Adiponectin expression in the porcine pituitary during the estrous cycle and its effect on LH and FSH secretion

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Kiezun M, Smolinska N, Maleszka A, Dobrzyń K, Szeszko K, Kaminski T. Adiponectin expression in the porcine pituitary during the estrous cycle and its effect on LH and FSH secretion. Am J Physiol Endocrinol Metab 307: E1038–E1046, 2014. First published October 14, 2014; doi:10.1152/ajpendo.00299.2014.—Female reproductive success is closely associated with nutritional status and energy balance. In this context, adiponectin appears to be a key hormone connecting reproductive system function and metabolism regulation. It is hypothesized that adiponectin expression in the pituitary depends on the phase of the estrous cycle. The effect of adiponectin on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion is also postulated. Changes in the adiponectin gene and protein expression in the porcine anterior (AP) and posterior (NP) pituitaries as well as the effect of in vitro administration of adiponectin on basal and gonadotropin-releasing hormone (GnRH)- and/or insulin-stimulated LH and FSH secretion were investigated on days 2–3, 10–12, 14–16, and 17–19 of the estrous cycle. Adiponectin gene was more pronounced on days 2–3 in AP but on days 10–12 in NP. Protein concentration in AP was the highest on days 10–12 and in NP on days 10–12 and 17–19 of the cycle. In vitro, adiponectin did not affect basal LH secretion but increased FSH release by AP cells. Adiponectin administration affected GnRH- and/or insulin-induced LH and FSH output in a manner dependent on the phase of the estrous cycle. In this study we indicated for the first time adiponectin expression in the porcine AP and NP that was dependent on the phase of the estrous cycle. In vitro studies indicated that adiponectin may affect gonadotropin secretion. The above suggests that the studied adipokine may influence female reproductive functions via its effect on LH and FSH secretion by gonadotrophs, but the cellular mechanism of its action remains unknown.

luteinizing hormone; follicle-stimulating hormone

Adiponectin is a 30-kDa protein, also termed Acrp30, apM-1, GBP28, or adipQ, was first identified by four independent research groups (4, 27, 30, 38). Adiponectin is highly abundant in human and mouse plasma, with a concentration corresponding to 0.01–0.05% of the total serum proteins, and shows an inverse correlation with the body mass index (5, 30, 54). In terms of structure, this protein is related to the complement 1q family and contains a COOH-terminal globular domain and an NH2-terminal collagenous domain. It is secreted in the form of a trimer as a low-molecular-weight form, a combination of two trimers as a middle-molecular-weight form, or as six trimers as a high-molecular-weight form (27, 50). Several studies have demonstrated that different multimeric forms can determine the activity of adiponectin (17, 47, 53). It is known for its protective role in obesity-related disorders, such as insulin resistance, type 2 diabetes mellitus, and atherosclerosis (21, 54) as well as in carcinogenesis (6). A beneficial role of adiponectin in the female reproductive system has been also suggested (8).

The discussed hormone action in the peripheral tissues is mediated principally through two receptors, AdipoR1 and AdipoR2, although a third receptor, T-cadherin, has also been identified (15, 55). AdipoR1 and AdipoR2 are 7-transmembrane receptors that belong to a recently discovered family of type 2 adiponectin receptor (46). AdipoR1 shows a higher affinity for the globular adiponectin, whereas AdipoR2 has an intermediate affinity for both globular and full-length forms (55). AdipoRs are widely expressed within peripheral tissues, which suggests the pleiotropic effect of adiponectin. Type 1 adiponectin receptor is mainly expressed in skeletal muscles of humans, mice, pigs, and chickens, whereas the highest expression of type 2 adiponectin receptor is observed in the liver of the above species (24, 35, 55). The cellular effects of adiponectin are mediated through an adenosine monophosphate-activated protein kinase pathway, a peroxisome proliferator-activated receptor-α pathway, or by activating a p38 mitogen-activated protein kinase (16).

