Immune Responses of the Bovine Fetus

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ABSTRACT

The bovine fetus is capable of mounting an antibody response when a bacterial antigen (killed *Escherichia coli*) or viral antigen (live reovirus) is deposited into the amniotic fluid. Time required for the fetus to respond to bacterial antigen given orally (amniotic fluid) is approximately 10 to 14 days and 8 to 10 days for viral antigen. Calves vaccinated prenatally with *E. coli* from 9 to 102 days before birth and deprived of colostrum survived oral challenge doses of viable *E. coli* which killed calves not vaccinated prenatally. One mechanism of protection was the local production of antibody in the gastrointestinal mucosa where immunofluorescent techniques showed immunoglobulins IgG, IgM, and anti-*E. coli* antibody in the duodenum, jejunum, and ileum as well as in the jejunal lymph node. Prenatal vaccination has been used in the field for prevention of colibacillosis. However, the occurrence of some stillbirths and premature births indicates the need for further research before there can be widespread field application of the technique.

INTRODUCTION

The relatively new concept of immunizing a fetus offers a number of interesting challenges and opens many avenues for basic as well as applied research. Ten years ago the immunologic capabilities of a bovine fetus were unknown. Today, knowledge concerning in utero vaccination of the bovine fetus has advanced to the point that such a procedure is conceivably practical for prevention of colibacillosis in neonatal calves.

Michigan State University (1, 2, 5, 6, 7, 8) has been one of the pioneers in vaccination of the bovine fetus. This along with work in Australia (3, 4) constitute much of the early work on immunity of the bovine fetus to *Escherichia coli*. Since the cost of research with pregnant cattle is high, there have been limitations on the number of studies. Consequently, questions are unanswered and potential uses of prenatal immunization have not been realized fully. For example, the degree of heterogenetic protection resulting from in utero injection of a single type of organism, the fetal response to organisms other than *E. coli* and reovirus, or the effect of antigenically stimulating a prenatally vaccinated calf are but a few of the important questions.

ABILITY OF THE FETUS TO DEVELOP IMMUNITY (ANTIBODY)

Ovine fetuses were used first in our immunization studies wherein unborn lambs, when injected intracardially with Brucella organisms from mid-gestation (80 days prenatally) up to near gestation, responded by producing humoral antibodies (5, 6). If the unborn lambs were given a second antigenic stimulus (Brucella organisms) in utero, they developed serum antibody concentrations as high as those attained when adult sheep were given primary and secondary stimulation. When secondarily stimulated at birth, neonatal lambs responded rapidly with high levels of antibody (5).

ORAL VACCINATION OF THE FETUS

Following the aforementioned preliminary studies, a finding of great practical importance was that the unborn lamb produced antibodies against Brucella organisms when antigen was deposited in amniotic fluid (7). This was an oral vaccination since amniotic fluid is being swal-
lowed constantly by the fetus. Furthermore, secondary responses were elicited in fetal lambs when a booster vaccination was administered intra-amniotically following a primary intra-amniotic vaccination.

The unborn calf is also capable of mounting an antibody response when vaccinated orally (amniotic fluid) with an *E. coli* organism (1). Not only did fetal calves have antibodies against *E. coli* but fetal lambs responded to *E. coli* antigen in a similar manner.

Gay (3, 4) also has demonstrated that calves vaccinated prenatally with *E. coli* (some were vaccinated intramuscularly, some intra-amniotically) had antibodies against *E. coli* at birth.

**PROTECTION AT BIRTH**

The presence of humoral antibodies at birth is only significant if the newborn is protected against a challenge exposure. Calves prenatally vaccinated (amniotic fluid) with *E. coli* were protected against an oral challenge dose of live *E. coli* organisms (homologous strain) given at birth (1) (Table 1). Although totally deprived of colostrum, these calves were normal, healthy animals during the 6-wk observation. The same dose of oral challenge organisms given to five calves that were not vaccinated in utero resulted in death within 2 to 10 days. The foregoing challenge procedure was used in prenatally vaccinated lambs (1) with results similar to those for calves.

**BREADTH OF PROTECTION**

Gay (3, 4) has reported heterogenic antibody or protection. In his first report (3) calves had antibodies against several serotypes of *E. coli* even though a single serotype was used in the prenatal immunizing inoculum. One such calf (vaccinated for the longest period before birth) had antibodies against a Salmonella organism. The second study (4), in which challenge exposures were used, indicated that in utero vaccination with a single serotype of *E. coli* could result in heterogenic protection against neonatal colisepticemia.

