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Development of a large commercial camel embryo transfer program: 20 years of scientific research☆

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ABSTRACT

Embryo transfer in camels was initiated to respond to demand from the camel industry particularly in the United Arab Emirates since 1990. This paper reviews the research performed in critical areas of reproductive physiology and reproductive function evaluation that constitute a pre-requisite for a successful embryo transfer program. A description of donor and recipient management as well as a retrospective evaluation of calf production in the embryo transfer program at Sweihan, UAE is provided. The program utilized two management systems for donors, with and without ovarian superstimulation. Non-stimulated donors are flushed every 14–15 days with a mean embryo production per year per female of 8.5 ± 3.1 (mean ± SEM). Response to gonadotropin stimulation is extremely variable. FSH doses and frequency of administration is often adjusted to a specific female. In the period of 1990–2010, 11,477 embryos were transferred to recipients. Transfers from 1990 to 2009 (n = 10,600) resulted in 2858 weaned calves, representing an overall efficiency (% weaned calves/transfer) of 27%. Pregnancy rates at 60 days post transfer varied from 19 to 44%. Pregnancy length following transfer is extremely variable. A major challenge in a large embryo transfer program is finding good quality recipients. Causes of pregnancy and neonatal losses are under study.

1. Introduction

Camel embryo transfer has seen tremendous development since 1990. This interest has been driven mainly by the camel racing industry and stems from several inherent and external factors that limit maximization of female genetic merit which is essential to the camel racing industry (i.e. the majority of racing animals and females). In the traditional rearing system, dromedary females have a long (18–30 month) calving interval (Tibary et al., 2005). In addition, the top racing females retire from competition at a relatively advanced age limiting the number of offspring they can have in their reproductive career. Multiple ovulation and embryo transfer (MOET) and associated reproductive biotechnologies allow shorting of generation interval, optimization of mating plans (i.e. more male choices during one season) and the potential of using females for reproduction while they are still in competition. In our laboratory, the production of calves by embryo transfer increased from 30 per year in the early 90s to >300 in 2010.

The objective of this paper is to describe the scientific program behind the development of a leading center for camel reproductive biotechnology. We describe

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contributions of our research team as well as others in this area, and discuss the development of reproductive biotechnology in the camels.

2. Physiological basis of in vivo embryo production

2.1. Follicular dynamics

Management of donors for MOET depends on understanding the follicular cycle, ovulation and early embryo development. Until the mid 1980s, most of our knowledge on the reproductive process in the female camel was based on clinical studies (mainly behavioral and per rectum palpation) and on postmortem observations (mostly slaughterhouse specimens; El Wishy and Hemeida, 1984; Musa and Abu Sineina, 1976; El Wishy and Ghoneim, 1986). Studies to characterize endocrine aspects of the reproductive pattern in the female camel started in the mid to late 80s through the purification and the characterization of camel gonadotropins particularly LH and FSH (Marie and Anouassi, 1986; Anouassi et al., 1987; Marie and Anouassi, 1987; Marie, 1987; Anouassi, 1991). Access to ultrasound imaging technology in the mid-1980s and early 1990s permitted more direct study of follicular activity by several authors (Tinson et al., 1992; Tibary and Anouassi, 1996).

The female camel has a seasonal reproductive pattern that is primarily controlled by nutritional condition and management (Tibary and Anouassi, 1997c; Sghiri and Driancourt, 1999; Tibary et al., 2007). Under optimal herd health management and nutritional conditions, camels display ovarian activity throughout the year. However, attempts to collect and transfer embryos during the hottest months of the year resulted in lower pregnancy rates after transfer, probably because of the effect of heat on the quality of embryo (Tibary and Anouassi, 1997c).

Follicular dynamics in the dromedary female has recently been reviewed in detail (Tibary et al., 2007; Skidmore, 2011). In the absence of mating or other ovulatory stimuli (i.e. GnRH or hCG treatment), there is a succession of overlapping follicular waves with variable rhythm showing 3 phases: growth, maturation and regression (Skidmore et al., 1996; Tibary and Anouassi, 1996; Tibary et al., 2007). The period of follicular growth lasts 10.5 ± 0.5 days during which follicles grow at a rate of 1 mm per day. Mature follicles continue to grow at a rate of 1.8 mm per day until they reach a maximum size which ranges from 10 to 25 mm. Duration of the mature follicle phase is 7.6 ± 0.8 days. Mature follicle stay at the maximum size for 2–3 days before undergoing regression which lasts about 11.9 ± 0.8 days. Follicular size is positively correlated with plasma estradiol 17ß concentration (Marie, 1987). In the absence of ovulation, the mature follicle continues to grow and reach sizes varying from 25 mm to 75 mm in 40–50% of the follicular waves. About one third of these anovulatory follicles become hemorrhagic and even undergo partial luteinization, sometimes causing challenges in the management of donors for superovulation. Anovulatory follicles usually regress over a period varying from 8 to 45 days and do not always inhibit development of a new follicular wave (Tibary and Anouassi, 1996; Tibary et al., 2007; Skidmore, 2011).

