Polish Journal of Veterinary Sciences Vol. 16, No. 1 (2013), 101–105

DOI 10.2478/pjvs-2013-0014

Original article

Ontogeny of the long form of leptin receptor gene expression in the porcine ovarian follicles

N. Smolinska, T. Kaminski, G. Siawrys, J. Przala

Department of Animal Physiology, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn, Oczapowskiego 1A, 10-719 Olsztyn, Poland

Abstract

Leptin is a polypeptide hormone produced predominantly in adipocytes. It has been found to be implicated in the regulation of satiety and energy homeostasis. A role for leptin in reproduction was later suggested by findings that this hormone may be involved in the regulation of the hypothalamic-pituitary-gonadal axis *via* endocrine, paracrine and/or autocrine pathways. The objective of the study was to investigate the ontogeny of the long isoform of leptin receptor (OB-Rb) gene in porcine ovarian follicles. The expression of OB-Rb gene was detected in porcine primordial, primary, secondary and antral follicles by *in situ* hybridization. In summary, our data suggest that leptin might have a direct effect on porcine follicles and plays an important role in the follicular development.

Key words: gene expression, ISH, leptin receptor, ontogeny, ovarian follicles, pigs

Introduction

Leptin is a 167-amino acid protein secreted by adipocytes. It has been recognized as an important hormone in appetite reduction and energy expenditure. Recent studies have revealed that leptin also plays a major role in reproduction (Zhang et al. 1994, Moschos et al. 2002). Leptin activities are mediated through the leptin receptor, a member of the class I cytokine receptor superfamily. The leptin receptor has been shown to have at least six splice variants. The isoforms can be classified into three types: long form (OB-Rb), short form (OB-Ra, -Rc, -Rd, -Rf), and secreted form (OB-Re). Only the OB-Rb isoform is thought to be capable of full intracellular signal transduction (Tartaglia et al. 1995, Bjorbaek et al. 1997).

It has been reported that leptin receptors are present in human and rat theca and granulosa cells (Cioffi et al. 1997, Ryan et al. 2003) and in theca and granulosa cells harvested from antral follicles of prepubertal gilts, in porcine corpora lutea (CL) and ovarian stroma (OS) (Ruiz-Cortes et al. 2000, Smolinska et al. 2007). These results suggest that leptin may have a direct effect on ovarian function. The identification of leptin mRNA and protein in human granulosa and theca cells, follicular fluid, oocytes and CL (Antczak and Van Blerkom 1997, Cioffi et al. 1997, Welt et al. 2003), in murine theca and granulosa cells, oocytes, CL and OS (Antczak and

Correspondence to: N. Smolinska, e-mail: nina.smolinska@uwm.edu.pl, tel.: +48 89 523 42 26

Van Blerkom 1997, Ryan et al. 2002, 2003, Archanco et al. 2003) as well as in porcine CL and OS (Smolinska et al. 2010) suggests that leptin is a product of the ovary. To date, ontogeny of the expression of the long form of leptin receptor gene in porcine ovarian follicles has not been previously examined. Therefore, the aim of the present study was to identify the localization of OB-Rb mRNA in porcine ovarian primordial, primary, secondary and antral follicles by *in situ* hybridization.

Materials and Methods

Experimental animals

The investigations were carried out in accordance with the principles and procedures of the Animal Ethics Committee at the University of Warmia and Mazury in Olsztyn (Poland). Cycling gilts (Large White x Polish Landrace; n=3), 7-8 months old, weighing 120-130 kg, were obtained from private breeders. The pigs were last fed in the afternoon of the day before their slaughter. Within a few minutes after slaughter, ovaries were collected. All the samples were frozen in liquid nitrogen and maintained at -80°C until *in situ* hybridization was performed.

