

# Mating management and embryo transfer in alpacas

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## Abstract

A simple mating protocol for alpacas is described, based on the unique reproductive physiology of South American camelids. Females should be mated at 12 months of age, or 15-20 days post-partum. They should be presented to the male every 7 days thereafter for up to 3 matings to ensure conception occurs as rapidly as possible. Non-surgical, trans-cervical embryo collection and transfer is also described.

## Key words

alpaca, reproduction, mating, pregnancy, embryo transfer

## Mating strategy for your females

Camelids are often regarded as less fertile than other domestic livestock. This is not true! At least 50 % of matings between fertile machos and hembras result in pregnancy, comparable rates to sheep, cattle and goats. However, there is a tendency for alpaca owners to focus on females that fail to conceive because of their individual value and persevere with attempts to get them pregnant. This is in comparison with other domestic livestock where females that fail to conceive after 3 matings are removed from the gene pool by culling.

A simple mating protocol for alpacas includes:

- First breeding at 12 months of age (maiden) when reached 65 % of estimated mature body weight OR 15-20 days after unpacking if the delivery was straightforward and unassisted.
- Breed once when receptive.
- Spit-off at 7 days to check for ovulation. If receptive, ovulation did not occur and female should be mated again. If non-receptive at 7 days ....
- Spit-off at 14 days to check for pregnancy. If receptive, conception did not occur and female should be mated again. Non-receptivity indicates presence of elevated plasma progesterone and a corpus luteum .... And probably pregnancy.
- Spit-off regularly (every 2-4 weeks) until ultrasound pregnancy test at (30 and) 60 days post-joining to observe foetus and therefore confirm pregnancy status.
- Spit-off intermittently throughout gestation as there is a 5 % chance of foetal loss after 60-days gestation.

A female should be given three rounds of this mating management. Ninety percent of females will conceive. The remaining 10 % of females that fail to conceive should ideally be removed from the breeding pool to maximize fertility in the herd. Veterinary advice may be sought to investigate reasons for conception failure. Ensure dates and findings are recorded to assist with further reproductive investigations.

## Reasons behind mating strategy

Understanding the reproductive biology of your females will lead to a better understanding of mating management on your farm.

## *Puberty*

Alpacas do not exhibit clear signs of oestrus, as they are induced ovulators and it is therefore difficult to assess when they attain an age at which conception may occur (Bravo 1994). Evidence of sexual receptivity is based on submission to a male for mating. However, young females may exhibit receptive behaviour yet still be too young to conceive (Smith et al 1994). Ovarian follicular growth is usually initiated as early as 5 to 6 months of age (Bravo 1997). Nutrition is recognised as a major environmental factor that influences the onset of ovarian activity in young females. As a rule-of-thumb, females need to

attain approximately 65 % of their mature weight in order to ensure a high likelihood of conception, whilst avoiding stunting and birthing difficulties (Smith et al 1994, Johnson 1989). In Peru, alpacas weighing at least 40 kg at 12 months of age were considered ready to reproduce (Novoa et al 1972). In Australia, this weight should be easily achievable by 12 months. Good nutrition after weaning together with monitoring of live weight and body condition score are essential for continued reproductive success.

### *Reproductive anatomy*

Alpacas have a bicornuate uterus with a single cervix. The body of the uterus is approximately 3 cm long and 3 cm in diameter, and the two uterine horns each about 8 cm long. In alpacas that have had a cria the left uterine horn is usually larger than the right as about 98 % of all pregnancies occur in the left horn (Fernandez-Baca et al 1973). The uterus resembles the letter “Y” and the tips of the uterine horns are blunt and rounded. Each uterine horn terminates in an oviduct which joins the uterine horn to the ovarian bursa. The latter is a membrane that surrounds the ovary and ‘captures’ the egg (oocyte) at ovulation and directs it into the oviduct for fertilisation if the female has been mated. The ovaries are approximately 1cm x 1cm x 1.5cm (peanut-sized). The cervix is usually 2 cm long and the vagina is relatively long (15 to 20 cm) compared with ruminants of similar size (Sumar et al 1997).

