Proceedings of the Third Annual Meeting for Animal Production Under Arid Conditions, Vol.1:108-121

Embryo Transfer in the Camel, Can it be Applied to Field Conditions with Realistic Costs?

A.H. Tinson and K. Singh

Embryo Transfer Research Center for Racing Camels, Private Department of H.H. Sheikh Khalifa Bin Zayed Al Nahyan, P.O. Box 17292, Al-Ain, United Arab Emirates.

ABSTRACT

Embryo Transfer has been used in many domesticated species to improve production and performance by increasing selection intensity of the desired trait. In the dromedary camel this has occurred almost exclusively in the racing camels of the United Arab Emirates. The technique is particularly useful in the camel given the long intercalving interval with traditional breeding and also the high value of the offspring when top racing animals are involved. The technique requires expensive equipment, drugs, and access to electricity and good handling facilities. In areas of North Africa and the Sub-continent, where camels can be a central part of agriculture, the opportunity to improve milk and meat production via Embryo Transfer(E.T). would not seem practical. Results using older, larger 9 day embryos in a camel E.T. program now suggest that field programs without the high costs might now be possible.

Key words: Embryo transfer, Megablast, Superovulation, Camel.

INTRODUCTION

With a gestation period of almost 13 months, a high incidence of early fetal resorption, abortion and variable birth to first estrus periods, the yearly calving rate for the camel can be lower than 40% (Wilson 1984). Breeding under range conditions, the genetic improvement in a herd can be a slow process. Camels will only produce one calf every 2 years. Previously published papers have discussed the improvements in the techniques and results since then (McKinnon and Tinson, 1992 and McKinnon et al., 1994).

The camels as an induced ovulator (Novoa 1970), required close monitoring techniques using ultrasound to check donor and

recipient ovarian development (Tinson and McKinnon, 1992; Tibary and Anouassi, 1996). Superovulation in the donors and ovulation in the recipients had to be closely monitored to allow the optimum handling regimes to be developed. When handling large numbers of animals the minimum number of examinations per animal per program to achieve pregnancy was one of our main goals.

This initially involved a significant investment in equipment, buildings, stables, stocks, etc. However, after intensive development of the techniques, coupled with monitoring of results over almost 10 yrs, one can more accurately predict responses and short cuts become possible.

Embryo transfer has been successfully applied to racing camels in the UAE to the extent that progeny testing via race results is giving good feedback to the selection intensity that has been applied to the process (Shereif and Tinson, 1998). However, while the cost relative to the value of a winning camel (often millions of Dirhams) is low, in the case of developing countries this cost is hard to justify.

Global warming and expanding deserts may increase the camel's potential worth in the long term to the many areas that depend on them for milk, and meat. Embryo transfer would allow more rapid improvement of herds but expense and expertise in many desert and marginal lands would be a drawback. In the case of the endangered wild Bactrian camel (Hare, 1997), a more economical form of E.T. in the camel would be of potential benefit to preserve genetic stock, but to set up a major facility in the Gobi desert is not economic or practical. However, if it was possible to run ET more like that have seen with commercial cattle herds, where the veterinary input is reduced from that seen in an in-house transfer center, and recipients were available on site, then it may be more feasible. In many desert areas, close monitoring with ultrasound and the use of microscopes would need portable generators on top of the cost of scientific equipment due to the lack of electricity. The following work proposes that the programs could be carried out without any equipment with a single visit of the veterinarian to collect and transfer the embryos. A technician would be needed to follow and administer the injection regime.

A pilot study in the 96/97 season suggested that the transfer of 9-day embryos could produce a better management of donors and recipients, as well as good pregnancy rates, and the ability to actually

be able to visualize the structure you were dealing with. The objectives of this is study were to look at results of single and multiple transfer of 9-day embryos, and to compare it to the pilot study and to normal 7 day collection results.

