INTRODUCTION

Neurotrophins (NTs) are small, homodimeric polypeptide growth factors that regulate the survival, maintenance, and differentiation of neurons in the nervous system (Ibáñez, 1995; Anderson et al., 2002; Paredes et al., 2004). They also act on non-neural cells, such as those in the reproductive system (Spears et al., 2003; Levanti et al., 2005; Kawamura et al., 2007). They include the nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins 3 and 4/5 (NT3 and NT4/5), which function through two different types of receptors, namely, tyrosine kinase (Trk) (i.e., TrkA for NGF, TrkB for BDNF and NT4, and TrkC for NT3) and p75 receptors. When the NTs bind to their receptors, they activate a specific Trk domain, resulting in a rapid increase in the phosphorylation of second messengers and other specific cellular components (Dissen et al., 1995; Fridman et al., 1999; Dissen et al., 2000).

The development of the mammalian ovary is initiated

Expression of Neurotrophin 4 and Its Receptor Tyrosine Kinase B in Reproductive Tissues during the Follicular and Luteal Phases in Cows*

Yongfeng Sun1,2,a, Chunjin Li1,a, Yanling Sun1, Lu Chen1, Zhuo Liu1, Yonghe Ma1, Chunqiang Wang1,3, Wei Zhang1 and Xu Zhou1,2,***

1College of Animal Science and Veterinary Medicine, Jilin Provincial Key Laboratory of Animal Embryo Engineering, Jilin University, Changchun, 130062, China

ABSTRACT : The neurotrophins, required for the survival and differentiation of the nervous system, are known to be important for the development of the reproductive tissues. However, the signals initiating the growth of follicles, gamete development, and transport and the development of zygote in the reproductive system of cows remain ambiguous. The purpose of the present study was to identify the transcripts and proteins of Neurotrophin 4 (NT4) and its receptor tyrosine kinase B (TrkB) in bovine reproductive tissues. The transcripts and immunoreactivity of NT4 and TrkB proteins were detected by reverse transcription polymerase chain reaction and western blot analysis. Using immunohistochemistry, the specific immunoreactivity of NT4 and TrkB were detected in the oocytes of primordial follicles and in the growing primary follicles. The NT4 and TrkB immunoreactivity was predominantly observed in granulosa cells, cumulus granulosa cells, cumulus oocyte complexes, theca cells of mature follicles, as well as in the oviduct epithelial cells, uterine gland cell, and epithelium cells of the uterus during the follicular and luteal phases in cows. Expressions of NT4 and TrkB mRNAs were not significantly different among the ovary, oviduct, and uterus of the follicular phase. For the luteal phase, the expression of NT4 mRNA in the ovary was significantly higher than that in the oviduct and uterus, and the expression of TrkB mRNA in the ovary was significantly higher than that in the ovary and uterus, as determined by fluorescence quantitative reverse transcription polymerase chain reaction. The expression of NT4 mRNA was significantly higher than that of TrkB mRNA in the ovary and uterus, whereas NT4 mRNA expression was lower than that of TrkB mRNA in the ovary during the luteal phase. The present study hypothesizes that NT4 participates in the regulation of both gonads and extra-gonadal reproductive tissues in cows. (*Key Words : Cows, Neurotrophin 4, Tyrosine Kinase B, Follicular Phase, Luteal Phase, Expression)
after migration of the primordial germ cells, which proliferate during and after migration (Witschi, 1948; Byskov, 1986). However, the factors and pathways involved are largely unknown. In the past few years, many results show that neurotrophins and their receptors play a critical role in the development of the mammalian ovary, oogenesis, folliculogenesis, and embryonic development, and in BDNF, NGF, and NT3 (via uterine tract immunolocalization) in rodents (Bjorling et al., 2002; Krizsan-Agbas et al., 2003; Shi et al., 2006), and in NGF in goats (Ren et al., 2005), identified at the mRNA or protein levels. Although much information on the reproduction of a mammal has been reported, there is no any study on the expression and distribution of NT4 and its receptor TrkB in the reproductive tissues of cows during the follicular and luteal phases. To elucidate further the role of NT4 in the female reproductive system and facilitate attempts to improve understanding of the mechanisms involved in infertility and embryo development, the presence of NT4 and TrkB in the ovaries, oviducts, and uteri of adult cows during the follicular and luteal phases was investigated.