2.2. Ovulation and luteal phase

The occurrence of an LH surge following mating by a vasectomized male, followed by a rise in serum progesterone levels, was a landmark in the study of ovulation in camels (Marie and Anouassi, 1986). A similar luteal phase is obtained after injection of GnRH or hCG at the peak of follicular development (Anouassi, 1984). Plasma LH level (determined every 10 min) surges within 80 min after mating and reaches a maximum level 4 h later. The LH peak is maintained for 2–3 h before decreasing and ovulation occurs in most females within 24–48 h after mating (Marie and Anouassi, 1987). Because of the relatively slow LH surger after mating, it was hypothesized that the mechanism of LH release is not through a neural reflex (Marie, 1987). The role of seminal plasma in induction of ovulation had been demonstrated in the Bactrian camel but our studies on artificial insemination showed that in utero semen deposition alone does not guarantee ovulation in the dromedary camel (Anouassi et al., 1992).

Purification of the major camel pituitary hormones (FSH, LH, Prl, and GH) allowed for development of specific homologous enzyme immunoassays for camel gonadotropins (Anouassi et al., 1987; Combarnous et al., 1989, 1990; Martinat et al., 1990a,b; Anouassi, 1991; Anouassi and Combarnous, 1991; Anouassi et al., 1991; Combarnous and Anouassi, 1994).

Spontaneous ovulations have been reported to occur in 1.4% and 14.3% of follicular waves in non-lactating and lactating camels, respectively (Nagy et al., 2005). Analysis of our database on regular examinations of more than 3000 recipients per year does not corroborate this observation. However, it is important to note that our animals are screened for any reproductive disorders and are not lactating when they enter the recipient herd.

The highest ovulatory response (85–100%) is obtained when the dominant follicle is between 12 and 16 mm in diameter and the uterus presents maximum tone and edema as assessed by transrectal palpation and ultrasonography (Anouassi et al., 1994; Tibary and Anouassi, 1996). The corpus luteum is easily recognizable by ultrasonography at 6 days post-mating. Its maximum diameter (15–25 mm) is reached at Day 7.2 ± 1.7 after ovulation. Luteolysis occurs on Day 10 ± 1.2 after mating (Marie and Anouassi, 1987; Tibary et al., 2007). On Day 14 post-ovulation, over 85% of the females have a mature follicle and are ready to ovulate again if mated. This 14-day cyclicity in ovulating females is used frequently in our laboratory for collection of embryos without ovarian stimulation (Tibary et al., 2007; Skidmore, 2011).

2.3. Hormonal induction of ovulation

Ovulation may be induced 26–48 h following treatment with GnRH (0.5–1 mg im or iv) (Bono et al., 1985; Anouassi et al., 1994) or its analog Busereline (15–20 μg, iv) (Cooper et al., 1990, 1992; McKinnon et al., 1992; Skidmore et al., 1992; Musa et al., 1993; Skidmore et al., 1995). Follicles

Table 1
Effect of copulation length on ovulation and embryo recovery in dromedary camels (Tibary and Anouassi, 1997a,b,c).

Mating duration (min)	Number of females	% non-ovulating females	% females with at least one embryo
Less than 1.5 min	45	35.6	44.9
1.5–3	232	13.8	53.5
4	102	13.9	56.8
4.5–5	102	16.7	65.9
5.5–6	57	14	77.6
>6	94	8.5	55.3

become responsive when they reach 9 mm in diameter. However, the maximum response is seen when follicular size is between 10 and 22 mm (Anouassi et al., 1994; Skidmore et al., 1995; Tibary and Anouassi, 1996). The proportion of non-ovulating females (10–15%) is comparable to that observed after natural mating and could be due to inadequate release of LH (Anouassi et al., 1994; Tibary and Anouassi, 1996).

Ovulation may be induced by administration of hCG (2500–4000 IU, im or iv). Response rates range from 85 to 100% in animals selected on the basis of follicular size and uterine tone (Anouassi and Ali, 1990; Cooper et al., 1990, 1992; McKinnon et al., 1992; Skidmore et al., 1992; Ismail et al., 1993; Anouassi et al., 1994; Skidmore et al., 1995).

2.4. Fertilization and embryo migration

Fertilization rate is very high and embryo development is rapid (Tibary et al., 2007). Embryos enter the uterine cavity approximately 6.5 days after ovulation at the hatching or early hatched blastocyst stage and start to elongate by Day 10 and the trophoblast establishes close contact with the endometrium by Day 14 (McKinnon et al., 1994; Tibary and Anouassi, 1997a,c; Tibary, 2001b). Nearly all pregnancies are established in the left uterine horn regardless of the side of ovulation. Differential luteolysis occurs in the left and right uterine horn which explains embryo migration from the right to the left uterine horn (Ghazi, 1981; Tibary and Anouassi, 1997c). In the dromedary, the early embryo (Day 10) exhibits high aromatizing activity and synthesizes large amounts of estrogens which may be involved in prevention of luteolysis (Skidmore et al., 1994).

3. Donor management

Although our donor females are predominantly retired racing animals, show and dairy animals have been increasingly presented by owners for embryo transfer in recent years. All donors undergo a thorough health screening before they are admitted to the facility. Donors are kept in quarantine for 4 weeks, during which they are screened for major contagious diseases (trypanosomiasis, camel pox, brucellosis). All animals receive prophylactic antiparasite and a trypanicide (quinapyramine sulfate) treatment during the period of observation. After initial health screening, donors undergo a breeding soundness examination which includes transrectal palpation and ultrasonography of the genital system and vaginal examination. Uterine cytology and culture are performed when indicated (Tibary et al., 2001). Overall, 20% of females presented as potential donors have reproductive disorders. The most common reproductive problems are uterine infection and ovario-bursal adhesions (Tibary and Anouassi, 2000, 2001a,b; Tibary et al., 2001, 2006). A priority is given to diagnosis and treatment of uterine infection as this can have a disastrous effect on a program that involves natural breeding where males may have up to 4 matings per day (Tibary et al., 2005).