### *Ovarian activity in the unmated, non-pregnant female*

Female alpacas that have reached an age at which conception may occur, but which have not been exposed to a male (i.e. neither mated, nor in close proximity), exhibit regular cycles of ovarian follicular growth which has been described as follicular waves (Vaughan 2001). Follicle waves involve synchronous emergence of several follicles (2 to 3 mm diameter) which grow together to 4 to 5 mm in diameter. By an unknown mechanism, one follicle in the cohort becomes the ‘dominant’ follicle at an average diameter of 5 to 6 mm and this follicle continues to grow; the other follicles in the cohort undergo regression. The dominant follicle takes about 3 to 6 days to reach its mature size (7 to 12 mm diameter) and can remain at this size for 2 to 8 days. If mating does not occur, the dominant follicle undergoes spontaneous regression over 3 to 6 days and this is associated with emergence of the next follicular wave (Bravo et al 1989, Sumar 1983, Adams et al 1990, Vaughan 2001).

The interval between emergence of successive follicular waves varies from 12 to 22 days in alpacas (Vaughan 2001). New wave emergence interval varies within individual females and among different females. Waves of follicular growth continue in early pregnancy and during subsequent lactation (Adams et al 1990).

Ovarian follicular waves do not necessarily alternate between ovaries and large follicles are equally distributed between ovaries (Bravo et al 1989, Bravo et al 1990b, San-Martin et al 1968). As noted above, a follicular wave emerges as soon as the dominant follicles starts to regress which means that both the regressing and emerging dominant follicles can be visualised on the ovaries at the same time (Bravo et al 1990b).

If females do not have contact with a male, ovarian follicular waves continue and females can show long periods of receptivity (San-Martin et al 1968, England et al 1971). In ruminants, a growing dominant follicle secretes increased amounts of the hormone oestradiol, until it reaches a threshold concentration in blood at which point oestradiol induces oestrous behaviour. This scenario does not operate in alpaca and females are receptive to males throughout different phases of follicular growth, with occasional and intermittent periods of non-receptivity lasting 1 to 2 days as a new follicle wave develops (Johnson 1989, Sumar et al 1993). It appears that behavioural oestrus in females is associated more with the absence in blood of the hormone, progesterone, rather than fluctuations in blood concentrations of oestradiol (Adam et al 1989, Sumar et al 1988b). If a dominant follicle ovulates and forms a corpus luteum it secretes progesterone which is known as the ‘hormone of pregnancy’. When females have blood progesterone concentrations of greater than 2 ng/mL they show strong rejection of males by spitting, kicking and running away (Adam et al 1989).

Alpacas are non-seasonal breeders. In South America, females are bred in summer when native pastures are green and plentiful; however, it was shown that ovarian follicular activity occurred throughout the year (Bravo et al 1989).

### *Mating and ovulation*

A sexually receptive female may readily assume the copulation position when approached by a male, get close to a mating couple and adopt the copulation posture, or stand in proximity to a mating couple. Occasionally, some females mount other females (Fernandez-Baca et al 1970d). Copulation takes place in a sitting position and usually lasts 20 to 25 minutes, with a range of 5 to 65 minutes (England et al 1971). In a study of paddock matings, the average duration of copulation in alpaca herds varied based on animal interactions; males in herds without other males averaged 20 minutes compared with 15 minutes in herds with several males. Interruption/short duration of copulation in multi-sire herds was attributed to normal male territorial fighting, since males are vulnerable to attack during mating (Escobar, 1984). Males mating yearling females averaged 15 minutes compared with 22 minutes with multiparous females. During copulation, the male penetrates the cervix with his penis, and deposits semen into both uterine horns (Franco et al 1981).

