DOI : 10.5958/2277-8934.2025.00038.7 *Vol 32 No 3, p 301-308*

MY JOURNEY TO CAMEL SCIENCE FROM CAMBRIDGE TO DUBAI

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ABSTRACT

My passion for adventure brought me to the Camel Reproduction Centre in Dubai where I was able to combine my love of animals with exciting research. Research as always presents many challenges, marked by periods of success and exhilaration, as well as periods of frustration and disappointment. However, whether it has been trying to relate oestrous behaviour to follicular wave patterns, working out the intricacies of embryo and semen collection, handling and cryopreservation or performing pioneering experiments hybridising New and Old World camelids it has been a time of fulfilment and satisfaction, as well as the realisation that determination and diligence can sometimes make the impossible possible! I will be forever grateful to the late Professor Roger Short who suggested I explore the world of camels, to the late Professor "Twink" Allen whose vision and encouragement has inspired me throughout my whole career and all my scientific friends and colleagues with whom I have had the pleasure of working over the years. The biology of camels will never cease to amaze me and I hope that the work we have done, and hopefully continue to do, will enable others to better understand the intricacies of camel reproduction and encourage them to explore it further.

Key words: Artificial insemination, camels, embryo transfer, embryos, semen

My first experience with camels was in 1989. At that time, I was working as a research assistant for the late Professor W.R. (Twink) Allen at the Equine Fertility Unit (EFU) in Cambridge/Newmarket when Twink was asked by His Highness Sheikh Mohammad bin Rashid Al Maktoum, now the Vice President and Prime Minister of the UAE and Ruler of Dubai, to visit Dubai to start an embryo transfer project in racing dromedary camels. I was working on embryo projects at the EFU and was therefore fortunate to be included in the project - an amazing opportunity. Together with Dr. Mike Cooper, a cattle vet from Staffordshire, we visited the Camel Reproduction Centre (CRC) in Dubai on several occasions during the camel breeding seasons of 1989 and 1990, and our exposure to these magnificent animals of the desert began.

The Camel Reproduction Centre was a small well-constructed building consisting of a camel handling area and lab space nestled amongst the sand dunes about 45 km from Dubai city and approachable only by 4WD vehicles but was further developed in 2012 to include more lab space (Fig 1). Together the three of us had several enjoyable visits to Dubai over the next two years but soon discovered that the camel held many mysteries reproductively. These we realised were going to take some time to unravel; however, we did succeed in obtaining some pregnancies from embryo transfer and, after teaching their camel vets how to recover and transfer embryos, our consultancy visits sadly drew to a close.

In 1991, whilst on a three month working trip in Australia, I met the world renowned (late) Professor Roger Short, a famous reproductive physiologist, who became a great friend and scientific mentor. After many fascinating and stimulating conversations with Roger he suggested I change from horses to camels and register for a Ph.D. in camel reproduction. I decided to take up the challenge; and it was a decision that changed my life! Thus, under the supervision of Prof Twink Allen, I registered at University of Cambridge (UK), and with the assistance of the late Dr. Billah, (Director of Zabeel Feedmill) returned to Dubai in October 1991 to start my Ph.D. entitled "Reproduction in the Dromedary Camel." It was a privilege, and a great help to me that Professor Twink Allen, Professor Roger Short and Professor Brian Heap agreed to be Governors of the CRC in those early years (Fig 2). There were many challenges in those first six months, not only with trying to unravel the intricacies of camel reproduction but also with living in Dubai (very different from the Dubai of today) as a single woman. However, with a great team at the CRC (Fig 3) these were soon overcome

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and although the initial plan was to stay three years in Dubai to complete my Ph.D., I am still here 33 years later.

