Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Domestic Animal Endocrinology 28 (2005) 74–84
DOMESTIC ANIMAL ENDOCRINOLOGY
www.journals.elsevierhealth.com/periodicals/dae

Progesterone receptors: their localization, binding activity and expression in the pig oviduct during follicular and luteal phases

L.E. Peralta [a], M. Roldán Olarte [a], M. Argañaraz [a], D. Ciocca [b], D.C. Miceli [a,*]

[a] *Faculty of Biochemistry, Chemistry and Pharmacy, Biology Institute, National University of Tucumán, National Research Council (CONICET), Química y Farmacia, UNT. Chacabuco 461, 4000 Tucumán, Argentina*
[b] *Laboratory of Reproduction and Lactation (LARLAC), Regional Center for Scientific and Technological Research (CRICYT), Mendoza, Argentina*

Received 2 December 2003; accepted 27 May 2004

Abstract

Estrogens (E) and progesterone (P) are known to require their respective steroid receptors in order to exert structural and functional effects on the oviduct. Cyclic changes in progesterone receptor (PR) localization in the oviductal tissue of female pigs were determined using an immunohistochemical technique with mouse monoclonal antibody mPRI against PR. The variations observed during the estrous cycle in the progesterone receptor (PR) intensity and proportion between ampulla and isthmus probably reflect different response of these regions to progesterone. Immediately before ovulation, during follicular phase, no staining was observed in either the ampulla or the isthmus stroma. However, a low expression of PR in the epithelium of the ampulla was observed. After ovulation, during luteal phase, PR immunostaining was more intense in the whole oviduct. According to immunohistochemical assays, the binding assays for nuclear and cytosolic PR (PRn and PRc, respectively), by using [^{3}H] R5020 at 4 °C for 15 h, also showed a higher specific binding during luteal phase. However, the PR mRNA in the oviduct, analyzed by RT-PCR, showed similar levels at both stages of the estrous cycle. Although this methods could not be quantitative, indicate the possibility that a post-transcriptional control could differentially regulate the PR in the pig oviduct.

Keywords: Pig; Oviduct; Progesterone receptor

* Corresponding author. Tel.: +54 381 4247752x389; fax: +54 381 4248025.
E-mail address: micelid@unt.edu.ar (D.C. Miceli).

0739-7240/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.domaniend.2004.05.008

1. Introduction

The porcine oviduct provides an important microenvironment for last steps of gametes capacitation as well as for fertilization and early development of the embryo. It has been shown that oviductal tissue culture synthesize and secrete de novo several proteins to the culture medium [1,2]. The relative importance of these proteins in fertilization and early pregnancy is not known, nor have many of them been identified. The infundibullum and ampulla, regardless of the day of the estrous cycle, have a biosynthetic activity significantly greater (2–3 times) than that of the isthmus [3]. When 17-β estradiol (E_2) concentration is at its highest level, the biosynthetic activity in the oviduct is also the highest one [4]. At present, it is known that sexual steroid hormones, E_2 and progesterone (P), differentially up- or down-regulate gene expression in the different regions of the oviduct [5]. The multiple effects of these hormones are mediated by binding to specific intracellular receptors and it is very well known the biochemical and structural properties of progesterone receptor (PR) in the chicken oviduct [6–11]. Although it seems likely that the oviductal function of all mammals is partially regulated by E_2 and P, only a few reports exist concerning the presence of E and P receptors in pig oviduct. "In vitro" binding and exchange methods have been used for investigating PR concentration at different stages of the estrous cycle indicating a relationship between the change in the levels of E_2 and P oviductal binding during early proestrus [12]. With the availability of specific monoclonal antibodies to PR [13], the "in situ" detection of the tissue location and content of receptors in individual cells has become feasible. In the present study, besides the binding activity of nuclear PR (PRn) and cytosolic PR (PRc), we investigated the cellular distribution of pig progesterone receptors in the oviduct immediately before ovulation, in the follicular phases, and post ovulation, during middle of the luteal phase, by using immunohistochemical techniques with PR monoclonal specific antibodies. However, this methods just give a gross estimation about PR concentration differences. The PR gene expression studied by mean of RNA isolation and reverse transcription (RT-PCR) assay, were used for provides us a relative concentrations of PR mRNA in different region of the oviduct [14].

