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LH/hCG RECEPTORS IN THE PORCINE UTERUS — A NEW EVIDENCE OF THEIR PRESENCE IN THE CERVIX

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High-affinity LH/hCG binding sites have been characterized in bovine, lepine, murine, human uteri and porcine myometrium and endometrium. In the present studies we analyzed these receptors in the porcine cervix. Radioreceptor ligand assays were performed with cell membrane preparations of the cervix which were analyzed for binding sites specificity, capacity and affinity. Corpus luteum and myometrium were used as positive control tissues. In the cervix there was little competition for receptor occupancy between hCG and porcine FSH (1.2%) or bovine TSH, porcine GH and porcine PRL (0.1%, 0.1% and $< 0.001\%$; respectively) but porcine LH could completely inhibit the binding of [^{125}I] hCG. There was not binding for LH/hCG in crude membrane preparations of kidney or skeletal muscle. The concentration (fmol/mg protein) of cervical LH/hCG receptor did not vary significantly during particular phases of the estrous cycle, except the early luteal phase (Days 6—7) when the level of LH receptors was very low ($p < 0.05$). The affinity of uterine LH/hCG binding sites in the cervix and the myometrium was not different from the affinity of LH/hCG binding sites in luteal cells. The porcine cervix as well as the myometrium contains a 75- and 48-kDa immunoreactive LH/cCG receptor proteins similar to corpus luteum. Southern blot of RT-PCR products performed to enhance the specificity and sensitivity of LH receptor transcripts determination in uterine tissues revealed that expected fragments of 740 and 470 bp were present in myometrium and corpus luteum. The cervix showed only 740 bp fragment. In situ hydridization showed the expression of mRNA for LH receptor in the epithelium of the cervix. Immunoreactive staining for LH/hCG receptors was also observed only in epithelial cells of the cervical tissue. Our studies are probably the first evidence demonstrating the specific LH/hCG binding sites in female cervix.

Key words: *LH/hCG receptor, cervix, pig.*

ABBREVIATIONS

BSA — bovine serum albumin, DNA — deoxyribonucleic acid, EDTA — ethylenediaminetetraacetate, FSH — follicle stimulating hormone, GH — growth hormone, hCG — human chorionic gonadotropin, LH — luteinizing hormone, mRNA — messenger ribonucleic acid, PBS — phosphate buffered saline, PRL — prolactin, RT-PCR — reverse transcription-polymerase chain reaction, SDS-PAGE — polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, TSH — thyrotropic hormone.

INTRODUCTION

The existence of specific, high-affinity, low-capacity binding sites for luteinizing hormone/human chorionic gonadotropin (LH/hCG) has been reported for the porcine myometrium (1, 2), endometrium (1, 3) and oviduct (4), rabbit uterus (5, 6), rat uterus (6, 7), bovine endometrium (8) and human endometrium and myometrium (9). LH/hCG receptors were also detected in the pig superficial smooth muscle layer of the broad ligament (2, 10) and in the umbilical cord (11). The amount of receptors in both human (9) and porcine (1) myometrium or porcine broad ligament (12) is higher during the luteal (secretory) phase than in the follicular (proliferative) phase of the estrous (menstrual) cycle — indicating possible regulation of these receptors by ovarian hormones. Recently Gawronska *et al.* (13) showed that combined estradiol and progesterone priming is necessary for LH-induced relaxation of the porcine oviduct. There are evidences that one of the physiological roles of the uterine LH receptors is its participation in the relaxation of myometrial smooth muscles in pig (12, 14) and women (15) and in porcine Fallopian tube (4).

The above findings turned our attention towards another part of the uterus hitherto unexamined for gonadotropin receptors — the cervix. The junction between the cervix and the uterine body is the internal os, and at this point the nature of the lining epithelium and the uterine wall changes. The cervix histologically differs from the uterine body. The cervical stroma is composed of smooth muscle fibres embedded in collagen, proportions of each vary according to age and parity. The lining of the lumen is a single layer of mucus-secreting epithelium. Normally the cervix is firm and rubbery and the cervical lumen is a narrow channel, but the cervix can be dilated under some circumstances (e.g. during estrus and parturition).

The present study was conducted: 1) to determine the existence of LH/hCG receptors in porcine cervix and 2) to compare their concentration in the cervix with the myometrium during the course of the estrous cycle.

MATERIALS AND METHODS

Reagents

Purified hCG (CR-127: 14 900 IU/g) and bovine TSH (bTSH 11-NIH) was provided by the National Hormone and Pituitary Program (NIDDKD), porcine LH (pLH B-1; 1.7 IU/mg), porcine FSH (pFSH B-1; 2.0 IU/mg), porcine PRL (pPRL B-1; 34 IU/mg) and porcine GH (pGH B-1; 1.54 IU/mg) were provided by USDA Hormone Program. The porcine LH receptor complementary DNA was obtained from Dr. H. Loosfelt of Hormones at Reproduction, Hospital de Biocentre, Kremlin Biocentre (Paris, France). Polyclonal antibodies raised against synthetic N-terminal rat LH/hCG receptor peptide sequence of 15—38 (anti-LHR 15—38) was obtained from Dr. P. Roche of the Mayo Clinic (Rochester, MN).

