Follicular dynamics and its importance in embryo transfer in dromedary camels (*Camelus dromedarius*)

J.A. SKIDMORE[1]

(Reçu le 10/04/2017; Accepté le 29/06/2017)

Abstract

Reproductive efficiency in camels is generally regarded to be low under natural pastural conditions and therefore understanding their ovarian dynamics and making use of assisted reproductive techniques, such as embryo transfer and artificial insemination, is paramount to improving their breeding potential. This review describes characteristics of the ovarian follicular wave pattern in camels and exogenous hormonal control of ovulation. It also summarizes the challenges involved in the transfer of fresh and frozen embryos to synchronized or ansynchrous recipients.

Keywords: Ovaries, follicular dynamics, follicular waves, ovulation, embryo transfer, dromedary camel

La dynamique folliculaire et son importance dans le transfert des embryons chez le dromadaire (*Camelus domedarius*)

Résumé

Les performances de reproduction du dromadaire en élevage pastoral sont généralement faibles. La caractérisation de la dynamique folliculaire est essentielle pour l'amélioration des performances de reproduction et l'établissement de protocole d'utilisation des techniques de reproduction assistée, telles que le transfert des embryons et l'insémination artificielle. Cet article est une synthèse de nos connaissances sur le développement des vagues folliculaires au cours du cycle et les techniques de contrôle de l'ovulation chez le dromadaire. L'article résume également les défis liés au transfert d'embryons frais ou congelés chez des receveuses synchronises ou non-synchronisées aux donneuses

Mots-clés: Ovaires, dynamique folliculaire, vagues folliculaires, ovulation, transfert d'embryon, dromadaire

INTRODUCTION

Dromedary and Bactrian camels share some unique aspects of reproductive physiology such as a short breeding season during the cooler winter months (Tibary and Anouassi, 1997a), induced ovulation in response to mating (Marie and Anouassi, 1986), a slow rise in peripheral serum progesterone concentrations after ovulation and a short luteal phase of only 9-10 days in the non – pregnant animal as well as a long gestation period of 13 months. In addition they exhibit a long (8-10 month) period of lactation- related anoestrus which leads to a long intercalving interval. These factors lead to a low reproductive efficiency, which could be increased by better control of the reproductive cycle and increased use of assisted reproduction techniques such as embryo transfer. This paper briefly describes ovarian follicular dynamics in camels and outlines methods that can be used to control their reproductive cycle and increase breeding efficiency.

FOLLICULAR WARE PATTERN

Camels are induced ovulators which means that they normally only ovulate when mated and therefore it is more accurate to describe their ovarian cycle as a follicular wave pattern rather than an oestrous cycle. The use of real-time ultrasonography has greatly enhanced the knowledge of ovarian dynamics and has shown that during the breeding season follicles pass through periods of growth, maturity and regression if ovulation is not induced by mating (Musa and Abusineina 1978; Elwishy, 1987). Although the follicular wave pattern seems to vary considerably between camels it can be divided into 3 phases: i) a growth phase lasting 6-10 (±0.5) days; ii) a mature phase of approximately 7-8 (± 0.8) days, and (iii) a regression phase of 11-12 (± 0.8) days. The follicular wave starts with a cluster of small follicles visible in both ovaries one of which is then selected to become the dominant follicle and continues to grow to a mature size of 1.3-1.7 cm in diameter. In all instances the new follicles become visible and start to grow before the mature follicle has completely regressed to give an interwave interval of 18.2 (± 1.0) days in dromedary camels (Skidmore *et al*., 1996) and 19 days in Bactrian camels (Niasari-Naslaji, 2008).

