Does prolactin influence the hypothalamo-pituitary GnRH-LH system in preovulatory-phase ewes?

Tomasz Misztal¹, Katarzyna Romanowicz, Marta Wańkowska, Anna Wójcik-Gładysz, Jolanta Polkowska.
Department of Endocrinology, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna near Warsaw, Poland

Received: 30 November 2004; accepted: 10 March 2005

SUMMARY

The effects of prolonged infusions of prolactin (PRL) into the third ventricle of the brain of cycling ewes on the secretory activity of hypothalamic GnRH neurons and pituitary LH cells in the pars distalis during the proestrous day were studied. Mature Blackhead ewes were infused with vehicle (control, n=5) or with prolactin (200 µg/day, n=5) during 4 consecutive days prior to the next spontaneous ovulation. The dose of PRL was infused each day in 4 series of 50 µg/100 µl/h at 30-min. intervals, from 8.30 to 14.00 h. The animals were slaughtered on the 16th (proestrous) day of the estrous cycle immediately after the last infusion and their brains were fixed in situ. Plasma samples were collected for 6 h at 10 min. intervals, on days 12 (before the infusions) and 16 of the cycle. The distribution pattern, number and morphology of GnRH neurons in vehicle- and PRL-infused ewes

¹Corresponding author: Department of Endocrinology, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna n/Warsaw, Poland; e-mail: t.misztal@ifzz.pan.pl

Copyright © 2005 by the Society for Biology of Reproduction
were found to be similar and typical for the proestrous phase of the cycle. The immunoreactive (ir) GnRH stores in the median eminence were high and similar in both groups. There were no differences between control and PRL-treated ewes in the number or features of irLH cells. The area fraction and optical density for irLH cells and mRNA LHβ-expressing cells did not differ between control and experimental groups. Irrespective of the kind of infusion, changes in LH secretion during the estrous cycle were similar in control and PRL-infused ewes. Mean plasma LH concentrations were higher (p<0.001) on day 16 compared to day 12 of the cycle. There were no differences in plasma LH concentrations or in the parameters of pulsatile LH secretion between groups. In conclusion, repeated, several-hour-long infusions of PRL into the CNS prior to the next spontaneous ovulation in ewes had no direct effect on the secretory activity of GnRH neurons, and/or the synthesis, accumulation, or tonic release of LH from the pituitary gonadotrophs. *Reproductive Biology* 2005 5(1): 31-49.

**Key words:** prolactin, GnRH, LH, GnRH neurons, gonadotrophic cells, estrous cycle

**INTRODUCTION**

In sexually active ewes, the changes in PRL secretion accompany the increasing activity of the GnRH-LH system during the follicular phase of the estrous cycle. The plasma PRL concentration increases gradually, reaching a maximum shortly before the preovulatory LH surge [15, 26, 28]. In contrast to its fundamental role in other reproductive processes (i.e. pregnancy, maternal behavior and lactation; [1]), the relation between PRL and GnRH-LH system activity during the follicular phase of the cycle in ewes has not been clearly determined. Pituitary PRL may reach the brain by retrograde blood flow from the anterior pituitary to the hypothalamus [22] and/or it is taken up from the circulation by the choroid plexus [20]. The presence of the PRL receptor gene in the sheep hypothalamus and pituitary gland [35, 36] suggests that PRL can regulate not only its own secretion [23], but may also affect the secretion of other pituitary hormones. Moreover, the close
associations between gonadotrophs and lactotrophs in the sheep pituitary gland [36] may predispose PRL to directly and/or indirectly affect LH secretion before oncoming ovulation.

The only data in this aspect come from studies in which pulsatile LH secretion was measured after acute infusions of PRL into the third ventricle of the brain in ovariectomized, estradiol-implanted [7] or intact, follicular-phase ewes [24]. Recently, we noticed that when PRL was infused intracerebroventricularly (icv.) for several days prior to the next spontaneous ovulation, it prolonged the estrous cycle and significantly delayed behavioral estrus [25]. Moreover, PRL strongly induced an increase in dopamine and noradrenaline turnover within the infundibular nucleus/median eminence (IN/ME; [25]), suggesting that the change in the activity of these catecholamines may block the preovulatory GnRH/LH surge. To describe more precisely the mode of prolactin action on the hypothalamo-pituitary-gonadotropic axis in cycling ewes, we tested the hypothesis that the prolonged PRL administration would inhibit GnRH and LH secretory activity within the hypothalamic GnRH neurons and pituitary gonadotrophs, respectively. Thus, the animals were subjected to a several-hour-long infusion of PRL into the third ventricle for 4 days prior to the next spontaneous ovulation. Immunohistochemistry, in situ hybridization, and radioimmunoassays (RIAs) were performed to examine the secretory activity of the GnRH hypothalamic neuronal system and the synthesis, storage and release of LH from the pituitary gonadotrophs during the expected proestrous day following PRL treatment. Plasma LH and progesterone concentrations were measured by RIAs to determine the changes in hormonal levels during the experiment.

