Expression of Nerve Growth Factor and Its Receptors, TrkA and p75, in Porcine Ovaries

Barbara JANA¹, Marlena KOSZYKOWSKA¹ and Joanna CZARZASTA¹

¹Division of Reproductive Endocrinology and Pathophysiology, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland

Abstract. The cellular localization of nerve growth factor (NGF) and its receptors (TrkA, p75) was investigated during the estrous cycle in gilts. Also, the levels of expression of these factors in walls of tertiary follicles and corpora lutea (CLs) were determined using Western blot. The ovaries from days 3, 7, 16 and 20 of the cycle revealed the presence of NGF and its receptors in oocytes of secondary and tertiary follicles, follicular cells of primary and secondary follicles, thecal and granulosa cells of tertiary follicles and steroidogenic cells of CLs. In wall cells of primary follicles, NGF, TrkA and p75 staining was strongest on day 16, while in secondary follicles, only p75 was more intensely stained on day 16 and 20. In walls of small (3 mm in diameter) and medium (4–6 mm in diameter) follicles, NGF staining was lower on day 16, and the p75 reaction was strongest on day 20. On day 20, NGF staining in large follicles (7–10 mm in diameter) was higher than in smaller follicles. The levels of NGF and p75 in small and medium follicles were highest on day 20. The contents of NGF and TrkA in large follicles on day 20 were higher than in smaller follicles. NGF and TrkA contents in CLs were highest on day 7. Our study demonstrates that NGF, TrkA and p75 are expressed in the ovary during the estrous cycle in gilts. These results suggest that NGF and its receptors may be important for ovarian function in cycling gilts.

Key words: Nerve growth factor, Ovary, Pig, p75, TrkA

Nerve growth factor (NGF) belongs to a family of neurotrophin (NTs) target-derived trophic factors required for survival and differentiation of neuronal populations in both the central and peripheral nervous system. Other NTs have been identified besides NGF, including brain-derived neurotrophic factor (BDNF) [1], NT-3 [2], NT-4/5 [3] and NT-6 [4]. NTs bind to two kinds of receptors with dissociation constants of $10^{-8}$ M and $10^{-11}$ M that denominate low- and high-affinity receptors, respectively, as reviewed by Friedman and Greene [5]. The low-affinity receptor is p75, that belongs to the tumor necrosis receptor family and binds all NTs with similar affinity [6]. TrkA is a high-affinity receptor, receptors TrkA, TrkB and TrkC. The Trk proteins bind preferentially to specific NTs. TrkA binds NGF, TrkB binds BDNF and NT-4/5 and TrkC binds NT-3 ligands [7, 8]. Most of the survival and growth properties elicited by NGF are mediated by TrkA [7]. The p75 can bind to TrkA and enhance responsiveness to NGF. Without TrkA, activation of p75 can induce apoptosis [9].

It is very well known that the action of NTs including NGF is not limited to the nervous system: NTs and their receptors have been found in the immune and endocrine systems [8–10]. Ovarian cells have been shown to express NGF and/or TrkA and p75 in a variety of mammals, including women [11–13], rats [14, 15], golden hamsters [16], cows [17, 18], sheep [19, 20] and Shiba goats [21]. NGF and its receptors regulate several ovarian functions, such as sexual development [22], follicular development and ovulation [23, 24], in an auto- and paracrine manner.

NGF activity and content were found to be increased by estradiol-17β (E2) in a glioma cell line culture [25]. A rise in NGF level was also revealed in the brain and spinal cord of testosterone-treated adult female mice [26]. In rats, estrogen administration altered TrkA mRNA content in the medium septum [27] and dorsal root ganglia (DRG) neurons [28]. The level of TrkA mRNA in the rat hippocampus widely fluctuated during the estrous cycle, while the expressions of NGF, TrkA and p75 in the rat ovary did not noticeably change during the estrous cycle [27]. In the ovaries of golden hamsters, stronger staining for NGF and its receptors in interstitial cells was revealed on the day of ovulation as compared with other days of the estrous cycle. This phenomenon may result from the stimulatory effect of a luteinizing hormone (LH) surge. Also, the atretic follicles on the day of proestrus displayed a more intensive p75 immunoreaction than on the rest of the days of the cycle [16].

