4-2020

Evaluation of Anterior Pituitary Leptin Receptor Expression in Pregnant and Non-pregnant Gilts

Juliana Pederson

Jeffrey Clapper

Evaluation of anterior pituitary leptin receptor expression in pregnant and non-pregnant gilts

[1]Juliana Pederson and [2]Jeffrey Clapper
Department of Biology & Microbiology[1]; Department of Animal Science[2]
South Dakota State University
Brookings, SD 57006

Acknowledgements: I would like to thank Dr. Jeffrey Clapper for his guidance throughout this study. This project was partially funded by the Orville and Enolia Bentley Undergraduate Research Award.

ABSTRACT

Successful reproduction in porcine depends on the coordinated synthesis and release of several hormones from the hypothalamus and anterior pituitary gland. Leptin, a hormone produced from adipocytes, interacts with other specific reproductive hormones and affects the reproductive success in swine. However, the full extent of leptin's impact is unclear. This experiment sought to examine the difference in expression between the anterior pituitary gland leptin receptors in pregnant and non-pregnant pigs to confer the impact leptin has on reproductive success. Twenty-four crossbred gilts of similar age and weight had their estrus cycles synchronized and then were bred via artificial insemination. The gilts were slaughtered to collect blood, anterior pituitary glands, and reproductive tracts 30 days after insemination. Reproductive tracts were examined. Anterior pituitary glands underwent RNA extraction to determine relative abundance of porcine leptin receptor, gonadotropin releasing hormone (GnRH) receptor, and luteinizing hormone (LHβ) expression compared to porcine β-actin. RNA was then reverse transcribed to produce cDNA to quantify porcine leptin receptor, GnRH receptor, LHβ expression in the mRNA. The results exhibit varying concentrations of GnRH receptor, LHβ, and leptin receptor expression in the anterior pituitary gland when pregnant and non-pregnant groups were compared. However, only LHβ expression in the anterior pituitary gland displayed statistical significance. Future experiments should include additional considerations such as animal adiposity which may influence leptin receptor expression. Increased anterior pituitary leptin receptor expression may be needed to maintain proper synthesis and release of gonadotropins throughout pregnancy in the pig. If true, leptin therapies could be devised and utilized to improve pregnancy rates as well as maintain or improve other hormones levels during the pregnancy period in porcine.

KEY WORDS

Anterior pituitary gland, gilts, gonadotropin-releasing hormone, leptin receptor, non-pregnant, pregnant.

INTRODUCTION

Successful reproduction in swine depends upon the coordinated synthesis and release of a number of hormones from the hypothalamus and anterior pituitary gland (AP). Gonadotropin releasing hormone from the hypothalamus stimulates the direct release of luteinizing hormone from the AP, which in turn, is responsible for causing ovulation and maintenance of the corpus luteum during pregnancy. Thus, other hormones that influence the release of GnRH and/or LH could influence reproductive success in the pig. Leptin is a hormone produced primarily from adipocytes and has been shown to regulate the LH release in the gilt at the level of the hypothalamus and the AP (Barb C.R. and Kraeling R. R., 2004).

Leptin is a 16-kDa protein encoded by the obese (ob) gene. Leptin was originally recognized as a regulator of food intake and body fat mass (Pelleymounter M.A. et al., 1995), but others have demonstrated it can also affect reproduction by regulating the hypothalamic-pituitary axis (Chehab F.F., 2014; Perez-Perez A.F. et al., 2015). Leptin exerts its effect by binding to and activating the leptin receptor (OB-Rb) which has been found to be expressed in the hypothalamus and AP of prepubertal and mature gilts and sows (Lin J. et al., 2000; Siawrys G. et al., 2009, 2007) and most recently in the AP of pregnant pigs (Siawrys and Gajewska, 2017).