For the duration of my Ph.D. (1991 – 1994), I spent the camel breeding season (October to March) in Dubai carrying out all the practical camel work and the non-breeding season (April to September) at the EFU in Newmarket doing the follow-up lab work and analysing results. As there was very limited literature published on camel reproduction we started by observing oestrous behaviour in female camels housed with a male. At the same time, we monitored ovarian follicular activity by ultrasonography and learnt that oestrous behaviour did not necessarily relate to follicular activity. We also confirmed that camels are induced ovulators, ovulating only when mated, and that follicle growth occurred in wave-like patterns with periods of growth, maturity and regression. In addition, we learnt that mature follicles capable of ovulating measured between 1.3 – 1.7 cm in diameter, but could continue to grow to as large as 4 – 6 cm if ovulation was not induced (Skidmore *et al,* 1995). We correlated this follicular activity with hormone profiles and discovered that the camel has a very short luteal lifespan of only 8 - 9 days, as evidenced by a rapid decline in serum progesterone concentrations from maximum concentrations on days 8 - 9 to basal concentrations by days 10 – 12 after ovulation. Although oestradiol concentrations increased as the follicle diameter increased, when the follicle reached 1.7 cm in diameter the oestradiol concentrations started to decline, even if the follicle continued to grow in size (Skidmore *et al,* 1996). This may account for why these large follicles do not ovulate.

We continued our studies looking at the pregnant camel and explored embryo development and placentation. We discovered that embryos enter the uterus between days 5 and 6 after ovulation as hatched, expanded blastocysts and start to elongate by days 10 – 12. As we had already concluded that luteolysis occurred around days 9 – 10 after ovulation, we tried to discover the potent maternal recognition of pregnancy (MRP) signal that passes between the embryo and maternal endometrium to signify pregnancy and inhibit luteolysis. We therefore cultured early embryonic tissue in M199 media with or without steroid precursors, (androstenedione and pregnenolone) to look for the ability of the embryonic tissue to synthesise either interferons or oestrogens. Interestingly, unlike ruminants, camel embryonic tissue does not synthesise interferon tau but, similar to the horse and pig, can synthesise oestrogens (Skidmore *et al,* 1994). This suggested that oestrogens could be involved in the MRP signal in camels. This hypothesis was further confirmed by the discovery of large, multinucleate cells at frequent but irregular intervals along the trophoblast that stained positively for the steroid- synthesising enzymes such as 3β – hydroxysteroid dehydrogenase, 17α- hydroxylase and aromatase.

We next turned our attention to embryo transfer in elite racing camels, the necessary prerequisites for which are superovulation (production of many follicles) in the donors and synchronisation of donors with recipient camels. Methods of superovulation involved trying different concentrations of equine chorionic gonadotropin (eCG, Folligon) with or without ovine FSH (Ovugen) or porcine FSH (Follitrophin or Stimufol) and we discovered that most follicles resulted from a combination of 2000 IU eCG and 400 mg of porcine FSH given over a period of four days.

Synchronising the donors and recipients presented more of a challenge as camels do not have the cyclical corpus luteum (CL) of other domestic animals. Therefore, the double prostaglandin injections given 11 days apart, used to synchronise cattle, did not work for camels. We enlisted the help of Professor Greg Adams (University of Saskatoon) and investigated methods using either a combination of oestradiol and progesterone, GnRH and prostaglandin (PG) or follicle ablation and discovered the easiest, and least time- consuming, method was to use a combination of GnRH injected every 15 days with PG injected on Day 7 after each ovulation (Skidmore *et al,* 2009). Not only is synchronisation necessary but the degree of synchronisation required between donor and recipient needed to be defined. Embryos were therefore transferred into recipients that had ovulated from one day before to three days after the donor was mated, and better pregnancy rates were achieved in recipients ovulating 24 – 48 hr after the donor (Skidmore *et al,* 2002). However, it is not always easy to tightly synchronise camels especially if you only have a small number of recipients. Therefore, we investigated methods to prolong the lifespan of the CL using meclofenamic acid, a prostaglandin synthetase inhibitor, so that embryos could be transferred as late as days 8, 10 or 12 after ovulation. By giving 1 g of meclofenamic acid orally from day 7 after ovulation to eight days after embryo transfer, we achieved pregnancy rates of 80%, 60% and 70%, respectively (Skidmore and Billah,

2005). Sometimes, however, follicles in the recipients matured more than 48 hr after the donor was mated, so we explored methods using progesterone injections to supplement the progesterone until the CL could take over production and maintain the pregnancy. We found that by injecting 75 mg of progesterone-in-oil from two days before to three days after embryo transfer we could achieve pregnancy rates between 50 – 60%, compared to 0% in control animals without progesterone supplementation (Skidmore and Billah, 2011). As part of our embryo transfer studies we were also successful in producing identical twins by bisecting an early day 6 blastocyst into two halves using a micromanipulator. Each half embryo was transferring into an individual recipient and Zahi and Bahi were born in 2008 (Fig 4).