Although many studies have examined the localizations and functions of NGF, TrkA and p75 in ovarian processes, little information is available concerning the expression of these factors in porcine gonads. So far, the only study on this subject has been that of Levanti et al. [18], who examined the cellular distribution TrkA and p75 in pig ovaries by immunocytochemical staining. Moreover, the ovaries used in that study were obtained from a slaughterhouse, and the phase of the estrous cycle was not determined. Therefore, the aim of the present experiment was to detect the cellular localization of NGF, TrkA and p75 in porcine ovaries throughout the estrous cycle. Additionally, the levels of NGF, TrkA and p75 protein expression were determined in tertiary folli-
Materials and Methods

Animals and experimental procedure

This study was performed on 24 crossbred gilts (Large White × Landrace), aged 7–8 months and weighing 90–110 kg, having two controlled subsequent estrous cycles. Behavioral estrus was detected using a boar tester. Three days before slaughter, the animals were transported from a farm to a local animal house and kept in individual stalls under natural light and temperature. They were fed a commercial grain mixture and tap water ad libitum. We followed the principles of animal care (NIH publication No. 86–23, revised in 1985) as well as the specific national law on animal protection.

The gilts were euthanized by electrical shock (ENZ 300 Meta- lowiec, Bydgoszcz, Poland) on days 3, 7, 16 or 20 of the estrus cycle (n=6 per day) and exsanguinated. The ovaries were immediately dissected out and weighed. Afterwards, the numbers of ovarian structures were estimated. The follicles were divided into three size classes: 1–3, 4–6 and 7–10 mm in diameter. From one ovary of each gilt, pieces of walls of follicles in the above size classes and CLs were dissected out, shock-frozen in liquid nitrogen and then stored at –70 °C in order to estimate the expression of NGF, TrkA and p75 proteins by Western blot analysis. The second ovary was fixed in Zamboni’s fixative to estimate the cellular localization of these factors by means of a routine single-immunofluorescence technique on cryostat sections (details see below).

Single-labelling immunofluorescence

Tissue blocks of ovaries fixed in Zamboni’s fixative for 30 min were washed in phosphate buffer and cryoprotected in 18% sucrose until sectioning. To investigate the localization of NGF, TrkA and p75, fragments of the ovaries were cut in a cryostat (Reichert-Jung, Nußlock, Germany) into 10-μm-thick sections and then subjected to the routine single-immunofluorescence technique described by Majewski and Heym [29]. Briefly, sections were incubated in a humid chamber overnight at room temperature (RT) with primary antibodies (all obtained from Santa Cruz Biotechnology, Santa Cruz, CA, USA), including rabbit anti-human NGF polyclonal antibody (H-20: sc-548), rabbit anti-human TrkA polyclonal antibody (C-14: sc-11) and rabbit anti-human p75 polyclonal antibody (N-20: sc-5634), which were all diluted 1:3000. The secondary antibodies were then visualized by streptavidin-CY3 complex (ICN Biomedicals; diluted 1:9000). Staining for each antibody was confirmed by carrying out the following negative controls: omission of the primary or secondary antibodies and neutralization of the primary antibodies by blocking peptides (all obtained from Santa Cruz Biotechnology, Santa Cruz, CA, USA). The localization of NGF and its receptor proteins was determined in the follicles and CLs. Stages of follicular development were defined as primordial, primary, secondary and tertiary [30, 31]. Additionally, tertiary follicles were divided into three size classes: small (up to 3 mm in diameter), medium (4–6 mm in diameter) and large (7–10 mm in diameter). The diameter of follicles, kind of ovarian cells displaying immunoreactivity (IR) for studied factors as well as intensity of staining were estimated using an Olympus BX51 microscope equipped with epifluorescence and appropriate filter sets and a Nikon image analysis software (Olympus Microimage) by two independent researchers. The results were expressed semiquantitatively (arbitrarily) as strong (+++), high (++), faint (+) or no reaction (−), as described previously by Levanti et al. [18].