In about 50% of follicular waves exhibited by females kept separately from male camels the dominant follicle continues to grow beyond the mature size of 1.3-1.7 cm to as large as 3.0-6.5 cm in diameter which is too large to ovulate. These overgrown follicles take as long as 18.4 ± 0.8 days (range = 11-33 days) to reach their maximum diameter, they remain at the same size for 4.6 ± 0.5 days and take 15.3 ± 1.1 days to regress (Skidmore *et al*., 1996). Even though other follicles will grow, mature and

[1] Camel Reproduction Centre, P.O. Box 79914, Dubai, United Arab Emirates. Correspondence: luluskidmore@yahoo.com

ovulate (if induced to do so) alongside these overlarge follicles their occurrence can cause problems in embryo transfer programmes. Various methods have therefore been used to try and hasten their regression, or prevent their development. For example, camels with follicles of >3.0 cm diameter have been treated with either a single injection of 20 µg buserelin (Receptal) or daily injections of 150 mg of progesterone-in-oil for 14 days. Whereas it took 22 (± 1.5) days for the large follicle to regress in untreated animals , regression occurred in 14.6 (± 1.3) days in animals injected with buserelin and 12.7 (± 1.5) days in those that received daily injections of progesterone. It is possible 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. However, since waiting for overlarge follicles to regress is very time consuming, especially when trying to synchronize groups of donor and recipient animals for embryo transfer, it is preferable to prevent their occurrence by inducing ovulation with an injection of GnRH when the follicle reaches 1.3-1.7 cm diameter.

INDUCTION OF OVULATION

Ovulation can be induced in camelids by mating to an intact or vasectomized male (Marie and Anouassi 1987; Anouassi *et al*, 1992) or by a single intramuscular (i.m.) injection of seminal plasma (Pan *et al*., 1992; Adams *et al*., 2005). However, ovulation must be controlled and synchronized when preparing animals for embryo transfer, so mating to a vasectomized male, or inseminating or injecting seminal plasma is not practical because of the difficulty of collecting camel semen and the risk of spreading venereal disease. Therefore, treatment with gonadotrophic hormones or a GnRH analogue at the optimal time in the follicular growth cycle is the most practical alternative. In a previous study injecting either 20 µg of the GnRH analogue, buserelin, or 3000 i.u. human Chorionic Gonadotropin (hCG) when the dominant follicle measures 1.0 -1.9 cm in diameter resulted in ovulation rates of 80-85 %. However if the follicle measured between 2.0-2.9 cm at the time of treatment the ovulation rate was reduced to < 20 % if and if it measured > 3.0 cm then no follicles ovulated at all (Skidmore *et al*., 1996).

LUTEAL LIFESPAN

The corpus luteum has a lifespan of approximately 8.5 days as reflected by the serum progesterone profiles of mated or GnRH treated camels. Progesterone concentrations remain low (<0.5 ng/ml) for the first 3 days after ovulation (day 0) and then rise steadily to reach peak values between 2.0-4.8 ng/ml on days 7 or 8 before falling sharply on day 9 or 10 to baseline mean values of <0.5 ng/ml again on days 10-12 (Skidmore *et al*, 1996).

EMBRYO TRANSFER

There are two essential prerequisites for an efficient embryo transfer programme, firstly ovarian stimulation of the donor and secondly efficient methods to synchronise large numbers of recipient animals.

Stimulation of donor animals

Various hormonal methods have been tested in camels to try and determine the best treatment protocol to produce multiple follicles in the camel ovary. Examples include i) a single injection of equine Chorionic Gonadotrophin (eCG: 1500 – 3000 iu), ii) 400 mg of porcine FSH, (pFSH; Follitrophin) given in gradually decreasing doses over 3-5 days (eg. Day 1: 2 x 80 mg; Day 2: 2 x 60 mg; Day 3: 2 x 40 mg; Day 4: 2 x 20 mg) or a combination of both ie 2000iu eCG (day 1) + 400 mg pFSH (over 4 days) with treatment starting on day 4 after ovulation. Follicular response varies between camels but the majority of camels produce between 4-30+ follicles which take between 7-10 days from the start of treatment to reach 1.3-1.7 cm in diameter when the camel is ready for mating. There is always a small proportion (10 – 20%) of camels that do not respond at all or only produce follicles that do not grow beyond 0.9 cm in diameter (McKinnon and Tinson 1992; McKinnon *et al*, 1994; Tibary and Anouassi, 1997b; Skidmore *et al*, 2002; Anouassi and Tibary, 2013).

