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Review: Unveiling the effect of beta-nerve growth factor on the reproductive function in llamas and cows

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ABSTRACT

The actions of the beta-nerve growth factor (**β-NGF**) on the neuroendocrine and reproductive system have challenged classical views on the control of reproductive function. After endometrial absorption, β-NGF triggers ovulation and promotes the development of functional corpora lutea in camelids. In this article, we review evidence showing that, in camelids, β-NGF exerts its actions by acting in both the hypothalamus and the ovary. In the hypothalamus, β-NGF may induce gonadotropin-releasing hormone (**GnRH**) release by interacting with neurons or glial cells expressing receptors for β-NGF. The LH surge occurs under the influence of ovarian estradiol and requires the release of GnRH into the portal vessels to reach the pituitary gland. In the ovary, β-NGF may be promoting the differentiation of follicular to luteal cells by modifying the steroidogenic profile of ovarian follicular cells in both camelids and ruminants. Although the mechanisms for these actions are largely undetermined, we aim to offer an update on the current understanding of the effects of β-NGF controlling reproductive function in camelids and ruminants.

Implications

The neurotrophin beta-nerve growth factor present in the semen of llamas and alpacas is the molecule responsible for ovulation induction in these species, originally classified as reflex ovulators. However, this factor is also expressed in the semen of spontaneous ovulatory species, cattle, horse, and pigs, but its potential role in spontaneous ovulators remains unknown. The ovulation induction in llamas is preceded by an LH surge after systemic administration of this factor resulting in the development of a corpus luteum that produces more progesterone (luteotrophic effect) than that of gonadotrophin-releasing hormone-treated females. However, recent evidence indicates that beta-nerve growth factor also exerts its actions directly on the ovarian tissue of llamas and cows.

Introduction

A decade ago, two independent studies conducted by Ratto et al. (2012) and Kershaw-Young et al. (2012) reported for the first time that the molecule responsible for inducing ovulation (previously known as ovulation-inducing factor) in llamas and alpacas is the neurotrophin beta-nerve growth factor (**β-NGF**), which is present in the seminal plasma of these species. Since then, many studies have investigated potential mechanisms of action on the hypothalamus-hypophysis-gonadal axis (Silva et al., 2011; Carrasco et al., 2018; 2021a; Berland et al., 2021) and also local effects on the ovary (Valderrama et al., 2019; 2020).

The β-NGF triggers a robust ovulatory response in llamas after parenteral administration (Ratto et al., 2010; 2012) but also exhibits a luteotrophic effect in llamas (Adams et al., 2005; Tanco et al., 2011; Ulloa-Leal et al., 2014) and cattle (Tanco et al., 2012; Tribulo et al., 2015; Stewart et al., 2018b). This protein is produced in the prostate gland of camelids (Bogle et al., 2018) and released during ejaculation. The semen of camelids is remarkably viscous and contains abundant quantities of β-NGF (~30% of total protein content). After mating, circulating concentrations of β-NGF and

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LH increase within 15 and 60 minutes (Berland et al., 2016), respectively, and llamas attain ovulation 26 hours after seminal plasma administration. Importantly, increasing doses of β-NGF are associated with larger LH surge responses, indicating a relationship (Tanco et al., 2012).

Although β-NGF is present in the semen of most species studied to date, its effects on LH release have been well characterized only in the female camelid. Recent studies have examined the actions of β-NGF on the ovary of both camelids and farm animal species. In this review, we aim to provide an overview of the current knowledge of the reproductive role of β-NGF in domestic species. We review potential routes for β-NGF to enter the brain and the mechanism of action of β-NGF in camelids. The direct ovarian effects of β-NGF in camelid and bovine species are also discussed.

The beta-nerve growth factor acting on the brain

An initial study conducted by Adams et al. (2005) reported that the administration of seminal plasma led to a rapid increase in circulating LH concentrations with a consequent corpus luteum that exhibited steroidogenic activity. Research conducted to determine the pathway of this β-NGF effect shows that the application of llama seminal plasma to cultured camelid and bovine pituitary cells leads to an increase in the LH content in the media (Bogle et al., 2012), similarly as in a previous report using alpaca semen and rat pituitary cells (Paolicchi et al., 1999), and thus supporting the hypothesis that the ovulation-inducing factor (i.e. β-NGF) acts on the pituitary. However, this conception was challenged by a study designed to elucidate the role of the hypothalamus in semen-induced ovulation (Silva et al., 2011): llamas pretreated with cetrorelix –a gonadotropin-releasing hormone (**GnRH**) receptor antagonist– failed to show LH release and ovulation in response to β-NGF administration, indicating that β-NGF actions involve GnRH signaling at the pituitary. The idea that β-NGF may act on the hypothalamus proposes a challenging hypothesis, as it requires the evaluation of whether this factor penetrates the blood–brain barrier and, if so, which cell populations are under its influence to elicit GnRH release for LH-dependent ovulation.

Does beta-nerve growth factor penetrate the brain?

The blood–brain barrier is characterized by the presence of tight junctions between non-fenestrated capillaries that are surrounded by projections of astrocytes (Ballabh et al., 2004). This arrangement provides a tight seal between the brain milieu (parenchyma/cerebrospinal fluid) and circulating compounds in the bloodstream, thus maintaining compartmentalization between peripheral and central compartments. However, the brain contains specialized structures called circumventricular organs that are located in the midline of the ventricular system and where dendrites or axon terminals of neurons project outside of the blood–brain barrier to sense and release compounds present in the blood (Rodríguez et al., 2010).

