

Manipulation of ovarian activity in camelids

Muhammad-Salman Waqas,^a Abdelhaq Anouassi,^b Ahmed Tibary^a

^aDepartment of Veterinary Clinical Sciences, College of Veterinary Medicine, and Center for Reproductive Biology, Washington State University, Pullman, WA, USA

^bVeterinary Research Center, Advanced Scientific Groups, Abu Dhabi, UAE

Abstract

Camelid production has increased in importance in the world in recent years. The high demand for genetically superior males and the implementation of reproductive technologies requires the synchronization of ovarian follicular waves. This paper describes the state of knowledge regarding seasonality and follicular wave patterns in camelids. Reproductive seasonality in camels can be manipulated using artificial photoperiod or melatonin treatment. As in other ruminants, protocols for follicular wave synchronization are based on the elimination of the dominant follicle (induction of ovulation) or progesterone treatment. These protocols are described for breeding management and ovarian superstimulation in embryo donors. Induction of ovulation and initiation of superstimulation 2-4 days later (emergence of a new follicular wave) results in higher response in terms of number of ovulations and collected embryos.

Keywords: Camels, alpacas, llamas, superovulation, follicular wave

Introduction

Camelids are a vital production animal in several areas of the world. The renewed interest in these species in the last 3 decades has increased our understanding of their reproductive biology. In camels, the importance of the racing industry and the intensification of camel dairy production have increased the demand for assisted reproductive technology. A similar situation is observed in South American camelids (SAC), particularly alpacas, where the show and fiber market increased the genetic selection pressure.

Efficient reproductive management and the implementation of reproductive technologies such as artificial insemination (AI) and embryo transfer (ET) in camelids require manipulation of ovarian activity. This paper reviews the mechanisms controlling ovarian follicular dynamics and methods to manipulate follicular dynamics and ovulation in camelids.

Follicular dynamics and ovulation in camelids

Endocrine and ultrasonographic studies in the mid to late 1990s helped to define follicular dynamics in several domestic camelid species.¹⁻⁸ All camelid species are induced ovulators. In the absence of an ovulatory stimulus (mating or hormonal induction), follicular waves occur in an overlapping manner

(Figure 1).^{6,9} Seasonal variation of ovarian activity of female camelids has been described in South American camelids (SAC)¹⁰ but is more pronounced in camels.^{6,11,12}

In camels, follicular activity (number and size) and oocyte quality are significantly lower during the nonbreeding season compared to the breeding season.^{12,13} However, a substantial proportion of females (20-60%) continue to have regular follicular activity outside the defined breeding season.^{6,14} Recent research has revealed that photoperiod is involved in the control of ovarian follicular dynamics in the dromedary.^{15,16}

Ovarian follicular activity is also affected by nutritional status (i.e. body condition score) in SAC^{17,18} and camels.¹⁹ Restricted nutrition decreases ovarian follicular growth and corpus luteum development, and lowers plasma progesterone and leptin concentrations. However, the interaction among photoperiod, temperature, and nutrition in the control of seasonality in camels remains poorly studied.^{12,20}

Remarkable difference exists between camels and SAC regarding the effect of lactation on follicular dynamics. Resumption of ovarian activity occurs within 5-10 days after parturition in SAC,^{21,22} whereas in camels, lactational anestrus can last from 45 days up to several months, and has substantial impact on the productivity of this species.^{6,23-26}

Ovarian follicular dynamics are regulated by follicle stimulating hormone (FSH) and luteinizing hormone (LH).^{1,27-29} Duration of follicular development phases, ovulation, corpus luteum development, and luteolysis are presented (Table). The presence of 2 or more codominant follicles is common in camelids and may occur in up to 40% of follicular waves.³⁰⁻³³ Follicles ovulate in response to mating or hormonal treatment (GnRH or hCG) when they reach an appropriate size (camels: 12 mm; SAC: 7-8 mm). Receptivity to the male is not strongly correlated with the size of the follicle and readiness for ovulation (camels;^{33,34} SAC³⁵⁻³⁸). Therefore, the best method for monitoring follicular dynamics is transrectal ultrasonography. At the peak of follicular development, the uterus is toned and has a characteristic edema pattern on ultrasonography (Figure 2).

A sharp rise in serum LH concentration occurs minutes after mating (camels;^{2,3,39} SAC^{28,29,40}). Ovulation occurs on average 30 hours (range: 26-72 hours) after mating. Ovulation is induced by the β nerve growth factor (β NGF) present in camelid seminal plasma (SAC;^{41,42} camels⁴³). The presence of β NGF and endometrial inflammation are required to maximize the ovulatory response.⁴¹ The action of the β NGF involves kisspeptin neuron activation^{44,45} and a local mechanism on the ovary;⁴⁶ β NGF treatment by various routes (intravenous [IV], intramuscular [IM], or intrauterine induces LH surge. However, the dose is higher with intrauterine route.⁴⁷ β NGF appears to have a luteotrophic effect on the CL.⁴⁸⁻⁵⁰ However, this effect was not observed after intrauterine treatment.⁵¹ Spontaneous ovulation has been described in up to 5% of the follicular waves in llamas and camels.⁵² This phenomenon appears to be more common in lactating animals.⁵³

Follicular recruitment starts 2-4 days after ovulation, and a mature follicle develops 4-6 days after completion of luteolysis, which occurs 9-10 days after a sterile mating. This short luteal phase results in a single follicular wave per cycle in most females. Preliminary data in our laboratory revealed that 90% of females (camels and alpacas) have only 1 follicular wave per ovulatory cycle. Thus, after a sterile mating or hormonal induction of ovulation, the average interval between 2 consecutive ovulatory follicles is 14 days (Figure 3).⁶

In the absence of mating or other ovulatory stimuli (i.e. GnRH or hCG treatment), follicles continue to grow and reach a maximum diameter (25 mm in camels, 12 mm in alpacas,

13 mm in llamas) then undergo atresia. Some follicles continue to grow and develop into large anovulatory follicles that may become hemorrhagic or luteinized (Figure 2). The incidence of anovulatory HAF is higher in camels,^{6,33} and llamas⁵⁵ than other camelid species. There is an individual predisposition for HAF in the absence of ovulation, but the exact pathophysiology of AHF is poorly understood.⁶

Manipulation of ovarian follicular activity is an important aspect of reproductive management and implementation of synchronization, timed-AI, and multiple ovulation and embryo transfer technologies. Several techniques have been used to control ovarian follicular dynamics, including inhibition of follicular activity, manipulation of seasonality, synchronization of follicular waves, and ovarian superstimulation.

Inhibition of follicular activity

Inhibition of follicular activity may be desired for management reasons (contraception) or to control the emergence of a new follicular wave. There are limited observations on hormonal inhibition of follicular activity in camelids. In alpacas, daily subcutaneous (SC) buserelin (50 μ g/female) treatment for 10 days suppressed follicular activity, starting on day 6 after treatment.⁵⁶ Immunization against GnRH has not been studied thoroughly in camelids, but preliminary experiments in our laboratory in males and females produced variable results.

Manipulation of seasonality

The seasonal reproductive pattern of camels is driven in large part by photoperiod. Peak reproductive activity in this species is observed during the short photoperiod.⁵⁷ The photoperiod effect on reproduction is confirmed by changes in the nocturnal melatonin secretion. Melatonin secretion peak is shorter under an artificially long photoperiod.^{15,16,58} Similar findings were reported in guanacos in the wild, indicating a circadian pattern of melatonin secretion.⁵⁹

Melatonin treatment advanced the breeding season in female dromedaries.⁶⁰ Dromedary females exposed to an artificial long photoperiod (16 light:8 dark) for 41 days and then treated with a subcutaneous melatonin implant (1 implant of 18 mg melatonin per 28 kg of body weight), displayed ovarian cyclicity 3.5 months earlier compared to females that did not receive melatonin.¹⁶

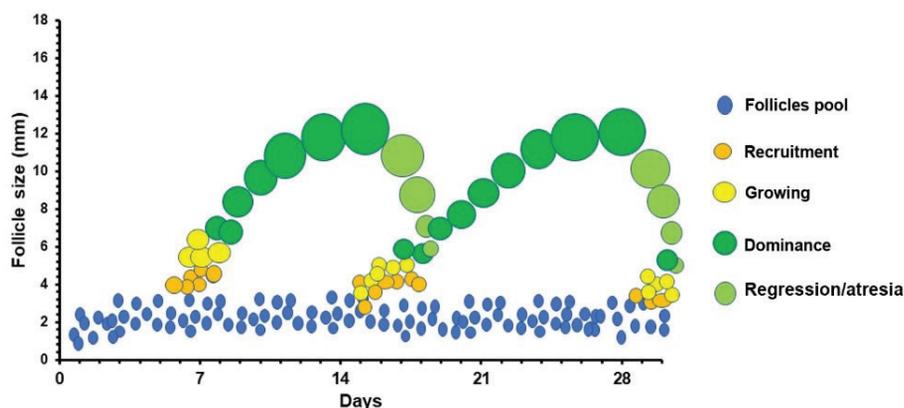


Figure 1. Schematic representation of follicular dynamics in camelids showing overlapping follicular waves in absence of ovulation

