REPRODUCTION
REVIEW

New insights of the role of β-NGF in the ovulation mechanism of induced ovulating species

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Abstract

The type of stimuli triggering GnRH secretion has been used to classify mammalian species into two categories: spontaneous or induced ovulators. In the former, ovarian steroids produced by a mature follicle elicit the release of GnRH from the hypothalamus, but in the latter, GnRH secretion requires coital stimulation. However, the mechanism responsible for eliciting the preovulatory LH surge in induced ovulators is still not well understood and seems to vary among species. The main goal of this review is to offer new information regarding the mechanism that regulates coitus-induced ovulation. Analysis of several studies documenting the discovery of β-NGF in seminal plasma and its role in the control of ovulation in the llama and rabbit will be described. We also propose a working hypothesis regarding the sites of action of β-NGF in the llama hypothalamus. Finally, we described the presence of β-NGF in the semen of species categorized as spontaneous ovulators, mainly cattle, and its potential role in ovarian function. The discovery of this seminal molecule and its ovulatory effect in induced ovulators challenges previous concepts about the neuroendocrinology of reflex ovulation and has provided a new opportunity to examine the mechanism(s) involved in the cascade of events leading to ovulation. The presence of the factor in the semen of induced as well as spontaneous ovulators highlights the importance of understanding its signaling pathways and mechanism of action and may have broad implications in mammalian fertility.
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Introduction

Ovulation in mammals involves the release of GnRH from the hypothalamus into the hypophyseal portal system followed by the release of LH from the pituitary gland into the systemic circulation (Karsch 1987, Karsch *et al.* 1997). Mammals have been classified as either spontaneous or induced (reflex) ovulators. In spontaneously ovulating females (e.g., woman, sheep, cow, mare, sow), increasing systemic concentrations of estradiol, under basal levels of progesterone, induces release of GnRH from the hypothalamus (Jaffe & Keys 1974, Knobil 1980, Kelly *et al.* 1988, Turzillo & Nett 1999). Hence, a luteal phase in these species is induced spontaneously and ovulation occurs at regular intervals. By contrast, in induced ovulators (e.g., rabbits, ferrets, cats, camelids), copulation is responsible for triggering GnRH secretion from the hypothalamus, followed by a pre-ovulatory release of LH from the pituitary (Bakker & Baum 2000). Therefore, females from these species do not display an estrous cycle per se, as observed in spontaneous ovulators.

Reasons for the evolution of a species-specific mechanism of ovulation among mammals remain unknown. Current concepts of phylogeny do not explain the presence of one type of ovulatory mechanism over the other, and the evolutionary drive toward an induced or spontaneous ovulation mechanism may be associated with social or environmental cues for a given species. Although the LH surge is a mandatory event for triggering ovulation in both types of ovulators, the specific signaling pathway to elicit the GnRH/LH surge is still poorly understood in induced ovulators.

The main goal of this review is to offer new information regarding the mechanism that regulates coitus-induced ovulation. Analysis of several studies documenting the discovery of β-NGF in seminal plasma and its role in the ovulatory mechanism in the llama and rabbit will be described. We also propose a working hypothesis regarding the site of action of β-NGF in the llama hypothalamus. Undoubtedly, gaps remain in our understanding of the role of β-NGF in the ovulation mechanism, but the results of recent research on the presence and effect of this seminal factor in both spontaneous and induced ovulators are exciting and may have a profound impact on fertility management in domestic animals and humans.

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General aspects of ovulation induction in induced ovulatory species

The first evidence of coitus-induced ovulation was documented more than 100 years ago by Heape (1905) who described the phenomenon in rabbits. In some induced ovulators, ovulation is triggered by penile intromission (mink and ferret; Carroll *et al.* 1985, Sundquist *et al.* 1988), mounting with or without copulation (rabbit; Ramirez & Soufi 1994), single copulation (camelids; Fernandez-Baca *et al.* 1970, England *et al.* 1971, Marie & Anouassi 1986) or multiple copulations (cat; Concannon *et al.* 1980). Although circulating estradiol concentration was positively correlated with increasing size of the dominant ovarian follicle in llamas and alpacas, the physiological increase did not result in ovulation in these species (Bravo *et al.* 1990*a*,*b*, 1991, 1992). Similarly, neither endogenous nor exogenous estradiol induced LH secretion in rabbits or ferrets (Sawyer & Markee 1959, Baum *et al.* 1990). However, estradiol in induced ovulators was reported to act on the female hypothalamus to induce the behaviors characteristic of the proceptive and receptive sexual stages (Baum & Schretlen 1978).

The rabbit is the only induced ovulator in which GnRH has been measured and was found to be released in a pulsatile manner using the push–pull perfusion technique (Pau *et al.* 1986, Ramirez *et al.* 1986). Coitus was associated with a dramatic increase in GnRH pulse-frequency from the medial basal hypothalamus (MBH) followed by an increase in circulating LH concentrations within 20–60 min (Pau *et al.* 1986, Ramirez *et al.* 1986). Neurotransmitter and peptidergic systems have been shown to affect GnRH secretion and ovulation in mammalian females (reviewed in McEwen & Parsons 1982). An increase in norepinephrine after coitus was deemed responsible for regulating GnRH secretion and subsequent ovulation in rabbits (reviewed in Kauffman & Rissman 2006). Norepinephrine neurons, A1, A2, A5 and A6 groups are located in several loci of the brainstem, including the medulla oblongata, solitary tract, lateral tegumentum of the pons and the locus coeruleus. The effects of neuropeptide Y and acetylcholine have also been reported to modulate ovulation in the rabbit (Ramirez & Soufi 1994).

Recent findings in camelids (llamas, alpacas, camels) offer new insight into the mechanism of induced ovulation (reviewed in Adams & Ratto 2013, Adams *et al.* 2016). An ovulation-inducing factor (OIF) in the seminal plasma of camelids has been isolated and identified as nerve growth factor (β-NGF, Kershaw-Young *et al.* 2012, Ratto *et al.* 2012, Kumar *et al.* 2013). Moreover, we have developed a llama model to document that genital-somatosensory stimuli of copulation is not the principal ovulation-inducing signal in this species (Berland *et al.* 2016). Whether or not the same mechanism is present in other species of induced ovulators is yet unknown.

The discovery of β-NGF in the semen of camelids and its role in ovulation induction

Seminal plasma is produced by the male accessory sex glands and its composition may vary among species according to the type of glands present. Seminal plasma has been considered primarily a vehicle for sperm transport; however, it contains proteins, growth factors, hormones and cytokines that may regulate important functions in the spermatozoa and in the female reproductive system (Bromfield 2016).

The first study to document a direct role of semen on the ovulatory mechanism was reported by Chen *et al.* (1985) where ovulation was observed after intra-vaginal semen deposition in Bactrian camels without any physical contact with the male. Also, seminal plasma was found to stimulate LH secretion from rat pituitary cells cultured *in vitro* (Paolicchi *et al.* 1999). The first study to document the association between systemic seminal plasma administration and LH secretion and ovulation in alpacas and llamas was reported by Adams *et al.* (2005); results demonstrated the presence of an ovulation inducing-factor (OIF) in the seminal plasma of both species. Intramuscular administration of seminal plasma induced ovulation in >90% of females by eliciting a sustained preovulatory LH surge.

