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Crosstalk between uterine serpin (SERPINA14) and pregnancy-associated glycoproteins at the fetal-maternal interface in pregnant dairy heifers experimentally infected with *Neospora caninum*

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ABSTRACT

Infection with *Neospora caninum* is the leading cause of abortion in cattle. In cows naturally infected with *N caninum*, plasma concentrations of pregnancy-associated glycoproteins (PAG) 1 and 2 indicate fetal-placental well-being, whereas an excess of progesterone in the second trimester of gestation has been related to high abortion rate. The immunosuppressive action of progesterone on the uterus during gestation has been attributed in part to the uterine serpins (SERPINA14). This study examines expression patterns of the genes *SERPINA14, PAG*, and *PAG2* at the fetal-maternal interface in dairy heifers experimentally infected with *N caninum* during the second trimester of pregnancy, when most abortions takes place in natural conditions. Irrespective of infection, expression of SERPINA14 was higher, and expression of PAG1 and PAG2 lower, for intercaruncular endometrium than for caruncles or cotyledons. Cotyledonary tissues showed the highest expression of both *PAG* genes but lowest expression of *SERPINA14*. The expression of *SERPINA14* was significantly higher in intercaruncular endometrium of control dams than for infected animals, pointing to potential disruption of modulation of maternal immune function during infection. Dramatically reduced *SERPINA14* was particularly apparent in infected dams with aborted fetuses. There was also a negative association between *N caninum* antibody titers with *SERPINA14* and *PAG* expression in infected animals, further suggesting that *N caninum* infection downregulates the uterine immunosuppressive function of SERPINA14.

1. Introduction

Neospora caninum is considered a main cause of abortion in cattle worldwide [1–4]. *Neospora*-seropositive cows carry a 12 to 19 times greater risk of abortion than seronegative cows; the incidence of abortion ranges from 30% to 44% in seropositive animals [5,6]. Further, the risk of repeat abortion persists in seropositive cows [7]. The major route of *N caninum* infection in dairy herds is transplacental, meaning that the parasite passes from dams to their fetuses during pregnancy [8,9]. Maternal immunity, host

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susceptibility, parasite strain diversity, and the stage of fetal development at which infection is acquired have all been related to transplacental infection and abortion [10–12]. Parasites may provoke lesions in the placenta that are severe enough to cause fetal death and pregnancy termination [3,4]. Among several cell-mediated immunity mechanisms, those induced by T helper 1 cells (Th1) have been described as the most important for reducing parasite multiplication in the host [2,12]. However, Th1 activity, although effective in nonpregnant animals, could play a role in the pathogenesis of fetal rejection during gestation [13].

Pregnancy-associated glycoproteins (PAGs) are a multigene family related to aspartic proteinases that are expressed in the placenta of artiodactyls. Ruminant PAGs are classified into two main groups: one of ancient origin (the PAG-II subgroup, including PAG2, PAG8, PAG10, PAG11, PAG12, PAG12, and PAG22), largely occurring at the placental fetal-maternal interface, and one produced by a more recent series of gene duplications (the PAG-I subgroup, including PAG1, PAG3, PAG15, PAG17, and PAG21), expressed primarily in trophoblast binucleate cells [14,15]. Plasma PAG-I concentrations are unaffected by chronic *N caninum* infection in dams although PAG-I and PAG-II concentrations in aborting animals are useful indicators of fetal-placental distress [16–18].