Animals and tissues

Tissues for the determination of capacity and affinity LH binding sites were collected from 20 gilts (8 months of age) at a local abattoir. The first day of behaviour estrus was established as Day 0 of the estrous cycle. Days of the estrous cycle were addinionally confirmed by morphological appearance of ovaries after slaughter (16). These parameters allowed the classification of the uterine tissues into four major groups (n = 4—6): 1) proestrus (Days 18—20); 2) early luteal phase (Days 6—7); 3) preluteolysis (Days 12—14) and 4) luteolysis (Days 15—16). Uteri and other tissues (corpora lutea, skeletal muscle, intenstine or kidney) were collected immediately after slaughter and transported on ice to the laboratory during 5 min.

Preparation of membrane fractions

Tissues (myometrium, cervix, corpora lutea, kidney and skeletal muscle) were dissected, minced with scissors and weighted. The cell membrane fractions were obtained using the procedure described by Ziecik *et al.* (1). Membrane fractions without BSA were assayed for protein determination by the method of Lowry *et al.* (17).

LH/hCG receptor assay

A ligand binding assay in membrane fractions was performed as previously decribed (1). Human CG (CR-127) was labeled according to Iodogen method (18). Na ^{125}I was purchased from Amersham International (Amersham, Buck's, UK). Separation of protein-bound and free ^{125}I was performed by chromatography on Sephadex G-50 (Pharmacia, Uppsala, Sweden) columns. The specific activity of labelled hCG was determined by self displacement analysis on corpus luteum membrane preparations in the radioligand receptor assay (19) and varied from 50 to 70×10^3 cpm/ng. Non specific binding was determined in the presence of 0.5 µg hCG and was less than 5% of the total ^{125}I label added. Non-specific binding was subtracted from total binding except for the time and temperature studies. The receptor concentration and equilibrium association constant (K_a) of unoccupied binding sites were determined by Scatchard analysis (20). Six to eight subsaturating doses (0.03—5.0 ng) of unlabelled hCG were used for each receptor preparation in duplicate.

To validate the time and temperature dependence of specific ^{125}I-hCG binding to the cervix membrane fractions, protein samples of Days 12—14 cervix extracts were incubated for 2—30 h at 4°C, 24°C and 37°C. It was found that 16—20 h incubation at room temperature was optimal for receptor determination in the cervix.

The specificity of ^{125}I-hCG binding in the cervix was determined by incubating increasing amounts of various nonradioactive hormones (hCG, pLH, pFSH, oPRL, bTSH) with a constant amount of radiolabelled hCG (25000 cpm/tube). Cross-reactivity was calculated from the relative amount of the tested hormone that inhibited ^{125}I-hCG binding by 50%. Specificity of ^{125}I-hCG binding to skeletal muscle and kidney was determined by incubating 1—2 mg of protein membrane preparation with constant amount of ^{125}I-hCG (25000 cpm/tube); corpus luteum tissue (0.5 mg of protein) similarly prepared served as a positive control.

Western blot analysis for LH receptor

Membrane fractions of myometrium, cervix, corpora lutea and skeletal muscle were prepared as described by Stepien *et al.* (3). Aliquots (70 µg) of protein were dissolved in double-strength loading buffer consisting of 50 mM Tris-HCl (pH 6.8), 4% SDS, 20% glycerol and 2% 2-mercaptoethanol, separated on 10% SDS-PAGE and electroblotted onto 0.45 µm nitrocellulose

membranes in 25 mM Tris-HCl buffer (pH 8.2), 192 mM glycine, containing 0.1% SDS. The non-specific binding sites were blocked with 5% non-fat dry milk in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.1% Tween-20 (TBST buffer) overnight at 4°C. The membranes were then incubated with 1 : 1000 dilution of the LH receptor antibody (anti-LHR 15—38) for 1.5 h at 22°C and washed three times with TBST. Antibody binding was detected with ABC complex (avidin-biotin-hoseradish peroxidase complex; Vectastain ABC kit, Vector Laboratories, Inc, Burlingame, CA). To develop colour, TBS containing 0.01% H_2O_2 and 0.04% 3, 3'-diaminobenzidine was used.