Studies in mice show poor brain uptake after the administration of radioactive NGF, (Angeletti et al., 1972; Pradier et al., 1994); however, in a study using mice (Pan et al., 1998), NGF brain uptake was reported to be receptor-mediated (Pan et al., 1998). Moreover, an autoradiographic study (Loy et al., 1994) in rats to evaluate the permeability of the brain to NGF revealed the existence of regional differences; circumventricular organs such as the organum vasculosum of the laminae terminalis, the subfornical organ, and the area postrema showed larger uptake of NGF than the iodinated bovine serum albumin-treated group. Thus, it is possible that circumventricular organs may act as a gateway for β-NGF acting on the llama GnRH systems (Fig. 1).

The hypothalamus as a site of action for the beta-nerve growth factor-induced ovulation in llamas

GnRH-producing neurons of the hypothalamus are known to control reproduction by finely tuning the pattern of GnRH release into the portal vessels (Karsch et al., 1987), which in turn modulate the release of LH and FSH. Thus, it has been thought that β-NGF acts directly at hypothalamic GnRH neurons llamas via high- or the low-affinity β-NGF receptors (Tyrosine kinase A [**TrkA**] or p75 neurotrophin receptor [**$P75^{NTR}$**], respectively). Nevertheless, a low proportion (2.5%) of llama GnRH neurons express the TrkA receptor, while $P75^{NTR}$ was not detected (Carrasco et al., 2018). Interestingly, TrkA and $P75^{NTR}$ are widely expressed in the llama hypothalamus, including the medial septum, preoptic area, and periventricular hypothalamus (Carrasco et al., 2018; 2021a), and $P75^{NTR}$ immunoreactivity has been detected in ependymal cells and tanycytes in the ventral portion of the third ventricle. Albeit the neurochemical identity of these cells was unknown, these findings propose the existence of β-NGF-responsive neurons and non-neuronal cells in the hypothalamus of llamas.

Sexual steroids are known to modulate the activity and response of GnRH cells and, consequently, LH release. Administration of β-NGF to llamas with different circulating concentrations of progesterone results in similar LH responses with no differences in the percentage of GnRH neurons expressing Fos protein (Carrasco et al., 2021b), a widely used marker for neuronal activation. Conversely, in the case of estradiol, administration of β-NGF to ovariectomized llamas results in a reduction of the LH release to one-third of that of intact llamas (Silva et al., 2011), and pretreatment with 17-beta estradiol or estradiol benzoate restored the response to β-NGF (Silva et al., 2011), indicating that estradiol also plays a role by modulating the β-NGF-induced LH release in induced ovulatory species and so sharing features with the modulation of LH release occurring in spontaneous ovulators.

Kisspeptin neurons located in the arcuate nucleus and the preoptic area project their axons in proximity to GnRH neurons (Clarkson and Herbison, 2006), which express the kisspeptin receptor GPR54 to elicit GnRH release. Research (Carrasco et al., 2020) conducted on camelids shows that intravenous administration of kisspeptin increase circulating LH concentrations in llamas, and this response can be blocked by administration of cetrorelix, indicating that the site of action of kisspeptin occurs at the hypothalamus via GnRH release. However, histological analysis failed to detect TrkA and $P75^{NTR}$ receptors in kisspeptin neurons of the arcuate nucleus or the preoptic area of the camelid hypothalamus (Carrasco et al., 2020). Whether kisspeptin plays a role in the β-NGF-induced ovulation in a physiological context in llamas still awaits to be determined.

How β-NGF exerts its actions that result in LH release that leads to ovulation, and subsequent luteinizing actions remains a puzzling question. The current pieces of evidence indicate that the LH response requires the release of GnRH, and this is possibly modulated by β-NGF signaling through the $P75^{NTR}$ receptor in tanycytes to facilitate GnRH release. As the median eminence is a circumventricular organ and so lacks a functional blood–brain barrier, this provides an accessible site for circulating β-NGF to reach the tanycytes **(**Fig. 2**).**

The luteotropic effect of beta-nerve growth factor

Llamas

It is known that in addition to the ovulatory effect, β-NGF also has a luteotropic effect: systemic or intrauterine infusion of the neurotrophin affects the diameter of the corpus luteum, and