After the success of these embryo transfer experiments, interest in embryo transfer grew quickly, not only for producing more of the elite racing or milking camels in the UAE, but also abroad in Iran where attempts were being made to try and preserve the Bactrian camel that was under threat of extinction. It was therefore a pleasure to accept the invitation from Professor Amir Niasari-Naslaji (a long- term friend and colleague) to visit Iran and assist him and his team (Fig 5) in projects to characterise follicular dynamics, multiple ovulation and embryo transfer in the Bactrian camel and then to perform interspecies embryo transfer between dromedary and Bactrian camels. As there were only 150 Bactrian camels in Iran at that time, the idea was to produce multiple embryos from the Bactrian donors and use the dromedary camels as the recipients. The project was successful with the birth of BEHNIA, the first Old World Camelid interspecies embryo transfer, in 2008 (Niasari-Naslaji *et al,* 2009).

In addition to embryo transfer, we have been investigating the use of artificial insemination (AI) in dromedary camels. Elite male racing camels are in high demand for breeding but as their libido and volume of semen produced is low, the use of AI would greatly enhance the number of females that could be covered by a single male. However, camel semen is not easy to work with: firstly, the male has to be trained to the artificial vagina (AV), secondly, the volume is low (usually only 2 – 8 ml per ejaculate) and thirdly, most problematic of all, the semen is very viscous, making mixing it with extender and getting accurate sperm concentration and motility valuations difficult. With the diligent work of my research assistant Mr. Tipu Billah, we trained some of our males to the AV so we could regularly collect semen. Firstly, we investigated several different commercial and home-made extenders but always achieved better results using Green Buffer produced by IMV Technologies (France) specifically for camel semen. Liquefying the semen was the next challenge, and gentle pipetting worked best as, although enzymes such as papain did liquefy the semen, they invariably caused damage to the acrosome or agglutination of the sperm heads. Once we could liquefy the semen we then had to determine the minimum number of sperm to inseminate to obtain pregnancies, where we should inseminate, (either the uterine body or into the tip of the uterine horn) and finally when to inseminate, (at the same time as the GnRH injection given to induce ovulation or 24 hr later, which is closer to the actual time of ovulation). From our studies we concluded that by inseminating 150 million live sperm into the uterine body or tip of the uterine horn, 24 h after injection of GnRH to induce ovulation could achieve a 50% pregnancy rate (Skidmore *et al,* 2006).

The ability to collect semen from bull camels led to another interesting project in trying to hybridise New and Old World camelids, a project instigated by Professor Short, who was fascinated by camelid evolution. The camel family originated in North America around 30 - 40 million years ago. Some camelids subsequently migrated north crossed the Bering Strait into China, Mongolia, the Arabian Peninsula and India and evolved into Old World Camelids (OWC) namely the Bactrian and dromedary camels, whereas others migrated south into South America and evolved into the New World Camelids (NWC) the guanaco, llama, vicuna and alpaca. Whereas all four species of NWC can readily hybridise with one another, and dromedaries and Bactrians can hybridise, NWC and OWC have not hybridised, due to their differences in size and location, even though they have the same diploid chromosome number of 2n =74. With the help of Paul and Sally Taylor, llama ranchers in Montana USA, we acquired some guanacos and llamas and learnt how to handle these somewhat flighty animals. After a few months we were able to collect semen from the male llamas and then inseminated female camels with llama semen and female llamas/guanacos with camel semen. After many attempts we announced the birth of "Rama the Cama" born from a guanaco dam and dromedary camel sire in 1998 (Fig 6) (Skidmore *et al,* 1999). Over the next few years six hybrids were born alive all of which were from llama dam and dromedary camel sire, but interestingly we did not

Fig 1. The Camel Reproduction Centre.

