Superovualtion Trials for Embryo Transfer in the Camel (*Camelus Dromedarius)*

S.T. Ismail , M. M. Al-Eknah , N. A. Hemeida

Dept. of Clinical Studies, College of Vet. Med. and Ani. Res.,
King Faisal University, Al-Ahsa, Saudi Arabia

Abstract:

The current investigation represents a successful attempt of superstimulation and embryo recovery in the dromedary camel in Saudi Arabia.Six mature non-pregnant female camels were used in this study. Camels were superstimulated by injecting 3000 IU equine chorionic gonadotropin (eCG) at the end of 10 days progesterone treatment. The development of ovarian follicles was monitored by ultrasound array scanner until the majority of follicles were considered sufficiently mature (1.3-1.9cm in diameter). Mating of the female camels with fertile male camel was allowed twice, 12 hours apart. Each female camel received 3000 IU human chorionic gonadotropin (hCG) just after the first mating. Embryo recovery, by the interrupted-syringe method, was carried out at day 7 to 7.5 post mating. All camels experienced oestrus 8 to 10 days post eCG administration. Four out of six camels responded to the superstimulatory treatment (66.70%). The mean number of ovulations (corpora lutea, CLs) produced by the camels responded to superstimulation was 8.75±4.80, with a range of 6 to 16 CLs. Among these camels, the percentage of ovulation was 97.22%. Three embryos, at hatched blastocyst stage, were collected from the four responded camels, one from each camel. In conclusion, despite the promising results of the current study concerning the superstimulatory response and ovulation rate, the embryo recovery rate needs more research to achieve similar success to superovuloation.

Key Words: Superstimulation, superovuloation, Embroy transfer, embryo recovery, Dromedary camel.

Introduction:

The number of offsprings likely to be produced by a prestigious female camel in her relatively short breeding life is inadequate to provide a good distribution of the desired genetic material (Musa *et al*., 1993). Also the restricted breeding season and the camel's long gestation period justify the use of embryo transfer for increasing the reproductive efficiency in this species (Skidmore, *et al,* 1992; Al-Eknah, 2000, 2001).

Stimulation of ovulation and induction of superstimulation in the donors are considered formidble challenge in camel embryo transfer technique (Mckinnon and Tinson, 1992). equine chorionic gonadotropin (eCG) has been successfully used in camels at various doses ranging between 1500 and 6000 IU to stimulate the ovaries for the production of multiple follicles (Anouassi

and Ali, 1990; Skidmore *et al*, 1992; Mckinnon and Tinson, 1992). Another method for superstimulation in camel is the use of 1-3 mg ovine FSH (follicle stimulating hormone) in a split dose regime over 3-6 days (Cooper, *et al*, 1990, 1992; Skidmore, *et al*, 1992; Mckinnon and Tinson, 1992). The latter investigators collected more embryos from donors stimulated with FSH than with eCG. The gonadotrophin treatments were performed just before or after the removal of the progesterone releasing intravaginal device (PRID), (Skidmore, *et al,* 1992; Cooper, *et al*, 1992), or on the last day of the progesterone therapy (Mckinnon and Tinson, 1992). Donor camels were mated once or twice 12 hours apart or artificially inseminated. Ovulation was enhanced with human chorionic gonadotropin (hCG) or gonadotropin releasing hormone (GnRH), (Cooper, *et al,* 1992; Mckinnon and Tinson, 1992). The superstimulatory response to the exogenous gonadotrophin therapy varied tremendously between individual donors according to the age and reproductive characteristics of the donors, the selected hormone therapy, and the time or season of the treatment (Anouassi and Ali, 1990; Skidmore, *et al*, 1992). However, embryo recovery per donor camel varies from 0-30 with current average 6 per donor (Tinson *et al,* 1998).

Flushing of embrys from donor camels has been tried on day 6 or 7 post mating (Anouassi and Ali, 1990; Cooper, *et al*, 1990; 1992). The embryos recovered on day 7 from the first mating ranged from compact morula to expanded blastocysts (Skidmore *et al,* 1992; Ismail *et al,* 1993). Embryo recovery has been performed in the camel using either a two-way or a three-way catheters during standing or sitting positions (Cooper, *et al*, 1990; Skidmore, *et al,* 1992).

The success rate of the mentioned techniques for camel superstimulation and embryo recovery are still far behind what had been accomplished in the other farm animals. Therefore, this study was designed to investigate the efficiency of eCG in inducing superstimulation and embryo recovery in the dromedary camel.