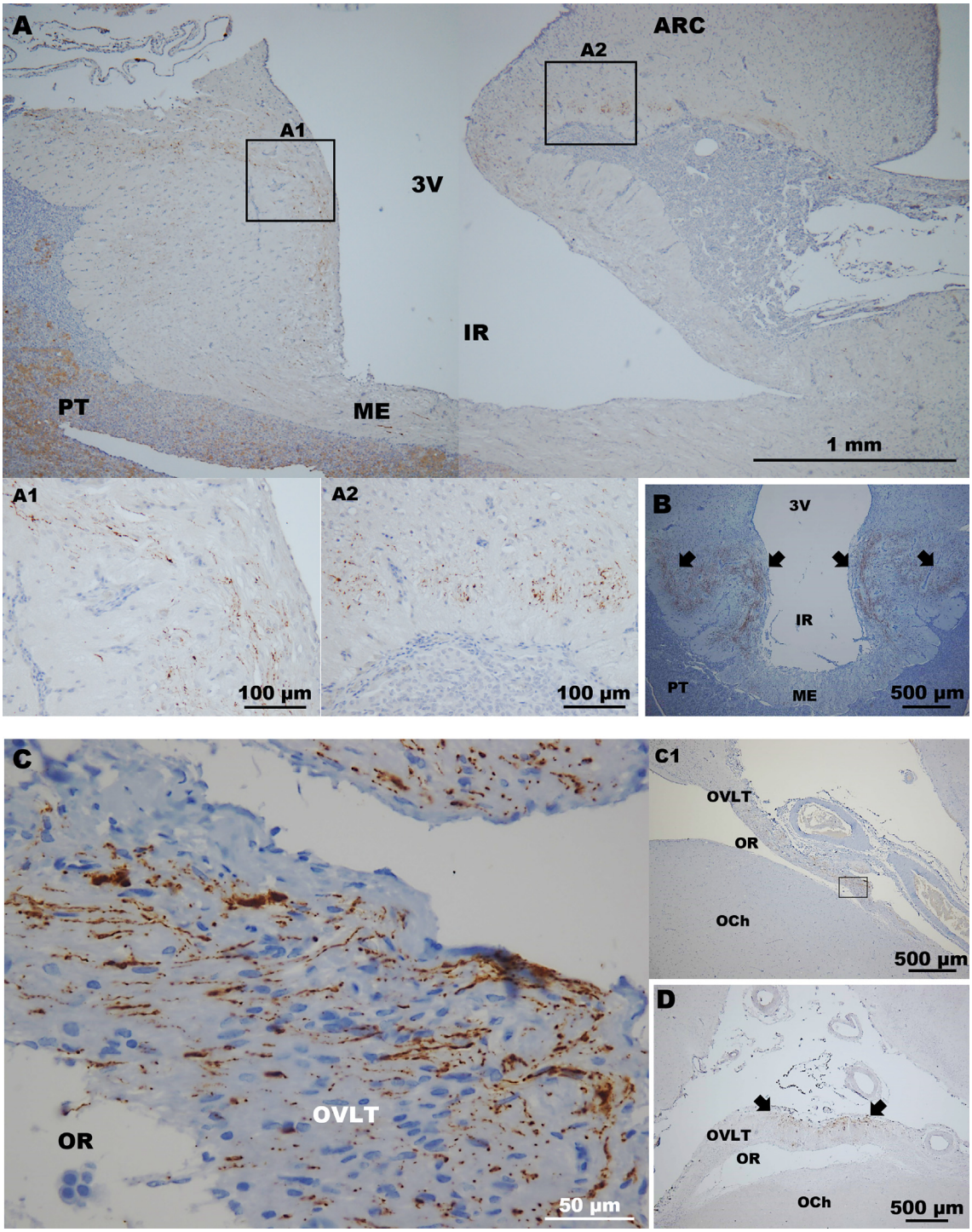

Fig. 1. Detection of gonadotropin-releasing hormone (GnRH) fibers in circumventricular organs of llamas. (A) In sagittal sections, GnRH fibers were found in (A1) rostral (preinfundibular) and (A2) caudal (postinfundibular) areas of the ME. (B) Coronal section showing GnRH fibers in the lateral walls (arrows) of the ME surrounding the infundibulum. Virtually no fibers were detected in the ventral wall of the ME. (C) High magnification image of (C1) a sagittal section showing GnRH fibers in the ventral area (square) of the OVLT. (D) Coronal section. Most of the fibers were found in the medial area (arrows) of the OVLT. Abbreviations: 3v = third ventricle; ARC = arcuate nucleus; GnRH = gonadotropin-releasing hormone; IR = infundibular recess; ME = median eminence; OCh = optic chiasm; OR = optic recess; OVLT = organum vasculosum laminae terminalis; PT = pituitary. From Berland et al. (2021).

increases the area of corpus luteum vascularization followed by a significant increase in progesterone secretion when comparing to GnRH-treated females (Adams et al., 2005; Ulloa-Leal et al., 2014). The luteotropic effect is linked to (i) a unique pattern of β-NGF-induced LH release, and (ii) a local effect on the preovulatory follicle that enhances the expression of steroidogenic enzymes and the angiogenic vascular endothelial growth factor (**VEGF**).

Different studies have shown that β-NGF exhibits a potent luteotropic effect when given systemically in llamas (Adams