Leptin binds to its cognate receptor and regulates the expression of its own receptor (Di Yorio M. P. et al., 2008) and thus, it may act in a paracrine or autocrine manner within the AP to stimulate the secretion of LH. All types of AP cells in the sheep (Iqbal J. S. et al., 2000) and the rat (Sone M. H. et al., 2001) express leptin receptors, including gonadotropes. However, to date, leptin receptors have not been localized to specific cell types of the porcine AP. Changes in leptin receptor expression within the porcine AP have been noted during stages of gestation and the estrous cycle which suggest it may be due to fluctuations in gonadal steroids (Kaminski T. et al., 2006). In humans and rats, circulating concentrations of leptin increase during pregnancy (Estienne M. J. et al., 2003; Metges C. C. et al., 2012), but serum concentrations of leptin did not change in pregnant sows during body weight gain. Therefore, leptin's influence on AP function and reproduction in the pig may be manifested by changes in leptin receptor expression within the AP of the pig.

BACKGROUND

Leptin's Role in Female Reproduction

Reproductive success is highly dependent on availability and consistency of energy sources. Any changes in the diet or body weight of the female can wreak havoc on the timing of sexual maturation, as well as fertility. In an animal's body, adipose tissue acts as a form of energy storage and as an endocrine organ that secretes adipokines such as leptin (Pérez-Pérez et al., 2015). Leptin has been proven to be involved in regulation of not only energy stores in the brain but also in physiological events such as inflammation, angiogenesis, hematopoiesis, immune function, and reproduction. Numerous studies have demonstrated that leptin excess, deficiency,

or even resistance can each be associated with abnormal reproductive function in females. Interestingly, leptin is also produced by the placenta during pregnancy (Pérez-Pérez et al., 2015).

Leptin Receptor Expression and Production Sites

Leptin is one of the main regulator molecules in many different types of tissues. However, the source of leptin's synthesis and location of the leptin receptor protein are unknown. Siawrys and his research team in Poland incorporated a Western-blot analysis approach to determine the relative expression of porcine leptin and leptin receptor protein in both the anterior and posterior pituitary glands. Furthermore, they used porcine samples collected during the mid and late luteal phases of the estrous cycle along with two stages of early pregnancy (Siawrys G. et al., 2007).

The Western-blot analysis showed that the leptin protein expression in the anterior pituitary was higher during the late luteal phase as compared to the mid luteal phase. However, during pregnancy, the leptin protein concentration in the anterior pituitary was higher on days 14-16 as compared to days 30-32. These experiments conclude that leptin is produced in the pituitary gland and then acts in an auto/paracrine way in the regulation of the pituitary gland during both the luteal phase of the estrous cycle and early pregnancy (Siawrys G. et al., 2007).

Additionally, the specific cell type where the leptin receptor is produced was thoroughly researched by Sone and his colleges based in the Diagnostic Pathology Center in Japan. Previously, leptin receptor production was found to take place in the pituitary, hypothalamus, and reproductive organs. However, the precise cell location of leptin receptor production had not been extensively researched. Therefore, researchers incorporated immunoblotting and

immunohistochemical staining methods during their study of leptin receptor production location (Sone M. et al., 2001). Their results showed that leptin receptor production is exclusively located in growth hormone secreting cells found in the anterior pituitaries of rats. Additionally, the experimental results suggest some type of paracrine system in the pituitary gland was organizing the production and interconnection of leptin receptor proteins from the growth hormone secreting cells (Sone M. et al., 2001).

Lastly, Iqbal and his team of researchers based out of Australia and Indiana, examined three different types of cells: corticotropes, somatotropes, and gonadotropes, to determine leptin receptor-like immunoreactivity in the ovine anterior pituitary gland via double-label immunofluorescence histochemistry. Results showed that in the pars distalis portion of the anterior pituitary gland, leptin receptor is expressed significantly more in the somatotropes compared to that of the gonadotropes or corticotropes. Additionally, a high concentration of leptin receptor was found in the pars tuberalis. Their findings further prove the exclusive nature of the gonadotrope cells in secretion of growth hormone. The varying expressional differences between the pars distalis and pars tuberalis could be a result of varying phenotypic expression between the two cells (Iqbal J. et al., 2000).

Obese Gene

The obese (*ob*) gene functions in the signaling pathway of adipose tissue and codes for the leptin protein. Mice that had a mutation in the *ob* gene were observed to be obese, diabetic, and demonstrate an overall reduced activity level in general (Pelleymounter MA. et al., 1995). Additionally, the mutated mice had reduced metabolism and body temperature. It is suggested

that the mutant mouse with the mutant *ob* gene lacked a blood-borne factor that regulates adiposity via moderation of metabolism and appetite.

