

Bovine Neosporosis: From Research to Clinic

“In clinical terms, it may be stated that IFN- γ production and cross-breed pregnancy can protect *Neospora*-infected dairy cows against abortion”

Sonia Almería

Universitat Autònoma de Barcelona
Spain

Fernando López-Gatius¹, Beatriz Serrano-Pérez^{2,3} and Sonia Almería^{4,5*}

¹Transfer in Bovine Reproduction SLu, Barbastro, Spain

²Department of Animal Science, University of Lleida, Lleida, Spain

³Agrotecnio Centre, University of Lleida, Lleida, Spain

⁴Centre de Recerca en Sanitat Animal (CRESA) - Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Campus UAB, Barcelona, Spain

⁵Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, Barcelona, Spain

COLUMN ARTICLE

Abstract

Bovine neosporosis is a main cause of abortion worldwide. In dairy cattle, the economic burden of neosporosis-associated abortion is so great that it might result in closure of a farm. However, not all infected cows abort and it is not yet understood why this occurs. Humoral immune responses are not protective against *N. caninum* but seropositivity and antibody level can be good markers for a diagnosis of bovine neosporosis and its associated abortion risk. Concentrations of PAG-2 can also be used to predict abortion. A partially protective immune response encompasses increased IFN- γ expression, which has to be counterbalanced by other cytokines such as IL-12 and IL-10, especially towards the end of pregnancy. IFN- γ is required to limit parasite proliferation, but a critical threshold of the IFN- γ response is required to limit adverse effects on pregnancy. In clinical terms, it may be stated that IFN- γ production and cross-breed pregnancy can protect *Neospora*-infected dairy cows against abortion.

Keywords: *Neospora caninum*; Abortion; Immune Responses; PAG; *Serpina*

Neospora caninum is an obligate intracellular protozoan parasite closely related to *Toxoplasma gondii* with a preference for cattle and dogs as hosts. Nowadays bovine neosporosis is a main cause of abortion worldwide. In dairy cattle, the economic burden of neosporosis-associated abortion is so great that it might result in closure of a farm. However, not all infected cows abort and it is not yet understood why this occurs. At present, there is no effective treatment or vaccine, so recommendations for disease control are very important.

In bovine neosporosis, abortion can be caused by cell death due to multiplication of *N. caninum* in the placenta but also by cytokines that are detrimental to the maintenance of pregnancy. Similarly, it has been suggested that placental infection and inflammation may trigger prostaglandin-induced luteolysis causing premature uterine contraction and foetal expulsion [1]. In cattle herds, most *N. caninum* associated abortions occur at mid gestation [1]. Studies performed in pregnant cattle at this time point (e.g. at 110 days of pregnancy) have reported a transient state of T-cell hypo responsiveness to the parasite with downregulation of cell proliferation, reduced IFN- γ responses and decreased lymphocyte subpopulations [2].

Immunity to neosporosis is mainly cell-mediated rath-

er than humoral. Presence of specific antibodies are indicative of parasite exposure and there is speculation as to what extent antibody titres might be associated with abortion risk or protection [3]. Several studies has shown that *Neospora*-seropositive cows are more likely to abort than their seronegative counterparts and a raised risk of abortion has been associated to elevated antibody titres in numerous studies [1,4]. The risk of abortion in our region can be some 19 times higher in *Neospora*-seropositive cows than in their seronegative peers. This increased risk of abortion among seropositive cows indicates a causal link between *N. caninum* infection and abortion, particularly in herds in which the presence of *N. caninum* has been confirmed in aborted fetuses. Endogenous transplacental infection route is associated with an acute increase in maternal antibodies, but antibody titres can fluctuate, especially during pregnancy. To date, neither serological tests nor markers for serodiagnosis have served to attribute abortion to *N. caninum* in an individual cow [4]. Despite this hurdle, at the herd level, yearly whole-herd serological screening for antibodies against *N. caninum* using ELISA tests is a very effective method both to detect *N. caninum* infection and to estimate the risk of abortion [5].

Cell-mediated immune mechanisms are ascribed a key role in controlling *N. caninum* in cattle [1]. Although Th1 type immunity involving pro-inflammatory cytokines such as gamma interferon (IFN- γ) is critical for the development of host protective immunity in non-pregnant *Neospora*-infected cows, it could be involved in the pathogenesis of foetal rejection during gestation. Consistently, it is accepted that abortion due to neosporosis can be the outcome of a shift from the beneficial Th2-type response towards an excessive Th1 immune response during the gestation period [6]. During a pro-inflammatory response, the only effective response against *N. caninum*, death by exaggerated immunological reactions has been observed in experimental *Neospora*-infections [3], particularly in the placenta. However, in *Neospora*-naturally infected cows, IFN- γ production was related to protection against abortion. 15.6 times higher risk of abortion was detected in seropositive cows not producing IFN- γ than in seronegative animals, whereas neosporosis was found to have no such effect in seropositive cows that produced IFN- γ [7]. These results showed

that IFN- γ is required to limit parasite proliferation, but a critical threshold of the IFN- γ response is required to limit adverse effects on pregnancy [4].