It has long been recognized that female reproductive success depends largely on nutritional status and energy balance. Even minor metabolic dysregulation may lead to reproductive abnormalities, for example, hyperinsulinemia is frequently associated with an inadequate luteinizing hormone (LH) secretion. Although insulin, as a key metabolic hormone, is often recognized as an important factor influencing reproductive functions, the effects of insulin on the pituitary gland are still relatively little known (1, 22, 51, 52). The regulating role of adipokines (i.e., leptin, ghrelin, and adiponectin) on the hypothalamic-pituitary-gonadal axis (HPG) is also documented (4, 10, 18, 31, 36, 40). Despite the conflicting evidence on adiponectin’s ability to cross the blood-brain barrier (24, 32, 43), the presence of AdiposRs in the human, pig, rat, murine, and chicken hypothalamus and pituitary suggests that adiponectin may be a factor modulating the secretory functions of central branches of hormonal axes and, indirectly, also their peripheral branches, including the HPG axis (19, 24, 26, 34, 37, 43, 44). Rodriguez-Pacheco et al. (37) revealed that adiponectin in vitro influences growth hormone and LH secretion by the isolated...
antior pituitary cells of rats. Such a study was done exclusively on a rat model. The possible effect of adiponectin on pituitary hormone secretion in pigs was completely unexplored. What is more, our recent studies have indicated that adiponectin can affect ovarian steroidogenesis in this species and that this effect is dependent on the phase of the estrous cycle (28). A single paper demonstrates changes in AdipoR expression in both the anterior (AP) and posterior (NP) pituitary, related to the hormonal status (19). Similarly, the plasma level of adiponectin is differentiated dependent on the phase of the estrous cycle (29). Data describing an inverse relationship, namely an influence of the sex steroids level, typical for each phase of the estrous cycle, on the pituitary adiponectin system expression, is very limited. Based on earlier research, it is hypothesized that the expression of adiponectin in the pituitary gland may depend on the phase of the cycle. Moreover, the influence of adiponectin on gonadotropin secretion is also postulated. Therefore, the aim of this study was to investigate adiponectin expression in AP and NP as well as in an in vitro effect of this hormone on the basal and gonadotropin-releasing hormone (GnRH) and/or insulin-stimulated secretion of LH (30–32).

GONADOTROPIH: INSULIN STIMULATED SECRETION OF LH

The hypothalamus may be considered to act as a nuclear regulator of the endocrine system. While the hypothalamus produces neurohormones that are released into the hypophyseal portal system, it is the pituitary gland that secretes certain hormones (33, 34). The gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) are two of the most important pituitary hormones. LH and FSH are released into the blood stream and act on the appropriate target organs, the ovaries and testes, respectively. LH and FSH regulate the production of androgens and estrogens, as well as the production of progesterone in the ovaries. LH is responsible for ovulation and is involved in the development of the corpus luteum (35). However, the balance between the two male sex hormones, testosterone and dihydrotestosterone, is determined by the ratio between LH and FSH levels (36). The effect of LH on the female reproductive tract is illustrated by the development of corpus luteum (37). FSH plays a crucial role in the development of ovarian follicles (38).

MATERIALS AND METHODS

Experimental animals and tissue collection. The studies were carried out in accordance with the principles and the procedures of the Animal Ethics Committee at the University of Warmia and Mazury in Olsztyn. Mature gilts (Large White × Polish Landrace) at 7–8 mo of age, with body weight of 120–130 kg, descended from private breeding, were used. The breed selected for this research is representative for the husbandry. It is commonly used in the studies as a lean-type animal model because of relatively low fat content (11, 24). Diets were balanced (crude proteins, metabolizable energy, exogenous amino acids, and minerals) in accordance with the nutrient requirements of domestic pigs. Individuals were given free access to water. To investigate adiponectin expression, the total of 20 animals was assigned to one of four experimental groups as follows: days 2–3 (early luteal phase), 10–12 (mid-luteal phase), 14–16 (late luteal phase), and 17–19 (follicular phase) of the estrous cycle (n = 5). For in vitro experiment, APs were pooled to assemble an essential number of cells. Females were monitored daily for estrus behavior in the presence of an intact boar. The onset of the second estrus was marked as day 0 of the estrous cycle. Phase of the estrous cycle was also confirmed on the basis of morphology of the ovary (2). Within a few minutes after slaughter, the pituitary gland and adipose tissue sample from the back subcutaneous fat depot were removed. The samples assigned both for quantitative real-time PCR and Western blotting were collected from the same individuals and at the same time. The pituitary gland was separated into AP and NP lobes. All of the samples were frozen in liquid nitrogen and stored at −80°C until processing for RNA and protein analysis. Pituitaries assigned for cell cultures were dissected immediately after slaughter. AP lobes were isolated, placed in chilled Dulbecco’s modified Eagle’s medium (DMEM) containing 0.1% BSA, and immediately transported to the laboratory.

