Peripheral lymphocytes response to progesterone during early pregnancy in pigs

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Resumen

El objetivo de este estudio fue investigar la respuesta proliferativa de linfocitos T periféricos a P₄ en el período peri-implantacional, momento en el que las concentraciones de la hormona son altas. El estudio se llevó a cabo en cerdas preñadas de 10 y 30 días de gestación y en no-preñadas. Se tomaron muestras de sangre con y sin heparina para la obtención de linfocitos y suero respectivamente. Los linfocitos periféricos se cultivaron sin y con P₄, Con-A, PHA-M. La concentración sérica de P₄ se determinó por RIA. No se encontraron diferencias significativas en la respuesta de los linfocitos a Con-A y PHA-M en los diferentes estados reproductivos de las cerdas. La respuesta de los linfocitos a P₄ en cerdas de 10 y 30 días de gestación fue significativamente menor que en cerdas no preñadas (p< 0.01). Por otra parte, hay una alta respuesta de los linfocitos en presencia de P₄ junto con mitógenos sin mostrar variaciones entre los diferentes estados reproductivos. Esto indicaría que la disminución de la respuesta a P₄ observada en cerdas preñadas se revierte. La P₄ se encuentra significativamente elevada (p<0.01) durante el período peri-implantacional. Estas altas concentraciones de P₄ coinciden con la disminución de la respuesta de los linfocitos periféricos a la hormona en ese mismo período. Estos resultados podría indicar el rol inmunomodulador de la P₄ a nivel sistémico durante la implantación en cerdos.

Palabras clave: cerdo | preñez temprana | progresetona | linfocitos periféricos | respuesta proliferativa.

Abstract

The aim of this study was to investigate the proliferative response of peripheral T lymphocytes to P₄ in the peri-implantational period, when serum concentrations of the hormone are high. This study was carried out on pregnant gilts of 10 and 30 days of gestation and non-pregnant gilts. Blood was collected with and without heparine, to obtain lymphocyte and serum respectively. Peripheral lymphocytes were cultivated without and with P₄, Con-A, PHA-M. The serum concentration of P₄ was determined by RIA. No significant differences were found in the lymphocytes response to Con-A and PHA-M in the different reproductive stages of the gilt. The lymphocytes response to P₄ in gilts of 10 and 30 days of gestation is significantly lower than in non-pregnant gilts (p< 0.01). There is a highly proliferative response of lymphocytes in the presence of P₄ and mitogen though this response shows no variation in the different reproductive stages which indicates that the decreased response to P₄ in the pregnant gilts is reverted. The P₄ is significantly high (p<0.01) during the peri-implantational. These high concentrations of P₄ coincide with the diminished

proliferative response of peripheral lymphocytes to the hormone in the same period. These results might be indicating the immunomodulator role of P₄ at systemic level during implantation swine.

Keywords: pig | early pregnancy | progesterone | peripheral lymphocyte | proliferative response.

Introduction

The successful implantation of allogenic embryo depends on adequate regulation of the immune response at the conceptus-maternal interphase and systemic level.

The Progesterone (P₄) is a crucial hormone for the maintenance of a normal pregnancy in most mammalian species as it regulates the endometrial functions to allow the early embryonic development, the implantation, the placentation and the successful culmination of pregnancy (Tellería et al, 1999; Arck, 2001; Joachim et al, 2003; Blois et al, 2004). One of the functions of this hormone is to act as immunesuppressant directly or indirectly through other factors. It has been shown that in human and murines pregnant female T lymphocytes have nuclear P₄ receptors while non-pregnant females do not (Szekeres-Bartho et al, 2001). In these species, the immunomodulatory effects of P₄ during pregnancy may be, at least in part, related to the induction of a 34 kDa protein known as progesterone induced blocking factor (PIBF) on the lymphocytes. The PIBF alters the balance of Th1/Th2 cytokines, inducing a shift toward the production of Th2 cytokines. These cytokines have a beneficial effect as they lead to decreased cell-mediated response and increase the synthesis of asymmetric antibodies. (Szekeres-Bartho and Wegmann, 1996; Check et al. 1997; Szekeres-Bartho et al. 1997; Kelemen et al. 1998; Szekeres-Bartho et al. 2001; Blois et al. 2004). In swine, little is known about the immunomodulation of P₄, either at local or systemic level. For this reason, the aim of this study was to investigate the proliferative response of peripheral T lymphocytes to P₄ in the peri-implantational period, when serum concentrations of the hormone are high.