Pelleymounter and his colleges in Washington devised an experiment that sought to determine the purpose of the OB protein by administering the OB protein to *ob*/*ob* mice. Their results showed that the OB protein has a vital role in body weight along with adiposity in *ob*/*ob* (mutant) mice. However, researchers speculate that metabolic and hormonal effects of the OB protein occur earlier and proceed any appetite suppression effects on the *ob*/*ob* mice since body temperature and serum glucose levels returned to baseline even though body weight and food intake was not dramatically reduced (Pelleymounter MA. et al., 1995).

Specifically, the Ob-R1 gene codes for leptin's receptor and is also the major form of the receptor mRNA involved in signal transduction. Lin J. and his teamed based in the southern United States, quantified mRNA expression of Ob-R1 in a variety of tissues from 105-day old prepuberal gilts and a 50-day old fetus. Their aim was to determine locations and relative concentrations of the mRNA from the Ob-R1 gene. The 105-day old prepuberal gilts demonstrated mRNA expression of the Ob-R1 gene in the hypothalamus, cerebral cortex, amygdala, thalamus, cerebellum, area postrema, and anterior pituitary. mRNA of the Ob-R1 gene was also found in the brain, intestine, muscle, fat, heart, liver, and umbilical cord of the 50-day old fetus. Since the Ob-R1 gene was found in such diverse tissues, the idea that leptin may play a larger role in the regulation of many physiological functions is supported (Lin J. et al., 2000).

Leptin is a key player in the overall metabolic and reproductive success of farm animals. Leptin carries out its function by attaching to the leptin receptor. Therefore, the expression of both leptin and the leptin receptor should be focused on to determine relative concentrations of leptin. Expression of leptin and the leptin receptor also varies in the cell specific location that they are expressed and produced in. Lastly, leptin has been proven to be encoded by the *ob* gene in mice. The previous literature provides good foundational knowledge; however, gaps exist where future experiments could further enhance the understanding of leptin's role in porcine reproduction. Relative expression and production of leptin and the leptin receptor, cell specific location of the expression and production of leptin and the leptin receptor, and mutational activity of the *ob* gene should all be considered when attempting to quantify relative concentrations of leptin and the leptin receptor.

Leptin receptor expression in the anterior pituitary gland increases during early stages of fertilization and pregnancy in porcine.

To test this hypothesis, we will carry out 4 specific aims:

- **Aim 1: Sync estrus cycles of gilts to artificially inseminate at the same time.**
- **Aim 2: Develop an aseptic technique of collecting blood, brain, and tissue samples.**
- **Aim 3: Evaluate serum samples based on estradiol-17β and progesterone concentrations.**
- **Aim 4: Reverse transcribe mRNA into cDNA to evaluate the amount of leptin receptor expression.**

MATERIALS AND METHODS

Twenty-four crossbred (Duroc x Large White x Landrace) gilts of similar age and weight (approximately 165 days of age; 225 kg BW) were used in this experiment. Estrus was synchronized by feeding gilts Matrix (altrenogest) for 16 days. Estrus was determined by exposure to a mature boar beginning 3 days following the cessation of Matrix. Blood samples were obtained by jugular venipuncture on the first day of boar exposure and continued for 4 days or until the gilt expressed estrus. Gilts were bred by artificial insemination when they first stood immobile in the presence of the boar (day 1) and again one day later. Approximately 30 days following the first insemination, gilts were slaughtered at the South Dakota State University Meat Lab. At slaughter, blood, AP glands, and reproductive tracts were collected. AP glands were trimmed of connective tissue, bisected midsagittaly, wrapped in aluminum foil, snap frozen in liquid nitrogen, and stored at -80º C. Reproductive tracts were examined for the presence of fetuses and the number of corpora lutea was recorded for each ovary.