Total RNA isolation, cDNA synthesis, and quantitative real-time PCR. Total RNA isolation, cDNA synthesis, and quantitative real-time PCR were conducted as described previously by Smolinska et al. (39). The entire total RNA was intact with high quality, i.e., optical density 260/280 and 260/230 ratios were between 1.8 and 2.0 and 1.8 or greater, respectively. In brief, quantitative real-time PCR analysis was performed using a PCR System 7300 (Applied Biosystems) with SYBR Green. Selected forward and reverse primers are presented in Table 1. Constitutively expressed genes, cyclophilin A and GAPDH, were used as the internal control to verify the quantitative real-time PCR. Neither cyclophilin A nor GAPDH showed any changes in the expression between the stages of the estrous cycle or between the studied tissues. The PCR reaction included 20 ng cDNA, 900 nM (adiponectin forward), 300 nM (adiponectin reverse, cyclophilin A forward and reverse), and 60 nM (GAPDH forward and reverse) primers, 12.5 μL SYBR Green PCR Master Mix (Applied Biosystems), and RNase-free water in a final volume of 25 μL. Quantitative real-time PCR cycling conditions were as follows: 50°C for 2 min, then 95°C for 10 min for initial denaturation and enzyme activation, followed by 40 cycles of denaturation at 95°C for 15 s, and annealing at 59°C for 1 min. Negative controls were performed using water as a substitute for cDNA, or reverse transcription was not performed before PCR. All samples were amplified in duplicate. The specificity of amplification was tested at the end of the PCR by melting-curve analysis. Product purity was confirmed by electrophoresis. Levels of gene expression were calculated using the ΔΔCt method and normalized using the geometrical means of reference gene expression levels: cyclophilin A and GAPDH.

Western blotting. Western blotting analysis was performed essentially as described by Smolinska et al. (41). Briefly, equal amounts of porcine pituitary lysates (AP and NP parts separately, 10 μg of total proteins) were resolved by SDS-PAGE (12.5%) for separating adiponectin and actin and transferred to nitrocellulose membranes (Whatman). Blots were blocked for 5 h at 4°C in Tris-buffered saline Tween 20 containing 5% skimmed milk powder and then incubated overnight at 4°C with the rabbit polyclonal adiponectin antibodies at a dilution of 1:150 (Santa Cruz) or rabbit polyclonal actin antibodies (Sigma) diluted 1:200, which were used as an internal control for equal loading and to quantify porcine adiponectin protein. To identify immunoreactive bands, membranes were incubated for 1.5 h at room temperature with mouse anti-rabbit IgG for adiponectin (diluted 1:2,000; Sigma) or goat anti-rabbit IgG for actin conjugated with alkaline phosphatase (diluted 1:5,000; Santa Cruz Biotechnology). Nonspecific fetal calf serum (MP Biomedicals) was used instead of primary antibodies to produce negative control blots. The immunocomplexes were visualized using 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate, according to the manufacturer’s protocol (Promega). The same procedures were used for preparing positive control adipose tissue. The quality of the experiment was confirmed using reference protein, actin, the expression of which did not change between the stages of the estrous cycle or between the studied tissues. The expression of adiponectin was confirmed using reference protein, actin, the expression of which did not change between the stages of the estrous cycle or between the studied tissues. The expression of adiponectin was confirmed using the geometrical means of reference gene expression levels: cyclophilin A and GAPDH.