2. Materials and methods

2.1. Reproductive tracts and blood collection

From March to December intact reproductive tracts from 1-year-old (15 ± 3 months) *Landrace* × *Yorkshire* × *Duroc Yersey* sows weighing between 85 and 110 kg were collected from a slaughterhouse in San Miguel de Tucumán (Argentina). Blood samples collected by cardiac puncture from recently sacrificed animals in 15 ml tubes and carried out to the laboratory at ambient temperature. After clotting, the samples were centrifuged at 3000 rpm for 10 min and serum was separated. Serum aliquots were used to measure E_2 and P by RIA techniques. Oviduct pieces were processed immediately when samples were going to be use for immunohistochemistry and others were transported to the laboratory in an ice bath for processing within the 3 h after collection. Oviducts were isolated from ovaries and uteri and their enveloping connective tissues were removed. The classification into two phases

of the estrous cycle, immediately before ovulation and after ovulation, during middle of the luteal phase, was done by direct observation of dominant follicles in the ovaries. The oviducts corresponding to ovaries with mature follicles (9.51 ± 1.01 mm) were selected as oviduct in follicular phase and those corresponding to ovaries with pink-yellow bodies, 10.06 ± 1.5 mm diameter, were classifies as oviducts in middle of the luteal phase. In all cases, samples with similar E_2 and P serum concentrations were selected for the different assays. Follicular phase: $E_2 = 32.5 \pm 2.5$ pg/ml; $P = 3.6 \pm 0.5$ ng/ml. Middle of the luteal phase: $E_2 = 3.8 \pm .0.4$ pg/ml; $P = 75 \pm 7$ ng/ml.

2.2. Sampling and processing for immunohistochemistry

Sections were taken from the mid-isthmus and the mid-ampulla of 8 left oviducts (4 corresponding at follicular and 4 at luteal phases) and fixed under appropriate conditions in the slaughterhouse immediately after the animals had been slaughtered. All the samples were fixed in 10% buffered formalin at room temperature for 24–48 h, dehydrated and imbibed in paraffin. 5 μm-thick deparaffinized tissue sections were stained with hematoxylin and eosin. Immunohistochemistry was performed on serial tissue sections mounted onto 3-aminopropyltriethoxy-silane (Sigma Chemical Co., St. Louis, MO, USA) coated slides as described in a previous work [15]. After dewaxing, endogenous peroxidase was blocked with 2% sodium azide in phosphate buffered saline (PBS) buffer containing 15% H_2O_2 for 30 min at room temperature. Then, an antigen retrieval protocol based on microwave oven heating was applied to the tissue sections to be immunostained for PR [16]. Sections were treated with mouse monoclonal antibody mPRI against PR (Transbio, SARL, France) [17] at 1:100 dilution, for 16 h at 4 °C and then washed with PBS. In staining control slides (absence of immunostaining), the tissue sections were incubated with blocking buffer (1% bovine serum albumin (BSA)) instead of the primary antibody. Rabbit anti-mouse IgG (whole molecule), biotin conjugated, was used as a second antibody (1:50), for 3 h at room temperature, followed by a peroxidase-labeled extravidin biotin detection system (LAB, 1:75) (Dako). 2 mg diaminobenzidine/ml 0.001% H_2O_2 was used as a chromogenic substrate. After immunostaining, the slides were stained with 0.5% methyl green to visualize nuclei. Specific staining was defined as staining that was present with the monoclonal antibody and absent in the negative control section. The immunohistochemical staining was evaluated semi-quantitatively using a double grading system following previous criteria based on the intensity and proportion of stained cells [18,19]. Oviductal ephitelial and stroma cells were assessed in ampulla and isthmus. Staining intensity was graded on a scale of 0 (no staining), 1 (weak), 2 (moderate) and 3 (strong). Proportion scores were expressed as percentage of reactive cells with respect to total amount of counted cells: 0 (0% positive cells), 1 (1–10%), 2 (11–33%), 3 (34–65%) and 4 (66–100%).