The fact that a fetus responds to an antigenic stimulus by production of a broad spectrum antibody is probably significant. The breadth of protection resulting from vaccination with a single type of organism has not been determined by challenge exposure. The effect of using two immunizing organisms is unknown.

**LOCAL IMMUNITY**

Calves vaccinated prenatally had protection against challenge exposures even though some had no detectable humoral antibodies (1). To define the protective mechanisms, immunofluorescent antibody examinations were made on 14 calves vaccinated in utero with *E. coli* or sterile saline (8) (Table 2). At birth, calves vaccinated prenatally with *E. coli* had antibody-producing cells (IgG, IgM, and anti-*E. coli*) in the mucosa of the duodenum, jejunum, ileum, and in the jejunal lymph node. This local cellular immunity may explain protection in the absence of humoral antibody. The one calf given an oral dose of antigen at birth had more immune-producing cells (also in the spleen) than calves not receiving a booster at birth. The heterogenicity of the antibodies produced at the local cellular level is not known.

**NEONATAL RESPONSE TO REOVIRUS**

Since reovirus is marketed as a preventative for enteritis in newborn calves, the response of the fetus to this virus was of interest. Recent studies in which calves were vaccinated prenatally with the commercial reovirus vaccine or a combination of this and *E. coli*, showed that there were serum antibodies against reovirus at birth (2). Although this was a pilot study involving six animals, results indicated that the fetus produced antibodies within 8 days after vaccination. There was also evidence that *E. coli* in the immunizing inoculum augmented the production of antibody against reovirus.

**FIELD STUDIES**

Several Michigan veterinary practitioners have performed in utero vaccination (*E. coli*) in herds where calfhood enteritis was a problem. For unknown reasons, an unexpected number of abortions occurred within 2 to 4 days after the vaccination. Aborted fetuses that lived and calves that were full-term appeared to be protected against enteritis. The occurrence of...
TABLE 1. Protection against challenge following in utero vaccination (oral route) of bovine fetuses, *E. coli* 026.

<table>
<thead>
<tr>
<th>Calf no.</th>
<th>Vaccinal antigen killed <em>E. coli</em> (no. of organisms)</th>
<th>Fetal vaccination (days before birth)</th>
<th>Serotest for agglutinin 0 at birth</th>
<th>Results of oral administration of challenge inoculum&lt;sup&gt;a&lt;/sup&gt; (1.5 × 10&lt;sup&gt;10&lt;/sup&gt; viable <em>E. coli</em>, 026)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>1 × 10&lt;sup&gt;10&lt;/sup&gt;</td>
<td>22</td>
<td>1:20</td>
<td>Survived</td>
</tr>
<tr>
<td>30</td>
<td>5 × 10&lt;sup&gt;10&lt;/sup&gt;</td>
<td>9</td>
<td>1:10</td>
<td>Survived</td>
</tr>
<tr>
<td>92</td>
<td>5 × 10&lt;sup&gt;10&lt;/sup&gt;</td>
<td>17</td>
<td>1:40</td>
<td>Survived</td>
</tr>
<tr>
<td>45</td>
<td>2 × 10&lt;sup&gt;10&lt;/sup&gt;</td>
<td>40</td>
<td>1:40</td>
<td>Survived</td>
</tr>
<tr>
<td>342</td>
<td>5 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>102</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Died at 10 days</td>
</tr>
<tr>
<td>C1</td>
<td>none</td>
<td>...</td>
<td>0</td>
<td>Died at 10 days</td>
</tr>
<tr>
<td>C2</td>
<td>none</td>
<td>...</td>
<td>0</td>
<td>Died at 3 days</td>
</tr>
<tr>
<td>C3</td>
<td>none</td>
<td>...</td>
<td>0</td>
<td>Died at 2 days</td>
</tr>
<tr>
<td>C4</td>
<td>none</td>
<td>...</td>
<td>0</td>
<td>Died at 4 days</td>
</tr>
<tr>
<td>C5</td>
<td>none</td>
<td>...</td>
<td>0</td>
<td>Died at 2 days</td>
</tr>
</tbody>
</table>

<sup>a</sup>All calves were totally deprived of colostrum.  
<sup>b</sup>ND = not done.

