

Neurotrophins and glial cell line-derived neurotrophic factor in the ovary: physiological and pathophysiological implications

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BACKGROUND: Neurotrophins [nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4)] and glial cell line-derived neurotrophic factor (GDNF) are soluble polypeptide growth factors that are widely recognized for their roles in promoting cell growth, survival and differentiation in several classes of neurons. Outside the nervous system, neurotrophin (NT) and GDNF signaling events have substantial roles in various non-neuronal tissues, including the ovary.

OBJECTIVE AND RATIONALE: The molecular mechanisms that promote and regulate follicular development and oocyte maturation have been extensively investigated. However, most information has been obtained from animal models. Even though the fundamental process is highly similar across species, the paracrine regulation of ovarian function in humans remains poorly characterized. Therefore, this review aims to summarize the expression and functional roles of NTs and GDNF in human ovarian biology and disorders, and to describe and propose the development of novel strategies for diagnosing, treating and preventing related abnormalities.

SEARCH METHODS: Relevant literature in the English language from 1990 to 2018 describing the role of NTs and GDNF in mammalian ovarian biology and phenotypes was comprehensively selected using PubMed, MEDLINE and Google Scholar.

OUTCOMES: Studies have shown that the neurotrophins NGF, BDNF, NT-3 and NT-4 as well as GDNF and their functional receptors are expressed in the human ovary. Recently, gathered experimental data suggest putative roles for NT and GDNF signaling in the direct control of ovarian function, including follicle assembly, activation of the primordial follicles, follicular growth and development, oocyte maturation, steroidogenesis, ovulation and corpus luteum formation. Additionally, crosstalk occurs between these ovarian regulators and the endocrine signaling system. Dysregulation of the NT system may negatively affect ovarian function, leading to reproductive pathology (decreased ovarian reserve, polycystic ovary syndrome and endometriosis), female infertility and even epithelial ovarian cancers.

WIDER IMPLICATIONS: A comprehensive understanding of the expression, actions and underlying molecular mechanisms of the NT/GDNF system in the human ovary is essential for novel approaches to therapeutic and diagnostic interventions in ovarian diseases and to develop more safe, effective methods of inducing ovulation in ART in the treatment of female infertility.

Key words: nerve growth factor / brain-derived neurotrophic factor / neurotrophins / glial cell line-derived neurotrophic factor / follicular development / diminished ovarian reserve / polycystic ovary syndrome / endometriosis / female infertility

Introduction

The mammalian ovary is a highly innervated organ that contains sympathetic and sensory nervous system fibers. These nerve fibers can reach most of the intraovarian compartments, including the ovarian artery, interstitial tissue and ovarian follicle (Malamed *et al.*, 1992). A rat ovary that was transplanted to an ectopic site was promptly reinnervated by both sympathetic and sensory nerve fibers (Lara *et al.*, 1991), indicating that the ovary could produce certain substances that promote the rapid ingrowth of such nerve fibers from the surrounding tissues. Indeed, evidence supported by animal studies showed that the innervated rat ovary was able to produce and secrete nerve growth factor (NGF), which was stimulated and regulated by the innervation (Lara *et al.*, 1990). In the rhesus monkey ovary, a two-fold increase in the density of ovarian innervation occurred during prepubertal development, suggesting that NGF exerts its trophic effects on the development of ovarian sympathetic neurons that contribute to ovarian development and reproductive capacity (Schulthea *et al.*, 1992). During mammalian development, the tissue-derived neurotrophic and neural guidance factors are essential to determine the proper directionality of the related tissues and organs, including heart, intestine and airways (Keast, 2013). Dysregulation of these neurotrophic and neural systems can cause defects of innervation that have been associated with various human

neurological diseases, including Alzheimer's disease, Huntington's disease, Parkinson's disease, neuroblastoma, multiple sclerosis-like model of experimental allergic encephalomyelitis, peripheral neuropathies and even motion sickness (Calissano *et al.*, 2010).

Neurotrophic factors are divided into the following main families: neurotrophins (NTs) and glial cell line-derived neurotrophic factor (GDNF; Airaksinen and Saarma, 2002; Chao, 2003). NTs were initially discovered as molecules that can promote the survival of developing neurons that newly innervate or terminate in their target peripheral tissues; these molecules constitute a class of polypeptide growth factors that are critical for the development, maintenance and regeneration of the central and peripheral nervous system (Hamburger, 1952). Since then, neurotrophic factors and their putative receptors have also been found to play substantial roles in various peripheral tissues, including the highly innervated ovary. Furthermore, experimental data gathered from clinical samples have demonstrated that these neurotrophic factors, including brain-derived neurotrophic factor (BDNF) and GDNF, are highly expressed in the human granulosa cells (GCs) in the absence of innervation (Zhao *et al.*, 2011). Similar to the bidirectional communication between neurons and glial cells, NTs, together with other hormones and growth factors, exert a considerable effect on both germ cells and somatic cells during the developmental stages of folliculogenesis (Davies and Wright, 1995; Dissen *et al.*, 2009). Over the past few

decades, the effects of NTs on growing ovarian follicles have been well studied with regard to follicular assembly, growth, survival and apoptosis (for reviews, see [Ojeda et al., 2000](#); [Dissen et al., 2002, 2009](#)). Furthermore, the involvement of NTs and GDNF has been demonstrated in the regulation of the final stage of folliculogenesis, which is characterized by oocyte maturation, steroidogenesis, ovulation and corpus luteum formation; the end point of this process is the acquisition of oocyte competence for embryo development following fertilization (for reviews, see [Linher-Melville and Li, 2013](#)). However, the functional roles of NTs and GDNF in the regulation of ovarian function remain poorly defined in humans because most information has been obtained from animal models, even though the fundamental process of ovarian folliculogenesis and oocyte maturation is highly similar across mammalian species. Therefore, in this review, we have summarized the functional roles of NTs and GDNF in human ovarian biology and ovarian disorders. Furthermore, we described and proposed the development of novel strategies for diagnosing, treating and preventing related abnormalities.

Methods

Comprehensive literature searches using either PubMed, MEDLINE or Google Scholar were performed to review articles written in English focusing on the role of NTs and GDNF in follicle assembly, primordial follicle activation, follicular growth and development, oocyte maturation, steroidogenesis, ovulation and corpus luteum formation, as well as ovarian disorders. The search included all relevant mammals, especially human studies published before August 2018.

NTs and functional receptors

The NTs are a family of soluble polypeptide growth factors that comprises five members, including NGF, BDNF, neurotrophin-3 (NT-3), neurotrophin-4 (NT-4, also known as NT-4/5 or NT-5) and neurotrophin 6 (NT-6), which share approximately 50% amino acid sequence identity ([Skaper, 2018](#)). To date, NT-6 has been identified only in non-mammalian species, such as fish ([Götz et al., 1994](#)). Initially synthesized as pre-pro-proteins in the endoplasmic reticulum, NT precursors are cleaved off the signal peptide and converted into pro-NTs. Prior to their release from the cell, pro-NTs are proteolytically processed by proprotein convertase subtilisin/kexin (PCSK) enzymes that dimerize to their mature forms in the *trans*-Golgi network ([Lessmann and Brigadski, 2009](#)). In certain tissues, the extracellular pro-BDNF can be cleaved by plasmin, which is activated by tissue plasminogen activator ([Pang et al., 2004](#)). Mature NTs are non-covalently linked homodimers that are formed by two identical peptide chains of approximately 120 amino acids. Each monomer contains six highly conserved cysteine residues that form a cysteine knot and stabilize the conformation of the proteins ([McDonald et al., 1991](#)).

Both pro- and mature NTs initiate their biological actions by binding to the following principal receptor types: high-affinity transmembrane tropomyosin receptor kinase (Trk) receptors and a low-affinity p75 neurotrophin receptor (p75NTR). High-affinity Trk receptors are encoded by members of the Trk proto-oncogene family that exhibit ligand-dependent activation of tyrosine kinase receptor activity, thus functioning as signaling receptors ([Barbacid, 1994](#); [Longo and Massa, 2013](#)). Trk receptors contain a tyrosine kinase intracellular

domain and extracellular domain (with a leucine- and cysteine-rich motif) that confers ligand binding ([Yano and Chao, 2000](#)). The intracellular action specificity of NTs is determined by the selective interaction with their cognate Trk receptors, TrkA (also known as NTRK1) for NGF, TrkB (also known as NTRK2) for BDNF and NT-4, and TrkC (also known as NTRK3) for NT-3 ([Yancopoulos et al., 1990](#); [Barbacid et al., 1991](#); [Raffioni et al., 1993](#); [Chao, 2003](#)) (Table I). Notably, NT-3 can also bind to non-cognate Trk receptors (TrkA or TrkB), although the physiological role of this ligand-receptor interaction is not clear ([Benedetti et al., 1993](#)) (Table I).

In contrast to the Trk family of receptors, the low-affinity NT receptor p75NTR (also known as the pan-neurotrophin receptor) binds all NTs with equal affinity ([Chao, 2003](#); [Kashyap et al., 2018](#)) (Table I). The p75NTR is a type I transmembrane glycoprotein receptor that is structurally related to the tumor necrosis factor (TNF) receptor family ([Chao, 2003](#)). Unlike Trk receptors, p75NTR lacks intrinsic enzyme activity but instead recruits signal adaptors that modulate Trk signaling ([Longo and Massa, 2013](#)). Ligand preferences of NT receptors are listed in Table I.

