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Induction of superovulation in South American camelids☆

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ABSTRACT

The development of assisted reproductive technologies such as embryo transfer (ET), artificial insemination (AI) and *in vitro* fertilization (IVF) in South American camelids is considerably behind that of other livestock species. Poor success of the embryo transfer technique has been related to a lack of an effective superstimulatory treatment, low embryo recovery rate, and the recovery of hatched blastocysts that are not conducive to the cryopreservation process. Superstimulation has been attempted using equine chorionic gonadotropin (eCG) and follicle stimulating hormone (FSH) during the luteal, or the sexually receptive phase, sometimes given at follicular wave emergence. The rationale for inducing a luteal phase prior to or during superstimulation in camelids is not clearly understood, but it may simply reflect an empirical bias to conventional methods used in other ruminants. The number of ovulations or CL varies widely among studies, ranging from 2 to more than 15 per animal, with the number of transferable embryos ranging from 0 to 4 per animal. The control of follicular growth combined with superstimulatory protocols has resulted in a more consistent ovarian response and a greater number of follicles available for aspiration and oocyte collection. Recent studies in llamas have demonstrated that the use of ovulation inducing treatments or follicle ablation can synchronize follicular wave emergence allowing the initiation of gonadotropin treatment in the absence of a dominant follicle resulting in a more consistent ovulatory response. Few studies in alpacas have been reported, but it appears from recent field studies that the ovarian response is more variable and that there is a greater number of poor responders than in llamas. A review of superstimulation protocols that have been used in llamas and alpacas in the last 15 years is provided, including a discussion of the potential of protocols designed to initiate treatment at specific stages of follicular growth.

1. Introduction

The development of assisted reproductive technologies in South American camelids is considerably behind that in other livestock species. Reports of studies on ovarian superstimulation, embryo transfer (ET) and in vitro embryo production in llamas and alpacas are scarce and represent the efforts of a small number of research groups around the world. Information is even more limited in wild camelids;

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there are only a few reports in the literature regarding the use superstimulatory protocols in vicunas, and no reports of the use of any of these biotechnologies in guanacos. An overview of the current state of ovarian superstimulation in llamas and alpacas is presented herein, with a focus on protocols developed in the last 15 years.

Poor success of the ET technique in South American camelidshas been related to high variability in the superstimulatory response and low embryo recovery rate. Another major limitation for the establishment of ET programs is the recovery of embryos at the hatched blastocyst stage; i.e., a stage that is sensitive to the cryopreservation process, thus limiting long term storage, transport or commercialization.

Embryo transfer technologies and superovulation treatments have been studied and developed intensively in other domestic livestock species, allowing their commercial use for the genetic improvement of the herds (i.e. cattle, sheep and goats). Most of the superstimulation protocols used in South American camelids have been extrapolated from those developed for cattle and sheep. However, South American camelids are induced ovulators rather than spontaneous ovulators; therefore, the wave pattern of follicle growth normally occurs in the absence of a luteal phase (Adams et al., 1990), and the dominant follicle remains viable for a relatively long period of time (several days) until ovulation is induced.

2. Ovarian superstimulation protocols

Ovarian superstimulation in llamas and alpacas has been achieved using equine chorionic gonadotropin (eCG), follicle stimulating hormone (FSH), or a combination of these. Treatment with eCG is usually given as a single intramuscular dose of 500–1500 IU (Bourke et al., 1994; Velásquez and Novoa, 1999). Higher doses have been related to the development of cystic follicles (Bravo et al., 1995). Porcine FSH is given intramuscularly in a constant or decreasing dose every 12 h for 4–5 days (Correa et al., 1997; Ratto et al., 1997; Sansinena et al., 2003). Ovine FSH has also been used successfully for the induction of follicular growth in llamas using a decreasing dose regime (Sansinena et al., 2007). After superstimulatory treatment, females may be mated to induce ovulation, but usually mating is followed by the administration of an ovulation-inducing agent such as gonadotropin-releasing hormone (GnRH), human chorionic gonadotropin (hCG) or luteinizing hormone (LH).

During the last decade, ovarian superstimulation has been attempted during 3 different physiological states: (a) the sexually receptive phase, (b) a natural luteal phase (generated by the induction of ovulation), or (c) an artificial luteal phase (induced by exogenous progesterone).

