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Role of nerve growth factor in the reproductive physiology of female rabbits: A review

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ABSTRACT

Rabbit does are reflex ovulators such that coitus is needed to release GnRH and elicit the LH surge that triggers the ovulation of mature oocytes. However, the mechanisms eliciting ovulation in this species remain unclear. One of the most promising recently discovered candidates with a role in female reproductive physiology is nerve growth factor beta (β-NGF). This neurotrophin and its high-affinity receptor TrkA and low affinity receptor p75, is present in all compartments of the ovary, oviduct and uterus suggesting a physiologic role in ovarian folliculogenesis, steroidogenesis, ovulation, luteogenesis and embryo development. Besides, evidence exists that β-NGF found in seminal plasma could exert a modulatory role in the female hypothalamus-pituitary-ovarian axis contributing to the adrenergic and cholinergic neuronal stimulus of GnRH neurons in an endocrine manner during natural mating. Probably, the paracrine and local roles of the neurotrophin in steroidogenesis and ovulation reinforce the neuroendocrine pathway that leads to ovulation. This review updates knowledge of the role of β-NGF in rabbit reproduction, including its possible contribution to the mechanisms of action that induce ovulation, and discusses perspectives for the future applications of this neurotrophin on rabbit farms.

1. Nerve growth factor system

The neurotrophin family comprises nerve growth factor (NGF) together with brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), neurotrophin 4/5 (NT4/5) and neurotrophin 6 (NT 6). The biological actions of these neurotrophins are mediated by two cell surface receptors: a specific high-affinity receptor known as tropomyosin-related kinase (Trk) of the tyrosine kinase receptor superfamily, and a non-specific low-affinity receptor named p75 of the cell death-promoting tumor necrosis factor family. Nerve growth factor beta (β-NGF) is the founding member of the neurotrophin family and its specific high-affinity receptor is TrkA [1]. Interaction between β-NGF and its TrkA receptor involves intracellular signaling cascades such as the ERK/MAPK and PI3K/AKT pathways [2].

Nerve growth factor was first found in tissues as a high molecular weight complex of ~140,000 KDa known as 7S NGF [3]. It can be dissociated into the active neurotrophic factor β-NGF (2.5S NGF) and two serine proteinases (α-NGF and γ-NGF) [4]. Nerve growth factor beta is a homodimer responsible for the factor's biological activity and consists of two identical subunits assembled in parallel of molecular weight of around 14,500 kDa each [5,6]. Nerve growth factor was first identified in the nervous system [7] and has been attributed many important regulatory functions such as the survival, development and maintenance of sympathetic and sensory neurons [8]. However, this neurotrophin is expressed and widely distributed in non-neuronal tissues including the reproductive system in both females [9] and males [10–12]. In effect, it has been detected in the seminal plasma of the species llama [13], alpaca [14], camel [15], bull [16], horse [17], deer [18], human [19] and rabbit [20–23], and in the follicular fluid of sheep [24], human [25] and mouse [26].

2. Roles of the β-NGF system in female reproduction

The β-NGF system has been ascribed physiological roles in reproduction involving endocrine, autocrine and paracrine cell

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signaling. In this regard, some data point to its physiological role in the ovary, oviduct and uterus, and a role in ovulation as it acts as an ovulation-inducing factor (OIF) in camelids [13,14,27].

2.1. Local effects of the β-NGF system on periovulatory events

The female reproductive tract is innervated by the sympathetic nervous system and peripheral sensory neurons. In the ovary, sympathetic innervation is prominent and nerve fibers may extend to the ovarian follicles [28]. Nerve growth factor beta is produced in the theca, granulosa and cumulus cells of the follicles in several species (reviewed in Ref. [29]), including rabbits [30,31]. The local production of this neurotrophin by the ovary indicates its participation in crucial events such as control of the folliculogenesis steps [32] follicle assembly, primordial follicle activation, follicular growth [33] and steroidogenesis [28,34]. The role of β-NGF in the events that take place around ovulation is also essential [35]. It has thus been observed that TrkA mRNA increases in the theca cells of periovulatory follicles and that this is accompanied by an increase in β-NGF mRNA content linked to the preovulatory release of LH in several species (rat: [35]; sheep: [36]; human [37]). Hence, when it interacts with the TrkA receptor, β-NGF may be one of the signals involved in the ovulatory cascade [38], as it has been incriminated in the disruption of gap junctions in periovulatory follicles [35]. Cellular dissociation of the follicular wall provokes the rupture of the follicle and release of the mature oocyte.