Here, the NT4 and TrkB transcripts and proteins were detected in the ovary, oviduct and uterus during the two phases, by using immunohistochemistry, fluorescence quantitative real-time polymerase chain reaction (FQ-RT-PCR) and Western blot.

**MATERIALS AND METHODS**

**Sample preparation**

The ovaries, oviducts and uteri of adult native yellow cows were obtained from a public slaughterhouse. The samples were collected within 15 min after slaughter, and then kept at -196°C. Groups were classified according to the follicle size or the presence of the corpus luteum. The follicular phase was defined as the period when the ovary lacks the corpus luteum, and the largest follicle size was more than 5 mm in diameter, whereas the luteal phase was defined as the period when the ovary already has a corpus luteum. For each phase, tissue samples were collected from five animals (n = 5). Tissue pieces were excised from the ovary, uterus, oviduct, and then fixed for 24 h in Bouin’s fixative, and subsequently processed for routine paraffin embedding.

**RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)**

Total RNA was extracted from the samples using Trizol reagent (Invitrogen Life Technologies Inc., USA) and cDNA was frozen until used. Specific primers for each gene are shown in Table 1. PCR was performed in reaction mixtures with a final volume of 25 μl containing 1 μl (100 ng) of cDNA, 2.5 μl of 10×Buffer, 0.2 μl of each primer, and 0.20 units of rTaq polymerase (TaKara Biotechnology, Otsu Shiga Japan). RNA samples incubated without reverse transcriptase served as negative controls. PCR products were separated in an agarose gel, eluted, and then sequenced using an ABI 377 DNA sequencer (Applied Biosystems, Foster, CA, USA), with M-13F/R primers (TransGen Biotech Co., Beijing, China).

**Quantitative real-time PCR**

Real-time PCR was performed using the ABI PRISM 7000 (ABI, America) according to the previously reported (Li et al., 2010). The primers and probes for RT-PCR are listed in Table 1. To normalize the amount of expressed NT4 and TrkB mRNAs, the internal housekeeping gene GAPDH was used, and each cDNA product was tested in triplicate. A standard curve was generated and used to evaluate the relative expression of the NT4 and TrkB genes in terms of the ratio (fold difference) of the target gene expression to the control gene expression.

**Immunohistochemistry**

The tissues were embedded in paraffin, and 5 μm-thick sections were cut. Slides were blocked with normal goat serum (10%) in PBS for 30 min at room temperature. They were then incubated with a primary antibody (anti-NT4 or

**Table 1. Details of primers and probes used for FQ-RT-PCR on bovine reproductive tissues**

| Gene type | Primer sequence 5'- 3' | Annealing temperature (°C) | Product length (bp) | Accession number |
|-----------|------------------------|-----------------------------|---------------------|------------------|
| NT4       | Forward :AGTCTCTACGTGCGGGCATT  
Reverse : CACAGGCCAGTGCAATTCGAA  
TAQMAN-PI:FCACCGATGCCAGGGCCGTP | 59 | 78 | XM_583142 |
| TrkB      | Forward :GGAAAGTAAAAATCGAGAAGGGTGT  
Reverse :CAAATGTTATGTGCTGCTGATGTCG  
TAQMAN-PI: CAATGATGATGACTCTGCCAGCCCA | 59 | 246 | NM_006180 |
| GAPDH     | Forward :GCCGCAAGAGGGACAT  
Reverse : GGTCGTCAGGAGGCCAT  
TAQMAN-PI:FTACTTCTCGTGGTTCAGCCCATCACA | 59 | 120 | NM_001034034 |

F = FAM, P = TAMRA.
TrkB) at 4°C for 24 h. Following several washes with PBS, the slides were exposed to goat anti-rabbit IgG, conjugated with fluorescein isothiocyanate (FITC, 1:200; BOSTER, China) or diaminobenzidine (DAB, 1:200; BOSTER, China) for 60 min at room temperature, and were then washed with PBS. Negative controls were prepared using bovine serum albumin (BSA) instead of primary antibody diluted with PBS. Finally, the slides were examined under a confocal laser microscope (Olympus, Japan) and optical microscope (Olympus, Japan). Sections of each sample were examined in triplicate for both positive antibody staining and negative controls.