Fig 3. The staff of the CRC.

Fig 5. My collaboration with Prof Amir Nasari-Naslaji and his team in Iran, 2007.

achieve any live births from the reciprocal cross. In terms of size hybrids were closer to the NWC but much stockier. They did not have a hump but did have the longer tail of the camel, the woolly coat of the llama and a footpad halfway between the cloven hooves of the NWCs and the single footpad of the camel. However, they were infertile so the project was not pursued further, but the fact that we have been able to obtain a viable hybrid between a New World and an Old World camelid after 30-40 million years of genetic isolation was truly amazing.

Once we were able to achieve consistently reliable pregnancy results after the transfer of fresh embryos and insemination of fresh semen, it seemed a natural progression to move onto the transfer/insemination of cooled and frozen embryos and semen. There are many advantages to being able to cool and freeze embryos and semen: namely they are

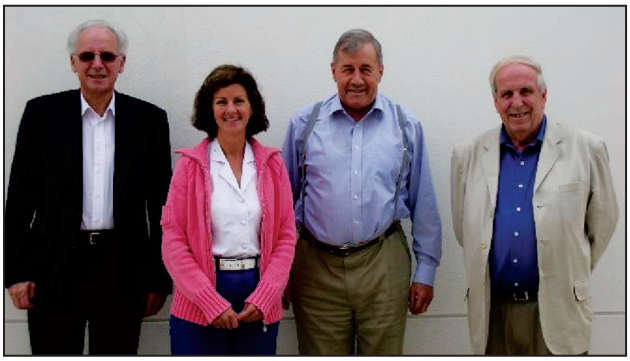

Fig 2. The Governors on the CRC with Dr Lulu Skidmore. (Left to right) Prof. Brian Heap, Dr Lulu Skidmore, Prof Twink Allen, Prof Roger Short.

Fig 4. Zahi and Bahi, genetically identical twins resulting from a bisected early blastocyst.

much easier to transport nationally or internationally than live animals, meaning that embryo recipients of females to be inseminated do not have to be in the same location as the embryo donors of male camel donate the semen. In addition, if more embryos are recovered than there are available recipients, or recipients are not synchronised with the donors, the embryos can be frozen, stored and only thawed when recipients are ready on their next natural cycle. However, we soon found that cryopreservation of camel embryos and semen had many challenges.

With the recruitment of more help – that of my good friend and colleague, Dr. Beth Crichton (Omaha Zoo, USA) on a seasonal basis from 2011-2019 and the full-time employment of Dr. Clara Malo in 2013, we made great progress in understanding how to handle and cryopreserve camel semen. Initial experiments investigated methods to improve sperm quality by single-layer centrifugation over a colloid developed by Dr. Jane Morell (Uppsala University, Sweden). This worked well to improve sperm quality and motility but reduced sperm concentration, as inevitably some spermatozoa was trapped in the colloid (Malo *et al,* 2017a). Next, sperm function was evaluated by developing a heterologous IVF system with goat oocytes. As camel oocytes are in

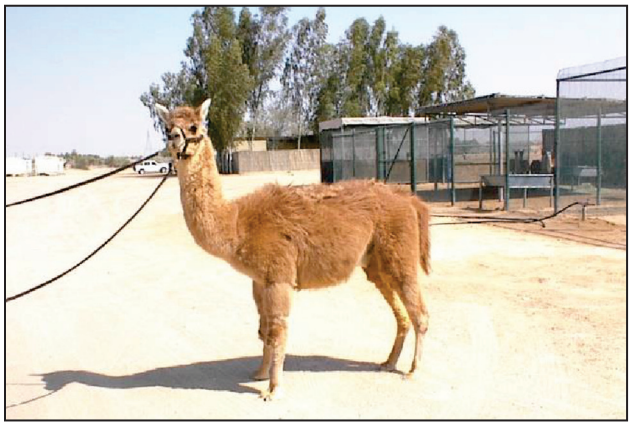

Fig 6. Rama the Cama the world's first camel guanaco hybrid.

