studies comparing the efficacy of natural mating with a single, i.v. injection of either 20 μg of the GnRH analogue, Buserelin, or 3000 IU human chorionic gonadotropin (hCG) for inducing ovulation at various stages of the follicular cycle have shown that there was no significant difference between the treatments for inducing ovulation. However, the stage of the cycle when mating or treatment occurred was important. An ovulation rate of approximately 80% was achieved when the dominant follicle measured between 1–1.9 cm in diameter, but it was dramatically reduced to < 20% if it measured between 2.0-2.9 cm and no follicles > 3.0 cm ovulated in response to any of the above treatments (Skidmore *et al*., 1996).

Control of luteolysis

Unlike most other large domestic animals, camels exhibit a relatively short luteal lifespan of only 8–10 d as indicated by progesterone concentrations in peripheral blood. Concentrations remain low for the first 3-4 d after ovulation and then rise steadily to a peak of around 2.7 ng/ml on Day 8 or 9 before falling sharply again on Days 10-11 to reach mean basal values of 0.5 ng/ml by Days 11 or 12 (Marie and Anouassi, 1987; Skidmore *et al*., 1995). The exact mechanism that controls luteolysis in camelids is still unknown, but there is firm evidence that prostaglandins (PG) are involved. Skidmore *et al*. (1998) demonstrated that there is an increase in PGF2α release at the time of luteolysis in non-pregnant camels but with no evidence of episodic secretion, and it was absent in pregnant animals in which luteostasis occurs. In addition, the daily oral administration of meclofenamic acid, a prostaglandin synthetase inhibitor, from Days 6–20 after ovulation prolonged the luteal lifespan for the duration of treatment, thereby giving further evidence for the involvement of PGF2α in luteolysis. However, i.v. administration of 20, 50, or 100 IU oxytocin on Day 10 after ovulation did not increase PGF2α release, either pulsatile or tonic, during the 3 hours after injection. This would suggest that although PGF2α plays a pivotal role in luteolysis in the dromedary camel, its release is probably not controlled by oxytocin.

Maternal recognition of pregnancy

In all domestic farm animal species, the developing conceptus must release a biochemical message to the maternal organism that prevents the normal cyclical release of PGF2α from the endometrium and thereby effectively prolongs the lifespan and secretory functions of the CL. In camels, this signal must be secreted before Day 8 if it is to prevent luteolysis occurring, which is much earlier than in other species. In ruminants, such as cattle and sheep that have a cotyledonary placenta, this maternal recognition of pregnancy signal is thought to be interferon tau (IFN-τ; Godkin *et al*., 1982; Godkin *et al*., 1988) whereas in pigs and horses, both of which are non-ruminants and have a diffuse, non-invasive epitheliochorial placenta, interferon-like molecules of embryonic origin are not secreted (La Bonnardiere *et al*., 1991; Baker *et al*., 1991). However, the embryonic tissues of both these species possess high aromatase activity, and they can synthesize large amounts of estrogens *in vitro* from as early as Day 10 after ovulation (Perry *et al*., 1973; Flood *et al*., 1979; Heap *et al*., 1982).

The camel ruminates but has a diffuse epithelial-chorial placenta like that of pigs and horses (van Lennep, 1961), and studies have been carried out to investigate the possible roles of either fetal estrogens or interferon-like proteins in the maternal recognition of pregnancy (Skidmore *et al*., 1994). Results of these incubation experiments of known weights of embryonic tissues convincingly showed that the young camel conceptus does not synthesize any interferon-like proteins that are similar to ovine IFN-τ. In contrast however, further incubation studies of camel embryonic tissue with tritiated estrogen precursors (i.e. androstenedione) showed clearly that embryos produce strikingly large amounts of both estradiol-17β and estrone from as early as Day 10 after ovulation.

However, in contrast to embryonic pig and horse tissue, the camel tissues synthesize considerably more estradiol than estrone from a C-19 precursor. In addition, incubation of endometrium of pregnant and non-pregnant camels with estrone and estradiol demonstrated a ready ability of this tissue to conjugate these estrogens and so diminish their biological potencies. This onset of estrogen synthesizing ability by camel embryonic tissues coincides well with the observed time of luteolysis following a sterile mating, thereby prompting the suggestion that these fetal estrogens may form an important component of the vital maternal recognition of pregnancy signal.

Use of assisted reproductive technology in camels

As mentioned previously, opportunities to improve the reproductive efficiency of camels are limited not only by the long gestation period and singleton births but also by the continuing use of traditional systems of reproductive management in most breeding herds. Artificial insemination and embryo transfer could be used to overcome these problems and provide the opportunity to produce more offspring from desirable sire and dam combinations.