RT-PCR/Southern blotting

Briefly, total RNA was isolated from the cervix, endometrium and both longitudinal and circular layers of myometrium, corpus luteum and intestine, according to Chomczynski and Sacchi (21) and Puissant and Houdebine (22). The purity of RNA was determined by the A_{260}/A_{280} ratio in spectrophotometry and its quality in agarose-formaldehyde gel electrophoresis (23). The isoforms of the pig LHR cDNA from Gene Bank (pLHRA, pLHRB, pLHRC, pLHRD) were aligned. The oligonucleotides used for RT-PCT were designed using the DNAStar Primer Selection program (DNASTAR Inc. Madison, WI). The specificity of the primers was confirmed by the BLAST Program (24). The sense primer (nucleotides 738—761, exon 9) 5'GACGCTAATTGCCACATCATCCTA was common to all of cDNA isoforms and antisense primer (nucleotides 1456—1475, exon 11) 5'ATTATGCTTGGAGGGTGGCT was related to full length isoform pLHRA and variant pLHRB. The alignment of the pig LHR sequence allowed to except two RT-PCR products of size of 740 (cDNA fragment derived from pLHRA isoform) and 470 bp (cDNA fragment generated by RT-PCR from pLHRB sequence).

RT-PCR was performed using two µg of total RNA per 100 µl reaction mixture. Reverse transcription at 60°C for 1 h, denaturation at 97°C for 3 min, was followed by PCR of cDNA at 40 cycles including denaturation at 96°C for 30 s, primer annealing at 55°C for 30 s and extension at 74°C. The DNA polymerase having both, reverse transcriptase and DNA polymerase activities (Epicentre Technologies, Madison, WI) was used. The PCR products (20 µl) were electrophoresed in 1% agarose and photographed under UV light.

The RT-PCR products (10 µl) were electrophoresed on 1.5% agarose gels and transferred on nylon filter (Hybond N, Amersham, UK) using standard procedure. The hybridization was performed according to Hybridization Protocols for nylon membranes (Hybond N, Amersham) with a pig cDNA probe (fragment 900—2081 of pig LHR cDNA) labelled with [α-^{32}P]dCTP using the random prime method Miltiprimer™ DNA Labelling System, GIBCO BRL) at 65°C for 12 h. After hybridization, blots were washed twice with 1 × SSC (150 mM NaCl, 15 mM sodium citrate; pH 7.0) containing 0.1% SDS for 15 min at room temperature, with 0.2 × SSC/0.1% SDS at 65°C for 15 min. Washed sheets were exposed to Amersham X-ray film at −80°C.

Immunoperoxidase staining for LH/hCG receptors

Cervical tissue was fixed for 6 h in 4% paraformaldehyde in 0.1 M PBS. Fixed tissue was stored in 18% sucrose in PBS with 0.01% sodium azide. Cryostat sections (8 µm) were air-dried, rinsed in 0.05 M TBS (pH 7.6), and then placed in ethanol ascending concentration series (50%, 70%, 96% of absolute alcohol). The sections were treated with 1% H_2O_2 in methanol for 30 min and then in 0.75% glycine in TBS for 30 min. After rinsing in TBS, the sections were incubated overnight with primary antibody (diluted 1:200). Antibody binding was detected with the ABC complex (Vectastain ABC kit). Peroxidase activity was revealed using 3, 3'-diaminobenzidine as a substrate. In the control sections the primary antibody was substituted with non-specific immunoglobulin G (IgG) during the procedure. The observations and photographs were made using a light microscope (Olympus IMP-2) equipped with Nomarsky optics.

In situ hybridization

The cRNA probes were synthesized in the presence of [^{35}S]-VTPαS (250 μCi; NEG-039H, POLATOM, Otwock-Świerk, Poland) using the cDNA corresponded to 76—491 bp fragment of the porcine LHR. An 415 bp fragment was subcloned into Ava I-RSA I sites of pGEM-4z vector. Restriction digest and subsequent in vitro transcription, using purified linearized cDNA templates, were performed to generate both antisense and sense probes. The probes were purified by centrifugation over a Sephadex G-50 column. The RNA probes were then frozen at −70°C and used for hybridization within 2 days. The RNA probes were diluted with hybridization buffer (1 × Denhardt's solution, 50% formamide, 0.3 M NaCl, 10 mM Tris-HCl [pH 8.0], 1 mM EDTA, 10 mM dithiotheitol, 500 μg of yeast RNA/ml and 10% dextran sulfate) to about 2×10^7 cpm/ml.

For hybridization, sections (8 μm) were removed from −70°C and kept at room temperature for 30 min. The sections were then put into 4% paraformaldehyde in 0.01 M PBS (pH 7.4) for 20 min, washed three times with 0.01 M PBS and put for 2 min in 2 × SSC at room temperature. The sections were acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0) for 10 min, rinsed in 2 × SSC, and then dehydrated in 50, 70, 80, 95 and 100% ethanol. The rinsed sections were air-dried.

