SUPEROVUALTION TRIALS FOR EMBRYO TRANSFER IN THE CAMEL (*CAMELUS DROMEDARIUS)*

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SUMMARY

Up to the best of our knowledge, the current investigation represents the first 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 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 was allowed twice, 12 hours apart. Each female camel received 3000 IU 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

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superstimulatory response and ovulation rate, the embryo recovery rate needs more research to achieve similar success.

INTRODUCTION

Embryo transfer technology offers many advantages to commercial animal breeding, and thus it has been considered as a routine practice in several species including cattle, pig, sheep, goats and horses. Embryo transfer increases the overall rate of progress in genetic improvement, improves the productivity of a particular female and shortens the genetic interval (Skidmore, 2000).

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). 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 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 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 hCG or 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 embryos from donor camels has been tried on days 6 to 8 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 whereas embryos collected later ranged from expanded to hatched blastocysts (Skidmore et al, 1992; Ismail et al, 1993 ; Tinson et al.,2000). 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 production in the dromedary camel.

MATERIALS AND METHODS

1. Camels

Six mature non-pregnant female camels were used in the present study. They were aged 8 to 14 years. 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 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 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. A camel embryo collection catheter (IMV technologies, France, fig. 1) was inserted through the cervix with the tip of the catheter at the body of the uterus. The balloon was inflated with 30 to 40 ml of air to seal the internal cervical os. The uterus was filled to moderate capacity with flushing medium (Dulbecco' s phosphate buffered saline containing 2 % Bovine Serum Albumin and 0.005% kanamycin sulphate; IMV technologies, France). A 60 ml-syringe was used for injection (Fig. 2). During the fluid injection, the operator's hand was passed in the rectum to monitor uterine filling. The medium was recovered by gravity flow and passed through an embryo filter (EmCon filter; Immuno Systems Ine. Wisconsin, USA, fig. 3).

The residual filtrate was searched for embryos with the aid of a stereoscopic binocular dissecting microscope (Labo Americal Inc., U.S.A., Fig.4). When embryos were allocated, they were assessed morphologically graded and washed in fresh flushing medium. Collected embryos were further evaluated under research microscope.

