Antibodies of aborted bovine fetuses respond to placental structures

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Summary. IgG isolated from aborted bovine fetuses was found to be directed not only to a diaplacental infectious agent but, in half of the cases, also to the site of infection, i.e. the placental tissue. The techniques used were immunohistochemistry and agarose immunoelectrophoresis. These findings suggest a hitherto unrecognized pathological mechanism of abortion.

Key words. Abortion; bovine; IgG; immunological response.

It is known that aborted bovine fetuses often have higher IgG concentrations than those seen in normally-born calves, before they get colostrum[1]. Further, we know of many optional abortion factors besides the well-known abortion-inducing agents like *Brucella abortus* Bang or the bovine infectious rhinotracheitis virus[2]. It is also known that the bovine fetus is capable of responding immunologically to a diaplacental infection[3].

Our aim was to demonstrate that the bovine fetus, in certain cases, not only responds to the infectious agent but also to the site of the fetal infection and that the immunological response could be a trigger for later abortion. Since the genus Bovidae have a placenta cotylodonaria, the usual place of fetal infection is the placentoma.

Materials and methods. Placentomas were collected from healthy pregnant cows either at slaughter or during cesarean section. Fetal serum was collected from aborted fetuses with known etiology (i.e. infection with *E. coli, Streptococci, Staphylococci,* Rickettsiae, Chlamydiae, bovine virus diarrhea, fungi, etc.) and unknown etiology. All sera used had elevated IgG values (higher than 0.3 g/l)[1]. Control sera were collected from normally-born calves before they had received colostrum.

Immunohistology was performed by a peroxidase-anti-peroxidase (PAP) technique[4]. The placental specimens were first incubated with the fetal serum as a source of primary antibody. As a secondary antibody we used a commercial rabbit-anti-bovine-IgG antiserum. The PAP technique was performed with a commercially available kit with a universal anti-rabbit antibody (Dako PAP-Kit 548, IG Zürich). Counterstaining was done with hematoxylin.

A crude extract of placentoma protein was prepared in analogy to a procedure devised by Hübscher et al.[5] for another purpose.

Antibody titration was performed by the immuno-dot-technique[6] using a commercially available rabbit-anti-bovine antibody linked to horseradish peroxidase. Immunoelectrophoresis was performed in 1% agarose according to Schuller et al.[7]. Statistical evaluation was performed by the Pearson chi-square test and the unpaired t-test.

Results. The immunohistological results showed that 50% of the aborted fetuses produced antibodies against placental structures compared to 12% in the control group (figs 1 and 2). The primary targets of these antibodies were the trophoblasts and the fibrinoid layer. In the immuno-dot-titration the sera from the aborted fetuses had, on average, a titer ten thousand-fold higher than the non-aborted control group. The immunoelectrophoresis indicated that the bovine serum was binding one protein in the placentoma (fig. 3). On the other hand, it could be shown that the recognizing protein in the fetal serum belonged to the IgG group (fig. 3).

Discussion. It is known that aborted bovine fetuses often have higher IgG concentrations than normally-born calves have before they receive colostrum[1]. Apart from *Brucella abortus* Bang or bovine infectious rhinotracheitis virus, a number of other infectious agents have been

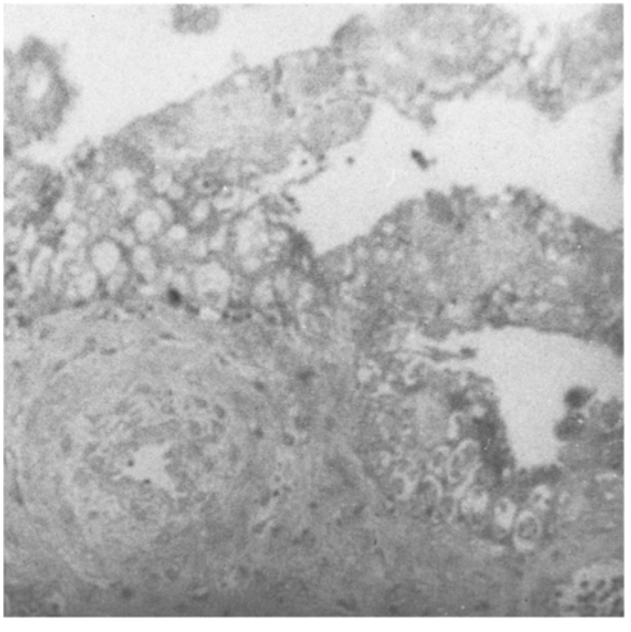

Figure 1. Immunohistology of a normal placentoma at term. The immunohistology was performed as outlined in the text, with a control serum from a non-aborted fetus (× 400).