Matings that occur in the absence of a dominant pre-ovulatory follicle do not induce ovulation, and conceptions do not occur. Matings that occur in the presence of a growing or mature follicle ( $\geq 6$  mm diameter) result in ovulation (Bravo et al 1991b, Sumar et al 1993). Alpacas are known as induced-ovulators because females require coital stimulation for the egg to be released from the dominant follicle on the ovary. It is thought that neural stimuli from the mating process and an ovulation-inducing factor in the semen are transmitted to the brain (hypothalamus) of the female where they induce the release of gonadotrophin releasing hormone (GnRH). GnRH released from the base of the brain stimulates the anterior pituitary gland to secrete luteinising hormone (LH), which is transported in the blood to the ovaries where it stimulates ovulation of a dominant pre-ovulatory follicle (Fernandez-Baca et al 1970d, Bravo et al 1991b). The leg clasp of the male and his orgling sounds may contribute to neural stimulation of the brain to release GnRH (Bravo 1994).

Ovulation occurs in females approximately 30 hours after mating. Ten to 25 % of females with an 'ovulatory size' follicle on the ovary may fail to ovulate. (San-Martin et al 1968, Sumar 1985, Sumar et al 1993). With regard to the latter group of females, it was not established whether the 'ovulatory size' follicle was in the growing phase, static phase, or regressing phase. It is likely that in the majority of these cases the follicle was in the late-static or early- regressing phase and might not be expected to ovulate. Ovulations occur with equal frequency from both ovaries (Fernandez-Baca et al 1970b, d).

A corpus luteum (CL) develops at the site of ovulation on the ovary 3 to 5 days after mating (2 to 3 days after ovulation) and secretes progesterone. The CL reaches a maximum size of 10 to 15 mm and maximum progesterone secretion 8 to 9 days after mating. Progesterone secretion by the CL starts to decline about 9 to 11 days after mating. If conception does not occur, prostaglandin is released from the uterus and induces regression (luteolysis) of the CL (Fernandez-Baca et al 1970c, Adams et al 1989, Adams et al 1990, Adam et al 1992, Sumar et al 1991). Females return to sexual receptivity approximately 14 days after mating if a corpus luteum develops but conception does not occur.

Females not completely deprived of visual, auditory and olfactory stimuli from a male can also ovulate. Spontaneous ovulation without coital stimulation or direct male influence can occur in 5 to 15% of females; however, many recorded cases included some form of male contact (England et al 1969; Bravo et al 1989). Multiple ovulations occur in up to 10 % of natural matings (Fernandez-Baca 1993).

### *Pregnancy*

The CL is the major source of progesterone throughout pregnancy and its presence is required to maintain pregnancy (Sumar 1988). Removal of the CL results in termination of pregnancy within 24 hours (Bravo 1994). Removal of the embryo induces CL regression 4 to 7 days later (Adam et al 1992). In pregnant females, a transient decline in blood concentration of progesterone occurs from Day 8 to 12 after mating, which coincides with the period of maternal recognition of pregnancy. The embryonic signal for maternal recognition of pregnancy must be transmitted as early as Day 10 after mating in order to 'rescue' the CL of pregnancy (Aba et al 1997). Thereafter, progesterone concentrations in blood increase and the diameter of the CL reaches a maximum of 10 to 19 mm around Day 21 of pregnancy (Adams et al 1991, Bravo et al 1993b). In alpacas, 98 % of pregnancies occur in the left uterine horn, even though the CL of

pregnancy is found equally on the left or right ovary (Fernandez-Baca et al 1973, 1979). Therefore, embryos derived from ovulation of the right ovary migrate into the left uterine horn. Gestation length varies from 330 to 350 days and is usually longer in spring than autumn.

Early embryonic death is common in alpacas and in Australia, about 10 % of embryos (up to 50 % in other countries) may be lost in the first 60 days of pregnancy (Fernandez-Baca et al 1970b, Adams et al 1991, Adams 1997). Factors responsible for this high attrition rate are unknown but nutritional constraints, hormonal imbalance or chromosomal aberrations may be principle causes (Sumar et al 1997). Certification of pregnancy by a veterinarian should be performed about 2 months after the last known mating date.