Table. Characteristics of the follicular wave, ovulation, and corpus luteum in camelids (adapted from⁵⁴)

	Dromedary camel (<i>Camelus dromedarius</i>)	Bactrian camel (<i>Camelus bactrianus</i>)	Llama (<i>Llama glama</i>)	Alpaca (<i>Vicugna pacos</i>)	Vicuna (<i>Vicugna vicuga</i>)	Guanaco (<i>Llama guanaco</i>)
Number of follicles at emergence	8-30	-	8-12	8-12	-	-
Growth phase (days)	9-12	9-12	3-9	3-9	3-6	5-9
Growth rate (mm/day)	1.6	1.6	0.7	1.4	1.8	1.0
Plateau (days)	5-9	5-11	3-8	3-8	2-4	2-4
Minimum ovulatory follicular size (mm)	8	9	8	7	7	8
Average ovulatory follicular size (mm)	15	15	10	9	8	10
Maximum ovulatory follicular diameter (mm)	25	22	14	12	10	13
Regression phase (days)	10-12	7-15	3-8	3-8	3-5	3-7
Hemorrhagic anovulatory follicle (HAF) incidence (%)	40-55	-	4-22	-	-	-
HAF size (mm)	30-90	30-50	14-35	13-35	-	-
HAF regression (days)	8-45	-	4-22	-	-	-
Codominance (%)	⁴⁵	-	10-30		25	12
Inter-wave interval (days)	18.2 ± 3.8	19.1 ± 0.6	15.8 ± 0.5	12-16	7.2 ± 0.5	12.6 ± 5.6
Ovarian alternance (%)	-	-	-	-	77	93
Interval mating to ovulation (hours)	32.-40	30-48	28-30	27-36	30	30
Corpus luteum size (mm)	15-25	15-25	11-18	11-15	11-15	11-15
Day at maximum size of corpus luteum	7.2±1.7	7.3	8	7-8	-	-
Day of luteolysis	10 ± 1.2	10.5	10-12	10-12	-	-
Return to receptivity (cycle-days)	12-16	12-16	12-15	12-15	-	-

Induction of ovulation

Ovulation can be reliably induced by GnRH (SAC buserelin 8 µg, camels 20 µg camels; SAC GnRH 20-50 µg, camels 100 µg) or hCG (SAC 500-750 IU IV; camels 1,500-3,000 IU IV) in the presence of a growing or mature follicle. The optimal ovulatory response after treatment is observed when the dominant follicle is 7-10 mm in alpaca, 8-12 mm in llamas, and 11-18 mm in camels.^{8,54,61-63} If given outside the optimal time, ovulation rate decreases. In llamas, there was no difference in ovulation rate, interval to ovulation, or luteal development when ovulation was induced by copulation or hormonal (LH or GnRH) treatment.⁶⁴

Synchronization of follicular waves

Synchronization of follicular waves is essential for timed-AI, superovulation, and embryo transfer.^{27,65} Methods used in other ruminants have been adapted to camelids with varying degrees of success. Several approaches have been used to control ovarian follicular dynamics and eliminate dominant follicles prior to gonadotropin treatment. These include manual

ablation (not recommended), ultrasound-guided aspiration of the dominant follicles, and hormonal treatments.^{54,63,66,67}

Follicular ablation

In the dromedary camel, a new follicular wave emerges 2.3 ± 0.5 days after ablation of the dominant follicle.⁶⁸ In SAC, a new follicular wave emerges 2-3 days after induction of ovulation of the dominant follicle. In camels, follicular wave emergence occurs 70.6 ± 1.4 h (range: 60-84 hours) after GnRH injection to induce ovulation, and follicular deviation occurs 58.6 ± 2.7 hours (range: 36-84 hours) after emergence.³¹ In dromedary camels, the percentages of females that ovulated within 14 days were: no treatment (47%), ultrasound-guided follicular ablation (40%), GnRH treatment (80%), or GnRH followed by a luteolytic dose of cloprostenol 1 week later (87%).⁶⁸ In llamas, elimination of the dominant follicle by ultrasound-guided aspiration or LH treatment is effective in inducing follicular wave synchronization.⁶⁹

Eliminating dominant follicles by aspiration or induction of ovulation allows initiation of gonadotropin treatment for

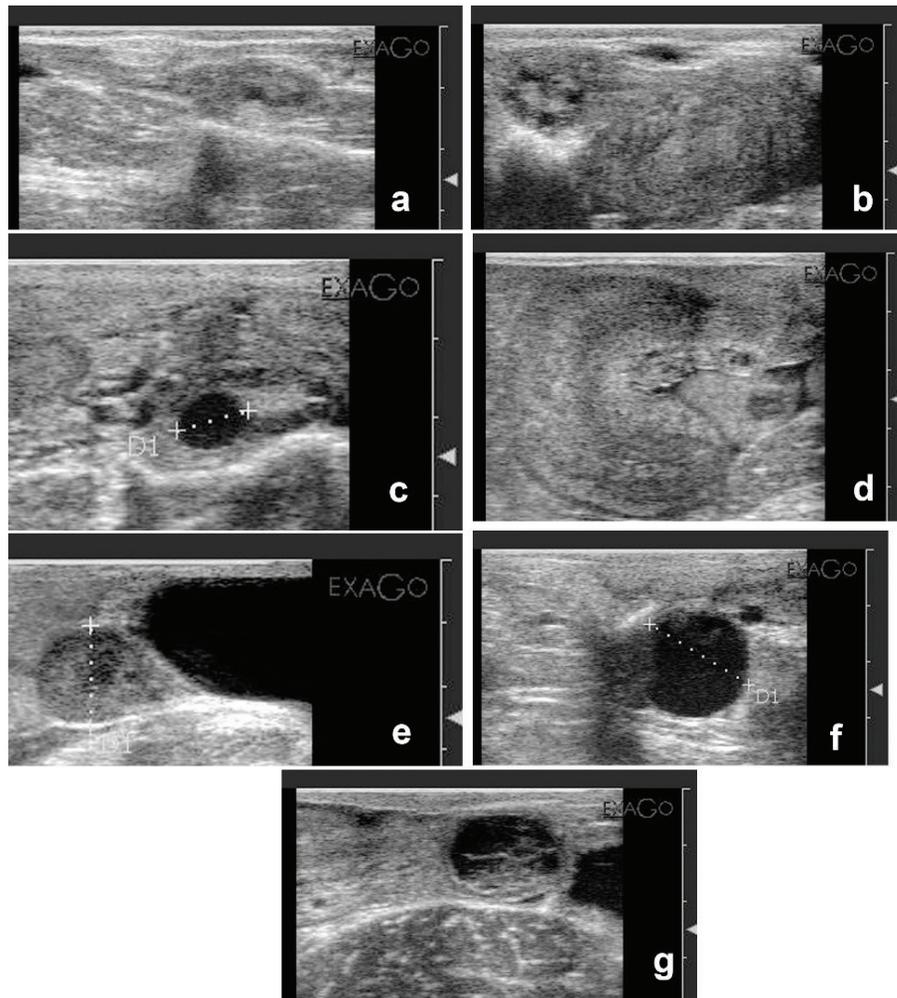


Figure 2. Ultrasonographic images of follicular dynamics in an alpaca: a. ovary quiescent stage; b. ovary with follicular recruitment and growth (follicles 3-4 mm in diameter); c. ovary with dominant follicle (9 mm in diameter); d. uterine horn during peak follicular growth (edema and tone); e. ovary with corpus luteum 7 days after ovulation; f. ovary with anovulatory follicle (19 mm) and g. ovary with hemorrhagic anovulatory follicle

superovulation that coincides with the emergence of a new follicular wave.⁷⁰ Embryo recovery rates are improved when gonadotropin treatment is started 2-4 days after induction of ovulation (SAC,^{71,72} camels⁷³⁻⁷⁵)

Synchronization with a combination of GnRH and PGF_{2α}

Ovulation synchronization using a combination of GnRH and PGF_{2α} was investigated in camelids (Figure 4). In camels, 2 GnRH injections given 14 days apart or 2 GnRH injections 14 days apart with PGF_{2α} 7 days after the first GnRH was effective in synchronizing ovulation.⁶⁸ Timed-breeding on day 22 after a hormonal treatment protocol consisting of GnRH on day 0, PGF_{2α} on day 7, GnRH on day 10, and PGF_{2α} on day 17 resulted in 46-60% pregnancy rates.⁷⁶⁻⁷⁸ However, several studies lacked a proper control group. Two injections of GnRH given 14 days apart followed by timed-mating (14 days after the second injection of GnRH) resulted in a 57.7% pregnancy rate.⁷⁹

In Bactrian camels, 2 injections of GnRH given 14 days apart resulted in better synchronization of follicular waves and

response to ovarian superstimulation with equine chorionic gonadotropin (eCG) or FSH.⁸⁰

In llamas, GnRH treatment on day 0 and PGF_{2α} after 7 days, then a second GnRH on day 10 resulted in a good synchronization of ovulation in females that ovulated after the first GnRH.⁸¹ There was no advantage in synchronization of the follicular wave, ovulation rate, or pregnancy rate using a treatment consisting of 2 injections of GnRH 1 week apart, followed by PGF_{2α} on day 14.^{54,82}