MATERIALS AND METHODS

Management of donors

In February 1998, camels were prepared and donors are selected by ability on the racetrack at our center. Donors are batched into groups of 3-4 animals. This number will vary depending on total donors and recipients available as well as how many bulls are available for mating. Generally camels over 12 years that have previously calved are the best potential embryo producers. All donors receive 14 days progesterone injections daily (100mg progesterone in oil I.M.) and are then checked ultrasonically for follicle development on both ovaries. Immediately following this a superovulatory regime of FSH(Follrophin-V Vetrepharm) and 2cc of prostaglandin (Estrumate Pitman Moore) is given. For first up donors, where likely response is unknown, FSH is preferred to eCG. (McKinnon et al., 1994). The response is also predictable with response in > 80% of first time donors.

The FSH is given as twice daily injections, over 7 days, in descending doses from 3 mg to 1 mg twice daily (BID). Camels are checked by ultrasound at 8 days and mated on day 9 and 10. With eCG (Pregnecol Heriot Aust) 4,500 IU are given as a single shot I.M.

This is also given at the end of the progesterone course when the ultrasound is completed. Camels are checked on day 2-3, post mating, to determine ovulation rates. In cases where the total mating time was less than 5 minutes or not deemed vigorous enough then 5cc (20 ug) of GnRH analogue, Receptal (Hoescht) was given I.M. to aid ovulation.

Flushing is done standing, with donors tranquilized with rompun ketamine mixture i.v. (30-50mg of each) with 5cc lidocaine epidural and disinfection of the perineum. Stocks are especially designed to protect operator and camel (Manefield and Tinson 1997).

Selection and management of recipients

Camels between the age of 5- 8 yrs that are either maidens or had 1 calf are optimum, but if large numbers are required (50+), we use 4-12 yrs with 1-3 calves. In each recipient group there were 12 camels prepared. Progesterone in oil (Bomagest, Bomac NZ) is also given I.M.at 100mg once daily (SID) but for only 10 days and terminates on the day the corresponding donor gets its third day of gonadotrophin.(Table 1). The following day the recipient gets 1,500 to 3,000 IU eCG (Pregnecol, Heriot Aust) as well as 2cc of Estrumate (Cloprostenol Pitman Moore) I.M.

Having been examined for follicle development the recipients receive hCG 3,000 IU 3 days (Marie, Anouassi 1987) after their donor group is mated. At this time an ovulatable follicle 10-20mm should be present. The age of the corpus luteum in the recipient will be 6 days on average, although depending on embryo recovery, 5.5 and 6.5 will also be used.

Table 1: Time involved between a synchronized donor and recipient for 9 day flush

Day	Donor Preparation	Day	Recipient Preparation
1	Progest daily for 14	8	Progest daily for 10
15	Start Superovluation	18	eCG inj.
24	Mate	27	HCG inj.
33	9 day flush	33	Transfer

Management of bulls

Only proven retired race bulls are used. Bulls are kept separate from the females and are given total sexual rest for at least one week

before a new program is commenced. If bulls are in shortage, then they mate daily in the morning and Receptacle is given to the donor if the mating is short or not vigorous. If there are enough bulls to be able to rotate, then the bull mates twice, 12hrs apart, and rests the following day.

Embryo recovery

The day of mating was termed day 0 and embryo collection was performed on day 9. Embryo recovery techniques have previously been described (McKinnon and Tinson 1992, Skidmore et al., 1992) but in the case of 9 day flushes, we use 2 liters of flushing solution (AB Tech bovine flush base). The fluid is introduced to the uterus slower as the larger 9 day embryos are much more fragile than the more commonly flushed 7 day embryos. The camel embryo increases in size after 7 days but tends to remain spherical till 9 days unlike the cattle embryo which tends to elomgate after 7.5 days (Elsden and Seidel 1990). During the second liter, the operator goes rectal to massage the uterus and ensure both horns are being flushed. Due to the age of the embryos, the donors do not receive a repeat flush. At the end of each liter, the fluid in the filter is placed in a separate petri dish. Before this happens, the large 9 day "Megablasts" are visualized and counted, to be compared to what was found searching the dishes with the microscope. In this experiment of 9 day flushes, we flushed 30 donor camels.

