Summary

- An extensive 32-month university research study compared the efficacy of 2 vaccination programs for providing fetal protection against challenge of pregnant cows with multi-strain bovine viral diarrhea viruses (BVDV) and bovine herpesvirus type 1 (BoHV-1; IBR).1
  - Heifers were vaccinated twice before breeding with Bovi-Shield® GOLD FP® 5, a vaccine containing modified-live viruses (MLV) (a control group was not vaccinated).
  - Half of the vaccinated heifers were transitioned to CattleMaSter® GOLD® FP 5 (a vaccine containing temperature-sensitive modified-live IBR virus and killed BVD viruses) during first pregnancy while the remaining vaccinates received Bovi-Shield GOLD FP 5 at pregnancy check (control animals remained non-vaccinated).
- When challenged with BVDV 11 months after vaccination at pregnancy check, animals in both vaccinated groups demonstrated reduced viremia, reduced BVD-associated pregnancy loss, and reduced persistently infected fetuses.
- When challenged with BoHV-1 at 3 months after another gestational vaccination, cows in both vaccinated groups demonstrated reduced viremia and BoHV-1-positive abortions.
- The CattleMaSter® GOLD FP 5 transition program showed no difference in fetal protection against BVDV challenge or BoHV-1 challenge compared to the Bovi-Shield GOLD FP 5 program, thus it offers new options for operations where circumstances prevent continuation of a MLV program with Bovi-Shield GOLD FP 5.

Comparison of BVDV and BoHV-1 Fetal Protection Provided by Bovi-Shield® GOLD FP® 5 and CattleMaSter® GOLD® FP 5 Vaccination Protocols for Pregnant Cows

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Bovine viral diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) are major viral diseases that perpetually threaten cattle productivity. The first is caused by BVD virus (BVDV), a common pathogen capable of causing respiratory, reproductive, and/or gastrointestinal disease in cattle. If a heifer or cow is infected during pregnancy, BVDV can jeopardize reproduction by causing abortion, fetal death/resorption, and/or congenital malformation of the fetus. In addition, if susceptible cows are infected between days 40 to 125 of gestation, their calves may be born persistently infected (PI) with the BVD virus. Though the prevalence of PI calves is estimated to be small (< 1%), these animals develop immunotolerance to their infective strain(s) and commonly shed large quantities of BVDV throughout life.2 As a result, PI cattle serve as a long-term source of infection that can continually erode herd health and thwart BVD control efforts.

IBR is caused by bovine herpesvirus type 1 (BoHV-1) and, in addition to other adverse health impacts, often triggers abortions in cattle infected during pregnancy. The virus can be harbored in latent sites of the
**BOVI-SHIELD GOLD FP 5** is a modified-live vaccine that provides protection against 5 major pathogens, with a 12-month duration of immunity against IBR abortions and BVDV-PI calves.

**CATTLEMASTER GOLD FP 5** has a temperature-sensitive live IBR virus that is labeled against IBR-induced abortion AND is safe to administer to any pregnant cow. It is also the **ONLY** killed BVD vaccine approved for BVDV PI fetal protection.

Vaccinations against BVD and IBR are common practices employed by cattle producers, and certain vaccines are designed for use in pregnant heifers and cows to specifically protect a developing fetus from these diseases. Zoetis markets 2 leading vaccines used in pregnant animals for targeted fetal protection and other general disease prevention:

- **BOVI-SHIELD GOLD® FP® 5** is a freeze-dried preparation of modified-live virus (MLV) strains that provides protection against 5 major pathogens (BVDV types 1 and 2, IBR, PI3, and BRSV; Table 1). In regard to BVD and IBR, BOVI-SHIELD GOLD FP 5 is for vaccination of healthy cows and heifers prior to breeding to prevent PI calves caused by BVDV types 1 and 2, and as an aid in preventing abortion caused by IBR. A 12-month duration of immunity has been demonstrated against IBR-induced abortion and PI calves caused by BVD types 1 and 2. BOVI-SHIELD GOLD FP 5 is also labeled for administration to pregnant cows provided they have been vaccinated according to label directions within the previous 12 months. Label directions state that heifers need to receive 2 doses of BOVI-SHIELD GOLD FP 5 between weaning and breeding.