Progesterone treatment

Several progestogens have been used to attempt to control follicular dynamics in camelids, including daily progesterone injection (50-100 mg in SAC and 100-150 mg in camels), intravaginal devices (PRIDs or CIDRs with 1.38 g or 1.9 g progesterone in camels, CIDRs 0.3 g progesterone and medroxyprogesterone acetate (MAP) sponges in llamas and alpacas) or SC implants of norgestomet (3 mg) in llamas and alpacas. In several studies, the length of treatment varied from 7 to 14 days.^{54,67,83,84}

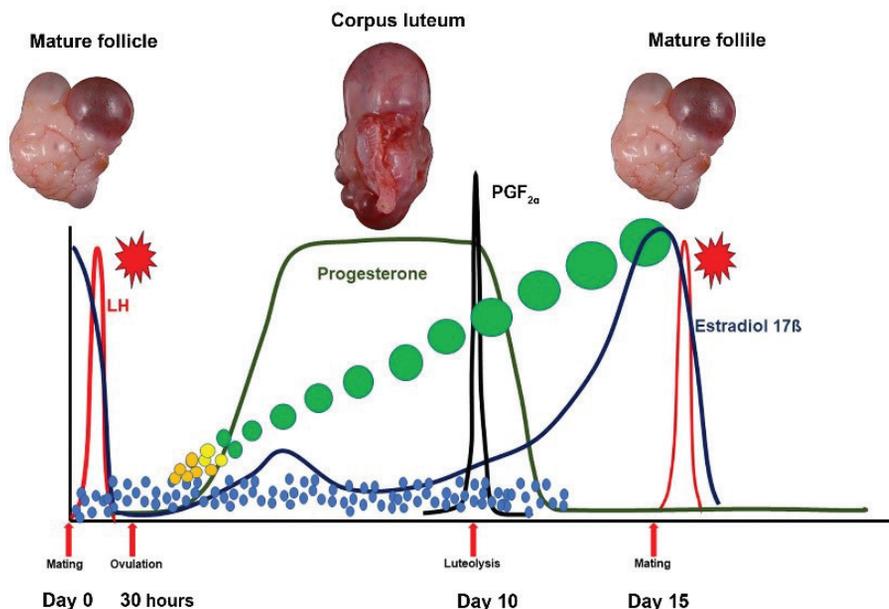


Figure 3. Camelid cycle in presence of an ovulation

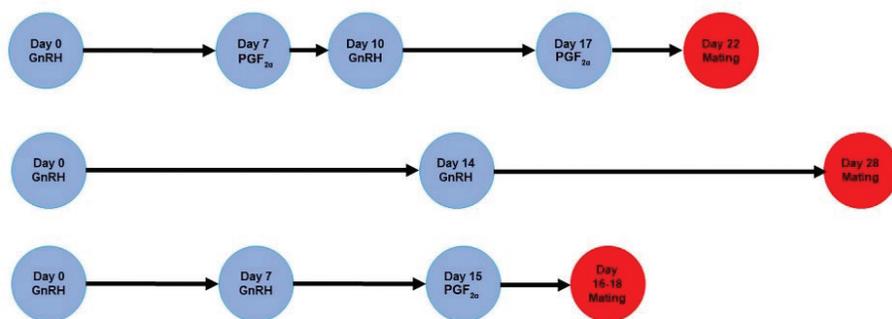


Figure 4. Examples of camelid follicular wave synchronization using GnRH and $PGF_{2\alpha}$

In camels, there are conflicting reports on the efficacy of PRIDs⁸⁵ and CIDRs^{19,86} for synchronization of follicular waves. In addition, some studies have reported an increase in spontaneous ovulation when these devices are used.⁸⁵ Treatment with PRIDs containing 1.55 g of progesterone for 7 days did not synchronize follicular waves.^{85,87} However, camels treated for 17 days with PRIDs containing 1.9 g progesterone and receiving a large dose of eCG (3,000 IU) had a better synchrony of follicular growth than those not treated with PRIDs.⁸⁸ Treatment with CIDRs containing 1.38 g of progesterone for 10 days did not synchronize follicular waves in the nonbreeding season.¹⁹ In dromedary camels, 70 and 75% had a preovulatory follicle on days 16 and 18, respectively, after treatment with CIDRs (1.9 g of progesterone) for 14 days.⁸⁹ However, this study has no control (untreated) group. Norgestomet implants were not efficacious in synchronizing follicular waves in Bactrian camels.⁸⁰ In dromedary camels, daily IM progesterone injections (100 mg/day) for 10-16 days were used with relatively good results to synchronize recipients in an embryo transfer program.⁹⁰ Long-acting progesterone injection can be used in camels and may be more advantageous than daily injections or intravaginal devices, but this compound still needs to be thoroughly investigated for synchronization of follicular waves.^{54,91} Daily IM injection of progesterone (50 mg) for 12 days inhibited follicular growth by day 7.⁹²

In llamas, 9 days of MAP treatment vaginal sponges (60 mg) synchronized follicular activity, resulting in the emergence of a pre-ovulatory follicle 6 days after treatment.⁹³ However, in another study, sponges containing 120, 240, or 480 mg MAP had no inhibitory effect on follicular development.⁹⁴ Treatment with CIDRs (0.33 mg progesterone) for 16 days reduced follicular diameter starting on day 5 of the treatment.⁹⁵ Similar results were obtained in our laboratory in llamas and alpacas with a 14-day treatment.⁵⁴ Intravaginal devices containing 0.5 mg of progesterone appears to provide better control of follicular activity and better response to superovulation with eCG. The shape and area of contact of the vaginal device for progesterone delivery may affect the absorption of progesterone.^{54,96} Vaginal devices containing 0.78 g progesterone (Cue-mate[®]) inserted for 7 days reduced follicular development. A new dominant follicle was available 6 days after the removal of the device.⁹⁷ Daily IM injections of progesterone (50 mg) for 12 days inhibited follicular growth by day 7.⁹²

In vicuñas, treatment with CIDRs for 5 days exerted a negative effect on follicular development and allowed a better superstimulation in response to eCG.⁹⁸

In summary, progesterone therapy in camelids reduces the growth of large follicles and inhibits LH release,³ but does not

completely suppress follicular activity; therefore, its use for synchronization of follicular wave emergence and timed-breeding remains questionable.^{6,54,63,83,87}

Combination of progesterone and estrogens

The combination of estradiol and progesterone is more effective in controlling follicular waves in some studies⁹⁹ but not in others.⁸⁴ In llamas, superstimulation at the end of a 5-day daily treatment with 100 or 150 mg progesterone after injection of estradiol benzoate (1 mg) resulted in a higher embryo recovery rate.¹⁰⁰ In another study, 1 injection of estradiol-17 β (1 mg) and progesterone (25 mg) provided some synchronization of follicular waves, but not as good as that accomplished by the induction of ovulation or by follicle aspiration.⁶⁹

In alpacas, daily estradiol benzoate (5 mg) and progesterone (50 mg) treatment for 7-10 days produced a more uniform follicular wave response; however, the ovulatory response was poor.¹⁰¹

In camels, 1 injection of estradiol benzoate (5 mg) and progesterone (100 mg) was ineffective for synchronizing follicular waves.⁶⁸

Ovarian superstimulation

In vivo and in vitro embryo production are important technologies to multiply genetically superior females and have become common place in camels.^{27,102} Additionally, these technologies are critical for the reproductive management and multiplication of endangered wild camelids using interspecies embryo transfer.^{73,103} Ovarian superstimulation is a crucial step for in vivo production of embryos and oocyte collection.

Protocols for ovarian superstimulation used in camelids have been largely adapted from those used in ruminants with variable success (camels;^{27,54,85,87,90,104} SAC^{65,84,105}).

The 2 main hormones used are FSH and eCG, either alone or in combination. As for other species, response to these hormones depends on the timing of treatment in relationship to follicular dynamics, dose, frequency of treatment, and individual animal variation. Although FSH and eCG treatments have been initiated during the receptive or luteal phase of the cycle with some success, the best results in camels are obtained when the treatment is initiated in the absence of any follicles > 3 mm in diameter (camel).²⁷

Follicle stimulating hormone

Ovine (oFSH) and porcine (pFSH) FSH have been used for ovarian superstimulation with variable success.^{6,27} Induction with camel FSH (cFSH), purified from camel pituitary extract, or equine pituitary extract, was not successful in the authors' experience. The manner of FSH treatment (dose, frequency, and timing during the cycle) has been investigated to some degree; however, detailed descriptions of the treatment protocols are not always provided in publications.