Embryo handling and transfer

All transfers were done non-surgically. Embryo handling and transfer has been previously described (McKinnon and Tinson 1992). However, with the large 9 day "Megablasts", the normal Drummond handling pipette cannot be used and a syringe with an IMV embryo straw is used to handle the embryos. Embryos are washed and held in Hec -2 holding solution (AB Tech). As previously reported by the author (Workshop 13th International Congress on Animal Reproduction), with the anatomy of the uterus, it is much easier to get into the right horn. A transfer to this side results in good pregnancy rates and a higher percentage of grade one transfers (side transfer rate left 41% 41/100, right 57% 101/177; grade transfer rate G1 64%, 72/113 and G1.5 43%, 49/115). So now

we tend not to try specifically for the left horn and, if anything, go for the right. Embryo migration ensures a left side pregnancy (El Wishy, 1987 and Arthur, 1992). Pregnancy in the recipient was assessed with ultrasound 10-12 days post transfer (Tinson and McKinnon, 1992; Tibary and Anouassi, 1996).

RESULTS

Donor preparation

Forty-seven donors were selected to be included in the program. This number was much higher than normal (usually 30) because it was the end of the season and we wanted to get some of the camels that had already given birth, and rest them next season. Since we also wanted to do a good number of multiple transfers, to compare to single transfers, we did not wish to risk being short of embryos. Of the 47 camels, 25 received a single shot of eCG (Pregnecol Heriot) and 22 received a course of FSH. If the camel has 5 or more follicles greater than 10mm at time of mating, the preparation is considered satisfactory and suitable for collection, should ovulation be complete.

The camels prepared with FSH 21/22 (95%) gave 5 or more follicles whereas with eCG 17/27 (63%) gave 5 follicles or more. With the FSH 19/21 were flushed post mating (90%) and 11/17 of eCG successful preparations were flushed (65%).

Recipient preparation

In this program we started with 118 potential recipients, of which 116 had a follicle with the potential of ovulation induction after the treatment with eCG (98%). Three days after induction of ovulation, the camel is checked for the presence of a corpus luteum. In this case, of the 116 camels that received hCG or GnRH, 109 ovulated (94%). The preparation percentage of original recipients was 109/118 (92%) which is similar to recent years' results. At time of transfer, the recipients are handled much as the donors, described previously. Of the 109 recipients, 108 received one or more embryos (one was showing discharge when presented for transfer.)

Embryo collection

Flushing 30 donors resulted in the collection of 188 embryos with an average of 6 embryos per camel. This is also similar to commercial results in cattle in on-farm E.T. in Australia (Harrison (1998)), but higher than previous averages in camels McKinnon et al., 1994; Tinson, (1998). When comparing the numbers visualized in the filter during collection, to total found after searching the dishes with microscope, the ratio of visualized to total was 149/188 (79%) were seen during estimations made at time of pouring cup into petri dish (not actual searching visualization).
With the FSH preparations, the embryo collection per flush was 5.6 (107 E's/19) and with eCG preparations, 7.4 (81 E's/11).
Of the 188 embryos collected, only 10 embryos graded worse than Grade 2; 53 were rated grade 1; 104 grade 1.5; and 21 grade 2 embryos.

Pregnancy rates

The embryos were transferred into 108 recipients and resulted in an overall pregnancy rate of 63% (68/108). This was an excellent result considering the age of the embryos and the number of recipients used. This is as good as our best smaller group 7day flush result and similar to that seen in cattle and camels in smaller groups (Skidmore et al 1996). The breakdown of the results is as follows in Table 2 with megablasts transferred either single or multiple, to the recipients.

Table 2: Transfer of "Megablasts"vs Pregnancy

Age of Embryo	# Transferred	Pregnancy/ Total	% Pregnant
9 Day	Single	29/52	56%
	Double*	23/32	72%
	Triple*	5/8	63%
	Quadruple*	½	50%
8 Day	Single	½	56%
	Double*	2/2	100%
	Triple*	3/6	50%
	Quadruple*	4/4	100%
Total S. Trans.		30/54	56%
Total M. Trans.		38/54	70%

(Note-*all multiple transfers resulted in single pregnancy at 60 days despite twins being detected in many cases on earlier examinations).