The effect of seminal plasma on ovulation may not be unique to induced ovulatory species. For instance, the preovulatory LH surge was advanced in cattle when mating was conducted within the first 6–8 h of behavioral estrus (Jochle 1975). Similarly, intrauterine infusion of boar seminal plasma accelerated ovulation in gilts (Waberski *et al.* 1995). Also, studies in humans suggested the presence of molecules with GnRH-like activity in semen (Izumi *et al.* 1985, Sokol *et al.* 1985).

Aside from the potent ovulatory effect of llama β-NGF, this molecule has a marked luteotrophic effect after intramuscular or intrauterine infusion in llamas and alpacas (Adams *et al.* 2005, Ratto *et al.* 2006). The CL that developed after ovulations induced by seminal plasma treatment tended to be larger and regress later than the CL resulting from GnRH treatment, and progesterone secretion was higher than that in GnRH-treated females (Adams *et al.* 2005). The magnitude of LH release and subsequent CL function in female llamas treated with seminal plasma suggest that the luteotrophic effect of β-NGF is mediated by LH. In this regard, the luteotrophic effects of seminal plasma has been confirmed in other studies using purified NGF from the seminal plasma of llamas (Ratto *et al.* 2011, Silva *et al.* 2011*a*,*b*, 2014, Tanco *et al.* 2012). Moreover, an increase in the vascular area of the CL was highly correlated to progesterone production in females treated with β-NGF compared to the GnRH-treated group (Ulloa-Leal *et al.* 2014).

Potential mechanism of seminal plasma β-NGF in ovulation induction in llamas

The circuits and basic mechanisms of stimulation and signaling for GnRH release and LH discharge have not been intensively investigated in induced ovulators. Although different stimuli such as olfactory, tactile, visual and auditory have been indicated as facilitators or modulators of ovulation in reflex ovulators, penile intromission during copulation has been considered the main factor associated with the preovulatory discharge of LH and subsequent ovulation in these species (Bakker & Baum 2000, Kauffman & Rissman 2006). However, more recent studies in llamas and alpacas have provided important and conclusive evidence to challenge the notion that physical stimulation of the genital tract is a necessary requirement to induce ovulation in these species (Adams & Ratto 2013, Adams *et al.* 2016, Berland *et al.* 2016).

Systemic administration of purified NGF from llama seminal plasma stimulates secretion of LH in both intact and ovariectomized llamas, and the magnitude of LH secretion was modulated by estradiol (Silva *et al.* 2012). In addition, the effect of OIF on LH secretion was inhibited in llamas pretreated with a GnRH antagonist (Silva *et al.* 2011*a*). Results document that NGF elicits a preovulatory LH surge by a direct or indirect action on GnRH neurons.

In a recent study (Berland *et al.* 2016), an urethrostomized male was used to discriminate between the effects of seminal plasma NGF and penile stimulation as the main stimulus to induce ovulation in llamas. Females mated with the urethrostomized male did not ovulate (0/6), but ovulation was observed in females mated with an intact male (6/7) or those given an intrauterine infusion of seminal plasma (5/6). Results indicated that intrauterine administration of seminal plasma was sufficient to elicit an LH surge and that circulating LH concentrations were positively correlated with a rapid systemic increase in β-NGF observed during the first hour after treatment. The results of this and previous studies (Ratto *et al.* 2005, Silva *et al.* 2011*a*, 2012, Berland *et al.* 2016) suggest that β-NGF from the seminal plasma is absorbed from the endometrium after copulation and enters systemic circulation to elicit GnRH secretion from the hypothalamus. However, the signaling pathway by which β-NGF elicits GnRH secretion remains unknown.

How then does β-NGF gain access at the central level to trigger GnRH secretion? What are the potential sites of action or binding of NGF in the hypothalamus? Is it necessary for NGF to cross the blood–brain barrier to reach the hypothalamus? Does NGF act directly or indirectly on GnRH neurons? There are still many questions to answer.

Gonadotrophin-releasing hormone is produced by hypothalamic neurons and transported to the terminal axons located at the median eminence (ME; Fink 1988). In mammals, GnRH nerve fibers are concentrated mainly in the lateral and medial post-infundibular regions, where their axon terminals are housed in the pericapillary space of the hypophyseal portal system in the ME (Baker *et al.* 1975, Barry & Dubois 1976, Rodríguez *et al.* 2010). As well, GnRH fibers located in the floor of the third ventricle are closely associated with highly specialized glial cells, the tanycytes, which have also been ascribed a role in the direct release of GnRH into the hypophyseal portal system (reviewed in Ojeda *et al.* 2008, 2010, Rodríguez *et al.* 2010).

In a recent study (Carrasco *et al.* 2018*a*), GnRH neurons in llamas were not equally distributed among hypothalamic areas and were not aggregated in hypothalamic nuclei, but rather had a more diffuse distribution. A high number of GnRH neurons were located in the medio-basal hypothalamus, twice as many as the second most-populated region, the anterior hypothalamus. A lower population of GnRH neurons was located in the diagonal band of Brocca and the arcuate and periventricular nuclei (Carrasco *et al.* 2018*a*).

Another component of the cellular network of GnRH release includes the neurons that produce Kisspeptin. In rodents, kisspeptin has been described as the most potent activator of LH secretion and the majority of GnRH neurons express receptors for kisspeptin (GPR54 or KISS1R; Herbison 2015). In rodents, the neurons that synthesize kisspeptin form clusters and are distributed in close association with GnRH neurons in the preoptic area and basal hypothalamus (Herbison 2015). Associations among GnRH neurons, kisspeptin neurons and hypothalamic tanycytes have been implicated in the preovulatory LH surge (Clarkson *et al.* 2010, Ojeda *et al.* 2010, Rodríguez *et al.* 2010).

The entry of peripheral peptides/proteins into the brain is regulated by the blood–brain barrier, a unique uninterrupted barrier that is located at the level of the endothelial cells of the cerebral capillaries (Rodríguez *et al.* 2010). However, this barrier is displaced at the circumventricular organs to specialized ependymal cells (Hawkins & Davis 2005, Rodríguez *et al.* 2010). The circumventricular organs have been described as windows that allow neural cells to sense the blood and exchange certain substances between blood and nerve tissue, which cannot otherwise cross due to the presence of the blood–brain barrier (reviewed in Prevot *et al.* 2010, Rodríguez *et al.* 2010, Spuch & Navarro 2010).