In the dromedary, a total dose of 20-30 mg of oFSH was given in 2 daily injections of decreasing doses over 6 days. The treatment starts 2 days before and continues up to 1 day after the completion of 7 days course of progesterone treatment by intravaginal

device (PRID).⁸⁵ FSH was also given in a single dose (3.3 units) followed the next day by 1 injection of 3,500 IU of eCG, resulting in an average of 7 embryos recovered per treated female.⁸⁷ In another study, oFSH was given twice daily (1-3 mg per injection) for 3-5 days after 10-15 days course of progesterone treatment (100 mg per day).⁹⁰ Single SC dose of oFSH has been tested with variable (5.7 ± 2.32 embryos recovered) results.¹⁰⁶

In the dromedary, ovarian superstimulation has been obtained by twice daily eFSH treatment in decreasing doses over 3, 5, or 7 days after 10-15 days progesterone treatment. (dromedary^{27,90,106-109}; Bactrian^{73,80}). In practice, superovulation protocols are modified on an individual basis, based on ultrasonographic monitoring of the follicular response. The interval from pFSH treatment to the development of mature follicles (10-16 mm in diameter) varies between 6 and 8 days (Figure 5).^{6,27,54,63} The number of FSH injections can be reduced using a slow-release FSH preparation (hyaluronan solution) resulting in the same superovulatory response and embryo collection rate.¹⁰⁹ A single epidural dose of FSH resulted in multiple ovulations in camels; however, the response was lower than for traditional protocols using multiple injections.¹¹⁰

A recent study reported an adequate embryo recovery response in dromedaries superovulated with a single dose of recombinant bovine FSH given IM (5.1 ± 3.6 embryos flushed per donor for 120 μ g FSH and 5.0 ± 2.9 for 100 μ g FSH) given 4 days after ovulation.¹¹¹

In llamas and alpacas, ovarian superstimulation with pFSH alone or in combination with eCG has been used after 12 days of progesterone treatment.^{84,112,113} The best results were obtained with pFSH treatment (IM) given twice daily for 5 days in decreasing doses (32, 27, 22, 17, and 12 mg)^{84,113} Superovulation was obtained in 95% of the alpaca cycles after treatment with FSH (decreasing doses) starting 2 days after induction of ovulation.¹¹⁴ The number of embryos (4.2 ± 3.3) obtained after pFSH stimulation is generally lower compared to the number of ovulations (10 ± 4.4).^{114,115} Generally, alpacas produce a more variable response to superstimulation protocols than llamas.⁸⁴

Equine chorionic gonadotropin

Ovarian superstimulation with eCG has been extensively used in camelids. In general, a single dose is given IM a day before or on the day of completion of progesterone treatment for 5-15 days. The dose of eCG used varies from 1,000 to 6,000 IU in camels,^{27,75,80,85,90,104,106,107,110,116,117} from 500 to 2,000 IU in llamas,^{72,84,85,93,96,99,100,113,118,119} and from 500 to 750 IU in alpacas^{84,113} and vicuñas.⁹³

In the dromedary, eCG is given as a single injection (2,000 IU, 2,500 IU, 3,000 IU, or 4,000 IU), 1 day before or 1 day after PRID removal, resulted in superovulation in 40% of treated animals. However, only 42% of ovulating females yielded > 1 embryo. The interval from PRID removal to mating was 5 and 4.5 days for females receiving 2,500 IU and 4,000 IU of eCG, respectively. This interval was 1 day shorter in females treated with eCG 1 day before the removal of PRID.^{85,87} When eCG (2,000-3,000 IU) is given to females at the beginning of a follicular wave (no follicle > 3 mm in diameter), the interval from treatment to mating (follicular diameter of 12 mm) is relatively constant (8 days).^{27,120} Follicular response is variable (0-19 follicles) and 20% of the females did not respond to eCG treatment.²⁷ Treatment of 2,500 IU eCG after a CIDR

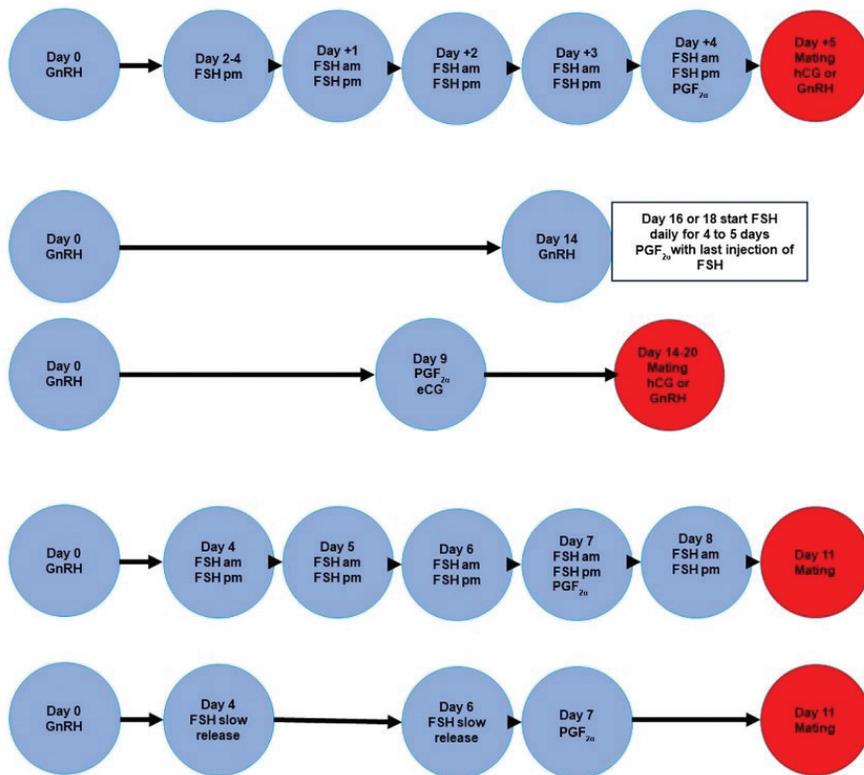


Figure 5. Examples of FSH ovarian superstimulation protocols

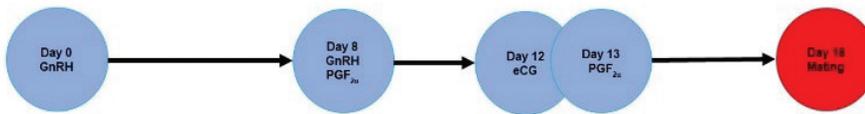


Figure 6. Example of eCG ovarian superstimulation protocol after induction of ovulation

(1.38 g progesterone for 3 days) produced superstimulation during the nonbreeding and transitional seasons, with follicles reaching ovulatory size 12-13 days after treatment.^{116,117} Treatment with eCG is on 2-4 days after induction of ovulation. The ovulation and embryo recovery rates are improved with doses ranging 3,000-4,000 IU. Higher doses (5,000-6,000 IU) of eCG produced a larger number of follicles; however, the ovulation rate and the number of transferrable embryos collected were lower.¹⁰⁹ A small number of animals had better response and fewer anovulatory follicles when 2,500 IU eCG was given at the end of a 13-day progesterone treatment in decreasing doses over 3 days compared to 1 dose.¹¹⁶ For the decreasing dose regimen, 2,500 IU eCG was splitted in 6 decreasing doses at 12-hour interval over 3 days as follows: 1,250, 600, 300, 200, 100, and 50 IU, respectively.

In llamas, eCG (1,000 IU) is given after progesterone priming (luteal phase after ovulation induction with hCG or GnRH, CIDR, or SC implants).^{72,96,119,121} Follicular response is variable (0-13 follicles), and the number of ovulations ranged from 0-7 with a mean of 1.3, and an average of 2.3 embryos collected per donor (range: 0-6). Follicles reached the mature size (9-13 mm) 5-11 days after eCG treatment. Several treated females had premature luteinization 7-9 days after eCG treatment. Increasing the dose of eCG to 2,000 IU increased the incidence of anovulatory follicles.⁵⁴ Supplementation with

exogenous progesterone during the eCG treatment in the luteal phase appears to inhibit excessive follicular growth and improve ovulation rate and embryo quality.¹²¹ Superstimulation with eCG and timed mating can be performed after synchronization with GnRH and PGF_{2α} (Figure 6).¹²²

As eCG is not available in the USA, the use of PG600 can result in ovarian superstimulation in alpacas but the ovulation rate was low.¹²³

In alpacas, the average number of recovered embryos per ovulating female was 3.7 after superovulation with 750 IU of eCG daily for 3 days.^{84,112}

The main disadvantage of eCG is the high incidence of follicular luteinization and failure of ovulation, most likely due to its long half-life. Additionally, females tend to become refractory to eCG after multiple use. This suggests that there is a risk of inducing anti-eCG antibodies.^{27,63}

Combination of FSH and eCG

Ovarian superstimulation protocols combining FSH and eCG have been published in alpacas, llamas,^{84,105,118} vicunas,³² and camels.^{27,80,110,124} These protocols consist of giving a

single dose of eCG followed by FSH injection twice daily in decreasing doses.^{124,125} Generally, these protocols do not provide much superiority compared to protocols using FSH alone.