Materials and Methods

1. Camels

Six mature, non-pregnant and non-lactating female camels were used in the present study. They were 8 to 14 years old. The camels were kept in open yard and fed on barley (2Kg/head/day). Rhodes grass hay and water were provided ad libitum.

2. Superstimulation

Each female camel received a daily intramuscular injection of 100 mg progesterone powder (Sigma, U.S.A.) prepared in 2 ml sesame oil for 10 consecutive days. At the last day of progesterone treatment, the animals were intramuscularily injected with 3000 IU eCG hormone (Folligon; Intrervet, Holland).

3. Mating and ovulation

The development of ovarian follicles was monitored by ultrasound array scanner. Scanning was daily performed for all female camels, starting 4 days from the commencement of the superstimulatory treatments until the majority of follicles were considered sufficiently mature (1.3-1.9cm in diameter). Mating of the females with one of two fertile male camels was allowed twice 12 hours interval. Each female camel received 3000 IU hCG (Chorulon; Intrervet, Holland) just after the first mating. Ovulation was confirmed by scanning.

4. Embryo recovery and evaluation

Embryo recovery, by the interrupted-syringe method, was carried out according to the technique described by Skidmore *et al*.(1992).The camel was restrained in the sitting position and given an epidural analgesia at the sacro-coccygeal vertebral space (10 ml 2% Lidocaine HCL; Lido-kel 02, Kelolab, Englad). The animal was sedated with a single intravenous injection of 3 ml Xylazine (Seton 2% Laboratories, Claire, Spain). The tail was wrapped and tied up aside. Rectal faeces was removed and the perinial region was cleaned. The process of embryo recovery, using camel collection catheter (IMV technologies, France) and an embryo filter (EmCon filter, Immuno System Inc., Wisconsin, U.S.A.), was conducted according to the method described by Skidmore (2000). Collected embryos were further evaluated under low and high power research microscope. Ovulation rate was expressed as:

$$\frac{\text{Number of Cls x 100}}{\text{Number of Cls + number of follicles}}$$

Whereas the embryo recover rate was expressed according to Mckinnon and Tinson (1992) as:

$$\frac{\text{Number of recovered embryos per attempts X 100}}{\text{Number of collection attempts}}$$

Results :

Induction of Oestrus:

Table (1) shows that all camels experienced oestrus as the result of the eCG treatment at the end of progesterone priming period. The interval from eCG treatment to oestrus, based on the signs of sexual receptivity and size of the ovarian follicles, ranged from 8-10 days.

Superstimulatory response:

Four of the six camels used responded to the superstimulatory treatment (66.70%), by developing more than 2 corpora lutea (Table 1). However, the other two camels (No. 2 and 6) did not respond to the superstimulation regimen and showed 1 and 0 CL, respectively.

The mean number of ovulations (CLs) given by the camels responded to superstimulation was 8.75±4.80. the highest number of ovulations (16 CLs) was given by camel No. 4 (Fig. 1). However, the least number of ovulations (6 CLs) was given by camels Nos. 3 and 5. Meanwhile, camel No. 1 produced 7 CLs (Fig. 2).

The number of anovulatory follicles among the camels responded to superstimulation was one, as estimated by rectal palpation, and ultrasonography, and confirmed by laparotomy.

Among the camel responded to superstimulatory treatment, the percentage of ovulation was accounted to be 97.22% (Table 1).

Embryo recovery and evaluation:

Trials for embryo recovery were carried out at day 7 to 7.5 post mating (0= day of oestrus). One embryo from each of camels Nos. 1, 4 and 5 was recovered. No embryos were recovered from camel No. 2.

The embryo recovery rate was 75%. The collected embryos were at hatched blastocyst stage (Fig. 3).