**MATERIAL AND METHODS**

**Animals and management**

The experimental procedures were approved by the Local Ethics Committee, according to the Polish Guide for the Care and Use of Animals (1997). Ten adult Blackface ewes, showing a normal 17-day estrous cycle, were used
in the experiment performed from September to the end of December. The animals were maintained indoors under natural lighting conditions (52°N, 21°E) and fed a constant diet of commercial concentrates, with hay and water available *ad libitum*.

Stainless steel guide canullae (1.2 mm o.d.) were implanted under stereotaxic control into the third ventricle of the brain through a drill hole in the skull [37] one month before the experiment. Canullae were fixed to the skull with stainless steel screws and dental cement. The external opening to the canal was closed with a stainless steel cap. The placement of the guide cannula was confirmed by the outflow of a small amount of cerebro-spinal fluid during surgery and after slaughtering.

The course of the estrous cycle was followed in all ewes by exposure to a vasectomized ram. In agreement with the note of Goodman [13], the preovulatory GnRH/LH surge was reflected in our ewes by clear estrous behavior (acceptance of a ram and mounting by a ram), which usually occurred during the early-morning hours on day 17 of the estrous cycle. Additionally, the plasma progesterone concentration was measured every third day. The obtained data were recorded as an individual graph for each ewe. The course of each curve allowed proper determination of the day of the individual estrous cycle. In follicular-phase ewes, the concentrations of progesterone were near sensitivity of assay, and during the luteal phase, progesterone ranged from 2 to 4 ng/ml. At least two estrous cycles were monitored before the experiment.

**Experimental design**

Ewes were randomly divided into two groups and the third ventricle of the brain of each was infused with: 1/ ovine PRL (20-50 IU/mg, Sigma) in a dose of 200 μg/day (PRL group, n=5) or 2/ a vehicle (control, n=5). Prolactin was dissolved in a small volume of sodium bicarbonate (10 mmol/l, pH 9.0) and diluted immediately before the infusion in physiological saline containing 1.0% (w/v) bovine serum albumin [7]. A similar solution, but without PRL, was prepared for control infusion as a vehicle. All infusions
were done with calibrated 1.0-ml gas-tight syringes and a BAS Bee™ microinjection pump (Bioanalytical Systems Inc., USA). During infusion the ewes were kept in comfortable cages where they could lie down and had unrestrained access to hay.

Infusions were performed over days 13-16 of the estrous cycle. The dose of PRL was chosen on the basis of our previous experiment [25] and administered each day in a series of four 1-h infusions (50 μg/100 μl/h) at 30-min. intervals, from 8.30 to 14.00 h. Immediately after the last infusion the animals were slaughtered by decapitation under pentobarbital anesthesia at a local licensed abattoir. Plasma samples were collected for 6 h, from 8.00 to 14.00 at 10 min. intervals, on day 12 (before the infusions) and on day 16 of the cycle through a catheter inserted into the jugular vein the day before the experiment. After centrifugation in heparinized tubes, plasma was stored at -20°C until hormone assay.

