Embryo Transfer - A Breeders Perspective

Background

The use of embryo transfer (ET) technologies has been a relatively recent advance in breeding technology for alpacas in Australia. The reasoning behind the development of the technology was to increase the use and allow great availability of genetically superior animals both locally and internationally. With long gestation periods for alpacas (11 months), conventional breeding results in slow genetic gain. Also information gained from these breedings is less reliable as it is difficult to compare results over various years due to the variations in season etc also influencing the results.

Reproductive technologies, Artificial Insemination (AI) and Embryo Transfer (ET) have been developed and used extensively to improve fleece and conformational characteristics in similar industries (Merino and Angora goats). However, the reproductive processes of alpacas are quite unique and are less understood, therefore the direct transfer of these technologies from ruminants to alpacas was not possible.

Protocols evaluated for embryo transfer in camelids have been adapted from protocols originally developed for cattle, sheep, pigs and horses. Embryo transfer can rapidly increase numbers of crias born to superior females. For example, it is possible to transfer the genes from the top 10 % of an alpaca herd (donors) into the bottom 90 % of females (recipients). Embryo transfer also allows breeders to determine optimal male/female combinations as multiple sires may be used over the same female in one year. Embryo transfer can give smaller breeders access to elite genes through purchase of embryos and will allow for inter-farm/state/national movement of superior genetics.

Alpaca Female Reproduction explained

The understanding of ovarian function in alpacas has been instrumental in the success of developing non-surgical, transcervical single and multiple ovulation ET. Females exhibit waves of ovarian follicular growth, with new waves emerging every 12 to 22 days (Vaughan *et al.* 2004). Females are induced ovulators, and ovulate 30 hours after copulation when they have a dominant follicle of at least 6 mm on either ovary (Adams *et al.* 2001; Bravo *et al.* 1991). A corpus luteum develops on the ovary at the site of ovulation 3-4 days after mating and secretes progesterone. If conception does not occur, prostaglandin is released from the uterus and induces regression of the corpus luteum 10-12 days after mating (Adams *et al.* 1989). The embryonic signal for maternal recognition of pregnancy must be transmitted as early as Day 9 or 10 after mating in order to ‘rescue’ the corpus luteum of pregnancy as the corpus luteum is the major source of progesterone throughout pregnancy.

Single ovulation versus multiple ovulation

Single-ovulation embryo transfer of alpacas does not require any hormonal treatment of donor females (Taylor *et al.* 2000). Donor females are mated once and flushed a week later. Approximately 7 of every 10 females flushed will produce an embryo. Follicle growth in the first 10 days after new wave emergence is consistent regardless of subsequent interwave interval (Vaughan *et al.* 2004), an observation integral to the success of single-embryo flushing of donor females every 10-12 days. More than 400 live births (50 % males, 50 % females) have occurred over the last 8 years in Australia, following single-embryo flushing performed by Dr Jane Vaughan and Dr David Hopkins in numerous

commercial alpaca herds. Donor females have since given birth to crias from matings performed soon after embryo flushing, indicating donor fertility was not interfered with during embryo collection.

Methods of multiple ovulation and embryo transfer (MOET or 'superovulation') are also being implemented in alpacas in Australia and other countries. Both equine chorionic gonadotrophin and follicle stimulating hormone are currently being used as agents to stimulate multiple ovulation. Techniques are producing an average of 2.5-3 embryos per flush (up to 21 embryos per individual) on most farms. Results have been less reliable on some farms, presumably due to variations in alpaca fertility, nutrition, environment and management. The number of studies on MOET in camelids remains low and further refinement of existing protocols is continuing to identify a MOET program that consistently yields an acceptable number of transferable embryos, and is associated with minimal risk of infertility to the elite donor female. Embryos have been yielded on many consecutive MOET programs in the last six years, without apparent effect on donor fertility as donor females have readily conceived within 2-4 weeks after their last MOET flush.