- **CATTLEMASTER® GOLD FP® 5** is a freeze-dried vaccine that provides similar protection against the same 5 pathogens, but is composed of inactivated (killed) BVDV (types 1 and 2) and chemically altered strains of IBR and PI3 viruses. The temperature-sensitive IBR vaccine strain has the safety characteristics of inactivated IBR with the immunogenicity of modified-live IBR vaccinal virus. Notably, CATTLEMASTER GOLD is the only killed BVD vaccine to offer a Fetal Protection Guarantee against IBR abortion and PI with BVD types 1 and 2.

MLV vaccines will generally offer a higher level of protection than killed products, but incorporating a MLV into an overall reproductive management plan may be too complex for some herds. Thus, some operations may struggle to maintain a pregnant cow vaccination program with conventional MLV IBR vaccines when compared to a temperature-sensitive IBR vaccine (e.g., purchasing pregnant cattle with unknown vaccination history; managing multiple breeding seasons or long breeding/calving seasons; not working cows prior to breeding). For such situations, the ability to transition from a MLV pregnant cow vaccination program to CATTLEMASTER GOLD FP would be helpful for providing fetal protection against reproductive diseases associated with BVD and IBR.

To address this need, research was conducted to determine if initial pre-breeding immune system priming with BOVI-SHIELD GOLD FP 5 will allow an annual application of BVDV and PI vaccine in pregnant cows and heifers without compromising the effectiveness of the fetal protection.
booster dose of CATTLEMASTER GOLD FP 5 to provide reproductive protection against BVD and IBR challenges.¹

Experiment Design

The study involved 75 fall-born commercial beef heifers from the Auburn University Experiment Station in Alabama. Heifers seronegative for BVDV and BoHV-1 were spring-weaned at 7 months of age on May 2, which constituted day 0 of the lengthy study eventually spanning approximately 2.7 years (32 months). As described in Figure 1, the heifers were destined to compose 3 treatment groups. Fifteen non-vaccinated heifers were randomly designated controls (group A) while 60 other heifers (groups B and C) were administered 2 pre-breeding doses of BOVI-SHIELD GOLD FP 5 in May (day 0) and November (day 183). Control animals were maintained on a pasture separate from vaccinates.

In late fall, heifers were prepared for fixed-time artificial insemination (FTAI) on day 214 using a melengesterol acetate (MGA®)-based estrus synchronization protocol.* Insemination was performed exactly 1 month after the second pre-breeding vaccination. After AI and exposure to 4 clean-up bulls for 60 days, heifers were pregnancy checked (ultrasound) the following April, with 84% (63/75) detected to be pregnant (all 15 controls and 48/60 vaccinated heifers). Only pregnant heifers were maintained on the study, with 24 vaccinates randomly assigned to each of groups B and C. Thus, the 3 pregnant heifer treatment groups (after pre-breeding vaccinations with BOVI-SHIELD GOLD FP 5, except for controls) were:

- Group A: controls (no vaccination), n = 15;
- Group B: BOVI-SHIELD GOLD FP 5 booster vaccination (‘BSG’; n=24);
- Group C: CATTLEMASTER GOLD FP 5 booster vaccination (‘CMG’; n=24).

Heifers calved the following autumn (1 heifer mortality in group C). In January of year 3, the cows were again bred (estrus synchronization and AI, plus 4 clean-up bulls for 3 months). Around mid-April of year 3, cows were transported approximately 150 miles to a BVD research facility where they were commingled and pregnancy checked (15 group A pregnant, 23 group B, 22 group C), and all cows were confirmed negative for BVDV. Challenge exposure to BVDV was initiated at this time (day 715, 11 months after the previous vaccination) by cohabiting cows for 16 days with 6 cattle persistently infected (PI) with BVDV (2 animals each PI with BVDV types 1a, 1b, and 2). Virus isolation for detection of viremia in the pregnant cows was performed on samples of whole blood (‘buffy coat’ white blood cells, WBC) and serum acquired at numerous times during the exposure period.