In high-producing dairy cows, *Neospora* infection affects endocrine patterns during gestation such that *Neospora* seropositivity has been associated with higher plasma progesterone (P4) concentrations [19]. Progesterone, a key pregnancy hormone, participates in the natural immunomodulation of gestation, reducing the Th1 response to induce maternal immunologic tolerance to the fetus [20,21]. However, excess P4 in the second trimester of gestation leads to a higher abortion rate in cows chronically infected with *N caninum* [22]. Thus, a threshold Th1 immune response such as gamma interferon production seems necessary to confer protection against abortion [23]. The maternal immune system can therefore tolerate the presence of paternal alloantigens without affecting antiinfection mechanisms during gestation. The uterine immunosuppressive actions of P4 have been attributed in part to uterine serpins [24]. These basic glycoproteins are members of the serpin superfamily of serine peptidase inhibitors. One such member, SERPINA14 [25], is expressed in response to P4 in the endometrium. According to its uterine expression and loss of proteinase inhibitory activity, a new function in establishing and maintaining pregnancy in ruminants has been suggested [26]. SERPINA14 inhibits lymphocyte function *in vitro* [27] and selectively interacts with other uterine proteins, such as PAGs [28], uteroferrin [29], IgM and IgA [30], and activin [31]. SERPINA14 is not only secreted by the endometrium of the pregnant ruminant it is also present in fetal fluids (allantoic and amniotic fluids) [32] and ovarian luteal and follicular structures [33].

The present study is one of a series of investigations performed in pregnant dairy heifers experimentally infected with *N caninum*. The objective was to examine expression of *SERPINA14*, *PAG1*, and *PAG2* genes at the fetal-maternal interface in the second trimester of gestation of infected animals. This stage of pregnancy was selected as the time when most abortions occur in field conditions. Also assessed were possible interrelations between expression patterns of *SERPINA14* and *PAG* with plasma *N caninum* antibodies and plasma concentrations of PAG-I and PAG-II in the infected dams.

2. Materials and methods

2.1. Animals and experimental design

A full description of the parasite inocula used and the characteristics of the experimentally infected heifers is provided by Almeria et al. (unpublished data, 2015). Briefly, ten 14 to 16-month-old Holstein-Friesian heifers that were seronegative against *N caninum* (CIVTEST, Spain) were synchronized and artificially inseminated. Seronegativity against the parasite was assessed before artificial insemination and on Days 60 and 90 of gestation. Heifers were previously vaccinated (6–8 months of age) against bovine viral diarrhea virus and infectious bovine rhinotracheitis virus. Pregnancy was assessed by ultrasound at 30, 45, 90, and 110 days after insemination. On Day 110 of gestation, six of the heifers were intravenously inoculated with 10^7 culture-derived tachyzoites of the *N caninum* isolate Nc-Spain7, kindly donated by Dr. L.M. Ortega-Mora (SALUVET, Universidad Complutense, Madrid, Spain). These six animals were euthanized on Day 152 of gestation. The four remaining heifers remained as uninoculated controls and euthanized at the same time as inoculated dams. After Day 110, heifers were visually inspected daily for possible abortion until their sacrifice.

2.2. Sample collection

Blood samples for antibody and placental protein determinations were collected from each heifer by tail vein puncture into heparinized vacuum tubes (BD Vacutainer, Becton-Dickinson and Company, Plymouth, UK) on Day 152 of gestation. Plasma obtained by centrifugation within 30 minutes of sampling was stored at −20 °C until analysis.

On Day 152 of gestation (6 weeks after infection), all animals were sedated with xylazine hydrochloride (Rompun; Bayer) and euthanized by an intravenous overdose of embutramide and mebezonio iodide (T61; Intervet). Immediately after death, heifers were necropsied. Amniotic and allantoic fluids were collected before the placenta was opened, and fetuses separated from the placenta. Fetal blood samples were obtained by cardiac puncture. Samples of nine selected placentomes (three cranial, three medial, and three caudal) were removed from each dam. Both the maternal side of the placenta (caruncle) and its corresponding fetal side (cotyledon) were carefully separated manually from each placentome. Intercaruncular tissue was also collected. Tissues collected from fetuses were CNS (brain and spinal cord), heart, lung, liver, skeletal muscle, spleen, and thymus.

2.3. Ethics

All procedures were approved by the Ethics Committees on Animal Experimentation of the Autonomous University