Embryo development in camelids
The embryos of camelids develop faster than in domestic ruminants and morulae have been recovered in the oviducts of llamas as early as 3 days after mating. The faster rate of embryo development in camelids is likely related to early maternal recognition of pregnancy, which needs to occur around Day 8 to 10 after mating to ensure persistence of the corpus luteum of pregnancy (Aba *et al.* 1997; Del Campo *et al.* 1995). Embryos are flushed from donor females a week after mating.

Non-surgical, trans-cervical collection and transfer of embryos
This method involves the introduction of a catheter through the cervix and placement of the catheter in the uterus. Medium is flushed through the catheter into the uterus, then allowed to drain, via gravity, into an embryo collection vessel. This method is relatively non-invasive and does not have the attendant risks of abdominal adhesions associated with surgical embryo collection. However, females with a narrow pelvis or excessive fat in their pelvis may not be suitable for non-surgical collection and there is also a risk of rectal trauma with this procedure. Dr Jane Vaughan uses the non-surgical method of embryo collection from alpacas and llamas.

The retrieved fluid is examined under a dissecting microscope for embryos. After collection and washing, single embryos are loaded into small plastic straws similar to those used for artificial insemination and then placed transcervically (non-surgically) into the uterus of the recipient female.

The success or failure of an ET program is influenced by many factors. Correct selection and preparation of females (both donor and recipient) is essential.

Registry Issues:

In Australia, the registration of ET cria is allowed. The process is as below.

The details of breeding sire and dam are certified by the veterinarian performing the transfer of embryos. Paperwork has to be signed off by the Vet on the sire and the dam of the embryo and the registration details of the recipient.

As all males and donor females used are DNA recorded, testing can be done to confirm the pedigree details of the embryo.

All recipients are required to be registered. The registry details are recorded by the Vet at the time of transfer and are also recorded against the embryo at the time of registration of the resultant cria.

Our Experience

Over the last 6 years, working with our veterinarian, Dr Jane Vaughan, we have become a major user of ET in Australia. We now have over 300 ET cria on the ground and have been very pleased with the results overall.

It has enabled us to reproduce our best genetics at a significantly faster rate than would be otherwise possible. Some of our best females have over 20 cria on the ground from a range of males (at 8 years of age!!) This allows us to then make these genetics available to our clients.

We have seen a rapid improvement in quality of our alpacas.

What have we learnt:

Soundness in all the animals used in the program is the foundation of a successful program. We may want to use a particular female that is stunning but fails to conceive naturally. However the hard truth is that if she is sub-fertile naturally, she will probably perform poorly in ET and also do you really want to produce more progeny from her as they are quite likely to also have fertility issues.

Data is King. It is essential to record and review the results of your ET program. To improve success, you must be willing to make hard decisions about dropping out donor females or males that do not perform in the process. We also spend a lot of time managing our recipients and their results. Taking into account the difficulty in producing embryos we want to maximise the results. Therefore if a female fails to hold an embryo greater than three times or if her cria is slow to grow out due to poor milk supply or mothering skills she is removed from the program.

As mentioned from our experience, not all females or males will perform in ET. Reproduction is a complex system and we have found that in the altered environment of super ovulation, some combinations will not produce embryo's. Therefore to improve success, decisions need to be made to alter the breedings or drop out females or males completely.

In any program, attention to detail will improve results. After every program we sit with Dr Jane Vaughan, to examine what our results have been and to see if something has changed that may have had a positive or negative result on the program. Major issues we have discovered are:

Nutrition:

All alpacas should be kept in good body condition (we work on a body score between 2.5 to 3 out of five). Donor females have a tendency to get fatter overtime. This will have a

negative impact and at times donors will need to be mated naturally to aid in the management of her weight.

Too much of a good thing can have negative impacts on the ET program. We try to have all animals involved on pasture that is a good mix of grasses and herbs. We try to minimise the amount of clover (due to the plant estrogen having a negative impact) and Rye grass also can have a negative impact on fertility.