Table 1. The sequences of oligonucleotide primer pairs

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<tbody>
<tr>
<td>Adiponectin</td>
<td>F: 5'-ATGATGTCACCTGCAACTG-GAATTC-3'</td>
<td></td>
<td>F: 514-536</td>
<td>24</td>
</tr>
<tr>
<td>Cyclophilin A</td>
<td>R: 5'-GACCTGGAATGCGAAGAGGAGA-3'</td>
<td></td>
<td>R: 565-584</td>
<td>24</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: 5'-CCTTCTGACCACTGCTTGCAGAGA-3'</td>
<td></td>
<td>F: 219-237</td>
<td>24</td>
</tr>
<tr>
<td>GAPDH</td>
<td>R: 5'-AGGATGCTATGCTTGCAGAGA-3'</td>
<td></td>
<td>R: 269-299</td>
<td>24</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: 5'-CCAAACATGAACTGACCCAGCATG-3'</td>
<td></td>
<td>F: 61-85</td>
<td>32a</td>
</tr>
<tr>
<td>GAPDH</td>
<td>R: 5'-CCAAACATGAACTGACCCAGCATG-3'</td>
<td></td>
<td>R: 219-243</td>
<td>32a</td>
</tr>
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F, forward; R, reverse.
not vary across the estrous cycle or between the studied tissues. The results of Western blotting were quantified by densitometric scanning of immunoblots with GelScan for Windows version 1.45 software (Kucharczyk, Poland). Data were expressed as ratio of adiponectin protein relative to actin protein in arbitrary optical density units.

Isolation of anterior pituitary cells and in vitro experiments. The procedure of AP tissue dissociation, preparation of cells, and in vitro culture was based on methods described previously by Bogacka et al. (7), with some modifications. Briefly, AP lobes were washed with fresh DMEM containing 0.1% BSA and antibiotics and minced into 1- to 2-mm pieces. Single cell suspension was obtained by several 0.25% trypsin (Biomed Lublin) digestions at 37°C for 10 min. Dispersed pituitary cells were, subsequently, transferred to plastic tubes, repeatedly centrifuged at 1,200 g for 8 min, and washed with DMEM three times. After the final wash, the cell suspension was passed through a nylon filter (60 µm mesh) to remove undigested fragments of the tissue. Cells were counted with a hemocytometer. Cell viability (90–97%) was determined by trypan blue dye exclusion. Finally, pituitary cells were resuspended in McCoy’s 5A medium containing 10% horse serum, 2.5% fetal calf serum, 240 U/ml nystatin, and 100 µg/ml gentamycin and supplemented with 0.1% MEM vitamins and 0.01% MEM nonessential amino acids. One milliliter of dispersed cell suspension (1 × 10⁶/ml) was transferred to each culture dish of 24-well plates and preincubated for 48 h at 37°C in a saturated atmosphere of 5% CO₂ and 95% air. Afterwards, 1 ml of McCoy’s 5A medium without serum was added to each well, and cells were incubated for another 24 h. After a total of 72 h of incubation, cells attached to the well’s surface were washed two times with 1 ml of fresh McCoy’s 5A medium without serum to remove undigested fragments of the tissue. After the final wash, cells were incubated (24 h at 37°C, 5% CO₂ and 95% air) in 1 ml of McCoy’s 5A medium containing bacitracin (2 × 10⁻⁵ M), without (control culture) or with the following agent’s combination: 1) adiponectin (1 µg/ml); 2) adiponectin (10 µg/ml); 3) GnRH (100 ng/ml); 4) insulin (10 ng/ml); 5) GnRH (100 ng/ml) and insulin (10 ng/ml); 6) adiponectin (1 µg/ml) and insulin (10 ng/ml); 7) adiponectin (10 µg/ml) and insulin (10 ng/ml); 8) adiponectin (1 µg/ml) and GnRH (100 ng/ml); 9) adiponectin (10 µg/ml) and GnRH (100 ng/ml); 10) adiponectin (1 µg/ml), GnRH (100 ng/ml), and insulin (10 ng/ml); or 11) adiponectin (10 µg/ml), GnRH (100 ng/ml), and insulin (10 ng/ml). The doses of adiponectin, GnRH, and insulin were established based on Maleszka et al. (28, 29), Bogacka et al. (7), and Gavin et al. (12), respectively. Each in vitro experiment was performed on a different day using separate pools of AP cells. The Alamar blue test revealed that neither adiponectin nor GnRH and insulin affected the viability of cultured cells. All incubations were performed in duplicate. At the end of the incubation, media were collected, centrifuged at 1,000 g, and stored at −20°C until ELISA analyses.

ELISA. The concentration of gonadotropins in culture media was measured using commercial ELISA kits for porcine LH (Cusabio) and porcine FSH (Cusabio). The data were linearized by plotting the log of LH/FSH concentrations vs. the log of the optical density, and the best-fit line was determined by regression analysis. According to the manufacturer, no significant cross-reactivity or interference between pig LH/FSH and analogs was observed.