GDNF and functional receptors

First identified as a trophic factor that promotes the survival of mid-brain dopaminergic neurons during embryonic development, GDNF is another neurotrophic factor that has been shown to play an essential role in the development and maintenance of the central and peripheral nervous system ([Lin et al., 1993](#); [Tomac et al., 1995](#)). As distant relatives of the transforming growth factor β (TGF- β) superfamily, the GDNF family comprises four structurally related members, including GDNF, neurturin, artemin and persephin ([Airaksinen and Saarma, 2002](#)). Despite low amino acid sequence identity (<20%), GDNF and members of the TGF- β superfamily have similar structural conformations that belong to the cysteine-knot protein family and function as homodimers ([Ibáñez, 1998](#)). Similar to NTs, GDNF is initially produced in the form of a precursor, pre-pro-GDNF, in which the signal sequence is cleaved on secretion, and activation of pro-GDNF most likely occurs through proteolytic cleavage by PCSK enzymes (specifically PCSK3, also known as furin; [Airaksinen and Saarma, 2002](#)). Animal studies have shown that GDNF can bind heparan sulfate proteoglycans of the extracellular matrix, which increases its local concentration by restricting diffusion in the tissue ([Hamilton et al., 2001](#)). The human GDNF gene contains six exons and generates two conserved isoforms by alternative splicing of exon 3, including a full-length pre- α -pro-GDNF (α -GDNF) and the shorter pre- β -pro-GDNF (β -GDNF) ([Grimm et al., 1998](#)) (Fig. I). Compared to the full-length isoform, the short isoform lacks 26 amino acids in the pro-region ([Airavaara et al., 2011](#)) (Fig. I). However, the lack of amino acids does not affect the proteolytic cleavage site, with both isoforms cleaved into mature GDNF. Recent studies have shown that both GDNF isoforms have a comparable biological effect and efficacy in the central nervous system ([Penttilinen et al., 2018](#)).

In vitro studies using overexpression approaches revealed that both GDNF isoforms are secreted from cells in two different ways. α -GDNF and its corresponding mature GDNF proteins are secreted constitutively, whereas the secretion of the β -GDNF (and its related mature GDNF) proteins appears to be activity-dependent ([Lonka-Nevalaita](#)

Table 1 Ligand preferences of neurotrophin/glial cell line-derived neurotrophic factor receptors.

Receptor	Ligand	References
TrkA	NGF, NT-3 ^a	Yancopoulos <i>et al.</i> (1990), Benedetti <i>et al.</i> (1993)
TrkB	BDNF, NT-4, NT-3 ^a	Yancopoulos <i>et al.</i> (1990), Benedetti <i>et al.</i> (1993)
TrkC	NT-3	Yancopoulos <i>et al.</i> (1990)
p75NTR	NGF, BDNF, NT-3, NT-4	Chao (2003)
GFR α 1	GDNF	Takahashi (2001)
RET	GDNF, NRTN, ARTN, PSPN	Takahashi (2001), Airaksinen <i>et al.</i> (1999)

^aLigand has lower affinity or is less commonly important for receptor activation *in vivo*.

Trk, transmembrane tropomyosin kinase; NGF, nerve growth factor; NT-3, neurotrophin-3, BDNF, brain-derived neurotrophic factor; NT-4, neurotrophin-4; p75NTR, p75 neurotrophin receptor; GDNF, glial cell line-derived neurotrophic factor; GFR α 1, GDNF receptor- α 1; NRTN, neurturin; ARTN, artemin; PSPN, persephin.

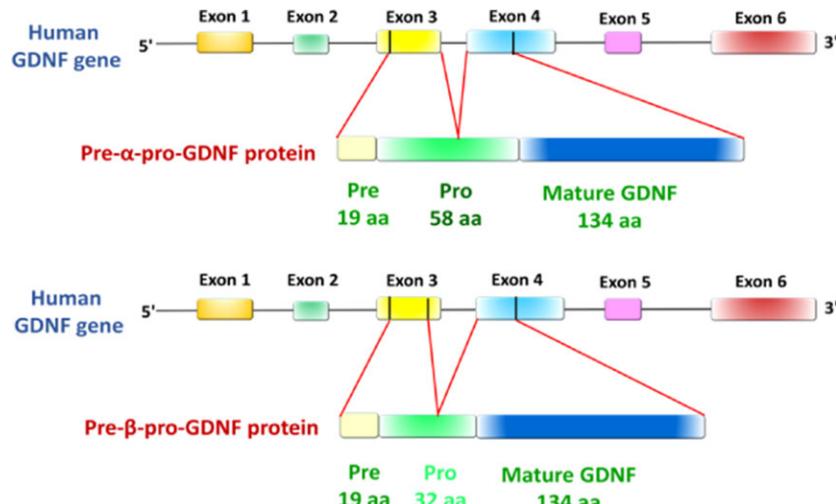


Figure 1 Schematic representation of the human GDNF gene and two splicing protein isoforms. The human glial cell line-derived neurotrophic factor (GDNF) gene has six exons (color boxes represent exons, although not to scale) that encode the pre- α -pro-GDNF isoform with a full-length 58 amino acid pro-region and the pre- β -pro-GDNF isoform with a shorter 32 amino acid pro-region. Adapted from (Penttinen *et al.*, 2018).

et al., 2010). Despite the different secretory patterns, the two major isoforms of GDNF are usually expressed in the same tissues but in varying proportions (Suter-Cazzolara and Unsicker, 1994). In the mammalian ovary, the LH surge dramatically induces the expression and secretion of GDNF, which promotes maturation of the oocyte (Zhao *et al.*, 2011). Furthermore, the treatment of human GCs with hCG alone or combined with FSH significantly increased GDNF mRNA levels *in vivo* and *in vitro*, indicating the positive regulatory role of LH and hCG in the production of GDNF (Zhao *et al.*, 2011). However, our most recent studies have shown a potential physiological function exerted by an intraovarian growth factor; bone morphogenetic protein 2 (BMP2), which negatively regulates the expression of GDNF in human granulosa-lutein cells (Zhang *et al.*, 2018).

GDNF signals through a unique multicomponent receptor complex that consists of the GDNF family receptor- α 1 (GFR α 1) and RET receptor tyrosine kinase (Takahashi, 2001). GFR α 1 receptor is usually linked to the plasma membrane by a glycosyl phosphatidylinositol anchor;

however, it can be cleaved by an unknown phospholipase or protease that produces soluble forms of these coreceptors (Paratcha *et al.*, 2001). GDNF binds to GFR α 1 and then forms a complex with RET as a signaling component (Table 1). First discovered as a proto-oncogene, RET is a single-pass transmembrane protein that contains two main cellular portions (Takahashi, 2001). The extracellular portion consists of four cadherin-like repeats, a cysteine-rich domain and a calcium-binding site, while the intracellular portion comprises a characteristic structural feature of tyrosine kinase domain (Airaksinen and Saarma, 2002).

NT signal transduction pathways

Upon NT binding, Trk receptors are tyrosine kinase receptors that subsequently trigger the activation of several intracellular signaling pathways, including Ras/mitogen-activated protein kinase (MAPK), phosphoinositide-specific phospholipase C- γ 1 (PLC γ 1) and phosphoinositide 3-kinase/protein kinase B-mammalian target of rapamycin

(PI3K/Akt-mTOR) pathways (Chao, 2003) (Fig. 2). Additionally, all four NTs bind to low-affinity p75NTR, which belongs to a family of transmembrane molecules that include receptors for the TNF family (Barbacid, 1994; Bibel et al., 1999) (Fig. 2).

The Ras/MAPK pathway

Following the binding of NTs to their corresponding Trk receptors, the activation of Ras protein occurs, which is an initial response required for cell survival and differentiation. The signal activation cascade is mediated by the binding of an adaptor molecule, Shc, to the phosphorylated tyrosine 490 where the phosphorylated Shc recruits an adaptor protein growth factor receptor-bound protein 2 (Grb2) and a guanine nucleotide exchange factor son of sevenless (SOS; Nimnuan et al., 1998; Robinson et al., 2005). The Grb2-SOS complex further increases Ras-GTP levels by catalyzing nucleotide exchange on Ras (York et al., 2000). This activated Ras molecule can stimulate a signaling cascade through various cytoplasmic kinase proteins, including a kinase specific for serine/threonine (Raf), MAPK kinase activator, MAPK and extracellular signal-regulated kinase 1/2 (ERK1/2; Wood et al., 1992; Xing et al., 1998). The activation of ERK1/2, which further phosphorylates and activates a variety of kinases and nuclear transcription factors, is critical for many Ras-induced cellular responses (Liebmann, 2001). Indeed, the Ras/MAPK pathway mediates various NT-induced regulations of ovarian function in humans (Dissen et al., 2009; Julio-Pieper et al., 2009).

The PLC γ 1 pathways

In addition to the Ras/MAPK pathway, one of the main mechanisms that mediates NT/Trk receptor interactions is driven by PLC γ 1, which is an intracellular messenger that transmits membrane receptor signaling to several molecules (Reichardt, 2006). The activation of PLC γ 1 leads to cleavage of the membrane lipid phosphatidylinositol 4,5-bisphosphate to form inositol triphosphate (IP3) and diacylglycerol (DAG), two key secondary messengers used in signal transduction in most cells (Reichardt, 2006). IP3 is a soluble molecule and is capable of diffusing to the rough endoplasmic reticulum where IP3 can bind to its receptor and trigger the release of Ca^{2+} into the cytoplasm (Taylor and Tovey, 2010). The increase in intracellular Ca^{2+} or DAG concentration may stimulate the activity of protein kinase C, which plays essential roles in several signal transduction cascades (Wilson et al., 2015; Ali et al., 2016).