Total RNA was isolated from one half of each AP using TriReagent (TR118, Molecular Research Company, Cincinnati, OH). Samples were treated with DNase according to the manufacturer's protocol (TurboDNA-free kit, Applied Biosystems, Foster City, CA, USA). Reverse transcriptase PCR was used to measure the abundance of porcine leptin receptor mRNA, GnRH receptor mRNA, and LHβ mRNA relative to the abundance of porcine β-actin in the total RNA isolated from AP tissue. Two micrograms of total RNA were reverse transcribed using random hexamer primers (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, Foster City, CA) to produce cDNA (Table 1). Reactions were measured using the Stratagene MX3000P quantitative real-time PCR instrument (Agilent Technologies, Foster City, CA) using

thermal cycling conditions recommended by the manufacturer. Unique forward and reverse primer pairs were used for the quantification of porcine leptin receptor, GnRH receptor, and LHβ expression.

Total RNA was isolated from one half of each AP using TriReagent (TR118, Molecular Research Company, Cincinnati, OH). Samples were treated with DNase according to the manufacturer's protocol (TurboDNA-free kit, Applied Biosystems, Foster City, CA, USA). Reverse transcriptase PCR was used to measure the abundance of porcine leptin receptor mRNA, GnRH receptor mRNA, and LHβ mRNA relative to the abundance of porcine β-actin in the total RNA isolated from AP tissue. Two micrograms of total RNA were reverse transcribed using random hexamer primers (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, Foster City, CA) to produce cDNA (Table 1). Reactions were measured using the Stratagene MX3000P quantitative real-time PCR instrument (Agilent Technologies, Foster City, CA) using thermal cycling conditions recommended by the manufacturer. Unique forward and reverse primer pairs were used for the quantification of porcine leptin receptor, GnRH receptor, and LHβ expression. Fold changes in relative expression of AP leptin receptor were determined using the Relative Expression Software Tool (Pfaffl M. W. et al., 2002).

RESULTS

The relative expression of LHβ was downregulated in the pregnant gilts by 0.813-fold versus non-pregnant gilts (Figure 1; P=0.001).

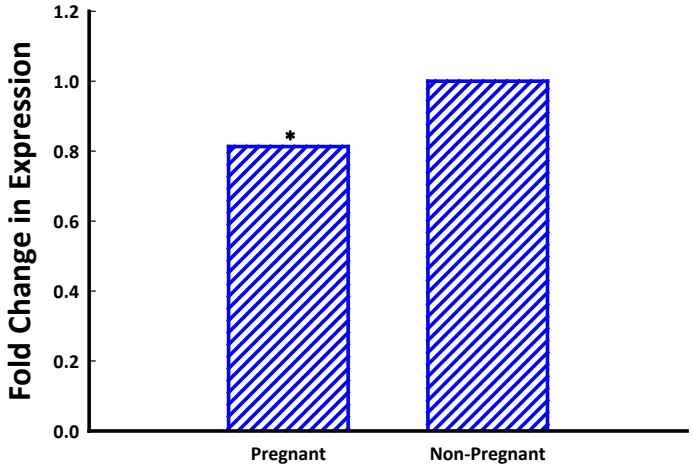

Fig. 1. Mean relative expression of LHβ in pregnant and non-pregnant gilts. Data are expressed as fold change in expression relative to the values in non-pregnant gilts. Means with * differed P=0.001.

There was no difference detected between the pregnant gilts and the non-pregnant gilts in the relative expression of GnRH receptor (Figure 2; P=0.4).

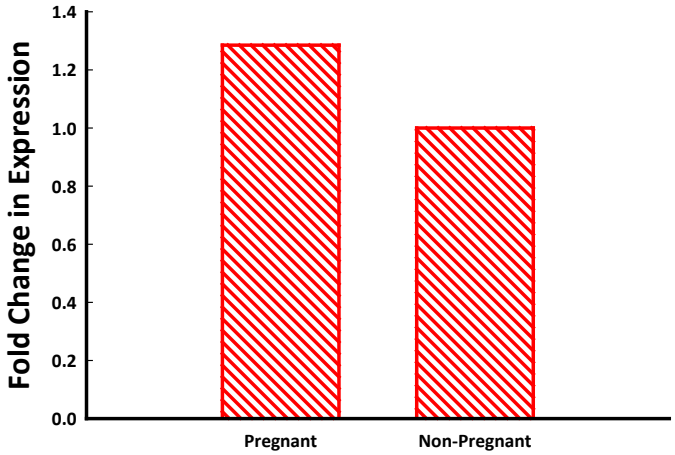

Fig. 2. Mean relative expression of GnRH receptor in pregnant and non-pregnant gilts. Data are expressed as fold change in expression relative to the values in non-pregnant gilts. Means did not differ P=0.4.