Hybridization was performed by covering sections with 50 μl diluted probes under Parafilm in a humified oven at 55°C for 24 h. After hybridization, slides were washed twice for 10 min in 2 × SSC plus 50% formamide at 52°C, then two times for 10 min in 2 × SSC in a shaking water bath at room temperature. The slides were then treated with ribonuclease-A (Sigma Chemical Co, St. Louis, MO; 100 μg/ml in 2 × SSC) for 30 min at 37°C. The slides were then washed twice for 1 min with 2 × SSC, 15 min in 1 × SSC plus 10 mM DTT and 15 min in 0.5 × SSC plus 10 mM DTT at room temperature. After that the slides were put into 0.1 × SSC plus 10 mM DTT for 30 min at 55°C and dehydrated with 50, 70, 80, 95 and 100% ethanol and air dried.

Air-dried slides were dipped in Kodak NBT-2 emulsion (Eastman Kodak, Rochester, NY) and exposed for 2 weeks at 4°C. The slides were then developed, stained with hematoxylin and eosin, and mounted for microscopic examination. The pictures were taken under darkfield microscopy (Olympus BX40, Olympus Company Ltd., Japan).

Statistics

Data were analyzed by ANOVA followed by Duncan's new range test. Results are expressed as mean ± SEM.

RESULTS

Concentration of LH/hCG receptors in the cervix

The number of LH/hCG receptors and their apparent association constant (K_a) were measured at equilibrium. The Scatchard plots prepared from cervical tissues were linear (*Fig. 1*). There was no statistical differences among K_a values for cervical tissues obtained at different days of the estrous cycle and the values varied from $3.02 \pm 0.76 \times 10^{10}$ M^{-1} to $4.48 \pm 0.62 \times 10^{10}$ M^{-1}. The affinity of porcine LH/hCG binding sites in the cervix was similar to the myometrium ($4.3 \pm 0.22 \times 10^{10}$ M^{-1}) and corpora lutea ($4.8 \pm 0.15 \times 10^{10}$ M^{-1}).

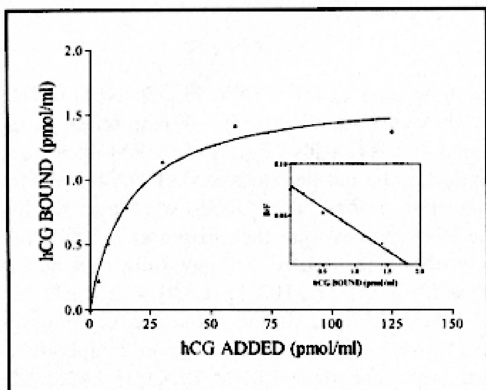

Fig. 1. Saturation curve and Scatchard plot of hCG binding to pig cervical cell membrane preparation. Affinity of LH/hCG binding sites in cervix membrane preparation from Days 12—14 of the estrous cycle is shown ($K_a = 4.37 \times 10^{10}\ M^{-1}$).

The specific binding of [^{125}I] hCG to cervical receptors (*Fig. 2*) was time and temperature dependent. The maximal binding at room temperature developed after 16 h of incubation. At 37°C the peak occurred after 4 h and then decreased. The hCG binding at 4°C was maintained at a constant, low level during the incubation time. The specificity of [^{125}I] hCG binding to cervical cell membrane preparations is shown in *Fig. 3*. There was very little competition between hCG and pFSH, bTSH, pGH and pPRL (1.2%, 0.1%, 0.01% and < 0.001%, respectively) but pLH could completely inhibit the binding of [^{125}I] hCG.

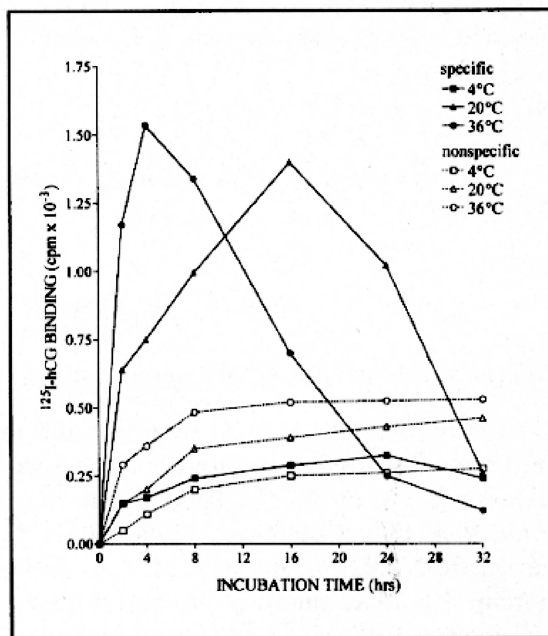

Fig. 2. Time and temperature dependence of specific (solid lines) and non-specific (dashed lines) ^{125}I-hCG binding to porcine cervix. Crude membrane preparations (1 mg protein) from Days 12—14 of the estrous cycle was incubated with 30000 cpm of ^{125}I-hCG at 4°C (squares), 20—24°C (triangles), or 37°C (circles).