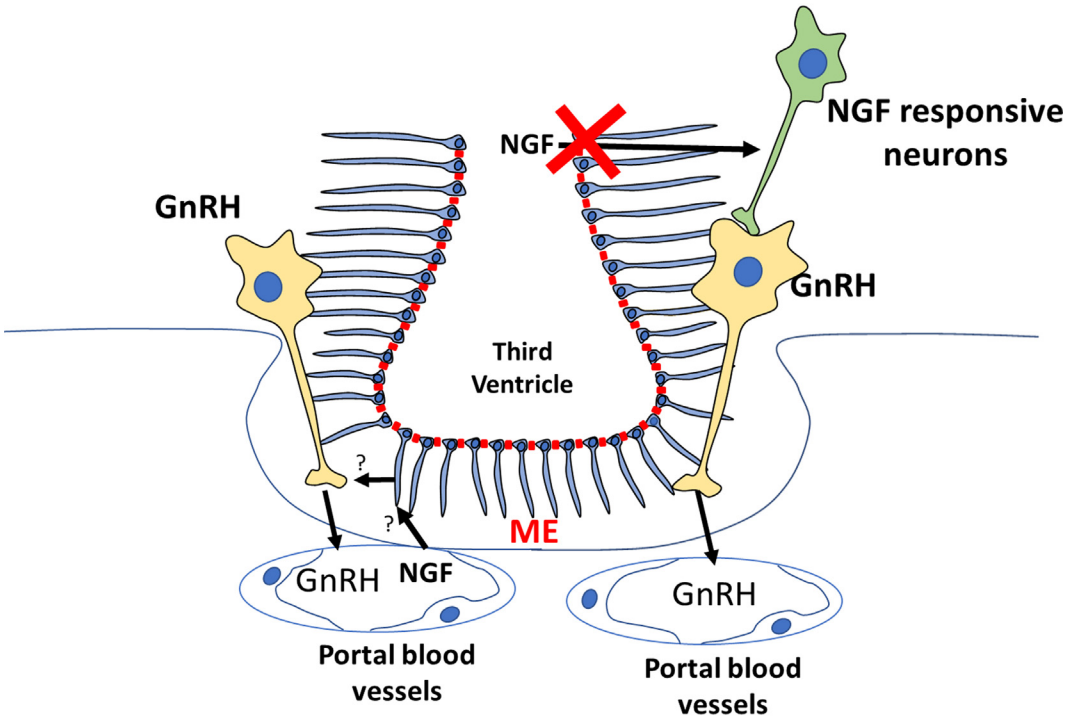

Fig. 2. Proposed pathway for β-NGF-induced ovulation at the hypothalamus. The diagram represents a transverse section of the ventral part of the third ventricle. Upon absorption from the endometrium, blood-borne β-NGF may facilitate GnRH release by signaling through P75-expressing tanycytes (elongated blue cells in the walls and floor of the third ventricle) in the median eminence of camelids. The transsynaptic route, represented by NGF-responsive neurons that influence GnRH neurons, might not be relevant for β-NGF-induced ovulation. Abbreviations: GnRH = gonadotropin-releasing hormone; ME = median eminence; NGF = nerve growth factor.

et al., 2005; Tanco et al., 2011; Ulloa-Leal et al., 2014). Intrauterine or intramuscular routes of administration of β-NGF that cause ovulation and subsequent corpus luteum formation consistently cause higher progesterone production during early corpus luteum development than the corpus luteum induced by GnRH administration. Also, a positive association between the extent of the LH peak and the subsequent luteal function has been found in llamas given either whole seminal plasma or purified β-NGF (Tanco et al., 2011; Silva et al., 2012). Thus, it has been hypothesized that the profile of the LH release resulting after β-NGF exerts a luteotropic effect in llamas (Fig. 3A and B).

The pattern of the LH surge induced by β-NGF provokes a rapid shift from estradiol to progesterone concentration ratio in the follicular fluid and changes in the expression of steroidogenic and angiogenic transcripts in granulosa cells from preovulatory follicles (Valderrama et al., 2019). In an *in vivo* study, granulosa cells collected by scrapping the follicular walls from dissected preovulatory follicles of ovariectomized llamas at 10 or 20 h after mating, GnRH, β-NGF, or phosphate buffer saline (control) treatments, showed a gene downregulation of cytochrome *CYP19A1* and upregulation of *CYP11A1*, *STAR* steroidogenic genes and also the angiogenic factor *VEGF* in response to β-NGF (Valderrama et al., 2019, Fig. 4).

The ovaries of induced and spontaneous ovulatory species show that follicles express the receptors to bind and transduce β-NGF actions, as both TrkA and $p75^{NTR}$ β-NGF receptors have been detected in granulosa and theca cells of cows (Dissen et al., 2000), humans (Salas et al., 2006), rabbits (Maranesi et al., 2018), gray squirrels (Maranesi et al., 2020) and rats (Dissen et al., 1996), indicating that the luteal effects of β-NGF are also exerted at the ovarian tissue. In this line, the application of β-NGF to a primary culture of granulosa cells collected from llama preovulatory follicles has been shown to enhance the expression of genes for the *VEGF* and enzymes involved in progesterone synthesis, resulting in the output of progesterone hormone (Valderrama et al., 2020; Figs. 5 and 6). The upregulation of genes of *VEGF* and *STAR* after 20 h of β-NGF application is greater than that detected in cells treated with LH. When β-NGF binds to its TrkA receptor results in the activation of the ERK1/2 signaling pathway (Huang & Reichardt, 2001; Van Kanegan & Strack, 2009), including in granulosa cells (Moore et al., 2001). In line with this, the sole application of purified llama β-NGF to primary cultured llama granulosa cells resulted in the activation of ERK1/2 signaling pathway**.** When granulosa cells were cultured with the ERK1/2 inhibitor, U0126, combined with β-NGF downregulated virtually all steroidogenic and *VEGF* transcripts in the llama granulosa cells, including a decrease in progesterone output (Valderrama et al., 2020; Fig. 6).

The physiological mechanisms possibly involved in the luteotropic effect of β-NGF have been indicated by *in vivo* studies. By the use of Power Doppler ultrasonography –a technique that gives detail of blood flow–, results obtained in llamas have shown that both the preovulatory follicle and the early corpus luteum display greater vascularization after β-NGF injection than that resulting from GnRH injection, but also in the production of higher progesterone concentrations in blood (Ulloa-Leal et al., 2014; Silva et al., 2017). Fernández et al. (2014) report that multiple administration of β-NGF during the periovulatory stage induces greater luteal vascularization and progesterone secretion than that observed in females treated with a single dose. Histological analysis has confirmed the increase in vascularization observed by ultrasonography (Silva et al., 2017). Besides, the administration of β-NGF also results in enhanced expression of the *VEGF* gene (Valderrama et al., 2019), which codifies the VEGF protein that is involved in angiogenesis through the induction of proliferation of endothelial cells (Dvorak et al., 1999). Thus, enhanced vascularization seems to play a critical role in the luteotropic effect *in vivo*.