The PI3K/Akt-mTOR pathway

The PI3K/Akt-mTOR signaling pathway integrates both extracellular and intracellular signals that serve as a principal regulator of various cell functions, including cell growth, proliferation, metabolism and survival (Laplante and Sabatini, 2009). In the mammalian ovary, this signaling pathway is the most studied pathway that governs the activation of primordial follicles (Adhikari and Liu, 2009). Recent studies have provided evidence showing that the PI3K/Akt-mTOR signaling pathway and KIT ligand play indispensable roles in the coordination of GCs and oocytes, which initiate the activation of primordial follicles in adult ovaries (Zhang and Liu, 2015). Notably, the achievement of this regulation over the reproductive lifespan involves several extracellular intraovarian factors, including NTs, KIT ligand,

BMP4, BMP7, vascular endothelial growth factor (VEGF) and other molecules (Adhikari and Liu, 2009; Hsueh, 2014).

In different subsets of tissues, PI3K is activated through Ras-dependent and Ras-independent signaling pathways (Reichardt, 2006). In most neuronal cells, the Ras-dependent activation of PI3K initiates the major signaling pathways that produce several cell survival signals (Vaillant et al., 1999). Additionally, the activation of PI3K can be induced through the Shc-Grb2-Gab1 (Grb2-associated binding protein-1) cascade. Specifically, this Ras-independent signaling pathway occurs when Shc is phosphorylated by TrkA and is associated with Grb2 to produce a complex, which further phosphorylates Gab1 adaptor protein and activates PI3K (Holgado-Madruga et al., 1997). In addition, the phosphorylation of Trk receptors activates PI3K by promoting the phosphorylation of insulin receptor substrate-1 in some neurons (Yamada et al., 1997). Once activated, either by a Ras-dependent or Ras-independent pathway, PI3K may phosphorylate several downstream effectors that are essential for NT-mediated cell function. Akt kinase is regarded as the most important intracellular protein that modulates the activity of certain transcription factors related to cell survival and apoptosis (Brunet et al., 2001) (Fig. 2). One of the major regulators of cellular growth and metabolism activated by the PI3K/Akt downstream pathway is the mechanistic target rapamycin complex 1 or 2 (mTORC1 or mTORC2) (Dibble and Cantley, 2015).

The p75NTR-mediated signaling pathway

All NTs bind to p75NTR with relatively low affinity (Fig. 2). Following the binding of NTs to p75NTR, several signaling pathways that are mediated through the binding of p75NTR to several adaptor proteins may be activated (Reichardt, 2006). One of the key pathways activated by NT-engaged p75NTR receptor binding is the Jun kinase signaling cascade, which leads to the activation of p53 and enhancement of cell apoptosis (Reichardt, 2006) (Fig. 2). Additionally, the binding of NTs to p75NTR may induce the activation of nuclear factor- κ B (NF- κ B), which contributes to NF- κ B-dependent neuronal survival (Hamanoue et al., 1999) (Fig. 2). Furthermore, the interaction of NTs with p75NTR can activate acidic sphingomyelinase and induce the formation of ceramide (Dobrowsky et al., 1994). Ceramide is responsible for the p75NTR-mediated amplification or inhibition effects on Trk-mediated cellular responses because ceramide modulates several signaling pathways, including ERK/MAPK, PI3K/Akt, Jun kinase and NF- κ B, as well as the activity of the TrkA receptor in a cell type-dependent manner (MacPhee and Barker, 1997; Müller et al., 1998; Zundel et al., 2000).

GDNF signal transduction pathway

Similar to other members of the GDNF ligand family, GDNF signals through two functional receptors, GFR α 1 and RET (for reviews, see Takahashi, 2001). The GDNF homodimer first binds to GFR α 1 (either a monomer or dimer), which then interacts with the extracellular domain of two RET molecules and induces their homodimerization and tyrosine autophosphorylation (Airaksinen et al., 1999). Upon phosphorylation, the intracellular domain of the activated RET acts as

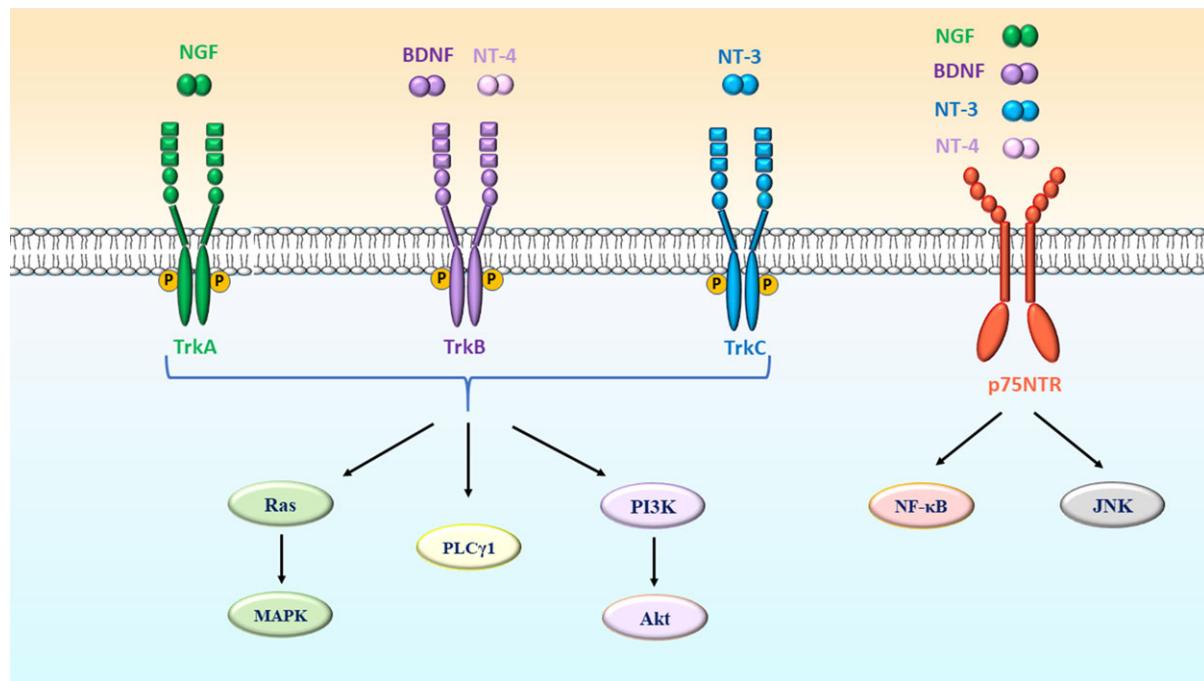


Figure 2 Neurotrophin receptors and their downstream signaling pathways. NGF binds to TrkA, BDNF and NT-4 bind to TrkB, and NT-3 binds to TrkC, which further induces the activation of Ras/MAPK, PLC γ 1 and PI3K/Akt pathways. All neurotrophins (NGF, BDNF, NT-3 and NT-4) also bind to p75NTR receptor, which initiates the recruitment of various adaptors that further activate NF- κ B and JNK pathways. BDNF, brain-derived neurotrophic factor; JNK, Jun kinase; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; NT-3, neurotrophin-3; NT-4, neurotrophin-4; NF- κ B, nuclear factor- κ B; PI3K, Phosphatidylinositol-3-kinase; PLC γ 1, phospholipase C γ 1.

high-affinity binding sites for several intracellular signaling proteins, including PI3K, MAPK and PLC- γ (for reviews, see [Airaksinen and Saarma, 2002](#)). However, there is evidence for RET-independent signaling mechanisms, as many tissues express GDNF and GFR α 1, but not RET ([Trupp et al., 1999](#)). Recent studies have revealed that in the absence of RET, GDNF can interact with other receptors, such as neural cell adhesion molecules and integrins (for reviews, see [Fielder et al., 2018](#)).

The GDNF signaling pathway may participate in crosstalk with other growth factors and receptors. In the peripheral nervous system, the endogenous TGF- β system is required as a cofactor for the exogenous GDNF-induced neuroprotective effect ([Airaksinen et al., 1999](#)). Furthermore, TGF- β can induce cell responsiveness to GDNF by recruiting GFR α 1 to the plasma membrane during several critical steps of signal transduction ([Peterziel et al., 2002](#)). Similar co-operation has been shown between GDNF and NT signaling, as both GDNF and BDNF are required for tissue survival in sensory neurons ([Erickson et al., 2001](#)). Indeed, a novel communication pathway between RET and NGF signaling has been demonstrated by using TrkA receptor ([Tsui-Pierchala et al., 2002](#)). In the mammalian ovary, these synergistic, co-operative mechanisms seem to be of great importance for the development and maintenance of female reproductive function as these growth factors (TGF- β , NTs and GDNF), and their functional receptors are highly expressed in ovarian follicles ([Roy and Kole, 1998](#); [Abir et al., 2005](#); [Farhi et al., 2010](#)).

Expression and localization in the human ovary

NTs and GDNF, as well as their cognate receptors, are expressed in preantral and antral follicles of the mammalian ovary. Additionally, data from clinical samples have shown that these neurotrophic factors are detectable in follicular fluid (FF; [Seifer et al., 2002, 2002; Kawamura et al., 2008; Palumbo et al., 2013](#)). Furthermore, an imbalance of any of these intraovarian growth factors may contribute to abnormal follicular development and ovarian dysfunction or pathology in humans. The expression and localization of the NT and GDNF system in the mammalian ovary has been reviewed in detail elsewhere ([Streiter et al., 2016](#)). In this review, we focused on the expression and potential function of these neurotrophic factors in the human ovary and their clinical connection to pathological conditions.