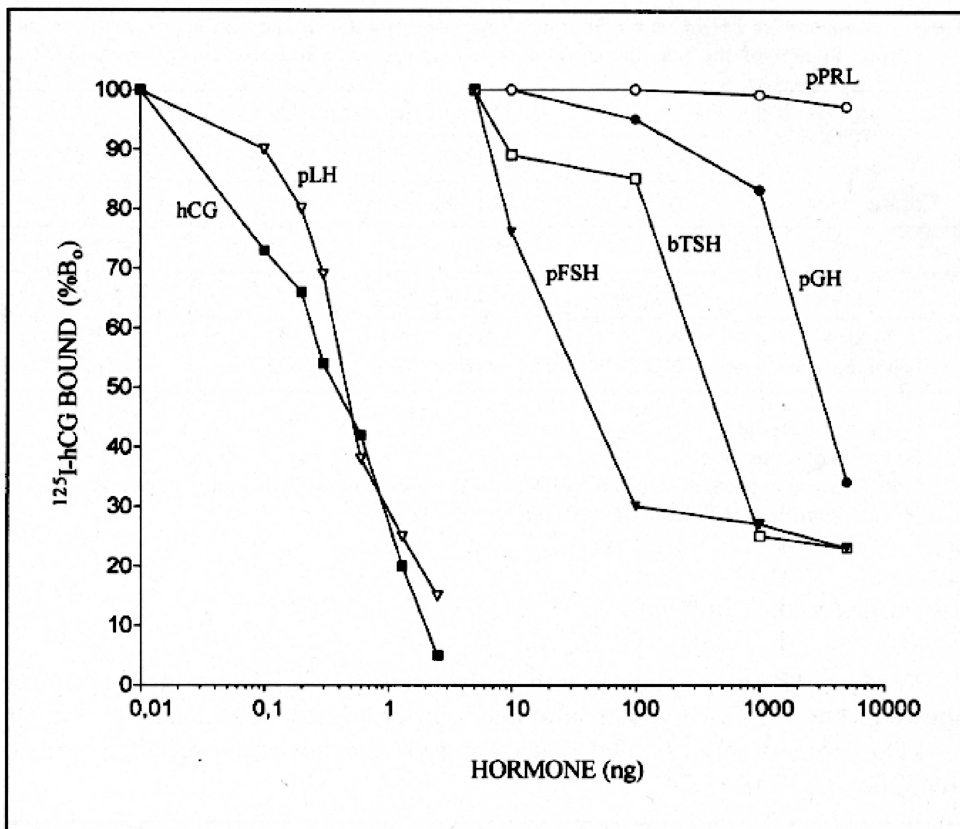

Fig. 3. Hormonal specificity of ^{125}I-hCG binding to porcine cervix. Crude membrane preparations (1 mg protein) from Days 12—14 of the estrous cycle was incubated with 30000 cpm of ^{125}I-hCG in the presence of increasing amounts of hormones. Means of duplicates from one representative of two experiments are shown. GH, growth hormone; PRL, prolactin; TSH, thyroid-stimulating hormone.

Estimation of the number of LH/hCG receptors in pig uterine tissues during the estrous cycle are shown in *Table 1*. The number of binding sites in the uterine tissues was 3—20 times less ($P < 0.01$) than the receptor capacity in the porcine corpora lutea (8.92 ± 1.61), when expressed per mg protein of crude membrane preparation. Concentrations of gonadotropin receptors in the cervix and myometrium did not vary significantly during the particular phases of the estrous cycle, except the early luteal phase (Days 6—7) when the level of LH receptors in the cervix was very low (0.45 ± 0.08; $P < 0.05$). LH/hCG receptors were not found in the negative control tissues — skeletal muscle and kidney.

Table 1. Capacity of LH/hCG binding sites (fmol/mg protein) in the cervix, myometrium and corpus luteum of the pig (kidney and skeletal muscle are negative control tissues)*.

Tissue	Days of the estrous cycle			
	6—7	12—14	15—16	18—20
Corpus luteum	(−)	8.92±0.06	(−)	(−)
Cervix	0.45±0.03 [a]	1.64±0.34 [b]	1.51±0.73 [b]	1.49±0.3 [b]
Myometrium	2.94±0.62	2.71±0.73	2.57±0.30	2.56±0.85
Kidney Skeltal muscle	ND ND	ND ND	ND ND	ND ND

(−) — not studied.
ND — not detectable.
* Results are expressed as mean±SEM; n = 4—6. Means with different superscripts within a row are significantly different ($P < 0.005$).

LH/hCG receptor protein

Western blotting studies with polyclonal LH/hCG receptor antibody showed that cervix, myometrium and corpora lutea contained 75 and 48 — kDa proteins (*Fig. 4*). The skeletal muscle did not show receptor protein (data not shown).