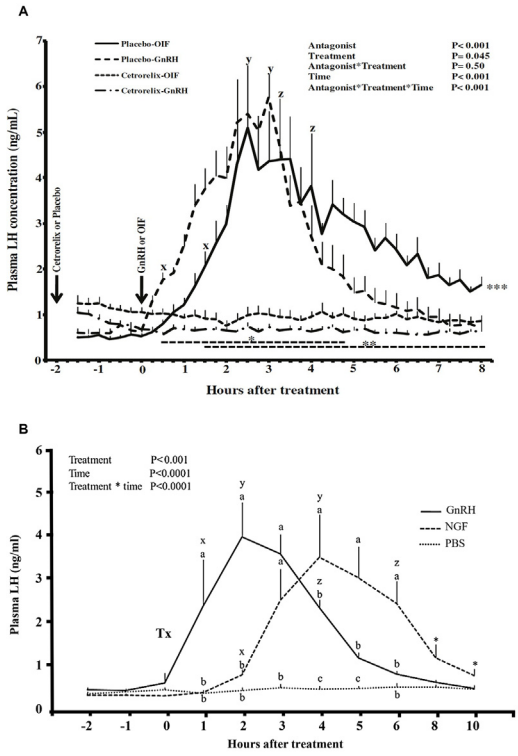

Fig. 3. (A) Effect of pretreatment with an antagonist of GnRH (cetrorelix) on plasma LH concentrations in llamas. The LH surge was observed only in the placebo-treated females. The LH surge was significantly higher and more sustained over the sampling period in the ovulation-inducing factor (NGF) than that of GnRH-treated females. From Silva et al. (2011). (B) Llama plasma LH concentration in females treated with GnRH, NGF or phosphate buffer saline. A higher increase in plasma LH concentration was observed in the NGF-treated females. Plasma LH concentration returned to basal levels at 6 hours after treatment with GnRH but remained elevated ($P < 0.05$) at 10 hours after NGF treatment. [a,b,c]For a given time, values without a common superscript are different among groups ($P < 0.01$), [x]Within group, the first significant increase from pretreatment concentration ($P < 0.05$), [y]Within group, the maximum concentration ($P < 0.05$), [z]Within group, the first significant decrease from maximum concentration ($P < 0.05$). Abbreviations: GnRH = gonadotropin-releasing hormone; NGF = nerve growth factor; OIE = ovulation-inducing factor; PBS = phosphate buffer saline. Modified from Ulloa-Leal et al. (2014).

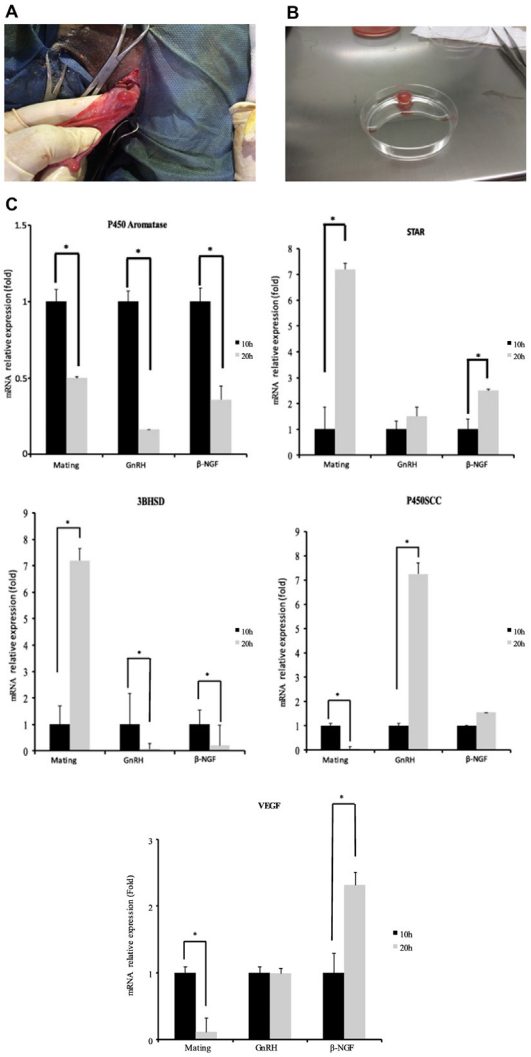

Fig. 4. (A) Preovulatory follicle of llama collected by ovariectomy and (B) dissected under a laminar flow hood, (C) Relative mRNA abundance (mean ± SEM, n = 6/per treatment) of cytochrome *CYP19A1* (P450 aromatase), *CYP11A1* (P450scc), *STAR*, *HSD3B* and the angiogenic factor *VEGF* of granulosa cells collected of preovulatory follicles at 10 or 20 h after mating, GnRH, or β-NGF treatment. *$P < 0.01$. Abbreviations: GnRH = gonadotropin-releasing hormone; NGF = nerve growth factor. Modified from Valderrama et al. (2019).

Apart from the effect on vascular development, female llamas treated with seminal plasma (which contains β-NGF) enhance the expression of genes related to enzymes implicated in steroid synthesis in luteal tissue, such as *STAR* and *CYP11A1* on days 4 and 8 of the luteal phase, resulting in enhanced progesterone concentration (Silva et al., 2017). Also, both systemic administration of purified llama β-NGF and natural mating induces increased expression of genes involved in progesterone production and a rapid shift from estradiol to progesterone synthesis (expressed as increased progesterone/estradiol ratio in the follicular fluid), indicating luteinization of preovulatory follicles (Valderrama et al., 2019). The changes in gene expression and progesterone production *in vivo* are greater when llamas are given β-NGF or submitted to mating than the administration of GnRH alone, indicating a direct effect of β-NGF on the ovary (Silva et al., 2017; Valderrama et al., 2019).