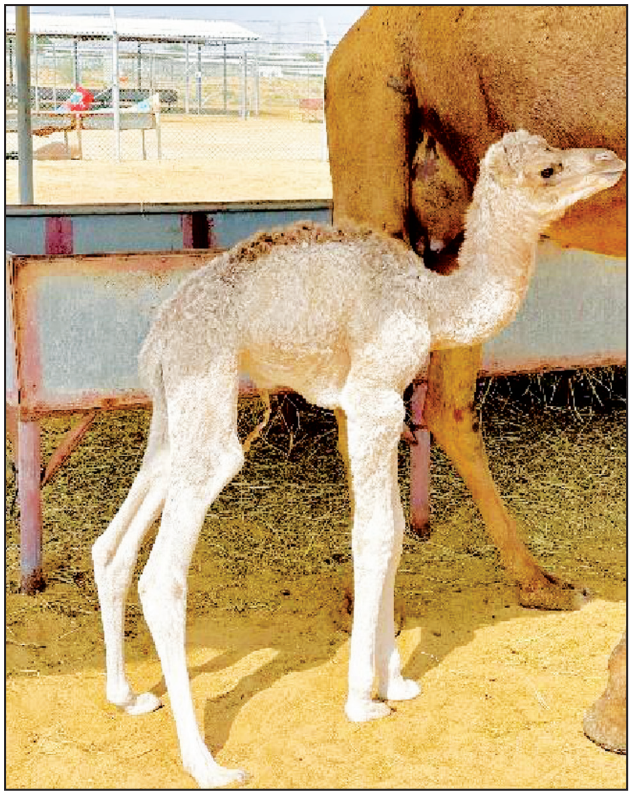

Fig 8. Our first IVF calf born April 2025.

short supply, denuded goat oocytes were incubated with camel sperm to assess the penetration ability of the sperm. Approximately 67% of the sperm successfully penetrated, decondensed and formed pronuclei indicating this is a novel and useful method to evaluate dromedary camel sperm function (Crichton *et al,* 2016). In addition, we investigated the application of cholesterol-loaded cyclodextrins, various cryoprotectants, different freezing and thawing rates and the use of antioxidants to improve post- thaw motility (Crichton *et al,* 2015, Malo *et al,* 2017b, 2018, 2019, 2020). Even though we obtained acceptable post- thaw motilities of around 40 - 45%

Fig 7. The lab staff of CRC. (Left to righ t- front) Dr Murren Herrid, Ali Ahmed, Dr Lulu Skidmore, Dr Clara Malo, Asif Rehman. (back row) Muqtador Billah, Abdel Rahman, Aijaz Hussain.

Fig 9. Participants of the first Short Course in Camel Reproduction held at CRC in 1999.

Fig 10. Lecturing to students.

by cryopreserving camel sperm using 3% glycerol as the cryoprotectant, and employing fast freezing and

thawing rates, pregnancy rates using frozen thawed semen were still disappointingly < 10%. Future research would do well to investigate the appropriate preparation and timing of the recipient for ovulation with respect to insemination of cryopreserved sperm, as synchrony between ovulation and sperm readiness for fertilisation are key to ultimate success.

Cryopreservation of embryos proved to be somewhat easier than cryopreserving semen. In our early studies, in collaboration with Dr. Naida Loskutoff (Omaha Zoo, USA), we were able to cryopreserve embryos by exposing them to 1.5M ethylene glycol for 5 – 10 min and using a slow, controlled rate of freezing (Skidmore *et al,* 2004). However, further studies concentrated on vitrification as it is a faster method that does not require expensive equipment and therefore could be used more easily in the field. In our initial vitrification studies, we achieved similar pregnancy rates to the slow-cooling method of around 37% (Skidmore *et al,* 2005), but this was further improved when Dr. Muren Herrid joined the CRC team in 2014 (Fig 7). After many studies optimising the vitrification method (Herrid *et al,* 2016, 2017), he developed a Camel Vitrification Kit, which we tried and tested at the CRC (Skidmore *et al,* 2020) and it is now commercially available from Minitube (Germany). Although pregnancy rates of 45 – 50 % can be achieved using this method of vitrification, results clearly depend on the quality and size of the embryos, with smaller, good- quality embryos yielding better results (Skidmore *et al,* 2021).