Western blot analysis
Total proteins were extracted according to the manufacturer’s instructions (APPLYGEN, China). Approximately 20 μg protein from each sample was separated by SDS-PAGE on a 12% polyacrylamide gel. The separated proteins were transferred onto PVDF membranes (Millipore) using an appropriate transfer system at 60 V for 2 h. Membranes were incubated with blocking solution (5% solution of nonfat dry milk in TBST) for 2 h at room temperature, and then with primary antibody (NT4 or TrkB) at 4°C overnight. The membranes were washed with TBST thrice for 15 min, and then incubated with HRP-conjugated goat anti-rabbit secondary antibody (1:50,000 in TBST) for 2 h at room temperature. The membranes were then washed several times until the proteins were detected using Super Signal substrate (Pierce) and exposure to x-ray film.

Statistical analysis
The experiments for RT-PCR, Western blot, and Immunohistochemistry were repeated at least four times, and data are expressed here as means±SEM. Statistical analyses were performed using one-way ANOVA (as implemented in SPSS 13.0. software), and Dunnet's test was applied for multiple comparison. A value of p<0.05 was taken to indicate a statistically significant difference between means. The number of all tissues and cDNA samples has been described in the materials and methods.

RESULTS
Expression of NT-4 and TrkB transcripts
The NT4 and TrkB mRNA were detected in the ovary, oviduct, and uterus during the follicular and luteal phases, and specific fragments of predicted sizes, 78 bp (NT4) and 246 bp (TrkB-fl) were obtained from the tissues (Figure 1). The NT4 and TrkB-fl genes were cloned by PMD-18T vector and detected by an automated DNA sequencer. Sequences of these products were found completely homologous to that of the corresponding region in NT4 (XM_583142) and TrkB (NM_006180). The absence of DNA was confirmed by the amplification of a single product from GAPDH with the expected size for RNA (120 bp) (Figure 1), as no staining was visible in the case of negative controls wherein the template was replaced by sterile water (Figure 1).

The FQ-RT-PCR results show no significant difference in the expressions between NT4 mRNA and TrkB mRNA in the ovaries, oviducts, and uteri in the follicular phase (Figure 2C). However, the expression of NT4 mRNA (Figure 2A) in the ovaries was predominantly higher than that in the oviducts and uteri, and the expression of TrkB mRNA (Figure 2B) in the oviducts was significantly higher than in the ovaries and uteri for the luteal phase (p<0.05). In addition, the expression of NT4 mRNA was significantly higher than that of TrkB mRNA in the ovaries and uteri, whereas NT4 expression was lower than TrkB in the

Figure 1. Results of RT-PCR analysis of NT4, TrkB-fl and GAPDH mRNA expression in bovine reproductive tissues. The products of NT4 (A), TrkB (B), and GAPDH (C) were detected in the ovary (lane 1), oviduct (lane 2), and uterus (lane 3) during the luteal phase; and the ovary (lane 4), oviduct (lane 5) and uterus (lane 6) during the follicular phase. The negative controls, for which no bands were detected in NT4 (D), TrkB (E), and GAPDH (F) (water as template).
Immunolocalization of NT4 and TrkB in cow reproductive tissues

NT4 and TrkB immunoreactivity were observed in the specimens during both phases. NT4 and TrkB are seen not only in the mature follicles of granulosa cells, cumulus granulosa cells, cumulus oocyte complexes, oocytes of primordial follicles, and growing-type primary follicles of the follicular phase (Figure 3), but also in the granulosa cells of mature follicles, single layer flat epithelials of primordial follicles, and vascular smooth muscle cells of the luteal phase (Figure 4). Their immunoreactivity were predominantly seen in the oviduct epithelium, and in the uterus mucosa epithelium cells and uterine gland at the follicular and luteal phases, respectively (Figures 3 and 4). The positive control was from the brain of adult cows. No staining was visible in the negative controls, in which primary antibodies were replaced by BSA (Figures 3 and 4).

