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Peripheral lymphocytes response to progesterone during early pregnancy in pigs

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Resumen

El objetivo de este studio fue investigar la respuesta proliferativa de linfocitos T periféricos a P_4 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 P_4, Con-A, PHA-M. La concentración sérica de P_4 se determine 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 P_4 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 P_4 junto con mitógenos sin mostrar variaciones entre los diferentes estados reproductivos. Esto indicaría que la disminución de la respuesta a P_4 observada en cerdas preñadas se revierte.
La P_4 se encuentra significativamente elevada ($p<0.01$) durante el período peri-implantacional. Estas altas concentraciones de P_4 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 P_4 a nivel sistémico durante la implantación en cerdos.

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

Abstract

The aim of this study was to investigate the proliferative response of peripherical T lymphocytes to P_4 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 P_4, Con-A, PHA-M. The serum concentration of P_4 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 P_4 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 P_4 and mitogen though this response shows no variation in the different reproductive stages which indicates that the decreased response to P_4 in the pregnant gilts is reverted. The P_4 is significantly high ($p<0.01$) during the peri-implantational. These high concentrations of P_4 coincide with the diminished

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proliferative response of peripheral lymphocytes to the hormone in the same period. These results might be indicating the immunomodulator role of P_4 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 (P_4) 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 P_4 receptors while non-pregant females do not (Szekeres-Bartho *et al, 2001*). In these species, the immunomodulatory effects of P_4 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 P_4, either at local or systemic level. For this reason, the aim of this study was to investigate the proliferative response of peripherical T lymphocytes to P_4 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.

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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 P_4 (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 P_4 were obtained by calibration curve in culture of non-pregnant gilts lymphocytes (data not shown).

In order to study the effect of P_4 on activated lymphocytes a culture was obtained with 5 + 0.2 μg/ml of Con-A and P_4 respectively and 25 + 0.2 μg/ml of PHA-M and P_4 respectively. Cells were incubated for 72 hs in a gas stove at 37 °C with 5% CO_2; 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= 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 P_4

P_4 was measured in serum for competitive Radioimmunoassay (RIA) using commercial kit Coat-A-Count® (Diagnostic Products Corporation) with solid phase ^{125}I for humans, given that the molecule in both species is similar. The serums were incubated in tubes coated with anti-progesterone, where ^{125}I labeled P_4 competes with P_4 in the serum. After incubation non-binding P_4 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 P_4 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.

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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 P_4 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 P_4 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 P_4 in the pregnant gilts reverts itself in cultures with mitogens (Figures 1 and 2).

Determination of P_4

As shown in Figure 3 P_4 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 ($X=40.55 \pm 1.14$ 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 P_4 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 P_4 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 P_4 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 P_4 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 P_4 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 P_4, there is no full

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immunosuppression 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 P_4 and mitogens differs from that of humans and murines.

During early pregnancy, P_4 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 P_4 were significantly high during pre and post-implantational period (40.55 ± 1.14 y 26.93 ± 0.82 ng/ml respectively; $p<0.01$) (Figure 3) (Ruíz López, 1997; Senger, 1999; Arck, 2001; Vivas *et al.* 2001). These high concentrations of P_4 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 P_4 at systemic level during the implantation in swine.

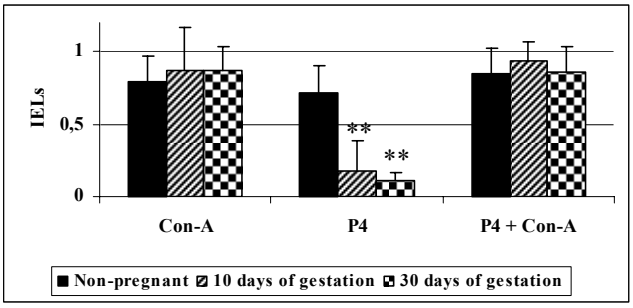

Fig. 1. *Values of IELs in response to 5 μg/ml Con-A, 0.2 μg/ml P_4 and 0.2 μg/ml P_4 + 5 μg/ml Con-A of peripheral lymphocytes from non pregnant gilts and pregnant gilts of 10 and 30 days. IEL ≥ 0.5 were considered as indicators of cell stimulation. The columns represent the mean ± SEM. (**p≤ 0.01)*

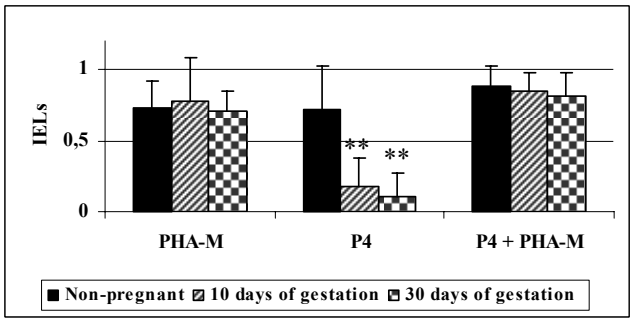

Fig. 2. *Values of IELs in response to 25 μg/ml PHA-M, 0.2 μg/ml P_4 and 0.2 μg/ml P_4 + 25 μg/ml PHA-M of peripheral lymphocytes from non pregnant gilts and pregnant gilts of 10 and 30 days gilts. IEL ≥ 0.5 were considered as indicators of cell stimulation. The columns represent the mean ± SEM. (**p≤ 0.01)*

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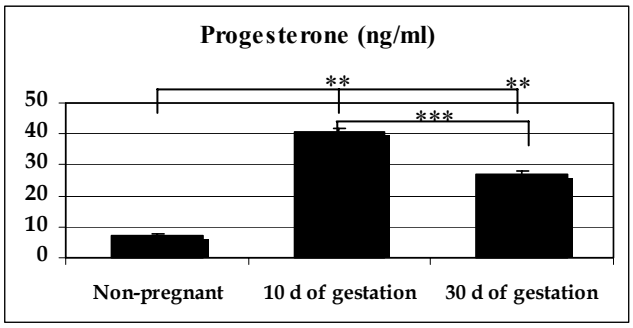

Fig. 3. Concentrations of progesterone (ng/ml) in serum of non-pregnant gilts and pregnant gilts of 10 and 30 days measured by RIA. The columns represent the mean ± SEM. (** $p<0.01$; *** $p<0.001$).

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