DISCUSSION

The idea of looking at 9-day transfers specifically came from a study in our center in the 96/97 season where camels were flushed at 7 to 13 days. Some were transferred and some kept for electron microscopy and the results for 9day singles and especially 9 day double transfers was high even though the numbers were low. Another program was done focusing on 9 day megablast transfers. The single transfers of 9 day megablasts were 43/91 (47%) but the double 9 day megablast transfers resulted in 15/21 pregnancies (71%). With a similar pregnancy rate to 7 day in the single transfer, and superior to 7 day doubles, we then were able to view 9 day flushes as a way of maximizing the use of donors. On days when the first camel gave a large number of embryos the other donor could be

delayed in collection which would save the need to freeze and maximize the use of recipients.

With delayed oviductal transport, associated with asynchronous ovulation's (McKinnon et al., 1994), there had always been the need to reflush camels at day 7.5 or 8.0 which increased the work load for the following flush day and made recipient management harder. When morulas appeared in 7 day collections, their pregnancy rates were always poor and we began to suspect that some of them had actually arrested their development. Rather than actually culture them, we decided to continue with 9 day flushes, which would allow for an extra 2 days' incubation under ideal uterine conditions. Any embryos still present at 9 days were viable megablasts (1-2 mm in diam.) and there was now no need to reflush. As long as the flushes were done carefully and there was no tearing of embryos during the flush, the technique was giving good pregnancy rates and work management advantages when handling large numbers of donors and recipients.

Oestradiol-17β and oestrone are detected in conceptus tissue as early as day 10 after ovulation (Skidmore et al., 1996). With early lysis of the corpus luteum in the camel showing precipitous drop of progesterone at 9-10 days (Skidmore et al., 1998), rapid strong signaling by the embryo to the CL is important whether by estrogens, interferon or other factors. The greater the trophoblastic mass involved, one would assume the stronger the signal. The results of our pilot study in 96/97 (unpublished data) showed that, in addition to 9 day doubles giving 71%, that 8day doubles gave 100% (3/3), but 7 day doubles only 54% (7/13), which is closer to single rates 45% (84/188) in the same season. The results of the multiple transfers in table 2 show an improvement in pregnancy as the trophoblastic mass increases.

The other interest in the 9 day transfer of megablasts is, as their nick name implies, they are big, being 1-2 mm in size. When flushing, you can see them coming out the flush line, in the filter cup and in the dish. This makes for very quick assessment of recipients needed as well as need for extra time flushing if a lot of embryos are liberated when one goes rectal on a multiparous camel during the second half of the flush.

The percentage visualized in the flush filter cup was done quickly while pouring into the petri dish. While 79% visualization

was good, if it was done in the petri dish in a more methodical manner, the actual recovery of embryos would be around 90%.
Donor preparation rates were high with FSH (95%) compared to eCG (63%). This is probably influenced by the fact that it was the third preparation of donors for the 97/98 season and those camels that were new or known FSH responders got FSH. On the other hand, camels that were poor responders to anything previously used were given a single dose of eCG in the hope of one or two follicles for a pregnancy. Embryo recovery compared to preparation was interesting in that eCG (7.4) showed higher average per flush than FSH (5.6). In our previous report (McKinnon and Tinson, 1994), we saw the opposite with 3.84 for FSH and 2.3 for eCG. Results here may have been skewed by one collection of 21 embryos with eCG since over the last 9 years, we have had consistently better results with FSH preparations. In 95/96 season FSH gave 310 embryos (310/73, 425%) and eCG gave 198 embryos (198/62, 319%). In the 96/97 season FSH again was superior with 257 embryos (257/61, 421%) and eCG only gave 68 embryos from 37 donors collected (68/37, 184%) (Tinson, 1998). It remains our first line preparation in a donor of unknown history. The lower antigenic response means it is more likely to give repeat responses.