Nerve growth factor beta effects on oocyte maturation have been scarcely explored in mammals. *In vitro* experiments in ovine oocytes have shown that supplementation with β-NGF [39] alone or combined with other paracrine factors [40] during oocyte maturation increases rates of cleavage and embryo development. However, it has not been possible to reproduce such effects in other species (cow: [41]; pig [42]) and so far this has not been attempted in rabbit. In does stimulated with 25 IU of eCG, our group immunolocalized β-NGF in the oocytes of primordial and primary follicles and in the granulosa and theca cells of secondary follicles [31]. However, we observed no immunoreaction in antral follicles (Fig. 1a). After ovulation (7 days after GnRH administration), the rabbits showed intense labeling for β-NGF in the corpus luteum (CL) (Fig. 1a), suggesting the local production of the neurotrophin in this structure. In addition, staining for TrkA was slightly more intense than before ovulation (Fig. 1a), also highlighting the importance of β-NGF in the process of luteogenesis. Effectively, a potent luteotrophic role of β-NGF in the developing CL through TrkA has been described for camelids (llamas: [43,44]; alpacas [45]) and cows [46]. It has been well established that the neurotrophin affects CL angiogenesis and vascularization [47] by producing an increase in vascular endothelial growth factor, and enhancing progesterone secretion [48] by upregulation of the expression of aromatase [44] and steroidogenic enzymes [48]. In the rabbit female, this luteotrophic effect is not as evident as in camelids [49,50] and information about vascularization has not been yet reported. Finally, we observed [31] the significant participation of p75 in ovarian function as it was immunolocalized before and after ovulation in the theca interna of secondary and preantral follicles, and scarcely in antral follicles. This neurotrophin receptor was also present in CL (Fig. 1a). Although p75 shows cell survival and apoptotic functions in other tissues, its effects in the rabbit ovary remain unknown.

After natural mating or artificial insemination (AI), the processes of capacitation of spermatozoa, fertilization, and early embryonic development take place in the oviduct. Different effects of β-NGF on sperm have been confirmed (see Refs. [51–53]). Further, this system is thought to play an autocrine/paracrine role in early stages of embryo development, since β-NGF and its receptors are expressed in the oviduct, including infundibulum, ampulla, and isthmus of several species (goat [54], hamster [55], cow [56], quail [57]), as well as rabbit ([31]) (Fig. 1b). In fact, prior work has also shown that β-NGF directly affects early embryo development *in vitro* in rabbit, and doses of 100 and 1000 ng/mL of NGF in the culture medium were found to improve the hatching blastocyst rate [58]. Therefore, another potential luteotrophic effect of β-NGF could be attributed to this direct effect on preimplantation embryos.

Finally, expression levels of β-NGF and TrkA are high in the lumen and stromal epithelial cells of the uterus in both unmated non-stimulated rabbits [59] and rabbits before and after ovulation [31] (Fig. 1c). Maranesi et al. [59] suggested that β-NGF has a physiological role in prostaglandin (PG) synthesis (PGF2α and PGE2) in the uterus that is mediated by oxide nitric synthase (NOS) and PGE2-9-ketoreductase (PGE2-9-K). Prostaglandins can influence luteotrophic/luteolysis events and PGE2 is a key mediator of ovulation and oocyte fertilization [60]. Further, the low-affinity receptor p75 has been found to show an uneven distribution in the basal and apical zone of some lining and glandular uterine epithelial cells that is more intense before ovulation [31] (Fig. 1c), though the role of p75 in the uterus still needs to be addressed.