In the human ovary, primordial follicles undergo a series of histological and functional changes during follicular development. Under the stimulation of multiple intraovarian factors, the primordial follicle activates and changes into primary and, later, into secondary follicles, followed by transitions to tertiary (antral) follicles ([Hsueh et al., 2015](#)). The development and growth of antral follicles is dependent on gonadotrophins, especially FSH ([Mihm and Bleach, 2003](#)). To discuss the expression and localization of neurotrophic factors and their receptors in the human ovary, we divided the ovarian follicles into the preantral stage (primordial, primary and secondary) and antral stage (Table II).

Table II Localization of neurotrophins/GDNF and receptors in the human ovary.

Ligands/Receptors	Localization	Expression	Detection method	References
NGF	Preantral			
	Follicles			
	Oocytes	Protein	IHC	Abir et al. (2005)
		Protein	IHC	Abir et al. (2005), Salas et al. (2006)
	Antral			
	Follicles			
	GCs	mRNA and Protein	RT-qPCR, IHC	Salas et al. (2006)
		Protein	IHC	Seifer et al. (2006)
		Protein	IHC	Salas et al. (2006)
		Protein	ELISA	Palumbo et al. (2013)
BDNF	(213.76 pg/ml, ranged from 100 to 360 pg/ml)			
	Preantral			
	Follicles			
	Oocytes	Protein	IHC	Harel et al. (2006)
		Protein	IHC	Harel et al. (2006)
	Antral			
	Follicles			
	GCs	mRNA and Protein	RT-qPCR, IHC	Zhao et al. (2011)
		Protein	IHC	Seifer et al. (2006)
		Protein	ELISA	Seifer et al. (2002)
NT-3	(645.2 ± 23.6 pg/ml) ^a			
	Preantral			
	Follicles			
	Oocytes	Protein	IHC	Oron et al. (2011)
		Protein	IHC	Oron et al. (2011)
	Antral			
	Follicles			
	GCs	Protein	IHC	Seifer et al. (2006)
		Protein	IHC	Seifer et al. (2006)
		Protein	ELISA	Seifer et al. (2002)
NT-4	(509 ± 97 pg/ml) ^a			
	Preantral			
	Follicles			
	Oocytes	mRNA and protein	ISH, IHC	Anderson et al. (2002), Harel et al. (2006)
		mRNA and protein	ISH, IHC	Anderson et al. (2002)
		Protein	IHC	Harel et al. (2006)
	Antral			
	Follicles			
	CCs	Protein	IHC	Seifer et al. (2006)
		Protein	ELISA	Seifer et al. (2002)
TrkA	(397 ± 71 pg/ml) ^a			
	Preantral			
	Follicles			
	Oocytes	Protein	IHC	Abir et al. (2005)
		Protein	IHC	Abir et al. (2005), Salas et al. (2006)
	Antral			
	Follicles			

Table II Continued

Ligands/Receptors	Localization	Expression	Detection method	References
TrkB	Oocytes	Protein	IHC	Seifer <i>et al.</i> (2006)
	GCs	mRNA and Protein	RT-qPCR, IHC	Salas <i>et al.</i> (2006)
	CCs	Protein	IHC	Seifer <i>et al.</i> (2006)
	TCs	Protein	IHC	Salas <i>et al.</i> (2006)
	Preantral			
	Follicles			
	Oocytes	Protein	IHC	Anderson <i>et al.</i> (2002), Harel <i>et al.</i> (2006)
	Pre-GCs	Protein	IHC	Anderson <i>et al.</i> (2002)
	GC	Protein	IHC	Harel <i>et al.</i> (2006)
	Antral			
TrkC	Follicles			
	Oocytes	Protein	IHC	Seifer <i>et al.</i> (2006)
	CCs	Protein	IHC	Seifer <i>et al.</i> (2006)
	Preantral			
	Follicles			
	Oocytes	mRNA and Protein	ISH, IHC	Oron <i>et al.</i> (2011)
	GCs	mRNA and Protein	ISH, IHC	Oron <i>et al.</i> (2011)
	Antral			
	Follicles			
	Oocytes	Protein	IHC	Seifer <i>et al.</i> (2006)
p75NTR	CCs	Protein	IHC	Seifer <i>et al.</i> (2006)
	Preantral			
	Follicles			
	Stroma	Protein	IHC	Anderson <i>et al.</i> (2002), Abir <i>et al.</i> (2005)
	Antral			
	Follicles			
	TCs	Protein	IHC	Anesetti <i>et al.</i> (2001)
	Preantral			
	Follicles			
	Oocytes	Protein	IHC	Farhi <i>et al.</i> (2010)
GDNF	GCs	Protein	IHC	Farhi <i>et al.</i> (2010)
	Stroma	Protein	IHC	Farhi <i>et al.</i> (2010)
	Antral			
	Follicles			
	Oocytes	Protein	IHC	Farhi <i>et al.</i> (2010)
	GCs	mRNA and Protein	RT-qPCR, IHC	Farhi <i>et al.</i> (2010), Zhao <i>et al.</i> (2011)
	FF	Protein	ELISA	Kawamura <i>et al.</i> (2008)
	(0.4–10.8 ng/ml)			
	Preantral			
	Follicles			
GFR α 1	Oocytes	mRNA and Protein	ISH, IHC	Farhi <i>et al.</i> (2010)
	GCs	mRNA and Protein	ISH, IHC	Farhi <i>et al.</i> (2010)
	Stroma	Protein	IHC	Farhi <i>et al.</i> (2010)
	Antral			
	Follicles			
	Oocytes	Protein	IHC	Farhi <i>et al.</i> (2010)
	GCs	Protein	IHC	Farhi <i>et al.</i> (2010)
	CCs	mRNA	RT-qPCR	Cui <i>et al.</i> (2018)

Continued

Table II Continued

Ligands/Receptors	Localization	Expression	Detection method	References
RET	Preantral			
	Follicles			
	Oocytes	Protein	IHC	Farhi et al. (2010)
	GCs	Protein	IHC	Farhi et al. (2010)
	Stroma	Protein	IHC	Farhi et al. (2010)
	Antral			
	Follicles			
	CCs	mRNA	RT-qPCR	Cui et al. (2018)

CCs, Cumulus cells; GCs, Granulosa cells; TCs, Theca cells; RT-qPCR, Quantitative real-time PCR; FF, Follicular fluid; ISH, *in situ* hybridization; IHC, Immunohistochemistry.

^aData are expressed as the mean \pm SD.

Expression of NTs and functional receptors in the human ovary

NGF mRNA transcripts and protein are expressed in both fetal and adult human ovaries; they are especially localized to the oocytes and GCs of preantral follicles (mainly from primordial to secondary follicles) (Anderson et al., 2002; Abir et al., 2005; Salas et al., 2006) (Fig. 3A). NGF protein has also been identified in both GCs (especially cumulus cells) and theca cells (TCs) of antral follicles (Salas et al., 2006; Seifer et al., 2006). Clinical samples obtained from infertile women undergoing IVF showed that NGF levels in FF are much higher than those in the serum (213.76 pg/ml vs. 46.47 pg/ml), indicating that NGF is locally produced in the ovarian follicle (Palumbo et al., 2013) (Table II).

BDNF is predominantly expressed in the somatic cells of primordial follicles in the human fetal ovary from 9 to 12 weeks gestation (Childs et al., 2010). BDNF is also detected in the oocytes and GCs (only in a minority of samples) of fetal and adult ovaries (Harel et al., 2006; Streiter et al., 2016) (Fig. 3B). Immunohistochemistry showed that BDNF was detected in cumulus cells (CCs) but at very low levels in the mural GCs of antral follicles (Seifer et al., 2006; Zhao et al., 2011; Streiter et al., 2016) (Fig. 3B). In mice, antral follicular levels of BDNF concomitantly increased with the LH surge during the preovulatory stage (Kawamura et al., 2005). In humans, the administration of LH or hCG increased the secretion of BDNF (up to a 4- or 14-fold increase, respectively) in the conditioned medium of cultured CCs obtained from IVF patients, indicating that BDNF is produced by CCs and is positively regulated by gonadotrophins (Feng et al., 2003). The mean FF (obtained from patients undergoing IVF) concentration of BDNF was 645.2 ± 23.6 pg/ml (Table II).

Transcripts of NT-4 have been identified in the extracts of all ovarian tissues obtained from fetuses and women (Harel et al., 2006). Similar to BDNF, NT-4 was primarily identified in oocytes and some GCs in 21-week-old fetal ovaries (Harel et al., 2006). Likewise, NT-4 has been detected in FF (mean concentration: 397 ± 71 pg/ml) obtained from women undergoing IVF (Seifer et al., 2002). NT-3 protein was identified in the oocytes and GCs of ovarian preantral follicles from fetuses, girls and women (Oron et al., 2011). In the antral follicles, NT-3 protein was identified by immunohistochemistry in GCs and CCs (Seifer et al., 2006), and the mature form of NT-3

protein was detected in the FF (mean concentration: 509 ± 97 pg/ml) (Seifer et al., 2002) (Table II).