In situ hybridization

Hybridization was carried out according to Smolinska et al. (2007). Briefly, paraformaldehyde-fixed sections (6 µm) from porcine ovarian samples were acetylated for 10 min in 0.25% acetic anhydride (Fluka, USA) in 0.1 M triethanolamine/0.9% NaCl and then dehydrated with ethanol series. The antisense oligonucleotide probes (5' – TTG GGA TGC TGA TCT GAT AA – 3') were labeled with [^{35}S]-α dATP (Perkin Elmer, USA) at the 3'-end using terminal deoxynucleotidyl transferase (Roche, France). The sense oligonucleotide probes (5' – AAC CCT ACG ACT AGA CTA TT – 3') were used as a negative control for hybridization specificity. Tissue sections were air-dried and incubated for 22 h with 100 µl of hybridization solution containing 50% formamide, 10% dextran sulfate, 1×Denhardt's solution, 4×SSC (saline sodium citrate), 0.5 mg/ml salmon sperm DNA, 0.25 mg/ml transfer RNA and appropriate probes (10^7 cpm/ml) under parafilm coverslips at 42°C in humidified chambers. After hybridization, the slides were subjected to several washes in 1×SSC for 10 min, 2×SSC/50% formamide at 42-45°C for 15 min, 1×SSC for 15 min, and finally in distilled water for 1 min. The slides were then serially dehydrated in ethanol and air-dried. The [^{35}S]-α dATP-labeled sections were dipped in LM-1 emulsion (Amersham Biosciences, UK), exposed for 9 days at 4°C, developed in D-19 (4 min), and fixed in Fixer (5 min; Eastman Kodak, USA). In addition, the sections were stained with hematoxylin/eosin, dehydrated in grade series of ethanol, and coverslipped with Entellan (Merck, Germany). All sections were observed under the CH30/CH40 microscope (equipped for bright-field and dark-field microscopy) and photographed by the C-5060 WZ digital camera (Olympus, Japan).

Results

The localization of OB-Rb mRNA was found in porcine oocytes and flattened/cuboidal granulosa cells of primordial follicles, in granulosa cells of primary follicles and in granulosa cells and theca cells of secondary ones. In antral stages, OB-Rb gene expression was detected in all follicular compartments, *i.e.*, the oocyte, cumulus cells, granulosa cells and theca cells. When the sense probes were used, no stain was detected in porcine ovarian follicles (Fig. 1).

Discussion

In this study we have shown that OB-Rb mRNA is present in porcine primordial, primary, secondary and antral follicles. To our knowledge, this is the first report showing *in situ* OB-Rb expression in the porcine follicles throughout ontogenesis. We have previously reported that, during the luteal phase and early pregnancy in the porcine CL and OS, leptin and OB-Rb mRNA and protein (Smolinska et al. 2007, 2010), and OB-Ra mRNA (Bogacka et al. 2006) are expressed. In humans, leptin gene and protein and OB-Rb gene and protein have been detected in oocytes of follicles from primordial stages onward, with lower levels in oocytes and higher levels in oocytes of regressing antral follicles (Loffler et al. 2001, Abir et al. 2005). In addition, leptin mRNA and protein have been found in human granulosa and theca cells, CL, oocytes, and follicular fluid (Antczak and Van Blerkom 1997, Cioffi et al. 1997, Loffler et al. 2001, Welt et al. 2003), in mouse granulosa and theca cells, CL, oocytes and OS (Antczak and Van Blerkom 1997, Ryan et al. 2002) and in rat theca cells, CL and oocytes (Archanco et al. 2003, Ryan et al. 2003). OB-Rb gene and protein expression has been identified in human granulosa and theca cells (Cioffi et al. 1997, Karlsson et al. 1997, Agarwal et al. 1999), in mouse follicular cells, CL, OS and oocytes (Ryan et al. 2002), in rat follicular cells, CL and oocytes (Zamarano et al. 1997,