Western blotting

The presence of NT4 and TrkB proteins in the specimens in both phases in were investigated by Western blotting with rabbit anti-human NT4 and TrkB polyclonal antibody. Specific bands corresponding to NT4 and TrkB were detected in the extracts of the reproductive tissue proteins (Figure 5). No bands were visible in negative controls where the antibody was replaced by normal goat serum (Figure 5).

**DISCUSSION**

The Tyrosine kinase receptors and their ligands are present in the genital tract of several mammals (Yeh et al., 1993; Gupta et al., 1997; Krizsan-Agbas et al., 2003; Levanti et al., 2005; Ren et al., 2005; Shi et al., 2006; Buratini et al., 2007) and promote oocyte developmental competence, early embryonic development, and implantation (Kawamura et al., 2003; De Sousa et al., 2004; Kawamura et al., 2005; Betancourt-Alonso et al., 2006). NT4 is a member of the neurotrophin protein family that plays important roles in the regulation of neuronal survival and differentiation mediated by TrkB. Increasing evidence has shown that neurotrophins may play a specific role in the...
development of reproductive tissues. Notably, NGF activation of TrkA in cultured thecal cells is reported to be involved in the disruption of gap junctions (Mayerhofer, 1996). Therefore, the possible communication between NT4 and TrkB in the reproductive tissues of cows under the two phases was explored. In the present study, evidence for the presence of NT4 and TrkB mRNAs and proteins in bovine ovary, oviduct, and uterus at the follicular and luteal phases, and immunological indications of their localization were obtained. In addition, BDNF and TrkB involves both autocrine and paracrine signaling within bovine oocyte-cumulus Cell Complex (COCs) (Martins et al., 2005), indicating that NT4 and its TrkB receptor may have a role in the development of these cells, and that the distribution of the TrkB receptor may contribute to the regulation of NT4 signaling in the immediate environment of germ and somatic cells in the bovine reproductive tract.

Oocytes are known to interact with somatic cells to form primordial follicles and survive (McLaren et al., 1991). In humans, NT4 protein was localized to the granulosa cells by immunohistochemistry, and at the early developmental stages of epithelioid cells, the TrkB receptor was also localized by immunohistochemistry to the germ cells, as observed from all examined gestations in a previous study (Richard et al., 2002). Both TrkA and TrkC receptors express immunoreactivity in the bovine follicular cells (Muñoz et al., 2009), and NGF and TrkA proteins are expressed in granulosa cells, where NGF and its receptors play an essential role in the ovulation process (Dissen et al., 2000). The results are consistent with a previous study demonstrating granulosa cells as possible sources of NGF (Mattioli et al., 1999). BDNF plays a role in conferring oocyte cytoplasmic competence to support early embryo development, and that this may involve both autocrine and paracrine signaling within the COCs (Martins et al., 2005). This study shows that tissue specificity of NT4 and its TrkB receptor was expressed in the ovary. Moreover, NT4 and its receptor TrkB expression traits are similar to the results of other neurotrophins in the mammals, such as rats (Dissen et al., 1995), monkey (Shimizu et al., 2002), mice (Dissen et al., 2001), and cow (Dissen et al., 2000). Furthermore, NT4 mRNA was lower in the ovary during the follicular phase.
than the luteal phase. The behavior of \( TrkB \) mRNA was contrary to that of \( NT4 \), by FQ-RT-PCR. BDNF is secreted by the granulosa and cumulus cells as an ovarian factor stimulated by the preovulatory LH surge, which enhances the first polar body extrusion of oocytes (Kawamura et al., 2005). Hence, \( NT4 \) may play a different regulatory role in the follicular development and ovulation through autocrine and paracrine pathways in bovine ovarian development.