In the preantral follicles of the human ovary, TrkA protein has been detected mainly in oocytes, some stroma cells and GCs of ovarian samples obtained from fetuses, girls and women (Abir et al., 2005). In the majority of fetal oocytes and oogonia TrkA was located in the cytoplasm but not in the nucleus (Abir et al., 2005). Additionally, TrkB mRNA transcripts were identified in all fetal and adult ovaries examined (Abir et al., 2005). Similarly, TrkB was mainly expressed in the oocytes/oogonia and some GCs of preantral follicles of the human fetal ovary (Anderson et al., 2002; Harel et al., 2006; Childs et al., 2010). TrkC protein was expressed in the oocytes and GCs of preantral follicles in ovarian samples from fetuses (at 19–33 weeks of gestation), girls and women (Oron et al., 2011). Using *in situ* hybridization, the same study showed that mRNA transcripts for full-length TrkC were detected in the oocytes of all samples examined and in GCs only in ovarian samples from girls and women (Oron et al., 2011). The expression of p75NTR in mammals is species-dependent. In the preantral follicles of human ovaries, the mRNA and protein of pan-specific p75NTR were detected in stroma cells and TCs of ovarian samples from young fetuses (at 19–21 weeks of gestation) but not in those from older fetuses, girls and women (Anesetti et al., 2001; Abir et al., 2005).

All of the NT receptors, including TrkA, TrkB, TrkC and p75NTR, have been detected in the antral follicles of human ovaries (Anesetti et al., 2001; Seifer et al., 2006). Many studies have demonstrated the expression of TrkA in the major compartments of human antral follicles, including oocytes, GCs and TCs (Shibayama and Koizumi, 1996; Salas et al., 2006; Seifer et al., 2006). Interestingly, the addition of hCG to cultured rat antral follicles rapidly increased the expression of TrkA, indicating that the increase in expression of TrkA during the preovulatory phase is LH dependent (Dissen et al., 1995). In contrast to the expression pattern in rodents (in oocytes only), TrkB was detected in oocytes and CCs of antral follicles in humans (Seifer et al., 2006). Similarly, TrkC protein has been identified in both oocytes and GCs in human antral follicles (Shibayama and Koizumi, 1996; Seifer et al., 2006). Using immunohistochemistry, p75NTR protein was identified in the TCs of human antral follicles (Anesetti et al., 2001) (Table II).

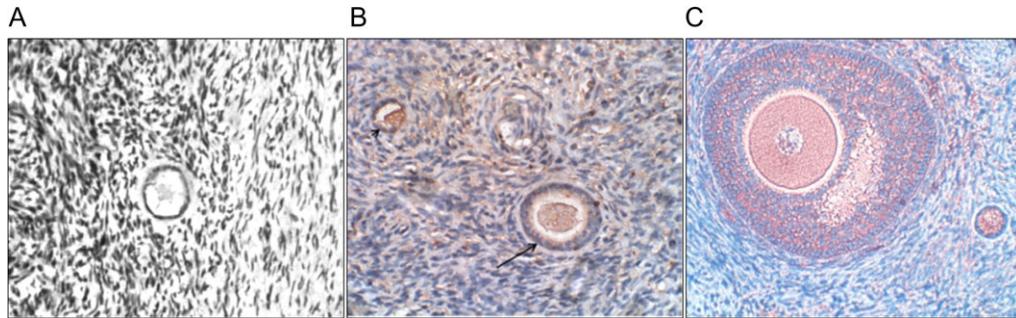


Figure 3 Immunohistochemical staining of NGF, BDNF and GDNF in the human ovary. All sections were stained with the immunoperoxidase staining and then counterstained with Mayer's haematoxylin. **(A)** Section of a human ovary from a 38-year-old woman. NGF is expressed in granulosa cells of the primordial follicle, and there is partial staining (with positive nuclear staining) in the oocyte. Original magnification X400. Reprinted with permissions from Abir *et al.* (2005). **(B)** Section of a human ovary from a 16-year-old girl. BDNF is expressed in the secondary (arrow) and primordial (arrow head) follicles. Original magnification X400. Reprinted with permissions from Streiter *et al.* (2016). **(C)** Section of a human ovary from a 22-year-old woman. GDNF is expressed in the oocytes and a portion of the granulosa cells and stroma cells. Original magnification X400. Reprinted with permissions from Farhi *et al.* (2010).

Expression of GDNF and functional receptors in the human ovary

In human preantral follicles, GDNF has been observed in the oocytes of all ovarian samples from fetuses and adult women, and in the GCs from half of all fetal samples and all adult samples (Farhi *et al.*, 2010) (Fig. 3C). Additionally, GDNF mRNA was identified in human GCs of antral follicles and the expression can be upregulated by the addition of hCG alone or in combination with FSH (Zhao *et al.*, 2011). Moreover, mature GDNF protein was detectable in human FF and ranged from 0.4 to 10.8 ng/ml, indicating a paracrine function in the development of ovarian follicles (Kawamura *et al.*, 2008) (Table II).

The two functional receptors for GDNF, GFR α 1 and RET, have been detected in the oocytes and GCs of human preantral and antral follicles (Farhi *et al.*, 2010). In particular, the mRNAs of GFR α 1 and two RET isoforms were found in the oocytes and GCs of all ovarian samples from fetuses, girls and women. Our recent studies also showed that the mRNAs of GFR α 1 and RET were detected in the CCs of mature follicles obtained from patients undergoing IVM (Cui *et al.*, 2018) (Table II).

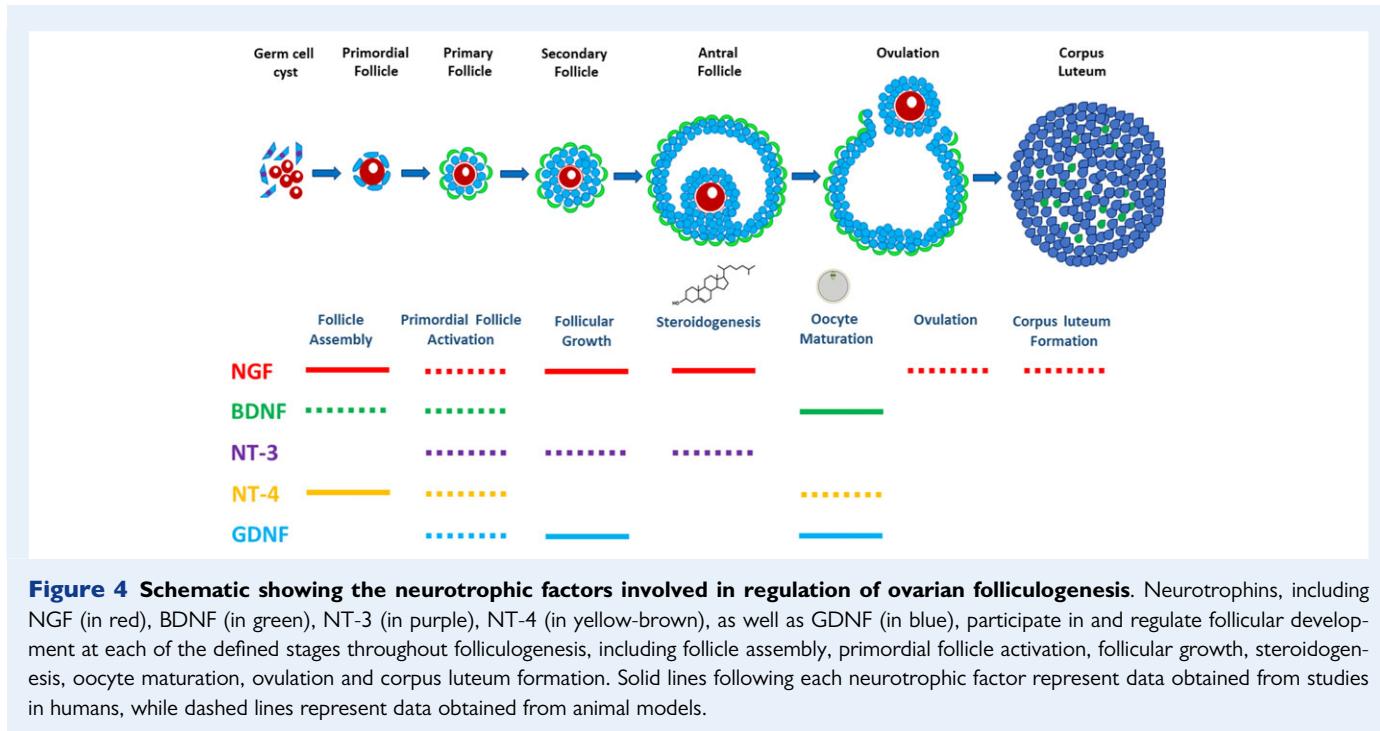
Regulation of ovarian follicle development

The detection of NTs, GDNF and their cognate receptors in the principal cells (oocytes, GCs and TCs) throughout the different follicular stages of the human ovary may suggest that these intraovarian growth factors are involved in various stages of human folliculogenesis. Indeed, recent studies using animal models, clinical samples and *in vitro* cell culture have indicated that neurotrophic factors play indispensable roles in the central and peripheral nervous system, as well as in various tissues, including the ovary under normal and pathological conditions (Streiter *et al.*, 2016; Skaper, 2018). Recent studies suggest an unexpected diversity of NT and GDNF actions in the regulation of ovarian and follicular development through nervous

system stimuli and cell–cell interactions in the mammalian ovary (Chaves *et al.*, 2013; Streiter *et al.*, 2016) (Fig. 4).