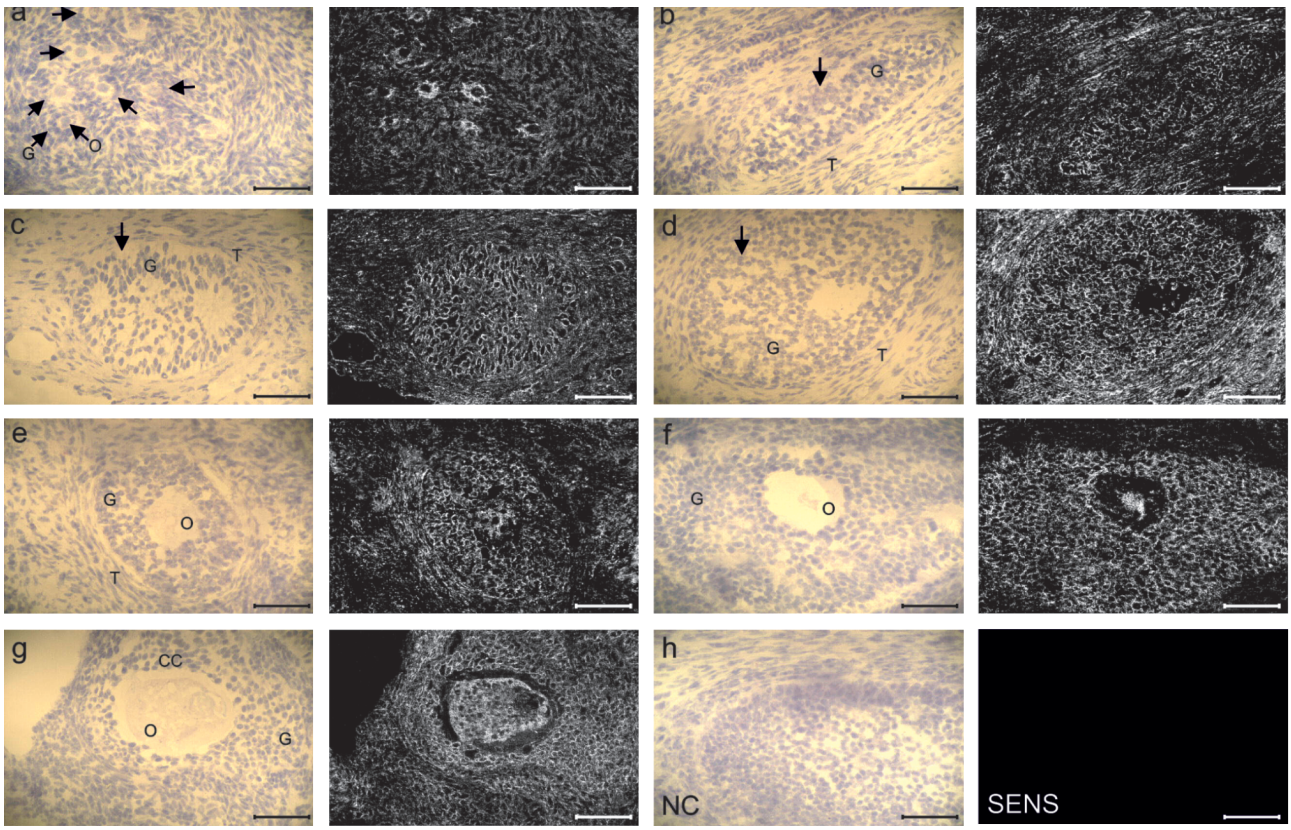

Fig. 1. Localization of OB-Rb mRNA determined by *in situ* hybridization (dark-field images) in porcine primordial and primary (a), secondary (b) and antral (c-g) follicles. Corresponding bright-field images depict hematoxylin/eosin staining. NC – Negative controls with the sense sequence (h). O – oocyte, G – granulosa cells, T – theca cells, CC – cumulus cells. Bar = 20 μm.

Ryan et al. 2003) as well as in porcine granulosa and theca cells, CL and OS (Ruiz-Cortes et al. 2000, 2003, Smolinska et al. 2007). This study coupled with our previous findings and results obtained in other species support the conclusion that the ovary is an important source of leptin and the hormone may act within the gland as an autocrine/paracrine factor directly modulating ovarian function.

In leptin deficient mice the number of primordial follicles and the total number of ovarian follicles have been reduced. In these animals apoptosis of GC and follicular atresia have been increased (Hamm et al. 2004). Moreover, leptin administration increased the number of primary follicles and the total number of ovarian follicles in leptin deficient mice (Barash et al. 1996). In immature rats, leptin accelerated the onset of puberty by attenuation follicular atresia and enhancement of follicular development (Almog et al. 2001). These results suggest that leptin might play a role in initiation of primordial follicle growth and the early stages of folliculogenesis, and in survival of primordial follicles.