2.1. Ovarian superstimulation during the sexually receptive phase

After 5 consecutive days of sexual receptivity, llamas were given 20 mg pFSH (NIH-FSH-P1) i.m. twice daily for 5 consecutive days (total dose of 200 mg) followed by the administration of 750 IU of hCG i.m. to induce ovulation (Correa et al., 1997; Ratto et al., 1997). Superstimulation during the sexually receptive phase has also been reportedin alpacas (Velásquez and Novoa, 1999), except that females were treated with a single dose of 1000 IU eCG (Day 0) followed by 1000 IU of hCG on Day 6 to induce ovulation (Table 1).

2.2. Ovarian superstimulation during the natural luteal phase

Females with a follicle at least 9 mm in diameter were treated with GnRH or hCG to induce ovulation (Day 0). After 7 days, 1000 IU of eCG was administered i.m. At Day 9, a luteolytic dose of prostaglandin was given, and finally, a dose of 750 IU of hCG was administered when follicles reached a diameter of 9–13 mm to induce a synchronous ovulation (Bourke et al., 1995a; Table 1).

2.3. Ovarian superstimulation during an artificial luteal phase

To mimic the luteal phase, progesterone/progestins have been administered by the application of vaginal sponges containing 60 mg of medroxyprogesterone acetate (MAP), 0.3 g of progesterone in a controlled intravaginal drug-releasing device (CIDR), subcutaneous implants of 3 mg of norgestomet (Crestar), or daily intramuscular administration of 50 mg of progesterone for 7 to 12 days (Bourke et al., 1992, 1994, 1995a; Correa et al., 1994; Velásquez and Novoa, 1999). Ovarian superstimulation has been achieved by either the administration of 20 mg pFSH (NIH-FSH-P1) i.m. twice daily for 5 days (total dose of 200 mg) or 1000 IU of eCG starting 48 h before progestagen removal. Finally, an ovulatory dose of hCG or GnRH is given. The need to induce a luteal phase prior to or during superstimulation in camelids is not clearly understood, but it may simply reflect an empirical bias to conventional methods used in other ruminants (Table 1).

3. Ovarian superstimulation after control of follicular growth

On the assumption that follicular dominance reduces the superstimulatory response, as it does in other ruminants, different approaches have been used to control follicular development in llamas and alpacas. With the use of daily ultrasonography, it is possible to start ovarian superstimulation in females at a time when follicular dominance is minimal (i.e., largest follicle is <7 mm diameter; Aller et al., 2002b; Carretero et al., 2010; Trasorras et al., 2009). However, regular ultrasonography in commercial herds may be not economical or practical. Hence, attempts have been made to inhibit follicular dominance by treating females with progesterone/progestins, with or without estradiol, or through the use of an ovulation-induction agent (GnRH or LH) or by follicular ablation. With successful development of protocols to control follicle development, the scope of possibilities under which superstimulation may be initiated is increased; i.e., superstimulation may then be started at a pre-scheduled time, regardless of the presence of a natural or artificial luteal phase.

Table 1
Ovarian superstimulation protocols given under different physiological status in llamas and alpacas.

Species	No. of donors	Physiologic status	Hormone	Viable embryos/donor	Reference
Llama	6	Luteal (hCG)	eCG	2.3	Bourke et al. (1992)
Llama	24	Luteal (GnRH)	eCG	1.4	Bourke et al. (1992)
Llama	5	Luteal (CIDR)	eCG	2.0	Bourke et al. (1992)
Llama	17	Luteal (norgestomet)	eCG	1.3	Bourke et al. (1992)
Llama	6	Luteal (norgestomet)	eCG	0	Bourke et al. (1994)
Llama	4/4	Sexually receptive	eCG	0	Correa et al. (1994)
Llama	4/4	Sexually receptive	FSH	0.5	Correa et al. (1994)
Llama	4/4	Sexually receptive	eCG+FSH	0.5	Correa et al. (1994)
Llama	12	Luteal (GnRH)	eCG	1.4	Bourke et al. (1995a)
Llama	15	Luteal (norgestomet)	eCG	1.3	Bourke et al. (1995a)
Llama	20	Sexually receptive	FSH	1.5	Ratto et al. (1997)
Alpaca	5	Sexually receptive	eCG + hCG	8.2[a]	Velásquez and Novoa (1999)
Alpaca	5	Luteal (CIDR)	eCG + hCG	17.8[a]	Velásquez and Novoa (1999)

Adapted from Ratto and Adams (2006).
[a] Average number of CL determined by laparotomy.