Recipient preparation success was high at 94% and is attributed to consistency of the program and close monitoring. As of the end of the 97/98 season, the center has flushed 640 donors, collected 2,375 embryos and performed 1,867 transfers (Tinson, 1998). When working with these sorts of numbers, the production of a set regime for preparation becomes more feasible.

Pregnancy rates previously recorded vary considerably, but with the short time E.T. has been used in the camel, the improvements have been rapid. McKinnon and Tinson, (1992) reported 28% from 121 non surgical transfers. In the 95/96 season 309 transfers produced 163 pregnancies (53%) (Manefield and Tinson, 1996). Tibary and Anouassi(1997) reported 42% pregnancy rate from embryo transfer with FSH preparation, 38% with eCG and 49 % with Inhibin. The results with the 9day embryo transfers, at 63%, show an improvement for our center since 95/96 and suggest the idea of increased trophoblastic mass being a factor. Besides the increased pregnancy rates, it is interesting to note the increase in embryos per donor and the quality of the embryos. It may be possible that the extra 2 days' incubation not only filters out arrested

embryos, but also brings on embryos that were in a fragile state at 7 days and that if collected at that time would not have resulted in a pregnancy. This would agree with the observation on cattle, sheep and other species (Adams, 1982) that the shock and trauma of transfer and collection may cause a slow down in development and possibly weaker signaling. Improved medias specifically for the camel may help to lessen this effect. The quality of the resultant embryos, coupled with the increase in trophoblastic mass, may be the main contributing factors to the improvement in embryo pregnancy rates.

CONCLUSION

With consistency in recipient and donor preparation, the concept of a fixed regime for donor and recipient preparation becomes feasible. Up until now, camel embryo transfer has revolved around close monitoring of the animals, examining each animal a minimum of three times with ultrasound, as well as close searching and evaluation of embryo at the time of collection. Collection at 9 days offers not only improved management flexibility of animals, but comparable or better pregnancy rates with good embryo recovery and quality.

It would now be possible to contemplate the following for camels in remote areas where the cost of E.T. would be prohibitive but improving milk production desirable. Firstly, once a few donors had been identified, along with a suitable number of recipients (depends solely on the number of initial donors), then a technician could follow an injection plan such as Table 1 and the operator would arrive at the site on day 33 to collect and transfer. If one was concerned, then rectal palpation (Tibary and Anouassi, 1997) could be done once on the donors on day 23 to determine super ovulation response. Embryos, once collected and visualized, could be transferred single or double depending on numbers of embryos and recipients. Pregnancy could be assessed by the strength of tail reflex in presence of bull at 10-12 days post transfer (Tibary and Anouassi, 1997).

By attempting the above, one does away with the need for expensive equipment such as ultrasound and microscope as well as the need for electricity. With cattle in Australia, the cost of E.T. per animal varies from $220 per transfer to $1300 depending whether the

recipient is on farm or on center (Harrison, 1998). The huge cost of maintaining recipients is done away with when using on farm recipients. The actual disposable and drugs to get embryos would be approximately 120 dirhams per embryo (based on 6 E's per collection) and another 140 dirhams per transfer (including recipient drugs).

This would include drugs and disposable and not include the labor cost or costs of feeding recipients and donors. These prices would be significantly lower in India due to low drug costs.

The final decision would depend on the value of the embryos involved. Comparing "dairy camel" embryos to the commercial situation in cattle, one could work on a value of $200-500 US per embryo (Harrison 1998) and so, slightly compromising the optimum situation in program design, could be offset with the benefit of faster herd improvement. When valuable racing camels are involved, where the offspring could be worth in excess of $500,000, then no risks or compromises can be tolerated.

ACKNOWLEDGMENTS

All work reported here was totally funded by H.H. Sheikh Khalifa Bin Zayed Al Nahyan.