It is conceivable that the effect of leptin on folliculogenesis is obtained through control of steroid hormones' production. However, the action of leptin on ovarian steroidogenesis is controversial. It has been found that leptin affects the secretion of steroids in a dose-dependent and species-dependent manner. In cultured human granulosa cells, low doses of leptin increased both basal oestradiol (E_2) and progesterone (P_4) secretion, but high doses inhibited the release of these steroids (Tsai et al. 2002, Karamouti et al. 2009). Similarly, in porcine granulosa cells, leptin at physiological doses increased the activity of steroidogenic acute regulatory protein and the accumulation of P_4, while a high dose inhibited them (Ruiz-Cortes et al. 2003). On the other hand, in mice, leptin at a high concentration significantly stimulated follicular E_2, P_4, and testosterone production (Swain et al. 2004). Moreover, it has been demonstrated that leptin inhibited the insulin-induced secretion of E_2 and P_4 by bovine granulosa cells (Spicer and Francisco 1997). Consistent with these findings, it has been reported that leptin attenuates LH-stimulated E_2 production in human granulosa cells (Karlsson et al. 1997). Agarwal et al. (1999) reported an inhibitory effect of this hormone on combined IGF-I- and FSH-stimulated E_2 production by human granulosa cells, and IGF-I (insulin-like growth factor I)- and LH-induced androstenedione secretion by cultured

theca cells. In addition, in porcine preovulatory follicles, leptin impaired basal and IGF-I- and GH-stimulated E_2 production (Gregoraszczuk et al. 2003, 2004). However, other studies revealed that leptin increased oestrogen secretion in cultured human granulosa cells (Kitawaki et al. 1999), and basal and IGF-I- and GH-stimulated P_4 release by porcine preovulatory follicles (Gregoraszczuk et al. 2003, 2004). Therefore, the effect of leptin on ovarian follicle steroidogenesis needs to be further elucidated.

In conclusion, our results indicate that OB-Rb transcripts are present in porcine primordial, primary, secondary and antral ovarian follicles. These findings suggest that leptin might directly affect porcine follicles and plays an important role in folliculogenesis.

Acknowledgements

This research was supported by the State Committee for Scientific Research (project nos. PBZ KBN-084/P06/2002JP, 0206.911 and 0206.0805) and The European Social Fund.

References

Abir R, Ao A, Jin S, Barnett M, Raanani H, Ben-Haroush A, Fisch B (**2005**) Leptin and its receptors in human fetal and adult ovaries. Fertil Steril 84: 1779-1782.

Agarwal SK, Vogel K, Weitsman SR, Magoffin DA (**1999**) Leptin antagonizes the insulin-like growth factor-I augmentation of steroidogenesis in granulosa and theca cells of the human ovary. J Clin Endocrinol Metab 84: 1072-1076.

Almog B, Gold R, Tajima K, Dantes A, Salim K, Rubinstein M, Barkan D, Homburg R, Lessing JB, Nevo N, Gertler A, Amsterdam A (**2001**) Leptin attenuates follicular apoptosis and accelerates the onset of puberty in immature rats. Mol Cell Endocrinol 183: 179-191.

Antczak M, Van Blerkom J (**1997**) Oocyte influences on early development: the regulatory proteins leptin and STAT3 are polarized in mouse and human oocytes and differentially distributed within the cells of the preimplantation stage embryo. Mol Hum Reprod 3: 1067-1086.

Archanco M, Muruzabal FJ, Llopiz D, Garayoa M, Gómez-Ambrosi J, Frühbeck G, Burrell MA (**2003**) Leptin expression in the rat ovary depends on estrous cycle. J Histochem Cytochem 51: 1269-1277.

Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK, Steiner RA (**1996**) Leptin is a metabolic signal to the reproductive system. Endocrinology 137: 3144-3147.

Bjorbaek C, Uotani S, da Silva B, Flier JS (**1997**) Divergent signaling capacities of the long and short isoforms of the leptin receptor. J Biol Chem 272: 32686-32695.

Bogacka I, Przała J, Siawrys G, Kaminski T, Smolinska N (**2006**) The expression of short form of leptin receptor gene during early pregnancy in the pig examined by quantitative real time RT-PCR. J Physiol Pharmacol 57: 479-489.

Cioffi JA, Van Blerkom J, Antczak M, Shafer A, Wittmer S, Snodgrass HR (**1997**) The expression of leptin and its receptors in pre-ovulatory human follicles. Mol Hum Reprod 3: 467-472.

Gregoraszczuk EL, Ptak A, Wojtowicz AK, Gorska T, Nowak KW (**2004**) Estrus cycle-dependent action of leptin on basal and GH or IGF-I stimulated steroid secretion by whole porcine follicles. Endocr Regul 38: 15-21.