**Immunohistochemistry**

Immediately after decapitation, each brain was perfused via both carotid arteries with 1000 ml 0.1 M phosphate buffered saline (PBS) and subsequently with 2000 ml 0.1 M PBS containing 4% paraformaldehyde and 15% saturated picric acid solution, pH 7.4. The hypothalami and pituitaries were removed 20 min after the start of perfusion and post-fixed for 72 h by immersion in the same fixative. Hypothalami were formed in blocks of the same dimension, containing the area between the septum and mammillary body, including the median eminence and the pituitary stalk. The hypothalami were cryoprotected in a 20% sucrose solution in 0.1 M PBS for at least two days and stored at -70°C. This material was cut into 30 μm sections in a cryostat (Jung CM 1500, Leica Instruments GmbH, Nussloch, Germany). Hypothalamic nuclei were identified using an atlas of the sheep brain [38]. The pituitaries were cut in the sagittal plane into two parts, dehydrated in graded alcohol, embedded in paraplast and cut in the sagittal plane at a thickness of 3 μm.
Hypothalamic and deparaffined pituitary sections were processed for immunohistochemistry. All sections were washed in 0.01 M PBS and incubated for 30 min. in 0.1% hydrogen peroxide in 0.01 M PBS and 30 min. in 2% preimmune lamb serum in 0.01 M PBS. Next, the pituitary and hypothalami sections were incubated with anti-oLHβ No.19526, diluted 1:400 and anti (2-10) GnRH No.1900 diluted 1:2000, respectively, for 48 h at 4°C [10, 11]. The antibodies were kindly donated by Dr. M.P. Dubois from INRA, Nouzilly, France. Incubation with the secondary antibody (sheep anti-rabbit Ig[H+L] labelled with peroxidase, The Pasteur Institute, France), diluted 1:40 in 0.1% normal lamb serum in PBS was performed for 2 h at room temperature. The color reaction was developed by incubating sections with 0.05% 3’3-diaminobenzidine tetrahydrochloride chromogen (Sigma) and 0.001% hydrogen peroxide in 0.05 M Tris buffer. Selected material was additionally stained by the silver intensification method of Lipolits et al. [19]. As a control reaction, the inhibition of anti-hormone serum with its homologous antigen was used. Antigens and antisera were mixed and preincubated for 24 h at 4°C before use. Preincubation of GnRH-antisera with 10 µg/ml synthetic GnRH (UCB Belgium) and the LH-antisera with 4 µg/ml synthetic rLH32V02 (National Hormone and Pituitary Program, USA) blocked the immunostaining.

**In situ hybridization**

LHβ sense and anti-sense RNA probes were produced, digoxigenin (DIG) labelled by *in vitro* transcription using SP6 and T7 RNA polymerase and the DIG labelling kit from Roche Molecular Biochemicals (Roche Diagnostics, Meylan, France) and pGEMTE as vectors (Promega, Charbonnieres, France) containing previously obtained 533 bp oLHβ-cDNA [8]. Paraffin-embedded pituitary sections (3 µm) mounted on slides coated with 3-aminopropyltriethoxysilane (2% in acetone, Sigma) were subjected to the non-radioactive hybridization procedure according to Breitschopf et al. [2], with minor modifications by Wańkowska et al. [39].
Image and statistical analyses

A Nikon type 104 projection microscope (Nikon Corporation, Yokohama, Japan) was used to analyze hypothalamic and pituitary sections. Staining was analyzed using a ‘Lucia’ version 3.5lab image analysis computer system (Laboratory Imaging Ltd, Prague, Czech Republic). Immunostained sections were projected by camera (Panasonic KR222, Matsushita Electric Industrial Co, Osaka, Japan) to a color monitor. Pictures were adjusted for optimal contrast, fixed at the same brightness levels, and saved in a buffering system. The analyses were performed with a 4x objective for GnRH-containing nerve terminals in the ME and a 40× objective for GnRH perikarya in the hypothalamus and for gonadotrophs in the adenohypophysis. Two parameters, (1) area fraction (percentage of total area that exhibited positive staining), and (2) integral density (the sum of individual optical densities of each pixel in the area being measured) were analyzed for adenohypophysial cells expressing irLHβ subunit and hybridizing with the anti-sense LHβ probe. Unfortunately, the GnRH data were not analyzed statistically because part of the hypothalamic sections of two controls and two experimental animals were destroyed. Therefore, the estimation was based on visual examination.