3.1. Embryo collection without superovulation

Embryo collection without ovarian stimulation is preferred for females that are refractory to superovulation treatment or when recipient availability is limited. This technique is also preferred by some owners because it allows production of calves from different sires in the same season without adding to the cost of recipient management.

Donors are monitored by transrectal palpation and ultrasonography and mated when the diameter of the follicle reaches 12–14 mm and the uterus shows maximum tone and edema. All mated females are given an injection of hCG or GnRH immediately following mating to insure ovulation. Although no direct experimentation was performed to determine the necessity for this treatment, our experience shows that it is helpful in some instances particularly when there is a high demand for a particular male as frequent use of the male results in a shorter duration of mating and possibly a lower ability for induction of ovulation (Table 1). In this system the donor can be flushed for embryo collection every 14 days (Table 2). The highest number of pregnancies obtained from a single female in a season using this approach was 29. Studies are underway to determine if repeated collection at short interval may affect conception rates.

Table 2
Embryo recovery in non-stimulated females following flushing on Day 7 to 7.5 days after mating.

Number of females	186
Ovulation rate	84.9%
Recovery rate	94.9%
Average embryo per female	1.16
Pregnancy rate after transfer	42%
Interval between collection and breeding	4.1 ± 2.1 days
Interval between two collections	14–16 days
Number of collection per season	10 ± 4.2
Average number of transferable embryos per season	8.5 ± 3.1
Mean number of pregnancies per season	4.1 ± 1.2

3.2. Embryo collection with superovulation

Ovarian stimulation protocols used in ruminants have been adapted to the female dromedary with variable success (Anouassi and Ali, 1990; Cooper et al., 1990, 1992; McKinnon et al., 1992; Skidmore et al., 1992). However, most reports do not provide complete descriptions of the superovulation protocol (dosage and monitoring of follicular activity) and embryo collection rate. Ovarian stimulation treatments include the use of ovine (oFSH), porcine (pFSH), camel (cFSH), eCG or a combination of FSH and eCG (Anouassi and Ali, 1990; Cooper et al., 1992; McKinnon et al., 1992; Skidmore et al., 1992). Treatment is initiated after synchronization of follicular wave with progesterone, elimination of the dominant follicle by GnRH or hCG treatment, or at the early stage of follicular wave (no follicle >2 mm). Ovarian stimulation by gonadotropin is influenced by season (Nowshari and Ali, 2005).

FSH of ovine or porcine origin has been used for superovulation in the dromedary. A total dose of 20–30 units of oFSH is given over 6 days (two injections daily) (Cooper et al., 1990, 1992; Skidmore et al., 1992). FSH treatment is started 2 days before and up to 1 day after completion of a 7 days course of progesterone treatment by intravaginal device (PRID). However, these authors did not specify whether the total dose was distributed in constant, increasing or decreasing fashion. The results of this treatment were very low in terms of embryo recovery. Of 11 females treated 8 did not yield any embryos, one female gave one embryo, one gave 4 embryos and another female gave 12 embryos. The interval from PRID removal to mating (sexual receptivity) was 7 and 8 days respectively for females treated with 20 or 30 units of oFSH on the day of PRID removal and 4.3 and 3 days when treatment was given one day before PRID removal (Cooper et al., 1990). Embryo recovery rates were very poor. FSH was also given in a single small dose (3.3 units) followed by an injection of 3500 IU of eCG and resulted in an average of 7 embryos recovered per treated female (Skidmore et al., 1992). However, these results are likely due to the effect of eCG rather to FSH. In another study, ovine FSH was given twice a day (1–3 mg per injection) during 3–5 days following a 10–15 day course of progesterone treatment (100 mg per day during 10–15 days).

Porcine FSH given in decreasing doses over 3, 5, or 7 days after a 10–15 day progesterone treatment also resulted in superovulation of dromedary females. The interval from treatment to development of mature follicles varied from 6 to 8 days. Superovulation can be obtained even without previous progesterone treatment. However, the best results are obtained when the treated females have no follicular structures on the ovary (Tibary and Anouassi, 1997a; Tibary et al., 2007).

Equine Chorionic Gonadotropin (eCG) was used for ovarian stimulation in the dromedary female at doses varying from 1500 to 6000 (Anouassi and Ali, 1990; Cooper et al., 1990; McKinnon et al., 1992, 1994). This hormone is generally administered in a single dose one day before or on the day of completion of a 5- to 15-day progesterone regime. A single injection of 2000 IU, 2500 IU or 4000 IU given one day before or one day after PRID removal resulted in ovulation in only 12 females out of 30 (40%) and only 5/12 (41.7%) ovulating females yielded at least one embryo (one = 1 embryos; 3 = 2–5 embryos and 1 = 6–12 embryos). The interval from PRID removal to mating was 5 and 4.5 days respectively for females receiving 2500 IU and 4000 IU of eCG. This interval was one day shorter in females treated with eCG one day before removal of PRID. Mating in this study was based on sexual receptivity which is not a good indicator of ovarian status and maturity of follicles. In our laboratory, the interval from eCG treatment to mating based on the presence of a mature follicle (diameter at least 12 mm) is relatively constant (8 days) (Cooper et al., 1990, 1992; Skidmore et al., 1992).