2.3. Cytosolic and nuclear fractions

All procedures for cytosolic and nuclear fractions were carried out at 0–4 °C unless otherwise noted. Excised tissues (4 oviducts corresponding to follicular and 4 to luteal phases) were weighed and homogenized in 1:4 (w/v) of TEDG buffer (50 mM Tris, 1.5 mM EDTA, 0.5 mM DTT and 10% glycerol; pH 7.4) with an Ultra-Turrax T-25 tissue homogenizer

at high speed. Four homogenization periods with 10 s intervals between them were done. The final homogenates were centrifuged at 1000 × *g* for 10 min. The pellet was separated to obtain the nuclear fraction while the cytosolic fraction (*) was obtained from the supernatant.

2.3.1. Nuclear fraction

First, the pellet was rinsed in ice-cold TEDG buffer by centrifuging at 1000 × *g* for 10 min. Then it was suspended in 1/4 (w/v) TMD-T buffer (10 mM Tris–HCl, 1.5 mM $MgCl_2$, DTT 1 mM, 0.25% Triton X-100; pH 7.4) and centrifuged again at 1000 × *g* for 10 min. This pellet was washed twice in TMD buffer, centrifuged at 1000 × *g* for 10 min and then suspended with TED-KCl buffer (50 mM Tris, 1.5 mM EDTA, 0.5 mM DTT and 0.7 M KCl) for 30 min for nucleus brooking. The nuclear suspension was centrifuged in a Sorvall OTD-50 B, T865 rotor (40,000 × *g* for 1 h) to obtain a nuclear soluble fraction in the supernatant.

2.3.2. Cytosolic fraction

The supernatant (*) was centrifuged at 40,000 × *g* for 1 h at 0–4 °C in an ultracentrifuge Sorvall OTD-50 B. The resulting supernatant was referred as cytosolic fraction. One aliquot of each sample was used to determinate protein concentration by Lowry's method.

2.4. Receptor binding assays

17α-Me-[^{3}H] R5020 (86.2 Ci/mmol) and non-radioactive R5020, were obtained from New England Nuclear (NEN, Boston, MA), T70 dextran from Pharmacia Fine Chemicals, Piscataway, NJ, and all the other chemicals from Sigma Chemical Co., St. Louis, MO. Cytosolic and nuclear PR binding and exchange assays were performed as follows: triplicate aliquots of the cytosolic or nuclear soluble fractions (100 μl) were incubated with 100 μl [^{3}H] R5020 (4 nM) and 800 μl TED-Glic-Mo buffer (50 mM Tris pH 7.4, 1.5 mM EDTA (Na''), 0.5 mM DTT, 20 mM MoO_4Na_2 and 10% glycerol). The specificity of the binding was examined by incubating samples with [^{3}H] R5020 alone and in the presence of the non-radioactive steroid (100×). Following the incubation period (15 h at 4 °C), the samples were treated with 100 μl of Dextran-coated charcoal (DCC) for 10 min and centrifuged to remove unbound ligands. The complexes [^{3}H] R5020 – receptor was measured in the supernatants and the radioactivity was referred to an standard of known radioactive hormone concentration and expressed as pmol of receptor per mg of proteins. Hormone concentration was selected according to pig PR reported by Stanchev.

2.5. RNA isolation and reverse transcription (RT)-PCR assays

Three oviducts in each selected sexual cycle phase were dissected in isthmic and ampullary region and then, epithelial and stromal tissues were separated by scrapping of the oviduct luminal face. The whole process was carried out in ice bath to avoid nucleic acids degradation. Total RNA was isolated according to Chomczynski et al. (1987) and its concentration was measured by absorbance at 260 nm. All the samples were subjected to reverse transcription (RT) and subsequently to cDNA amplification by PCR at the same time to avoid