Western blot analysis

The expression of NGF, TrkA and p75 proteins in the follicular walls and CLs were estimated with the method described by Jana et al. [32]. Briefly, the tissues were homogenized on ice with a cold buffer (50 mmol/l Tris-HCl, pH 8.0; 150 mmol/l NaCl; 1% Triton X-100, 10 μg/ml aprotinin, 52 μmol/l leupeptin, 1 mmol/l pepstatin A, 1 mmol/l EDTA, 1 mol/l PMSF) and centrifuged (10 min, 2,500 × g, 4°C). The supernatants were centrifuged (1 h, 17,500 × g, 4°C), and pellets were stored at –80 °C for further analysis. The Bradford method was used to estimate protein level [33]. Equal amounts (20 μg) of tissue lysates and β-actin (Abcam, UK) were dissolved in sodium dodecyl sulphate (SDS), a gel-loading buffer, heated (95°C, 4 min) and separated by 10% SDS-polyacrylamide gel electrophoresis. Separated proteins were electroblotted onto a 0.45-μm nitrocellulose membrane in a transfer buffer. The nonspecific binding sites were blocked by incubation with 5% fat-free dry milk in TBS-T buffer at RT for 1.5 h. The nitrocellulose membrane was incubated overnight at 4°C with primary antibodies, the same as in single-labelling immunofluorescence, against NGF, TrkA or p75 (all diluted 1:1000). NGF, TrkA and p75 were detected by incubating the nitrocellulose membranes for 1.5 h at RT with secondary biotinylated goat anti-rabbit antibodies (diluted 1:2,500; Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA). Visualization of the immune complex was performed by incubation with a freshly prepared mixture of 3.3′-diaminobenzidine tetrahydrochloride and H2O2 in Tris-buffered saline (pH 7.2) for 3–4 min. Each analysis was repeated three times. To provide antibody specificity, neutralization of the primary antibodies by blocking peptides (all obtained from Santa Cruz Biotechnology, Santa Cruz, CA, USA) was performed as a negative control. The proteins isolated from the porcine DRG were loaded as positive controls. The NGF, TrkA and p75 protein concentrations were quantitated by measuring optical density using the Kodak 1D Image Analysis Software (USA). Data were expressed as a ratio of NGF, TrkA and p75 proteins relative to β-actin protein in arbitrary optical density units.

Statistical analysis

In Western blot analysis, the mean (±SEM) of the intensity of band staining (arbitrary units) was calculated for each type of follicle and CLs on the particular studied days. The mean (±SEM) for follicular walls and CLs represents the values obtained from 6 ovaries (n=6). The Bonferroni test was applied to compare the mean values for the same structure between the particular days and mean values for follicles of different sizes on the same day (ANOVA, GraphPad InStat, GraphPad Software, San Diego, CA, USA). The level of significance was set at P≤0.05 for all analyses.
Results

Macroscopic evaluation of ovaries

In the ovaries collected on day 3 of the estrous cycle, only small (1–3 mm in diameter) follicles and CLs were found, while in the ovaries collected on day 7, small and medium (4–6 mm in diameter) follicles as well as CLs were found. In the ovaries from day 16, small and medium follicles, as well as CLs were also present. The ovaries on day 20 contained small, medium and large follicles (7–10 mm in diameter; Table 1).

Immunohistochemistry

No immunostaining for NGF, TrkA or p75 was detected when either the primary or secondary antibodies were omitted and neutralization of the primary antibodies was performed (Figs. 1J–L). NGF staining was not observed in the oocytes of the primordial and primary follicles from days 3, 7, 16 and 20 of the estrous cycle. NGF expression in the cells of primary follicles was faint on days 3 and 7 and strong on days 16 and 20. In the secondary follicles, the IR on each of the four days was high in the follicular cells and faint in the oocytes. The oocytes of small and medium tertiary follicles on all studied days were also faintly stained. The oocytes of large follicles on day 20 showed faint or high expression (Fig. 1A). In the walls of small follicles, NGF staining was high on days 3, 7 and 20 and faint on day 16. In the walls of medium follicles, IR for NGF was high on days 7 and 20 and faint on day 16. On day 20, IR for NGF was high or strong in the thecal cells and strong in the granulosa cells of large follicles (Fig. 1B). The steroidogenic cells of CLs on days 3, 7 (Fig. 1C) and 16 displayed strong NGF expression. On day 7, a larger number of these cells were stained than on the other studied days.