In llamas, progesterone treatment apparently inhibited follicular development temporarily and synchronized the emergence of a new dominant follicle approximately 7 days after the beginning of treatment (Alberio and Aller, 1996; Chaves et al., 2002). The use of progesterone alone or associated with the administration of 1 mg EB at the beginning of progesterone treatment reportedly resulted in the emergence of a new follicular wave 4.5 and 6.5 days after the beginning of treatment, respectively (Aller et al., 2010). Additionally, authors reported that treatment with gonadotropins after EB + progesterone resulted in a higher embryo recovery rate than in llamas treated with progesterone alone.

The synchronizing effect of progesterone and estradiol treatment, however, was not observed in other studies (Ratto et al., 2003; Vaughan, 2006). Ovarian follicular wave emergence in llamas treated with progesterone in combination with 17β-estradiol was no better than in untreated controls; a new follicular wave emerged 4.5 ± 0.8 and 5.5 ± 1.0 (mean ± SEM) after treatment, respectively (Ratto et al., 2003). Other approaches, involving administration of LH to induce ovulation of the extant dominant follicle, or transvaginal ultrasound-guided ablation of all follicles ≥5 mm,were more effective for synchronizing the emergence of a new follicular wave (2 days after treatment) and the development of an ovulatory-sized follicle (5 days after treatment; Ratto et al., 2003). Manual transrectal rupture of the dominant follicle has also been described as an effective method for the suppression of follicular dominance (Sansinena et al., 2003, 2007), with superstimulatory treatment started after follicle rupture, but has the inherent risk of rectal wall damage with inexperienced operators (Table 3).

In a recent study in llamas (Aller et al., 2010), embryo recovery was not improved when superstimulatory treatment was started at the time of follicular wave emergence induced by EB plus intravaginal sponges of MPA. However, other studies in llamas (Huanca et al., 2009) and alpacas (Huanca, 2008) have demonstrated an improved embryo recovery rate when superstimulatory treatments are started at the time of follicular wave emergence induced by LH administration (Table 2). As stated previously, there appears to be no physiologic basis for inducing a luteal phase prior to or during superstimulation in camelids, and this practice may simply be a reflection of conventional methods used in other species. In this regard, eCG with or without MPA effectively induced a superovulatory response and multiple embryo production in llamas; i.e., addition of progestin during superstimulation provided no beneficial effects (Huanca et al., 2009).

Field studies conducted in alpacas in our laboratory (unpublished) suggest that the ovarian response is more variable than that observed in llamas, and that there is a greater proportion of non-responders. These findings are supported by results of a study in which only 53% (18/34) of alpacas produced embryos after superovulation, with a recovery rate of 41% (number of embryos/number of CL detected by ultrasound examination; Huanca, 2008). In the same study, only 6% of the superstimulated females produced an average of 7 transferable embryos and 45% of the females produced between 1 and 3 embryos.

Despite efforts to improve superovulation protocols in llamas and alpacas, the ovarian response is still frustratingly variable. The total number of CL reported ranges from 0 to 17 per female, and the embryo recovery rate does not exceed 45% (i.e., 0–4 transferable embryos per female), with a proportion (~20%) of females rendering no embryos at all. Further investigation is required to determine the extent to which follicular status contributes to the variability in the superstimulatory response.

4. Ovarian superstimulation for the collection of cumulus–oocyte complexes by follicular aspiration

The control of follicular growth combined with superstimulatory protocols has resulted in a consistent ovarian response with a large number of follicles available for follicular puncture and oocyte collection (Table 3). However, it appears that when ovarian superstimulation treatments are used for superovulation and embryo collection, ovulation, in vivo fertilization or gamete transport may be compromised, resulting in a poor embryo recovery rate; e.g., the mean number of CL was 11.5 per female after eCG treatment and the mean number of embryos recovered was 4.8 (Huanca et al., 2009). Therefore the collection of viable cumulus–oocytes complexes by follicular aspiration

Table 2
Ovarian superstimulation protocols in llamas and alpacas after control of follicular growth.