2.2. Ovulation-inducing effect of β-NGF in the rabbit: recombinant rabbit β-NGF (rrβNGF)

One of the reproductive features of rabbit species is that females are reflex ovulators like camelids, cats, voles and ferrets. In all these species, ovulation is triggered by a neurohormonal stimulus provoked by natural mating or AI. In rabbits, neural pathways, in which noradrenergic neurons and cholinergic neurons of the brainstem are major participants, elicit the pulsatile release of gonadotropin-releasing hormone (GnRH) from the GnRH neurons in the mediobasal hypothalamus, which are mainly located between the anterior commissure and the optic chiasm [61]. As occurs in spontaneous ovulating species, GnRH release is followed by an immediate preovulatory LH surge from the pituitary gland, resulting in final maturation of the oocyte (resumption of meiosis), ovulation and CL formation. Usually, mating and GnRH administration in does induce the rapid release of plasma LH, which plateaus over 15 min [62]- 30 min [63], as GnRH rapidly reaches the pituitary gland.

The role of β-NGF in inducing ovulation in the rabbit has not been clearly established. However, recent evidence suggests its possible participation in this event since we and others have confirmed its abundant presence in rabbit seminal plasma at concentrations ranging from 2 ng/mL [20–22,64] to 150 μg/mL [30]. Further, the presence of β-NGF in seminal plasma has been previously detected in other induced ovulation species (llama: [13]; alpaca: [14]; camel [15]). Notwithstanding, according to the effects of homologous and heterologous seminal plasma in rabbits and camelids, some authors suggest that the ovulation process may be species-specific [23,64].

As indicated in Table 1, the intramuscular (i.m) administration of rabbit seminal plasma was able to induce ovulation in llamas and led to the development of a functional CL producing progesterone, as observed in llamas treated with homologous seminal plasma [65]. However, the i.m administration of homologous seminal plasma in rabbit does did not induce ovulation [65,66], although numerous hemorrhagic follicles were observed. Cervantes et al. [67] reported that the i.m. administration of rabbit seminal plasma induced ovulation in group-housed but not individually-housed rabbits. These findings suggest that the sensorial stimulus of social and physical interaction could be important in this species. In addition, neither ovulation nor CL formation were detected when rabbits were treated with heterologous camel seminal plasma [68].