One week after removal of the PI cattle (mid-May, day 738), all cows received another booster vaccination according to their respective treatment group assignment. Three months later in mid-August (day 830), each animal received BoHV-1 challenge by intravenous inoculation (Colorado strain; all cows confirmed negative for BoHV-1 prior to inoculation). Samples of blood and nasal secretions were collected at various times for 4 weeks post-challenge to detect viremia and viral shedding.

Cows were monitored for abortions after each challenge exposure/infection. Tissues were collected from all abortions and analyzed for BVDV and BoHV-1 isolation (indicative of fetal infection). Live calves born during the October to December calving period were evaluated for BVD and IBR status (viremia).

Cow and/or calf samples of whole blood (WBC), serum, ear notches, and placenta were analyzed for BVDV using reverse transcription nested polymerase chain reaction (RT-nPCR). BVDV virus isolation was also performed on tissues from aborted fetuses when available (spleen, thymus, liver, kidney, lung, placenta). Skin samples were collected for BVDV immunohistochemistry (IHC) and antigen-capture ELISA, and placenta was also tested by IHC. Sequencing of BVDV-positive samples was performed on aborted fetuses, live-born BVDV-PI calves, and the WBC-positive samples from cows. For BoHV-1 detection, blood and nasal samples from

* MGA fed for 14 days; gonadotropin-releasing hormone (GnRH) administered 12 days after MGA withdrawal; prostaglandin (LUTALYSE®) administered 7 days after GnRH. Insemination performed 72 hours after prostaglandin with GnRH administered at the time of AI.
cows, blood and placenta samples from calves, and tissues from aborted fetuses were cultured on Madin-Darby bovine kidney cells and examined for cytopathic effect. Confirmation of BoHV-1 as the pathogenic cause was performed by immunoperoxidase staining and/or PCR. Collected data were statistically analyzed using appropriate standard methods, with significance between treatments declared when $P \leq 0.05$.

**Results**

**BVD outcomes — Viremia**

Because BVDV can travel widely throughout the body of cattle, a key to preventing reproductive or respiratory BVD is stopping the onset of viremia. A vaccine capable of preventing BVD viremia will also help prevent the spread of the viral pathogen to other tissues and organs, impede secondary viral replication, and help reduce viral shedding, all of which helps protect the fetus in pregnant cows. Therefore, viremia prevention is a clinically relevant measure of vaccine efficacy and value.

Results of viremia detection in pregnant parity-2 cows are summarized in Table 2 and Figure 2 (serum and WBC virus isolation). None (0%) of the vaccinated cows (groups B or C) developed serum viremia following exposure to PI herdmates. In contrast, 47% (7/15) of control cows experienced BVDV viremia in serum. These findings are notable since serum viremia is most closely associated with BVD fetal infection. Rates of viremia associated with WBC were higher in all groups, but still much lower in vaccinates compared to controls.

Even though vaccinated cattle experienced prolonged and effective levels of BVDV exposure from the PI animals introduced to the herd (evidenced by titers of PI cattle plus disease impacts in controls), Both **BOVI-SHIELD GOLD FP 5** and **CATTLEMASTER GOLD FP 5** protected 100% of the heifers from developing BVDV serum viremia after exposure to PI herdmates.

**BVD outcomes — Offspring**

BVDV-positive abortions occurred for 67% (10) of the non-vaccinated control cows (Table 3, Figure 3), and 27% (4) gave birth to live calves that were positive for BVDV (PI). None of the live calves from the control group cows were negative for BVDV. One abortion in the controls was BVDV-negative, from a cow that had inexplicably seroconverted prior to challenge. Only 1 abortion and 1 live birth from vaccinated cows was positive for BVDV (both in group B). No BVDV-positive abortions or PI live births occurred for group

<table>
<thead>
<tr>
<th>Group</th>
<th>BVD-positive abortions</th>
<th>BVD-positive live births</th>
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<tbody>
<tr>
<td>A (control)</td>
<td>67% (10/15)</td>
<td>27% (4/15)</td>
</tr>
<tr>
<td>B (BOVI-SHIELD GOLD FP 5)</td>
<td>4%* (1/23)</td>
<td>4%* (1/23)</td>
</tr>
<tr>
<td>C (CATTLEMASTER GOLD FP 5)</td>
<td>0%* (0/22)</td>
<td>0%* (0/22)</td>
</tr>
</tbody>
</table>

* Significantly reduced compared to controls ($P < 0.0001$).