Data analysis. Data are presented as means ± SE from five different observations. Differences between groups within each phase of the estrous cycle separately were analyzed by one-way ANOVA followed by least-significant differences post hoc test. Statistical analyses were performed using Statistica Software (StatSoft). Values of P < 0.05 were considered statistically significant.

RESULTS

Adiponectin gene expression in the AP and NP. In AP, the highest adiponectin mRNA levels were noted on days 2–3 (P < 0.05; Fig. 1A) but on days 10–12 in NP (P < 0.05; Fig.
Adiponectin gene expression was more pronounced in AP than NP in all studied phases of the estrous cycle \((P < 0.05; \text{Fig. 1C})\).

**Adiponectin protein expression in the AP and NP.** Adiponectin protein concentration in AP was the highest on days 10–12 and the lowest on days 2–3 and 14–16 of the estrous cycle \((P < 0.05; \text{Fig. 2A})\). In NP, adiponectin protein levels were higher on days 10–12 and 17–19 than on days 2–3 and 14–16 of the cycle \((P < 0.05; \text{Fig. 2B})\). Adiponectin protein was more abundant in NP than in AP on days 14–16 and 17–19 \((P < 0.05)\). In the other studied phases the differences in protein expression were negligible (Fig. 2C).

**Effects of adiponectin on basal and GnRH- and/or insulin-stimulated LH secretion by the isolated anterior pituitary cells.** Administration of adiponectin at both doses \((1 \text{ and } 10 \mu g/ml)\) did not affect basal LH secretion during the whole estrous cycle (Fig. 3, A, C, E, and G). Similarly, during the early luteal phase (days 2-3 of the estrous cycle), LH concentrations in GnRH- insulin-, as well as GnRH- and insulin-stimulated probes were not changed in relation to controls (Fig. 3B). On days 10 to 12 of the estrous cycle (midluteal phase), the stimulatory effect of GnRH alone on LH production was sligter than in combination with insulin \((P < 0.05)\). At this stage of the cycle, insulin-stimulated LH secretion was increased in response to 1 \(\mu g/ml\) of adiponectin \((P < 0.05)\), whereas GnRH- and insulin-stimulated LH production was reduced by this adipokine \((1 \text{ and } 10 \mu g/ml; P < 0.05; \text{Fig. 3D})\). During the late luteal phase (days 14-16 of the estrous cycle), adiponectin at concentrations of 10 \(\mu g/ml\) and 1 and 10 \(\mu g/ml\) caused a decrease in insulin- as well as GnRH- and insulin-stimulated LH secretion, respectively \((P < 0.05; \text{Fig. 3F})\). On days 17 to 19 of the cycle (follicular phase), adiponectin \((1 \text{ and } 10 \mu g/ml)\) reduced GnRH- and insulin-stimulated LH secretion \((P < 0.05; \text{Fig. 3H})\).

**Effects of adiponectin on basal and GnRH- and/or insulin-stimulated FSH secretion by the isolated anterior pituitary cells.** The addition of adiponectin at both concentrations \((1 \text{ and } 10 \mu g/ml)\) caused an increase in basal FSH secretion during the whole stages of the estrous cycle. Stronger effect of a lower dose of the adipokine was observed on days 2 to 3 of the estrous cycle \((P < 0.05; \text{Fig. 4A})\), whereas the effect of a higher dose was more pronounced in other phases of the cycle \((P < 0.05; \text{Fig. 4, C, E, and G})\). On days 2 to 3 of the cycle, GnRH-stimulated FSH secretion was enhanced by adiponectin at the concentration 1 \(\mu g/ml\), whereas the addition of this treatment at a dose of 10 \(\mu g/ml\) significantly diminished FSH production by the porcine anterior pituitary cells \((P < 0.05; \text{Fig. 3H})\).
0.05; Fig. 4B). During the midluteal phase (days 10–12 of the estrous cycle), the stimulatory effect of GnRH alone on FSH production was slighter than in combination with insulin (P < 0.05). Adiponectin at the dose of 1 μg/ml decreased insulin-stimulated FSH release (P < 0.05; Fig. 4D). On days 14 to 16 of the estrous cycle, adiponectin treatment at both doses caused an increase in GnRH-stimulated and a decrease in GnRH- and insulin-stimulated FSH secretion (P < 0.05; Fig. 4F). During the follicular phase, adiponectin (1 μg/ml) caused an increase in GnRH-stimulated secretion of FSH (P < 0.05). Under the influence of 10 μg/ml of adiponectin a significant increase in FSH concentration was observed in both insulin- and GnRH- and insulin-stimulated cultures (P < 0.05; Fig. 4H).
DISCUSSION