any potential experimental variation. Total RNA (2 μg) plus 0.5 μg oligo dT primer were incubated at 70 °C for 5 min and then set on ice. Then the mixture was reverse transcribed in a 25 μl reaction buffer (50 mM Tris–HCl, pH 8.3 at 25 °C; 75 mM KCl; 3 mM $MgCl_2$; 0.5 mM of each dNTP; 10 mM DTT; 25 U of rRNasin Ribonuclease Inhibitor; 200 U of Moloney Murine Leukemia Virus Reverse Transcriptase, M-MLV RT) and incubated at 42 °C for 90 min. The heat inactivation of M-MLV reverse transcriptase was carried out at 94 °C for 2 min. For PCR, the primers were designed by means of *primer3* software from the Gene Bank AJ245450 PR sequence and were upstream (5-AACACCAAACCCGACACTTC-3) and downstream (5-CCAAAGGCCGCAAGTTCTAC-3). The β-actin protein (Gene Bank AF054837) primers were upstream (5′-CGTGGGCCGCCCTAGGCACCA-3′) and downstream (5′-TTGGCCTTAGGGTTCAGGGGGG-3′). For each primer set the optimal cycle number was previously determined, and adjusted to work in the exponential phase of the PCR assays. Aliquots of RT reactions (1.5 μl) were added to the final PCR reaction mixture in a final volume of 25 μl containing 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 9.0 at 25 °C), 0.1% Triton X-100, 1 mM of PCR primers, 200 mM of each dNTP, and 2 U *Taq* DNA polymerase (Promega). After a first cycle of denaturation at 94 °C for 1 min, reactions were carried out for 30 cycles at 94 °C for 30 s, at 50 °C for 90 s and at 72 °C for 40 s, with a final extension period at 72 °C for 5 min, using a DNA thermo cycler (GeneAmp® PCR System 2400, Perkin-Elmer). Reaction products were resolved on 2% agarose gels and visualized by ethidium bromide staining. The images were captured under UV transillumination and the intensity of the bands was quantified using Molecular Analyst® Software (*Bio-Rad*). Signal intensities of the PR products were normalized with those of β-actin products to obtain arbitrary units of relative abundance.

2.6. Statistical analysis

Statistical analysis of binding PR activity and mRNA relative concentration was performed using the Sigma Plot 2001. Differences were interpreted as significant for $P < 0.05$.

3. Results

3.1. PR distribution among cells of different region of the pig oviduct

The immunohistochemical analysis with specific PR monoclonal antibodies allowed us to visualize progesterone target cells in pig oviducts by mean of the detection of their receptors. PR staining was mainly localized in the nuclei of the epithelial cells. The staining intensity for PR varied in the epithelium along the length of the tube according to the estrous cycle. During follicular phase the ampulla showed moderate staining while the isthmus showed none. Oviducts in luteal phase showed high intensity in both ampulla and isthmus epithelial cells. Stroma cells of both regions of the oviduct showed no PR during follicular phase but they showed a high PR increase after ovulation take place, in the middle of the luteal phase. (Figs. 1 and 2).

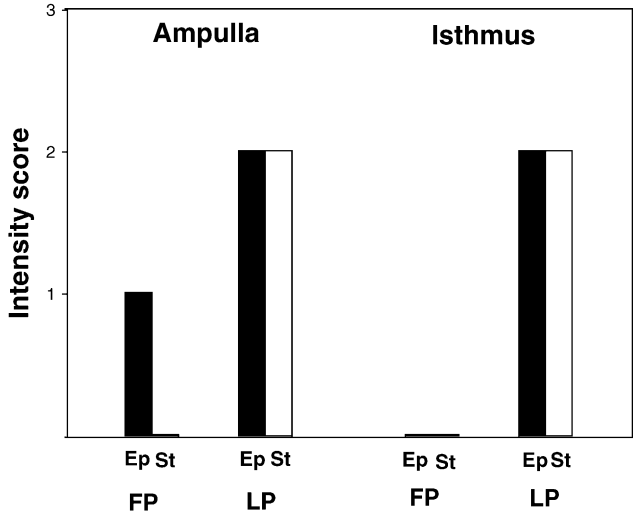

Fig. 1. Intensity score of specific immunohistochemical localization of PR in the pig oviduc. Ep: epithelial cells; St: stromal cells; FP: follicular phase; LP: luteal phase. The intensity of the staining was expressed semi-quantitatively in arbitrary units according to Ciocca et al. [32] as follows: 0 (negative), 1 (weak), 2 (moderate), 3 (strong).