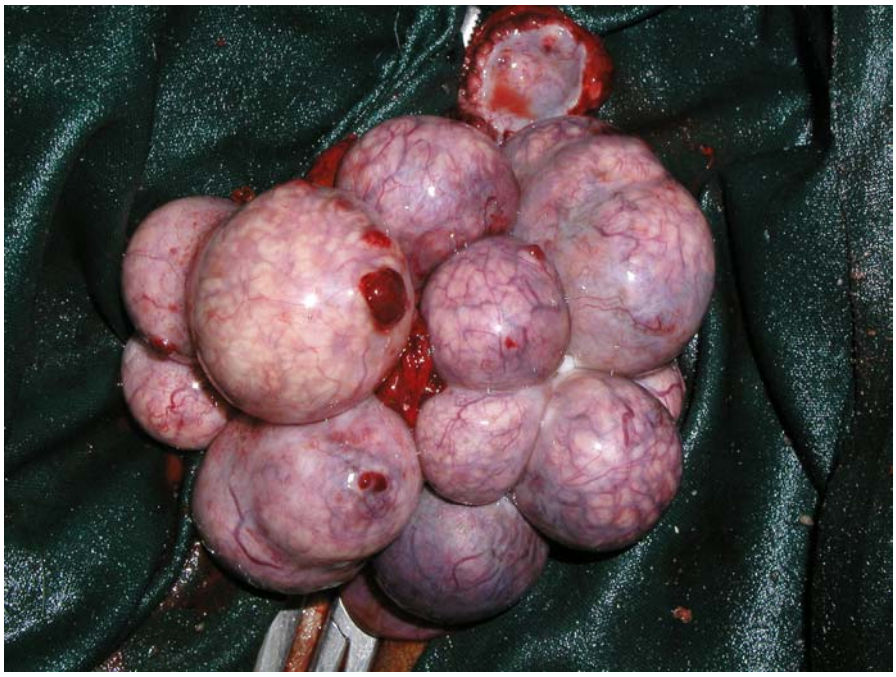

Fig. 1 Ovaries of camel number 4 containing 16 corpora lutea.

Table (1)
Superstimulatory response and embryo recovery of the camels

Number of treated camels	6
Number of camels experienced estrus (%)	6 (100 %)
Interval from eCG treatment to mating	8-10 days
Number of camels responded to superstimulation (%)	4 (66.7 %)
Average number of ovulatios in responded camels	8.75 ± 4.8 CL
Range of ovulations	6-16 CL
Ovulation rate	97.22 %
Day of embryo recovery	7 to 7.5
Number of recovered embryos	3
Embryo recovery rate	75%
Stage of embryo development	Hatched blastocyst

Fig. 2 Ovaries of camel number 1 containing 7 corpora lutea.

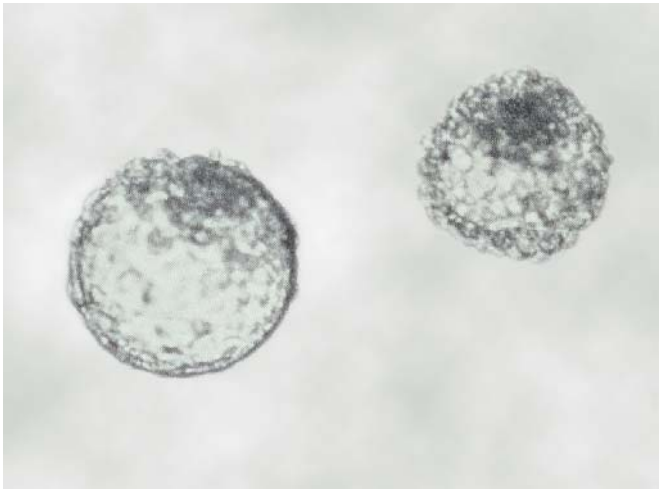

Fig. 3: A collapsed hatched blastocyst (right) and expanded hatched blastocyst (left).

Discussion:

The current investigation represents a successful attempt of superstimulation and embryo recovery in the dromedary camel in Saudi Arabia.

Despite the use of eCG in the present investigation, which is known to have low superstimulatory effect in comparison to FSH (purohit, 1999), the obtained results heren are considered truly prosmising. The mean number of ovulations obtained in this study (8.75 CLs) is much higher than the means of 4.6 to 5.7 CLs reported by several authors, used the same hormone (Anouasi and Ali, 1990; Ismail *et al*., 1993; Mc Kinnon *et al*., 1994; Vyas, 1998).

In this aspect, the higher superovulatry response observed here can be attributed to the absence of palpable follicles at the time eCG treatment (Tibary and Anouassi, 1997; Skidmore, 2000) This can be related to the higher progesterone level predominating at this time following the period of progesterone treatment which precedes the eCG administration. Jensen et al. (1982), Donaldson (1985) and Callesen *et al*. (1988) emphasized that higher levels of progesterone at the commencement of superstimulation is favorable in terms of ovarian response and embryo quality.

The results of the current study demonstrate that about 67% of the camels respond to the superstimulatory treatment. Similar results have been reported by Cooper *et al*. (1992), Mckinnon and Tinson (1992), Skidmore et al. (1992) and Vyas (1998). Skidmore (2000) stated that one of the most important problems in superstimulation of the camel is the high incidence of non-responsive females (Approximately 20-30 %) which fails to produce follicles.