To our knowledge, this is the first study to demonstrate adiponectin mRNA and protein expression in both AP and NP, as well as adiponectin influence on LH and FSH secretion by the isolated AP cells of cyclic gilts. The level of adiponectin mRNA varied throughout the estrous cycle, with a marked increase on days 2–3 in AP and 10–12 in NP. Unlike the level of adiponectin mRNA, the content of the protein was elevated on days 10–12 in AP and 10–12 and 17–19 in NP. The lack of similarity in the patterns of adiponectin gene and protein expression may result from the complex regulation of transcriptional and posttranscriptional processes, differences in the stability of mRNA and protein, as well as physiological feedbacks influencing transcript and protein concentration (13, 49). What is more, the amount of locally produced adiponectin protein might be complemented by this produced outside the brain. The above suggests that tissue mRNA and protein levels are determined mainly by the physiological state, and they are rarely correlated.

There is a general scarcity of data regarding adiponectin system component (the hormone and its receptors) expression from the complex regulation of transcriptional and posttranscriptional processes, differences in the stability of mRNA and protein, as well as physiological feedbacks influencing transcript and protein concentration (13, 49). What is more, the amount of locally produced adiponectin protein might be complemented by this produced outside the brain. The above suggests that tissue mRNA and protein levels are determined mainly by the physiological state, and they are rarely correlated.

There is a general scarcity of data regarding adiponectin system component (the hormone and its receptors) expression...
in the pituitary. To date, the presence of mRNA for adiponectin and its receptors has only been reported in rats (20, 37, 44), humans (37), and chickens (35). Adiponectin receptor gene expression was noted in AP and NP of cyclic gilts (19). AP of female rats (20), somatotroph cells isolated from transgenic green fluorescent protein-expressing mice, the GH3 cells (rat pituitary tumor cell line) (44), and LBT2 (immortalized mouse gonadotrophs) (20, 25). Psilopanagioti et al. (34) observed the localization of AdipoRs in gonadotrophs, somatotrophs, and thyrotrophs but not in corticotrophs or lactotrophs (34). This may suggest the influence of adiponectin on the secretory functions of these cells. Our earlier studies indicated that the AdipoR protein expression varied both during different phases of the estrous cycle as well as between AP and NP (19). Namely, the AérioR1 protein content was elevated in AP during the luteal phase, which seems to correlate with the plasma adiponectin levels reported by Maleszka et al. (29).

Interestingly, AdipoR2 protein was elevated on days 10–12 in AP and on days 10–12 and 17–19 in NP (19). The same time-course pattern for adiponectin protein expression was observed in the current study. These findings seem to support the hypothesis that adiponectin may play an important role in the auto/paracrine regulation of the pituitary secretory functions, including LH and FSH secretion. The mechanism of adiponectin action may include also up- and/or downregulation of its own receptors, which was also noticed in a study by Rodriguez-Pacheco et al. (37).

The earlier studies provide evidence that the abundance of adiponectin receptor gene transcripts is dependent on the phase of the estrous cycle (19, 20). Observed in the present study, the abundance of adiponectin mRNA and the presence of adiponectin protein in the porcine pituitary confirms the thesis that the hormone may be produced locally in the pituitary cells. What is more, the changes in the level of adiponectin gene transcript and protein during the estrous cycle suggest that the animal hormonal milieu, especially the concentration of sex steroid hormones, may affect adiponectin expression in the pituitary. Kim et al. (20) in studies on the rat pituitary found that adiponectin expression pattern was positively correlated with the progesterone level, which is in line with the results presented in this paper. It is also possible that the rapidly increasing level of progesterone during the early luteal phase of the estrous cycle (days 2–3) may be responsible for a higher level of adiponectin mRNA content and protein during pregnancy corresponding to an increasing adiponectin mRNA content. In addition to progesterone, estradiol could be yet another ovarian hormone that may be involved in the regulation of adiponectin expression. A study by Takemura et al. (45) suggested that the increasing concentrations of estradiol in the human endometrium during the late proliferative phase (late follicular phase) may cause a decrease in adiponectin expression. Furthermore, Maleszka et al. (28, 29) indicated that both the level of adiponectin in the porcine plasma and adiponectin expression in the porcine ovary are lower during the follicular phase than in the luteal phase, which clearly indicates the influence of sex steroid hormones.