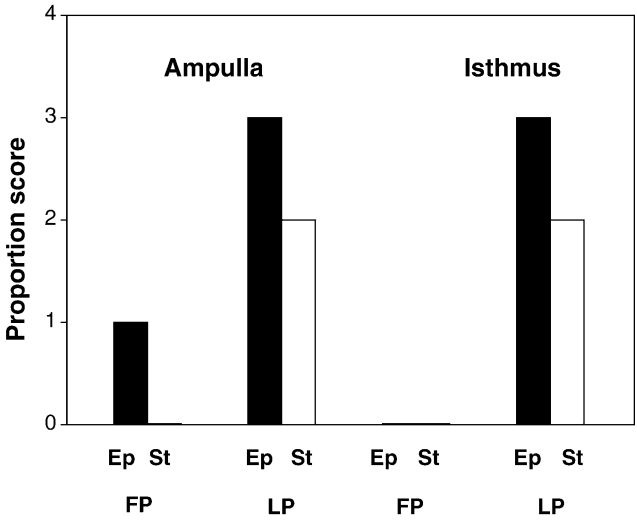

Fig. 2. Proportion score of specific immunohistochemical localization of PR in the pig oviduct. Ep: epithelial cells; St: stromal cells; FP: follicular phase; LP: luteal phase. The proportion is expressed as percentage of reactive cells with respect to total amount of counted cells [32] as follows: 0 (0% of positive cells), 1 (10%), 2 (11–33%), 3 (34–65%) and 4 (66–100%).

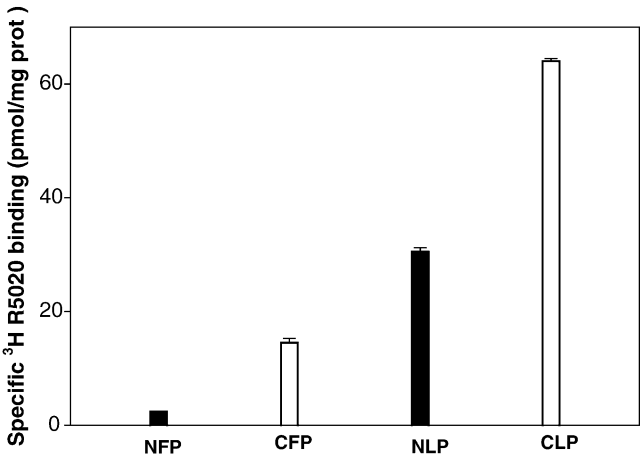

Fig. 3. [^{3}H] R5020 specific binding in the nuclear and cytosolic fractions from ampulla in pig oviduct. Nuclear fraction in follicular phase (NFP), cytosolic fraction in follicular phase (CFP), nuclear fraction in luteal phase (NLP) and cytosolic fraction in luteal phase (CLP) were assayed for [^{3}H] R5020 (4 nM) binding activity as described in Section 2.

3.2. Binding of progesterone to specific sites in cytosolic and nuclear fractions

In the nuclear and cytosolic fractions of the ampulla, specific [^{3}H] R5020 binding was remarkably lower during follicular than during luteal phase. Specific binding in the nuclear fraction was lower than in the cytosolic fraction in both periods, the main binding occurring in the cytosolic fraction during luteal phase (Fig. 3).

3.3. Expression of oviductal PR mRNA

Total RNA obtained as described above was analyzed for steady-state PR mRNA levels using RT-PCR assays. As an internal control, constitutive expressed β-actin mRNA was amplified in each sample. PR primers were specific for PR sequence of pig oviduct transcripts (Gene Bank AJ245450), a partial sequence which included the region that corresponds to a ligand-binding domain of the human nuclear hormone receptor. The primers of the β-actin correspond to a conserved domain that amplified *Sus scrofa* β-actin cDNA.

Samples were obtained before ovulation, in the follicular phase and in the middle of the luteal phase, with the object of studying the possible differential expression of the PR gene under E_2 and P predominance, respectively. Semiquantitative RT-PCR analyses revealed that PR mRNA was expressed at comparable high levels in the ampullary and isthmic epithelial and stromal cells of the pig oviduct. Histograms (Fig. 4) show that the relative expression of PR transcript levels is constant in the two kinds of tissue of the oviduct under the influence of either E_2 or P.

The expected size of amplification for PR and β-actin were 268 and 243 bp, respectively (Fig. 5). The primer pair of the β-actin spans an intron, which helps distinguish mRNA reverse-transcribed to cDNA from contaminating genomic DNA, which would generate a second PCR product larger than the one expected