The oviduct can secrete many growth factors, such as EGF, TGF, IGF, and activin (Schell et al., 1994; Gandolfi et al., 1995). In this experiment, the immunoreactions for \( NT4 \) and \( TrkB \) were also detected in the epithelium of the oviduct. This is the first report of the expression of \( NT4 \) in the cow oviduct under the two phases. \( BDNF \) significantly increased the proportions of MII oocytes at both 10 ng/ml in \textit{in vitro} mature (Hong et al., 2009). In addition, \( BDNF \) plays a role in conferring oocyte cytoplasmic competence to support early embryo development, independently of nuclear maturation. This may involve both autocrine and paracrine signaling within the COCs (Martins et al., 2005). The

![Figure 4. Detection of the \( TrkB \) in the bovine ovary, oviduct, and uterus during the two phases. \( TrkB \) immunoreactivity is seen in the granulosa cells of mature follicles (A), oocyte (G1, long arrows), cumulus granulosa cells, COCs (G1, short arrows), oocytes of primordial follicles (H, short arrows), and in growing-type primary follicles (H, long arrow) during the follicular phase. \( TrkB \) is detected in the granulosa cells of mature follicles (D), single layer flat epithelial of the primordial follicles (I, short arrows), and vascular smooth muscle cells (I, long arrows) during the luteal phase. \( TrkB \) immunoreactivity is predominantly seen in the oviduct epithelium during the follicular (B) and luteal phases (E). It is also found in the uterus mucosa epithelium cells (I, C) and uterine gland (K, F) during the follicular and luteal phases, respectively. Positive control of bovine brain from adult cows (L) is also shown. Section incubated with \( TrkB \) antibodies preadsorbed with the \( TrkB \) peptide used to raise the antibodies is shown in (M). A-F: Original magnification \( \times 40 \). G1, H-M: Original magnification \( \times 400 \). G: Original magnification \( \times 100 \).](image)

![Figure 5. Western blot analysis of the \( NT4 \) and \( TrkB \) protein. Protein extracts from bovine uterus (lane 1), ovary (lane 2), and oviduct (lane 3) during the follicular phase; and the uterus (lane 4), ovary (lane 5), and oviduct (lane 6) during the luteal phase.](image)
present results indicate that NT4 may play a role in the capacitation of spermatozoa and early embryonic development in the oviduct during the follicular phase. They have shown that BDNF promotes the in vitro development of zygotes into preimplantation embryos (Kawamura et al., 2005). However, there no statistically significant difference in the blastocyst development in in vitro culture was found (Hong et al., 2009). In this study, the low expression of NT4 during the luteal phase, suggests that BDNF is not the only factor that functions during the transport and early embryonic development, but also TrkB has another ligand, NT4, that participates in the regulation of the bovine oviduct during the luteal phase.

In the present study, immunoreactivity for NT4 and TrkB were observed in the endothelial and uterine gland cells of the uterus, which is similar to NGF and TrkA responses in the Shiba goat (Ren et al., 2005). The identification of NT4 in the uterus raises the question of its role in the function of this organ. The receptivity detected in the endometrium is the main factor that influences embryo implantation. However, the state of receptivity detected in the endometrium prior to embryo implantation is coordinated and regulated by estrogen, progesterone, and other reproductive regulation factors. Given that NT4 primarily acts as a target-derived neurotrophic factor, one of its possible roles is to attract and maintain the sympathetic and sensory innervation of the uterus. In this study, the NT4 and TrkB mRNA expression diversity in the bovine uterus under the follicular and luteal phases, indicate that NT4 may play a role in the embryonic development, transport, and embedding implantation by autocrine or paracrine signaling. Other autocrine/paracrine functions suggested for the neurotrophins in the other tissues (Zettler et al., 1991; Donovan et al., 1995) should be considered as well.

In conclusion, NT4 and its receptor are expressed in the ovary, oviduct and uterus of the native cow during the follicular and luteal phases. The information provided in this study may strongly aid in understanding the potential roles of NT4/TrkB in the mammalian reproductive mechanism.