The most striking result in the current investigation is the high percentage of ovulation (97.22%); only one out of 36 follicles failed to ovulate. This Indicates that the regimen used here, which allows mating the female camels twice, 12 hours apart with injection of 3000 IU hCG after the first mating, can successfully induce ovulation in the camel. Skidmore (2000) reported that in order to achieve a good ovulation rate, donors must be monitored by ultrasonography throughout the superstimulation treatment period and bred when the follicles reach a size between 13 and 16 mm in diameter.

The low embryo recovery rate, observed here has been reported by many authors. Cooper *et al*. (1990,1992), using FSH for superstimulation in the camel, obtained good superstimulation but poor embryo recovery of 1.5 embryo/donor (3 out of 11 donors responded to superstimulation and yielded

1,4 and 11 embryos). Skidmore et al. (1992), in their early trials, failed to collect embryos in 63% of the treated donors. Similarly, Vyas (1998) failed to recover any embryo from superstimulated Indian camels using 3000 IU eCG. On the other hand, the recent results obtained by Tinson *et al*. (2000) indicate that the embryo recovery rate is improved to give 5.6 and 7.4 embryos per donor for camels superovualted by eCG and FSH, respectively.

The variability in embryo recovery rates can be ascribed to the type of superstimulatory treatment used (Purohit, 1999) and the delayed oviductal transport or asynchronous ovulations (Mc Kinnon *et al*., 1994). The latter assumption is supported by occurrence of pregnancy in many donors despite flushing once or even twice, 24 hours interval (Mc Kinnon *et al*., 1994).

In conclusion, despite of the promising results obtained in this study concerning the average ovulations per donor (8.75 CLs) and ovulation rate (97.22%), embryo recovery rate needs more efforts to reach a similar success.

This research project has been financially supported by the Deanship of Scientific Research at King Faisal University, Saudi Arabia

References:

1. Al-Eknah, M.M. (2000). Reproduction in Old World camels. Animal Reproduction Science, 60-61: 583-592.
2. Al-Eknah, M.M. (2001). Camel reproduction. In: Arthur's Veterinary Reproduction and Obstetrics. Eds. Noakes, D.E., Parkinson and England, G. Harcourt Health Science, UK**.**
3. Anouassi, A. and Ali, A. (1990). Embryo transfer in camel (Camelus dromedarius). Proc. Unite de Coordination pour L`Elevage Camelin. In: Is it possible to improve the reproductive performance of the camel ? Proceeding UCDEC Workshop, Paris, pp 327-331.
4. Callesen, H., Greve, T. and Hyttel, P.(1988). Preovulatory evaluation of the superstimulatory response in donor cattle. Theriogenology, 30: 477-488.
5. Cooper, M.J., Skidmore, J., Ali, M., Billah, A., Wensvoort, S., Billah, M. and Allen, W.R. (1990). An attempt to induced and synchronize ovulation and superstimulation in dromedary camels for embryo transfer. Proc. Unite de Coordination pour L`Elevage Camelin. In: Is it possible to improve the reproductive performance of the camel? Proceeding UCDEC Workshop, Paris, pp 313-326.
6. Cooper, M.J., Skidmore, J., Allen, W.R., Wensvoort, S., Billah, M., Chaudhary, M.A. and Billah, A.M. (1992). Attempts to stimulate and synchronise ovulation and superstimulation in dromedary camel for embryo transfer. In: Proceedings of the First International Camel Conference, pp. 187-191. Eds W.R. Allen, A.J.

Higgins, I.G. Mayhew, D.H. Snow and J.F. Wade, R&W Publications, Newmarket.