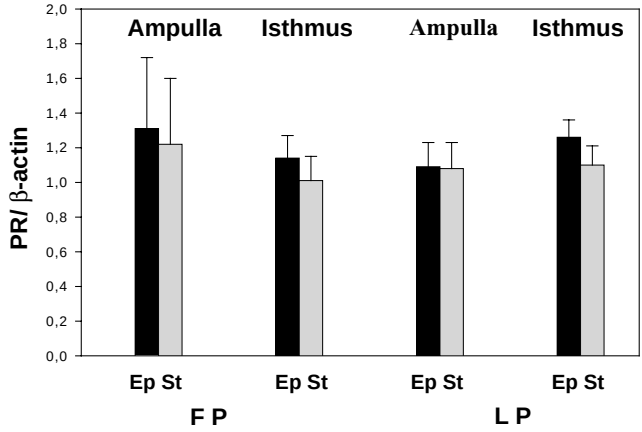

Fig. 4. Relative PR transcription levels in ampullary and isthmic epithelium and stroma. Ep: epithelium; St: stroma in follicular phase (FP) and luteal phase (LP). Histograms are the mean relative abundance of PR mRNA values in arbitrary units ± S.D. of three separate experiments.

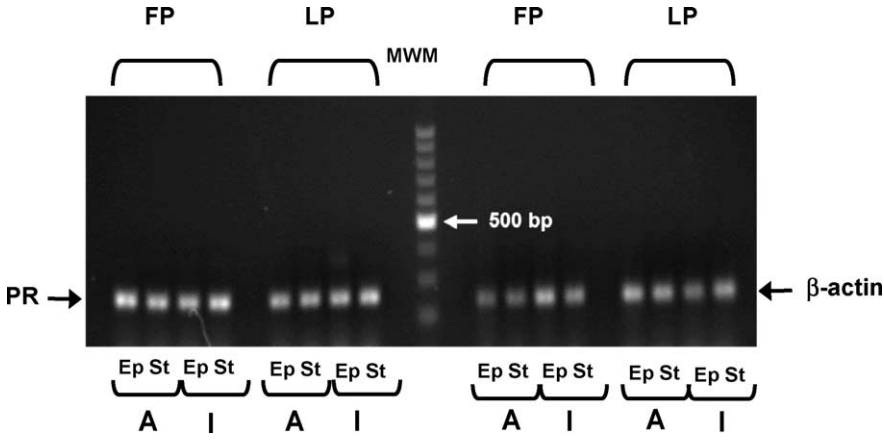

Fig. 5. Pattern of expression of PR by semi-quantitative RT-PCR in pig oviduct. A: ampulla; I: isthmus; Ep: epithelium; St: stroma; MWM: molecular weight marker; FP: follicular phase; LP: luteal phase. Total RNA was isolated and reverse-transcribed. Each RT reaction was PCR amplified with PR and β-actin primers. An ethidium bromide-stained agarose gel of RT-PCR products from one set of samples is presented. PR expression is shown as single bands of expected size, PR (268 bp) and β-actin (242 bp)

4. Discussion

The major physiological modulators of PR concentration are the ovarian hormones E_2 and P, which bind to their receptors in order to exert their effect. Although the total role of these hormones in the reproductive tract remains unclear [20], it has become increasingly evident that steroid hormones regulate the concentration not only of their own receptors

[21,22] but also of those of other hormones [23]. For example, E_2 up-regulates estrogen receptors (ER) in ewe endometrium but down-regulates them in liver [24]. E_2 can either induce a significant increase in PR gene expression in the uteri of many mammalian species [25,26] or decrease PR expression in lamb uterus [27]. The positive regulatory action of E_2 on PR has been observed in many other tissues and cell lines except in the ovary, where PR regulation is primarily steroid independent [27]. Probably, P could control PR levels either by a modulation of E_2 action [28], by an increase in PR expression [5], or by down-regulation in most tissues which have two PR isoforms [8]. The presence of PR in the pig oviduct has been reported previously by Stanchev et al. [12], who found that the Kd for R5020 lay in the range 2.4×10^{-9} to 3.4×10^{-9} for both oviductal nuclear and cytosolic PR (PRn and PRc, respectively); these data being similar to those reported for human oviducts [29].

In all samples whatever the stage of the sexual cycle in which they were obtained, we found higher PR activity in the cytosolic than in the nuclear fraction. In both, specific-binding activities showed important differences during the estrous cycle. It was observed that PRc and PRn concentrations were low during the follicular phase and high in the luteal phase. These results were confirmed through immunohistochemical techniques. Coincidentally, when the staining intensity and proportion of positive cells detected by immunohistochemistry were maximum in luteal phase, the binding of tritiated hormone to PR was also high. Stanchev et al. [12] also found that, in pig, PRc in the ampulla segment was high during the luteal phase, concurrently with the presence of significant blood plasma levels of P in the same period.