Ovarian follicle assembly

The assembly of primordial follicles in the human ovary is a complex developmental process that involves the extensive co-ordination of many growth factors; among these factors, NTs are the major players (Maheshwari and Fowler, 2008; Grive and Freiman, 2015; Streiter *et al.*, 2016). The involvement of NTs in ovarian follicle assembly was supported by the identification of NTs and pan-specific p75NTR in the human fetal ovarian cortex during this process (Anderson *et al.*, 2002; Abir *et al.*, 2005). In particular, both NGF mRNA and protein are expressed in the human ovary prior to formation of the first primordial follicle (Abir *et al.*, 2005). NT-4 localizes to oocytes and pre-GCs in the fetal ovaries of humans from 13 weeks until the completion of gestation (Anderson *et al.*, 2002; Harel *et al.*, 2006). Furthermore, p75NTR mRNA and proteins were detected in the pre-GCs that surround oocytes only in fetal ovaries at gestational ages 13–21 weeks, representing the time before and during human follicle assembly (Anderson *et al.*, 2002; Abir *et al.*, 2005). Compared to the addition of neutralizing antibodies against NT-4, the addition of NT-4 to cultured human fetal ovaries enhanced follicle assembly *in vitro* (Farhi *et al.*, 2011). Animal studies also support a critical role of NTs in regulating ovarian follicle assembly. Depletion of both *Bdnf* and *Nt-4* in mice led to impaired follicular organization, increased oocyte death and grossly abnormal ovaries (Ojeda *et al.*, 2000; Spears *et al.*, 2003) (Table III). Mice lacking *Ngf* or *Trka* had a phenotype of reduced primordial follicle number (Kerr *et al.*, 2009) (Table III). *Trkb* knockout mice exhibited an increased number of non-encapsulated oocytes (Kerr *et al.*, 2009) (Table III). Similarly, the addition of NGF to the cultured ovaries of *Ngf*-null mice resulted in an increased number of primordial follicles (Lee *et al.*, 1992; Ojeda *et al.*, 2000). Collectively, these results indicate that ovarian follicle assembly is most likely induced by the synergistic and complementary effects of these NTs and their corresponding receptors (Fig. 4).



Activation of primordial follicles

Following follicle assembly, the human ovary contains the maximum number of primordial follicles at birth (a few days after birth in rodents), which is defined as the ovarian reserve (Findlay *et al.*, 2015). These primordial follicles remain in a quiescent state in a primordial pool for decades until activation by certain stimuli in the surrounding microenvironment. Upon activation, the primordial follicle transforms into the primary follicle along with oocyte growth, TC recruitment and GC differentiation (from flat to cuboid shape) (Kerr *et al.*, 2013). Among the members of the NT family, NGF seems to be involved in the activation of primordial follicles as NGF is expressed in rat follicles at the primordial stage. Knockout mice lacking NGF had a reduced number of both primary and secondary follicles (Dissen *et al.*, 2001, 2002). Moreover, a functional study confirmed that the addition of NGF to cultured neonatal ovaries enhanced the activation rate of primordial follicles in mice (Paredes *et al.*, 2004). NT-3 activates the progression of primordial follicles to growing follicles in cultured rat ovaries (Nilsson *et al.*, 2009). NT-3 promotes the primordial to primary follicle transition by upregulating several target genes related to formation of the cytoskeleton and plasma membrane (Nilsson *et al.*, 2009). In sheep, GDNF improves the activation rate of primordial follicles in cultured ovaries (Esmaielzadeh *et al.*, 2013).

Recent studies using transgenic mouse models have indicated that the intraovarian PI3K/Akt-mTOR pathway is the key signaling pathway that directs the transition from primordial to primary follicles as mice lacking several genes [v-akt murine thymoma viral oncogene homolog 1 (*Akt1*), forkhead box O3 (*Foxo3*), phosphatase and tensin homolog (*Pten*), ribosomal protein S6 (*RpS6*) or tuberous sclerosis complex 1/tuberous sclerosis complex 2 (*Tsc1/Tsc2*)] related to this pathway had a phenotype of premature depletion of primordial follicles (Adhikari and Liu, 2009). A recent study using a rodent model

demonstrated that localized ovarian trauma or ovulation can induce the activation of primordial follicles, which was completely blocked by the mTOR inhibitor rapamycin (He *et al.*, 2017). A trauma-induced transient increase in locally produced NGF was proposed to trigger the activation of dormant primordial follicles through the PI3K/Akt-mTOR signaling pathway from pre-GCs to oocytes (He *et al.*, 2017). This proposal may provide a molecular mechanism by which the signaling changes in the surrounding intraovarian environment affect the selective recruitment of a cohort of primordial follicles during the menstrual period. Indeed, the injury-induced activation and development of follicles can be used to explain the beneficial effects of ovarian wedge resection and laparoscopic ovarian diathermy drilling for treating women with polycystic ovary syndrome (PCOS; Lebbi *et al.*, 2015).

Ovarian follicular growth and development

NTs are crucial growth factors for the survival, maintenance, and development of neurons in the central and peripheral nervous system (Huang and Reichardt, 2001). These neuronal growth factors also provide sympathetic innervation and trophic support for the ovary, which is essential for follicular development (Dissen *et al.*, 2002). In humans, the addition of Trk receptor blockers to fetal ovaries *in vitro* (obtained at early gestation: 13–16 weeks) decreased oogonia survival, indicating an important role for NTs in maintaining germ cell survival (Spears *et al.*, 2003). *In vitro* studies have demonstrated that treatment with NGF decreased cell apoptosis and increased the follicular growth of mouse ovaries by downregulating apoptosis-related genes (Roh and Pi, 2013). Additionally, NGF promoted follicular development and increased cell responses to FSH (and the formation of cAMP) by upregulating FSH receptors in neonatal rat ovaries, indicating crosstalk between this NT and the endocrine signaling system (Romero *et al.*, 2002). Similarly, NGF also upregulated the expression of the FSH receptor in human GCs *in vitro* (Salas *et al.*,

Table III Genetic mutations in the neurotrophin/GDNF system that cause ovary-specific phenotypes.

Type of mutation	Ovarian phenotype	References	
NGF knockout mice	Targeted deletion of the <i>Ngf</i> gene	Reduced population of primordial, primary and secondary follicles, oocytes failed to be incorporated into follicular structure	Dissen <i>et al.</i> (2001)
NGF knockin mice	Transgenic overexpression of the <i>Ngf</i> gene	Reproductive and metabolic alterations characteristic of PCOS, arrested follicular development and increased apoptosis of antral follicles	Wilson <i>et al.</i> (2014), Dissen <i>et al.</i> (2009)
NT-4 knockout mice	Targeted depletion of the <i>Nt-4</i> gene	Normal ovarian folliculogenesis	Ojeda <i>et al.</i> (2000)
Double NT-4/BDNF knockout mice	Target depletion of both <i>Nt-4</i> and <i>Bdnf</i> gene	Impaired follicular organization, increased oocyte death and grossly abnormal ovaries	Ojeda <i>et al.</i> (2000), Spears <i>et al.</i> (2003)
GDNF knockout mice	Targeted depletion of the <i>Gdnf</i> gene	Reversal in the orientation of the ovary in relation to the abdominal viscera	Moore <i>et al.</i> (1996)
TrkA knockout mice	Targeted depletion of the <i>Trka</i> gene	Reduced number of primordial follicles, reduced number of FSH receptors	Kerr <i>et al.</i> (2009)
TrkB knockout mice	Targeted depletion of the <i>Trkb</i> gene	Reduced number of primordial follicles, reduced number of FSH receptors, an increased number of non-encapsulated oocytes	Kerr <i>et al.</i> (2009)

PCOS: polycystic ovary syndrome.

2006). In addition to the effects on germ cells and GCs, NGF has been shown to participate in promoting TC proliferation. In an *in vitro* culture of TCs from antral follicles, transfection with TrkA-expressing plasmids in the presence of NGF resulted in an increase in the bovine TC proliferation rate (Dissen *et al.*, 2000). The addition of NT-3 to the culture medium also increased the cell proliferation rate in bovine TCs *in vitro* (Dissen *et al.*, 2000).

GDNF and its cognate receptors are expressed in human fetal and adult ovaries and promote follicular development in rat ovaries (Dole *et al.*, 2008; Farhi *et al.*, 2010). Rat ovaries cultured in the presence of GDNF showed an increased number of developing follicles without an increased total number of ovarian follicles (Dole *et al.*, 2008). Additionally, the combined treatment of cultured ovaries with GDNF, epidermal growth factor (EGF), kit ligand, and fibroblast growth factor enhanced the differentiation and viability of primordial follicles in sheep (Esmaielzadeh *et al.*, 2013). Moreover, the most recent studies have shown that GDNF inhibited cell apoptosis in human CCs *in vitro* (Cui *et al.*, 2018).

Ovarian steroidogenesis

Ovarian steroidogenesis products are the key components for maintaining normal ovarian function, including follicular development, oocyte maturation, ovulation and embryo implantation (Chang *et al.*, 2016a). In mature human follicles, NGF is apparently involved in the modulation of steroid hormone production. For instance, NGF inhibited the differentiation of GCs into luteal cells by suppressing the production of progesterone in isolated human GCs (Salas *et al.*, 2006). Alternatively, NGF directly promoted the production of estradiol and indirectly enhanced the cell response to FSH (FSH increases the production of estradiol in GCs) by upregulating expression of the FSH receptor in human GCs, which occurred through the activation of high-affinity tyrosine kinase NGF receptor TrkA (Romero *et al.*, 2002; Salas *et al.*, 2006). In contrast, the addition of antibodies blocking NGF reversed the inducing effect of NGF on estradiol secretion

(Salas *et al.*, 2006). In bovine antral follicles, NGF increased the production of both androgen and progesterone in freshly plated TCs through the TrkA receptor-mediated signaling pathway (Dissen *et al.*, 2000). NT-3 is another intragonadal molecule that is involved in promoting follicular steroidogenesis (Waraksa *et al.*, 1995). When NT-3 (at 100 ng/ml but not higher doses) was added to hamster follicles *in vitro*, NT-3 induced a two-fold increase in estradiol production (Waraksa *et al.*, 1995).

