EXPRESSION OF PROGESTERONE RECEPTOR RELATED TO POLYMORPHISMS IN THE PROGESTERONE RECEPTOR GENE

Peiró R.[1]*, Santacreu M.A.[1], Argente M.J.[2], García M.L.[2], Muelas R.[2], Merchán M.[3], Folch J.M.[3], Herrler A.[4], Blasco A.[1]

[1]Institute for Animal Science and Technology, Polytechnic University of Valencia, P.O. Box 22012, 46071 Valencia, Spain
[2]Departamento de Tecnología Agroalimentaria, Universidad Miguel Hernández de Elche, 03312 Orihuela, Spain
[3]Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[4]Department of Anatomy and Reproductive Biology, Medical School, RWTH University of Aachen, Wendlingweg 2, 52057 Aachen, Germany
*Corresponding author: ropeibar@dca.upv.es

ABSTRACT

The effect of the 2464G>A SNP found in the promoter region of the progesterone receptor gene on progesterone receptor (PR) expression was evaluated by Western blot analysis. This SNP was associated to two lines divergently selected for uterine capacity. The GG genotype was the genotype more frequent in the line selected to increase uterine capacity. Two isoforms were obtained: the PR-B, previously described in rabbits, and the PR-A isoform, not described in rabbits before. The GG genotype showed lower PR-B and PR-A expression than the AA genotype in the oviduct. Higher both PR expressions were obtained at day 3 of gestation in the oviduct with respect to day 2. Similar PR-A expression was observed in the ampulla and isthmus, meanwhile a higher PR-B expression was found in the isthmus.

Key words: PR-B and PR-A expression, *PGR* genotype, Oviduct, Uterus.

INTRODUCTION

Most physiological effects of progesterone take place through its interaction with the specific progesterone receptor (PR). This receptor belongs to the superfamily of intracellular receptors mediating the nuclear effects of steroid hormones. In humans, mice and pigs, nuclear PR consist of two distinct isoforms, PR-A and PR-B, derived from a single gene independently regulated by separated promoters (Kastner *et al.,* 1990; Schott *et al.,* 1991; Conneely *et al.,* 2001), but up to now, only one isoform (PR-B) has been described in rabbits (Loosfelt *et al.,* 1984). Different PR-B expression has been observed at the first days of gestation in the rabbit oviduct (Gutierrez-Sagal *et al.,* 1993; Anzaldúa *et al.,* 2007).

A diallelic 2464G>A SNP has been located in the promoter region of rabbit progesterone receptor (*PGR*) gene using the GenBank accession no. X06623. This SNP explains part of the difference in early embryo survival and development at day 3 of gestation between two lines divergently selected by uterine capacity, while no effect was observed in these traits at day 2 of gestation (Peiró *et al.,* 2007). The aim of this work is to determine the PR expression among *PGR* genotypes in the rabbit oviduct in early gestation.

MATERIALS AND METHODS

Animals and experimental design

A total of 49 pregnant intact F_2 does were slaughtered at day 2 or 3 of gestation. From 7 to 9 does were analyzed per genotype and day of gestation. The F_2 population came from the reciprocal cross of

animals selected divergently for uterine capacity. Does were sacrificed by intravenous injection of sodium thiopental in a dose of 50 mg/kg body weight (Tiobarbital, B. Braun Medical S. A., Barcelona, Spain) at least one month after their fourth parity. After the slaughter, the total reproductive tract was removed.

The oviduct was flushed with 5 ml of 150 mM ammonium bicarbonate buffer and then it was split into ampulla and isthmus. The samples were submerged in 1.5 ml0.15 M ammonium bicarbonate buffer and snap-frozen in liquid nitrogen. Samples were stored at -20ºC prior analyses.

Protein content was determined by DC Protein Assay (Bio-Rad, Munich, Germany). One hundred and five µg of protein was used in the oviductal samples, diluted in loading buffer [5% (v/v) β-mercaptoethanol; 30 mM Tris, pH 6.8; 1.5 urea; 7.5% (v/v) glycerol; 0.5% (w/v) sodium dodecyl sulphate (SDS); 0.05% (w/v) Bromophenol Blue]. Samples were heated to 100º for 3 min and subjected to 1D SDS-PAGE using 5% stacking gel and 7.5% resolving gel under reducing conditions. For size determination, a prestained molecular weight marker (PageRuler, Fermentas, Munich, Germany) was used. After electrophoretic separation, the proteins were transferred to a polyvinylidene diflouride membrane (Millipore, Schwalbach, Germany) by semi-dry blotting for 30 min at 100 mA. Following transfer, the membrane was blocked for 1 h with 5% (w/v) milk powder in TBS/Tween-20 (0.1% w/v) at room temperature. A primary antibody against mouse progesterone receptor was used (mouse anti-rabbit progesterone receptor; hPRa-1, Dianova, Hamburg, Germany) at a concentration of 33 pg protein per µL TBS containing 5% milk powder. The blot was incubated at 4º for 12 h. After three washes in TBS/0.1% Tween, the second antibody was added and left at room temperature for 1 h. After three washes, detection of the second antibody (Goat anti-mouse HRP, DAKO, Hamburg, Germany; 1:200) was performed using enhanced chemiluminescence; ECLplus (Amersham, Little Chalfont, UK). The blot was exposed to an X-ray film (X-Omat UV Plus, Kodak New York, USA) for 1 min. The intensities of the immunoreactive bands were converted into digitalized signals using a computerized densitometry system and quantified by a gel documentation program (Image Master 1D, Amersham, Germany). Signal intensities of PR isoforms were normalized to those of ß-actin and positive control (T47D cells) as ratios to produce arbitrary densitometry units (ADU) of relative abundance. The protocol for the detection of ß-actin was as following: monoclonal anti ß-actin (Clone AC15, Abcam Limited, Cambridge, UK: 1:50000 in TBS/0.1% Tween; goat anti-mouse HRP 1:5000; ECL).