Pro-inflammatory cytokines may be counter-regulated by IL-10, IL-4 and transforming growth factor beta (TGF- β), which are expressed at the materno-foetal interface to avoid foetal rejection. However, they at the same time render the pregnant host unable to control *Neospora* infections by allowing parasite proliferation and vertical transmission. Several studies have demonstrated the concomitant production and/or upregulation of both Th1 cytokines (mainly IFN- γ) and Th2- and Treg-cytokines during the course of experimental *N. caninum* infection in both aborting and non-aborting dams. This can occur upon infection at any point of the pregnancy, early, mid gestation, or late gestation [3,4]. Mixed Th1-T reg-Th2 patterns were also evident in studies in the placenta [4]. These results indicate that a partially protective immune response encompasses increased IFN- γ expression, which has to be counterbalanced by other cytokines such as IL-12 and IL-10, especially towards the end of pregnancy. Our recent study showed up-regulated expression of Th1, Th2 and Treg in infected dams with live fetuses and in their fetuses. On the other hand, down-regulation of Th2 immune responses and Treg cytokines were observed in infected dams which had aborted or had non-viable fetuses at euthanasia, suggesting an immunological recovery of cytokine gene expression levels in dams a few weeks after an abortion occurred [8].

The immune response of the foetus also plays a crucial role for the outcome of the infection. The capacity of the foetus to mount an immune response to an invading pathogen increases progressively with time as the immune system develops. Early in infection significant cytokine expression were observed in fetuses of experimentally infected heifers at 110 days of gestation [2]. Moreover, in cows infected last in the pregnancy, abortion and lesions were not observed in the fetuses, suggesting a control of the infection by the maternal as well as foetal immune responses [4]. At this time in pregnancy a high rate of vertical transmission is usually observed.

Pregnancy-associated glycoproteins 1 (PAG-1) and 2 (PAG-2) are useful for pregnancy diagnosis and as a marker

of placental/foetal well-being. Both PAG-1 and PAG-2 patterns are modified by *N. caninum* infection, levels undergo a sudden drop in aborting cows, thus acting as a useful marker of foetus-placental status [4,9] whilst some PAG-2 concentrations may be a useful indicator of abortion risk in *N. caninum*-infected cows [4]. As markers of placental/foetal well-being, higher plasma concentrations of PAG-1 were observed in cross-breed gestations compared to pure-breed dairy cattle gestations. Cross-breed pregnancies, with their higher plasma PAG-1 concentrations, have a beneficial effect on placental function probably due to higher PAG-1 and IFN- γ thresholds for inflammation than produced in pure-breed pregnancies [4]. This could explain why the use of beef bull semen in dairy herds, especially Limousin semen, leads to significantly reduced *Neospora*-associated abortions [5].

Interestingly, the immunosuppressive action of progesterone on the uterus during gestation has been attributed in part to the uterine serpins. In a recent work, expression of one such member of serpins, SERPINA14, was significantly higher in inter caruncular endometrium of control dams than for *Neospora*-infected animals, pointing to potential disruption of modulation of maternal immune function during infection [10]. These data suggest that *N. caninum* infection down-regulates the uterine immunosuppressive function of SERPINA14. A negative correlation was observed between inter caruncular SERPINA14 expression and IFN γ expression in cotyledon samples and with IL4 expression in UTLN, while no relationships were detected between cytokine gene expression at the foetal-maternal interface and SERPINA14 expression in the luteal samples. These findings suggest that gene expression of the uterine serpin SERPINA14 correlates negatively with the expression of Th1 and Th2 cytokines at the foetal-maternal interface but not in the corpus luteum [11].

In conclusion, humoral immune responses are not protective against *N. caninum* but seropositivity and antibody level can be good markers for a diagnosis of bovine neosporosis and its associated abortion risk. Concentrations of PAG-2 can also be used to predict abortion. A partially protective immune response encompasses increased IFN- γ expression, which has to be counterbalanced by other cyto-

kines such as IL-12 and IL-10, especially towards the end of pregnancy. IFN- γ is required to limit parasite proliferation, but a critical threshold of the IFN- γ response is required to limit adverse effects on pregnancy. In clinical terms, it may be stated that IFN- γ production and cross-breed pregnancy can protect *Neospora*-infected dairy cows against abortion.

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