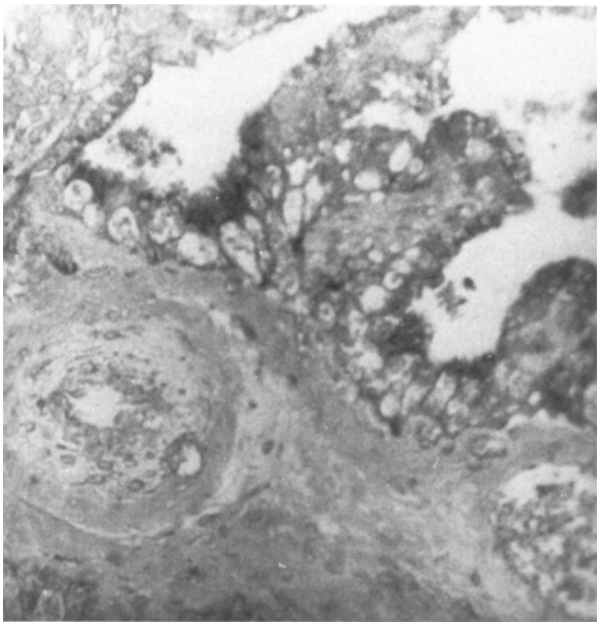

Figure 2. Immunohistology of the placentoma shown in figure 1. The immunohistology was performed as outlined in the text, i.e. with a serum isolated from an aborted fetus (×400).

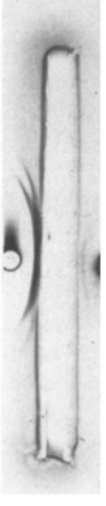

Figure 3. Immunoelectrophoresis of an aborted bovine fetal serum: center slit: commercially available anti-bovine-IgG (light and heavy chain, ICN Immunobiologicals, No. 641401, Lisle, IL, USA). Left-hand well: commercial available bovine IgG (ICN Immunobiologicals, No. 641401, Lisle, IL, USA). Right-hand well: serum of an aborted bovine fetus.

implicated in abortions[2]. The bovine fetus begins to be immunologically competent at 120 days of pregnancy[3]. It was already shown in 1949 that most abortions occur in the last trimester of gestation[8], i.e., at a time when the fetus is immunologically competent[1]. There are precedents in man for the existence of epitopes shared by host and viral proteins, e.g. the sequence homology between the myelin basic protein encephalitogenic site and the hepatitis B virus polymerase[9]. An immune response

Immunohistological findings and results of serological titrations: Evaluation of immunohistological staining specifity was done by three independent persons. Antibody titration by the immuno-dot-test was performed by reacting fetal bovine serum with 90 ng of curde placentoma protein adsorbed to each of several nitrocellulose disks placed in wells of a microtiter plate. Statistical evaluation was done by the Pearson chi-square test for the immunohistology ($p = 0.00266$) and by the unpaired t-test for the antibody titration ($p = 0.0001$).

	Sera from aborted fetuses	Sera from non-aborted fetuses
Immunohistology		
Number of sera tested	26	26
Staining of trophoblasts and fibrinoid layer	13 (50%)	3 (12%)
No staining	13 (50%)	23 (88%)
Serological titration		
Number of sera tested	19	21
Average of titer	$10^{6.57}$	$10^{2.84}$
SD	$10^{2.27}$	$10^{1.46}$
Range of titer	$10^{4}-10^{11}$	$10^{1}-10^{5}$

against a determinant shared by host and virus can induce a tissue-specific immune response and lead to tissue destruction. In this instance, the infectious agent and host determinants must be sufficiently similar to induce a cross-reactive response. Therefore, although an infectious agent may initiate a disease, the likelihood of detecting it in the affected tissue by the time of abortion is small. This fits in with clinical observations showing that in up to 70% of cases studied, the causative agent of abortion remains uncertain[3].

Our findings demonstrate that the bovine fetus is capable of mounting an immunological response not only to a diaplacental infectious agent but also to the site of the infection. These findings suggest a previously unrecognized cause of abortion.

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