Quantitative analysis was performed for each pituitary gland in the subarea of the adenohypophysis using a threshold function to select a range of gray values that was identified as positive staining. All other values were referred to as nonstaining. Before measurements, the images were processed by subtraction of background and removal of artifacts. Frame size was kept constant for the duration of the image analysis. The analyses of immunoreacted and/or hybridized cells were made in four sections of each adenohypophysis, using every 40th mounted and stained section (16 fields of 0.5595 mm² measured in each section). The data from each section were averaged to obtain a mean estimate for each adenohypophysis within each animal. The mean data were pooled to represent PRL- or vehicle-treatment groups. The data were analyzed by the non-parametric Wald-Wolfowitz test (STATISTICA®). The data are reported as the mean % ± SEM of the total area that exhibited positive staining and mean relative units ± SEM of integral density. Significance was defined at the p<0.001 level.
Hormonal assays and statistical analyses

Plasma LH concentration was assayed by a RIA using anti-ovine LH and anti-rabbit gammaglobulin antisera and bovine LH standard (NIH-LH-B6; [33]). The assay sensitivity was 0.3 ng/ml, and the intra- and interassay coefficients of variations were 8.2 and 12.5%, respectively. The progesterone concentration, used to monitor the estrous cycle, was assayed by a direct RIA method, routinely used in our laboratory [34], with a sensitivity of 6.2 pg/ml.

Plasma LH and progesterone concentrations are expressed as a mean±SEM. The effects of the treatments on plasma hormone concentrations were analyzed within groups (between day 12 and 16 of the estrous cycle) and between groups (on day 12 or 16 of the cycle) by analysis of variation followed by the Tukey test (STATISTICA®).

The frequency and amplitude of LH pulses were determined by the PC-PULSAR computer program according to the method of Merriam and Wachter [21] with cut-off G parameters: G1 = 3.98; G2 = 2.40; G3 = 1.68; G4 = 1.24 and G5 = 0.93. Analysis was performed individually for every ewe and encompassed the entire sampling period. The frequency of LH pulses was defined as the number of identified pulses per collecting period. The significance of differences in LH pulse frequency within groups was assayed by the Wilcoxon test and between groups by the Mann-Whitney test. The differences in the amplitude of LH pulses within and between groups were assayed by the nonparametric ANOVA rank Kruskal-Wallis test.

RESULTS

Immunocytochemistry of GnRH in the hypothalamus

In both vehicle- (fig. 1a, c, e) and PRL-treated (fig. 1b, d, f) ewes the distribution pattern and morphology of irGnRH neurons were similar and characteristic of the proestrous phase of the estrous cycle. The greatest number of perikarya that stained positively for GnRH (approximately
Fig. 1. Immunoreactive (ir) GnRH neurons in the medial preoptic area of representative sheep from vehicle- (a, c, e) and prolactin-infused (b, d, f) groups. Scale bars: a, b = 500 µm, c, d = 100 µm, e, f = 50 µm. Note: 1. numerous GnRH perikarya and dense network of ir axons (thin black arrows) in both groups (c, d); 2. variety of morphological types of GnRH perikarya – multipolar (white arrow) and unipolar with expansive dendrite (black arrow) and clearly visible axon (gray arrow, e, f). AP – area preoptica, B – base of the brain.

20-30 cells per slide) was concentrated in the medial preoptic area at the level of the organum vasculosum of the lamina terminalis (OVLT; fig. 1a-d). They were irregular and fusiform in shape, generally bi- or multipolar, with extensive dendrite-like processes irregular in outline and branched (fig. 1e, f). A small cluster of cell bodies was found in the lateral preoptic area and in the region of the recessus supraopticus. Some
single cell bodies were sporadically seen in the nucleus infundibularis. An excessively dense network of irGnRH fibers was observed within the preoptic area and the anterior hypothalamic area in the control as well in the prolactin–infused sheep. There were no differences between both groups in the distribution and concentration of the irGnRH stores situated in the median eminence (ME; fig. 2). A high accumulation of irGnRH nerve terminals located in the external palisade layer of the rostral and medial parts of the ME was seen in the control as well in prolactin-infused sheep (fig. 2). Neither of the control stainings exhibited any specific staining (data not shown).

**Immunocytochemistry of the LH cells**

The characteristics and the number of irLH cells in the pituitaries of both control and prolactin-treated ewes were typical of the phase of the cycle that they represented (proestrous day). They showed a similarly intense immunocytochemical reaction and similar numbers of irLH cells (fig. 3 a, b). This was reflected in the lack of differences in the percentage area occupied by the irLH cells and in the density of the ir material within the adenohypophyses between both groups (fig. 4a, b).
Fig. 3. Immunoreactive (ir) LHβ (a, b) and hybridizing LHβ (c, d) cells in the adenohypophyses of two representative sheep from vehicle- (a, c) and prolactin-infused (b, d) groups. Scale bar: 50 µm. Note no changes detected in LHβ-ir and LHβ-hybridizing cells.