Synchronisation of follicular waves

Pregnancy rates of 65-75% can now be routinely achieved in camels after transfer of fresh Day 7 embryos into synchronized recipients on Day 5 or Day 6 after ovulation (McKinnon *et al*., 1994; Skidmore *et al*., 2002). However, if the level of asynchrony between donor and recipient increases to +1 day ahead of the donor (transfer into a day 8 recipient) or up to 3 days behind (transfer into a day 4 recipient) then pregnancy rates reduce to approximately 10%. Synchronizing ovulation and follicular growth poses particular problems in camels because they lack the cyclical corpus luteum of spontaneous ovulators, which means the conventional methods used in cattle that involve giving two injections of prostaglandin 11 days apart are unsuitable for use in camels (Cooper *et al.*, 1976).

Synchronization of ovulation between donor and recipient camels can be achieved by selection of recipients from a random group of camels. This involves serial ultrasound examination of the ovaries of all the recipient camels and injection of all those with a mature follicle in their ovaries with GnRH 24 -48 h h after the donor is mated. This method works well but it is labour intensive and is only feasible when a large number of recipient camels are available.

Alternatively, recipients can be treated daily with progesterone-in-oil (100 mg/day) for 10-15 days followed by administration of 1500-2500 i.u. eCG. The eCG treatment is scheduled for the day after the donor receives eCG and should guarantee the presence of mature follicles in the recipient 24-48 h after the donor has ovulated. However, although the progesterone treatment reduced the rate of follicular growth it did not inhibit it completely, so response to the eCG and time taken for the next follicle to reach a mature size was variable (McKinnon *et al.*, 1994). More recently two injections of GnRH given 14 days apart, with the second GnRH scheduled to be administered the day after the donor is mated, has given promising results with ovulation rates of over 80% after the second GnRH injection (Skidmore *et al.*, 2009; Nikjou *et al.*, 2008).

METHODS TO BROADEN RECIPIENT AVAILABILITY FOR EMBRYO TRANSFER

As discussed above it can be difficult and time consuming to accurately synchronize donors and recipients. It would therefore be of great value to be able to establish pregnancies using non-synchronised camels as recipients. A number of experimental approaches have been tested.

For example, if the recipients ovulate too late (ie they are only on day 3 or day 4 after ovulation at the time of transfer) pregnancy rates of 50% (ov+3) and 62% (ov+4) have been achieved if the recipients are treated with a daily i.m. injection of 75 mg progesterone-in-oil from 2 days before embryo transfer to 9 days after ovulation (Figure 1).

Conversely a further study has shown that if recipients ovulate too early (are on day 8, 10 or 12 after ovulation) at the time of embryo transfer, pregnancies can be achieved if the recipients are treated with meclofenamic acid. Meclofenamic acid is a prostaglandin synthetase inhibitor, which prevents both the luteolytic action of exogenous PGF2α and the normal increase in peripheral plasma PGFM concentrations in late dioestrus, and thereby prolongs the luteal phase. If meclofenamic acid is administered orally to camels from Day 7 after ovulation until 7 days after embryo transfer pregnancy rates of between 60-80% have been obtained (Table 1: Skidmore and Billah, 2005).

Table 1: Pregnancy results in meclofenamic-acid treated recipients.

Stage of Cycle (ov+)	No of camels treated	No. of pregnancies (%)
8 (control)	10	1 (10)
8	10	8 (80)
10	10	6 (60)
12	10	7 (70)

Pregnancies rates of 44% can also be achieved in non-ovulated progesterone treated recipients if they are injected with 150 mg of progesterone–in-oil from 2 days before embryo transfer. However, as these animals would require daily injections of progesterone for the entire 13 month gestation period (because camels rely on the progesterone produced from the CL to maintain their pregnancy state) this is not a practical method for large numbers of recipients. This method can be improved once the camel is confirmed pregnant, by injecting 2000 iu eCG on day 25 of gestation to induce follicular development, whilst still receiving progesterone treatment. Once a follicle of suitable size (1.3 cm) matures approximately 7-10 days later, ovulation can be induced with GnRH, and once the subsequent CL has developed exogenous progesterone treatment is stopped (Figure 2: Skidmore and Billah, 2011).