Species	No. of donors	Follicular growth status	Method to control follicular growth	Hormone	Follicles ≥7 mm	Viable embryo/donor	Reference
Llama	12[a]	Inhibition of DF	EB + CIDR	eCG	4.2 ± 1.9	1.8	Aller et al. (2002b)
Llama	26	Wave emergence	LH induced ovulation	eCG	16.6 ± 5.3	4.8	Huanca et al. (2009)
Llama	27	Wave emergence	LH induced ovulation + MPA	eCG	12.9 ± 3.7	3.5	Huanca et al. (2009)
Llama	18	Wave emergence	MPA	eCG	9.4 ± 1.0	1.1	Aller et al. (2010)
Llama	18	Wave emergence	EB + MPA	eCG	12.4 ± 1.0	2.4	Aller et al. (2010)
Llama	16[b]	Inhibition of DF	EB	eCG	4.4 ± 0.9	1.6	Carretero et al. (2010)
Llama	14[c]	Inhibition of DF	EB + Progesterone	eCG	4.8 ± 0.7	1.9	Carretero et al. (2010)
Llama	10[d]	Absence of DF	Checked by ultrasound	eCG	4.6 ± 0.6	2.2	Carretero et al. (2010)
Llama	22	Absence of DF	Checked by ultrasound	eCG	Not reported	3.0	Trasorras et al. (2010)
Alpaca	23	wave emergence	LH induced ovulation	eCG	10.7 ± 1.3	2.7	Huanca (2008)
Alpaca	22	wave emergence	LH induced ovulation	FSH	6.0 ± 1.5	2.7	Huanca (2008)

EB: estradiol benzoate; DF: dominant follicle; CIDR: controlled intravaginal drug release of 0.33 g of progesterone; MPA: medroxyprogesterone acetate.
[abcd]Proportion of donors that failed to respond to superovulation: 3/12, 7/16, 4/14 and 1/10, respectively.

for *in vitro* fertilization, could be an alternative method to overcome the difficulties observed during the in vivo embryo production procedure.

In llamas and alpacas, ovarian stimulation with eCG and FSH provides a uniform population of oocytes (Ratto et al., 2005; Brogliatti et al., 2000). The best response to gonadotrophins was obtained when treatment was initiated after inhibition of the dominant follicle or synchronization of follicular wave emergence (Ratto et al., 2005, 2007; Berland et al., 2011). These treatments also had the advantage of providing more expanded cumulus–oocyte complexes (COC; Ratto et al., 2005; Conde et al., 2008; Trasorras et al., 2009). In llamas, superstimulation with eCG was associated with a slightly greater proportion of expanded COC and COC in metaphase II compared to superstimulation with FSH (Ratto et al., 2005); however, in another study (Berland et al., 2011), superstimulatory treatments with FSH and eCG were equally effective.

Although, a great number of cumulus–oocyte complexes can be obtained from in vivo animals, there are not many studies related to in vitro embryo production in these species. Successful llama in vitro embryo production has been reported after control of follicular growth by EB + progesterone administration (Conde et al., 2008) or follicle ablation (Berland et al., 2011) and the use of either eCG (Conde et al., 2008; Berland et al., 2011) or FSH (Berland et al., 2011) to obtain in vivo mature cumulus–oocyte complexes. Reported blastocyst rates range from 17 (Conde et al., 2008) to 21.9% (Berland et al., 2011) of presumptive zygotes developing into early to expanded blastocysts. Moderate success has also been reported for llama embryo production using ICSI (Sansinena et al., 2007; Conde et al., 2008) and nuclear transfer (Sansinena et al., 2003).

In light of these results, and considering the low embryo recovery rates observed in conventional ET programs, ovarian superstimulatory protocols could be used more effective as a potential method to obtain mature COC for in vitro fertilization and embryo production. The improvement of in vitro embryo production will be a challenge before this technique may be available in commercial farms.