Human menopausal gonadotropin

Human menopausal gonadotropin (hMG) is a hormone with equal FSH and LH activity. Its use for superovulation in camelids has been limited. Although hMG produced superovulation in camels, ovulation rate (FSH: 22.4 ± 2.25 ; eCG + FSH: 11.6 ± 2.58 ; hMG: 7 ± 3.19) and embryo yield (FSH: 16.2 ± 2.72 ; eCG + FSH: 7.2 ± 3.1 ; hMG: 1.6 ± 1.17) were less predictable and lower than those obtained with superovulation protocols based on FSH or FSH + eCG.^{106,107}

Immunization against inhibin

Immunization against inhibin, inhibin subunits or recombinant DNA vaccines result in high concentrations of circulating FSH, and consequently, an increase in the number of recruited and mature follicles. The vaccination was followed by a booster 28-231 days later, depending on the species and study. Immunization against inhibin has been used successfully to improve ovarian superovulation in several species (cattle,^{126,127} horses,¹²⁸ goats,¹²⁹ sheep¹³⁰). In the dromedary, a trial on immunization against inhibin gave encouraging results. An increase in ovulation number (4-10) was observed in 60% of the immunized females.⁶³ Further studies reported a high rate of triple ovulations up to 5 months after the initial immunization.^{131,132}

Problems with superovulation in camelidae

Major problems encountered in superovulation of camelids are the high incidence of non-responsive females (20-30%), high incidence of follicular luteinization, overstimulation in some females, and loss of efficacy after multiple treatments. Additionally, ovulation response and embryo yield remain highly variable.^{6,27,65,84} The recovery rate (number of embryos recovered/number of corpora lutea) is also variable, ranging from 30-90%. Initiation of gonadotropin treatment 2 days after induction of ovulation or after a progesterone treatment results in the best superovulation and embryo recovery outcomes (Figure 6).

Failure of ovulation may be due to premature regression of follicles and may be associated with inappropriate FSH dose or method of delivery.⁷⁴ Luteinization of follicles before ovulation may be due to increased concentrations of LH in response to high serum estradiol concentrations. Preliminary trials in our laboratory using recombinant highly purified FSH are encouraging. Most females with overstimulated ovaries do not produce embryos, possibly because of alteration of gamete transport. Finally, some females become refractory to superovulation with either FSH or eCG, perhaps because of the development of antibodies against these hormones.

Sources of variations in the response to superovulation in camelids that need to be investigated include species, breed, and individual animal. In alpacas, ovarian superstimulation response is positively correlated to serum AntiMüllerian hormone concentrations.¹³³ A similar trend was reported recently in dromedary camels.¹³⁴

Conclusion

Research on camelid reproductive endocrinology and clinical monitoring of the reproductive function in female camelids led to the adaptation of several hormonal strategies to alter this function positively or negatively. Knowledge gained on factors governing seasonality in camels, in particular photoperiod, allowed the use of artificial photoperiod or melatonin treatments to extend the breeding season in camels. Multiple approaches to synchronize follicular waves have been adapted from other species, with variable results. Progesterone therapy alone or in combination with estradiol has some efficacy for the control of follicular waves; however, it is still not optimal for timed-AI. Synchronization based on induction of ovulation followed by natural or induced luteolysis provides better synchronization of follicular dynamics and allows optimization of ovarian superstimulation protocols. Ovarian superstimulation treatments were adapted mainly from ruminant protocols. However, these treatments are still far from being optimized and need to be adjusted on an individual animal basis. Factors affecting ovarian response to superstimulation treatment remain poorly studied; however, recent observations indicate that serum AntiMüllerian hormone concentrations could be used to predict this response.

Conflict of interest

Authors declare no conflict of interest.

References

1. Anouassi A: Hormones antéhypophysaires du dromadaire (*Camelus dromedarius*): purification, caractérisation, dosages et utilisation pour l'induction de la superovulation. PhD Thesis, Université Paris VI, France, 1999.
2. Marie M, Anouassi A: Mating-induced luteinizing hormone surge and ovulation. *Biol Reprod* 1986;35:792-798. doi: 10.1095/biolreprod35.4.792
3. Marie M, Anouassi A: Induction of luteal activity and progesterone secretion in the nonpregnant one-humped camel (*Camelus dromedarius*). *J Reprod Fert* 1987;80:183-192. doi: 10.1530/jrf.0.0800183
4. Marie M: Bases endocriniennes de la fonction sexuelle chez le dromadaire (*Camelus Dromedarius*). Thèse de doctorat de L'Université Paris 6, France, 1987.
5. Skidmore JA: Reproductive physiology in female Old World Camelids. *Anim Reprod Sci* 2011;124:148-154. doi: 10.1016/j.anireprosci.2010.08.023
6. Tibary A, Anouassi A, Sghiri A, et al: Current knowledge and future challenges in camelid reproduction. *Soc Reprod Fertil Suppl* 2007;64:297-313. doi: 10.5661/rdr-vi-297
7. Vaughan JL: Ovarian function in South American camelids (alpacas, llamas, vicunas, guanacos). *Anim Reprod Sci* 2011;124:237-243. doi: 10.1016/j.anireprosci.2010.08.031
8. Vaughan JL, Tibary A: Reproduction in female South American camelids: A review and clinical observations. *Small Rumin Res* 2006;61:259-281. doi: 10.1016/j.smallrumres.2005.07.015
9. Cavilla MV, Bianchi CP, Maistruarena C, et al: Ultrasonographic and endocrine characterization of follicular waves in llamas with a special reference to the overlapping phenomenon during successive waves. *Reprod Dom Anim* 2013;48:923-930. doi: 10.1111/rda.12187

10. Powell SA, Smith BB, Timm KI, et al: Estradiol production by pre-implantation blastocysts and increased serum progesterone following estradiol treatment in llamas. *Anim Reprod Sci* 2007;102:66-75. doi: 10.1016/j.anireprosci.2006.10.002
11. Ennassiri A. Ennassiri A: Activite folliculaire chez la chamelle (*Camelus dromedarius*). These de doctorat vétérinaire de l'Institut Agronomique et Veterinaire Hassani II, Rabat, Maroc 1985.
12. Sghiri A, Driancourt MA: Seasonal effects on fertility and ovarian follicular growth and maturation in camels (*Camelus dromedarius*). *Anim Reprod Sci* 1999;55:223-237. doi: 10.1016/s0378-4320(99)00017-2
13. Abdoon ASS: Factors affecting follicular population, oocyte yield and quality in camels (*Camelus dromedarius*) ovary with special reference to maturation time in vitro. *Anim Reprod Sci* 2001;66:71-79. doi: 10.1016/S0378-4320(01)00078-1
14. Vyas S, Rai AK, Sahani MS, et al: Use of real-time ultrasonography for control of follicular activity and pregnancy diagnosis in the one humped camel (*Camelus dromedarius*) during the non-breeding season. *Anim Reprod Sci* 2004;84:229-233. doi: 10.1016/s0378-4320(01)00078-1
15. El Allali K, Achaaban MR, Vivien-Roels B, et al: Seasonal variations in the nycthemeral rhythm of plasma melatonin in the camel (*Camelus dromedarius*). *J Pineal Res* 2005;39:121-128. doi: 10.1111/j.1600-079X.2005.00224.x
16. El Allali K, Sghiri A, Bouâouda H, et al: Effect of melatonin implants during the non-breeding season on the onset of ovarian activity and the plasma prolactin in dromedary camel. *Front Vet Sci* 2018;5:44. doi: 10.3389/fvets.2018.00044
17. Norambuena MC, Silva M, Urra F, et al: Effects of nutritional restriction on metabolic, endocrine, and ovarian function in llamas (*Lama glama*). *Anim Reprod Sci* 2013;138:252-260. doi: 10.1016/j.anireprosci.2013.01.019
18. Norarnbuena MC, Hernandez F, Maureira J, et al: Effects of leptin administration on development, vascularization and function of corpus luteum in alpacas submitted to pre-ovulatory fasting. *Anim Reprod Sci* 2017;182:28-34. doi: 10.1016/j.anireprosci.2017.04.006
19. Monaco D, Lacalandra GM, El-Bahrawy KA: Ovarian monitoring and effects of Controlled Intravaginal Drug Releaser (CIDR) on vaginal environment and follicular activity in dromedary camels, during non-breeding season in Egypt. *Emirates J Food Agric* 2013;25:296-300. doi: 10.9755/ejfa.v25i4.15498
20. El Allali K, Achaaban MR, Bothorel B, et al: Entrainment of the circadian clock by daily ambient temperature cycles in the camel (*Camelus dromedarius*). *Am J Physiol Regul Integr Comp Physiol* 2013;304:1044-1052. doi: 10.1152/ajpregu.00466.2012
21. Bravo PW, Fowler ME, Lasley BL: The postpartum llama – fertility after parturition. *Biol Reprod* 1994;51:1084-1087. doi: 10.1095/biolreprod51.6.1084
22. Bravo PW, Lasley BL, Fowler ME: Resumption of ovarian follicular activity and uterine involution in the postpartum llama. *Theriogenology* 1995;44:783-791. doi: 10.1016/0093-691X(95)00265-A
23. Derar R, Ali A, Al-Sobayil FA: The postpartum period in dromedary camels: Uterine involution, ovarian activity, hormonal changes, and response to GnRH treatment. *Anim Reprod Sci* 2014;151:186-193. doi: 10.1016/0093-691x(95)00265-a
24. Tibary A, Anouassi A: *Theriogenology in Camelidae*. Mina, Abu Dhabi, UAE; Abu Dhabi Printing Press: 1997. p. 227.
25. Tibary A, Anouassi A, Sghiri A: Factors Affecting Reproductive Performance of Camels at the Herd and Individual Level. *NATO Sciences Series, I: Life and Behavioural Sciences*. Clifton, VA: IOS Press; 2005. Vol. 362. p. 97-114.
26. Juhasz J, Nagy P: Follicular wave emergence after GnRH induced ovulation at the beginning of the breeding season in dromedaries (*Camelus dromedarius*). *Reprod Dom Anim* 2012;47:575-575.
27. Anouassi A, Tibary A: Development of a large commercial camel embryo transfer program: 20 years of scientific research. *Anim Reprod Sci* 2013;136:211-221. doi: 10.1016/j.anireprosci.2012.10.012
28. Bravo PW, Stabenfeldt GH, Lasley BL, et al: The Effect of ovarian follicle size on pituitary and ovarian responses to copulation in domesticated South American camelids. *Biol Reprod* 1991;45:553-559. doi: 10.1095/biolreprod45.4.553
29. Bravo PW, Stabenfeldt GH, Fowler ME, et al: Pituitary response to repeated copulation and/or gonadotropin-releasing hormone administration in llamas and alpacas. *Biol Reprod* 1992;47:884-888. doi: 10.1095/biolreprod47.5.884
30. Campbell AJ, Pearson LK, Spencer TE, et al: Double ovulation and occurrence of twinning in alpacas (*Vicugna pacos*). *Theriogenology* 2015;84:421-424. doi: 10.1016/j.theriogenology.2015.03.027
31. Manjunatha B, Al-Bulushi S, Pratap N: Ultrasonographic characterization of follicle deviation in follicular waves with single dominant and codominant follicles in dromedary camels (*Camelus dromedarius*). *Reprod Domest Anim* 2014;49:239-242. doi: 10.1111/rda.12260
32. Miragaya MH, Aba MA, Capdevielle EF, et al: Follicular activity and hormonal secretory profile in vicuna (*Vicugna vicugna*). *Theriogenology* 2004;61:663-671. doi: 10.1016/s0093-691x(03)00238-3
33. Tibary A, Anouassi A: Ultrasonographic changes of the reproductive tract in the female camel (*Camelus dromedarius*) during the follicular cycle and pregnancy. *J Camel Pract Res* 1996;3:71-90.
34. Ghoneim IM, Waheed MM, Adam MI, et al: Relationship between the size of the dominant follicle, vaginal electrical resistance, serum concentrations of oestradiol and progesterone and sexual receptivity during the follicular phase of the dromedary camel (*Camelus dromedarius*). *Anim Reprod Sci* 2015;154:63-67. doi: 10.1016/j.anireprosci.2015.01.003
35. Padalino B, Rateb SA, Ibrahim NB, et al: Behavioral indicators to detect ovarian phase in the dromedary she-camel. *Theriogenology* 2016;85:1644-1651. doi: 10.1016/j.theriogenology.2016.01.027
36. Pollard JC, Littlejohn RP, Scott IC. The effects of mating on the sexual receptivity of female alpacas. *Anim Reprod Sci* 1994;34:289-297. doi: 10.1016/0378-4320(94)90024-8
37. Sumar J, Bravo PW, Foote WC: Sexual receptivity and time of ovulation in alpacas. *Small Rum Res* 1993;11:143-150. doi: 10.1016/0921-4488(93)90147-A
38. Vaughan JL, Macmillan KL, D'Occhio MJ: Ovarian follicular wave characteristics in alpacas. *Anim Reprod Sci* 2004;80:353-361. doi: 10.1016/j.anireprosci.2003.08.002
39. Chen BX, Yuen ZX, Pan CW: Factors inducing ovulation in the bactrian camel. In: Cockrill RW: editor. *The Camelid: An*