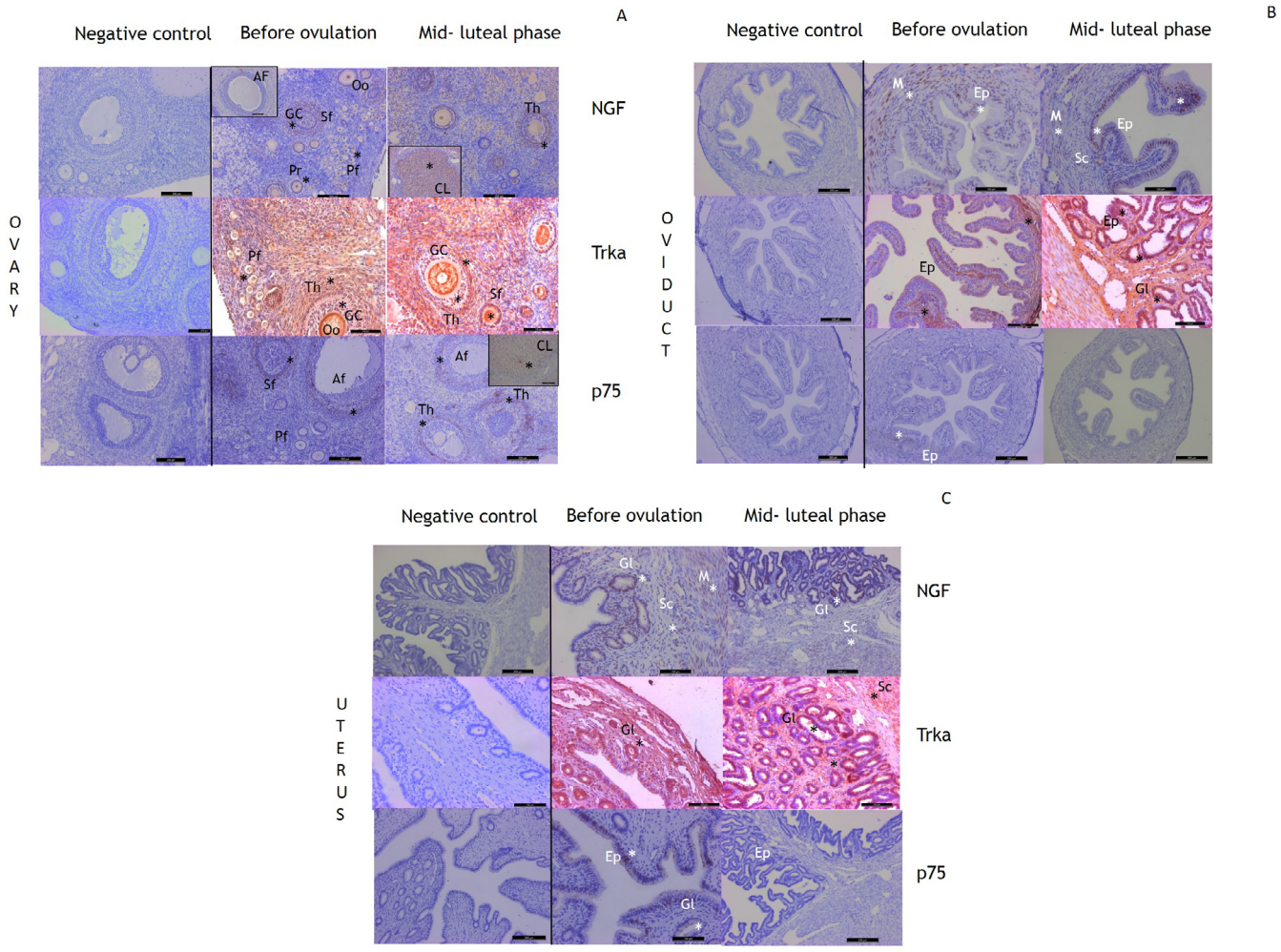

Fig. 1. Representative images showing immunolocalization of a) β-NGF, b) TrkA receptor and c) p75 receptor in the ovary, oviduct and uterus of rabbit females stimulated with ecG 48 h before ovulation (developing follicles) and 7 days after GnRH administration (mid-luteal phase). Scale bar: 50 μm. NC: negative control, Oo: oocyte, GC: granulosa cells, Th: theca cells, CL: corpus luteum, Pf: primary follicles, Sf: secondary follicles, Af: antral follicles, Ep: epithelial cells, ES: stroma cells, Gl: glandular cells. * indicates immunoreaction.

After injecting murine β-NGF i.m in rabbits [21], we observed a slight ovulatory response. Only 17% of treated does ovulated in the absence of catheter stimulation but a high rate of anovulatory hemorrhagic follicles was recorded. Further, the LH surge could not be detected at 2 h post-treatment. Based on the results of these studies [21,49], we hypothesized that the presence of different β-NGF isoforms in mouse species, showing variations in amino acid sequences, might affect receptor binding [69]. Although β-NGF protein is highly conserved among species [13], the orientation of β-NGF in the signaling complex with TrkA is important [70]. A

Table 1
Ovulation rates (%) in response to different OIF doses (specified in parentheses) used in llama and rabbit females according to the administration route (intramuscular, intrauterine or intravaginal) and OIF used (homologous seminal plasma (SP) = green, heterologous SP = pink, β-NGF purified from SP = blue, recombinant mouse β-NGF = gray or recombinant rabbit β-NGF = orange). References:[1] [101],[2] [65],[3] [66],[4] [68],[5] [102],[6] [89],[7] [103],[8] [91],[9] [21],[10] [49],[11] [80].

Route	Intramuscular	Intrauterine	Intravaginal
	Ovulation rate % and dosage ()		
Llama	SP 80-100%[1,2,5] 90-100% (2mg)[6,7,8]	100% (4ml)[5] 100% (2ml)[4] 0% (2mg)[6] 83% (10mg)[5] 100% (20mg)[6]	
Rabbit	0-8.3% (2ml/14mg)[2,3] 0% (2ml)[4] 16.7% (14 μg)[9]		50-60%[11,12]

Homologous SP
Heterologous SP
β-NGF purified from SP
Recombinant mouse β-NGF
Recombinant rabbit β-NGF