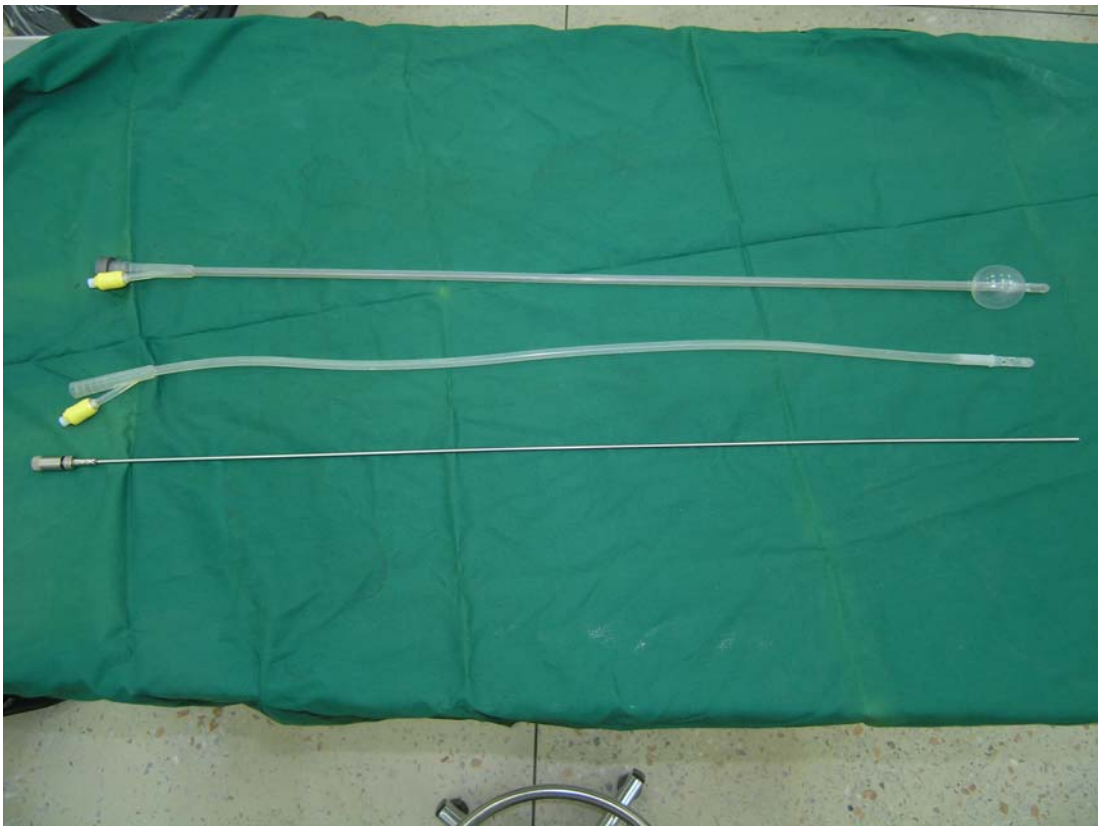

Fig. 1 A camel embryo collection catheter.

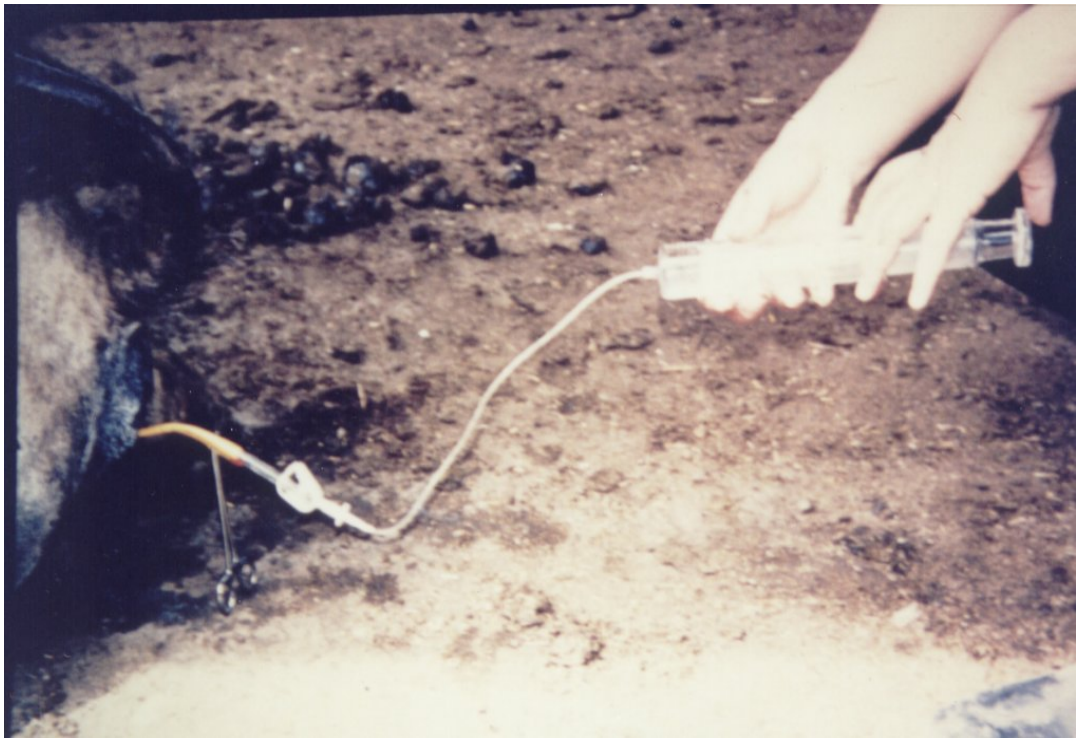

Fig. 2 A 60 ml-syringe used for embryo recovery.

Fig. 3 EmCon embryo filter.

Fig. 4 A stereoscopic binocular dissecting microscope connected to a computer.

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 (2 and 6) did not respond to the superstimulation regimen and showed 1 CL and 0, 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 4 (Fig. 5). However, the least number of ovulations (6 CLs) was given by camels 3 and 5. Meanwhile, camel 1 produced 7 CLs (Fig. 6).

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 ovulation rate 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 1, 4 and 5 was recovered. No embryos were recovered from camel 2.