- All-purpose Animal. Uppsala; Scandinavian Institute of African Studies: 1985. p. 387-398.
40. Aba MA, Forsberg M, Kindahl H, et al: Endocrine changes after mating in pregnant and non-pregnant llamas and alpacas. *Acta Vet Scand* 1995;36:489-498. doi: 10.1186/BF03547663
 41. Adams GP, Ratto MH. Ovulation-inducing factor in seminal plasma: a review. *Anim Reprod Sci* 2013;136:148-156. doi: 10.1016/j.anireprosci.2012.10.004
 42. Kershaw-Young C, Druart X, Vaughan J, et al: β -Nerve growth factor is a major component of alpaca seminal plasma and induces ovulation in female alpacas. *Reprod Fertil Dev* 2012;24:1093-1097. doi: 10.1071/RD12039
 43. Sanjay K, Sharma VK, Sudhuman S, et al: Proteomic identification of camel seminal plasma: purification of β -nerve growth factor. *Anim Reprod Sci* 2012;136:289-295. doi: 10.1016/j.anireprosci.2012.11.001
 44. El Allali K, El Bousmaki N, Ainani H, et al: Effect of the camelid's seminal plasma ovulation-inducing factor/ β -NGF: a kisspeptin target hypothesis. *Front Vet Sci* 2017;4:99. doi: 10.3389/fvets.2017.00099
 45. Ainani H, Chhaibi H, Achaaban MR, et al: Induced-ovulation in female dromedary camel involves kisspeptin neuron activation by β nerve growth factor. *Biol Reprod* 2022;107:1490-1502. doi: 10.1093/biolre/iaoc170
 46. Ratto MH, Paiva L, Carrasco R, et al: Review: unveiling the effect of beta-nerve growth factor on the reproductive function in llamas and cows. *Animal* 2023;17:100754. doi: 10.1016/j.animal.2023.100754. doi: 10.1016/j.animal.2023.100754
 47. Silva M, Fernandez A, Ulloa-Leal C, et al: LH release and ovulatory response after intramuscular, intravenous, and intrauterine administration of beta-nerve growth factor of seminal plasma origin in female llamas. *Theriogenology* 2015;84:1096-1102. doi: 10.1016/j.theriogenology.2015.06.006
 48. Fernandez A, Ulloa-Leal C, Silva M, et al: The effect of repeated administrations of llama ovulation-inducing factor (OIF/NGF) during the peri-ovulatory period on corpus luteum development and function in llamas. *Anim Reprod Sci* 2014;149:345-352. doi: 10.1016/j.anireprosci.2014.08.001
 49. Silva M, Ulloa-Leal C, Norambuena C, et al: Ovulation-inducing factor (OIF/NGF) from seminal plasma origin enhances corpus luteum function in llamas regardless the preovulatory follicle diameter. *Anim Reprod Sci* 2014;148:221-227. doi: 10.1016/j.anireprosci.2014.05.012
 50. Ulloa-Leal C, Bogle OA, Adams GP, et al: Luteotrophic effect of ovulation-inducing factor/nerve growth factor present in the seminal plasma of llamas. *Theriogenology* 2014;81:1101-1107. doi: 10.1016/j.theriogenology.2014.01.038
 51. Silva M, Urrea F, Ulloa-Leal C, et al: A comparative study of the effects of intramuscular administration of gonadorelin, mating and intrauterine infusion of either raw seminal plasma or seminal plasma purified -NGF on luteal development in llamas. *Reprod Dom Anim* 2017;52:625-631. doi: 10.1111/rda.12958
 52. Nagy P, Jutka J, Wernery U: Incidence of spontaneous ovulation and development of the corpus luteum in non-mated dromedary camels (*Camelus dromedarius*). *Theriogenology* 2005;64:292-304.
 53. Adams GP, Ratto MH, Carrasco RA: Natural and controlled ovulation in South American camelids. *Anim Reprod* 2018;15:996-1002. doi: 10.21451/1984-3143-AR2018-0033
 54. Tibary A. Monitoring and controlling follicular activity in camelids. *Theriogenology* 2018;109:22-30. doi: 10.1016/j.theriogenology.2017.12.011
 55. Adams GP, Sumar J, Ginther OJ: Hemorrhagic ovarian follicles in llamas. *Theriogenology* 1991;35:557-568. doi: 10.1016/0093-691x(91)90452-j
 56. Echevarria L, Smitz J: Follicular arrest in ovaries induced by Busereline in alpacas (*Lama pacos*). *Reprod Dom Anim* 2012;47:575-575.
 57. Ainani H, Achaaban MR, Tibary A, et al: Environmental and neuroendocrine control of breeding activity in the dromedary camel. *Rev Maroc Sci Agron Vét* 2018;6:143-157
 58. El Allali K, Sinitskaya N, Bothorel B, et al: Daily Aa-nat gene expression in the camel (*Camelus dromedarius*) pineal gland. *Chronobiol Int* 2008;25:800-807. doi: 10.1080/07420520802384085
 59. Riveros JL, Schuler G, Bonacic C, et al: Ovarian follicular dynamics and hormonal secretory profiles in guanacos (*Lama guanicoe*). *Anim Reprod Sci* 2010;119:63-67. doi: 10.1016/j.anireprosci.2009.11.005
 60. Dholpuria S, Vyas S, Purohit GN, et al: Sonographic monitoring of early follicle growth induced by melatonin implants in camels and the subsequent fertility. *J Ultrasound* 2012;15:135-141. doi: 10.1016/j.jus.2012.02.008
 61. Anouassi A, Tibary A, Adnani M, et al: Preovulatory phase characterization in *Camelus dromedarius* and induction of ovulation. *Proceedings of Conference in Niama, Niger* 1994.
 62. Manjunatha BM, Pratap N, Al-Bulushi S, et al: Characterization of ovarian follicular dynamics in dromedary camels (*Camelus dromedarius*). *Theriogenology* 2012;78:965-973. doi: 10.1016/j.theriogenology.2012.05.011
 63. Tibary A, Anouassi A: Artificial breeding and manipulation of reproduction in Camelidae. In Tibary A, Anouassi A: editors. *Theriogenology in Camelidae: Anatomy, Physiology, BSE, Pathology and artificial breeding*. Arles: Ed. Actes; 1997. p. 413-452.
 64. Ratto M, Huanca W, Singh J, et al: Comparison of the effect of natural mating, LH, and GnRH on interval to ovulation and luteal function in llamas. *Anim Reprod Sci* 2006;91:299-306. doi: 10.1016/j.anireprosci.2005.03.015
 65. Vaughan JL, Mihm M, Wittek T: Factors influencing embryo transfer success in alpacas-A retrospective study. *Anim Reprod Sci* 2013;136:194-204. doi: 10.1016/j.anireprosci.2012.10.010
 66. Sansinena MJ, Taylor SA, Taylor PJ, et al: Production of nuclear transfer llama (*Lama glama*) embryos from in vitro matured llama oocytes. *Cloning Stem Cells* 2003;5:191-198. doi: 10.1089/153623003769645857
 67. Anouassi A, Tibary A, El Allali K: Hormonal manipulation of reproduction. In Tibary A, Anouassi A: editors. *Theriogenology in Camelidae*. 2nd edition, Abu Dhabi, UAE: Advanced Scientific Group; 2014. p. 621-642.
 68. Skidmore JA, Adams GP, Billah M: Synchronisation of ovarian follicular waves in the dromedary camel (*Camelus dromedarius*). *Anim Reprod Sci* 2009;114:249-255. doi: 10.1016/j.anireprosci.2008.08.024