Pregnancy rates of approximately 50 % have been achieved using this method which has the advantage of the recipient not needing daily injections of progesterone throughout gestation.

These results indicate that the degree of synchrony between embryo age and that of the recipient's uterus is perhaps less important so long as there is a sufficient level of progesterone in the blood and therefore these methods would relieve the need for tight synchrony between the donor and recipient.

Although relatively high pregnancy rates were achieved at day 20 of gestation this is not necessarily reflected in the calving rate. Anouassi and Tibary (2013) reported pregnancy rates of between 50-85% at days 14-25 in their embryo transfer programme, but they had reduced to 19-44% by day 60. Over a period of 10 years the overall efficiency of their transfers (% weaned calves/transfer) was 27%. These losses could be due to the reduced nutritional input, health care and vaccination programme the recipient camels receive once they return to their original herd. Even under natural mating conditions where conception rate might be as high as 75-85% the overall calving rate can drop dramatically to around 40% (Djellouli and Saint Martin, 1992), although with improved nutrition and health care birth rate can improve to 55-80 % (Mutugi *et al*, 1992).

EMBRYO CRYO-PRESERVATION

Reliable methods for the cooling and cryo-preservation of embryos has greatly facilitated a more widespread application of embryo transfer for genetic management and improvement without the need for transporting live animals.

Cooled embryos. Pregnancies can be achieved after transfer of cooled embryos that have been kept at 4ºC for 24 h. Embryos are placed in a sealed eppendorf completely

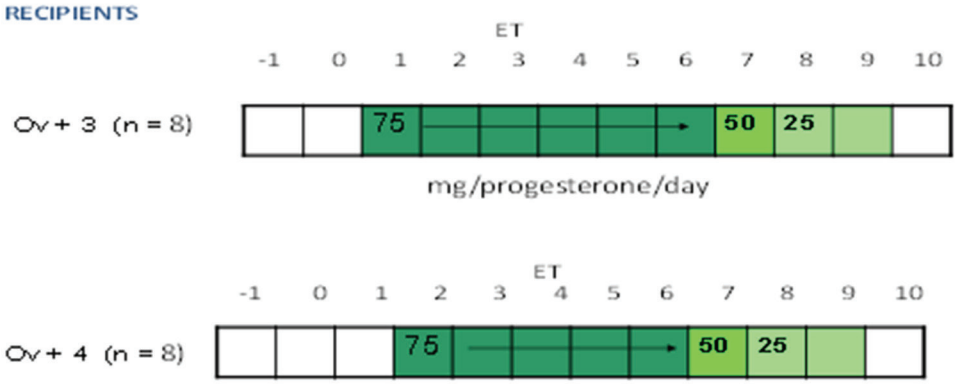

Figure 1: Progesterone treatment protocol for ov + 3 and ov + 4 recipients (from Skidmore and Billah, 2011)

filled with holding media and placed in an Equitainer (Hamiton Thorn, Massachusetts, USA) with two freezer cans designed to cool the contents to 4ºC and maintain this temperature for 24-36 h (Cook *et al*, 1989). Two thermal ballast bags kept at room temperature are wrapped around the tubes containing the embryos before they are deposited in the Equitainer. Cooled embryos that are recovered from the eppendorf 24 h later, washed in fresh holding media and transferred to day 5 or 6 recipients achieve pregnancy rates of approximately 60%.

Another important advantage of cryopreservation is that recipients do not have to synchronized by exogenous hormone treatment with, or even be in the same location as, the donor animal because the embryos can be stored and then thawed and transferred to recipients after timing of natural ovarian cycles. There are two methods of cryopreservation of embryos, firstly, controlled rate (slow) freezing and secondly, vitrification.