Recent immunocytochemical studies of nerve tissue from llamas treated with seminal plasma (Berland 2017) revealed strong immunoreactivity for trkA (high-affinity receptor) and its ligand β-NGF in the choroid plexus at the roof of the third ventricle (Fig. 1). An interesting aspect of this finding is that the expression of the receptor in the choroid plexus has not been described in any species previously. In rats, Timmusk *et al.* (1995) reported the presence of NGF, NT-4, NT-3 and BDNF mRNAs in the

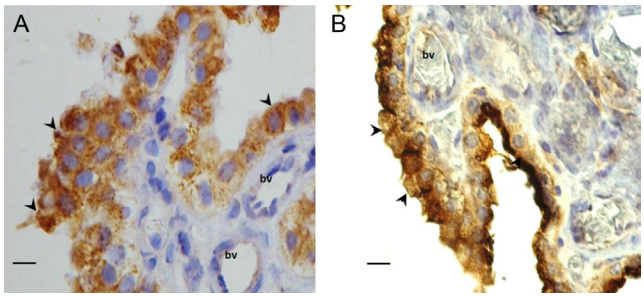

Figure 1 Immunostaining of trkA receptor (A) and β-NGF (B) in the choroid plexus of the llama encephalon. (A) Shows granular immunolabeling of trkA receptor in the cytoplasm of the epithelial cells in the choroid plexus at the roof of the third ventricle. (B) Shows immunolabeling of β-NGF in the choroid plexus. Immunlabelling of controls for trkA and β-NGF is presented in the Supplementary Figure 1 (see section on Supplementary data given at the end of the article). Scale bars = 10 µm. bv, blood vessels. Arrow heads show immunolabeling. Specific antibodies: β-NGF, sc-548 and sc-118 polyclonal, from Santa Cruz Biotechnology.

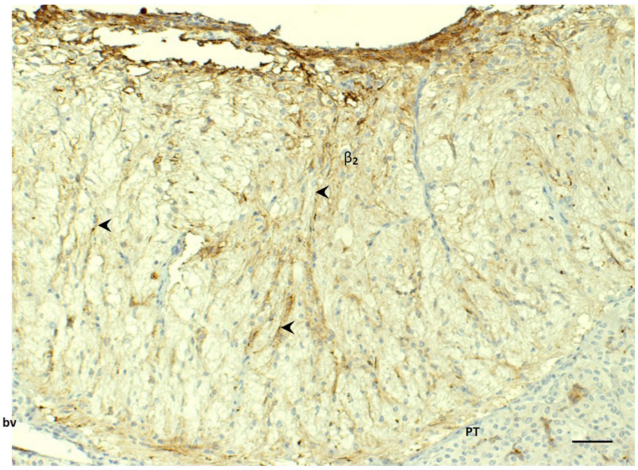

Figure 2 Immunostaining of β-NGF labeling of β_2 tanycyte processes in the median eminence. Negative control for immunostaining of β-NGF is presented in Supplementary Figure 2. Scale bars = 50 µm. bv, blood vessels; PT, pars tuberalis. Specific antibodies: β-NGF sc-548, rabbit polyclonal from Santa Cruz Biotechnology.

choroid plexus, as well as high mRNA levels for trkB, undetectable levels of trkA and trkC mRNA. Although the presence of β-NGF and its specific receptor in the choroid plexus does not necessarily indicate that β-NGF is transported from circulation to the CSF by this route, this feature may represent a unique particularity associated with induced ovulating species. Transport of other growth factors, peptides, neurotrophins and hormones through their corresponding specific receptors at the choroid plexus have been demonstrated (Redzic & Segal 2004, Rodríguez *et al.* 2010), suggesting that this route may be a potential window for NGF to access the brain via cerebrospinal fluid.

We have also identified β-NGF immunoreactivity in the tanycytes of the median eminence in llamas (Fig. 2), where GnRH nerve terminals are concentrated. Tanycytes in the median eminence contribute to the development of an anatomical link between ventricular CSF and portal blood (Rodríguez *et al.* 2005). This anatomical arrangement allows bidirectional passage of macromolecules between the portal blood and CSF (Rodríguez *et al.* 2005, 2010, Prevot *et al.* 2010) potentially providing signals to the GnRH or kisspeptin neurons. In rats, removal of tanycytes prevented GnRH release into the portal blood, the peak of luteinizing hormone and ovulation in rats (Rodríguez *et al.* 1979). The mechanism of tanycyte in leptin transport from the portal blood into the ventricular CSF has been demonstrated (Balland *et al.* 2014). In addition, a greater relative abundance of kisspeptin fibers was detected in association with GnRH fibers in the median eminence region (Fig. 3).

Therefore, we propose two potential pathways by which β-NGF from seminal plasma may signal at the level of the hypothalamic region (Fig. 4): (1) by entering into the cerebrospinal fluid of the third ventricle through the choroid plexus or via tanycyte transport from portal capillaries at the median eminence or (2) by acting directly or indirectly on GnRH neurons present at the level of median eminence without entering into the third ventricle.

In this context, another potential site of signaling of NGF for GnRH release, may be the organum vasculosum laminae terminalis (OVLT), a structure where a significant population of GnRH neurons have been found in rodents (Silverman *et al.* 1994, Herbison 2006, Herde *et al.* 2011). Although it is not yet known if neurons of the OVLT are involved in the control of the hypothalamic–hypophyseal axis, we cannot rule out that systemic β-NGF may transduce its signal at this site to initiate the ovulatory cascade in llamas. Herde *et al.* (2011) showed that GnRH neurons at the OVLT

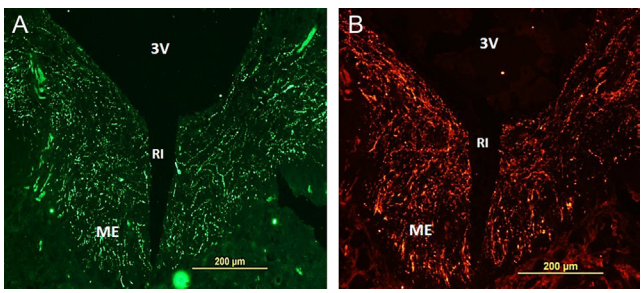

Figure 3 Immunolabeling of GnRH and kisspeptin neuronal fibers in median eminence of coronal sections of the llama hypothalamus. Low magnification of the llama median eminence where abundant fibers of GnRH (A) and kisspeptin (B) neurons are observed. Immunostaining of negative controls for GnRH and Kisspeptin are presented in Supplementary figure 3. 3V, third ventricle; ME, median eminence; RI, infundibular recess. Specific antibodies: GnRH mouse polyclonal, contributed by Dr S Ojeda; kisspeptin AB9754 are rabbit polyclonal, Millipore.