The embryo recovery rate was 75%. The collected embryos were at hatched blastocyst stage (Fig. 7).

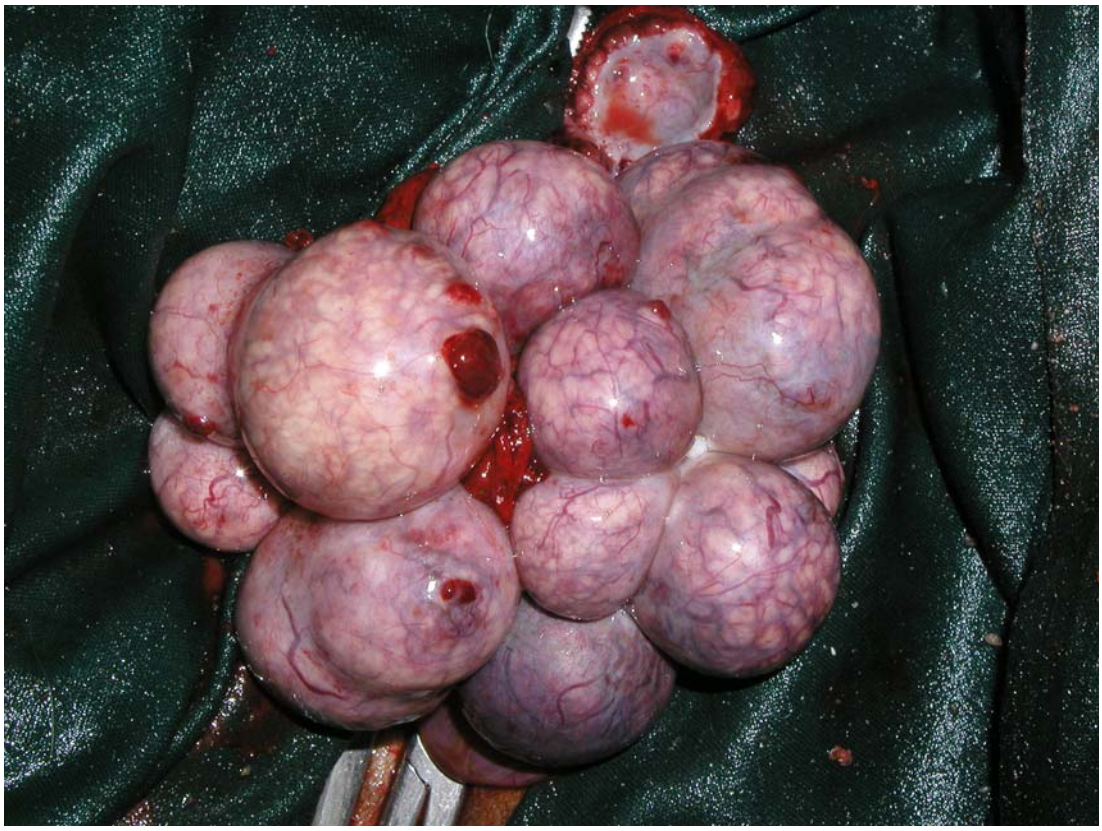

Fig. 5 Ovaries of camel 4 containing 16 corpora lutea.

Fig. 6 Ovaries of camel 1 containing 7 corpora lutea.

Table 1, Superstimulatory response 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 %

Table 2: Results of embryo recovery

Day of embryo recovery	7 to 7.5
Number of recovered embryos	3
Embryo recovery rate	75%
Stage of embryo development	Hatched blastocyst

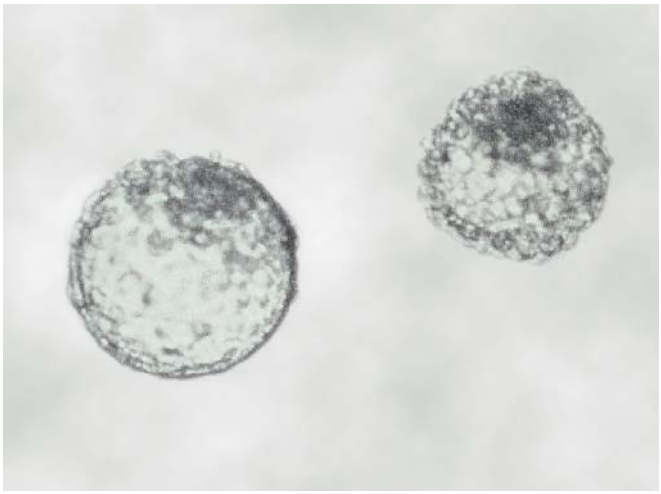

Fig. 7 A collapsed hatched blastocyst (right)
and expanded hatched blastocyst (left).