Artificial insemination

The use of AI has been reported in camels and most studies have been carried out with Bactrian camels (Zhao, 1994). In dromedaries, several researchers have studied semen preservation but insemination trials are rare, which could be due to the difficulty in collecting and handling semen and its subsequent analysis.

Collection of semen

The accepted methods of semen collection in camels are by artificial vagina (AV) or electro-ejaculation. For electro-ejaculation, the male camel has to be fully anaesthetized and specialized equipment is necessary (Musa *et al*., 1992), so it is preferable to use an AV. For collection by AV, a modified bull AV (30 cm in length and 5 cm internal diameter) with an imitation cervix has yielded the best results (Bravo *et al*., 2000).

Semen of camels is grey to milky white in color and has a very viscous consistency immediately after collection. Unlike horse semen, the gel fraction cannot be separated from the sperm-rich fraction which makes spermatozoal motility very difficult to assess and highly variable. However, if it is allowed to stand for 15–20 min, it will partially liquefy and become easier to mix with the extender, and motility can be more readily assessed. Deen *et al*. (2003) have looked at the effect of α-chymotrypsin (1% α-chymotrypsin in Tris buffer) and caffeine (0.2 mM caffeine supplemented in Tris extender), added to split samples of semen at a ratio of 1:1, on spermatozoal motility and found that the addition of caffeine, but not of α-chymotrypsin, improved motility of individual spermatozoon. In another recent study, samples of spermatozoa were diluted to 50 x 10^6 spermatozoa/ml with sodium citrate (2.9%) seminal extender and spermatozoal concentrations, motility percentages, and velocity measurements were evaluated with a new computerized cell motion analyzer (CMA, Medical Technologies Montreux, SA, Switzerland). It was found that spermatozoal concentrations and kinematic variables, in particular percentages of progressive motility, amplitude of lateral head displacement and linearity percentage, were strongly correlated ($P < 0.01$) with fertility rates of dromedary bulls. The CMA-derived measurements of velocity straight line (VSL), velocity curved line (VCL), and velocity averaged line (VAP) were significantly affected by the type of spermatozoal tracks. These results indicated that the CMA was a reliable system for determining spermatozoal concentration, motility percentages, and velocity measures and is considered as an accurate and rapid method for evaluating and predicting fertility in camel bulls (Al Qarawi *et al*., 2002).

Use of fresh and liquid-stored camel semen

For fresh and liquid storage of semen, a number of extenders have been used (Seime *et al*., 1990), but the best results to date have been achieved when the semen has been diluted (1:1) in either: (i) commercially available Green buffer (I.M.V., L'Aigle, France) plus 20% egg yolk (v:v; 50% pregnancy rate; Bravo *et al*., 2000; Skidmore and Billah, 2006), (ii) extender containing 11% lactose plus 20% egg yolk (v:v; 50% pregnancy rate; Anouassi *et al*., 1992), or (iii) a Tris egg-yolk extender (40% pregnancy rate; Deen *et al*., 2003). Artificial insemination in camels also requires the induction of ovulation. Because ovulation occurs between 28–36 h after GnRH or hCG injections, the optimum time for insemination seems to be 24 h after treatment. Good results have also been obtained with fresh semen when insemination occurred 24 h after mating with a vasectomized male (Anouassi *et al*., 1992), however, more detailed studies on the minimum number of spermatozoa to use to establish pregnancies are required. Initial studies have indicated that a minimum of 100 x 10^6 spermatozoa are needed (Anouassi *et al*., 1992) although fertility could be improved when as many as 300 x 10^6 are inseminated (Bravo *et al*., 2000). In a more recent study, insemination of 150 x 10^6 live spermatozoa into the uterine body or just 80 x 10^6 into the tip of the uterine horn ipsilateral to the ovary containing the dominant follicle have both yielded pregnancy rates of 40-50% (Skidmore and Billah, 2006).