Importantly, relative expression of leptin receptor did not differ between the pregnant gilts and the non-pregnant gilts (Figure 3; P=0.52).

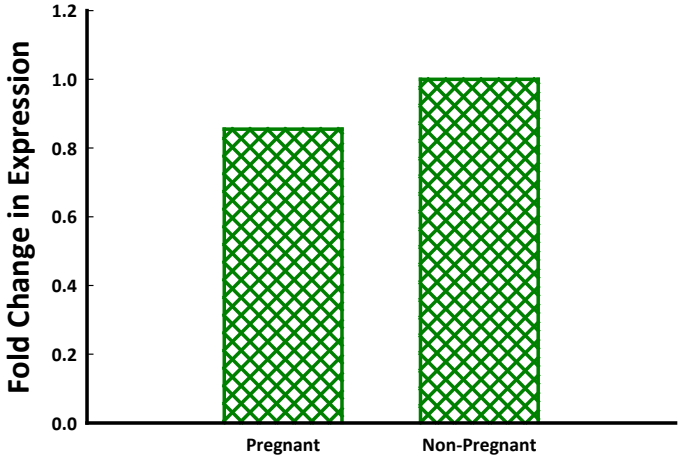

Fig. 3. Mean relative expression of leptin receptor in pregnant and non-pregnant gilts. Data is expressed as fold change in expression relative to the values in non-pregnant gilts. Means did not differ P=0.52.

Table 1. Forward and reverse primers for Real-Time PCR for porcine mRNA

Gene & Accession Number	Primer	Amplicon Size
GnRH Receptor (NM_214273.1)[a]	Forward 5' – AGCCAACCTGTTGGAGACTCTGAT – 3' Reverse 5' – AGCTGAGGACTTTGCAGAGGAACT – 3'	101 bp
pβ-actin (U07786.1)[b]	Forward 5' – TCGCCGACAGGATGCAGAAGGA – 3' Reverse 5' – AGGTGGACAGCGAGGCCAGGAT – 3'	129 bp
LHβ (NM_214080.1)[c]	Forward 5' – ATGCTCCAGAGACTGCTGTTGT – 3' Reverse 5' – TGCTGGTGGTAAAGGTGATGCAGA – 3'	151 bp
Leptin Receptor (NM_001024587.1)[d]	Forward 5' – CCTGGGCACAAGGACTTAAT – 3' Reverse 5' – TGGCACCATCTCATCCTTATTT – 3'	254 bp

[a] Porcine Gonadotropin Releasing Hormone Receptor; [b] Porcine Beta-Actin; [c] Porcine Luteinizing Hormone Subunit beta; [d] Porcine Leptin Receptor

DISCUSSION

Successful reproduction in swine depends upon the coordinated synthesis and release of a number of hormones from the hypothalamus and anterior pituitary gland. However, little is known about leptin's role in female reproduction. GnRH directly simulates the release of LH from the anterior pituitary gland. LH causes ovulation as well as maintains the corpus luteum throughout pregnancy of the pig. Therefore, other hormones that control or influence the release of GnRH and/or LH have the capacity to influence the overall reproductive success of the pig.

Barb and his team argue in their 2005 review on leptin that adipose tissue acts more like an endocrine organ as more research uncovers the tissue's complete role. Overall changes to the pig's body weight and/or state of nutrition manifest themselves in the fluctuating levels of hormones and growth factors within the serum of such animals (Barb C.R. et al., 2005). Additionally, Barb and his team found that circulating levels of leptin concentrations have a direct relationship with adiposity; as animal adiposity increases, leptin concentrations also increase. Therefore, they found that circulating concentrations of leptin both in the plasma and adipose tissue drastically decreased because of food deprivation.