Table 3
Results of MOET using FSH (400 mg divided in decreasing doses) and eCG (single injection of 3000 IU).

	FSH	eCG
Number of treatments	176	153
% responding to stimulation	69.9	69.3
% ovulating	79.7	79.2
Number of embryos per collection (range)	8.2 ± 6.1 (0–36)	7.1 ± 4.3 (0–19)
Stage of embryo at collection		
Hatched blastocysts (%)	90	90
Morula or early blastocysts with zona pellucid (%)	8	6
Unfertilized (%)	2	4
Pregnancy rate at 25 days	46.2	38
Pregnancy rate for excellent embryos	60	61
Pregnancy rate for medium quality embryos	22	21

Follicular response following treatment with 1500 IU or 2000 IU of eCG was respectively 4.4. ± 3.2 (range 0–10) and 5.7 ± 5.4 (range 0–19). The proportion of females with 4 or more follicles after treatment was 6/9 and 11/21 respectively for females receiving 1500 IU eCG and those receiving 2000 IU eCG. The proportion of females that did not show any follicular growth following injection of 1500 IU and 2000 IU of eCG was respectively 2/9 and 4/21(Anouassi and Ali, 1990).

Our earlier observations showed that progesterone implants have an inhibitory effect on LH release but do not inhibit follicular development (Anouassi, 1984). Therefore, we developed a protocol for ovarian stimulation based on initiation of gonadotropin treatment 2–4 days after induction of ovulation or when the female did not have any follicle greater than 2 mm on the ovaries. In our laboratory, treatment with cFSH and oFSH failed to produce ovarian stimulation and multiple embryo recovery in a reliable fashion. Stimulation with eCG (1500–3000 IU) gave variable results (Table 3). The interval from eCG administration to breeding is on average 8–9 days. However, we observed several problems with eCG treatment including development of two different generation of follicles, premature luteinization of follicles and failure of ovulation and development of large anovulatory follicles. Additionally, repeated use of eCG in the same females induced a refractoriness to the treatment. Our preferred treatment for superovulation presently is the use of pFSH twice daily

in decreasing doses. The total dose and number of days of treatment is tailored (400–600 mg of pFSH) to each donor female based on ultrasonographic monitoring of her response. The number of days of treatment ranges from 4 to 7 with the majority of females (80%) treated for only 4–5 days. It is important to note that camels can become refractory to pFSH. Donors are never superovulated at the beginning and end of the breeding season and the number of superovulation treatments is kept to two per season with a minimum of 60 days period of rest between treatments. Superovulation problems are of two types: lack of response (20%) or overstimulation (15–20%) (Table 3) (Tibary and Anouassi, 1997a).

3.3. Problems with superovulation in camelidae

Major problems that need to be addressed are a high incidence of superstimulation failure, premature luteinization of follicles, variability in ovarian response among females, and apparent refractoriness to repeated superstimulation treatments. Between 20 and 30% of donors given superstimulatory treatments do not develop multiple large follicles. Premature regression of the follicles subsequent to FSH treatment may be associated with inappropriate dosage or method of delivery. Luteinization of follicles before ovulation may be due to the LH activity of superstimulatory treatments and, results of a preliminary trial with highly purified FSH are encouraging. Regarding variability in response, some females do not respond to treatment at all and others "over-stimulate" (i.e., more than 30 large follicles per ovary). Most of the females with over-stimulated ovaries do not yield any embryos, perhaps because of disturbance of gamete transport or fertilization processes. Lastly, some camels appear to become refractory to superovulation with either FSH or eCG, perhaps as a result of developing an antibody response to these hormones. We have observed a complete arrest of ovarian activity in 5 females that have been repeatedly treated with these hormones.

3.4. Donor insemination and verification of ovulation

All breedings in our program are scheduled based on ultrasonographic evaluation of follicular size and uterine tone. Donors are bred once when the largest follicle(s) are 12–16 mm in diameter and uterine tone is high. Follicles are generally 5–6 mm in diameter 3–5 days after initiating superstimulatory treatment and will continue to grow at a rate of 1.8 mm per day; hence breeding is performed about 7–9 days after initiation of treatment.

Ovulation is confirmed by ultrasonography or progesterone assays (Anouassi and Ali, 1990; Cooper et al., 1990; Tinson et al., 1992; Anouassi et al., 1994; Tibary and Anouassi, 1996). Visualization of the corpora lutea is difficult in the first 3 days post ovulation but becomes easy thereafter. Plasma progesterone levels start to increase 2–3 days after ovulation and reach high levels (>2 ng/ml) by Day 5 after ovulation. Plasma progesterone levels are highly correlated with the number of corpora lutea but can also be elevated in the case of luteinization (Tibary and Anouassi, 1997a).