Diluted semen can be stored in a refrigerator or in an Equitainer (Hamilton Thorn, Canver, AM, USA)

at 4°C for 24 h and providing it has a motility of at least 35-40% after 24 h it can be used for insemination. However, whilst pregnancy rates of around 50% have been reported for camels inseminated with fresh diluted semen, conception rates decreased to 25–30% in camels inseminated with cooled semen. All of these pregnancies were achieved using cooled semen diluted in Green Buffer + 20% egg yolk (Bravo *et al*., 2002). Further studies have been carried out by Niasari-Naslaji *et al*. (2005) comparing the use of Green Buffer with their novel extender, SHOTOR diluent (2.6 g Tris, 1.35 g citric acid, 1.2 g glucose and 0.9 g fructose in 100 ml ionized water), with or without 10% lactose (w:v) for the preservation of Bactrian camel semen. The results showed that although the initial percentage of live spermatozoa was comparable in all three extenders, the initial forward progressive motility was better in Green Buffer and SHOTOR without lactose than for SHOTOR with lactose (60.5%, 65.5%, and 31%, respectively). However, by 12 h the forward progressive motility of the spermatozoa had significantly decreased in both the SHOTOR diluent with lactose (8.5%) and Green Buffer (33%) but not in the SHOTOR diluent without lactose (44.5%). Further decreases in motility with all extenders had occurred by 24 h, so it was concluded that the SHOTOR diluent without lactose was the better extender for chilling Bactrian semen for up to 12 h.

Frozen storage of camel semen

Seime *et al*. (1990) used split samples of semen to compare different freezing methods by assessing post-thaw morphology, motility, and viability of spermatozoa. They found that the best method for dromedary semen was a modification of the technique developed for boar semen by Westendorf *et al*. (1975). This involved initial dilution of the semen in 11% lactose- egg yolk (20%) buffer, cooling to 15°C over 2.5 h, then diluting further with freezing extender (lactose-egg yolk buffer containing 6% glycerol and 1.5% Equex (Nova Chemical Sales Inc., Scituate, MA, USA) to yield a sperm concentration of 150 x 10^6 /ml and cooling to 5°C over 1.5 h. Finally, a further dilution with freezing extender was carried out to give a concentration of 100 x 10^6 spermatozoa/ml and a final glycerol concentration of 2%. Freezing of the 4 ml straws was achieved by suspending them over liquid nitrogen vapor for 20 min prior to plunging into liquid nitrogen. Although these authors reported a post-thaw motility of 70%, which is promising, the pregnancy results to date in dromedary camels do not reflect this (J.A. Skidmore, unpublished observations) although remarkable results have been reported for Bactrian camels inseminated with frozen/thawed semen (Zhao *et al*., 1996a; Zhao *et al*., 1996b).

For freezing Bactrian semen, different glucose-, sucrose-, and lactose-based diluents were examined by Chinese researchers, and the extender SYG-2 (73 ml of 12% sucrose + 20 ml egg yolk + 7 ml glycerol) yielded the best results regarding post-thaw motility, survival, and acrosome integrity (Zhao *et al*., 1996b). Initially, non-glycerolated extender is added to the semen (1:3), and the diluted semen is held at 20°C for 2 h before cooling and holding at 4°C for 4 h. The glycerolated extender is then added (1:1) and the semen is held at 4°C for a further 10 min before being transferred into 2 ml ampoules and frozen over liquid nitrogen vapor as described above.

Thawing of camel semen is best carried out in a water bath. Small straws (0.25 ml) should be thawed at 40°C for 10 s, large straws (4 ml) at 50°C for 40 s, and ampoules at 50–55°C for 1–2 min. More work needs to be carried out to determine the optimum size of straw for camel semen.

Fertility rates could be improved by more detailed studies on the timing of insemination in relation to ovulation as well as the method of insemination, that is, just inseminating through the cervix with an inseminating pipette verses hysteroscopic insemination using a video-endoscope to deposit much smaller numbers of spermatozoa directly at the uterotubal junction. The insemination of fresh and frozen-thawed semen would greatly improve the reproductive efficiency of camels and could lead to the genetic improvement of camelidae worldwide, but more research is required to improve the pregnancy rate and maximize the use of each ejaculate.

Embryo transfer

The technique of embryo transfer provides the opportunity to produce more offspring from desirable sire and dam combinations. However, there are two essential prerequisites for successful embryo transfer programs: first, the induction of superovulation in the donor animals and second, simple methods of preparing groups of synchronized recipients.