Reproductive success is highly dependent on availability and consistency of energy sources. Any changes in the diet or body weight of the female can wreak havoc on the timing of sexual maturation as well as fertility. Pérez-Pérez and his team support Barb's previous previous findings in that adipose tissue has dual function acting as a form of energy storage and as an endocrine organ that secretes adipokines such as leptin (Pérez-Pérez A.F. et al., 2015). Leptin has been proven to be involved in regulation of not only energy stores in the brain but also in

physiological events such as inflammation, angiogenesis, hematopoiesis, immune function, and reproduction. Numerous studies have demonstrated that leptin excess, deficiency, or even resistance can each be associated with abnormal reproductive function in females (Perez-Perez A.F. et al., 2015).

Specifically, the Ob-R1 gene codes for leptin's receptor and is also the major form of the receptor mRNA involved in signal transduction. Lin and his team, based in the southern United States, quantified mRNA expression of Ob-R1 in a variety of tissues from 105-day old prepuberal gilts and a 50-day old fetus. Their aim was to determine locations and relative concentrations of the mRNA from the Ob-R1 gene. The 105-day old prepuberal gilts demonstrated mRNA expression of the Ob-R1 gene in the hypothalamus, cerebral cortex, amygdala, thalamus, cerebellum, area postrema, and anterior pituitary. mRNA of the Ob-R1 gene was also found in the brain, intestine, muscle, fat, heart, liver, and umbilical cord of the 50-day old fetus. Since the Ob-R1 gene was found in such diverse tissues, the idea that leptin may play a larger role in the regulation of many physiological functions is supported (Lin J. et al., 2000).

Leptin is one of the main regulator molecules in many different types of tissues. However, the source of leptin's synthesis and location of the leptin receptor protein are unknown. Siawrys and his research team in Poland incorporated a Western-blot analysis approach to determine the relative expression of porcine leptin and leptin receptor protein in both the anterior and posterior pituitary glands. Furthermore, they used porcine samples collected during the mid and late luteal phases of the estrous cycle along with two stages of early pregnancy (Siawrys G. et al., 2007).

The Western-blot analysis showed the leptin protein expression in the anterior pituitary increased during the late luteal phase as compared to the mid luteal phase. However, during pregnancy, the leptin protein concentration in the anterior pituitary increased on days 14-16 as compared to days 30-32. Their experiments conclude that leptin is produced in the pituitary gland and then acts in an auto/paracrine way in the regulation of the pituitary gland during both the luteal phase of the estrous cycle and early pregnancy (Siawrys G. et al., 2007).

Leptin has been proven to act through the hypothalamic-pituitary axis. Previous studies cited by Barb and his team demonstrated that leptin increased LH secretion from porcine pituitary cells in addition to increasing GnRH release from hypothalamic tissue in vitro (Barb C.R. et al., 2005). Barb and his team dug further and found that intracerebroventricular application of leptin suppressed food intake but did not stimulate LH secretion. However, leptin affects LH secretion during the stage of sexual maturation in the pig. Moreover, Barb and his team theorize that leptin provides a metabolic signal that may ultimately activate the reproductive axis in the pig, but not necessarily trigger the start of puberty (Barb C.R. et al., 2005).

In the present study, LHβ, GnRH receptor, and leptin receptor expression displayed varying concentration levels when pregnant and non-pregnant groups were compared. However, only LHβ expression displayed statistical significance. The lack of statistical significance when analyzing the leptin receptor and comparing the pregnant and nonpregnant groups could be a result of adiposity differences between the gilts. Barb's 2005 article previously mentioned discusses how adipose tissue can have dual function, a form of energy store and an endocrine

organ. Pérez-Pérez's 2015 article also supports Barb's claim. Additionally, Barb and his team presented that leptin increased LH secretion from porcine anterior pituitary cells and increased GnRH release from hypothalamic tissues *in vitro*. However, one must keep in mind that *in vitro* is a more controlled environment compared to *in vivo*.

CONCLUSIONS

The results demonstrate that expression of leptin receptor in the anterior pituitary did not differ in pregnant versus non-pregnant gilts. However, determination of the expression of other anterior pituitary hormones is needed to delineate possible differences in anterior pituitary function in pregnant versus non-pregnant gilts. Further experiments regarding anterior pituitary leptin receptor expression in the pig should include additional considerations such as animal adiposity, as previously discussed, in order to mitigate variability across samples.

REFERENCES

Barb, C. R., G. J. Hausman, and K. Czaja. 2005. Leptin: A metabolic signal affecting central regulation of reproduction in the pig. The Journal of endocrinology 29: 186-192.