Although the results concerning adiponectin’s ability to cross the blood-brain barrier are divergent, one cannot exclude the effect of both local pituitary-produced and systemic-produced hormone on the reproductive functions. Accumulating evidence suggests that this adipokine may affect the estrous cycle directly via the modulation of sex steroid hormone secretion (28), as well as indirectly, through the influence on gonadotropin secretion (37). In this study, we noted that adiponectin increases basal FSH secretion in vivo throughout the estrous cycle but does not affect basal LH release by the isolated AP cells. The influence of adiponectin on GnRH- and/or insulin-stimulated LH and FSH secretion in vitro seems to depend on the stage of the estrous cycle and the dose of adiponectin. Rodríguez-Pacheco et al. (37) indicated that the short-time (4 h) exposure of AP cells isolated from male rats resulted in a decrease in the basal and GnRH-stimulated LH secretion. Interestingly, long-time adiponectin administration did not affect LH release, irrespective of the adipokine dose. Studies on LBT2 cells (mouse gonadotroph cell line) also revealed that adiponectin acutely (30 min) inhibited LH secretion, but chronic (48 h) adipokine treatment had no effect (25). Despite the species- and gender-specific differences, the above seem to be in agreement with the results obtained in this study, since we observed no changes in either basal or GnRH-stimulated LH secretions after 24-h incubation of AP cells in the presence of adiponectin. The fact that short-time exposure to adiponectin caused a significant decrease in GnRH receptor expression in the isolated AP cells of male rats may suggest that adiponectin inhibits LH secretion through reduced gonadotroph sensitivity to GnRH (37). The studies of Maleszka et al. (29) revealed that the plasma adiponectin level depends on the phase of the estrous cycle. In detail, the concentration of the adipokine remained stable throughout the luteal phase, whereas the significant drop during the follicular phase was observed. It may suggest the regulative role of sex steroid hormones, with the upregulating effect of progesterone and/or downregulating action of estrogens. On the other hand, one cannot exclude that the variations in the level of plasma adiponectin may modulate LH and FSH secretion. Plasma LH and FSH levels, occurring about 12 h before ovulation (3, 48), are preceded by a decrease in plasma adiponectin level.

It is postulated that insulin could be another important modulator of gonadotroph secretory functions (1, 22, 42, 51, 52). In this study we observed the influence of insulin on the porcine pituitary secretory functions during the estrous cycle. The earlier reports indicated a stimulatory effect of insulin on GnRH-stimulated LH and/or FSH secretion in a dose- and/or time-dependent manner in rats (1, 42) and in pigs during the follicular phase (22). Our findings suggest that the effect of insulin on LH and FSH secretion depends on animal hormonal milieu. We observed that adiponectin modulation of insulin-stimulated LH and FSH secretion by the isolated porcine AP cells also alters within the estrous cycle. This may suggest that AP cells show different sensitivity to adiponectin, which may be because of the stage of the estrous cycle and the dose of the adipokine. One also cannot exclude a specific cross talk between adiponectin and insulin, but the exact mechanism is unexplored.

In conclusion, our study indicates the expression of adiponectin in the porcine AP and NP lobes of the pituitary gland, depending on the phase of the estrous cycle. The presence of all components of the adiponectin system in the pituitary of cyclic gilts suggests that this hormone may directly affect gland secretory functions via auto/paracrine regulation. What
is more, adiponectin may indirectly influence female reproductive functions via its effect on LH and FSH secretion by gonadotrophs. The results of this study confirm the potential role of adiponectin as an important factor connecting energy homeostasis and reproduction, although the mechanisms underlying the described processes need further research.

GRANTS
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DISCLOSURES
The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS
Author contributions: M.K., N.S., and T.K. conception and design of research; M.K., A.M., K.D., and K.S. performed experiments; M.K. analyzed figures; M.K. drafted manuscript; M.K., N.S., K.D., and K.S. approved research; M.K., A.M., K.D., and K.S. performed experiments; M.K. analyzed valuables in the described processes need further research.

REFERENCES
E1046

ADIPONECTIN FUNCTION IN THE PITUITARY GLAND