Superovulation

Earlier attempts to induce superovulation in camels used either FSH (20 IU ovine FSH or 400 mg porcine FSH) or equine chorionic gonadotropin (eCG; 2000–6000 IU) administered to the camel when there was minimum follicular activity in the ovaries. The response to the treatments varied greatly between individual camels, but they resulted in the recovery of 2–12 embryos (Skidmore *et al*., 1992; McKinnon *et al*., 1994). More recently, Vyas *et al*. (2004) compared two commercially available porcine FSH preparations. Although the follicular response was not reported, no significant difference between the two preparations was found, and a total of 30 embryos were recovered from 17 donors. Other methods using a combination of eCG and FSH have shown more promise. A total of 2500 IU eCG injected as a single i.v. injection on Day 1 and a

total dose of 400 mg porcine FSH administered twice daily in gradually decreasing doses over 4 d, also beginning on Day 1, produced an average of 19.7 ± 5.3 follicles. The majority of these follicles had reached a mature size of between 1.3–1.8 cm in diameter approximately 10 d after the start of treatment at which time the donor was mated (Skidmore *et al*., 2002).

There are several problems associated with superovulation treatments. For example, about 20% of camels do not respond to the treatment at all, or in some cases the ovaries become overstimulated and contain many generations of follicles of different sizes, many of which do not mature enough to ovulate. In addition, some camels can become refractory to the treatment with FSH and eCG that may lead to a complete arrest of ovarian activity after repeated treatments over a few years (Tibary and Anouassi, 1997). Also, in some animals, there is a high incidence of follicle luteinization or ovulation just before breeding which could be due to the LH-like activity of eCG.

Embryo collection and transfer

Embryos are generally recovered from camels on Day 7 after ovulation using non-surgical, transcervical uterine lavage as described by McKinnon *et al*. (1994) and Skidmore *et al*. (2002). In brief, an 18 French gauge, flexible, two-way Gibbon balloon catheter (Benkat Instruments, Herts, UK.) was passed through the cervix, and the uterus was filled with flushing medium (Embryo Flushing Medium; I.M.V. L' Aigle, France) that was then recovered by gravity flow into sterile beakers. This procedure was repeated a total of three times before the recovered medium was passed through a sterile embryo filter (EmCon Filter; Immuno Systems Inc., Wisconsin, USA), and the residual filtrate was searched for embryos using a stereoscopic binocular dissecting microscope. When embryos were located, they were assessed morphologically and graded 1 to 5 (Grade 1 = excellent, Grade 2 = good/fair, Grade 3 = poor, yet expanded, Grade 4 = collapsed and degenerate, Grade 5 = fragmented and degenerate).

For transfer, a single embryo of Grade 2 or above was aspirated into a 0.25 ml straw that was then loaded into a sheathed bovine/equine embryo transfer gun (I.M.V.,). The transfer gun was guided through the cervix (per rectum) and preferably into the left uterine horn before the embryo was deposited. Pregnancy in the recipients was diagnosed by ultrasonography of the uterus initially between Days 18 and 20 after ovulation followed by confirmation of a viable conceptus between Days 25 and 30, as described by Tinson and McKinnon (1992).

Synchronization of ovulation

Results of previous embryo transfer studies in camels indicate that the best recipient to use should have ovulated on the same day or up to 48 h after the donor (McKinnon *et al*., 1994; Skidmore *et al*., 2002). However, as camels do not have a cyclical corpus luteum (CL) that would be present in spontaneously ovulating species such as the horse and cow, this poses a particular problem. Cattle can be synchronized either by two injections of PGF2α administered at an interval of 11 days (Cooper *et al*., 1976) or by administration of exogenous progesterone or progestagens (Roche, 1976); neither of these treatments are really suitable for camels however, because they are induced ovulators and usually only have a CL when they are pregnant. However, a CL can be created by injecting GnRH when a mature follicle is present, and PGF2α will shorten the luteal lifespan (from 8 to 6 d) if injected after Day 4 after ovulation. Since the inter-wave interval is only shortened by a couple of days though, the usefulness of this form of treatment is limited (Skidmore, 2003). Treatment with progesterone-releasing intravaginal devices (PRIDs) has also been found to be unreliable because not only did several animals developed a vaginal discharge, but only about 33% of the females ovulated after receiving hCG or GnRH when the PRID was removed (Cooper *et al*., 1992).

Synchronization of donor and recipient camels can be achieved by selection of recipients from a random group of cyclic camels or by treatment using a combination of progesterone-in-oil and eCG. Selection of recipients from a random group of cyclic camels involves examining their ovaries regularly and injecting all those with a mature follicle in their ovaries with GnRH 24 h after the donor has been mated. This method is labor intensive and only feasible if there are a large number of recipients available. Better results for synchronization have been obtained if the recipients are treated with progesterone-in-oil (100 mg/day) for 10–15 d followed by administration of 1500 IU eCG (McKinnon *et al*., 1994). Progesterone treatment should stop on the day of injection of eCG in the donor. The eCG treatment guarantees the presence of mature follicles in the recipient at the same time or 24–48 h after the donor has ovulated. However, this method is also time consuming as it involves daily handling and injecting of the camels, in addition to being expensive.