69. Ratto MH, Singh J, Huanca W, et al: Ovarian follicular wave synchronization and pregnancy rate after fixed-time natural mating in llamas. *Theriogenology* 2003;60:1645-1656. doi: 10.1016/S0093-691X(03)00176-6
70. Berland MA, Ulloa-Leal C, Barria M, et al: Seminal plasma induces ovulation in llamas in the absence of a copulatory stimulus: role of nerve growth factor as an ovulation-inducing factor. *Endocrinology* 2016;157:3224-3232. doi: 10.1210/en.2016-1310
71. Huanca W: Reproductive biotechnologies in domestic South American camelids as alternatives for genetic improvement. *Archivos Latinoamericanos de Producción Animal* 2015;23:1-4.
72. Huanca W, Cordero A, Huanca T, et al: Ovarian response and embryo production in llamas treated with equine chorionic gonadotropin alone or with a progestin-releasing vaginal sponge at the time of follicular wave emergence. *Theriogenology* 2009;72:803-808. doi: 10.1016/j.theriogenology.2009.05.019
73. Niasari-Naslaji A, Nikjou D, Skidmore JA, et al: Interspecies embryo transfer in camelids: the birth of the first Bactrian camel calves (*Camelus bactrianus*) from dromedary camels (*Camelus dromedarius*). *Reprod Fertil Dev* 2009;21:333-337. doi: 10.1071/rd08140
74. Rodríguez J, Pearson L, Campbell A, et al: Comparison of two superovulation treatments in the dromedary camel (*Camelus dromedarius*). *Proceedings of the Society for Theriogenology and American College of Theriogenologists Annual Conference, Portland, Aug 6-9, 2014. Clinical Theriogenology* 2014;6:371.
75. Skidmore JA, Billah M: Embryo transfer in the dromedary camel (*Camelus dromedarius*) using non-ovulated and ovulated, asynchronous progesterone-treated recipients. *Reprod Fertil Dev* 2011;23:438-443. doi: 10.1071/RD10136
76. Manjunatha BM, Al-Bulushi S, Pratap N: Synchronisation of the follicular wave with GnRH and PGF(2 alpha) analogue for a timed breeding programme in dromedary camels (*Camelus dromedarius*). *Anim Reprod Sci* 2015;160:23-29. doi: 10.1016/j.anireprosci.2015.06.023
77. Manjunatha BM, Al-Hosni A, Al-Bulushi S: Resynchronization of synchronized follicular wave in dromedary camels of unknown pregnancy status (*Camelus dromedarius*). *Theriogenology* 2018;119:208-213. doi: 10.1016/j.theriogenology.2018.07.001
78. Manjunatha BM, Al-Hosni A, Al-Bulushi S: Effect of advancing the breeding season on reproductive performance of dromedary camels. *Theriogenology* 2022;179:230-236. doi: 10.1016/j.theriogenology.2021.12.007
79. Nagy P, Juhasz J: Fertility after ovarian follicular wave synchronization and fixed-time natural mating compared to random natural mating in dromedary camels (*Camelus dromedarius*). *Anim Reprod Sci* 2012;132:223-230. doi: 10.1016/j.anireprosci.2012.05.010
80. Nikjou D, Niasari-Naslaji A, Skidmore JA, et al: Synchronization of follicular wave emergence prior to superovulation in Bactrian camel (*Camelus bactrianus*). *Theriogenology* 2008;69:491-500. doi: 10.1016/j.theriogenology.2007.10.020
81. Bianchi CP, Benavente MA, Simonetti M, et al: Synchronization of time of ovarian follicular development in llamas (*Lama glama*) using a protocol based on GnRH and PGF2 α . *Anim Reprod Sci* 2018;192:200-205. doi: 10.1016/j.anireprosci.2018.03.011
82. Bowman J: Synchronization of ovarian activity in South American camelids. MS Thesis, Washington State University, 2005.
83. Hussein FM, Metwelly KK, Mona, et al: Effect of CIDR application duration (7-10-14 days) on circulating estrogen and progesterone during breeding and non-breeding season in she-camels. *Alex J Vet Sci* 2015;44:125-129. doi: 10.5455/ajvs.1224
84. Ratto MH, Silva ME, Huanca W, et al: Induction of superovulation in South American camelids. *Anim Reprod Sci* 2013;136:164-169. doi: 10.1016/j.anireprosci.2012.10.006
85. Cooper MJ, Skidmore JA, Allen WR, et al: Attempts to stimulate and synchronise ovulation and superovulation in dromedary camels for embryo transfer. *Proc 1st Int Camel Conf, Dubai, UAE 1992*. p. 187-191.
86. Mohamed RH, Khalphallah A, Ali F, et al: Impact of vitamin ADE treatment before application of controlled intra-vaginal drug releaser on clinical and hematologic findings, ovarian hormones, and calving rates in she-camels. *Open Vet J* 2022;12:965-974. doi: 10.5455/OVJ.2022.v12.i6.24
87. Skidmore J, Allen WR, Cooper MJ, et al: The recovery and transfer of embryos in the dromedary camel: results of preliminary experiments. *Proc 1st int Camel Conf, Dubai, UAE 1992*. p. 137-142.
88. Al-Sobayil K: The use of estrus synchronization and timed artificial insemination in dromedary she-camels in Saudi Arabia. *J Agric Vet Sci Qassim Univ* 2008;1:3-9.
89. Swelum AAA, Alowaimier AN: The efficacy of controlled internal drug release (CIDR) in synchronizing the follicular wave in dromedary camels (*Camelus dromedarius*) during the breeding season. *Theriogenology* 2015;84:1542-1548. doi: 10.1016/j.theriogenology.2015.08.003
90. Mckinnon AO, Tinson AH, Nation G: Embryo-transfer in dromedary camels. *Theriogenology* 1994;41:145-150. doi: 10.1016/S0093-691X(05)80060-3
91. Chhaibi H, Campbell A, Pasha K, et al: Pharmacokinetics of a long-acting progesterone formulation in female camels. *Camelid Reproduction Satellite Symposium, July 1-3, 2016. Tours France Pages 23-26*. 2016.
92. Alberio RH, Aller JF: Control y sincronización de la onda folicular mediante la aplicación de progesterona exógena en llamas. *Rev Arg Prod Anim* 1996;16:325-329.
93. Aba MA, Miragaya MH, Chaves MG, et al: Effect of exogenous progesterone and eCG treatment on ovarian follicular dynamics in vicunas (*Vicugna vicugna*). *Anim Reprod Sci* 2005;86:153-161. doi: 10.1016/j.anireprosci.2004.06.003
94. Ferrer MS, Agüero A, Flores M, et al: Control de la dinámica folicular en la llama (*Lama glama*) utilizando esponjas intravaginales con medroxiprogesterona. In: *Proceeding of the II Congreso Mundial sobre Camélidos, Cusco, Perú, 1999*. p. 173.
95. Chaves MG, Aba M, Aguero A, et al: Ovarian follicular wave pattern and the effect of exogenous progesterone on follicular activity in non-mated llamas. *Anim Reprod Sci* 2002;69:37-46. doi: 10.1016/S0378-4320(01)00173-7
96. Aller JF, Abalos MC, Acuna FA, et al: Embryo yield in llamas synchronized with two different intravaginal progesterone-releasing devices and superovulated with eCG. *Span J Agric Res* 2015;13:e04SC01. doi: 10.5424/sjar/2015133-7308