Controlled rate freezing

Using this method embryos are equilibrated at lower concentrations of cryoprotectant (10%) for longer periods (5-10 min) and cooled at a slower rate (0.3-0.5ºC/min). Previous studies have shown that pregnancy rates of 33-37% (7/19) were achieved from slow-cooled, frozen/thawed camel embryos when the embryos were exposed to 1.5 M ethanediol as the cryoprotectant for 10 min. After exposure the embryos were loaded in 0.25 ml straws and placed in embryo freezing machine at -7ºC and after seeding, and 10 min equilibration time, the temperature was lowered in steps of 0.5ºC/min to -33 ºC at which time they were plunged into liquid nitrogen and stored. The embryos were later thawed by holding in air for 8 sec, followed by swirling in a 32 ºC water bath for 2 min before rehydrating in holding media or holding media containing 0.2 M sucrose for 10 min (Skidmore *et al.*, 2004). However further refinement of this method is needed to improve pregnancy rates from slow-cooled cryopreserved camel embryos. Two of these pregnancies were allowed to go to term and resulted in normal live births. The remaining 5 were given prostaglandin after day 60, when a visible fetus with heart beat was well established, as they were part of a research herd that were not required to remain pregnant.

Vitrification

Using this method, embryos are equilibrated at higher concentrations of cryoprotectant (20-40 %) for much shorter periods (10-20 sec) and then rapid cooled and warmed. Nowshari *et al.,* (2005) achieved 3 pregnancies from 49 camel embryos that had been vitrified/thawed using a simple 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 0.5 M sucrose in PBS. Aller *et al*., (2002) were able to successfully vitrify llama embryos (pregnancy rates of 50%; 2/4) after exposing embryos to vitrification solution (20% glycerol +20% ethanediol + 0.3M sucrose + 0.375 M glucose + 3% polyethylene glycol) in three steps, loading into 0.25 ml straws and plunging into liquid nitrogen. Embryos were thawed in air for 6 s, warmed in a 25ºC water bath for 1 min, rehydrated in 0.5 M sucrose in PBS +20% FCS (5 min) followed by 0.25 M sucrose solution (5 min) and finally PBS +20% FCS prior to transfer. This method has also been successful in dromedary camels but the size of the embryo was found to be important if the embryo was to be successfully vitrified. Better pregnancy rates (38%; 8/21) were achieved with smaller day 6 embryos (≤ 350 µm) than with larger day 7 embryos, as between 50-80% of the bigger embryos were fractured or torn after warming (Skidmore *et al*., 2005). More recently Herrid *et al.,* (2016, 2017) have achieved promising pregnancy results combining ethylene glycol and glycerol as the cryoprotectants and using the open pulled straw (OPS) method of vitrification. Conversely, however, they found that day 7 embryos survived better than day 6 embryos when using the OPS method. This stage dependant sensitivity of the embryos is a major obstacle to a practical application of the vitrification method.

CONCLUSIONS

The increasing necessity to improve camel production has led to a more scientific approach to management of these animals. Ovulation can be controlled by using GnRH or gonadotrophic hormones if administered at the correct stage of the cycle. Hormonal methods using a combination of progesterone and eCG or 2 injections of GnRH

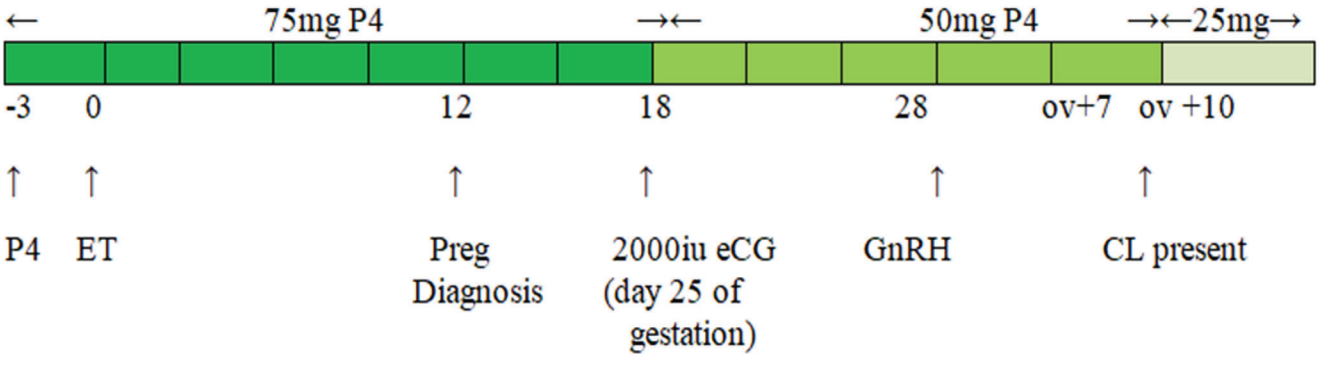

Figure 2: Treatment schedule for non-ovulated, progesterone – eCG treated recipients. P4 progesterone; ET embryo transfer; eCG equine chorionic gonadotrophin. (Skidmore and Billah, 2011)