**Pituitary LHβ gene expression**

No detectable hybridization signal was observed in sections hybridized with sense probes for LHβ (data not shown). *In situ* hybridization with an anti-sense probe for LHβ showed that the number of hybridized cells and intensity of DIG-labeling were similar in ewes from the control and PRL-infused groups (fig. 3c, d). Microscopic observations were supported by quantitative computer estimation of the percent of hybridized cells (percentage of area occupied by hybridizing cells) and the intensity of hybridization signals, which did not differ between treatments (fig. 4c, d).

**Hormone concentrations**

Irrespective of the kind of infusion, the mean plasma LH concentrations were significantly higher (p<0.001) on day 16 than on day 12 of the estrous cycle. In
control group, mean plasma LH concentrations on day 16 and 12 were 3.76±0.06 vs. 3.44±0.05 ng/ml respectively. In PRL-infused ewes, mean plasma LH concentrations on day 16 and 12 were 3.68±0.04 vs. 3.37±0.03 ng/ml, respectively. There were no significant differences between groups in LH concentration noted on particular days of the cycle. No significant differences were found in LH pulse frequency or in LH pulse amplitude when values from day 12 and 16 of the estrous cycle were compared within each group as well as from the particular days of the cycle between groups (data not shown).

The mean plasma progesterone concentrations were significantly lower (p<0.001) on day 16 than on day 12 of the estrous cycle in both control (0.23


\[ \pm 0.02 \text{ vs. } 2.05 \pm 0.09 \text{ ng/ml} \) and PRL-infused \((0.26\pm0.05 \text{ vs. } 1.71\pm0.12 \text{ ng/ml})\) ewes.

**DISCUSSION**

The results of the study demonstrate that repeated, several-hour-long infusions of PRL into the CNS, prior to the next spontaneous ovulation in ewes, has no direct effect on the secretory activity of GnRH neurons in the hypothalamus and/or the LH-producing cells in the adenohypophysis and has no effect on the tonic secretion of LH.

The early reports of Polkowska et al. [27] and Lehman et al. [18] established the distribution and morphology of GnRH neurons in ewes during various reproductive stages. The present data, concerning irGnRH perikarya and storage of irGnRH in the nerve terminals of the ME during the proestrus stage, before ovulation, are in agreement with these observations. To our knowledge, there is only one article by Harris et al. [14], showing the temporal changes in GnRH mRNA expression during the periovulatory period in ewes. These authors reported that cellular GnRH mRNA content falls prior to the preovulatory GnRH surge [14]. Although this may result from increased degradation of the mRNA molecule, the other possibility is that an abundance of GnRH, sufficient for the generation of the pituitary LH surge, is stored much earlier prior to the preovulatory surge. That particular situation may be seen on the hypothalamic images of our PRL-infused and control sheep. Therefore, the lack of differences between the GnRH neurons of both groups may lend support to the supposition that prolactin does not exert a direct inhibitory effect on some secretory activities of GnRH neurons, i.e. on the synthesis and storage of the hormone. As described by Polkowska et al. [27], GnRH output escalated at the time of the preovulatory gonadotropin surge until the disappearance of irGnRH from the ME after ovulation.

In our experiment, the features of LH-producing cells were also typical of the proestrus phase of the cycle in both groups, indicating that the stores of the hormone had accumulated in the gonadotropic cells before the
preovulatory LH surge. A day after the onset of the LH surge both serum and pituitary LH levels fall dramatically [6, 17, 27]. A high number of cells hybridizing for LHβ and a high intensity of hybridization signals in both our groups indicate enhanced synthesis of LHβ subunits. It was reported that LHβ mRNA amounts began to increase after day 12 of the cycle [17]. This predisposes gonadotropes to increase LH synthesis and pulsatile hormone secretion throughout the next follicular phase of the cycle. The RIA data on LH concentration and the pattern of pulsatile LH secretion confirmed that the preovulatory LH surge did not occur before decapitation in either PRL- or vehicle-infused ewes. However, it was found that plasma LH concentrations were significantly higher on day 16 (expected proestrous, end of the experiment) than on day 12 (luteal phase, beginning of the experiment). This reflects a gradual increase in tonic LH secretion typical of the period studied. Thus, in this model, contrary to expectations, the prolonged administration of prolactin into the CNS did not inhibit the secretory activity of LH-producing cells, i.e. synthesis, storage, or release of the hormone. It is noteworthy that the effectiveness of our PRL dose within the ewe CNS was shown in a previous experiment [25], in which the PRL level in the peripheral circulation was significantly reduced following the infusions. Another of our studies [24] showed that only a small amount of the infused prolactin crossed to the circulation. In view of the health of animals and for technical reasons, it was not possible, however, to infuse the hormone and collect the cerebro-spinal fluid after the infusion to determine the rate of prolactin degradation within the brain. Additionally, the changes in plasma progesterone concentrations, which dropped to values near 0 on the expected proestrous day, indicate that the ovaries were properly functioning.