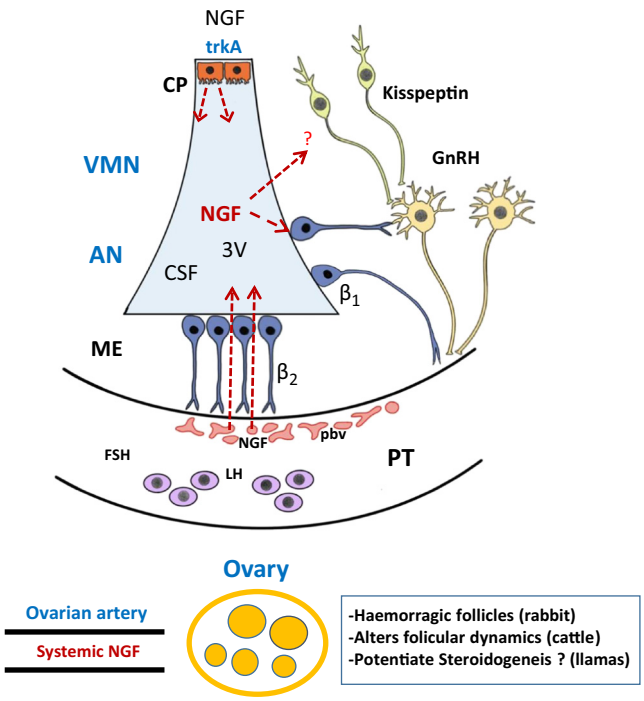

Figure 4 Proposed model for the potential access and signaling pathways of β-NGF for triggering ovulation in llamas. Seminal plasma β-NGF is absorbed from the uterus and transported through the circulation to the hypothalamic region, where it may (1) enter into the cerebrospinal fluid of the third ventricle through the choroid plexus or via tanycyte-transport from the portal capillaries at the median eminence, or (2) act directly or indirectly on GnRH neurons at the median eminence. Additionally, ovarian actions of β-NGF are described for rabbit, cattle and llama. 3V, third ventricle; AN, arcuate nucleus; β1, β2, tanycyte; CSF, cerebrospinal fluid; CP, choroid plexus; FSH, follicle-stimulating hormone; LH, luteinizing hormone; ME, median eminence; PG, pituitary gland; pbv, portal blood vessels; trk-A, β-NGF receptor; VMN, ventromedial nucleus.

have dendritic terminals that can be activated by local administration of kisspeptin, and thereby stimulate LH secretion without crossing the blood–brain barrier (Messager *et al.* 2005, Navarro *et al.* 2005). These results are particularly interesting light of a study in which exogenous administration of kisspeptin induced ovulation in the *Musk shrew* (an induced ovulator; Inoue *et al.* 2011).

According to a recent llama study (Carrasco *et al.* 2018*b*), an indirect effect of β-NGF on GnRH neurons was postulated since only a 2.5% of GnRH neurons expressed the high-affinity receptor trkA, and no immunoreactivity was detected for the low affinity receptor p75.

Potential mechanism of seminal plasma β-NGF on ovulation induction in rabbits

Similar to camelids, the presence of a factor capable of inducing ovulation in the seminal plasma of rabbits was also suggested nearly a decade ago (Niño 2010, Silva *et al.* 2011*b*). Niño (2010) documented the expression of β-NGF in rabbit semen by western blot, and when female llamas were used in an *in vivo* bioassay, intramuscular administration of rabbit seminal plasma induced and LH surge and ovulation (Silva *et al.* 2011*b*). Curiously, however, neither the administration of rabbit nor the administration of llama seminal plasma induced LH release or ovulation in rabbits (Silva *et al.* 2011*b*, Cervantes *et al.* 2015), suggesting that a species-specific response exists between these two induced ovulators.

Recently, several studies (Maranesi *et al.* 2015, Casares-Crespo *et al.* 2016, García-García *et al.* 2017, Maranesi *et al.* 2018) have confirmed the presence of NGF in rabbit semen. The molecule was found at relatively high concentrations in rabbit seminal plasma (about 1894 ± 277 pg/mL and 151.9 ± 9.25 µg/mL; Maranesi *et al.* 2015, 2018); however, concentrations were lower than those described in camelids (Tanco *et al.* 2012). Moreover, different authors have suggested that the prostate gland is the main source of this neurotrophin in rabbit semen (Maranesi *et al.* 2015, García-García *et al.* 2017), similar to reports in llamas (Bogle *et al.* 2013, 2018).

Despite the abundance of NGF in rabbit semen, the ovulatory responses after treatment with seminal plasma or NGF have not been as consistent as in camelids. A recent study (García-García *et al.* 2017) reported that only 1 of 6 female rabbits treated intramuscularly with 24 µg of murine β-NGF, ovulated, and no preovulatory elevation in LH was detected. In the same study, mechanical stimulation of the vagina increased the ovulation rate to 2/4 in β-NGF-treated females (García-García *et al.* 2017), suggesting that physical stimulation may be required to complement the effect of ovulation-inducing factor in this species. In contrast, Rebollar *et al.* (2012) reported 75% ovulation rate in rabbits does artificially inseminated with raw semen delivered by intravaginal deposition, but again, no increase in plasma LH concentration was observed. In the same study, the use of lumbar anesthesia blocked ovulation in the inseminated female rabbits. Similarly, Maranesi *et al.* (2018) reported ovulation rates of 67% (4/6) and 17% (1/6) in female rabbits inseminated with raw semen in non-anesthetized and lumbar-anesthetized females, respectively, and ovulations were preceded by an increase in plasma LH concentration during the 2 h after artificial insemination. These last studies highlight the role of efferent nervous pathways in the induction of ovulation in rabbits.

Strikingly similar ovulation rates and changes in blood concentrations of β-NGF and LH were described in a recent report in rabbits (Maranesi *et al.* 2018) as in an earlier report in llamas (Berland *et al.* 2016) after natural mating or artificial insemination with raw semen. However, in rabbits, administration of lumbar anesthesia or COX inhibitors before artificial insemination blocked

the increase in plasma LH concentration, attenuated the systemic rise of β-NGF and reduced the ovulation rate. Based on these findings, the authors proposed that in rabbits (a) semen-derived NGF stimulates *de novo* synthesis of NGF in the uterine wall, (b) both seminal NGF and uterine NGF are absorbed into the bloodstream and act directly on the ovary rather than on the pituitary or hypothalamus and (c) semen-derived and locally synthesized NGF stimulate uterine/cervix sensory neurons which trigger GnRH neurons in the hypothalamus.

The local effect of NGF in the rabbit ovary was based on immunodetection of NGF and its high- (trkA) and low- (p75) affinity receptors in the oocyte, and the granulosa and theca cells of ovarian follicles at different stages of development (Maranesi *et al.* 2018). Interestingly, in rabbits, i.m. treatment with homologous seminal plasma (Silva *et al.* 2011*b*) or with murine β-NGF (García-García *et al.* 2017) resulted in a significant increase in the number of large hemorrhagic anovulatory follicles. Similar hemorrhagic follicles have been observed after superovulation of does with eCG (García-Ximénez & Vicente 1990, Mehaisen *et al.* 2005) or treatment with high doses of hCG (Bomsel-Helmreich *et al.* 1989), suggesting a direct local effect of these molecules with LH-like action on LH receptors at the follicular wall. Whether the formation of large hemorrhagic follicles observed after treatment with seminal plasma (Silva *et al.* 2011*b*) or NGF (García-García *et al.* 2017) is due to a direct action of NGF on its own receptors or by means of an indirect action through LH receptors at the follicular wall remains to be investigated.