Cattle

Although the ovulatory effect that β-NGF exerts in camelids has not been replicated in cattle (Tanco et al., 2012), several pieces of evidence suggest that this molecule has other effects on the female bovine. For example, the administration of llama β-NGF to prepuberal (Van Steelandt et al., 2008; Tanco et al., 2012) and cyclic (Tanco et al., 2012) heifers does not induce ovulation but influences follicular wave dynamics. Most interesting, β-NGF treatment has been shown to have a luteotrophic effect in cyclic heifers, stimulating progesterone production of preexisting corpus luteum (Tanco et al., 2012).

The effects of β-NGF in bovines may imply practical applications on cattle reproductive management and therefore have led to a series of studies focused on the isolation of the molecule from bull semen and its evaluation as a promoter of cattle fertility. In this regard, Stewart et al. (2018b) after evaluating semen from over 100 bulls from different breeds reported a consistent association between seminal plasma β-NGF concentration and conception rates.

Intramuscular administration of 296 μg of bull β-NGF to beef cows given at the time of artificial insemination results in greater

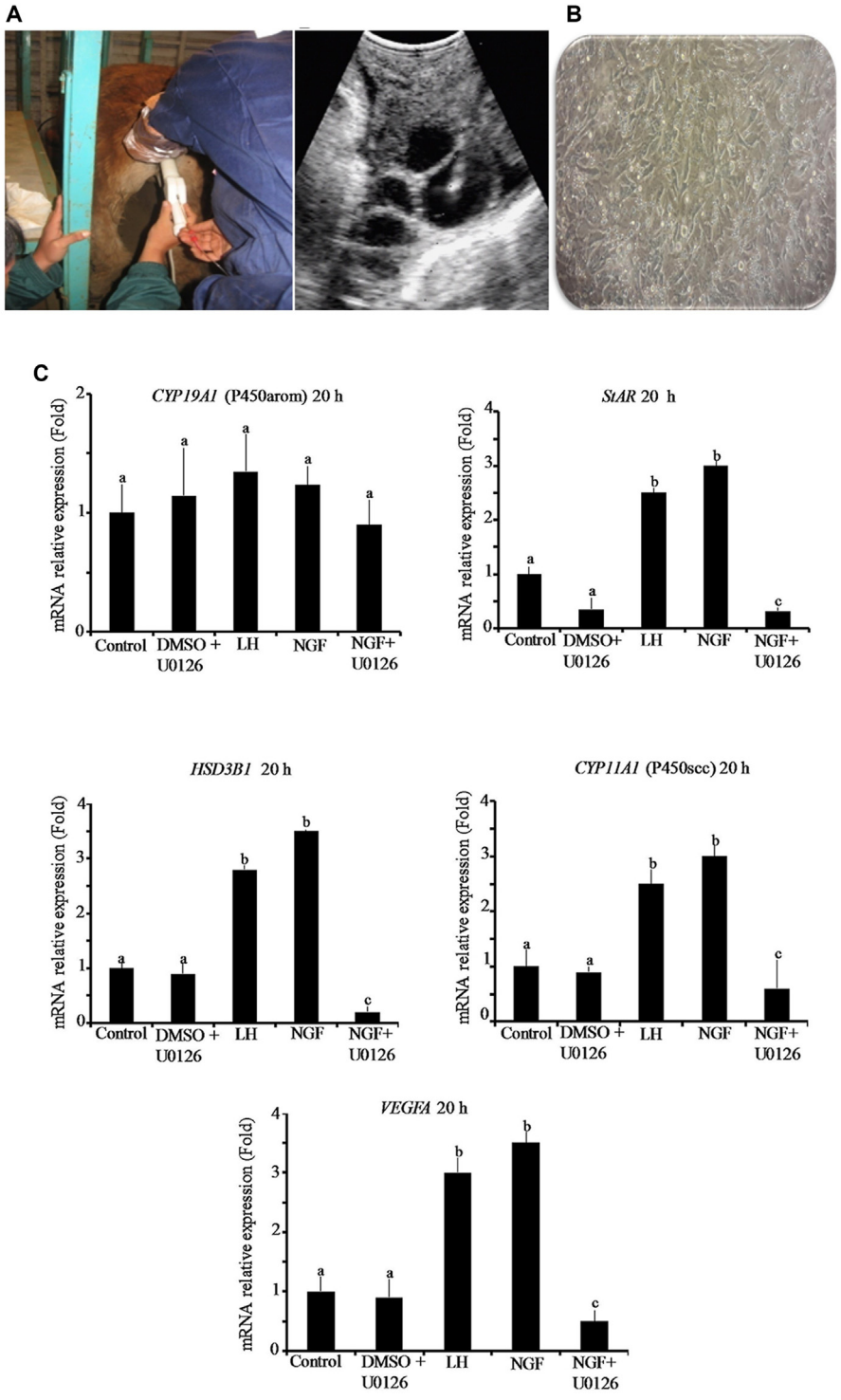

Fig. 5. (A) Collection of granulosa cells by ultrasound-guided follicular aspiration from llamas. (B) *In vitro* primary culture of llama granulosa cells for 48 hours, (C) Relative mRNA expression of *CYP19A1* (P450arom), *STAR*, *HSD3B1*, *CYP11A1* (P450scc) steroidogenic enzymes, and *VEGFA* angiogenic factor in primary llama granulosa cell culture treated with LH, NGF or NGF plus the ERK inhibitor U0126 for 20 h. Mean + SEM; n = 5 biological samples, within each biological sample, four experimental replicates were performed; a, b, c superscripts indicate significant differences ($P \leq 0.01$) between control and other groups. Abbreviations: DMSO = dimethyl sulfoxide; NGF = nerve growth factor; U0126 = ERK pathway inhibitor. Modified from Valderrama et al. (2020).