97. Cavilla MV, Bianchi CP, Aguilera F, et al: Hormonal changes and follicular activity after treatment with intravaginal progesterone-releasing devices in llamas. *Reprod Dom Anim* 2016;51:930-939. doi: 10.1111/rda.12762
98. Aba MA, Quiroga MA, Auza N, et al: Control of ovarian activity in llamas (*Lama glama*) with medroxyprogesterone acetate. *Reprod Dom Anim* 1999;34:471-476. doi: 10.1111/j.1439-0531.1999.tb01406.x
99. Aller JE, Cancino AK, Rebuffi GE, et al: Effect of estradiol benzoate used at the start of a progestagen treatment on superovulatory response and embryo yield in lactating and non-lactating llamas. *Anim Reprod Sci* 2010;119:322-328. doi: 10.1016/j.anireprosci.2010.02.001
100. Carretero MI, Miragaya M, Chaves MG, et al: Embryo production in superstimulated llamas pre-treated to inhibit follicular growth. *Small Rumin Res* 2010;88:32-37. doi: 10.1016/j.smallrumres.2009.11.006
101. Picha Y, Nielsen S, Rodriguez JS, et al: Synchronization of follicular waves with progesterone/estrogen combination before superstimulation with pFSH in alpacas. *Clinical Theriogenology* 2009;1:218.
102. Anouassi A, Tibary A: Chapter 26. Assisted reproductive technologies. In Tibary A, Anouassi A: editors. *Theriogenology in Camelidae*. 2nd edition, Abu Dhabi, UAE: Advanced Scientific Group; 2005. p. 687-714. ISBN 978-9948-751-97-7.
103. Sumar JB: Embryo transfer in domestic South American camelids. *Anim Reprod Sci* 2013;136:170-177. doi: 10.1016/j.anireprosci.2012.10.029
104. Anouassi A, Ali A: Embryo transfer in camel (*Camelus dromedarius*). Proc workshop – is it possible to improve the reproductive performance of the camel? – Paris 1990. p. 327-332.
105. Campbell A, Tibary A: Embryo transfer in camelids. *Clinical Theriogenology* 2017;9:321-327.
106. Ararooti T, Niasari-Naslaji A, Razavi K, et al: Comparing three superovulation protocols in dromedary camels: FSH, eCG-FSH and hMG. *Iran J Vet Res* 2017;18:249-252.
107. Ararooti T, Niasari-Naslaji A, Asadi-Moghaddam B, et al: Superovulatory response following FSH, eCG-FSH and hMG and pregnancy rates following transfer of hatched blastocyst embryos with different diameter and shape in dromedary camel. *Theriogenology* 2018;106:149-156. doi: 10.1016/j.theriogenology.2017.10.017
108. Azizi-Moghadam A: Superovulatory response and embryo recovery after treatment with different gonadotrophin hormones during induced luteal phase in Iranian camel (*Camelus dromedarius*). *Philipp J Vet Anim Sci* 2010;36:73-80.
109. Manjunatha BM, Al-Hosni A, Al-Bulushi S: Simplified superovulation protocols in dromedary camels (*Camelus dromedarius*). *Theriogenology* 2019;126:214-221. doi: 10.1016/j.theriogenology.2018.12.006
110. Olsson O, Sakaguchi K, Tinson AH, et al: Singular epidural follicle-stimulating hormone administration on follicular growth in camels. *Jpn J Vet Res* 2022;70:33-37.
111. El-Shazly M, Mansour N, Karen A, et al: Evaluation of a long-acting recombinant bovine FSH for multiple ovulation and embryo transfer in dromedary camels. *Anim Reprod Sci* 2024;261:107398. doi: 10.1016/j.anireprosci.2023.107398
112. Correa JE, Ratto MH, Gatica R: Oestrus activity and ovarian response in llamas and alpacas treated with progesterone and PMSG or FSH. *Archiv Med Vet* 1994;26:59-64.
113. Correa JE, Ratto MH, Gatica R: Superovulation in llamas (*Lama glama*) with pFSH and equine chorionic gonadotrophin used individually or in combination. *Anim Reprod Sci* 1997;46:289-296. doi: 10.1016/s0378-4320(96)01615-6
114. Forshey BS, Moraes CR, Lakritz J, et al: Embryo production by superovulation and dual siring in alpacas (*Vicugna pacos*). *Small Rumin Res* 2018;162:63-68. doi: 10.1016/j.smallrumres.2018.03.006
115. Palomino JM, Jones L, Vanhanen T, et al: Alpaca embryo transfer on a private Canadian farm. *Can Vet J* 2018;59:631-634.
116. Khalifa MA, Rateb SA, El-Bahrawy KA: Fixed-time induction of ovulation in camels superovulated by different eCG modalities during the transition period in Egypt: superovulation in camels during the transition period. *Trop Anim Health Prod* 2016;48:823-829. doi: 10.1007/s11250-016-1037-2
117. Khalifa MA, Abd El-Hamid IS, Rateb SA: Induction of synchronized multiple ovulation in dromedary camels during the early non-breeding season. *Small Rumin Res* 2020;182:67-72. doi: 10.1016/j.smallrumres.2019.106028
118. Agüero A, Chaves MG, Capdevielle EF, et al: Superovulation in llamas: comparison of two treatments. *In Vet* 2001;3:13-18.
119. Bourke DA, Kyle CE, McEvoy TG, et al: Superovulatory responses to eCG in llamas (*Lama glama*). *Theriogenology* 1995;44:255-268. doi: 10.1016/0093-691x(95)00175-8
120. Tibary A, Pearson LK, Campbell AJ: Embryo transfer in camelids. *Spermova* 2015;5:234-252. doi: 10.18548/aspect/0002.44
121. Aller JE, Abalos MC, Acuña E, et al: Plasma steroid profiles and ovarian response in llamas treated with eCG for superovulation combined with exogenous progesterone during early luteal phase. *Anim Reprod Sci* 2019;208. doi: 10.1016/j.anireprosci.2019.106108
122. Zampini EG, Veiga ME, Fumuso FG, et al: Development of a GnRH-PGF based synchronization and superstimulation protocol for fixed-time mating in llama embryo donors. *Front Vet Sci* 2020;7:595889. doi: 10.3389/fvets.2020.595889
123. Arroyo E, Patino C, Ciccarelli M, et al: Evaluation of ovarian response to PG600 in alpacas. Proceedings of the Society for Theriogenology and American College of Theriogenologists Annual Conference, Omaha, NE July 21-24, 2021. *Clinical Theriogenology* 2021;13:333.
124. Karen A, Mansour N: Factors affecting pregnancy rates and pregnancy losses after embryo transfer in dromedary camels. *Anim Reprod Sci* 2020;221:106580. doi: 10.1016/j.anireprosci.2020.106580
125. Karen A, Abd-Elfattah A, Nasef M, et al: Factors affecting outcomes of embryo transfer in dromedary camels: A retrospective study. *Reprod Dom Anim* 2022;57:402-417. doi: 10.1111/rda.14078
126. Ma L, Li Z, Ma Z, et al: Immunization against inhibin promotes fertility in cattle: a meta-analysis and quality assessment. *Front Vet Sci* 2021;7:687923. doi: 10.3389/fvets.2021.687923
127. Yan L, Li H, Shi Z: Immunization against inhibin improves in vivo and in vitro embryo production. *Anim Reprod Sci* 2015;163:1-9. doi: 10.1016/j.anireprosci.2015.11.001

128. Roser JF, Meyers-Brown G: Superovulation in the mare: a work in progress. *J Equine Vet Sci* 2012;32:376-386. doi: 10.1016/j.jevs.2012.05.055
129. Ge L, Wu C, Dai J, et al: Effects of inhibin immunization on superovulation in Chongming white goats. *Acta Agric Shanghai* 2018;34:96-100.
130. Khan SU, Jamal MA, Su Y, et al: Towards improving the outcomes of multiple ovulation and embryo transfer in sheep, with particular focus on donor superovulation. *Vet Sci* 2022;9:117. doi: 10.3390/vetsci9030117
131. Rateb SA, Khalifa M, El-Bahrawy KA: The influence of active immunization against inhibin on dromedary camel ovarian and hormonal dynamics. *Small Rumi Res* 2015;132:32-36. doi: 10.1016/j.smallrumres.2015.10.003
132. Rateb SA, El-Bahrawy KA, Khalifa MA: The prolonged reproductive response to immunization against inhibin and manipulating ovarian hyperactivity for timed ovulation in camels. *Small Rumin Res* 2016;137:53-58.
133. Patino C, Arroyo E, Ciccarelli M, et al: Serum anti-Müllerian hormone concentrations in female alpacas: variations during the reproductive cycle and correlation with ovarian superstimulation response. *Clinical Theriogenology* 2022;14:91-97. doi: 10.58292/ct.v14.9139
134. Seyedasgari F, Vidales LM, Souza A, et al: Anti-Mullerian hormone in female dromedary camel and its association with super-ovulatory response in embryo donors. *Domest Anim Endocrinol* 2024;86:106818. doi: 10.1016/j.domaniend.2023.106818