Materials and Methods

Animals

This study was carried out on crossbred gilts (Landrace x Yorkshire), young and healthy, whose approximate weight was 100 to 170 kg, taken from a local breeding farm. The study comprised two groups, formed according to gestational age: twenty two gilts of 10 days of gestation, (pre-implantational period) and twenty two gilts of 30 days of gestation (post-implantational period). In all the cases pregnancy was determined by ultrasound (Draminski Pregnancy Detector) on 18 days after mating. Twenty non-pregnant gilts in the luteal phase of the cycle were used as control.

All gilts were bled by a cava cranial venipuncture (20 ml), with and without heparine, to obtain lymphocyte and serum respectively.
Peripheral lymphocytes response

Peripheral blood mononuclear cells were isolated by Histopaque δ=1077g/ml (Sigma®). One-hundred microliter of cells suspension (10^5 cells/well) were pipetted in triplicate into 96-well flat-bottomed cell culture plates NUNCLON® without and with 0.2 μg/ml of P₄ (20 μg/ml en etanol) Sigma®. As proliferation control, lymphocytes were simultaneously stimulated with 5 μg/ml of Concanavalin A (Con-A) Sigma® and 25 μg/ml of Phytohemagglutinin M (PHA-M) Sigma®.

The optimal concentrations of each mitogen and P₄ were obtained by calibration curve in culture of non-pregnant gilts lymphocytes (data not shown).

In order to study the effect of P₄ on activated lymphocytes a culture was obtained with 5 + 0.2 μg/ml of Con-A and P₄ respectively and 25 + 0.2 μg/ml of PHA-M and P₄ respectively. Cells were incubated for 72 hs in a gas stove at 37 °C with 5% CO₂; at 48 hs, 1 μCi of [3H]-TdR (PerkinElmer®) was added to each well. At 72 hours, the cells were harvested onto filter paper (Whatmann®) using a cell harvester (Siem) and transferred to a scintillation vial with 1 ml OptiPhase II in 24 h. The radioactivity was counted in a liquid scintillation beta-counter (Bekman). The results were expressed as the Index of lymphocytes stimulation (IEL) calculated according to the formula:

\[ IEL = \frac{DPM-E - DPM-C}{DPM-E} \]

DPM-E: disintegrations per minute in cultures of stimulated cells.
DPM-C: disintegrations per minute in cultures of control cells (without stimulation).
IEL ≥ 0.5 were considered as indicators of cell stimulation.

Determination of P₄

P₄ was measured in serum for competitive Radioimmunoassay (RIA) using commercial kit Coat-A-Count® (Diagnostic Products Corporation) with solid phase ^125I for humans, given that the molecule in both species is similar. The serums were incubated in tubes coated with anti-progesterone, where ^125I labeled P₄ competes with P₄ in the serum. After incubation non-binding P₄ was removed by decanting the supernatant and the radioactivity was measured in a gamma counter (Automatic Gamma Counter, Wallac 1470 Wizard™). The sensibility of method is approximately 0.05 ng/ml. The coefficients of variation inter and intraassay were 7.93 % y 7.2 % respectively. The RIA Coat-A-Count is highly specific for P₄ with a low crossreactivity to other steroid hormones.

Statistic analysis

The results were measured by ANOVA using software STATISTICA® 6.0. The values of p < 0.05 were considered significant.
Results

Proliferative response of peripheral lymphocytes

No significant differences were found in the peripheral lymphocytes response to Con-A and PHA-M in the different reproductive stages of the gilt (as shown in Figures 1 and 2). In the presence of P4 the lymphocytes of non-pregnant gilts respond with an IEL average value of 0.73 ± 0.18, whereas the average value of lymphocyte response in gilts of 10 and 30 days of gestation is significantly lower (p< 0.01) (in Figures 1 and 2).

Besides, there is a highly proliferative response of peripheral lymphocytes in the presence of P4 and mitogens (for both Con-A and PHA-M) though this response shows no variation in the different reproductive stages which indicates that the decreased response to P4 in the pregnant gilts reverts itself in cultures with mitogens (Figures 1 and 2).