Taken together, results of recent studies of the effects of seminal plasma lead us to re-evaluate the mechanism of reflex ovulation stated many years ago. For instance, β-NGF in camelid semen is the main driver of ovulation, and mechanical stimulation of the female genitalia appears to have little or no role in this species. Conversely, although the presence of β-NGF may be involved in the ovulatory mechanism in rabbits, it appears that physical stimulation is required to induce a high rate of ovulation.

Potential mechanism of seminal plasma β-NGF in spontaneous ovulators

Several studies confirmed the presence of β-NGF in seminal plasma of spontaneous ovulators (e.g., bovine, equine and porcine; Ratto *et al.* 2012, Bogle *et al.* 2018). It was also documented that the administration of llama seminal plasma or purified β-NGF influenced ovarian function in spontaneous ovulators. For instance, llama seminal plasma induced ovulation in a pre-pubertal mouse model (Bogle *et al.* 2011), and systemic administration of purified β-NGF from llama seminal plasma altered ovarian follicular growth and had a luteotrophic effect in cattle (Tanco *et al.* 2012). In the last study, intramuscular administration of 4–5 mg/animal of purified β-NGF induced neither a preovulatory LH surge nor ovulation in prepubertal heifers, but increased FSH secretion and hastened the emergence of the next follicular wave. Also, the same authors found that llama β-NGF treatment induced a greater growth of the CL resulting in higher plasma progesterone concentration compared to GnRH-treated post-pubertal heifers. The high-affinity NGF receptor, trkA, has also been detected in bovine granulosa cells of preovulatory follicles and in luteal cells of bovine CL (Carrasco *et al.* 2016), and the preovulatory LH surge seems to upregulate the expression of trkA receptor in these cells. In a further study, the administration of bovine seminal plasma in heifers pre-treated with LH induced a more synchronous interval from treatment to ovulation than in non-pretreated heifers, and plasma progesterone concentrations tended to be higher than those of the control group, reinforcing the notion of a potential luteotrophic effect of NGF in cattle (Tribulo *et al.* 2015). In a recent bovine study (Stewart *et al.* 2018*a*), administration of purified β-NGF from bovine seminal plasma intramuscularly at the time of artificial insemination increased pregnancy rate up to 16%. Treated females displayed a greater plasma progesterone concentration, improving conceptus development and ultimately conception rate. In addition, concentration of NGF in bovine seminal plasma has been correlated with fertility in dairy and beef bulls (Stewart *et al.* 2018*b*).

Conclusions

Among induced ovulators, there appears to be a differential species-specific mechanism that, contrary to the established notion that mechanical stimulation from copulation is the pivotal stimulus to trigger ovulation, relies on the presence of seminal factors that chemically induce endocrine changes that ultimately induce the ovulatory response. The discovery of β-NGF in the seminal plasma of camelids, and recently in rabbits, and its central role as the main chemical compound inducing ovulation in these species without the need of copulation, allow us to challenge the notion that reflex ovulation depends merely upon physical stimulation of the female genital tract. Surprisingly, β-NGF appears to act on different target tissues in these two species, suggesting different evolutionary adaptations to arrive as present-day induced ovulators. Evidence documented in cattle suggests that β-NGF may have a role as a luteotrophic factor in spontaneous ovulators.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/REP-18-0305.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Adams GP & Ratto MH 2013 Ovulation-inducing factor in seminal plasma: a review. *Animal Reproduction Science* **136** 148–156. (https://doi.org/10.1016/j.anireprosci.2012.10.004)

Adams GP, Ratto MH, Huanca W & Singh J 2005 Ovulation-inducing factor in the seminal plasma of alpacas and llamas. *Biology of Reproduction* **73** 452–457. (https://doi.org/10.1095/biolreprod.105.040097)

Adams GP, Ratto MH, Silva ME & Carrasco RA 2016 Ovulation-inducing factor (OIF/NGF) in seminal plasma: a review and update. *Reproduction in Domestic Animals* **51** 4–17. (https://doi.org/10.1111/rda.12795)

Baker BL, Dermody WC & Reel JR 1975 Distribution of gonadotropin releasing hormone in the rat brain as observed with immunocytochemistry. *Endocrinology* **97** 125–135. (https://doi.org/10.1210/endo-97-1-125)

Bakker J & Baum MJ 2000 Neuroendocrine regulation of GnRH release in induced ovulators. *Frontiers in Neuroendocrinology* **21** 220–262. (https://doi.org/10.1006/frne.2000.0198)

Balland E, Dam J, Langlet F, Caron E, Steculorum S, Messina A, Rasika S, Falluel-Morel A, Anouar Y, Dehouck B *et al*. 2014 Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain. *Cell Metabolism* **19** 293–301. (https://doi.org/10.1016/j.cmet.2013.12.015)

Barry L & Dubois MP 1976 Immunoreactive LRF neurosecretory pathways in mammals. *Acta Anatomica* **94** 497–503. (https://doi.org/10.1159/000144582)

Baum MJ & Schretlen PJM 1978 Oestrogenic induction of sexual behavior in ovartectomised ferrets housed under short or long photoperiods. *Journal of Endocrinology* **78** 295–296. (https://doi.org/10.1677/joe.0.0780295)

Baum MJ, Carroll RS, Cherry JA & Tobe SA 1990 Steroidal control of behavioral, neuroendocrine and brain sexual differentiation: studies in a carnivore, the ferret. *Journal of Neuroendocrinology* **2** 401–418. (https://doi.org/10.1111/j.1365-2826.1990.tb00425.x)

Berland MA 2017 *Acción central del factor inductor de ovulación (OIF/NGF) presente en el plasma seminal de llamas*. Thesis, Doctorate on Veterinary Sciences, Faculty of Veterinary Sciences, Universidad Austral de Chile, Valdivia, Chile.

Berland MA, Ulloa-Leal C, Barría M, Wright H, Dissen GA, Silva ME, Ojeda SR & Ratto MH 2016 Seminal plasma induces ovulation in llamas in the absence of a copulatory stimulus: role of nerve growth factor as an ovulation-inducing factor. *Endocrinology* **157** 3224–3232. (https://doi.org/10.1210/en.2016-1310)

Bogle OA, Ratto MH & Adams GP 2011 Evidence for the conservation of biological activity of ovulation-inducing factor (OIF) in the seminal plasma. *Reproduction* **142** 277–283. (https://doi.org/10.1530/REP-11-0042)

Bogle OA, Singh J & Adams GP 2013 Distribution of ovulation-inducing factor in male reproductive tissues of llamas. *Reproduction, Fertility and Development* **25** 272 (abstract). (https://doi.org/10.1071/RDv25n1Ab248)

Bogle OA, Carrasco RA, Ratto MH, Singh J & Adams GP 2018 Source and localization of ovulation-inducing factor/nerve growth factor in male reproductive tissues among mammalian species. *Biology of Reproduction* **99** 1194–1204: doi:10.1093/biolre/ioy149.