Methods to improve pregnancy rate after embryo transfer

To date, embryo transfer studies in camels have reported pregnancy rates of between 50–70% when Day 7 embryos are transferred into recipients that are either precisely synchronized or have ovulated 24–48 h after the donor (McKinnon *et al*., 1994; Skidmore *et al*., 2002). To try and improve these pregnancy rates, groups of recipients have been treated with progesterone, flunixin meglumine, GnRH, and meclofenamic acid. (Skidmore *et al*., 2002; Skidmore and Billah 2005)

Progesterone

Intramuscular injections of 150 mg of progesterone-in-oil were given daily to ovulated and non-ovulated recipients beginning 1 d before embryo transfer and continuing until at least Day 25 after transfer when the pregnancy could be accurately diagnosed by ultrasonography. Seven pregnancies from the 16 embryos transferred (44%; 3 pregnancies in ovulated recipients and 4 in non-ovulated recipients) were established. The disadvantage of this method is that if no CL is present in the ovaries, then the recipients need daily injections of progesterone for the duration of pregnancy (13 months). 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.

Flunixin meglumine

It has been suggested that manipulation and distention of the vagina and cervix can stimulate endogenous PGF2α release from the endometrium, and it is thought this may induce premature luteolysis and loss of the pregnancy. To investigate this, 12 camels were injected with 500 mg of the prostaglandin synthetase inhibitor, flunixin meglumine, 15 min before embryo transfer, and blood samples were taken at regular intervals. The results showed that stimulation of the cervix did cause a brief release of PGF2α that was suppressed by flunixin meglumine. However, premature luteolysis did not occur indicating that the amount of PGF2α released was either insufficient, or the rise was not prolonged enough to cause any harm to CL function. In fact, the pregnancy rate was reduced in flunixin meglumine treated recipients (16%; 2/12) compared with control animals (67%; 10/15). This may have resulted from flunixin meglumine suppressing uterine motility, thereby reducing embryo movement throughout the uterine lumen and preventing the embryo from secreting enough of its maternal recognition of pregnancy signal over sufficient area of the endometrium to prevent luteolysis from occurring.

GnRH

In cattle, treatment with GnRH results in a transient increase in peripheral estradiol-17β concentration followed by a fall in estrogen concentrations that lasts 3–4 d because of GnRH-induced ovulation or atresia of the follicles (Mann and Lamming, 1995; Mann *et al*., 1995). This in turn reduces the stimulus for the development of oxytocin receptors in the endometrium, which is the key event in the development of the luteolytic mechanism in cattle (Mann and Lamming, 1995) and has been shown to improve pregnancy rates (Mann *et al*., 1995). To investigate this phenomenon in camels, 12 animals were injected with GnRH on Day 5 after ovulation, 24 h before embryo transfer. The results indicated however, that although there was a transitory rise in estradiol-17β for about 24 h after GnRH administration, this increase was not followed by a decline in oestradiol levels as has been shown in cattle. In fact, a more sustained increase in plasma estradiol-17β concentrations occurred from around Day 8 after ovulation in all treated and untreated camels. This may have been caused by the development of the new wave of ovarian follicles that would occur in the non-pregnant animal before luteolysis (Skidmore *et al*., 1995) and partly by embryonic estrogens that have been shown to be synthesized by the camel conceptus from as early as Day 8 (Skidmore *et al*., 1994). However, only 4 pregnancies resulted from the 12 embryos transferred (33%) to treated recipients; therefore, GnRH treatment did not improve pregnancy rate above that of the untreated recipients (67%; Skidmore *et al*., 2002).

Meclofenamic acid (Arquel)

As mentioned previously, luteal lifespan in camels can be prolonged if they are treated daily with meclofenamic acid, a prostaglandin synthetase inhibitor, starting on Day 6 after ovulation (Skidmore *et al*., 1998). To investigate whether camels treated with meclofenamic acid during the luteal phase could be used as asynchronous recipients in an embryo transfer program, meclofenamic acid was administered orally to camels from Day 7 after ovulation until 7 d after embryo transfer. Embryos were transferred into these treated recipients on Days 8, 10, or 12 after ovulation and the pregnancy rates were 80%, 60%, or 70%, respectively, 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). Treatment with meclofenamic-acid could reduce the need for tight synchrony between donors and recipients because if the recipients ovulate 4–5 d before the donor, they could be maintained on meclofenamic acid until the donor is flushed. This method has the added advantage that the CL is maintained by the conceptus once it is established, and further daily administration of exogenous progesterone or progestagens throughout gestation is unnecessary.