5. Ovarian superstimulation in wild South American camelids

Genetic conservation of vicuna and guanaco populations is of importance because of their high quality fiber production and threatened status. However, considerably less information has been produced for these wild species than for domesticated llamas and alpacas. Two studies (Aller et al., 2002a; Aba et al., 2005) have demonstrated that ovarian superstimulatory protocols used in llamas are effective in vicunas (Table 4). However, these studies involved a very small number of animals and only data of follicular development after ovarian superstimulation were reported; i.e., no information is available regarding embryo recovery or ET. Therefore, the use of ET and ovarian superstimulatory treatments in wild South American camelids is still an unexplored field of research.

Table 3
Ovarian superstimulation protocols intended for cumulus–oocyte complexes (COC) collection in llamas and alpacas.

Species	No. of donors	Follicular growth status	Method to control follicular growth	Hormone	No. of follicles ≥6/donor	No. of COC/donor	Reference
Llama	9	Inhibition of DF	Manual rupture DF	pFHS	34.1	33.1	Sansinena et al. (2003)
Llama	20	Wave emergence	Follicle ablation	eCG + LH	17.7	11.2	Ratto et al. (2005)
Llama	20	Wave emergence	Follicle ablation	pFSH + LH	17.9	10.7	Ratto et al. (2005)
Alpaca	7	Absence of DF	Checked by ultrasound	pFSH + hCG	20	26.2	Ratto et al. (2007)
Alpaca	7	Absence of DF	Checked by ultrasound	eCG + hCG	27	23.3	Ratto et al. (2007)
Llama	21	Inhibition of DF	EB + CIDR	eCG + GnRH	10.6	9.2	Conde et al. (2008)
Llama	11	Inhibition of DF	Manual rupture DF	oFSH	14.2	13.1	Sansinena et al. (2007)
Llama	20	Inhibition of DF	EB + CIDR	eCG + GnRH	11.8	9.7	Trasorras et al. (2009)
Llama	16	Wave emergence	Follicle ablation	pFSH + LH	16	11.5	Berland et al. (2011)
Llama	16	Wave emergence	Follicle ablation	eCG + LH	14	9.7	Berland et al. (2011)

DF: dominant follicle.

Table 4
Ovarian superstimulation protocols in vicunas (*Vicugna vicugna*).

Species	No. of donors	Follicular growth status	Control follicular growth	Hormone	No. of follicles	Embryos/donor	Reference
Vicuña	24	Inhibition of DF	EB + MPA Sponge	eCG	Not reported	Not reported	Aller et al. (2002a)
Vicuña	2	Absence of DF	Checked by ultrasound	eCG	16.5	Not reported	Aba et al. (2005)
Vicuña	2	Inhibition of DF	CIDR treatment for 5 days	eCG	29	Not reported	Aba et al. (2005)

DF: dominant follicle; EB: estradiol benzoate; MPA: medroxyprogesterone acetate; CIDR: controlled intravaginal device release of 0.33 g of progesterone.

Table 5
Pregnancy and live birthing rates after embryo transfer in South American camelids from 1968 to 2010.

Species	No. of donors	Superstimulation	No. of recipients	No. of conceptions	Crias born	Reference
Alpaca	3	eCG	3	0	0	Novoa and Sumar (1968)
Alpaca	15	NR	44	4	1	Sumar and Franco (1974)
Llama	2	NR	2	1	1	Wiepz and Chapman (1985)
Alpaca	2	NR	3	3	2	Palomino et al. (1987)
Llama	33	NR	11	3	2	Bourke et al. (1992)
Llama	24	GnRH + eCG	7	3	3	Bourke et al. (1992)
Llama	17	Progesterone implant + eCG	4	0	0	Bourke et al. (1992)
Llama	1	NR	2	1	1	Gatica et al. (1994)
Llama/Guanaco	12	eCG	10	5	4	Bourke et al. (1995b,c)
Alpaca	8	pFSH/eCG	30	23	19	Palomino (2000)
Llama	22	No treatment	49	18	NR	Taylor et al. (2000)
Llama	12	eCG/pFSH	5	2	NR	Aller (2001)
Llama/Alpaca	1	No treatment	2	2	2	Taylor et al. (2001)
Llama	15	CIDR-EB-eCG	10	4	0	Aller et al. (2002b)
Llama/Alpaca	NR	NR	34/13	25/4	21/4	Huanca et al. (2006)
Alpacas	34	pFSH/eCG	32	14	NR	Huanca (2008)
Llama	22	eCG	50	12[a]	NR	Trasorras et al. (2010)