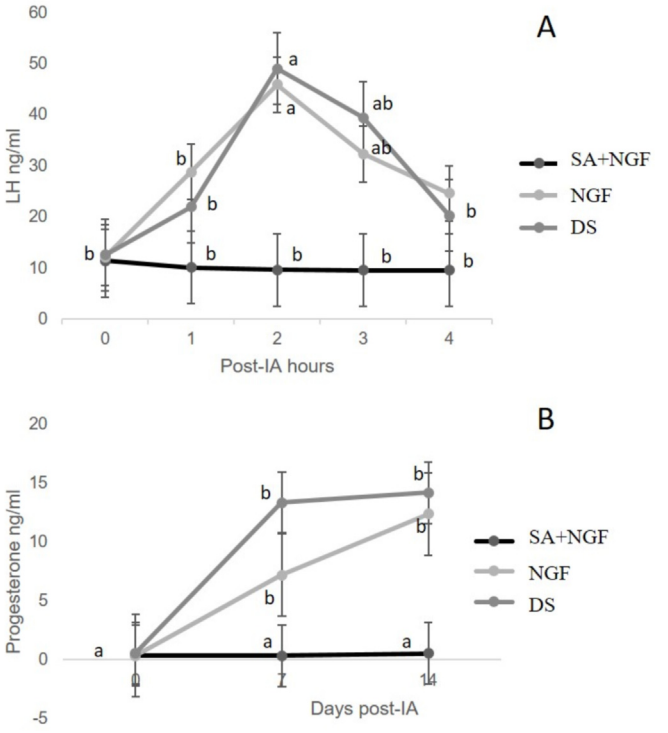

Fig. 2. Plasma concentrations of LH (A) or progesterone (B) in rabbit does inseminated with 1 μL of rrβ-NGF in the seminal dose (β-NGF; n = 8); inseminated with semen diluted 1:5 (DS; n = 8); or sedated and anesthetized and inseminated with 1 μL of rrβ-NGF in the seminal dose (SA+ β-NGF; n = 9). Different letters indicate significant differences between groups (P < 0.05).

detailed comparative analysis of amino acid sequences between induced- and spontaneously-ovulating females has revealed essential differences between rabbits and other species in some receptor binding sites to the neurotrophin [49]. In rabbits, serine is replaced by alanine and proline at the TrkA binding site, and there is a new lysine replacing asparagine and methionine at the site where p75 binds. These modifications could affect the binding of exogenous neurotrophin (e.g., mouse or llama) to the receptors in rabbits. However, in other species such as llama this seems not so relevant, as rabbit seminal plasma can elicit ovulation in this species (Table 1).

In this context, our group recently sequenced, produced and purified a recombinant protein from rabbit prostate (rrβ-NGF; Gene Bank Accession number KX528686) [49]. When added to the insemination dose, we showed that rrβ-NGF can induce ovulation in rabbit does in a dose-dependent manner [71]. It was found that 1 μg/mL of rrβ-NGF given via the vaginal route induced ovulation (in 60% of does, Table 1), and there was a delayed LH surge (peaking at 2 h) and lower progesterone levels at 7 days compared to GnRH-treated does that was consistent with embryo development. In effect, healthy offspring were delivered and there was no difference in the number of live and stillborn pups compared to the GnRH group.

3. Mechanisms of action of β-NGF in ovulation in rabbit reproduction

While the mechanism of action of β-NGF remains unknown both in camelids and rabbits, it seems to differ between these species. In camelids, it has been speculated that the role of β-NGF is carried out mainly by the endocrine pathway after its i.m. and intravenous (i.v.) administration or absorption from the uterus/vagina [27,72]. In llamas, the presence of TrkA near the periventricular area of the third ventricle of the hypothalamus could indicate passage of β-NGF to the cerebrospinal fluid through the blood brain-barrier [73]. From here it would pass to the central nervous system, where the main site of action is located at the anterior and mediobasal hypothalamus [74]. However, it seems not to act directly on GnRH-secreting neurons since these cells were not immunoreactive to TrkA and there was no co-localization with p75 [74]. The regulation of neuronal GnRH secretion depends on numerous inputs [75], and an intermediate step before GnRH activation has been suggested [27,72]. One of these inputs could be provided by interneurons after their interaction with β-NGF [74] and it has been proposed that in camelids this input could be kisspeptins. Further, recent advances have located β-NGF at the center of a homeostatic loop between target and non-target neurons, microglia and astrocytes [76]. Glial-neuronal interactions with GnRH cell bodies in the median eminence of the hypothalamus seem to be involved in the regulation of GnRH release to the portal system [77]. Further studies are needed to elucidate this neural circuitry and its relationship with β-NGF.