Determination of P4

As shown in Figure 3 P4 is significantly high (p<0.01) during the peri-implantational period in relation to the non-pregnant gilts. Furthermore, at tenth day of gestation (pre-implantational period) there is a significant increase in relation to the post-implantational period (X=40.55 ± 1.14 ng/ml) (p<0.001).

Discussion

In swine, as in most mammals, the implantation of the embryo depends on a adequate modulation of the immune and endocrine response from the mother. Although it is recognized that this modulation occurs at the implantation site, it has not been described at systemic level.

In the study we report in this paper we found that proliferative response of peripheral lymphocyte from pregnant gilts to mitogens, Con-A and PHA-M, was the same for both pregnant and non-pregnant gilts (Figures 1 and 2).

On the contrary, Matthiesen et al. (1996) and Shirshev and Kunklina (2002) working with humans found that during pregnancy the proliferative activity of T lymphocytes is significantly reduced in the presence of mitogens.

We also observed that the proliferative response to P4 was significantly lower in pregnant gilts lymphocytes during peri-implantational period than in non-pregnant gilts (p<0.01) (Figures 1 and 2). In humans, Szekeres-Bartho et al. (2001) observed that peripheral lymphocytes of pregnant women have a higher density of P4 receptors than the lymphocytes of non-pregnant women. Therefore, this same phenomenon could be occurring in the swine species, suggesting the presence of specific binding sites for P4 in pregnant gilt lymphocytes. The binding of the hormone to the receptor has an effect on T cells, blocking their activation during pregnancy. Szekeres-Bartho et al. (2001) also showed that P4 indirectly blocks the production of IL-2, cytokine required by T lymphocytes to be able to proliferate.

The analysis of the proliferative response of peripheral lymphocytes in gilts in the peri-implantational period to P4 and mitogens (Con-A and PHA-M) revealed high values of IELs, similar to those of non-pregnant gilts (Figures 1 and 2). This shows that though the peripheral lymphocytes of pregnant gilts do not respond to P4, there is no full...
imunosuppression because this response reverts in the presence of mitogens. This means that the peripheral lymphocytes retain their capacity of response to antigen exogenous stimuli, such as infections agents. This is another evidence that the swine lymphocytes response to P₄ and mitogens differs from that of humans and murines.

During early pregnancy, P₄ coordinates a series of complex events that ultimately lead to the synchronized development of the embryo and differentiation of uterine cells and plays an important role in the local immunomodulation at the moment of implantation (Telleria et al. 1999; Sengupta and Ghosh, 2000; Joachim et al. 2003). Like most authors, we found that serum values of P₄ were significantly high during pre and post-implantational period (40.55 ± 1.14 y 26.93 ± 0.82 ng/ml respectively; p<0.01) (Ruiz López, 1997; Senger, 1999; Arck, 2001; Vivas et al. 2001). These high concentrations of P₄ coincide with the diminished proliferative response of peripheral lymphocytes to the hormone in the same period. These results might be indicating the immunomodulator role of P₄ at systemic level during the implantation in swine.

Fig. 1. Values of IELs in response to 5 µg/ml Con-A, 0.2 µg/ml P₄ and 0.2 µg/ml P₄ + 5 µg/ml Con-A of peripheral lymphocytes from non pregnant gilts and pregnant gilts of 10 and 30 days. IEL ≥ 0.5 were considered as indicators of cell stimulation. The columns represent the mean ± SEM. (***p ≤ 0.01)

Fig. 2. Values of IELs in response to 25 µg/ml PHA-M, 0.2 µg/ml P₄ and 0.2 µg/ml P₄ + 25 µg/ml PHA-M of peripheral lymphocytes from non pregnant gilts and pregnant gilts of 10 and 30 days gilts. IEL ≥ 0.5 were considered as indicators of cell stimulation. The columns represent the mean ± SEM. (***p ≤ 0.01)
Peripheral lymphocytes response to progesterone during early pregnancy in pigs. **Fig. 3.** Concentrations of progesterone (ng/ml) in serum of non-pregnant gilts and pregnant gilts of 10 and 30 days measured by RIA. The columns represent the mean ± SEM. (** p<0.01; *** p<0.001).

Acknowledgements
This research was supported by SECYT of UNRC, FONCyT and CONICET, Argentina.

References

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Trabajo recibido el 30/08/2006, nº de referencia 110608_REDVET. Enviado por su autor principal. Publicado en Revista Electrónica de Veterinaria REDVET®, ISSN 1695-7504 el 01/11/06.

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