7. Donaldson, L.E. (1985): LH and FSH profiles at superstimulation and embryo production in the cow. Theriogenology, 23, 441-447.
8. Jensen, A.M., Greve, T., Madei, A. and Edquist, L. (1982). Endocrine profiles and embryo quality in the PMSG – PGF2α treated cow. Theriogenology, 18, 33-44.
9. Ismail, S.T., Azab, G.A., Abdoon, A.S.S. and El-belely, M.S. (1993). New approach for superstimulation and embryo recovery in the one-humped camel. Vet. Med. J., 41, 35-39.
10. Mckinnon, A.O. and Tinson, A.H. (1992). Embryo transfer in dromedary camel. In: Proceedings of the First International Camel Conference, pp. 203-208. Eds W.R. Allen, A.J. Higgins, I.G. Mayhew, D.H. Snow and J.F. Wade, R&W Publications, Newmarket.
11. McKinnon, A.O., Tinson, A.H. and Naion, G. (1994). Embryo transfer in dromedary camels. Theriogenology, 41, 145-150.
12. Musa, B.E., Siema, H., Merkt, H., Hago, B., Cooper, M., Allen, W. and Jochle, W. (1993). Manipulation of reproductive functions in male and female camels. Anim. Reprod. Sci., 33, 289-306.
13. Purohit, G.N. (1999). Biotechnologies in camelid reproduction: Current status and future prospectives. J. Camel Prac. Res., 6, 1-13.
14. Skidmore J.(2000). Embryo transfer in the Dromedary camel (Camelus dromedarius).In: Recent Advances in Camelid Reproduction, Skidmore L. and Adams G.P. (Eds.). International Veterinary Information Service , Ithaca NY.
15. Skidmore, J.A., Allen, W.R., Cooper, M.J., Ali Chaudhry, M., Billah, M. and Billah, A.M. (1992). The recovery and transfer of embryos in the dromedary camel: results of preliminary experiments. In: Proceedings of the First International Camel Conference, pp. 137-142. Eds W.R. Allen, A.J. Higgins, I.G. Mayhew, D.H. Snow and J.F. Wade, R&W Publications, Newmarket
16. Tibary, A. and Anouassi, A. (1997). Theriogenology in Camelid. Abu-Dhabi Printing and Publishing Co., Abu-Dhabi, U.A.E..
17. Tinson, A., Singh, K. and Kuhad, K.S. (1998). Large scale management of camels for embryo transfer. Int. Sem. Camel App. Res. Dev. Bikari, India.
18. Tinson, A.H., Singh, K. and Kuhad, K.S. (2000). Large scale management of camels for embryo trasfer. J. Camel Prac. Res., 7, 143-147.
19. Vyas S.(1998). Final report. Development of embryo transfer technology in Indian camel . National Research Centre on Camel, Bikaner, India.

محاولات إحداث التبويض المتعدد من أجل نقل الأجنة في الإبل

سيد طه إسماعيل، مرزوق بن محمد العكنه، نبيل عبدالمنعم حميدة

قسم الدراسات الإكلينيكية، كلية الطب البيطري والثروة الحيوانية
جامعة الملك فيصل، الأحساء، المملكة العربية السعودية

الملخص :

تمثل هذه الدراسة محاولة ناجحة لإحداث التبويض المتعدد وتجميع الأجنة في المملكة العربية السعودية. تم تحفيز التبويض المتعدد في ٦ نوق بالغة غير حوامل، بحقن ٣٠٠٠ وحدة دولية من هرمون الفرس المشيمائي المحفز للمناسل عند اليوم العاشر من حقن هرمون البروجستيرون (الذي تم حقنه لمدة عشرة أيام متوالية. تم متابعة نمو جريبات المبيض عن طريق جهاز الموجات فوق الصوتية حتى وصلت الحجم المناسب للتبويض (قطر ١٣- ١٩ مم). عندها تم تلقيح النوق مرتين بينهما ١٢ ساعة باستخدام جمل عالي الخصوبة. ثم حقنت الحيوانات بعد أول تلقيح، باستخدام ٣٠٠٠ وحدة دولية من الهرمون الآدمي المشيمائي المحفز للمناسل. تم تجميع الأجنة بالطريقة غير الجراحية في اليوم السابع والسابع والنصف من التلقيح. وضحت مظاهر الشياع على كل النوق بعد ٨- ١٠ أيام من حقن هرمون الفرس المشيمائي المحفز للمناسل. استجابت ٤ نوق من ٦ للتبويض المتعدد. بلغ متوسط عدد الأجسام الصفراء للنوق الأربع ٤,٨ ± ٨,٧٥، وتراوحت بين ٦- ١٦ جسم أصفر. بلغ معدل التبويض لهذه النوق ٩٧,٢٢٪. وقد تم جمع ٣ أجنة، واحد من كل ناقة. خلصت الدراسة أنه على الرغم من النتائج المشجعة في مجال إحداث التبويض المتعدد ومعدل التبويض، إلا إن معدل تجميع الأجنة ما زال في حاجة إلى دراسات مستقبلية لتحقيق نجاح مشابه للتبويض المتعدد.