Adapted from Ratto and Adams (2006); Miragaya et al. (2006).
EB: estradiol benzoate; CIDR: controlled intravaginal device release of 0.33 g of progesterone; NR, not reported.
[a] Embryos transferred ipsi or contralateral to the side of the CL.

6. Pregnancy and live birthing after embryo transfer in SAC

Since the first birth of an alpaca cria reported in 1968 by surgical embryo collection and transfer (Novoa and Sumar, 1968), the development of ET in llamas and alpacas has been slow. During the last 35 years, less than 60 live births have been reported throughout the world, with successful events reported in only 4 countries (Perú, Chile, USA and UK). Although in early studies, a non-surgical approach was feasible for the successful collection and transfer of embryos in these two species (Wiepz and Chapman, 1985; Palomino et al., 1987), improvements on pregnancy and birthing rates have been marginal. Successful interspecies ET in camelids was reported a decade ago (Taylor et al., 2001) where two alpaca crias were born using llama recipients.

The diameter of the embryos at the time of the transfer into recipients appears to be crucial for the successful development of pregnancy. In this regard, an early llama study (Bourke et al., 1995a) reported that successful pregnancies were achieved with the transfer of embryos that ranged from 600 to 1200 μm in diameter. Similarly, alpaca pregnancies were achieved only after transfer of embryos 400–1000 μm in diameter (Huanca, 2008). The low success rate of ET in South American camelids has also been attributed to the narrow window of time that transferred embryos have to suppress luteolysis, increased $PGF_{2\alpha}$ release due to manipulation of the cervix, and donor-recipient asynchrony. However, recent data suggests that cervix manipulation during ET procedures induces a short-lived increase in PGF2α which is not enough to affect CL function or pregnancy (Trasorras et al., 2010). Pregnancy rates and live births after ET in llamas and alpacas are summarized in Table 5.

Conflict of interest

The authors declare that they have no competing interests.

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References

Aba, M.A., Miragaya, M.H., Chaves, M.G., Capdevielle, E.F., Rutter, B., Agüero, A., 2005. Effect of exogenous progesterone and eCG treatment on ovarian follicular dynamics in vicunas (*Vicugna vicugna*). Anim. Reprod. Sci. 86, 153–161.

Adams, G.P., Sumar, J., Ginther, O.J., 1990. Effects of lactational status and reproductive status on ovarian follicular waves in llamas (*Lama glama*). J. Reprod. Fertil. 90, 535–545.

Alberio, L.H., Aller, J.F., 1996. Control y sincronización de la onda folicular mediante la aplicación de progesterona exógena en llamas. Rev. Arg. Prod. Anim. (Argentina) 16, 325–329.

Aller, J.F., 2001. Cryopreservation of semen and artificial insemination in South American camelids. In: International Foundation for Sciences Final Report.

Aller, J.F., Rebuffi, G.E., Cancino, A.K., 2002a. Superovulation response to progesterone-eCG treatment in vicuna (*Vicugna vicugna*) in semicaptive conditions. Theriogenology 57, 576 (Abstract).

Aller, J.F., Rebuffi, G.E., Cancino, A.K., Alberio, R.H., 2002b. Successful transfer of vitrified llama (*Lama glama*) embryos. Anim. Reprod. Sci. 73, 121–127.

Aller, J.F., Cancino, A.K., Rebuffi, G.E., Alberio, R.H., 2010. 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. 119, 322–328.

Berland, M.A., von Baer, A., Ruiz, J., Parraguez, V.H., Morales, P., Adams, G.P., Ratto, M.H., 2011. In vitro fertilization and development of cumulus oocytes complexes collected by ultrasound-guided follicle aspiration in superstimulated llamas. Theriogenology 75, 1482–1488.