Table 2
Estradiol, progesterone and β-NGF serum concentrations in rabbit does before ovulation (females with developing follicles stimulated with 25 IU of eCG 48 h earlier) and in the mid-luteal phase (7 days after GnRH administration when a corpus luteum is present).[a,b] P < 0.05. From Ref. [31].

	Before ovulation	Mid-luteal phase
Estradiol (pg/mL)	44.85 ± 8.2	45.70 ± 6.02
Progesterone (ng/mL)	0.67 ± 0.11[a]	22.28 ± 3.73[b]
β-NGF (pg/mL)	345.42 ± 77.45	301.72 ± 69.66

Studies in rabbits have nevertheless reinforced the notion that the neural pathway is crucial for ovulation in this species, and that β-NGF could exert a modulatory role in the hypothalamus-pituitary-ovarian axis. Several studies have shown that a component of semen, probably β-NGF, can trigger ovulation in rabbits. After the intravaginal deposition of raw semen without the administration of a GnRH analog, ovulation occurs in a high percentage of females: 75% [63] and 67% [30]. Further, when semen diluted 1:5 was introduced, ovulation rates between 30 and 50% were recorded (30%: [78]; 32.5%: [79]; and 50% [80]). In all these studies, intravaginal deposition of semen was performed with the help of a catheter, which could act as a physical stimulus. Thus, when the influence of a mechanical stimulus was examined, our group and others determined that the nervous stimulus (by both sensory and sympathetic neurons) in rabbits seems essential to induce ovulation. An empty rigid seminal cannula introduced in the vagina without GnRH administration provoked ovulation in 37.5% of females [63] and this proportion was increased (64%) through the use of a long flexible cannula [78]. Unexpectedly, ovulation did not occur in does inseminated with raw semen after lumbar intraepidural anesthesia [63] or occurred at a low rate (16.7%) [30], although β-NGF concentrations increased in plasma even if there was no ovulation [30]. Thus, while epidural anesthesia produced sensory and motor blockade at the spinal level, it did not affect β-NGF release because it was probably derived from uterine synthesis [30]. The sympathetic innervation of the uterus arises from different origins: the upper part of the uterus only receives axonal projections from the ovarian nerves, whereas the lower uterus and cervix arise from lumbar paravertebral ganglia [81]. Perhaps, β-NGF is locally secreted in the upper part of uterus and ovary as epidural anesthesia only affects the lower uterus.

Blockage of the adrenergic system through a mixture of 60 μg/kg medetomidine +10 mg/kg ketamine +0.2 mg of midazolam given