Absence of changes in LH secretion was also demonstrated after acute icv. PRL administration in both OVX estradiol-implanted ewes [7] and intact follicular-phase ewes [24]. In contrast, findings on pigs have shown that PRL might positively affect GnRH/LH secretion during the periovulatory period. Ciereszko et al. [5] demonstrated that treatment of sows with PRL, begun shortly after the preovulatory surge, increased in vitro GnRH release by hypothalamic explants. GnRH-stimulated LH production was also enhanced in PRL-treated sows as compared with controls [5]. The opposite
effects were documented in rats, where PRL suppressed LH release from pituitary fragments [3] and reduced the percentage of pituitary LH-secreting cells \textit{in vitro} [32]. Moreover, the \textit{in vivo} and \textit{in vitro} LH responses to exogenous GnRH administration were reduced in hyperprolactinemic rats [3, 30, 31]. It has been shown, in this aspect, that chronic PRL administration decreased pituitary GnRH receptor content [12].

To make the picture of the relationship between PRL and the secretory activity of the GnRH-LH system in cycling ewes more complete, it is necessary to recall data from our previous experiment with a similar design. Ewes infused for several days with PRL showed a prolonged estrous cycle and a significant, 2-4-day, delay of estrous behavior [25]. Classically, in ewes the onset of estrus is coupled with a preovulatory GnRH/LH surge [13]. Unlike here, the assessment of the extracellular activity of some hypothalamic catecholamines was made in the area of the IN/ME using high performance liquid chromatography [25]. The IN/ME has been shown to be a site of GnRH release and of synapses between GnRH- and DA-containing neurons [9, 16]. The onset of the GnRH/LH surge was, in turn, reported to be strongly related to the reduction of the inhibitory tone of DA on IN/ME-GnRH neurons [4]. We found that prolonged PRL treatment prior to the next spontaneous ovulation evoked a significant increase in DA and noradrenaline turnover in this hypothalamic area on day 16 of the estrous cycle, thus, the change in the activity of these catecholamines in response to PRL might be the main reason for delayed preovulatory events [25]. Similar results were also obtained in rats by Wise [40], who showed that preovulatory LH surges and ovulation were completely blocked and the median eminence DA turnover rate was elevated in response to PRL treatment. Possible involvement of beta-endorphin in the PRL inhibitory action on GnRH in rats was suggested by Sarkar and Yen [29]. Taken together we conclude that PRL does not directly influence the GnRH-LH system in preovulatory ewes. Rather, its action could be mediated by DA which could act at the level of GnRH stores in the nerve terminals of the ME by blocking the release of GnRH to the portal circulation. Since an increase in PRL secretion precedes ovulation in normally cycling ewes [15, 26, 28], it is reasonable to suggest that the attenuation of the PRL-DA feedback at the end of the follicular phase
of the estrous cycle may be one of the critical factors for the initiation of the preovulatory GnRH/LH surge.

ACKNOWLEDGEMENTS

We wish to thank Józef Rutkowski for help in brain surgery and the other members of the Department of Endocrinology for help in the collection of blood samples and technical assistance with the radioimmunoassays. This study was supported by the State Committee for Scientific Research (KBN, grant No. 5P06D 02319).

REFERENCES


17. Landefeld T, Maurer R, Kepa J 1985 Luteinizing hormone beta-subunit mRNA amounts increase during the preovulatory surge of luteinizing hormone in the ewe: the highest levels are observed at the completion of the peak. *DNA* **4** 249-254.


27. Polkowska J, Dubois MP, Domański E 1980 Immunocytochemistry of luteinizing hormone releasing hormone (LHRH) in the sheep hypothalamus during various reproductive stages. *Cellular Tissue Research* 208 327-341.