TABLE 2. Antibody production, detected by immunofluorescence, at birth following prenatal vaccination.

<table>
<thead>
<tr>
<th>In utero vaccination</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Jejunal L. node</th>
<th>Ileum</th>
<th>Ileal L. node</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline in amniotic fluid</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td><em>E. coli</em> in amniotic fluid</td>
<td>none</td>
<td>IgM++</td>
<td>IgG++</td>
<td>IgM+</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Fluorescent cells per microscopic field (×25): + = 1; ++ = 2 to 3; +++ = >3.
premature birth in calves vaccinated in utero has prevented widespread field use of the technique. Field experiences are in contrast to those on campus where there have only been four abortions in approximately 90 cows vaccinated in utero. The reason for abortions in the field has not been determined.

VACCINATION PROCEDURE

The ovine fetus requires 15 days following injection of Brucella to achieve maximum antibodies (6). A similar time is required for maximums in lambs stimulated at birth. Antibody which was protective against colisepticaemia appeared in the serum of colostrum-deprived calves at 10 to 14 days after birth (3).

Based upon these two reports, the bovine fetus should be vaccinated at least 14 days prenatally for maximum antibody protection at birth. Since parturition dates in cows may be variable, we advocate fetal vaccinations 3 to 4 wk before anticipated date of birth. While the fetus is capable of mounting antibody responses considerably earlier than 4 wk before birth, antibody at birth may be lower if antigenic stimulation occurs too early in the gestation period (5).

The fetus is vaccinated orally by injection through the dam’s right flank. Injection site is where the fetus is closest to the abdominal wall as determined by ballotment, care being taken to avoid abomasal or duodenal areas. Following clipping of hair and scrubbing the skin with a surgical antiseptic, local anesthetic is infiltrated into subcutaneous tissue, abdominal muscles, and peritoneum at the proposed injection site. A 12 gauge hypodermic needle, 5 cm long, is inserted into the peritoneal cavity via a stab incision in the skin. This short needle serves as a cannula for passage of a 25 or 30 cm 16 gauge needle which is directed toward the uterus and gently pushed through the uterine wall and fetal membranes until the fetus is touched, but not pierced. Presence of the needle in the amniotic cavity is confirmed by aspirating amniotic fluid prior to the injection; however, on rare occasions fluid can not be aspirated. The volume of bacterial antigen injected is 2 to 3 ml containing approximately $2 \times 10^{10}$ cells per ml. Aseptic techniques are used throughout the vaccination procedure. With one assistant, the vaccination can be performed in 5 min.

The immunizing inoculum, E. coli, 026:K60, was prepared at the University. The organism was seeded on bottles of trypticase soy agar and incubated 20 to 24 h at 37 C. The growth was washed twice with sterile saline solution and examined by bacteriologic culture technique to determine purity. To kill the cells, 4% formalin was added, and the suspension was incubated in a shaking water bath (37 C) for 18 to 24 h. The formalin-killed cells were then washed twice with sterile saline and a cell count was obtained. The suspension was then diluted with sterile saline containing 0.002% thimerosal to a desired concentration, approximately 1.5 or 2 $\times 10^{10}$ cells/ml, stored at 4 C and used within 20 days.

POSSIBLE APPLICATION OF IN UTERO VACCINATION

The potentially useful applications of prenatal vaccination are dependent upon expanded research efforts. However, it appears feasible that such a procedure could provide protection against diseases for which there are inadequate or unsatisfactory immunization procedures after birth. For example, viral diseases such as bovine viral diarrhea (BVD) or infectious bovine rhinotracheitis (IBR) might be prevented by fetal vaccination. Postnatal vaccination for these diseases is not always effective and, if given at the wrong time, results in abortions and fetal aberrations. There are at least two viruses (reovirus, coronavirus) responsible for enteric disorders of the newborn calf. Although it may be possible to immunize the neonatal calf against these diseases, prenatal vaccination conceivably could provide the newborn with a more effective immunization, because protective antibodies would be present at birth.

An application of importance is protection of calves against colibacillosis, an enteric disease that kills many calves before 10 days of age. Since this disease affects calves before they develop their own defensive mechanisms, protection at the time of birth is of great value.

If there is heterogenetic protection, as has been reported in calves vaccinated prenatally, this phenomenon would increase interest in and significance of the technique.

Department of Microbiology and Public Health, G. R. Carter.
REFERENCES