Gregoraszczuk EL, Wojtowicz AK, Ptak A, Nowak K (**2003**) In vitro effect of leptin on steroids' secretion by FSH- and LH-treated porcine small, medium and large preovulatory follicles. Reprod Biol 3: 227-239.

Hamm ML, Bhat GK, Thompson WE, Mann DR (**2004**) Folliculogenesis is impaired and granulosa cell apoptosis is increased in leptin-deficient mice. Biol Reprod 71: 66-72.

Karamouti M, Kollia P, Kallitsaris A, Vamvakopoulos N, Kollios G, Messinis IE (**2009**) Modulating effect of leptin on basal and follicle stimulating hormone stimulated steroidogenesis in cultured human lutein granulosa cells. J Endocrinol Invest 32: 415-419.

Karlsson C, Lindell K, Svensson E, Bergh C, Lind P, Billig H, Carlsson LM, Carlsson B (**1997**) Expression of functional leptin receptors in the human ovary. J Clin Endocrinol Metab 82: 4144-4148.

Kitawaki J, Kusuki I, Koshiba H, Tsukamoto K, Honjo H (**1999**) Leptin directly stimulates aromatase activity in human luteinized granulosa cells. Mol Hum Reprod 5: 708-713.

Loffler S, Aust G, Kohler U, Spanel-Borowski K (**2001**) Evidence of leptin expression in normal and polycystic human ovaries. Mol Hum Reprod 7: 1143-1149.

Moschos S, Chan JL, Mantzoros CS (**2002**) Leptin and reproduction: a review. Fertil Steril 77: 433-444.

Ruiz-Cortes ZT, Martel-Kennes Y, Gévry NY, Downey BR, Palin MF, Murphy BD (**2003**) Biphasic effects of leptin in porcine granulosa cells. Biol Reprod 68: 789-796.

Ruiz-Cortes ZT, Men T, Palin MF, Downey BR, Lacroix DA, Murphy BD (**2000**) Porcine leptin receptor: molecular structure and expression in the ovary. Mol Reprod Dev 56: 465-474.

Ryan NK, Van der Hoek KH, Robertson SA, Norman RJ (**2003**) Leptin and leptin receptor expression in the rat ovary. Endocrinology 144: 5006-5013.

Ryan NK, Woodhouse CM, Van der Hoek KH, Gilchrist RB, Armstrong DT, Norman RJ (**2002**) Expression of leptin and its receptor in the murine ovary: possible role in the regulation of oocyte maturation. Biol Reprod 66: 1548-1554.

Smolinska N, Kaminski T, Siawrys G, Przala J (**2007**) Long form of leptin receptor gene and protein expression in the porcine ovary during the estrous cycle and early pregnancy. Reprod Biol 7: 17-39.

Smolinska N, Kaminski T, Siawrys G, Przala J (**2010**) Leptin gene and protein expression in the ovary during the oestrous cycle and early pregnancy in pigs. Reprod Domest Anim 45: e174-e183.

Spicer LJ, Francisco CC (**1997**) The adipose obese gene product, leptin: evidence of a direct inhibitory role in ovarian function. Endocrinology 138: 3374-3379.

Swain JE, Dunn RL, McConnell D, Gonzalez-Martinez J, Smith GD (**2004**) Direct effects of leptin on mouse reproductive function: regulation of follicular, oocyte, and embryo development. Biol Reprod 71: 1446-1452.

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI (**1995**) Identification and expression cloning of a leptin receptor, OB-R. Cell 83: 1263-1271.

Tsai EM, Yang CH, Chen SC, Liu YH, Chen HS, Hsu SC, Lee JN (**2002**) Leptin affects pregnancy outcome of in vitro fertilization and steroidogenesis of human granulosa cells. J Assist Reprod Genet 19: 169-176.

Welt CK, Schneyer AL, Heist K, Mantzoros CS (**2003**) Leptin and soluble leptin receptor in follicular fluid. J Assist Reprod Genet 20: 495-501.

Zamorano PL, Mahesh VB, De Sevilla LM, Chorich LP, Bhat GK, Brann DW (**1997**) Expression and localization of the leptin receptor in endocrine and neuroendocrine tissues of the rat. Neuroendocrinology 65: 223-228.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (**1994**) Positional cloning of the mouse obese gene and its human homologue. Nature 372: 425-432.