Both vaccinated groups had reduced incidences of BVDV-positive abortions and PI calves vs controls.

Both **BOVI-SHIELD GOLD FP 5** and **CATTLEMASTER GOLD FP 5** protected 100% of the heifers from developing BVDV serum viremia after exposure to PI herdmates.
C cows (CATTLEMASTER), and the rates of infected offspring did not significantly differ between the 2 vaccinated groups ($P = 0.4894$). Thus, both vaccination programs provided significant improvement ($P < 0.0001$) in BVDV fetal protection and reduction of PI calves compared to controls. Sequencing of BVDV strains implicated in abortions revealed that the majority were type 1. Of the 10 aborted control fetuses, 4 were type 1a, 4 were type 1b, and 2 were type 2; and the lone abortion from vaccinated cows was type 1a. Of the 5 live-born PI calves, 4 were type 1a, and 1 control calf was type 2.

**BoHV-1 (IBR) outcomes**

Viremia and reproductive outcomes related to the BoHV-1 challenge infection are summarized in Table 4 and Figure 4. The incidence of IBR serum viremia and nasal shedding in non-vaccinated control cows ranged from 73% to 93%, respectively. Vaccinated cows, in contrast, demonstrated significantly reduced ($P < 0.0001$) incidences of viremia and nasal shedding.

Both vaccinated groups experienced significant ($P < 0.0001$) protection against BVDV. Whereas there were no live calves born BVDV negative in the control group. These outcomes for viremia and shedding incidence were mirrored in the rate of abortions positive for BoHV-1. Eight of the abortions by control cows were positive, reflecting 53% of the cows. However, BoHV-1-positive abortion incidence was significantly ($P = 0.0013$) reduced for vaccinates, ranging from 0% to 9% of the

**Table 4 – Incidence of BoHV-1 (IBR) viremia and shedding for challenged cows, and abortions positive for BoHV-1.**

<table>
<thead>
<tr>
<th></th>
<th>Group A (Control)</th>
<th>Group B (BOVI-SHIELD GOLD FP 5)*</th>
<th>Group C (CATTLEMASTER GOLD FP 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum viremia</td>
<td>73% (11/15)</td>
<td>8%* (2/24)</td>
<td>0%* (0/23)</td>
</tr>
<tr>
<td>Nasal shedding</td>
<td>93% (14/15)</td>
<td>13%* (3/24)</td>
<td>26%* (6/23)</td>
</tr>
<tr>
<td>BoHV-1-positive abortions</td>
<td>53% (8/15)</td>
<td>9%* (2/23)</td>
<td>0%* (0/22)</td>
</tr>
</tbody>
</table>

* Significantly reduced compared to controls ($P \leq 0.0013$).
*24 cows challenged, 23 pregnant at time of challenge.
*23 cows challenged, 22 pregnant at time of challenge.
The same 2 BOVI-SHIELD GOLD FP 5-vaccinated cows were positive for BoHV-1 in serum and aborted fetus.

![Figure 3](image) – Incidence of BVDV-positive abortions, PI live-born calves, and normal BVDV-negative calves.

![Figure 4](image) – Incidence of BoHV-1 (IBR) viremia and shedding for challenged cows, and abortions positive for BoHV-1.
Implications

This dual-challenge study is of interest to many practitioners because no previous research had evaluated the efficacy of pre-breeding vaccination with BOVI-SHIELD GOLD FP products followed by transition to annual vaccination with CATTLEMASTER GOLD FP during pregnancy. Under typical production conditions, a double challenge of the scope and severity used in this study would rarely occur. Still, even under these extreme circumstances, study results offer evidence and confidence that the CATTLEMASTER GOLD FP 5 transition program can deliver protection similar to that afforded by annual administration of BOVI-SHIELD GOLD FP 5.