Bomsel-Helmreich O, Huyen LVN & Durand-Gasselin I 1989 Effects of varying doses of HCG on the evolution of preovulatory rabbit follicles and oocytes. *Human Reproduction* **4** 636–642. (https://doi.org/10.1093/oxfordjournals.humrep.a136957)

Bravo PW, Fowler ME, Stabenfeldt GH & Lasley BL 1990*a* Ovarian follicular dynamics in the llama. *Biology of Reproduction* **43** 579–585. (https://doi.org/10.1095/biolreprod43.4.579)

Bravo PW, Fowler ME, Stabenfeldt GH & Lasley B 1990*b* Endocrine response in the llama to copulation. *Theriogenology* **33** 891–899. (https://doi.org/10.1016/0093-691X(90)90824-D)

Bravo PW, Stabenfeldt GH, Lasley BL & Fowler ME 1991 The effect of ovarian follicle size on pituitary and ovarian responses to copulation in domesticated south american camelids. *Biology of Reproduction* **45** 553–559. (https://doi.org/10.1095/biolreprod45.4.553)

Bravo PW, Stabenfeldt GH, Fowler ME & Lasley BL 1992 Pituitary response to repeated copulation and/or gonadotropin-releasing hormone administration in llamas and alpacas. *Biology of Reproduction* **47** 884–888. (https://doi.org/10.1095/biolreprod47.5.884)

Bromfield JJ 2016 A role for seminal plasma in modulating pregnancy outcomes in domestic species. *Reproduction* **152** R223–R232. (https://doi.org/10.1530/REP-16-0313)

Carrasco R, Singh J & Adams GP 2016 The dynamics of trkA expression in the bovine ovary are associated with a luteotrophic effect of ovulation-inducing factor/nerve growth factor (OIF/NGF). *Reproductive Biology and Endocrinology* **14** 47–57. (https://doi.org/10.1186/s12958-016-0182-9)

Carrasco RA, Singh J & Adams GP 2018*a* The relationship between gonadotrophin releasing hormone and ovulation inducing factor/nerve growth factor receptors in the hypothalamus of the llama. *Reproductive Biology and Endocrinology* **16** 83–92. (https://doi.org/10.1186/s12958-018-0402-6)

Carrasco RA, Singh J & Adams GP 2018*b* Distribution and morphology of gonadotrophin releasing hormone neurons in the hypothalamus of an inducing ovulator – the llama (*Lama glama*). *General and Comparative Endocrinology* **263** 43–50. (https://doi.org/10.1016/j.ygcen.2018.04.011)

Carroll RS, Erskine MS, Doherty PC, Lundell LA & Baum MJ 1985 Coital stimuli controlling luteinizing hormone secretion and ovulation in the female ferret. *Biology of Reproduction* **32** 925–933. (https://doi.org/10.1095/biolreprod32.4.925)

Casares-Crespo L, Talaván AM & Viudes-de-Castro MP 2016 Can the genetic origin affect rabbit seminal plasma protein profile along the year? *Reproduction in Domestic Animals* **51** 294–300. (https://doi.org/10.1111/rda.12680)

Cervantes MP, Palomino JM & Adams GP 2015 *In vivo* imaging in the rabbit as a model for the study of ovulation-inducing factors. *Laboratory Animals* **49** 1–9. (https://doi.org/10.1177/0023677214547406)

Chen BX, Yuen ZX & Pan GW 1985 Semen induced ovulation in the Bactrian camel (*Camelus bactrianus*). *Journal of Reproduction and Fertility* **73** 335–339. (https://doi.org/10.1530/jrf.0.0740335)

Clarkson J, Han S, Liu X, Lee K & Herbison AE 2010 Neurobiological mechanisms underlying kisspeptin activation of gonadotropin-releasing hormone (GnRH) neurons at puberty. *Molecular and Cellular Endocrinology* **324** 45–50. (https://doi.org/10.1016/j.mce.2010.01.026)

Concannon P, Hodgson B & Lein D 1980 Reflex LH release in estrous cats following single and multiple copulations. *Biology of Reproduction* **23** 111–117. (https://doi.org/10.1095/biolreprod23.1.111)

England BG, Foot WC, Cardozo AG, Matthews DH & Riera S 1971 Oestrus and mating behaviour in the llama (*Lama glama*). *Animal Behaviour* **19** 722–726. (https://doi.org/10.1016/S0003-3472(71)80176-8)

Fernandez-Baca S, Madden DHL & Novoa C 1970 Effect of different mating stimuli on induction of ovulation in the alpaca. *Reproduction* **22** 261–267. (https://doi.org/10.1530/jrf.0.0220261)

Fink G 1988 Gonadotropin secretion and its control. In *The Physiology of Reproduction*, 3rd ed, pp 1349–1377. Eds E Knobil & JD Neill. New York: Raven Press.

García-García RM, Masdeu M, Sánchez-Rodriguez A, Millán P, Arias-Alvarez M, Sakr OG, Bautista JM, Castellini C, Lorenzo PL & García-Rebollar P 2017 β-Nerve growth factor identification in male rabbit genital tract and seminal plasma and its role in ovulation induction in rabbit does. *Italian Journal of Animal Science* **17** 442–453.

García-Ximénez F & Vicente JS 1990 Effect of PMSG treatment to mating interval in the superovulatory response of primiparous rabbits. *Journal of Applied Rabbit Research* **13** 71–73.

Hawkins BT & Davis TP 2005 The blood-brain barrier/neurovascular unit in health and disease. *Pharmacological Reviews* **57** 173–185. (https://doi.org/10.1124/pr.57.2.4)

Heape W 1905 Ovulation and degeneration of ova in the rabbit. *Proceedings of the Royal Society B* **76** 260–268. (https://doi.org/10.1098/rspb.1905.0019)

Herbison AE 2006 Physiology of the GnRH neuronal network. In *The Physiology of Reproduction*, 3rd ed, pp 1415–1482. Ed JD Neill. San Diego: Elsevier Academic Press.

Herbison AE 2015 Physiology of the adult gonadotropin-releasing hormone neuronal network. In *The Physiology of Reproduction*, 4th ed, pp 399–467. Eds TM Plant & AJ Zeleznik. San Diego: Elsevier Academic Press.

Herde MK, Geist K, Campbell RE & Herbison AE 2011 Gonadotropin-releasing hormone neurons extend complex highly branches dendritic trees outside the blood-brain barrier. *Endocrinology* **152** 3832–3841. (https://doi.org/10.1210/en.2011-1228)

Inoue N, Sasagawa K, Ikai K, Sasaki Y, Tomikawa J, Oishi S, Fujii N, Uenoyama Y, Ohmori Y, Yamamoto N *et al*. 2011 Kisspeptin neurons mediate reflex ovulation in the musk shrew (*Suncus murinus*). *PNAS* **108** 17527–17532. (https://doi.org/10.1073/pnas.1113035108)

Izumi S, Makino T & Iizuka R 1985 Immunoreactive luteinizing hormone-releasing hormone in the seminal plasma and human semen parameters. *Fertility and Sterility* **43** 617–620. (https://doi.org/10.1016/S0015-0282(16)48506-7)

Jaffe RB & Keys WR Jr 1974 Estradiol augmentation of pituitary responsiveness to gonadotropin-releasing hormone in women. *Journal of Clinical Endocrinology and Metabolism* **39** 850–855. (https://doi.org/10.1210/jcem-39-5-850)

Jochle W 1975 Current research in coitus-induced ovulation: a review. *Journal of Reproduction and Fertility Supplement* **22** 165–207.