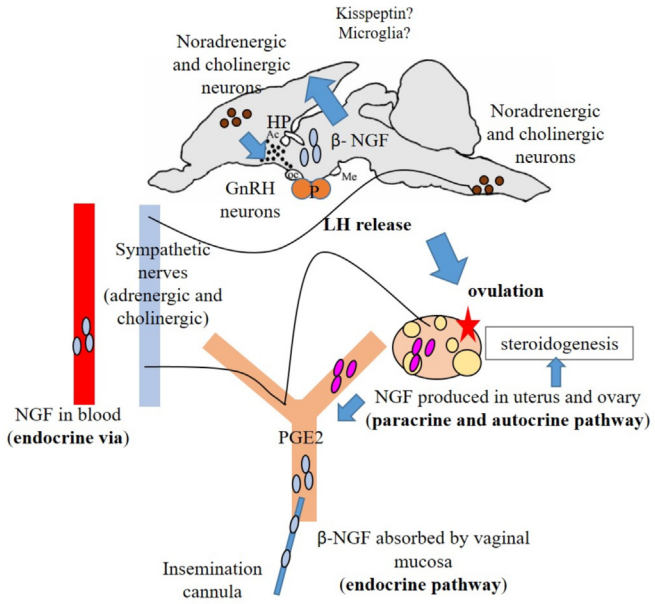

Fig. 3. Hypothesis of modulatory role of β-NGF in rabbit ovulation. rrβ-NGF introduced in the seminal dose is absorbed rapidly from the vaginal mucosa into the bloodstream and through the endocrine pathway reaches the third ventricle of the hypothalamus. Via an unknown mechanism (stimulation of kisspeptin neurons by neuropeptide Y or substance P, or microglia cells), β-NGF regulates GnRH secretion in the mediobasal hypothalamus and LH release by the pituitary gland. The nervous stimulus provoked by the insemination cannula seems crucial in this species, and β-NGF could modulate the activation of GnRH neurons by noradrenergic and cholinergic sympathetic neurons in the hypothalamus and midbrain. In addition, β-NGF could promote a paracrine and local effect in the uterus (producing PGE2, which participate in luteogenesis) and in the ovary (promoting steroidogenesis and thus estradiol, essential for GnRH release and estrous behavior). HP: hypothalamus, P: pituitary, Ac: anterior commissure, Me: median eminence, Oc: optic chiasm. Black dots represent GnRH neurons and brown dots noradrenergic and cholinergic neurons. Blue ellipses represent β-NGF in the seminal plasma or administered by the intravaginal route; pink ellipses indicate locally produced β-NGF in the female reproductive tract. (Brain diagram adapted from Ref. [61]).

via the epidural route in rabbits inseminated with semen diluted 1:5 plus 1 μg/mL rrβ-NGF avoids the LH surge (analyzed over 4 h) and also prevents the progesterone rise at 7 days compared to rrβ-NGF-treated does without this blockade (Fig. 2) [80]. Coitus in rabbits activates norepinephrine (NE) neurons and the release of hypothalamic NE [82]. In addition, the mammalian ovary has noradrenergic receptors in granulosa and theca cells that play a role in steroidogenesis [83]. Medetomidine could reduce the release and reuptake of NE in the central nervous system [84]. As mentioned earlier, sympathetic innervation is very significant in the female reproductive tract. In the ovary, oviduct [85] and upper uterus [81], adrenergic innervation is the most abundant and synapses with the hypothalamus. Interruption of noradrenergic inputs to the hypothalamus, depletion of catecholamine stores or synthesis, or blockade of adrenergic receptors modify gonadotropin secretion in rabbits [84]. Thus, in our study in which the mechanical stimulus was blocked by epidural anesthesia, locally produced or exogenously administered β-NGF could not elicit the LH peak, since sympathetic neurons were blocked by demetomidine. However, β-NGF and its receptors do not occur in noradrenergic neurons of the SNC in mammals [86], and cholinergic neurons expressing TrkA and p75 receptors are the main β-NGF-responsive neurons in the basal forebrain [86]. Sensory nerves compete with sympathetic nerves for β-NGF [87]. We therefore propose that, together, noradrenergic and cholinergic neurons may be more responsible than sensory nerves for GnRH activation.

Another facilitating role of β-NGF at the uterine and ovarian level could be related to endocrine, autocrine and/or paracrine mechanisms. Systemic β-NGF concentrations increase significantly up to 3–6 fold 15 min after natural mating or following the artificial deposition of raw semen in the vagina of receptive females [30]. In camelids, β-NGF can rapidly reach the nervous system via the endocrine pathway after its absorption from the vaginal mucosa. After the i.v. administration of an OIF it increases in the cerebrospinal fluid, suggesting that OIF can also cross the blood–brain barrier reaching the hypothalamus in this species [88]. However, the subsequent mechanism eliciting LH is unknown. According to Valderrama et al., the ovary can participate in a local manner by producing β-NGF thus enhancing oocyte ovulation and steroidogenesis [48].