These results indicate that pregnancies can be maintained in non-ovulated recipients treated with progesterone or in non-synchronous recipients treated with meclofenamic acid. However, treatment with GnRH or with flunixin meglumine does not improve the pregnancy rate above that of control animals.

Cryopreservation of camel embryos

Reliable methods for the cryopreservation of embryos have been well established for many mammalian species, and this has enabled the more

widespread application of embryo transfer for genetic improvement as there is no need to transport live animals or artificially synchronize recipient animals with the donors. Embryos can be stored, thawed, and then transferred to recipient animals based on the timing of natural ovarian cycles.

Controlled rate (slow cooling)

Until recently there has been very limited data on the cryopreservation of camel embryos. However, Skidmore and Loskutoff (1999) conducted some preliminary studies to determine the most appropriate cryoprotective agent (CPA) for use with camel embryos. Of the four CPA's tested (glycerol, ethanediol, propandiol and DMSO), only those embryos cryopreserved in ethanediol with or without sucrose survived; therefore, ethanediol was the CPA of choice. Subsequent experiments exposed embryos to 1.5 M ethanediol in holding medium (HM) for 1, 5, or 10 min before loading embryos into the freezing machine. After 1 min equilibration at –7°C, the straws were seeded, held for a further 10 min at –7°C, and then cooled to –33°C at a rate of 0.5°C/min. Thawing was carried out in a 32°C water bath for 2 min before all embryos were rehydrated by expelling them directly into HM or HM containing 0.2 M sucrose for 5 or 10 min. Finally, all embryos were transferred into HM without sucrose prior to transfer.

Embryo survival rate immediately post thaw was high in the majority of cases (75–100%) but dramatically decreased to as low as 30-40% in some groups after 1-2 h in culture. This would suggest that although the embryonic cells were not acutely damaged by the osmotic effects of freezing and thawing or ice crystal formation, they were damaged by a latent intracellular response to the permeating CPA. The majority of pregnancies resulted from embryos equilibrated in ethanediol for 5 or 10 min indicating that perhaps a 1-min equilibration time was insufficient for the CPA to permeate and protect the inner cell mass. In addition, those embryos rehydrated using HM containing sucrose and incubated for 5 min before being transferred to medium without sucrose showed improved survival rates compared with embryos decanted directly into HM or into 0.2 M sucrose in HM and incubated for 10 min. This could indicate that a more gradual rehydration process using sucrose in the medium to prevent excessive osmotic shock is beneficial for embryos, but excessive exposure is detrimental (Table 1; Skidmore *et al*., 2004).

Table 1. Viability of cryopreserved camel embryos after controlled rate (CR) freezing.

Group	Cryopreservation method (exposure duration to 1.5 M ethanediol)	Method of rehydration	Total no. embryos	No. (%) embryos* surviving after		No. embryos transferred	No. (%) fetuses detected
				0 h	1 – 2 h		
1a	1 min	HM direct	16	12 (75)	6 (31)	5	0
b		SM (10)- HM	11	5 (45)	4 (36)	4	0
c		SM (5) - HM	13	12 (92)	6 (46)	6	1 (17)
2a	5 min	HM direct	21	21 (100)	12 (57)	12	2 (17)
b		SM (10)-HM	17	14 (82)	10 (59)	10	0
c		SM (5) - HM	13	13 (100)	12 (92)	12	1 (8)
3a	10 min	HM direct	28	28 (100)	12 (43)	12	4 (33)
b		SM (10)-HM	18	18 (100)	12 (67)	12	3 (25)
c		SM (5) - HM	21	21 (100)	19 (90)	12	7 (37)

HM: Holding medium;
SM; 0.2M sucrose in HM for 10 min (10) or 5 min (5) at room temperature.
* Embryos graded as C quality or better were transferred (Embryos are graded as A, B, C, D and F; A = excellent, B = good/fair, C = poor yet expanded, D = collapsed and degenerated, F = fragmented).
Source: Table modified from Skidmore *et al*., 2004.

Vitrification of hatched camel blastocysts

Vitrification methods are much quicker and simpler than slow-cooling methods because they do not require specialized equipment or training. When embryos are vitrified, ice crystal formation is prevented by the use of a high concentration of cryoprotectants and high cooling and warming rates (Vajta *et al*., 1997a, b) although they still can be damaged by cryoprotectant toxicity, osmotic stress, and chilling injuries.