progesterone production between day 10 and day 19 after artificial insemination (Stewart et al., 2018a), and also a significant increase in pregnancy rate when compared to control females (75 vs 59%, respectively). Moreover, the concentration of the pregnancy-specific protein B (produced by the conceptus) of β-NGF-treated cows was also significantly greater, and mRNA expression of interferon-stimulated genes (i.e., *ISG15*, *MX1*, *MX2* and *RTP4*) at Day 24 postartificial insemination, demonstrating a

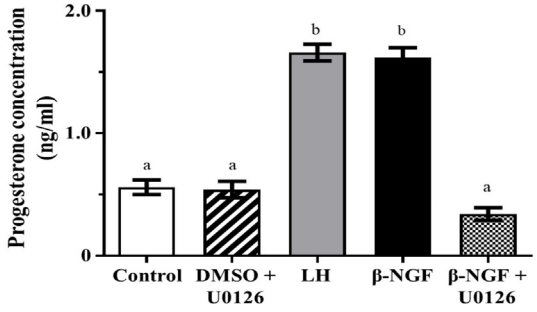

Fig. 6. Progesterone secretion from primary culture of llama granulosa cells after 48 h. Mean ± SEM; n = 6 biological samples, within each biological sample, four experimental replicates were performed; a, b superscripts indicate significant differences ($P \leq 0.01$) between control and other groups. Abbreviations: DMSO = dimethyl sulfoxide; NGF = nerve growth factor; U0126 = ERK pathway inhibitor. From Valderrama et al. (2020).

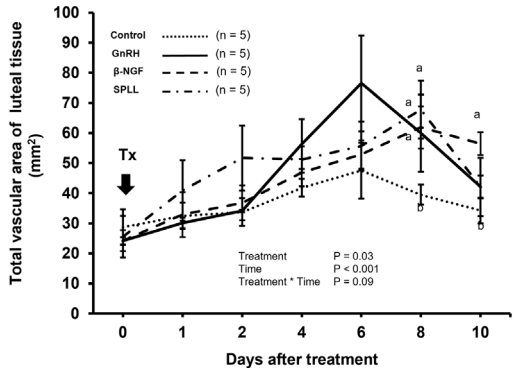

Fig. 7. The effect of systemic administration of GnRH, raw llama seminal plasma, purified llama β-NGF or phosphate buffer saline (control) given on Day 5 after ovulation in lactating Holstein-Friesian cows. There was a higher luteal tissue vascularization in those cows treated with GnRH, llama seminal plasma and purified llama β-NGF than that of the control group at Day 8 after treatment. [a,b]For a given time, values without a common superscript are different among groups ($P < 0.01$). Abbreviations: GnRH = gonadotropin-releasing hormone; NGF = nerve growth factor; SPLL = seminal plasma from llama; Tx = treatment.

beneficial effect on early embryo development and survival, and so positively impacting rate pregnancy. Conversely, no significant effects on the relative abundance of interferon-stimulated genes or pregnancy rate per artificial insemination were found in larger scale study using Holstein-Friesian dairy cows, as only primiparous cows showed a slight increase in plasma progesterone concentration, presumably due to milk production differences with multiparous females (Hubner et al., 2022).

Interestingly, recent studies on heifers have documented both bull and llama β-NGF potential role in corpus luteum functionality (Stewart et al., 2020; Gajardo et al., 2021). Stewart et al. (2020) have reported that administration of 350 μg of purified bull β-NGF increased the preovulatory follicle diameter and systemic LH concentration, and heifers had an increase in serum progesterone concentration during the subsequent luteal phase. These effects were not linked to changes in the vascularization area of preovulatory follicle or corpus luteum, but rather to increases in small luteal cell number and steroidogenic *STAR* mRNA abundance in the corpus luteum. Consistently, a similar enhancement of corpus luteum function was observed by Gajardo et al. (2021) when Holstein-Friesian heifers were treated with heterologous llama β-NGF given during the preovulatory surge of LH, females exhibited a significant increase in corpus luteum vascularization area and progesterone concentration at Days 9, 12 and 15 after ovulation confirming a potential effect of β-NGF on the hypothalamus-hypophysis-gonadal axis in the bovine model.

In an *in vitro* bovine study (Stewart et al., 2022), pieces of follicular wall from preovulatory follicles collected from heifers incubated with bovine β-NGF stimulated testosterone production, however, estradiol production by granulosa cells was not affected, indicating that this molecule acts directly on granulosa and theca cells. As suggested recently in llamas (Silva et al., 2017; Valderrama et al., 2019), the luteotrophic effect of β-NGF in cattle could affect ovarian function either through a direct action on follicular wall cell populations or/and indirectly through the increase of preovulatory LH surge (Stewart et al., 2020).