Soils may be lacking in some essential nutrients. Australia soils are often low in Selenium and this will have a negative impact on fertility. We therefore supplement with Selenium (via injection) where necessary. We are very active in improving our soil health, so routinely take soil samples and address any deficiencies as part of our farm management.

Donors:

Donors are selected based on their superior genetic qualities. Also they must be free of all known heritable genetic faults.

They need to be sound both conformationally and in regards to fertility. It is easy to fall in love with their fleece but they need to be sound in all other respects before considering to reproduce them in ET.

As mentioned earlier, donors over time tend to put on excess weight. This will have a negative impact on their performance. We therefore try to maintain their weight and ensure they are fit. If they do gain too much weight, we would mate them naturally and let them have a cria then re-assess them for further use in our ET program.

Another issue that may require the females to be mated naturally is their uterine volume. To remove the embryos we flush the donors uterus with commercial embryonic solutions. Over time, the volume of the donors uterus can decrease and if they no longer flush well, we would breed them and let them carry a normal pregnancy and then use them again after they have had their cria.

We have not observed any negative impacts on the future fertility of the donor females by them being used in our ET program.

Males:

Males used as sires in ET must of course have the characteristics that you are wanting to reproduce. They must also be fertile. We only use proven males in our program.

As with donors and recipients, the males must be in good physical condition. Leading up to a program, we also ensure that the selected males are not overworked with other breedings.

We have found that over heating will have negative impacts of male fertility. We therefore keep the males in a well shaded area leading up to breedings to minimise these affects.

Also some males, for some reason do not perform in ET though they are getting pregnancies in our regular breedings. Once this characteristic has been identified, we stop trying to use him in ET and just keep him for our natural breedings.

Recipients:

Selection of recipients is a key factor in the success of your program. We select females that have been proven as good mothers. They need to be fit and healthy. Some females will not work as recipients and if they fail to carry an embryo more than three times we remove them from our program.

Any female that fails to mother their cria well or whose cria is slow to grow out is removed from the program.

Stress:

We try to minimize stress in our animals so as soon as we start to plan a program, we organise our groups so that the alpacas are able to develop a hierarchy within their groups. These groups are maintained as long as possible.

The animals are used to being run into our barn to be weighed monthly, so the routine of running them into the barn for their treatments is not a stressful event.

Conclusion:

The use of ET has had a significant impact on the success of our breeding program and business. It has allowed us to rapidly improve the quality of our herd. It has also allowed us to make available these leading genetics to our clients. These results would not have been possible without the close working relationship between Canchones and Dr Jane Vaughan (Cria Genesis)

Acknowledgements:

We thank Dr Jane Vaughan for allowing us to use excerpts from her published works in this article.

Background:

Dr Jane Vaughan:

Dr Jane Vaughan, has been working with alpacas and llamas since 1991. She studied the control of ovarian follicular function as part of her PhD studies. She was also the chief investigator of a study into the development of artificial insemination technology in alpacas. She now performs commercial ET in alpacas throughout Australia as well as in New Zealand and the United Kingdom. She regular presents lectures to farmers, veterinarians and veterinary students on alpaca reproduction and nutrition in Australia and internationally. More information is available about Dr Jane Vaughan, Cria Genesis and ET at www.criagenesis.cc

Canchones:

Canchones is a specialist black breeder based in Victoria, Australia. The ranch was established in 1998 and is owned and managed by Peter Kennedy and Robert Gane. The ranch is run on a 400 acre property in the north east of Victoria, Australia.

Robert has been involved in many aspects of the alpaca industry in Australia. He has held position at the regional (affiliate) and national levels as well as being a director of the Australian Alpaca Co-operative. He has developed and presented seminars on various including Marketing and Sales and herd management both throughout Australia and internationally.

Peter is an alpaca judge in both Australia, New Zealand and the USA. He successfully completed the International Alpaca Judging School in Peru in 2004. He subsequently certified as both an Australia Alpaca Association (AAA). Alpaca Association of New Zealand and Alpaca Owners and Breeders Association (AOBA) judge. He has since judged throughout Australia and the USA as well as in Canada and New Zealand.