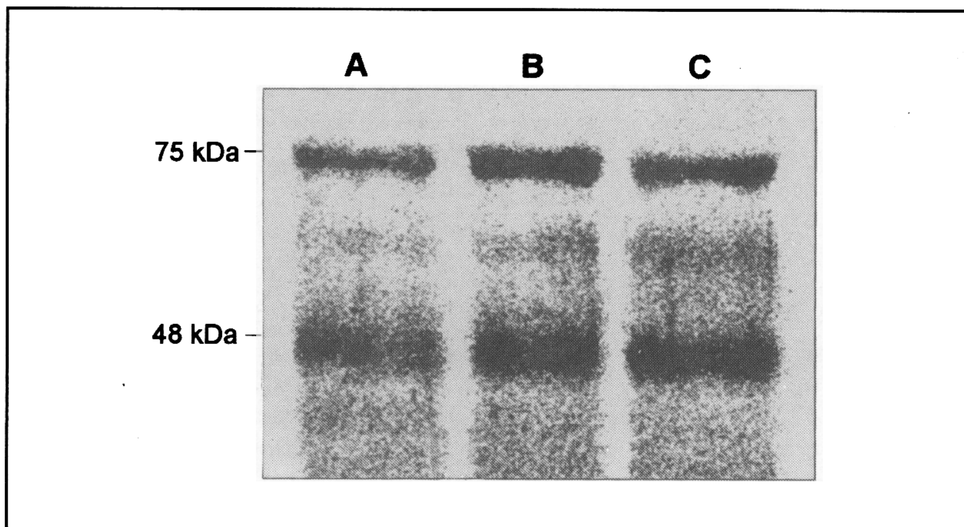

Fig. 4. Western immunoblot for LH/hCG receptors in the porcine cervix (lane A), corpus luteum (lane B) and myometrium (lane C), Day 12—14 of the estrous cycle, 70 μg protein of each tissue were used.

LH/hCG receptor mRNA

Southern blot of RT-PCR products was performed to enhance the specificity of determinations LH receptor transcripts in investigated tissues (*Fig. 5*). Those techniques revealed that expected fragments of 740 and 470 bp were present in corpus luteum and showed additional product of 670 bp as well as 440 bp fragment visible on X-ray film after longer exposition. The myometrium layers: longitudinal and circular presented all 4 products. Products of 740 and 470 bp were amplified from endometrial RNA as predicted from pLHR A and B isoforms, respectively. Only one RT-PCR product of 740 bp was found in the cervix. The negative control tissue e.g. intestine and kidney (data not shown) did not showed presence of any RT-PCR products.

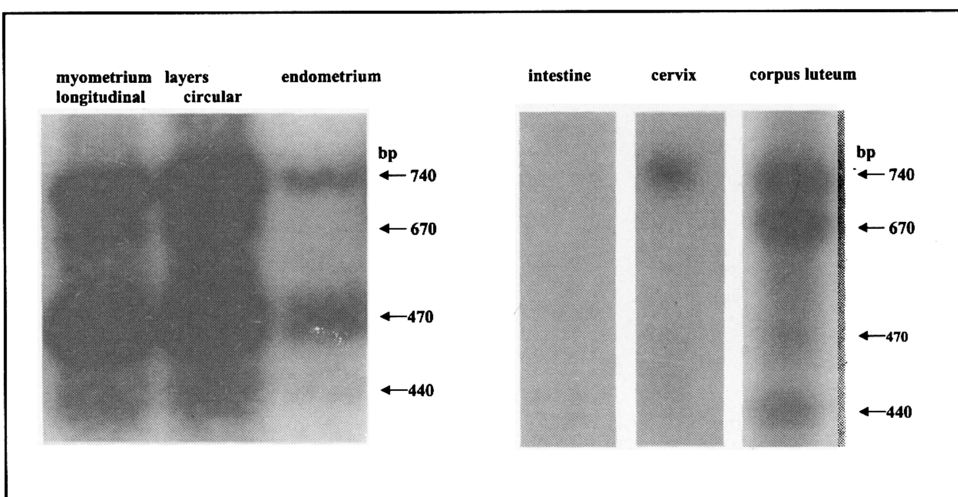

Fig. 5. Southern blot of RT-PCR products of pig LH receptor in the uterus and the cervix. Two µg of total RNA was reverse transcribed and amplified in PCR. Line of 1.5% agarose gel were loaded with 10 µl of RT-PCR products, electrophoresed overnight, transferred on nylon membrane and hybridized with pig LH receptor fragment (nucleotides 900—2081 of pig LH-R cDNA). Subsequently membrane was exposed to Amersham X-ray film 3 days for the cervix and negative control tissue (intestine), 24 h for uterus and 2 h for corpus luteum (positive control.

Immunoperoxidase staining of LH/hCG receptors

The immunostaining of the porcine cervix for the LH/hCG receptors is shown in *Fig. 6*. Immunoreactivity for LH receptors in the cervix was observed in epithelium, but not in smooth muscle cells (*Fig. 6 A* and *C*). The immunostaining was absent when the receptor antibody was omitted or substituted with non-specific IgG during the procedure (*Fig. 6 B* and *D*). Control sections were prestained with hematoxylin and eosin for better slides visibility.