14 days apart will successfully synchronize follicular waves in camels, which has always been considered a necessary pre-requisite for embryo transfer. In addition, recipient availability has been broadened by treatment of ovulated, asynchronous recipients with progesterone or meclofenamic acid so that pregnancies can be successfully achieved in recipients that have ovulated up to 4 days before or 3 days after the donor. Even recipients that do not ovulate can be used if treated with a combination of progesterone, eCG and GnRH. These methods allow the embryo an extra 48-72 h to become established and secrete enough maternal recognition of pregnancy signal to maintain the CL itself.

These results show that controlled breeding and strategic use of hormone treatments should increase the efficiency of embryo transfer programmes in camels and therefore improve their reproductive potential.

REFERENCES

Adams G.P., Ratto M.H., Huanca W., Singh J. (2005). Ovulation inducing factor in the seminal plasma of alpacas and llamas. *Biol. Reprod.,* 73: 452 – 457.

Aller J.F., Rebuffi G.E., Cancino A.K., Alberio R.H. (2002). Successful transfer of vitrified Ilama (*Lama glama*) embryos. *Anim. Reprod. Sci*., 73:121-7.

Anouassi A., Adnani, M., Raed E.L. (1992). Artificial insemination in the camel requires induction of ovulation to achieve pregnancy. . *Proc. 1st Int. Camel Conf.* pp.175-177. Eds. W.R. Allen, A.J. Higgins, I.G. Mayhew, D.H. Snow and J.F. Wade., R & W Publications (Newmarket) Ltd. UK.

Anouassi A., Tibary A. (2013). Development of a large commercial camel embryo transfer program: 20 years of scientific Research. *Anim. Reprod. Sci*., 136: 211- 221.

Cook V.M., Squires E.L., McKinnon A.O., Bailey J., Long P.L. (1989). Pregnancy rates of cooled, transported equine embryos. *Equine Vet. J.,* (Suppl) 8: 80-81.

Cooper M.J., Hammon D., Harker DB, Jackson PS (1976). Control of the bovine oestrous cycle with ICI 80996 (cloprostenol). Field results in 3810 beef cattle. *Proc. 8th Int. Congr. Anim. Reprod. AI, Krakow, Poland*., 3: 449-451.

Djellouli M.S., Saint-Martin G. (1992). Productivitiy and econiomy of camel breeding in Tunisia. In *Proc. 1st Int. Camel Conf.* pp.209 - 212. Eds. W.R. Allen, A.J. Higgins, I.G. Mayhew, D.H. Snow and J.F. Wade. , R & W Publications (Newmarket) Ltd. UK.

El Wishy A.B. (1987). Reproduction in the female dromedary *(Camelus dromedarius)*. A Review. *Anim. Reprod. Sci.,* 82: 587 – 593

Herrid M., Billah M., Malo C., Skidmore, J.A. (2016). Optimization of a vitrification protocol for hatched blastocysts from the dromedary camel (*Camelus dromedarius*). *Theriogenology,* 85: 585-590.

Herrid M . Vajta G., Skidmore J.A. (2017). Current status and future direction of cryopreservation of camelid embryos. *Theriogenology*, 89: 20-25.

Marie M., Anouassi A. (1986). Mating induced luteinizing hormone surge and ovulation in the female camel (*Camelus dromedarius*). *Biol. Reprod.,* 35: 792-798.

Marie M., Anouassi A. (1987). Induction of luteal activity and progesterone secretion in the non-pregnant one-humped camel (*Camelus dromedarius*). *J. Reprod. Fert.,* 80: 183-192.