To date three different methods for vitrifying camel embryos have been investigated. Skidmore *et al*. (2005) compared the open pulled straw method (OPS), developed by Vajta *et al*. (1997a, b) for bovine and porcine embryos, with the French straw method developed by Aller *et al*. (2002) for llama embryos. For

the OPS method, Day 6 and Day 7 camel embryos were subjected to concentrations of either 10% and 20% or 20% and 40% ethanediol as the cryoprotectant before being loaded into open pulled straws and plunged into liquid nitrogen.

Embryos were thawed and rehydrated by expelling them directly into holding medium or holding medium containing 0.2M sucrose for 5 or 10 min before being transferred into just holding medium prior to transfer. Again, survival rate immediately post thaw was high (70–100%) but had dramatically reduced to approximately 50–55% after 1-2 h in culture, and none of the embryos transferred resulted in a pregnancy. Somewhat more success was achieved using the vitrification method of Aller *et al*. (2002). These embryos were exposed to vitrification solution (20% glycerol + 20% ethanediol + 0.3M sucrose + 0.375M glucose + 3% polyethylene glycol) in three steps and after loading into 0.25 ml straws were plunged into liquid nitrogen. These embryos were thawed in air for 6 s and warmed in a 25ºC water bath for 1 min, before being rehydrated in 0.5M sucrose in PBS + 20% FCS (5 min) followed by 0.25M sucrose solution (5 min) and finally into just PBS + 20% FCS prior to transfer. A much greater number of the Day 7 and Day 8 embryos were fractured or torn after warming, and none of the 12 embryos transferred resulted in a pregnancy. Better survival rates were achieved with the smaller Day 6 embryos (94%), which resulted in 8 pregnancies from the 21 embryos transferred. From these results, it was concluded that although it is possible to vitrify camel embryos, it is currently limited to blastocysts smaller that 350 μm in diameter. Nowshari *et al*. (2005) achieved 3 pregnancies from 49 embryos vitrified/thawed using a simpler combination of cryoprotectant and sugars. Embryos were exposed to ethanediol (7.0 mol/L) with sucrose (0.5 mol/L) in two steps, transferred to 0.25 ml straws prior to plunging in liquid nitrogen, thawed in a water bath at 25°C for 10 s, and then rehydrated in 0.5M sucrose in PBS.

In vitro maturation and fertilization

The goal of any *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) program is to produce a high number of good quality embryos capable of resulting in live births after transfer to recipient animals. Much less is known about IVM and IVF in camelids in comparison with some other large domestic animals, but just recently the first live birth of an IVF produced dromedary camel calf was reported (Khatir *et al*., 2005).

Initial studies investigated the chronological events and optimal time for *in vitro* maturation of oocytes collected from the follicles of slaughter-house ovaries. Wani *et al*. (2004) cultured the oocytes in maturation medium comprising TCM 199 supplemented with 0.6 mg/ml calcium lactate, 0.1 mg/ml L-glutamine, 0.8 mg/ml sodium bicarbonate, 1.4 mg/ml HEPES, 0.25 mg/ml pyruvate, 50 μg/ml gentamicine, 10 μg/ml FSH, 10 μg/ml LH, 1 μg/ml estradiol, and 10% estrous camel serum and incubated at 38.5°C in 5% CO2 for 4–48 h. At 4-h intervals, some oocytes were denuded, fixed, and examined with phase contrast microscopy, and they concluded that 40-44 h of *in vitro* maturation yielded the highest proportion of M-II stage oocytes (52%, 103/198) suitable for further use in assisted reproductive technologies in camels. Kafi *et al*. (2005) also aspirated oocytes from slaughter-house derived camel ovaries but cultured them in Hams F10 medium supplemented with 10% (v:v) FCS and 0.35 IU/ ml hCG for up to 48 h. They found that the percentage of matured oocytes (M II stage) at 30 and 42 h were 66.5% and 71%, respectively. Further examination of the oocytes under transmission electron microscopy indicated that after 12 h of culture, disruption of the junctions between the cumulus cell process endings (CCPEs) and the oolemma had occurred together with the breakdown of the germinal vesicle. The first polar body was extruded between 12–24 h, and by 30 h of maturation, the oocytes had a well developed perivitelline space containing numerous finger–like microvilli in an erected status accompanied by disrupted cumulus cell process endings. Cortical granules had moved towards the peripheral areas of the ooplasm and formed a lining in the suboolemmal area, and cytoplasmic vesicles and lipid droplets were intermixed and normally distributed throughout the oocyte. However, there was a tendency towards a higher percentage of degenerate oocytes when the culture period was increased to more than 30 h; therefore, they concluded that 30 h was the optimal culture time for maturation of slaughter-house derived oocytes in Hams F10 medium.