In this study we confirm the presence of specific PR in the pig oviduct and demonstrate the existence of a direct relation between the variations in PR concentrations and the differences in PR binding activity in oviductal ampulla and isthmus during follicular and luteal phases. However, when a semiquantitative relative RT-PCR technique was used to examine PR gene expression, we found that PR seems to be expressed in oviductal pig cells during follicular and luteal stages of the sexual cycle with no significant changes. No statistical differences between relative PR transcript levels in epithelium and stroma were found. Taking into account the results presented in this work, it seems reasonable to suggest the possible presence of a post-transcriptional control that differentially regulates the synthesis of the PR. At present it is known that PR synthesis regulation seems to be more complex than previously suspected [30] because in some reproductive tissues PR appeared to be constitutive [31]. It has been reported that PR protein is a target for ubiquitylation and that the abundance of ubiquitylated PR increases by P treatment [9]. The degradation of PR by the ubiquitin-proteasome system would constitute a post-transcriptional regulation of PR in the different stage of the sexual cycle.

Acknowledgements

ANPCYT and CIUNT (Research Council of the National University of Tucumán) funded this research. The authors thank Frigorífico Industrial del Norte S.A. for allowing reproductive tracts collection from their slaughtered sows.

References

[1] Buhi WC, Alvarez IM, Sudhipong V, Dones-Smith MM. Identification and characterization of de novo-synthesized porcine oviductal secretory proteins. Biol Reprod 1990;43(6):929–38.
[2] Buhi WC, O'Brien B, Alvarez IM, Erdos G, Dubois D. Immunogold localization of porcine oviductal secretory proteins within the zona pellucida, perivitelline space, and plasma membrane of oviductal and uterine oocytes and early embryos. Biol Reprod 1993;48(6):1274–83.
[3] Buhi WC, Alvarez IM, Kouba AJ. Oviductal regulation of fertilization and early embryonic development. J Reprod Fertil Suppl 1997;52(1):285–300.
[4] Buhi WC, Vallet JL, Bazer FW. De novo synthesis and release of polypeptides from cyclic and early pregnant porcine oviductal tissue in explant culture. J Exp Zool 1989;252(1):79–88.
[5] Meikle A, Garofalo EG, Rodriguez-Pinon M, Tasende C, Sahlin L. Regulation by gonadal steroids of estrogen and progesterone receptors along the reproductive tract in female lambs. Acta Vet Scand 2001;42(1):161–9.
[6] Gasc JM, Renoir JM, Radanyi C, Joab I, Tuohimaa P, Baulieu EE. Progesterone receptor in the chick oviduct: an immunohistochemical study with antibodies to distinct receptor components. J Cell Biol 1984;99(4 Pt 1):1193–201.
[7] Tuohimaa P, Joensuu T, Isola J, Keinanen R, Kunnas T, Niemela A, et al. Development of progestin-specific response in the chicken oviduct. Int J Dev Biol 1989;33(1):125–34.
[8] Bouchard P. Progesterone and the progesterone receptor. J Reprod Med 1999;44(2 Suppl):153–7.
[9] Syvala H, Vienonen A, Zhuang YH, Kivineva M, Ylikomi T, Tuohimaa P. Evidence for enhanced ubiquitin-mediated proteolysis of the chicken progesterone receptor by progesterone. Life Sci 1998;63(17):1505–12.
[10] Gonzalez-Moran G, Camacho-Arroyo I. Histomorphometric and progesterone receptor immunohistochemical analysis in the oviduct of newly hatched chicks treated with follicle-stimulating hormone during embryonic development. Anat Histol Embryol 1998;27(4):277–82.
[11] Isola JJ. Distribution of estrogen and progesterone receptors and steroid-regulated gene products in the chick oviduct. Mol Cell Endocrinol 1990;69(2–3):235–43.
[12] Stanchev P, Rodriguez-Martinez H, Edqvist LE, Eriksson H. Oestradiol and progesterone receptors in the pig oviduct during the oestrous cycle. J Steroid Biochem 1985;22(1):115–20.
[13] Greene GL, Press MF. Immunochemical evaluation of estrogen receptor and progesterone receptor in breast cancer. New York: Plenum; 1987.
[14] Ochman H, Gerber AS, Hartl DL. Genetic applications of an inverse polymerase chain reaction. Genetics 1998;120:621–3.
[15] Fanelli MA, Vargas-Roig LM, Gago FE, Tello O, Lucero De Angelis R, Ciocca DR. Estrogen receptors, progesterone receptors, and cell proliferation in human breast cancer. Breast Cancer Res Treat 1996;37(3):217–28.
[16] Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J Histochem Cytochem 1991;39(6):741–8.
[17] Perrot-Applanat M, Groyer-Picard MT, Lorenzo F, Jolivet A, Vu Hai MT, Pallud C, et al. Immunocytochemical study with monoclonal antibodies to progesterone receptor in human breast tumors. Cancer Res 1987;47(10):2652–61.
[18] Wagner RK, Jungblut PW. Quality control in steroid hormone receptor analyses. Cancer 1980;46(12 Suppl):2950–2.
[19] Elledge RM, Ciocca DR, Langone G, McGuire WL. Estrogen receptor, progesterone receptor, and HER-2/neu protein in breast cancers from pregnant patients. Cancer 1993;71(8):2499–506.
[20] Heikinheimo O, Mahony MC, Gordon K, Hiu JG, Hodgen GD, Gibbons WE. Estrogen and progesterone receptor mRNA are expressed in distinct pattern in male primate reproductive organs. J Assist Reprod Genet 1995;12(3):198–204.
[21] Vermeirsch H, Simoens P, Hellemans A, Coryn M, Lauwers H. Immunohistochemical detection of progesterone receptors in the canine uterus and their relation to sex steroid hormone levels. Theriogenology 2000;53(3):773–88.
[22] Scott RE, Wu-Peng X, Pfaff DW. Regulation and expression of progesterone receptor mRNA isoforms A and B in the male and female rat hypothalamus an pituitary following oestrogen treatmen. J Neuroendocrinol 2002;14(3):175–83.