Karsch FJ 1987 Central actions of ovarian steroids in the feedback regulation of pulsatile secretion of luteinizing hormone. *Annual Review of Physiology* **49** 365–382. (https://doi.org/10.1146/annurev.ph.49.030187.002053)

Karsch FJ, Bowen JM, Caraty A, Evans NP & Moenter SM 1997 Gonadotropin-releasing hormone requirements for ovulation. *Biology of Reproduction* **56** 303–309. (https://doi.org/10.1095/biolreprod56.2.303)

Kauffman AS & Rissman EF 2006 Neuroendocrine control of mating-induced ovulation. In *The Physiology of Reproduction*, 3rd ed, pp 2283–2326. Ed JD Neill. San Diego: Elsevier Academic Press.

Kelly CR, Socha TE & Zimmerman DR 1988 Characterization of gonadotrophic and ovarian steroids hormones during the periovulatory period in high ovulating select and control line gilts. *Journal of Animal Science* **66** 1462–1474. (https://doi.org/10.2527/jas1988.6661462x)

Kershaw-Young CM, Druart X, Vaughan J & Maxwell WMC 2012 β-Nerve growth factor is a major component of alpaca seminal plasma and induces ovulation in female alpacas. *Reproduction, Fertility and Development* **24** 1093–1097. (https://doi.org/10.1071/RD12039)

Knobil E 1980 The neuroendocrine control of the menstrual cycle. *Recent Progress in Hormone Research* **36** 53–88.

Kumar S, Kumar-Sharma V, Singh S, Hariprasad GR, Mal G, Srinivasan A & Yadav S 2013 Proteomic identification of camel seminal plasma: purification of β-nerve growth factor. *Animal Reproduction Science* **136** 284–295.

Maranesi M, Zerani M, Leonardi L, Pistilli A, Arruda-Alencar J, Stabile AM, Rende M, Castellini C, Petrucci L, Parillo F *et al*. 2015 Gene expression and localization of NGF and its cognate receptors NTRK1 and NGFR in the sex organs of male rabbits. *Reproduction in Domestic Animals* **50** 918–925. (https://doi.org/10.1111/rda.12609)

Maranesi M, Petrucci L, Leonardi L, Piro F, García-Rebollar PG, Millán P, Cocci P, Vullo C, Parrillo F, Moura A *et al*. 2018 New insights on a NGF-mediated pathway to induce ovulation in rabbits (*Oryctolagus cuniculus*). *Biology of Reproduction* **98** 634–643. (https://doi.org/10.1093/biolre/ioy041)

Marie M & Anouassi A 1986 Mating induced luteinizing hormone surge and ovulation in the female camel (*Camelus dromedarius*). *Biology of Reproduction* **35** 792–798. (https://doi.org/10.1095/biolreprod35.4.792)

McEwen BS & Parsons B 1982 Gonadal steroid action on the brain: neurochemistry and neuropharmacology. *Annual Review of Pharmacology and Toxicology* **22** 555–598. (https://doi.org/10.1146/annurev.pa.22.040182.003011)

Mehaisen GMK, Vicente JS, Lavara R & Viuedes-de-Castro MP 2005 Effect of eCG dose and ovulation induction treatments on embryo recovery and in vitro development post-vitrification in two selected lines of rabbit does. *Animal Reproduction Science* **90** 175–184. (https://doi.org/10.1016/j.anireprosci.2005.01.015)

Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, Lomet D, Carlton MBL *et al*. 2005 Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *PNAS* **102** 1761–1766. (https://doi.org/10.1073/pnas.0409330102)

Navarro VM, Castellano JM, Fernández-Fernández R, Tovar S, Roa J, Mayen A, Nogueiras R, Vazquez MJ, Barreiro ML, Magni P *et al*. 2005 Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. *Endocrinology* **146** 156–163. (https://doi.org/10.1210/en.2004-0836)

Niño A 2010 *Determinación de la presencia de un factor inductor de ovulación en el plasma seminal de conejo*. Thesis, Master in Clinical Sciences, Faculty of Veterinary Sciences, Universidad Austral de Chile, Valdivia, Chile.

Ojeda SR, Lomniczi A & Sandau US 2008 Glial–gonadotrophin hormone (GnRH) neurone interactions in the median eminence and the control of GnRH secretion. *Journal of Neuroendocrinology* **20** 732–742. (https://doi.org/10.1111/j.1365-2826.2008.01712.x)

Ojeda SR, Lomniczi A & Sandau US 2010 Contribution of glial–neuronal interactions to the neuroendocrine control of female puberty. *European Journal of Neuroscience* **32** 2003–2010. (https://doi.org/10.1111/j.1460-9568.2010.07515.x)

Paolicchi F, Urquieta B, Del Valle L & Bustos-Obregon E 1999 Biological activity of the seminal plasma of alpacas: stimulus for the production of LH by pituitary cells. *Animal Reproduction Science* **54** 203–210. (https://doi.org/10.1016/S0378-4320(98)00150-X)

Pau KYF, Orstead KM, Hess DL & Spies HG 1986 Feedback effects of ovarian steroids on the hypothalamic-hypophyseal axis in the rabbit. *Biology of Reproduction* **35** 1009–1023. (https://doi.org/10.1095/biolreprod35.4.1009)

Prevot V, Hanchate NK, Bellefontaine N, Sharif A, Parkash J, Estrella C, Allet C, de Seranno S, Campagne C, d'Anglemont de Tassigny X *et al*. 2010 Function-related structural plasticity of the GnRH system. *Frontiers in Neuroendocrinology* **31** 241–258. (https://doi.org/10.1016/j.yfrne.2010.05.003)

Ramirez VD & Soufi W 1994 The neuroendocrine control of the rabbit ovarian cycle. In *The Physiology of Reproduction*, 2nd ed, pp 585–611. Eds E Knobil & JD Neill. New York: Raven Press Ltd.