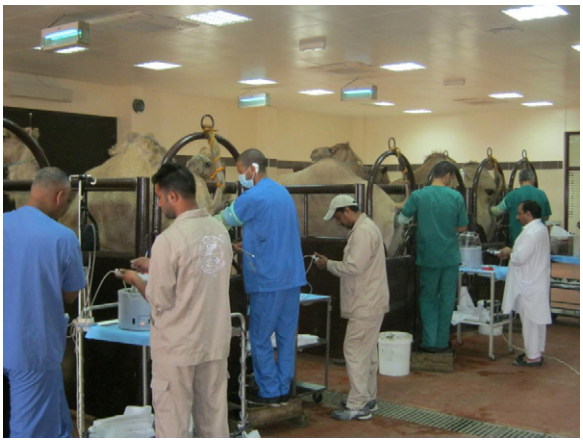

Fig. 1. Embryo collection facility at the Sweihan laboratory.

4. Embryo collection and evaluation

Camelid embryos enter the uterus 6–6.5 days after ovulation. For maximum embryo recovery, flushing is performed 7–8 days after mating. Embryo recovery rates (embryos recovered/ovulations) are highly variable and depend on many factors including superovulation treatment, fertility of the donor and the male, management, collection date and technician experience (McKinnon et al., 1994; Tibary and Anouassi, 1997a; Tibary, 2001a). Recovery rate from the dromedary is 85% in single ovulators and 165% in double ovulators (Tibary and Anouassi, 1996). Only hatched blastocysts are transferable.

All procedures for embryo collection and embryo transfer in our laboratory are performed on the standing animal in specially designed stocks (Fig. 1). The use of stocks is extremely important for a large embryo transfer program not only for its practicality and ease when hundreds of females are examined daily but also to reduce risk of contamination.

Embryo flushing is performed using a non surgical technique similar to that described for the bovine. The donor is placed in palpation stocks; the rectum is cleaned from all feces; the tail is wrapped or held high. The perineal area and the vulva are scrubbed. An 18–22 gauge Foley catheter with a stylet is threaded through the cervix by recto-vaginal manipulation (Anouassi and Ali, 1990; Tibary and Anouassi, 1997a). This technique is extremely important because dromedary females do not tolerate vaginal manipulation very well and the risk of contamination of the uterus and vagina is reduced. Passage of the cervix by rectovaginal manipulation is somewhat difficult for the inexperienced practitioner especially in older and superovulated females. Some authors suggest the use of low epidural anesthesia prior to embryo collection (McKinnon et al., 1992; Skidmore et al., 1992; McKinnon et al., 1994) but this is rarely used in our laboratory.

Although the whole uterus may be flushed at one time by placing the cuff just cranial to the cervix, we prefer

individual horn flushing by placing the cuff at the base of the horn in order to avoid loss due to cervical dilation (Anouassi and Ali, 1990; McKinnon et al., 1992; Skidmore et al., 1992; McKinnon et al., 1994). The catheter cuff is inflated with 20–30 ml of air and the uterine horn is flushed repeatedly with 30–70 ml of commercial medium (DPBS with 1% FCS and 0.2% BSA). A total of 500–1000 ml of medium is used per female. The uterus is manipulated transrectally during the flushing to evaluate distention and ensure fluid recovery. Initially we recovered fluid in sterile siliconized cylinders and allowed it to settle at 37 °C for 20 min before siphoning using a tube fitted with 75 μm embryo filter to reduce the volume to 30 ml. More recently we have been recovering the flushing fluid directly into an embryo filter.

At the beginning of the program all embryos were evaluated using a 5 grade system based morphological characteristics and stage of development (Tibary and Anouassi, 1997a). All embryos were photographed before transfer. However, this system was simplified in recent years to include only 2 categories: transferable embryos (hatched blastocysts with normal appearance) and non-transferrable embryos (unfertilized and with obvious degenerative changes such as severe collapse and darkened appearance). Our earlier observations showed that normal appearing morulas or young blastocysts with a zona pellucida recovered from the uterus are be considered arrested and non-transferable (Tibary and Anouassi, 1997a).

5. Recipient selection and management

There are two contracts used for the programs. For resident donors embryos are transferred into females selected from our own herd of recipients which includes 800–1000 females. For visiting donors, owners are requested to provide at least 10 recipients per donor. All animals are identified and screened as describe above for the major contagious diseases before introduction to the center.

5.1. Animal Identification

With nearly 2500 females in our facility, we have developed an identification system to help maintain an electronic database for individual record-keeping. Camels are fitted with a neck-strap with a unique number that is easy to read by the handler, and an electronic microchip identification in implanted intramuscularly (Trovan®, Trovan electronic identification system, Koln, Germany). We have found this system to be reliable and safe, and is presently being updated to include a Bluetooth-enabled reader to allow direct recording of examinations into the computerized database. The electronic identification is also linked to the DNA parentage verification certificate. During the peak of the breeding season, between 24 and 30 donors are flushed and regular ultrasound monitoring activity is performed on about 120 females daily. Data are entered into the database and a list of females to be examined is generated daily.

5.2. Health screening

Recipient females are selected on the basis of their reproductive and health history. We encourage owners to bring in females between 5 and 13 years of age which have recently weaned a calf. A breeding soundness examination protocol was developed to screen for common reproductive problems and includes transrectal evaluation by palpation and ultrasonography, vaginoscopy and endometrial cytology. Uterine culture and biopsy is performed on select cases (Tibary et al., 2001, 2005).