Genotyping of the progesterone receptor gene

Blood samples were collected by veinpuncture. Genomic DNA was extracted following the protocol of the ABI PRISM™ 6100 Nucleic Acid PrepSation (Applied Biosystems). A PCR-RFLP was developed for genotyping animals and the designed primers were forward 5´-GAAGCAGGTCATGTCGATTGGAG-3´ and reverse 5´-CGCCTCTGGTGCCAAGTCTC-3´. The PCR-RFLP assay yielded two bands of 416 and 142–bp (genotype GG), a single 558–bp band (genotype AA) and all three bands (genotype GA).

Statistical Analysis

The model used to analyze PR expression in the oviduct sample was:

$$y_{ijkl} = \mu + D_i + G_j + T_k + (D*G)_{ij} + (G*T)_{jk} + (D*T)_{ik} + (D*G*T)_{ijk} + e_{ijkl}$$

where y_{ijkl} is the signal intensity of each PR expression, D_i is the effect of the day of gestation (with 2 levels: day 2 and 3 of gestation), G_j is the effect of *PGR* gene genotype (with three levels: GG, GA and AA) and T_k is the effect of tissue (with 2 levels: ampulla and isthmus).

The analysis was based on Bayesian methods. Data were conditionally distributed as:

$$\mathbf{y} \mid \mathbf{b}, \sigma^2_e \sim N(\mathbf{Xb}, \mathbf{I}\sigma^2_e)$$

where **b** contains the effects described above, **X** is the incidence matrix and **I** is the identity matrix. Bounded flat priors were used for all unknowns. Marginal posterior distributions of all unknowns were estimated by Gibbs Sampling. After some exploratory analyses we used one chain of 100,000 samples discarding the first 20,000 and saving every 10 thereafter. Convergence was tested using the Z criterion of Geweke and Monte Carlo sampling errors were computed using time-series procedures.

RESULTS AND DISCUSSION

The expression of two PR isoforms, PR-B and PR-A, was observed in the oviduct of pregnant rabbit at day 2 and 3 of gestation by Western blotting using a commercial monoclonal antibody. The molecular weight of the PR-B was 110 kDa, similar to the isoform described by Gutierrez-Sagal *et al.* (1993), while the molecular weight of the PR-A was 81 kDa. To our knowledge, this is the first time in which the rabbit PR-A isoform has been described. A similar band was previously found by Gutierrez-Sagal *et al.* (1993) although they described this band as a degradation of the PR-B isoform.

Features of the estimated marginal posterior distributions of the ratio for arbitrary densitometry units for both PR isoforms in the oviduct are presented in Table 1. All Monte Carlo standard error were lower than 0.005 and lack of convergence was not detected by the Geweke test.

Table 1: Features of the estimated marginal posterior distributions of the ratio of the arbitrary densitometry units (ADU) of PR isoforms for several effects in the oviduct

PR-B	Median	$HPD_{95\%}$	P (%)	**PR-A**	Median	$HPD_{95\%}$	P (%)
day2/day3	0.42	0.29 , 0.53	100	day2/day3	0.61	0.45 , 0.80	100
A/I	0.50	0.38 , 0.61	100	A/I	1.05	0.86 , 1.29	70
GG/AA	0.81	0.58 , 1.02	94	GG/AA	0.73	0.51 , 0.98	98

day2, ADU at day 2 of gestation; day3, ADU at day 3 of gestation; A, ADU of ampulla; I, ADU of isthmus; GG, ADU of GG genotype; AA, ADU of AA genotype; $HPD_{95\%}$, highest posterior density region at 95% of probability; P, P(ratio>1) when median >1 or P(ratio<1) when median <1

An increase of both isoforms through the first days of gestation was observed in the oviduct, since the day2/day3 ratios were lower than 1 (Table 1, P=100%). The PR-B expression at day 3 of gestation was 2.4 times the expression at day 2 of gestation and the PR-A expression was 1.6 times.

In the oviduct, the PR-B expression in the isthmus was twice than in the ampulla. Similar result was found by Anzaldúa *et al.* (2007). However, similar PR-A expression was found in both tissues.

Regarding the genotypes, both PR expressions in the AA genotype were higher than in the GG genotype, being the PR-B and the PR-A expression 1.2 and 1.4 times in the AA genotype, respectively. The AA genotype was the genotype more frequent in the line selected to decrease uterine capacity whereas the GG genotype was the genotype more frequent in the line selected to increase uterine capacity. Both genotypes showed similar early embryo survival and development at day 2 of gestation; however, the GG genotype had a higher early embryo survival and development at day 3 of gestation (Peiró *et al.,* 2007). At day 3 of gestation, the genotype with a higher survival had lower PR expression in the oviduct.

CONCLUSIONS

We found two different progesterone receptor isoforms in the oviduct and uterus in the first stages of gestation. An SNP located in the promoter region was associated to PR expression in the oviduct.

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