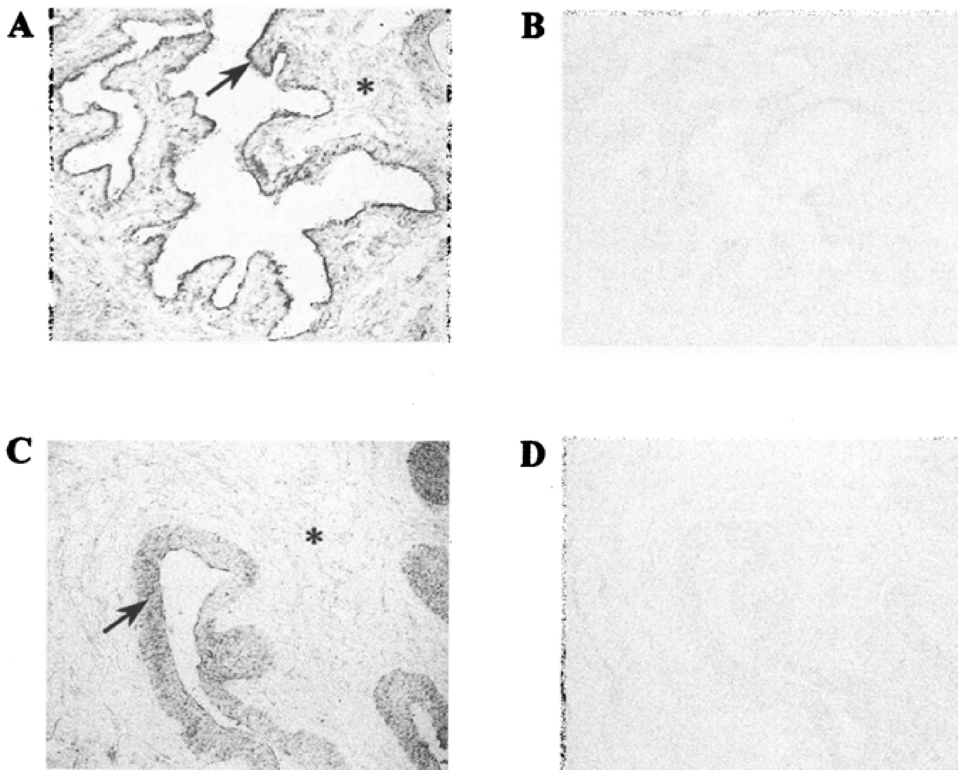

Fig. 6. Immunocytochemistry for LH/hCG receptors in the porcine cervix (A, C). Control in which non-specific IgG was substituted for primary antibody (B, D). Scale bars represent (A, B) × 100 and (C, D) × 250. Arrows = positive reaction in epithelium; asterisk = stroma (smooth muscle fibres embedded in collagen).

In situ hybridization

Figure 7 presents the results of in situ hybridization in the porcine cervix. mRNA for LH receptor was expressed in epithelial cells of the cervix (*Fig. 7 A*). There was no specific hybridization in the mucosa layer. Background, nonspecific hybridization was present in the control section (*Fig. 7 B*) incubated with the sense probe for porcine LH receptor.

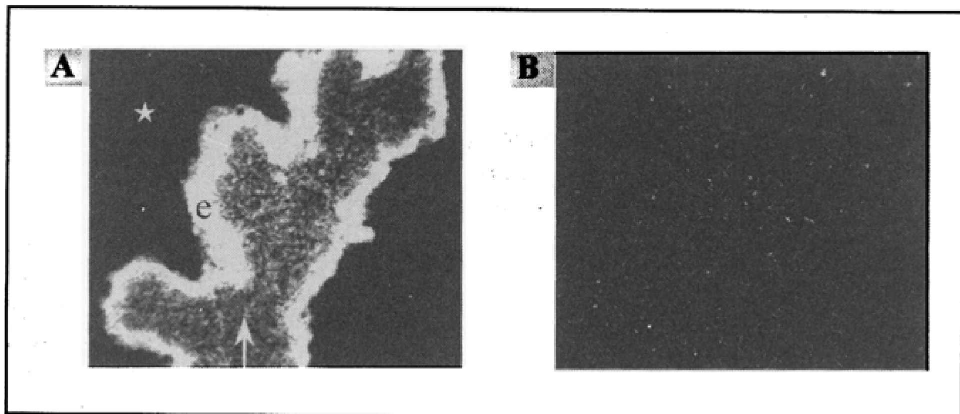

Fig. 7. In situ hybridization with a [^{35}S]cRNA antisense probe spanning 415 bp fragment of LH/hCG receptor reveals positive hybridization in epithelial cell (A). In the control section (B) hybridization with the sense probe for LH receptor was performed. Darkfield photographs are presented. Arrow = lumen of the cervix; asterisk = stroma; e = positive reaction in the epithelium.