5.3. Nutrition

Nutritional management of donors and recipients is of paramount importance for the breeding program. However, there are very few studies on nutrient requirements in camels. Animals at the center receive daily rations of Rhodes and alfalfa hay as well as vitamin and mineral supplementation. Concentrate is given to thin animals particularly donors. Body condition of animals is monitored at regular intervals according to a system we developed internally (Tibary and Anouassi, 1997b; Tibary et al., 2005).

5.4. Selection of recipients for transfer

The first attempts of embryo transfer at our center showed that the best pregnancy rates are obtained in recipients that have ovulated one or two days after the donor (Anouassi and Ali, 1990). Synchronization of follicular development in donors and recipients has been attempted using progestagen with variable degrees of success. We select females on the basis of follicular size from a large pool of recipients and induce ovulation with GnRH or hCG (Tibary and Anouassi, 1997a). Progesterone assay the day before transfer was used in our laboratory previously but has been abandoned because it did not provide any additional information compared to clinical evaluation. Presently all recipients are examined by ultrasonography on the day of transfer and selected based on presence and size of the corpus luteum.

All transfers are done non-surgically using the recto-vaginal technique as in the bovine using a side delivery transfer gun fitted with a chemisette to prevent contamination of the uterus. Although we have shown that transfer of embryos into the uterine horn ipsilateral to the corpus luteum is preferable in order to achieve the maximum pregnancy rates, this advantage is offset by lengthy manipulation during transfer. Therefore we emphasize speed of transfer rather than deposition of the embryo ipsilateral to the corpus luteum to avoid any damage to the endometrium (Anouassi and Tibary, 2010) (Table 4). The time from collection to transfer of embryos including evaluation, washing of embryos and loading of embryos into 0.5 ml straws is less than 2 h.

6. Embryo yield

Embryo recovery rates are highly variable and depend on several factors. Embryo recovery rates in the dromedary vary from 114% to 384% and are affected by several factors

Table 4
Pregnancy rate (PR %) obtained following transfer of embryo to the horn ipsilateral or opposite horn to the CL bearing ovary (Khatir et al., 2005).

CL side/Horn side	Right/Right	Left/Left	Right/Left	Left/Right
Year 1 PR (*n*)	69.6a (102)	47.8b (90)	53.3b (30)	50.0b (28)
Year 2 PR (*n*)	61.5a (39)	57.1a (21)	52.6a (78)	47.4b (97)
Year 3 PR (*n*)	68.6a,b (70)	71.8a,b (32)	57.1a (28)	72.6b (62)
Total PR (*n*)	67.8a (211)	54.5b (143)	53.7b (136)	56.1b (187)

Different letters within the same row represent significant difference ($P < 0.05$).

such as superovulation treatment, fertility, management, collection date and experience (McKinnon et al., 1992, 1994; Tibary and Anouassi, 1997a). In non stimulated females embryo recovery rate is 85% in single ovulators and 165% in double ovulators (Table 2).

The type of superovulation treatment can have a great effect on the embryo recovery rate. In a retrospective study, FSH treated dromedary females yielded more embryos (417% for natural mating, 188% for artificially insemination) than eCG treated females (227% natural mating, 69% AI) (McKinnon et al., 1994). The low recovery rates obtained in eCG superovulated females can be due to an increase in spontaneous luteinization associated with the high LH activity in eCG and its long lasting effect (McKinnon et al., 1992, 1994). Similar results have been observed by our group (Table 3).

6.1. Effect of timing of flushing on embryo recovery rate

Studies on the timing of embryo recovery from the uterus in the dromedary female have been rather confusing until recently. Some studies refer to day of mating while others refer to day of ovulation. In addition, the use or lack of use of hormonal induction of ovulation following mating may have further confounded this issue. Early reports suggested that the embryo reaches the uterus on Day 6 to 6.5 after ovulation (Anouassi and Ali, 1990; McKinnon et al., 1994). In practice, the uterus is flushed 7–8 days after mating and treatment with hCG or GnRH (Anouassi and Ali, 1990; Cooper et al., 1990; McKinnon et al., 1992; Skidmore et al., 1992; McKinnon et al., 1994). The best embryo recovery rates are obtained when the donors are flushed on Day 8 post-mating. In two separate studies from the same group, the recovery rates were 114%, 179% and 267% after flushing on Day 6, Day 6.5 and Day 7 after mating, and 60%, 157%, 175%, 200% and 300% for collections at Day 6, Day 6.5, Day 7, Day 7.5 and Day 9, respectively (McKinnon et al., 1992, 1994). The effect of collection date on embryo recovery rates is even more pronounced in superovulated females because of possible delay in oviductal transport and fertilization. This observation is substantiated by the high frequency of pregnancies observed in donors after embryo collection. Several donors become pregnant even after two flushing at 12–24 h interval (McKinnon et al., 1992, 1994).

6.2. Flushing technique

Inexperience of the operator can account for many unsuccessful flushings. In our laboratory, recovery rates on single ovulators increased from 60% to 85% with increased experience of the operator. Most inexperience practitioners have difficulties determining the position of the cuff and can go too far into the uterine horn. In addition, an error in position of the cuff results in loss of fluid in the vaginal cavity. In individual horn flushing, insertion of the catheter into the left horn may be difficult for left handed palpators and vice versa. Harsh manipulation can also lead to bleeding which makes embryo identification very difficult (Tibary and Anouassi, 1997a).