DISCUSSION

Up to the best of our knowledge, the current investigation represents the first 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).However, Anouassi and Ali (1990) and Skidmore, et al.(1992) reported that 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.

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 responded 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.

REFERENCES

Al-Eknah, M.M. (2000). Reproduction in Old World camels. Animal Reproduction Science, 60-61: 583-592.

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.**

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.

Callesen, H., Greve, T. and Hyttel, P.(1988). Preovulatory evaluation of the superstimulatory response in donor cattle. Theriogenology, 30: 477-488.

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.

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 Coference, pp. 187-191. Eds W.R. Allen, A.J. Higgins, I.G. Mayhew, D.H. Snow and J.F. Wade, R&W Publications, Newmarket.

Donaldson, L.E. (1985): LH and FSH profiles at superstimulation and embryo production in the cow. Theriogenology, 23, 441-447.

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.

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.

Mckinnon, A.G. and Tinson, A.O. (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.

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

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.

Purohit, G.N. (1999). Biotechnologies in camelid reproduction: Current status and future prospectives. J. Camel Prac. Res., 6, 1-13.

Skidmore L.(2000). Embryo transfer in the Dromedary camel (Camelus dromedaries).In : Recent Advances in Camelid Reproduction, Skidmore L. and Adams G.P. (Eds.) . International Veterinary Information Service , Ithaca NY.

Skidmore, J.A., Billah, M. and Allen, W.R. (1992). Ultrasonography and videoendoscopic monitoring of early fetal development in the dromedary camel. In: Proceedings of the First International Camel Coference, pp. 273-284. Eds W.R. Allen, A.J. Higgins, I.G. Mayhew, D.H. Snow and J.F. Wade, R&W Publications, Newmarket

Tibary, A. and Anouassi, A. (1997). Theriogenology in Camelidae. Abu-Dhabi Printing and Publishing Co., Abu-Dhabi, U.A.E..

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.

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.

Vyas S.(1998). Final report. Development of embryo transfer technology in Indian camel . National Research Centre on Camel, Bikaner, India.

الملخـ ـص

محاولات إحداث التبويض المفرط من أجل نقل الأجنة في الإبل

تمثل هذه الدراسة – حسب معلوماتنا – أول محاولة ناجحة لإحداث التبويض المفـرط وتجميع الأجنة في المملكة العربية السعودية. تم تحفيز التبويض المفرط في ٦ نوق بالغة غير حوامل، بحقن ٣٠٠٠ وحدة دولية من هرمون الفرس المشيمائي المحفز للمناسل فى اليـوم الأخير من العلاج .رمون 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 باسـتخدام جمل عالي الخصوبة. ثم حقنت الحيوانات بعد أول تلقيح، باستخدام ٣٠٠٠ وحدة دولية من الهرمون الآدمي المشيمائي المحفز للمناسل. تم تجميع الأجنة بالطريقة الغير جراحية في اليوم السابع إلى السابع والنصف من التلقيح. وضحت مظاهر الشياع على كل النـوق ٨-١٠ أيام من حقن هرمون الفرس المشيمائي المحفز للمناسل. استجابت ٤ نوق من ٦ للتبويض المفرط (٦٦ز٧%). بلغ متوسط عدد الاجسام الصفراء للنوق الأربـع ٤,٨ ± ٨,٧٥، وتراوحت بين ٦-١٦ جسم أصفر. بلغ معدل التبويض لهذه النوق ٩٧,٢٢%. وقد تم تجميع ٣ أجنة، واحد من كل ناقة. خلصت الدراسة أنه على الرغم من النتـائج المشجعة في مجال إحداث التبويض المفرط ومعدل التبويض، إلا إن معدل تجميع الأجنة ما زال في حاجة إلى دراسات مستقبلية لتحقيق نجاح مشابه.