The excellent fetal protection against both BVDV and BoHV-1 achieved by the transition program provides new options for those operations that struggle to maintain a pregnant cow vaccination program with conventional MLV IBR vaccines when compared to a temperature-sensitive IBR vaccine. In addition, this CATTLEMASTER GOLD FP 5 transition program is backed by the Zoetis Fetal Protection Guarantee. Under this program, Zoetis guarantees all calves born to be 100% BVD PI-free and guarantees against IBR abortions, provided that all cows and heifers are vaccinated with BOVI-SHIELD GOLD FP, CATTLEMASTER GOLD FP, or PREGGUARD® GOLD FP according to label or program indications. This includes the transition program, in that heifers receiving 2 doses of BOVI-SHIELD GOLD FP or PREGGUARD GOLD FP prior to breeding may be annually revaccinated with CATTLEMASTER GOLD FP and qualify for the guarantee.

Abortion overview

An overall summary of abortions occurring after either of the 2 challenge exposures/infections appears in Table 5. Of the 60 pregnant cows subjected to challenge, 15 aborted their fetuses, with rates ranging from 5% and 13% for vaccinates to 73% for non-vaccinated controls. Notably, most abortions (12/15) occurred after BoHV-1 challenge, with only 3 occurring after BVDV exposure. In the absence of the BoHV-1 challenge, the many BVDV-positive abortions in the control group would have likely been live births PI with BVDV.

In the absence of the BoHV-1 challenge, BVDV-positive abortions in controls would have likely been live births PI with BVDV.

cows. The 2 abortions in group B occurred for the same 2 cows that were detected to be viremic. Still, the rates of positive abortions between the vaccinated groups did not significantly differ (P = 0.4889). All live calves born during the study were free of BoHV-1, regardless of treatment group.

Table 5 – Incidence of abortions after BVDV and BoHV-1 challenges of cows.

<table>
<thead>
<tr>
<th></th>
<th>Group A (Control)</th>
<th>Group B (BOVI-SHIELD GOLD FP 5)</th>
<th>Group C (CATTLEMASTER GOLD FP 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant at challenge onset</td>
<td>15/15</td>
<td>23/24</td>
<td>22/23</td>
</tr>
<tr>
<td>Abortions after BVDV but before BoHV-1 challenges</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Abortions after BoHV-1 challenge</td>
<td>9</td>
<td>2</td>
<td>1*</td>
</tr>
<tr>
<td>Total abortion incidence</td>
<td>73% (11/15)</td>
<td>13% (3/23)</td>
<td>5% (1/22)</td>
</tr>
</tbody>
</table>

*Fetus negative for both BVDV and BoHV-1.
Conclusions

Results of this extensive 32-month university research study confirmed that annual administration of CATTLEMASTER GOLD FP 5 or BOVI-SHIELD GOLD FP 5 to heifers/cows vaccinated pre-breeding with a MLV vaccine achieved excellent and comparable maternal and fetal protection against BoHV-1 and BVDV types 1a, 1b, and 2. Heifers vaccinated with BOVI-SHIELD GOLD FP 5 (MLV) twice pre-breeding and then subsequently vaccinated with either CATTLEMASTER GOLD FP 5 (killed BVD vaccine) or BOVI-SHIELD GOLD FP 5 (MLV) during mid-gestation were protected against multiple-strain BVDV challenge 11 months later (reduced viremia and BVDV-positive abortions compared to non-vaccinated controls). Pregnant cows were also protected against fetal challenge with intravenous BoHV-1 administered 3 months post-vaccination (reduced IBR abortions vs controls).

Study outcomes suggest that veterinarians and producers can enjoy more options and flexibility in designing pregnant cow vaccination programs. The ability to transition to an effective temperature-sensitive IBR and BVD killed-vaccine program using CATTLEMASTER GOLD FP 5 can help ensure optimal, uninterrupted disease control and fetal protection for operations where circumstances prevent continuation of a MLV program with BOVI-SHIELD GOLD FP 5.
References

1. Data on file, Study Report No. 1ICARGBIO02, Zoetis Inc.