McKinnon A.O.,Tinson A.H. (1992). Embryo transfer in dromedary camels. *Proc. 1st Int. Camel Conf.* pp. 203-208. Eds. W.R. Allen, A.J. Higgins, I.G. Mayhew, D.H. Snow and J.F. Wade., R & W Publications (Newmarket) Ltd. UK.

McKinnon A.O., Tinson A.H., Nation G. (1994). Embryo transfer in dromedary camels. *Theriogenology,* 41: 145-150.

Musa B.E., Abusineina M.E. (1978). The oestrous cycle of the camel (*Camelus dromedarius*.) *Vet. Rec.*, 102: 556-557.

Mutugi M.W., Olaho-Mukani W., Kuto B., Alushula N. A.R. (1992). Some reproductive parameters in a Kenyan population of *Camelus dromedarius*. *Bull. Anim. Health. Prod. Afr*., 40: 215-217.

Niasari-Naslaji A. (2008). An update on Bactrian camel reproduction. *J. Camel Pract. Res*., 15: 1-6.

Nikjou D., Niasari-Naslaji A., Skidmore J.A., Mogheiseh A., Razavi K. (2008). Synchronization of follicular wave emergence prior to superovulation in Bactrian camel (*Camelus bactrianus*). *Theriogenology,* 69: 491-500.

Nowshari M.A., Ali S.A., Saleem S. (2005). Offspring resulting from transfer of cryopreserved embryos in camel (*Camelus dromedarius*). *Theriogenology*, 63: 2513-22.

Pan G.W., Zhao X.X., Chen B.X., Jiang S, Huang YM, Zu Y.S., Wang H.Y. (1992). The ovulation-inducing effect of seminal plasma in the Bactrian camel *Proceedings of 1st International Camel Conference* pp 159–162 Eds WR Allen, AJ Higgins, IG Mayhew, DH Snow & JF Wade. R &W Publications (Newmarket) Ltd UK.

Skidmore J.A., Billah M. (2005). Embryo transfer in the dromedary camel (*Camelus dromedarius*) using asynchronous, meclofenamic acid-treated recipients. *Reprod. Fertil. Dev.,* 17: 417-421.

Skidmore J.A., Billah M. (2011). Embryo transfer in the dromedary camel (*Camelus dromedarius*) using non-ovulated and ovulated asynchronous progesterone-treated recipients. *Reprod. Fertil. Dev*., 23: 438-443

Skidmore J.A., Billah M., Allen W.R. (1996). The ovarian follicular wave pattern and induction of ovulation in the mated and non-mated one-humped camel (*Camelus dromedarius). J. Reprod. Fertil.,* 106: 185-192.

Skidmore J.A., Billah M., Allen W.R. (2002). Investigation of factors affecting pregnancy rate after embryo transfer in the dromedary camel. *Reprod. Fertil. Dev.* 14: 109-116.

Skidmore J.A., Billah M., Loskutoff N.M. (2004). Developmental competence *in vitro* and *in vivo* of cryopreserved, hatched blastocysts from the dromedary camel (*Camelus dromedarius*). *Reprod. Fertil. Dev*., 16: 605-9.

Skidmore J.A, Billah, M., Loskutoff N.M. (2005). Comparison of two different methods for the vitrification of hatched blastocysts from the dromedary camel (*Camelus dromedarius*). *Reprod. Fert. Dev*., 17: 523-527.

Skidmore J.A., Adams G.P., Billah M. (2009). Synchronization of ovarian follicular waves in dromedary camels (*Camelus dromedarius*). *Anim. Reprod. Sci*., 114: 249 -255.

Tibary A., Anouassi A. (1997a). Reproductive physiology in the female camel. in: Institut Agronomique et Vétérinarie Hassan II, Rabat, Maroc. (Eds.), Theriogenology in *camelidae*. Abu Dhabi Printing and Publishing Company. U.A.E. pp 169-241.

Tibary A., Anouassi A. (1997b). Artificial breeding and manipulation of reproduction in camelidae. in: Institut Agronomique et Vétérinarie Hassan II, Rabat, Maroc. (Eds.), Theriogenology in *camelidae*. Abu Dhabi Printing and Publishing Company. U.A.E. pp. 413 - 452.