6.3. Ovulation failure

Ovulation failure occurs in 15–20% of stimulated donors even when hCG or GnRH is administered (Anouassi and Ali, 1990; Tibary and Anouassi, 1996). In some cases, the follicles regress after breeding instead of ovulating. Poor ovulation response can also occur when breeding decision is based only on receptivity of the female. This is due to the fact that superovulated females can be receptive even in the presence of small immature follicles which will fail to ovulate. This may be the reason why early work on embryo transfer in the dromedary resulted in poor embryo recovery results (Cooper et al., 1990, 1992). In eCG treated female, ovulation failure may be due to early luteinization of the follicles.

6.4. Fertilization failure

Reduced or complete failure of fertilization has been documented in some males with low quality semen. In one study all females mated to a male that had low sperm concentration (<20 million/ml) failed to produce an embryo (McKinnon et al., 1992, 1994).

Several pathological problems such as salpingitis and endometritis affect fertilization and embryo survival and reduced embryo yields in donors. In the case of endometritis, the flushing fluid is usually opaque or cloudy and contains cellular debris and pus (Tibary and Anouassi, 1997a). Fertilization and embryo survival may also be affected by the age of the donor. In one study on the dromedary, it has been found that embryo yield is higher in donors 12 years or older than in younger female (285% vs. 149%). Also, multiparous females yielded more embryos than nulliparous females (353% vs. 121%) (McKinnon et al., 1992, 1994). The effect of age could also be due to increased incidence of overstimulation of the ovaries in younger females.

Fertilization or ova and embryo transport can also be hindered by the presence of large anovulatory follicles. Embryo recovery rate was found to be higher in females without (220%) than in females with anovulatory follicles (148%) (McKinnon et al., 1992, 1994). The incidence of

anovulatory follicles is 8–10 times higher in females superovulated randomly (43%) than in females superovulated following a 10–15 day progesterone treatment course (4%) (McKinnon et al., 1992, 1994).

Lower embryo recovery rates probably due to reduced fertilization are also observed when donors are artificially inseminated. Recovery rates in donors bred naturally are consistently higher (299%) than in females inseminated with fresh raw (199%) or extended semen (124%) (McKinnon et al., 1992, 1994). Whole ejaculate insemination performed at our center results in better fertilization and embryo recovery rates (Anouassi et al., 1992). In non-stimulated females, deep horn insemination with diluted semen containing as little as 12 million spermatozoa has resulted in adequate fertilization and pregnancy rates (Anouassi and Tibary, 2010). Embryo recovery rate was severely affected by the use of frozen thawed semen despite good post-thaw motility (Tibary and Anouassi, 1997a).

7. Embryo quality

Embryos recovered from the uterus are at the hatched blastocyst (Cooper et al., 1990; Anouassi et al., 1992; Cooper et al., 1992; McKinnon et al., 1992; Skidmore et al., 1992; McKinnon et al., 1994). The embryos recovered from the dromedary camel 7 days after mating are extremely variable in size and have a diameter ranging from 175 to 500 μm (Skidmore et al., 1992). This variability of the stage of development is probably due to the wide spread of ovulations in superovulation animals. Hatched embryos continue to grow rapidly and become easily visible to the naked eye as they expand. They start losing their spherical form by Day 8.5 or 9 post ovulation (Anouassi and Ali, 1990; McKinnon et al., 1994; Tibary and Anouassi, 1997a; Tibary et al., 2007).

8. Embryo preservation

With the exception of a few trials most of the transfers in the dromedary and particularly in commercial embryo transfer operations are done with fresh embryos. In our laboratory, MOET has been practiced since 1990. During the period between 1992 and 1998, a total of 2653 fresh embryos were transferred, resulting in an overall pregnancy rate of 62% at 35 days. Pregnancy rates improved steadily from 30% to 70% over that period. It is not uncommon to achieve a pregnancy rate of 100% with some batches of embryos. Low pregnancy rates and high early pregnancy loss rates are observed when transfers are performed during the hottest months of the year (McKinnon et al., 1994; Tibary and Anouassi, 1997a).

Short term preservation of camel embryos is possible through cooling or in vitro culture and offers an alternative to freezing if recipients are not available (Skidmore et al., 2002). Pregnancy rates following short term preservation by in vitro culture are slightly lower than following transfer of fresh embryos but still within acceptable range (Khatir et al., 2009).

Cryopreservation of camel embryos using slow freezing methods results in poor (0–15%) pregnancy rates (McKinnon et al., 1994; Tibary and Anouassi, 1997a), probably due to the stage of development at which they are collected (i.e. hatched blastocyst) and to their size (i.e. 400–2500 μm). More recently the use of vitrification techniques, thought on small numbers of animals, resulted in higher pregnancy rates particularly for smaller embryos (Day 6 post-ovulation (Nowshari et al., 2005; Skidmore et al., 2005, 2009).