### *Receptivity After unpacking*

The interval from parturition to resumption of ovarian follicular activity is about 5-7 days in South American camelids and females can be ready to ovulate by 10 days post-partum. The uterus only takes about 20 days to involute, probably because of the diffuse (microcotyledonary) nature of placentation.

Some owners have described difficulties in getting some females pregnant again if mated later than 3 or 4 weeks post-partum. These females generally conceive easily once their cria has been weaned. Explanations for this observation include:

- Females of other species of domestic livestock produce certain hormones during lactation that may reduce/inhibit ovarian function. This may also occur in camelids.
- Females reach peak lactation approximately 4 weeks post-partum. Lactation is the most metabolically demanding time for alpacas, and nutrients are diverted preferentially to the udder, possibly to the detriment of ovarian function.

### **Embryo transfer in alpacas**

The reproductive physiology of alpacas differs to that of other domestic livestock and remains poorly understood, therefore hindering the direct transfer of artificial insemination (AI) and embryo transfer (ET) technologies from ruminants to alpacas. Generation intervals are relatively long in alpacas because males are slow to sexually mature and females exhibit an extended gestation (11.5 months), so conventional breeding results in slow genetic gain. The development of embryo transfer in alpacas in Australia is increasing the use of genetically superior animals.

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 will give smaller breeders access to elite genes through purchase of embryos and will allow for inter-farm/state/national movement of superior genetics.

### *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. 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 180 live births (50 % males, 50 % females) have occurred over the last 4 years in Australia, following single-embryo flushing performed by the author 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 examined in alpacas in Australia and other countries. Both equine chorionic gonadotrophin and follicle stimulating hormone are currently being tested as agents to stimulate multiple ovulation. Techniques are producing an average of 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 three years, without apparent effect on donor fertility as donor females have readily conceived within 2-4 weeks after their last MOET flush.

#### *Preparation of donors and recipients*

Females that are to be used as **donors** need to be reproductively sound (owners must resist the temptation of preparing females that have been difficult to get pregnant in the past), of superior genetic quality, have good conformation, and be free of all known inherited genetic disorders. Females that are to be used as embryo **recipients** must also be reproductively sound in order to optimise the chances of successful embryo implantation and birth of a cria. Demonstrated good mothering ability is an advantage. Females with physical and/or genetic abnormalities (carpal valgus, luxating patellae, fused toes, extra toes, wry face) can be used as recipients since these characteristics will not be transferred to the embryo and gestating foetus.

Attention to detail and thorough preparation of donor and recipient females and males is essential for successful embryo transfer. Four factors appear to be important for *all* alpacas participating in an ET program: normal fertility, body condition score 2.5 to 3 (out of 5), appropriate nutrition and selenium supplementation.

#### *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). A week after mating, embryos have migrated to the uterus and are usually in the form of a hatched blastocyst. At this point, embryos are flushed from donor females. Ninety percent of embryos recovered between 6.0 and 7.5 days after ovulation had hatched from the zona pellucida, the protective coat around the outside of the oocyte and early embryo (Del Campo 1997; Bourke *et al.* 1992). The zona pellucida is protective to embryos during freezing and thawing procedures of cattle and sheep embryos.

#### *Non-surgical, trans-cervical collection and transfer of embryos*

This method involves the introduction of a Foley 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. The author 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. Pregnancy diagnosis using transrectal ultrasonography can be performed from approximately Day 25 after embryo transfer to assess pregnancy (Parraguez *et al.* 1997).

Future developments include the continued refinement of multiple ovulation protocols and the freezing of embryos to allow indefinite storage and easy transport of genetic material. Pregnancies have been achieved in camels (Skidmore *et al.* 2004) and llamas (Skidmore *et al.* 2004; Aller *et al.* 2002) following vitrification, thawing and transfer of embryos, but this success has not yet been translated to alpacas.

## **References**

Available on request.