[23] Meikle A, Sahlin L, Ferraris A, Masironi B, Blanc JE, Rodriguez-Irazoqui M, et al. Endometrial mRNA expression of oestrogen receptor alpha, progesterone receptor and insulin-like growth factor-I (IGF-I) throughout the bovine oestrous cycle. Anim Reprod Sci 2001;68(1–2):45–56.
[24] Zou K, Ing NH. Oestradiol up-regulates oestrogen receptor, cyclophilin, and glyceraldehyde phosphate dehydrogenase mRNA concentrations in endometrium, but down-regulates them in liver. J Steroid Biochem Mol Biol 1998;64(5–6):231–7.
[25] Spencer TE, Bazer FW. Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biol Reprod 1995;53(6):1527–43.
[26] Ing NH, Tornesi MB. Estradiol up-regulates estrogen receptor and progesterone receptor gene expression in specific ovine uterine cells. Biol Reprod 1997;56(5):1205–15.
[27] Meikle A, Forsberg M, Sahlin L, Masironi B, Tasende C, Rodriguez-Pinon M, et al. A biphasic action of estradiol on estrogen and progesterone receptor expression in the lamb uterus. Reprod Nutr Dev 2000;40(3):283–93.
[28] Kraus WL, Katzenellenbogen BS. Regulation of progesterone receptor gene expression and growth in the rat uterus: modulation of estrogen actions by progesterone and sex steroid hormone antagonists. Endocrinology 1993;132(6):2371–9.
[29] Punnonen R, Lukola A. Binding of estrogen and progestin in the human fallopian tube. Fertil Steril 1981;36(5):610–4.
[30] Graham JD, Roman SD, McGowant E, Sutherland RL, Clarke CL. Preferential stimulation of human progesterone receptor B expression by estrogen in T-47D human breast cancer cells. J Biol Chem 1995;270(51):30693–700.
[31] Kurita T, Lee KJ, Cooke PS, Lydon JP, Cunha GR. Paracrine regulation of epithelial progesterone receptor and lactoferrin by progesterone in the mouse uterus. Biol Reprod 2000;62(4):831–8.
[32] Ciocca DR, Vargas-Roig LM, Fanelli MA. Estrogen receptor in the human female genital tract in normal and pathological conditions. Biocell 1994;18(3):1–21.