On days 3, 7, 16 and 20 of the estrous cycle, IR for TrkA and p75 was not present in the oocytes of primordial and primary follicles. The cells of primary follicles on each of the four days displayed high staining for both receptors, except on day 16 when the reaction was strong. On all studied days, the follicular cells (Fig. 1D) and oocytes of secondary follicles showed faint and high IR for TrkA, respectively. Expression of TrkA in the oocytes and the thecal and granulosa cells of small, medium and large (Fig. 1E) tertiary follicles was high on each of the four days. However, IR for TrkA in the granulosa cells of large follicles on day 20 was also strong (Fig. 1E). A faint p75 reaction was detected in the oocytes of secondary follicles on each of the four days (Fig. 1G) and in the wall cells of these follicles on days 3 and 7. On days 16 (Fig. 1G)

Table 1. Mean (±SEM) number of the follicles and corpora lutea in porcine ovaries on days 3, 7, 16 and 20 of the estrous cycle per ovary

<table>
<thead>
<tr>
<th>Day of the estrous cycle</th>
<th>Follicles (mm)</th>
<th>Corpora lutea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–3</td>
<td>4–6</td>
</tr>
<tr>
<td>3</td>
<td>21.0 ± 3.0</td>
<td>l.s.</td>
</tr>
<tr>
<td>7</td>
<td>18.0 ± 8.0</td>
<td>8.5 ± 0.5</td>
</tr>
<tr>
<td>16</td>
<td>24.0 ± 3.5</td>
<td>8.5 ± 1.6</td>
</tr>
<tr>
<td>20</td>
<td>15.3 ± 0.9</td>
<td>5.5 ± 1.3</td>
</tr>
</tbody>
</table>

l.s.: Lack of structure.

Fig. 1. Immunohistochemical localization of NGF (A–C), TrkA (D–F) and p75 (G–I) in porcine ovaries during the estrous cycle. A) Faint and high NGF staining in the oocyte of the large tertiary follicle on day 20. B) High and strong immunoreactivity for NGF visible in the thecal and granulosa cells, respectively, of large tertiary follicle on day 20. C) Note the intense reaction in the steroidogenic cells of the CL on day 7. D) Moderate staining for TrkA in follicular cells of secondary follicles on day 16. E) High and strong TrkA expression visible in the thecal and granulosa layers, respectively, of large tertiary follicle on day 20. F) Intense reactivity of TrkA in the steroidogenic cells of CL on day 16. G) Faint p75 reaction in the oocytes and high in follicular cells of secondary follicles on day 16. H) High p75 staining of the thecal and granulosa cells of medium tertiary follicles on day 7. I) Note the high and strong p75 expression in the thecal and granulosa cells, respectively, of large tertiary follicles on day 20. Negative controls for NGF (J), TrkA (K) and p75 (L). arrow head—oocyte, arrow—thecal cells, asterisk—granulosa cells, × 200.
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and 20, IR for p75 was high in the walls of secondary follicles. The oocytes in tertiary follicles of all size classes were mostly stained highly for p75. In the walls of small and medium (Fig. 1H) tertiary follicles on days 3, 7 and 16, high IR for p75 was revealed, and strong IR was revealed on day 20. In the thecal cells of large follicles on day 20, high or strong IR for p75 was visible, while the granulosa cells showed strong staining (Fig. 1I). The single steroidogenic cells of CLs from days 3, 7 and 16 displayed strong TrkA (Fig. 1F) and p75 expression (Table 2).