9. Effect of recipient synchrony of success of ET

Because of the small window of opportunity to transfer hatched blastocysts before initiation of luteolysis, we focused mainly on using asynchronous recipients for our program. Preliminary results in our laboratory between 1990 and 1994 showed that the best pregnancy rates are achieved when recipients ovulated one or two days after the donors. Later we observed that pregnancy rate were similar for synchronous and asynchronous females (-1 or -2 days). Similar results have been reported recently (Khatir et al., 2005). Early pregnancy loss due to failure to prevent of luteolysis was considered a big problem. In attempt to circumvent this problem, recipients were given progesterone one day before transfer and progesterone treatment was continued until pregnancy diagnosis. Females that were pregnant but had no corpus luteum were given a dose of eCG in attempt to create new or accessory corpora lutea. Although some success was achieved using this technique we did not think it was good enough to adopt for a large scale embryo transfer (Tibary and Anouassi, 1999). Of interest, recipients that had luteinized anovulatory follicles could be used for embryo transfer and could maintain pregnancy. Oral administration of meclofenamic acid between days 7 and 9 after ovulation has been shown to prevent luteolysis and allow use of females that have ovulated up to 5 days before the donor as embryo recipient with acceptable pregnancy rate (Skidmore and Billah, 2005).

Transfer of embryos into estradiol/progesterone-treated, bilaterally ovariectomized recipient resulted in a 30% pregnancy rate (Tibary and Anouassi, 2001a). However, daily progesterone treatment is thereafter required throughout pregnancy. Contrary to what has been reported earlier (Skidmore et al., 1992, 2002), camels that are maintained pregnant by daily exogenous progesterone treatment do not calve spontaneously and are at increased risk of dystocia from failure of cervical dilation. Moreover, progesterone treatment seems to increase the risk for inadequate milk production.

10. Overall embryo transfer program performance

Transfers at the center are performed from September until April. The mean gestation length after transfer is 379.4 days (SD = 10.72; range = 349–420 days). Factors influencing pregnancy length are still under study.

The number of calves produced by embryo transfer per year in our transfer program is shown in Fig. 2. Although the number of calves produced is increasing, the efficiency of the embryo transfer program in terms of weaned claves per transfer remains relatively low compared to other species (Table 5). Pregnancy rates at 60 days vary from 21.5% to

Table 5
Overall performance of the embryo transfer program at Sweihan Laboratory during the period of 1992–2010.

Season	# of transfers	Pregnancy rate at 2 months	Weaning rate for pregnant females	Weaning rate for all transfers
1992–1993	28	92.8	100	92.8
1993–1994	219	34.7	88.2	30.6
1994–1995	268	33.6	91.1	30.6
1995–1996	438	38.1	74.3	28.3
1996–1997	433	34.6	77.2	29.8
1997–1998	459	40.7	60.4	24.6
1998–1999	437	44.4	84.0	37.3
1999–2000	674	32.0	91.7	29.4
2000–2001	701	36.1	85.4	30.8
2001–2002	1130	21.5	74.5	16
2002–2003	998	26.5	73.1	19.3
2003–2004	992	24.5	85.2	20.9
2004–2005	534	42.5	84.1	35.8
2005–2006	750	35.5	82.7	29.3
2006–2007	859	37.4	75.7	28.3
2007–2008	858	36.8	85.1	31.4
2008–2009	822	41.0	70.0	28.7
2009–2010	877	36.5	–	–

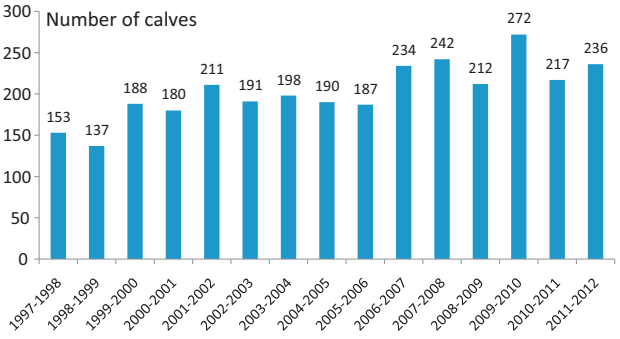

Fig. 2. Number of calves produced by embryo transfer from 1997 to 2011.

92.8%. This reflects a variation in the rate of pregnancy loss in the period between transfer and 60 days. Pregnancy rates at 14–25 days are systematically between 50 and 85%. Although efforts have been made to select recipients based on reproductive health, severe losses are registered particularly in situations of trypanosomiasis outbreaks. Also, with increasing demand for embryo transfer, most recipients are returned to their original herds after transfer and therefore to health and nutrition systems that are often less adequate that what is provided within our center. Abortion and neonatal mortality also take a heavy toll on the overall performance of the program for the same reason. Improvement in weaning rates in recipients that are directly under the veterinary control of the center has been achieved through systematic vaccination of the dams against clostridial diseases, *Escherichia coli* and pasteurellosis and insuring passive transfer of immunity through colostrum intake and better biosecurity measures in the calving pens.

11. Conclusion

Dromedary commercial embryo transfer presents several challenges due to the peculiar reproductive physiology of the species. The knowledge gained since 1990 has allowed development of MOET programs but these are still not very efficient. Ovarian stimulation treatment merits more studies. In our conditions, the most challenging aspect of a large scale embryo transfer operation is dealing with the infertile recipient and donor. In addition, results of MOET in term of number of calf production remain limited by the high incidence of early and late pregnancy loss.

Conflict of interest statement

No conflict.

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