Bourke, D., Adam, C., Kyle, C., Young, P., Mc Evoy, T.G., 1992. Ovulation, superovulation and embryo recovery in llamas. In: Proceedings 12th International Congress on Animal Reproduction, vol. 1, The Hague, Netherlands, pp. 193–195.

Bourke, D., Adam, C., Kyle, C., Mc Evoy, T.G., Young, P., 1994. Ovarian response to PMSG and FSH in llamas. In: First European Symposium on South American Camelids, Bonn, Germany, pp. 75–81.

Bourke, D.A., Kyle, C.E., Mc Evoy, T.G., Young, P., Adam, C.L., 1995a. Superovulatory responses to eCG in llamas (*Lama glama*). Theriogenology 44, 255–268.

Bourke, D.A., Kyle, C.E., McEvoy, T.G., Young, P., Adam, C.L., 1995b. Recipient synchronization and embryo transfer in South American camelids. Theriogenology 43, 171.

Bourke, D.A., Kyle, C.E., McEvoy, T.G., Young, P., Adam, C.L., 1995c. Advanced reproductive technologies in South American camelids. In: Proccedings of the Second European Symposium on South American Camelids, Camerino (MC), Italy, pp. 235–243.

Bravo, P.W., Tsutsui, T., Lasley, B.L., 1995. Dose response to equine chorionic gonadotropin and subsequent ovulation in llamas. Small Rumin. Res. 18, 157–163.

Brogliatti, G.M., Palasz, A.T., Rodriguez-Martinez, H., Mapletoft, R.J., Adams, G.P., 2000. Transvaginal collection and ultrastructure of llama (*Lama glama*) oocytes. Theriogenology 54, 1269–1279.

Carretero, M.I., Miragaya, M., Chaves, M.G., Gambarotta, M., Agüero, A., 2010. Embryo production in superstimulated llamas pre-treated to inhibit follicular growth. Small Rumin. Res. 88, 32–37.

Chaves, M.G., Aba, M.A., Agüero, A., Egey, J., Berestin, V., Rutter, B., 2002. Ovarian follicular wave pattern and the effect of exogenous progesterone on follicular activity in non-mated llamas. Anim. Reprod. Sci. 69, 37–46.

Conde, P.A., Herrera, C., Trasorras, V.L., Giuliano, S.M., Director, A., Miragaya, M.H., Chaves, M.G., Sarchi, M.I., Stivale, D., Quintans, C., Agüero, A., Rutter, B., Pasqualini, S., 2008. In vitro production of llama (*Lama glama*) embryos by IVF and ICSI with fresh semen. Anim. Reprod. Sci. 109, 298–308.

Correa, J., Ratto, M.H., Gatica, R., 1994. Actividad estral y respuesta ovárica en alpacas en llamas tratadas con progesterona y gonadotrofinas. Arch. Med. Vet. 26, 59–64.

Correa, J., Ratto, M., Gatica, R., 1997. Superovulation in llamas with pFSH and equine chorionic gonadotrophin used individually or in combination. Anim. Reprod. Sci. 46, 289–296.

Gatica, R., Ratto, M., Schuler, C., Ortiz, M., Oltra, J., Correa, J.E., 1994. Cría obtenida en una llama (Lama glama) por transferencia de embriones. Agric. Tec. (Chile) 54, 68–71.

Huanca, T., 2008. Efecto de la administración de gonadotropinas exógenas (FSH y eCG) en la respuesta ovárica y en la producción de embriones en alpacas (Vicugna pacos), PhD Thesis. Universidad de Santiago de Compostela, Lugo, España, pp: 78.

Huanca, W., Ratto, M., Cordero, A., Santiani, A., Huanca, T., Cárdenas, O., Adams, G.P., 2006. Respuesta ovárica y transferencia de embriones en alpacas y llamas en la zona altoandina del Perú. Resumen V Congreso Mundial de Camélidos, Catamarca, Argentina, pp. 105.

Huanca, W., Cordero, A., Huanca, T., Cardenas, O., Adams, G.P., Ratto, M.H., 2009. 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 72, 803–808.

Miragaya, M.H., Chaves, M.G., Agüero, A., 2006. Reproductive biotechnology in South American camelids. Small Rumin. Res. 61, 299–310.