Western blotting

Western blots of the porcine DRG (as a positive control) showed immunoreactive bands at 13, 140 and 75 kDa and were interpreted to be NGF, TrkA and p75 proteins, respectively. In turn, after neutralization of the primary antibodies against these factors, the bands were absent (data not shown). All porcine ovarian tissue collected on days 3, 7, 16 and 20 of the estrous cycle expressed NGF, TrkA and p75 proteins at satisfactorily detectable levels (Fig. 2). The level of NGF protein in small follicles on day 20 was higher compared with the levels on days 3 (P<0.05), 7 (P<0.01) and 16 (P<0.01). On day 20, the level of this protein in medium follicles was higher (P<0.05) than on days 7 and 16. NGF protein expression in the large follicles was higher than in the small (P<0.05) and medium (P<0.01) follicles. Compared with day 3, the content of TrkA in CLs was enhanced (P<0.05) on day 7 (Fig. 2B). The levels of p75 protein in the small and medium follicles on day 20 were higher (P<0.05) than on days 3 and 7, respectively (Fig. 2C).

Discussion

The present study is the first to show the cellular localization of NGF as well as its receptors TrkA and p75 in porcine ovaries during the estrous cycle. We also demonstrated the levels of protein expression of these factors in the walls of tertiary follicles and CLs.

Microscopic observations of porcine ovaries obtained on days 3, 7, 16 and 20 of the estrous cycle revealed the localization of NGF, TrkA and p75 in follicular cells of primary and secondary follicles and in thecal and granulosa cells of small, medium and large tertiary follicles as well as in oocytes of all types of follicles except primordial and primary follicles. The distribution of both receptors in follicle walls is in agreement with data reported earlier in pigs and cows [18]. Moreover, the localization of NGF and its receptors is similar to that found in golden hamsters [16] and Shiba goats [21]. It is worth mentioning that, in women, only NGF and TrkA were found in the walls of preantral and antral follicles [34], while in rats, both of these proteins were present in the granulosa layer [23]. In mice, the granulosa layer expressed only p75, and expression of NGF and TrkA occurred in both thecal and granulosa layers

Table 2. Distribution of NGF, TrkA and p75 in porcine ovaries on days 3, 7, 16 and 20 of the estrous cycle

<table>
<thead>
<tr>
<th>Factor</th>
<th>NGF</th>
<th>TrkA</th>
<th>p75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of the cycle</td>
<td>3</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Ovarian structure</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Primordial follicles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oocytes</td>
<td>––</td>
<td>––</td>
<td>––</td>
</tr>
<tr>
<td>Primary follicles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oocytes</td>
<td>––</td>
<td>––</td>
<td>––</td>
</tr>
<tr>
<td>Follicular cells</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Secondary follicles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oocytes</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Follicular cells</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Small tertiary follicles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oocytes</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cells: Thecal</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Granulosa</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Medium tertiary follicles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oocytes</td>
<td>L.s.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cells: Thecal</td>
<td>L.s.</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Granulosa</td>
<td>L.s.</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Large tertiary follicles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oocytes</td>
<td>L.s.</td>
<td>L.s.</td>
<td>L.s.</td>
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<tr>
<td>Cells: Thecal</td>
<td>L.s.</td>
<td>L.s.</td>
<td>L.s.</td>
</tr>
<tr>
<td>Granulosa</td>
<td>L.s.</td>
<td>L.s.</td>
<td>L.s.</td>
</tr>
<tr>
<td>Corpora lutea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steroidogenic cells</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Staining intensity: ++++, ++, + and – represent strong, high, faint and lack of staining, respectively. L.s.: Lack of structure.
The distribution of NGF and its two receptors in oocytes observed in the present study matched those found in pigs but contradictory to those found in cows [18]. These discrepancies are probably due to species differences.

The presence of NGF, TrkA and p75 in porcine follicular cells of primary and secondary follicles implies the possibility that NGF acting via both kinds of receptors affects the development of preantral follicles. NGF null mutant mice have a markedly reduced number of primary and secondary follicles [24]. In women and rodents, NGF facilitates the development of newly formed follicles by promoting the initial differentiation and subsequent growth of primordial follicles [11, 15, 35, 36]. These actions are related to the ability of NGF to sustain the proliferation of both mesenchymal and follicular cells and to induce the synthesis of follicle stimulating hormone (FSH) receptors [15, 36].