REFERENCES

Anderson, R. A., L. L. Robinson, J. Brooks and N. Spears. 2002. Neurotrophins and their receptors are expressed in the human fetal ovary. J. Clin. Endocrinol. Metab. 87:890-897.
Betancourt-Alonso, M. A., F. I. Flores-Pérez, C. Rosas-Velasco and M. Pérez-Martinez. 2006. Role of cytokines in embryo implantation in domestic mammals. Vet. Mex. 37:335-350.
Björling, D. E., M. Beckman, M. K. Clayton and Z. Y. Wang. 2002. Modulation of nerve growth factor in peripheral organs by estrogen and progesterone. Neuroscience 110:155-167.
Buratiní, J. Jr., M. G. Pinto, A. C. Castilho, R. L. Amorim, I. C. Giometti, V. M. Portela, E. S. Nicola and C. A. Price. 2007. Expression and function of fibroblast growth factor 10 and its receptor, fibroblast growth factor receptor 2B, in bovine follicles. Biol. Reprod. 77:743-750.
Byskov, A. G. 1986. Differentiation of mammalian embryonic gonad. Physiol. Rev. 66:71-117.
De Sousa, P. A., S. J. da Silva and R. A. Anderson. 2004. Neurtrophin signaling in oocyte survival and developmental competence: a paradigm for cellular toti-potency. Cloning Stem Cells 6:375-385.
Dissen, G. A., A. N. Hirshfield, S. Malamed and S. R. Ojeda. 1995. Expression of neurotrophins and their receptors in the mammalian ovary is developmentally regulated: changes at the time of folliculogenesis. Endocrinology 136:4681-4692.
Dissen, G. A., J. A. Parrott, M. K. Skinner, D. F. Hill, M. E. Costa and S. R. Ojeda. 2000. Direct effects of nerve growth factor on theclic cells from antral ovarian follicles. Endocrinology 141:4736-4750.
Dissen, G. A., C. Romero, A. N. Hirshfield and S. R. Ojeda. 2001. Nerve growth factor is required for early follicular development in the mammalian ovary. Endocrinology 142:2078-2086.
Donovan, M. J., R. C. Miranda, R. Kraemer, T. A. McCaffrey, L. Tessarollo, D. Mahadeo, S. Sharif, D. R. Kaplan, P. Tsorfas, L. Parada, C. D. Toran-Allerand, D. P. Hajjar and B. L. Hempstead. 1995. Neurotrophin and neurotrophin receptors in vascular smooth muscle cells. Regulation of expression in response to injury. Am. J. Pathol. 147:309-324.
Fridman, W. J. and L. A. Greene. 1999. Neurotrophin signaling via Trks and p75. Exp. Cell Res. 253:131-142.
Gandolfi, F., S. Modina, T. A. Brevini, L. Passoni, P. Artini, F. Petraglia and A. Lauria. 1995. Activin beta A subunit is expressed in bovine oviduct. Mol. Reprod. 40:286-291.
Gupta, A., F. W. Bazer and L. A. Jaeger. 1997. Immunolocalization of acidic and basic fibroblast growth factors in porcine uterine and conceptus tissues. Biol. Reprod. 56:1527-1536.
Hong, S. G., G. Jang, H. J. Oh, O. J. Koo, J. E. Park, H. J. Park, S. K. Kang and B. Lee. 2009. The effects of brain-derived neurotrophic factor and metformin on in vitro developmental competence of bovine oocytes. Zygote 17:187-193.
Ibáñez, C. F. 1995. Neurotrophin factors: from structure-function studies to designing effective therapeutics. Trends Biotechnol. 13:217-227.
Kawamura, K., N. Kawamura, J. Fukuda, J. Kumagai, A. Hsueh and T. Tanaka. 2007. Regulation of preimplantation embryo development by brain-derived neurotrophic factor. Dev. Biol. 311:147-158.
Kawamura, K., N. Kawamura, S. M. Mulders, M. D. Sollewijn Gelpke and A. J. Hsueh. 2005. Ovarian brain-derived neurotrophic factor (BDNF) promotes the development of oocytes into preimplantation embryos. Proc. Natl. Acad. Sci. USA 102:9206-9211.
Kríasán-Agbas, D., T. Pedchenko, W. Hasan and P. G. Smith. 2003. Oestrogen regulates sympathetic neurite outgrowth by modulating brain derived neurotrophic factor synthesis and release by the rodent uterus. Eur. J. Neurosci.18:2760-2768.
Levanti, M. B., A. Germana, F. Abbate, G. Montalbano, J. A. Vega and G. Germana. 2005. TrkA and p75NTR in the ovary of adult cow and pig. J. Anat. 207:93-96.
Li, C. J., L. W. Zheng, C. Q. Wang and X. Zhou. 2010. Absence of
nerve growth factor and comparison of tyrosine kinase receptor A levels in mature spermatozoa from oligoasthenozoospermic, asthenozoospermic and fertile men. Clin. Chim. Acta. 411:1482-1486.