In a recent study conducted in our group (unpublished data), multiparous lactating Holstein-Friesian cows (n = 20) synchronized with a traditional fixed-time artificial insemination protocol based on progesterone/estradiol at 45 days postpartum were randomly assigned at Day 5 (Day 0 = ovulation) to receive an intramuscular

Table 1
Main achievements and conclusions in the study of the nerve growth factor (NGF) in cattle.

Reference	Source of semen and/or NGF	Achievements or conclusions
Harper et al., 1982	Bulls (no breed informed)	First isolation and biochemical characterization of NGF molecule in bull semen. Determined that bull semen is a rich source of NGF.
Ratto et al., 2006	BT	Used the llama bioassay to demonstrate the potential ovulatory effect of bull seminal plasma on llamas.
Tanco et al., 2012	LG	Concluded that llama β-NGF does not induce ovulation in beef heifers but hastened new follicular wave emergence in prepubertal heifers and influenced follicular dynamics in mature heifers. Concluded that llama β-NGF exerted a luteotrophic effect in preexisting corpus luteum in heifers.
Bogle et al., 2012	LG	Demonstrated that llama β-NGF stimulates *in vitro* LH release from llama and bovine adenohypophysis cultured cells.
Tribulo et al., 2015	LG, bulls (no breed informed)	First report suggesting a luteotrophic effect of bovine seminal β-NGF in cattle
Carrasco et al., 2016	N/A	Suggested that luteotrophic effect of β-NGF in cattle is mediated, entirely or in part, by an increase in TrkA expression in the ovulatory follicle and early corpus luteum.
Bogle et al., 2018	LG, VP, bulls (No breed informed)	Conclude that β-NGF is a common and abundant protein present in the male accessory glands of camelids, bovids and cervids.
Stewart et al., 2018a	BT	Demonstrated that intramuscular administration of β-NGF from bull semen to cows on the day of artificial insemination improves corpus luteum function and early embryo development.
Stewart et al., 2018b	BT	Determined that β-NGF is produced in the ampulla and vesicular glands in bulls. Suggested a positive association between β-NGF semen concentration and bull fertility.
Stewart et al., 2019	BT	Determined that supplementation of semen freezing extender with β-NGF had minimal effects on postthaw sperm quality in bulls.

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Table 1 (*continued*)

Reference	Source of semen and/or NGF	Achievements or conclusions
Stewart et al., 2020	BT	Demonstrated that intramuscular administration of β-NGF from bull semen to periovulatory heifers significantly increased pituitary LH release and corpus luteum function.
Stewart et al., 2022	BT	Demonstrate that β-NGF isolated from bull seminal plasma acts directly on granulosa and theca cells of the bovine preovulatory follicle stimulating testosterone production. Suggest β-NGF action on hastening the onset of follicle wall cellular remodeling occurring during early luteal development.
Gajardo et al., 2021	LG	Demonstrated that the systemic administration of llama β-NGF at the end of synchronization protocol in dairy heifers enhances corpus luteum vascularization and progesterone secretion.
Hubner et al., 2022	BT	Showed that the administration of β-NGF purified from bull semen to Holstein-Friesian cows at the time of artificial insemination had minor effects on progesterone concentration only in primiparous cows and did not alter the expression of interferon-stimulated genes or the pregnancy rate per artificial insemination
Rimayanti et al., 2022	BI	Showed that seminal concentration of NGF has a strong correlation with testosterone levels, sperm motility, viability, and concentration in bulls.

Abbreviations: BT = *Bos taurus*, BI = *Bos indicus*, LG = *Lama glama*, VP = *Vicugna pacos*, N/A = *Not applicable*.

administration of (i) 50 μg of GnRH agonist (gonadorelin acetate), (ii) 4 ml of raw llama seminal plasma, (iii) 4 mg of purified llama β-NGF, or (iv) 4 ml of phosphate buffer saline (control); only in GnRH-treated females, formation of an accessory corpus luteum was observed, but the total area of corpora lutea/corpus luteum vascularization were similar in the GnRH, seminal plasma, and purified β-NGF and these were higher than the observed in the PBS treated females, thus β-NGF or seminal plasma treatment may enhance the function of the corpus luteum induced by the synchronization protocol (Fig. 7).

The potential effects of β-NGF on bovine reproduction are still in the initial stages and warrant further investigation. Table 1 shows a chronological summary of the most relevant research undertaken in the study of the effects of seminal NGF on bovine reproductive physiology and the association between seminal NGF in bulls with male fertility traits.

Conclusions

The pieces of evidence reviewed here strongly support that β-NGF present in the semen of camelids is a key molecule in modulating the hypothalamus-hypophysis-gonadal axis in llamas. Evidence suggest that β-NGF may not penetrate the brain to stimulate GnRH neurons, but this factor can be sensed from the systemic circulation by circumventricular organs such as the median eminence which expresses receptors for β-NGF and receive GnRH processes. The particular pattern of LH secretion observed in llamas after β-NGF administration influence the development of a corpus luteum resulting in more production of progesterone. However, it has been found that the luteotropic actions of β-NGF also occurs locally in the ovary of llamas and cows. Despite β-NGF does not induce ovulation in cows, it may influence the ovulatory process as changes in LH release affect the differentiation of granulosa and luteal cells following systemic administration of this neurotrophin. Further field studies are required to confirm the impact of the luteotrophic effect of β-NGF on pregnancy rate and embryo survival in cattle in artificial insemination programs.

Ethics approval

Not applicable.

Data and model availability statement

Data or models were not deposited in an official repository. No new datasets were created.

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