Our results show the patterns of NGF, TrkA and p75 protein expression in porcine follicles throughout the estrous cycle. In the cells of primary follicles, these three components stained more strongly on day 16 (NGF also on day 20) than on other days. A stronger staining for p75 was found in the wall cells of secondary follicles on days 16 and 20 of the cycle than on days 3 and 7, while the intensity of NGF and TrkA staining in these cells was similar during the estrous cycle. The patterns of NGF and its receptor expression were also altered in small and medium tertiary follicles (except for TrkA in small follicles) during the estrous cycle, although, the staining of these factors in the cells not always matches the levels estimated by Western blot analysis. The levels of NGF protein in small and medium follicles and p75 expression in these structures were higher on day 20 of the cycle than on the other days. In contrast, TrkA protein levels decreased on days 16 and 20 in medium follicles. In golden hamsters, p75 staining in

Fig. 2. Western blot analysis of NGF (A), TrkA (B) and p75 (C) proteins in the follicles (F) and corpora lutea (CL) in porcine ovaries on days 3, 7, 16 and 20 of the estrous cycle. NGF, TrkA and p75: day 3 – 1–3 mm F (line 1), CL (line 2); day 7 – 1–3 mm F (line 3), 4–6 mm F (line 4), CL (line 5); day 16 – 1–3 mm F (line 6), 4–6 mm F (line 7), CL (line 8); day 20 – 1–3 mm F (line 9), 4–6 mm F (line 10), 7–10 mm F (line 11). A densitometric analysis of NGF, TrkA and p75 presented in arbitrary units; a and b indicate differences (P<0.05, P<0.001) between the particular days for the same structure, and x and y indicate differences (P<0.05, P<0.01) among follicles of different sizes on the same day of the estrous cycle.
granulosa cells of follicles rose in proestrus, and p75 and NGF were found to be expressed in interstitial cells in estrus [16]. The expression of NGF and its receptor in the uterus of the golden hamster was highest in proestrus, when E2 production is greatest [39]. These above findings suggest that an increase in NGF and/or its receptor expression in porcine follicles, especially on day 20, may relate with an auto- and/or paracrine effect of E2. The increase in expression of NGF and TrkA in large follicles found in our study on day 20 may result also from an LH effect. This assumption is supported by results showing a decrease in the intensity of immunostaining for NGF and its receptors in interstitial cells of the golden hamster ovary after treatment with anti-estrogen against luteinizing hormone-releasing hormone [16] as well as by the study of Dissen et al. [23] demonstrating that juvenile rats exhibited markedly elevated TrkA mRNA levels after the first preovulatory LH surge. It should also be added that LH may increase both NGF and p75NTK in the ovary of adult cow and pig. [21].

In porcine ovaries, we determined also that NGF and its receptors are expressed in steroidogenic cells of CLs derived from days 3, 7, 16 of the estrous cycle. These factors have previously been localized in CLs of golden hamsters [16] and Shiba goats [21]. However, Levanti et al. [18] observed expression of TrkA, but not p75, in porcine CLs. The difference in the distribution of p75 between the two studies is probably due to a difference in the day of the estrous cycle on which the ovaries were collected. We observed that the intensity of staining for NGF and its receptors in steroidogenic cells of CLs was similar (strong) during the estrous cycle. In turn, increased expression of NGF and TrkA protein levels in CLs on day 7 compared with day 3 were found. In golden hamsters, CL cells displayed a stronger reaction for NGF and its receptors in metestrus than in estrus and diestrus [16]. Our finding of parallel increases in NGF and TrkA protein levels in the CLs suggests that NGF affects CL function acting by this kind of receptor. The higher content of NGF and TrkA protein expressions in CLs on day 7 than on day 3 may imply that NGF plays a role in development and/or maintenance of luteal function. This supposition is supported by the findings that NGF can stimulate of P4 and oxytocin release [40] and acetylcholine production [41] in bovine CLs as well as maintenance of luteal vasculature in rats [38].

In conclusion, we demonstrated that NGF, TrkA and p75 proteins are expressed in the ovary during the estrous cycle in gilts. These results suggest that locally produced NGF in the ovary may affect follicular and luteal function in cycling gilts.

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References