Oocyte maturation

Oocyte maturation is a critical reproductive event that involves a series of functional and morphological changes, including nuclear maturation, cytoplasmic reorganization, meiotic resumption, cytoskeletal dynamics and gamete-somatic cell interactions (Coticchio *et al.*, 2015). Oocyte nuclear maturation is characterized by germinal vesicle breakdown and extrusion of the first polar body (PBI), whereas oocyte cytoplasmic maturation is characterized by cytoplasmic changes essential for monospermic fertilization, and the subsequent preparation for development into preimplantation embryos (Eppig, 1996).

In addition to the endocrine system driven by the hypothalamic-pituitary-ovarian axis, oocyte maturation relies on several locally produced intraovarian factors (Hsueh *et al.*, 2015; Chang *et al.*, 2016b). NTs (especially BDNF and NT-4) and GDNF appear to be the crucial intrafollicular molecules that play a supporting role in oocyte maturation. In mice, oocytes cultured in the presence of BDNF showed increased oocyte maturation and PBI extrusion (an indicator of oocyte nuclear maturation) rates, which improved the competence of oocytes to complete preimplantation development (Seifer *et al.*, 2002; Kawamura *et al.*, 2005). Similarly, *in vitro* culture of mouse ovaries with a high concentration (100 ng/ml) of NT-4 resulted in an increased rate of PBI extrusion (Seifer *et al.*, 2002). In humans, BDNF FF levels obtained from women undergoing IVF are positively correlated with the number of mature oocytes (Wang *et al.*, 2011).

Additionally, combined treatment with BDNF, EGF and insulin-like growth factor-I (IGF-I) improved the maturation rates of human oocytes and subsequent embryo development (Yu et al., 2012).

GDNF has been identified in the GCs/CCs of antral follicles in several species, including mice, pigs and humans (Linher et al., 2007; Dole et al., 2008; Zhao et al., 2011). In addition, the mature form of GDNF protein was highly detectable in porcine and human FF (Linher et al., 2007; Zhao et al., 2011). Animal studies using porcine oocytes have shown that GDNF significantly enhanced CC expansion and increased the extrusion of the PBI (Linher et al., 2007). In humans, functional studies have demonstrated the role of GDNF in enhancing the developmental competence of oocytes (Zhao et al., 2011). Consistent with these results, our recent studies showed that exogenous GDNF promoted the maturation *in vitro* of cultured immature oocytes to metaphase II (MII) stage (Cui et al., 2018). Human microRNA (miRNA) analyses revealed that the addition of GDNF to culture medium induced several differentially downregulated miRNAs among *in vitro* matured CCs, which most likely mediated GDNF-induced maintenance of CC viability and enhancement of oocyte maturation (Cui et al., 2018). Intriguingly, mice lacking *Gdnf* had an abnormal ovarian topology that these transgenic mice exhibited a reversal in orientation of the ovary in relation to their abdominal viscera (Moore et al., 1996) (Table III).

Ovulation

Based on the mechanisms that initiate the ovulatory cascade, mammals are classified as either spontaneous (e.g. mice, pigs, horses and cattle) or induced (e.g. cats, rabbits, koala and camelids) ovulators (Silva et al., 2015). In spontaneous ovulators, ovulation is regularly triggered by an increase in the circulating concentration of estradiol, whereas in induced ovulators, ovulation is induced by stimulation of the female genital tract during the copulation (Silva et al., 2015). Animal studies have provided evidence that NGF is involved in the process of mammalian ovulation. In rats, both NGF and TrkA receptor are dramatically increased in the cells of the follicular wall and interstitial tissue of the ovary, reaching their peak levels \sim 5 h before ovulation (Dissen et al., 1996). *In vivo* administration of antibodies against NGF attenuated the release of ovarian prostaglandin E₂ and subsequently suppressed ovulation, indicating the role of NGF in triggering rodent ovulation (Dissen et al., 1996). Furthermore, NGF-induced activation of TrkA receptors can disrupt cell-cell communication by affecting the integrity of the connexin 43-based gap junctions, which further induced cellular dissociation of the follicular wall that precedes ovulatory rupture in rats (Mayerhofer et al., 1996).

NGF (especially β -NGF) has been regarded as the ovulation-inducing factor that is present in the seminal plasma of some male mammals (Ratto et al., 2012). Indeed, intramuscular, intravenous or intrauterine administration of NGF consistently induced ovulation in a dose-dependent manner in several induced ovulatory mammals, such as llamas, camelids and alpacas (Ratto et al., 2005; Silva et al., 2015; El Allali et al., 2017). A recent study using a camel model proposed that NGF in the seminal plasma was absorbed through the endometrium to the bloodstream and transferred to the hypothalamus where this growth factor stimulates neurons to produce kisspeptin (a potent stimulator of GnRH), which eventually regulates the preovulatory LH surge (El Allali et al., 2017). At present, the functional role of NTs in the regulation of ovulation in humans remains unknown.

Corpus luteum formation

In addition to the functional role of NGF in regulating ovulation, NGF was associated with enhanced tissue vascularization during the early stage of corpus luteum development in llamas (Ulloa-Leal et al., 2014). Several studies using animal models have revealed that the preovulatory LH surge induced by NGF can be sustained remarkably longer than that induced by GnRH administration, indicating that the luteotropic effect is most likely triggered by NGF due to prolonged LH secretion (Adams et al., 2005; Silva et al., 2011; Ulloa-Leal et al., 2014). Subsequent studies have confirmed the luteotropic role of NGF in regulating corpus luteum function through direct or indirect effects on GnRH neurons in the hypothalamus (Silva et al., 2011; Fernández et al., 2014; El Allali et al., 2017).

Roles for NTs in ovarian pathology and diseases

Given the important roles for NTs in regulating follicular development and oocyte competence, we can speculate that dysregulation of the NT system may negatively affect ovarian function, leading to reproductive pathology or female infertility. Clinical data obtained from fertile women revealed a cyclic change in plasma BDNF levels, which were positively correlated with estradiol and progesterone and negatively correlated with age (Begliuomini et al., 2007; Pluchino et al., 2009). Additionally, the concentrations of BDNF steadily declined after menopause (Begliuomini et al., 2007). Interestingly, in menopausal women, hormone replacement therapy restored their plasma BDNF to levels comparable to those in premenopausal women (Begliuomini et al., 2007). In mice, chronic unpredictable stress decreased the expression of BDNF in the antral follicles and reduced the number of mature oocytes and formation of blastocyst embryos, which was reversed by exogenous treatment with BDNF (Wu et al., 2012). In three goat breeds, a single nucleotide polymorphism (SNP; A705G) that was associated with larger litter size was detected in the NGF gene, indicating that NGF could potentially be used as a marker for goat litter size (An et al., 2013).

Diminished ovarian reserve

Altered FF levels of NTs have been identified in women with different etiologies of infertility undergoing IVF treatment, indicating the association of dysregulated NTs with infertility (Buyuk and Seifer, 2008). Compared to women with normal ovarian function, women with diminished ovarian reserve have higher FF levels of NGF and lower (although not statistically significant) FF levels of BDNF (Buyuk and Seifer, 2008). Lower expression levels of BDNF in women with diminished ovarian reserve may reflect their ovarian ages and a gradual decline in follicle quantity. Although many studies have demonstrated the decline in BDNF levels with advancing chronological age in women (Begliuomini et al., 2007; Ziegenhorn et al., 2007; Buyuk and Seifer, 2008), BDNF (unlike anti-Müllerian hormone) does not reflect ovarian function or serve as an indicator for cancer in women receiving chemotherapy (Aslam et al., 2011).

In humans, NGF levels were significantly higher (\sim 4- to 5-fold) in FF than in serum, indicating local production of NGF in the ovarian follicle (Palumbo et al., 2013). Since NGF is expressed in the oocytes

and GCs from primordial to secondary follicles in human fetal and adult ovaries, higher expression levels of NGF may accelerate the activation of primordial follicles that eventually leads to poor ovarian reserve. Another study using clinical samples obtained from women undergoing IVF treatment also showed that an increased FF level of NGF was correlated with a lower ovary response (especially in older women) (Palumbo *et al.*, 2013). However, the high expression levels of NGF may be the byproduct of a lower response of germ cells or follicular cells to NGF receptors. Indeed, an *in vitro* study demonstrated that decreased expression levels of the NT receptors TrkA and p75NTR in human CCs were associated with diminished ovarian reserve in women undergoing ART (Buyuk *et al.*, 2011). At present, studies using genetic approaches have not detected any naturally occurring mutations in genes of NTs or their receptors that are associated with women with primary ovarian insufficiency.