Despite recent advances in cryopreservation of camel embryos, widespread application of this technique is still limited, as transport involving liquid nitrogen is problematic. Therefore, our more recent studies have investigated various cooling and culture methods for short- term preservation of embryos. When my research colleague, Brendan Mulligan, joined us at CRC in 2022, we compared cooling (4°C) and culture (37°C) of varying morphological grade embryos for up to 72 hr. Pregnancy rates of 53.3 and 50% were achieved for good quality, cooled and cultured embryos, respectively after 72 hr. However, although cooling or culture could maintain the viability of good- quality embryos, only embryo culture preserved low morphological grade embryos, as they had poor tolerance to cooling and did not survive 72 hr at 4°C (Mulligan and Skidmore, 2023).

Our lastest achievement was the development of a successful IVF culture system that resulted in the birth of an IVF calf in April 2025 (Fig 8). *In vitro* fertilisation and the production of embryos in camels is difficult due to the lack of a regular supply of oocytes and the challenges involved in collecting good- quality semen, both of which hindered our progress.

Over the years it has been important to us to share the knowledge we have gained from our research, not only in publication of papers in international journals, but also by organising short courses in camel reproduction. The first one was held in 1999 with many national and international participants (Fig 9) and these courses have become so popular now that in 2025 we held two courses (Fig 10).

It has been a great privilege to work with these amazing animals, the "ships of the desert" for the last 33 years and discover some of their reproductive mysteries. The interest in camels is certainly growing worldwide as evidenced by the United Nations declaring 2024 the Year of the Camelid. It was therefore a great honour for me to be invited to speak on our camel reproduction research at a UN meeting in Vienna. I was also invited as a keynote speaker to give lectures about our research at several conferences during "The Year of the Camelid", namely in Morocco, Kuwait, Saudi Arabia (KSA) and United Arab Emirates. Whilst in KSA I visited the Salam Veterinary Group to discuss various possibilities for collaboration between our two laboratories.

I have also acted as a consultant for the Food and Agricultural Organisation (FAO) on a project to protect the Bactrian camel population in Iran. In 2023 we hosted Mr Tavasoli, Mr Veysehzadeh and Mr Markdaneh, (together with Ms Sahereh Joezy as their translator) for a three-week training period at CRC and then in 2024, I advised on the setting up of a mobile laboratory to visit their rural farms and ran a series of online lectures about assisted reproductive techniques in camels. More recently, in June 2025, I was invited by Professor Esengali and his team to visit Kazakhstan to lecture at their summer school held at the Kazakh National Agrarian Research University and to act as a consultant on their camel breeding and genetics project. They were very kind and generous hosts and I thoroughly enjoyed my visit.

With all my recent travels it has been encouraging to see the growing interest in camels worldwide, especially as the many uses of camels and the health benefits of camel milk, for example, become more widely known. I sincerely hope all the work we have carried out at the CRC will continue to inspire people to continue the research and ensure that these amazing animals get the recognition they deserve.

Acknowledgements

I would like to sincerely thank His Highness Shaikh Mohammad bin Rashid Al Maktoum, Vice President and Prime Minister of United Arab Emirates and Ruler of Dubai for his continued support, interest and funding of all our work at the CRC and Dr Ali Redha our Director General. Also particular thanks to the late Prof Roger Short for inspiring me to work with camels and the late Prof Twink Allen for his enthusiasm, encouragement and never ending support throughout my career studying reproduction in horses and camels. In addition, huge thanks to Mr. Tipu Billah and Mr. Aijaz Hussain my research assistant and technician who have been standing alongside me for the last 33 years, to Ali Ahmed, Asif Rehman and all the camel hands who maintain and care for the animals and facilities at the CRC and to Dr. Jane Vaughan (Cria Genesis, Australia), for her invaluable help assisting with veterinary care and embryo transfers during the camel breeding seasons from 2015 – 2020. Also many thanks to Dr. Clara Malo, Dr. Muren Herrid, Dr. Beth Crichton, Dr. Jane Morell, Dr. Budhan Pukazhenthi and Dr. Gregg Adams for their hard work and scientific input that have made the publication of our many scientific papers possible.

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