DISCUSSION

Until now the porcine extragonadal LH/hCG receptors have been studied mainly in the myometrium and endometrium. The ovariectomy of gils causes the attenuation of myometrial receptors and their maintenance at a very low, subdetectable level (25). It is not clear at this moment whether a small number of LH/hCG binding sites reflects a high amount of occupied receptors or their regression through elevated concentrations of endogenous LH in the blood circulation. Estradiol and progesterone independently stimulate the appearance of unoccupied LH/hCG receptors in the porcine myometrium (25) and oviduct (13). Ziecik *et al.* (14) showed that LH can inhibit the electromyographic activity of the myometrium in estrogen-primed pigs.

Our latest data (3) showed that endometrium is also a target for LH. Using Western blot analysis we were able to detect functional LH/hCG receptors in endometrial cells throughout the estrous cycle with its higher concentrations before and during the time of luteolysis.

The present experiment was conducted to study the existence of LH/hCG receptors in another part of uterus — the cervix. The data of our studies revealed the presence of gonadotropin receptors in the cervix. LH/hCG receptors were found in all preparations of the cervix. There was no difference in the affinities between uterine LH/hCG receptors (cervix and myometrium) and the mid-cycle corpora lutea. Similarly, Ziecik *et al.* (26, 27) and Freidman *et al.* (8) demonstrated no differences in the affinities of porcine uterine, bovine endometrial or ovarian LH/hCG binding sites. However Sawitzke and Odell

(6) reported that in the rat uterus, the LH binding site affinity was 25 times greater than the ovarian binding site.

The specificity of [^{125}I] hCG binding to the cervix tissues was similar to the hormonal specificity of LH/hCG binding to porcine myometrium, endometrium (1) and oviduct (4), endometrium of heifers (8) and corpora lutea of pigs (26), sheep (28) and cows (29).

The cervical LH receptors were more numerous on Days 12—20 of the estrous cycle and dropped to very low levels after ovulation i.e. during the early luteal phase. There were no differences in LH receptor concentration in myometrium at particular days of the estrous cycle. However, Rzucidlo *et al.* (30) observed that number of LH binding sites was higher during mid-luteal phase ofthe estrous cycle (Days 6—7 and 10—12). It is interesting that the same radioreceptor assay allowed to detect LH/hCG receptors in endometrium only from Days 12 to 16 of the estrous cycle (3).

Our studies showed that porcine corpora lutea, myometrium as well as cervix contain main 75-kDa receptor species what agrees with a calculated molecular mass for LH receptor in the pig (31). We suggest, however, that found in our studies 75-kDa band is rather glycosylated 68-kDa form described by Vu Hai-Luu Thi *et al.* (32) in porcine testis. The 48-kDa receptor variant detected in porcine corpus luteum, myometrium and cervix is apparently a form lacking transmembrane or intracellular domain. RT-PCR and Southern blot techniques revealed the existence of two expected fragments of cDNA in corpora lutea and myometrium, but only one in the cervix. We suggest that the differences in various patterns of LH receptor transcripts found in uterine tissues are caused by the tissue-specific alternative splicing of primary transcript. In situ hybrydization and immunohistochemical staining showed the cell-specific expression of LH receptors. Both methods revealed the presence of LH/hCG binding sites in epithelial cells of the cervix. Neither mRNA nor LH receptor protein was observed in mucosal and connective tissue.

Prostaglandins are throught to be one of the key-modulators of biochemical and structural changes in the connective tissue of the cervix (33). PGE_2 has been found to cause softening and ripening of the cervix (33, 34) by increasing collagenolysis in the connective tissue (35). However, since both stimulatory and inhibitory subtypes of PGE_2 receptor are present in the reproductive tract, PGE_2 could cause either contraction or relaxation of the cervical muscle depending on the subtype of the receptor (36). LH was shown to increase prostaglandin production in such reproductive tissues as porcine (3) and bovine endometrium (8), human endometrium (37) and Fallopian tube (38). However, LH action on PGE_2 production in the cervix needs to be examined.

On the other hand the prevulatory LH surge causing ovulation can also, *via* cervical LH receptors, exert a relaxing effect on the cervix. LH is strongly

involved in the control of collagen turnover and the changing activity of the collagenases (39). The cervix is characterized by a thick wall and constricted lumen. The primary function of the cervix is to prevent intruders from entering the uterine lumen. It is tightly closed except during estrus — at which time it relaxes slightly to permit spern to enter the uterus. We hypothesize that the presence of LH/hCG receptors in the cervix facilitate its relaxation during estrus for better sperm penetration and ejaculate deposition in some species.

In conclusion, we found that LH/hCG receptors are present in porcine cervix. The characteristic of hCG binding to cervical receptors and receptor protein are similar to myometrium. The LH receptor RNA is found in the cervix as well as in the myometrium, although it shows different pattern of LH transcripts. The presence of LH/hCG receptors in the cervix is limited to epithelial cells. Physiological significance of LH receptor in the cervix remains unknown and requires further studies.

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