REFERANCES

Adams, C.E. 1982. Mammalian eggs transfer C.R.C. Press. Florida, USA.

Arthur, G.H. 1992. An overview of reproduction in Camelids. Proc 1st Int. Camel Conf. Eds. Allen, W.R., Higgins, A.J., Snow, D. and Wade, J.F. Newmarket, Suffolk, UK., R&W Publications NewmarketLtd., 109-113

Elsden, R.P. and G.E. Seidel. 1990. Procedures for recovery, bisection, freezing and transfer of bovine embryos. Animal Reproduction and Biotechnology Laboratory, Bulletin No.2, Colorado State University.

El Wishy, A.B. 1987. Reproduction in the female dromedary Camelus dromedarius: A Review. Animal Reproduction Sci. 15:273-297.

Hare, J.N. 1997. Status and distribution of wild Bactrian camels Camelus Bactrianus ferus in China. Camelids: Science and Productivity. Journal of Camel Practice and Research4 (2):107-110.

Harrison, B.T. 1998. Cattle in on-Farm E.T. in Australia Personal Communication.

Manefield, G.W. and A.H. Tinson. 1997. 'Camels' A Compendium. Vade Mecum Series C, No. 22 Post Grad. Foundation. Sydney University.

Marie, M. and A. Anouassi. 1987. Induction of luteal activity and progesterone secretion in the non-pregnant one-humped camel Camelus dromedarius. J. Reprod. Fert., 80: 182-192.

McKinnon, A.O. and A.H. Tinson. 1992. Embryo transfer in dromedary camels. Proc. 1st Int. Camel Conf. Eds. Allen, W.R., Higgins, A.J., Snow, D. and Wade, J.F. Newmarket, Suffolk, UK, R&W Publications NewmarketLtd., 203-208.

McKinnon, A.O., A.H. Tinson and G. Nation. 1994. Embryo transfer in dromedary Camels. Theriogenology 41:145-150.

Novoa, C. 1970. Review: Reproduction in Camelidae. J. Reprod. Fert. 22:3-20.

Shereif, N. and A.H. Tinson. 1998. Genetics and breeding methods in camels. In: Selected Topics in Camels. Published by Camel Publishing House, Bikaner, India.In press.

Skidmore, J.A., W.R. Allen, M.J. Cooper, M. A. Chaudhry, M. Billah and A.M. Billah. 1992. The recovery and transfer of embryos in the dromedary camel: Results of preliminary experiments. Proc. 1st Int. Camel Conf. Eds. Allen, W.R.,

Higgins, A.J., Snow, D. and Wade, J.F. Newmarket, Suffolk, UK, R&W Publications Newmarket Ltd., 137-142.

Skidmore J.A., W.R. Allen and Heap. 1997. Maternal recognition of pregnancy in dromedary camel. Camelids: Science and Productivity. Journal of Camel Practice and Research. 4, (2), In press.

Skidmore, J.A., G. Starbuck, G.E Lamming and W.R. Allen. 1998. The control of luteolysis in the dromedary camel camelus dromedarius. J.Reprod Fert.114: 201-209

Tibary, A. and A. Anouassi. 1996. Ultrasonographic changes of the reproductive tract of the reproductive tract in the female camel Camelus dromedarius during follicular cycle and pregnancy. J. Camel Pract. and Res. 3 (2): 71-89.

Tibary, A. and A. Anouassi. 1997. Theriogenology in Camelidae. Abu Dhabi Printing and Publishing Co. Mina, Abu Dhabi, UAE.

Tinson, A.H. and A.O. McKinnon. 1992. Ultrasonography of the female dromedary camel reproductive tract. Proc. 1st Int. Camel Conf. Eds. Allen, W.R., Higgins, A.J., Snow, D. and Wade, J.F. Newmarket, Suffolk, UK, R&W Publications Newmarket Ltd., 109-113.

Tinson, A.H. 1998. Embryo transfer in the dromedary camel. Masters Degree. Melbourne University.

Wilson, R.T. 1984. The Camel. Longmon Group Limited, U.K.