Novoa, C., Sumar, J.,1968. Colección de huevos in vivo y ensayo de transferencia en alpaca. In: 3er Boletín extraordinario IVITA. Universidad Nacional Mayor de San Marcos, Lima, Perú, pp. 31–34.

Palomino, H., 2000. Biotecnología del transplante y micromanipulación de embriones de bovinos y camélidos de los Andes. A.F.A. Editores Importadores, Perú. Capítulo, 11, p. 266.

Palomino, H., Tabacchi, L., Avila, E., Eli, O., 1987. Ensayo preliminar de transferencia de embriones en camélidos Sud Americanos. Rev. CamélidosSud Am. (Perú) 5, 10–17.

Ratto, M.H., Gatica, R., Correa, J.E., 1997. Timing of mating and ovarian response in llamas (*Lama glama*) treated with pFSH. Anim. Reprod. Sci. 48, 325–330.

Ratto, M.H., Singh, J., Huanca, W., Adams, G.P., 2003. Ovarian follicular wave synchronization and pregnancy rate after fixed-time natural mating in llamas. Theriogenology 60, 1645–1656.

Ratto, M., Berland, M., Huanca, W., Singh, J., Adams, G.P., 2005. In vitro and in vivo maturation of llama oocytes. Theriogenology 63, 2445–2457.

Ratto, M.H., Adams, G.P., 2006. Embryo technologies in South American camelids. In: Youngquist, R.S., Threlfall, W.R. (Eds.), Current Therapy in Large Animal Theriogenology, vol. 2. Saunders Elsevier, St. Louis, Missouri, USA, pp. 900–905.

Ratto, M., Gómez, C., Berland, M., Adams, G.P., 2007. Effect of ovarian superstimulation on COC collection and maturation in alpacas. Anim. Reprod. Sci. 97, 246–256.

Sansinena, R.J., Taylor, S.A., Taylor, P.J., Denniston, R.S., Godke, R.A., 2003. Production of nuclear transfer llama (*Lama glama*) embryos from in vitro matured llama oocytes. Cloning Stem Cells 5, 191–198.

Sansinena, R.J., Taylor, S.A., Taylor, P.J., Schmidt, E.E., Denniston, R.S., Godke, R.A., 2007. In vitro production of llama (*Lama glama*) embryos by intracytoplasmatic sperm injection: Effect of chemical activation treatments and culture conditions. Anim. Reprod. Sci. 99, 342–353.

Sumar, J., Franco, E., 1974. Informe Final IVITA-La Raya. Universidad Nacional Mayor de San Marcos, Lima, Perú, p. 35.

Trasorras, V., Chaves, M.G., Miragaya, M.H., Pinto, M., Rutter, B., Flores, M., Agüero, A., 2009. Effect of eCG superstimulation and Buserelin in cumulus–oocytes complexes recovery and maturation in llamas (*Lama glama*). Reprod. Domest. Anim. 44, 359–364.

Trasorras, V., Chaves, M.G., Neild, D., Gambarotta, M., Aba, M., Agüero, A., 2010. Embryo transfer technique: factors affecting the viability of the corpus luteum in llamas. Anim. Reprod. Sci. 121, 279–285.

Taylor, S., Taylor, P.J., James, A.N., Godke, R.A., 2000. Successful commercial embryo transfer in the llama (*Lama glama*). Theriogenology 53, 344.

Taylor, P.J., Taylor, S., James, A.N., Denniston, R.S., Godke, R.A., 2001. Alpaca offspring born after cross species embryo transfer to llama recipients. Theriogenology 55, 401.

Vaughan, J., 2006. Ovarian synchronization and induction of ovulation. In: Youngquist, R.S., Threlfall, W.R. (Eds.), Chapter in Current Therapy in Large Animal Theriogenology. , 2nd ed. Saunders Elsevier, St. Louis MO, pp ¿??

Velásquez, C., Novoa, M.C., 1999. Superovulación con PMSG aplicada en fase folicular y fase luteal en alpacas. Rev. Invest. Vet. (Perú) 10 (1), 234–238.

Wiepz, D.W., Chapman, R.J., 1985. Non-surgical embryo transfer and live birth in a llama. Theriogenology 24, 251–257.