Barb, C. R., and R. R. Kraeling. 2004. Role of leptin in the regulation of gonadotropin secretion in farm animals. Animal reproduction science 82-83: 155-167.

Chehab, F. F. 2014. 20 years of leptin: leptin and reproduction: past milestones, present undertakings, and future endeavors. The Journal of endocrinology 223: T37-48.

Clapper, J., and A. Taylor. 2011. Components of the porcine anterior pituitary insulin-like growth factor system throughout the estrous cycle. Domestic animal endocrinology 40: 67-76.

Di Yorio, M. P., M. G. Bilbao, M. C. Pustovrh, J. P. Prestifilippo, and A. G. Faletti. 2008. Leptin modulates the expression of its receptors in the hypothalamic-pituitary-ovarian axis in a differential way. The Journal of endocrinology 198: 355-366.

Estienne, M. J., A. F. Harper, D. M. Kozink, and J. W. Knight. 2003. Serum and milk concentrations of leptin in gilts fed a high- or low-energy diet during gestation. Animal reproduction science 75: 95-105.

Iqbal, J., S. Pompolo, R. V. Considine, and I. J. Clarke. 2000. Localization of leptin receptor-like immunoreactivity in the corticotropes, somatotropes, and gonadotropes in the ovine anterior pituitary. Endocrinology 141: 1515-1520.

Kaminski, T., N. Smolinska, A. Gajewska, G. Siawrys, S. Okrasa, K. Kochman, and J. Przala. 2006. Leptin and long form of leptin receptor genes expression in the hypothalamus and pituitary during the luteal phase and early pregnancy in pigs. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society 57: 95-108.

Lin, J., C. R. Barb, R. L. Matteri, R. R. Kraeling, X. Chen, R. J. Meinersmann, and G. B. Rampacek. 2000. Long form leptin receptor mRNA expression in the brain, pituitary, and other tissues in the pig. Domestic animal endocrinology 19: 53-61.

Metges, C. C., I. S. Lang, U. Hennig, K. P. Brussow, E. Kanitz, M. Tuchscherer, F. Schneider, J. M. Weitzel, A. Steinhoff-Ooster, H. Sauerwein, O. Bellmann, G. Nurnberg, C. Rehfeldt, and W. Otten. 2012. Intrauterine growth retarded progeny of pregnant sows fed high protein:low carbohydrate diet is related to metabolic energy deficit. PloS one 7: e31390.

Pelleymounter, M. A., M. J. Cullen, M. B. Baker, R. Hecht, D. Winters, T. Boone, and F. Collins. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269: 540-543.

Perez-Perez, A., F. Sanchez-Jimenez, J. Maymo, J. L. Duenas, C. Varone, and V. Sanchez-Margalet. 2015. Role of leptin in female reproduction. Clinical chemistry and laboratory medicine 53: 15-28.

Pfaffl, M. W., T. M. Georgieva, I. P. Georgiev, E. Ontsouka, M. Hageleit, and J. W. Blum. 2002. Real-time RT-PCR quantification of insulin-like growth factor (IGF)-1, IGF-1 receptor, IGF-2, IGF-2 receptor, insulin receptor, growth hormone receptor, IGF-binding proteins 1, 2 and 3 in the bovine species. Domestic animal endocrinology 22: 91-102.

Siawrys, G., and A. Gajewska. 2017. In vitro effect of leptin on anterior pituitary cells LH secretory activity during early pregnancy in pig. Polish journal of veterinary sciences 20: 67-76.

Siawrys, G., T. Kaminski, N. Smolinska, and J. Przala. 2007. Expression of leptin and long form of leptin receptor genes and proteins in pituitary of cyclic and pregnant pigs. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society 58: 845-857.

Siawrys, G., T. Kaminski, N. Smolinska, and J. Przala. 2009. Expression of leptin and long-form leptin-receptor proteins in porcine hypothalamus during oestrous cycle and pregnancy. Reproduction in domestic animals = Zuchthygiene 44: 920-926.

Sone, M., H. Nagata, S. Takekoshi, and R. Y. Osamura. 2001. Expression and localization of leptin receptor in the normal rat pituitary gland. Cell and tissue research 305: 351-356.