Ramirez VD, Ramirez AD, Slamet W & Nduka E 1986 Functional characteristics of the luteinizing hormone-releasing hormone pulse generator in conscious unrestrained female rabbits: activation by norepinephrine. *Endocrinology* **118** 2331–2339. (https://doi.org/10.1210/endo-118-6-2331)

Ratto MH, Huanca W, Singh J & Adams GP 2005 Local versus systemic effect of ovulation-inducing factor in seminal plasma of alpacas. *Reproductive Biology and Endocrinology* **3** 29. (https://doi.org/10.1186/1477-7827-3-29)

Ratto MH, Huanca W, Singh J & Adams GP 2006 Comparison of the effect of ovulation-inducing factor (OIF) in seminal plasma of llamas, alpacas,

and bulls. *Theriogenology* **66** 1102–1106. (https://doi.org/10.1016/j.theriogenology.2006.02.050)

Ratto MH, Delbaere LTJ, Leduc YA, Pierson RA & Adams GP 2011 Biochemical isolation and purification of ovulation-inducing factor (OIF) in seminal plasma of llamas. *Reproductive Biology and Endocrinology* **9** 24. (https://doi.org/10.1186/1477-7827-9-24)

Ratto MH, Leduc YA, Valderrama XP, van Straaten KE, Delbaere LTJ, Pierson RA & Adams GP 2012 The nerve of ovulation inducing factor. *PNAS* **109** 15042–15047. (https://doi.org/10.1073/pnas.1206273109)

Rebollar PG, Dal Bosco A, Millán P, Cardinali R, Brecchia G, Sylla L, Lorenzo PL & Castellini C 2012 Ovulating induction methods in rabbit does: the pituitary and ovarian responses. *Theriogenology* **77** 292–298. (https://doi.org/10.1016/j.theriogenology.2011.07.041)

Redzic ZB Segal MB 2004 The structure of the choroid plexus and the physiology of the choroid plexus epithelium. *Advanced Drug Delivery Reviews* **56** 1695–1716.

Rodríguez EM, González CB & Delannoy L 1979 Cellular organization of the lateral and postinfundibular regions of the median eminence in the rat. *Cell and Tissue Research* **201** 377–408.

Rodríguez EM, Blázquez JL, Pastor FE, Peláez B, Peña P, Peruzzo B & Amat P 2005 Hypothalamic tanycytes: a key component of brain–endocrine interaction. *International Review of Cytology* **247** 89–164. (https://doi.org/10.1016/S0074-7696(05)47003-5)

Rodríguez EM, Blázquez JL & Guerra M 2010 The design of barriers in the hypothalamus allows the median eminence and the arcuate nucleus to enjoy private milieus: the former opens to the portal blood and the latter to the cerebrospinal fluid. *Peptides* **31** 757–776. (https://doi.org/10.1016/j.peptides.2010.01.003)

Sawyer CH & Markee JE 1959 Estrogen facilitation of release of pituitary ovulating hormone in the rabbit in response to vaginal stimulation. *Endocrinology* **65** 614–621. (https://doi.org/10.1210/endo-65-4-614)

Silva M, Smulders JP, Guerra M, Valderrama XP, Letelier C, Adams GP & Ratto MH 2011*a* Cetrorelix suppresses the preovulatory LH surge and ovulation induced by ovulation-inducing factor (OIF) present in llama seminal plasma. *Reproductive Biology and Endocrinology* **9** 74.

Silva M, Niño A, Guerra M, Letelier C, Valderrama XP, Adams GP & Ratto MH 2011*b* Is an ovulation-inducing factor (OIF) present in the seminal plasma of rabbits? *Animal Reproduction Science* **127** 213–221. (https://doi.org/10.1016/j.anireprosci.2011.08.004)

Silva ME, Recabarren MP, Recabarrren SE, Adams GP & Ratto MH 2012 Ovarian estradiol modulates the stimulatory effect of ovulation-inducing factor (OIF) on pituitary LH secretion in llamas. *Theriogenology* **77** 1873–1882. (https://doi.org/10.1016/j.theriogenology.2012.01.004)

Silva M, Ulloa-Leal C, Norambuena C, Fernández A, Adams GP & Ratto MH 2014 Ovulation-inducing factor (OIF/NGF) from seminal plasma origin enhances corpus luteum function in llamas regardless the preovulatory follicle diameter. *Animal Reproduction Science* **148** 221–227. (https://doi.org/10.1016/j.anireprosci.2014.05.012)

Silverman A, Livne I & Witkin JW 1994 The gonadotrophin-releasing hormone (GnRH), neuronal systems: immunocytochemistry and in situ hybriditization. In *The Physiology of Reproduction*, 2nd ed, pp 1683–1706. Eds E Knobil & JD Neill. New York: Raven.

Sokol RZ, Peterson M, Heber D & Swerdlof RS 1985 Identification and partial characterization of gonadotropin-releasing hormone-like factors in human seminal plasma. *Biology of Reproduction* **33** 370–374. (https://doi.org/10.1095/biolreprod33.2.370)

Spuch C & Navarro C 2010 Transport mechanisms at the blood-cerebrospinal-fluid barrier: role of megalin (LRP2). *Recent Patents on Endocrine, Metabolic and Immune Drug Discovery* **4** 190–205. (https://doi.org/10.2174/1872214811004030190)

Stewart JL, Canisso IF, Ellerbrock RE, Mercadante VRG & Lima FS 2018*a* Nerve growth factor β production in the bull: gene expression, immunolocalization, seminal plasma constitution, and association with sire conception rates. *Animal Reproduction Science* **197** 335–342. (https://doi.org/10.1016/j.anireprosci.2018.09.006)

Stewart JL, Mercadante VRG, Diaz NW, Canisso IF, Yau P, Imai B & Lima FS 2018*b* Nerve growth factor-beta, purified from bull seminal plasma, enhances corpus luteum formation and conceptus development in *Bos taurus* cows. *Theriogenology* **106** 30–38. (https://doi.org/10.1016/j.theriogenology.2017.10.007)

Sundquist C, Ellis LC & Bartke A 1988 Reproductive endocrinology of the mink (*Mustela vison*). *Endocrine Reviews* **9** 247–266. (https://doi.org/10.1210/edrv-9-2-247)

Tanco VM, Van Steelandt MD, Ratto MH & Adams GP 2012 Effect of purified llama ovulation-inducing factor (OIF) on ovarian function in cattle. *Theriogenology* **78** 1030–1039. (https://doi.org/10.1016/j.theriogenology.2012.03.036)

Timmusk T, Mudò G, Metsis M & Belluardo N 1995 Expression of mRNAs for neurotrophins and their receptors in the rat choroid plexus and dura mater. *NeuroReport* **6** 1997–2000. (https://doi.org/10.1097/00001756-199510010-00011)

Tribulo P, Bogle O, Mapletoft RJ & Adams GP 2015 Bioactivity of ovulation inducing factor (or nerve growth factor) in bovine seminal plasma and its effects on ovarian function in cattle. *Theriogenology* **83** 1394–1401. (https://doi.org/10.1016/j.theriogenology.2014.12.014)

Turzillo AM & Nett TM 1999 Regulations of the GnRH receptor gene expression in sheep and cattle. *Journal of Reproduction and Fertility Supplement* **54** 75–86.

Ulloa-Leal C, Bogle OA, Adams GP & Ratto MH 2014 Luteotrophic effect of ovulation-inducing factor/nerve growth factor present in the seminal plasma of llamas. *Theriogenology* **81** 1101–1107.e1. (https://doi.org/10.1016/j.theriogenology.2014.01.038)

Waberski D, Sudhoff H, Hahn T, Jungblut PW, Kallweit E, Calvete JJ, Ensslin M, Hoppen HO, Wintergalen N & Weitze KF 1995 Advanced ovulation in gilts by the intrauterine application of a low-molecular mass pronase-sensitive fraction of boar seminal plasma. *Journal of Reproduction and Fertility* **105** 247–252. (https://doi.org/10.1530/jrf.0.1050247)

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