PCOS

NGF and BDNF are two neurotrophic factors that are most likely involved in the pathogenesis of PCOS. The BDNF levels in both FF and plasma were higher in women with PCOS than in healthy women (Russo *et al.*, 2012). In human GCs, the addition of hCG to culture medium increased the levels of BDNF up to 3- to 4-fold, indicating that locally produced BDNF is mainly regulated by LH or hCG (Feng *et al.*, 2003; Zhao *et al.*, 2011). Therefore, the high levels of LH in women with PCOS likely contribute to increased expression of BDNF in GCs, resulting in increased secretion of BDNF in the FF and plasma (Russo *et al.*, 2012). These findings suggest that BDNF could be a candidate neurotrophic factor capable of promoting follicular development. Indeed, the levels of BDNF in FF and plasma have been identified as potential predictors of IVF outcomes (Monteleone *et al.*, 2007; Wang *et al.*, 2011).

In rats, exogenous administration of steroids induced an increase in intraovarian synthesis of NGF and p75NTR, which led to polycystic ovaries that were characterized by the appearance of multiple follicular cysts (Lara *et al.*, 2000). In mice, transgenic overexpression of *Ngf* targeted to the ovary caused an ovarian cystic morphology that represented both the reproductive and metabolic features of human PCOS (Dissen *et al.*, 2009; Wilson *et al.*, 2014) (Table III). Morphological evaluation of the ovaries in these mice showed that overproduction of NGF led to arrested follicular development and increased apoptosis of antral follicles (Dissen *et al.*, 2009). Collectively, these findings support the hypothesis that sympathetic neuron hyperactivity of the ovary may contribute to the development and progression of PCOS (Wilson *et al.*, 2014). Indeed, clinical data obtained from patients undergoing IVF revealed that women with PCOS had an approximate 2-fold increase in the FF level of NGF and a 6-fold increase in NGF expression in GCs compared with those in women without PCOS (Dissen *et al.*, 2009). In contrast, another clinical study did not show a similar result regarding the increased FF levels of NGF in patients with PCOS (Buyuk and Seifer, 2008).

Endometriosis

Affecting ~10% of reproductive-aged women, endometriosis is a chronic gynecological disease that is defined as the appearance of endometrial fragments outside the uterine cavity (Vigano *et al.*, 2004). Despite extensive investigations, the precise pathogenesis and

detailed molecular mechanisms of this common disease remain elusive (Bulun *et al.*, 2015). Emerging evidence has suggested a critical role for NTs in uterine physiology and endometrial pathology (Barcena de Arellano *et al.*, 2013; Wessels *et al.*, 2014). The immunointensity of NGF and TrkA was higher in both the endometriotic stroma and epithelium obtained from women with endometriosis and deep dyspareunia than that in those obtained from women with endometriosis without deep dyspareunia, indicating that NGF signaling may play an important role in endometriosis-associated sexual pain (Peng *et al.*, 2018). A clinical study demonstrated that women with endometriosis had higher levels of NGF in their FF than did patients with male factor infertility (Buyuk and Seifer, 2008).

Proteomic analyses have identified the presence of both BDNF and NT-4 in the eutopic endometrium of women with endometriosis (Browne *et al.*, 2012). Additionally, estrogen receptor, BDNF and TrkB mRNA and protein were detected in the ectopic endometrium of women with ovarian endometriosis (Yu *et al.*, 2016). Furthermore, the estrogen-induced upregulation of BDNF and TrkB that enhances the proliferation of endometrial cells in uterine tissue has been proposed to explain the pathogenesis of estrogen-dependent diseases, including endometriosis (Wessels *et al.*, 2015; Dong *et al.*, 2017). Indeed, recent clinical studies have reported that plasma concentrations of BDNF were significantly higher in women with endometriosis, especially in Stages I and II disease, indicating that BDNF is a potential clinical marker for this painful disorder during its early stage (Wessels *et al.*, 2016; Rocha *et al.*, 2017; Perricos *et al.*, 2018). Targeted genetic association analysis of a case-control study including 425 patients with endometriosis revealed the presence of a BDNF SNP that was linked to an increased severity of endometriosis (Stage III and Stage IV) (Zhang *et al.*, 2012). Interestingly, higher FF levels of BDNF were detected in women with unexplained infertility than in those with known causes of infertility (Sadeu *et al.*, 2012). Future studies will be of great interest to determine whether NTs are involved in the pathogenesis of endometriosis or whether they are merely disease-related byproducts.

Epithelial ovarian cancer

Epithelial ovarian cancer (EOC) represents approximately 90% of total ovarian cancer cases; EOC is a deadly cancer type with a low 5-year survival rate (Siegel *et al.*, 2011). In mammals, NGF and its receptor TrkA might be involved in the regulation of morphological changes in ovarian surface epithelium during the breeding season (Bao *et al.*, 2014). In humans, NGF and TrkA are highly expressed in EOC and are associated with the processes that are crucial for EOC development, including cancer cell proliferation, migration, invasion and angiogenesis (Vera *et al.*, 2014). TrkA-mediated NGF-induced angiogenesis in endothelial cells promotes the development and progression of EOC (Julio-Pieper *et al.*, 2006; Vera *et al.*, 2014). *In vitro* studies have demonstrated that NGF upregulated the expression of VEGF, which contributes to the metastatic potential of a tumor (Julio-Pieper *et al.*, 2006; Tapia *et al.*, 2011). In addition to VEGF, other molecules regulated by NGF that are involved in the crucial process required for the progression of EOC include calreticulin (CRT), cyclo-oxygenase-2, disintegrin, metalloproteinase domain-containing protein 17 and several miRNAs (for reviews see (Retamales-Ortega *et al.*, 2017)). These studies suggest that NGF may be applied as a biomarker for ovarian cancer.

Clinical applications and therapeutic potential

In many mammals, including humans, NGF, BDNF and GDNF are associated with oocyte maturation and embryo development (Seifer et al., 2002; Kawamura et al., 2005; Wang et al., 2011; Linher-Melville and Li, 2013; Cui et al., 2018). In clinical application, both FF and plasma levels of BDNF are reportedly potential predictors of pregnancy outcome during IVF/ICSI treatment (Monteleone et al., 2007; Wang et al., 2011). Furthermore, the addition of BDNF and GDNF to cultured immature human oocytes and human CCs promoted total yields of MII oocytes and CC viability, respectively (Zhao et al., 2011; Cui et al., 2018). Similarly, combined treatment with BDNF, EGF and IGF-I improved the maturation rates of human oocytes and subsequent embryo development (Yu et al., 2012). Collectively, these findings have provided insights into the roles of NTs and GDNF in the regulation of oocyte maturation and CC viability. Supplementation of the IVM culture medium with these intraovarian growth factors (alone or in combination) may eventually be applied to enhance the developmental potential of immature oocytes obtained from patients undergoing ART.

Given the indispensable role of NGF-induced PI3K/Akt-mTOR signaling in the activation of dormant primordial follicles, a potential novel infertility therapy has been proposed by using PTEN inhibitors and PI3K stimulators to promote the PI3K/Akt-mTOR-mediated activation of residual follicles in patients with primary ovarian insufficiency (Kawamura et al., 2013). Based on animal studies showing that treatment with the mTOR inhibitor rapamycin can preserve the size of rat follicles (Adhikari et al., 2013), we speculate that blocking agents targeting ovary-specific mTOR signaling or NGF function could be a good option in the future for fertility preservation in women, who now have an extended life expectancy. However, future studies on NGF and human primordial follicle activation are needed.

Data obtained from clinical samples showing that plasma concentrations of BDNF were significantly higher in women with endometriosis (Stages I and II) (Wessels et al., 2016; Rocha et al., 2017; Perricos et al., 2018) may indicate the potential use of BDNF in diagnosing this chronic disease in its early stage. The participation of NGF signaling in the development of ovarian cancer and the findings that NGF and TrkA are highly expressed in women with EOC suggest that NGF may be applied as a biomarker for ovarian cancer. In human ovarian surface epithelium and EOC cell lines, NGF increased the expression of chaperone protein CRT, which was reversed by the addition of a specific TrkA receptor inhibitor (Vera et al., 2012), suggesting that blocking NGF activity could potentially be used as a therapeutic approach or adjuvant therapy in treating patients with EOC.

Conclusion

Recently, there has been substantial interest in locally produced growth factors and intrafollicular signaling between the oocyte and its supporting follicle cells. Evidence supported by animal studies and clinical trials in humans has suggested that intraovarian NTs and GDNF play crucial roles in regulating ovarian function, ranging from ovarian follicle assembly, primordial follicle activation, follicular

growth and development, to steroidogenesis, oocyte maturation, ovulation and corpus luteum formation (Fig. 4). Normal ovarian function and follicular development rely on the synergistic and complementary effects of these neurotrophic factors and their corresponding receptors. Dysregulation of these intraovarian growth factors may lead to the development of infertility or ovarian diseases, such as diminished ovarian reserve, PCOS, endometriosis and even ovarian cancer. As our understanding of the expression, actions and underlying molecular mechanisms of these neurotrophic factors in the human ovary expands, the advancement of new diagnostic and therapeutic applications for the management of patients with infertility and ovarian pathology, as well as improvement in oocyte quality, will become plausible.

Authors' roles

H.M.C. collected the information, designed the pictures and wrote the manuscript. H.C.W. collected the information and designed the pictures. Z.G.S. collected the information and wrote the manuscript. F.L. collected the information and critically revised the manuscript. P.C.K.L. collected the information, designed the pictures and critically revised the manuscript. All the authors have seen and approved the final version.

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Conflict of interest

We declare that H.C., H.W., Z.S., F.L. and P.C.L. have no conflict of interest with the contents of this manuscript.

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