Improvements in culture conditions for maturation, fertilization, and culture of *in vitro* produced (IVP) embryos is essential for the success of any IVF program. In camelid species, embryos have been successfully produced *in vitro* using cumulus-oocyte complexes (COCs) recovered post mortem, fertilized with either epididymal spermatozoa in the llama (Del Campo *et al*., 1994) or with fresh ejaculated semen in the dromedary (Khatir *et al*., 2004), and co-cultured *in vitro* with their respective epithelial oviductal cells. However, only 10% of fertilized oocytes developed to blastocyst stage in the dromedary (Khatir *et al*., 2003, 2004), and in llamas, only 11.5% and 4.7% of zygotes reached the morula/early blastocyst and hatched blastocyst stages respectively (Del Campo *et al*., 1994). More recent studies by Khatir *et al*. (2005) have investigated the developmental competence and pregnancy rate of *in vitro* produced (IVP) dromedary embryos in two different culture systems: (i) modified potassium simplex-optimized medium (KSOM - developed initially for mouse embryos; Lawitts and Biggers, 1991) supplemented with Eagle's essential and non-essential amino acids (mKSOMaa) and (ii) camel epithelial oviductal cell co-culture. The COCs were

matured by incubation in TCM 199 medium supplemented with 10% (v:v) FCS, 10 ng/ml epidermal growth factor, 1 μg/ml FSH, 1 μg/ml estradiol, and 500 μM cysteamine for 30 h at 38.5°C under 5% CO2 and fertilized using fresh semen (0.5 x 10^6 spermatozoa/ml in modified TALP solution). Fertilized COCs were denuded then cultured at 38.5°C in either mKSOMaa (with 10% FCS added 24h post IVF), in 5% CO2, 5% oxygen, and 90% nitrogen (Group 1) or with dromedary epithelial oviductal cell monolayers in TCM 199 with 10% FCS in 20% oxygen (Group 2). Results showed that the rate of cleavage was higher for Group 1 embryos than for those in Group 2, and there was a slight but not significant superiority of the semi-defined mKSOMaa medium over the somatic cell co-culture system in terms of blastocyst formation (21% vs 16.5%) and hatchability (21% vs 14%, respectively). Pregnancy rates were similar for the first 60 days in both groups, but all pregnancies were lost after 60 days except for 2 of 6 in Group 1. It was therefore concluded that although both culture systems support *in vitro* production of dromedary embryos by IVM/IVF of oocytes, embryos obtained by culture in the mKSOMaa medium appear to have a better *in vivo* developmental ability. The causes of pregnancy loss following transfer of IVM/IVF produced embryos needs further investigation.

In conclusion, although camels are seasonal breeders and do not reach puberty until 3-4 yr of age, the increasing necessity to improve camel production has led to the development of a more scientific reproductive management of these animals. The ovarian cycle can be monitored by ultrasonography, and when mature follicles are present, ovulation can be controlled by injections of GnRH, gonadotropic hormones, or seminal plasma.

The use of assisted reproduction techniques is being developed in camelidae, but the use of AI still requires further research to define the best extender to use for fresh, cooled, and frozen/thawed semen and to determine the minimum number of spermatozoa required to estabilish a pregnancy, thereby maximizing the use of each ejaculate. Superovulation can be induced by using eCG and/or FSH and embryos recovered non-surgically on Day 7 after ovulation. Pregnancy rates of 65-70% can be achieved when fresh embryos are transferred into recipients that have ovulated 24–48 h after the donor, but this is not improved by the use of progesterone, GnRH, or flunixin meglumine. However, meclofenamic-acid can be used in asynchronous recipients to maintain their CL until embryo transfer is carried out. Embryos have been successfully frozen/thawed and rehydrated by using slow-cooling and vitrification techniques, but further work is needed to improve the 30-40% pregnancy rates reported to date. The first *in vitro* produced (IVP) camel calf has been born, which is very encouraging, but much more research is required to develop reliable methods for IVM and IVF of camel oocytes.

All these results show that with good management, controlled breeding, and strategic use of hormone treatments, it is possible to increase the reproductive efficiency of camels.

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