As in spontaneously ovulating species, elevated systemic estradiol concentrations are required for estrous behavior and receptivity in induced ovulation females [61]. In both camelids and rabbits, the quantity of estradiol seems critical. It is known that estradiol levels affect pituitary LH secretion triggered by β-NGF [89] and GnRH neurons respond to estrogen because they express ERα and ERβ in several species [90]. In camelids, El Allali et al. [72] hypothesized that in kisspeptin-secreting neurons, β-NGF along with estradiol could promote the release of kisspeptin when β-NGF binds to TrkA and estradiol to its ERα receptor. These authors argued that kisspeptin could then bind to the G-protein coupled receptor GPR54 on GnRH neurons to trigger the release of GnRH inducing LH secretion. In rabbits, estradiol may also be a key factor. When we inseminated non-receptive females with rrβ-NGF in the seminal dose, the LH surge was delayed 2 h [49] yet was not prolonged in time as occurs in camelids [91]. This delay was not observed in ovulating sexual receptive females with high plasma estradiol levels inseminated with raw semen [30]. Nerve growth factor beta seems to participate in the steroidogenesis process in the ovary and in estrogen secretion [92]. Accordingly, when rabbit females inseminated with rrβ-NGF are not stimulated with eCG and presumably are not receptive, estradiol concentrations from non-mature follicles could be locally enhanced by β-NGF in the ovary. This process could mediate the stimulatory actions of ovarian steroids that lead to GnRH release [61]. However, as follicles are not mature, estradiol levels would have to increase to reach the threshold to trigger action potential firing of GnRH neurons [90] and this could perhaps explain the delay in the LH peak. In addition, ovarian estradiol binds interneurons [90]. In rabbits, these interneurons could be kisspeptin neurons modulated by neuropeptide Y or substance P with a known role in LH release in rabbits [93]. These hypotheses need further clarification in studies in rabbits.

Nerve growth factor beta may be also regulated by sex hormones (estradiol and progesterone) through modulation of TrkA expression [94]. Nonetheless, circulating β-NGF concentrations in rabbit does stimulated with eCG before and after ovulation were reported similar (345.42 ± 77.45 and 301.72 ± 69.66 pg/mL, respectively) and no correlation was found with serum steroid levels (Table 2) [31]. In rabbits, estrogens are also essential for physiological luteal function [95].

Based on all these available data for rabbits, we here propose a plausible modulatory role of β-NGF in ovulation induction in rabbit females (Fig. 3).

4. Perspectives and future applications

Artificial insemination is common practice in commercial rabbit farms, and requires the use of exogenous hormones such as GnRH and its analogs to elicit the LH peak and thus induce ovulation. These compounds are routinely given by the i.m. route. However, i.m. administration is time-consuming, invasive and stressful with

known effects on the longevity and welfare of does [96]. Further, it can have detrimental effects on ovarian physiology including oocyte maturation [97]. Research efforts have been therefore targeted at avoiding the i.m. route by including GnRH analogs in the seminal dose. This practice, nevertheless, also has drawbacks such as the high doses needed to induce ovulation [63] along with enzyme degradation of the protein [98].

Adding β-NGF to the semen extender to prepare ready-to-use seminal doses could be an interesting strategy to avoid such hormone treatments. Besides, β-NGF could be a natural ovulation-inducing factor in rabbit breeding as it is naturally present in semen. However, when semen is used for AI, the concentration of this factor is reduced when the semen dose is diluted with extender. Growth factors usually have a short half-life when administered *in vivo* and it is necessary to protect them. Thus, additives in the semen extender (enzyme inhibitors) could help [99]. Another promising option is nanoencapsulation such as, for example, the slow release of β-NGF incorporated in chitosan microspheres [100]. Besides, the dose, source or application method of rrβ-NGF along with sexual receptivity and mechanical stimulus for ovulation induction in rabbits could be intriguing targets of investigation for further research.

Finally, the effect of β-NGF on oocyte maturation, steroidogenesis, and embryo development in rabbits could be examined using *in vitro* culture systems providing an exciting model to understand the potential local and paracrine effects of β-NGF on ovulation, oocyte competence and early embryo development.

5. Concluding remarks

The role of β-NGF in rabbit reproduction remains unclear. According to recent findings, it could play a modulatory role in inducing ovulation through endocrine, paracrine or autocrine pathways. However, it seems that the nervous stimulus, mainly via sympathetic neurons, is the factor responsible for eliciting GnRH release in this species. Local β-NGF production in the ovary could promote steroidogenesis, which is likely an important mechanism for sufficient estradiol production to trigger the LH/GnRH surge. Available data further indicate that the adrenergic system may also contribute to steroidogenesis, determining that both β-NGF and adrenergic mechanisms could be necessary for the regulation of ovulation besides the physical stimuli that play a major role in the female rabbit.

Based on all these lines of evidence, the use of rrβ-NGF in the seminal dose combined with new cannulas to stimulate the nervous system could be a novel strategy for rabbit reproduction on farms. In future studies, the mechanisms whereby β-NGF intervenes in reproduction will need to be elucidated.

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