Martins, da Silva, S. J., J. O. Gardner, J. E. Taylor, A. Springbett, P. A. De Sousa and R. A. Anderson. 2005. Brain-derived neurotrophic factor promotes bovine oocyte cytoplasmic competence for embryo development. Reproduction 129:423-434.

Mattioli, M., B. Barboni, L. Gioia and P. Lucidi. 1999. Nerve growth factor production in sheep antral follicles. Domest. Anim. Endocrinol. 17:361-371.

Mayerhofer, A., G. A. Dissen, J. A. Parrott, D. F. Hill, D. Mayerhofer, R. E. Garfield, M. E. Costa, M. K. Skinner and S. R. Ojeda. 1996. Involvement of nerve growth factor in the ovulatory cascade: trkA receptor activation inhibits gap junctional communication between thecal cells. Endocrinology 137:5662-5670.

McLaren, A. 1991. Development of the mammalian gonad: the fate of the supporting cell lineage. BioEssays 13:151-156.

Muñoz, M., A. Rodríguez, C. Díez, J. N. Caamaño, M. T. Fernández-Sánchez, A. Pérez-Gómez, C. De Frutos, N. Facal and E. Gómez. 2009. Tyrosine kinase A, C and fibroblast growth factor-2 receptors in bovine embryos cultured in vitro. Theriogenology 71:1005-1010.

Paredes, A., C. Romero, G. A. Dissen, T. M. DeChiara, L. Reichardt, A. Cornea, S. R. Ojeda and B. Xu. 2004. TrkB receptors are required for follicular growth and oocyte survival in the mammalian ovary. Dev. Biol. 267:430-449.

Ren, L., M. S. Medan, Q. Weng, W. Jin, C. Li, G. Watanabe and K. Taya. 2005. Immunolocalization of nerve growth factor (NGF) and its receptors (TrkA and p75NGFR) in the reproductive organs of Shiba goats. J. Reprod. Dev. 51:399-404.

Schell, D. L., P. A. Mavrogiannis, A. T. Fazleabas and H. G. Verhage. 1994. Epidermal growth factor, transforming growth factor-alpha, and epidermal growth factor receptor localization in the baboon (Papio anubis) oviduct during steroid treatment and the menstrual cycle. J. Soc. Gynecol. Investig. 1:269-276.

Shimizu, K., W. Jin, C. Kojima, Z. Shi, M. Kondo, M. Itoh, G. Watanabe and K. Taya. 2002. Cellular localization of nerve growth factor (NGF) and its receptors TrkA and p75NGFR in the ovary of the Japanese monkey (Macaca fuscata fuscata). J. Reprod. Dev. 48:377-381.

Shi, Z., K. Y. Arai, W. Jin, Q. Weng, G. Watanabe, A. K. Suzuki and K. Taya. 2006. Expression of nerve growth factor and its receptors NTRK1 and TNFRSF1B is regulated by estrogen and progesterone in the uteri of golden hamsters. Biol. Reprod. 74:850-856.

Spears, N., M. D. Molinek, L. L. Robinson, N. Fulton, H. Cameron, K. Shimoda, E. E. Telfer, R. A. Anderson and D. J. Price. 2003. The role of neurotrophin receptors in female germ-cell survival in mouse and human. Development 130:5481-5491.

Witschi, E. 1948. Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal fold. Contrib. Embryol. 32:67-80.

Yeh, J. and R. Osathanondh. 1993. Expression of messenger ribonucleic acids encoding for basic fibroblast growth factor (FGF) and alternatively spliced FGF receptor in human fetal ovary and uterus. J. Clin. Endocrinol. Metab. 77:1367-1371.

Zettler, C., R. J. Head and R. A. Rush. 1991. Chronic nerve growth factor treatment of normotensive rats. Brain. Res. 538:251-262.