What can the BioPYRN blood-based pregnancy test tell you?

- Iowa goat producer provides data for a comparative study -

Mark Hostetler, Bethel Farm
Chuck Passavant & Doug Pals, BioTracking, LLC

A study was completed with Mark Hostetler on his "Bethel Farm" in Iowa comparing BioPRYN with transabdominal ultrasound in dairy goats in November 2008. BioPRYN is a blood-based pregnancy test which detects in presence of Pregnancy Specific Protein B (PSPB), and is accurate at 30 or more days post breeding. BioPRYN is manufactured by BioTracking, LLC located in Moscow, Idaho. The Hostetlers live on a 160-acre farm near Parnell, Iowa. Mark and Gwen and their family (Anna, Marie, Micah and Danielle) have been in the dairy goat business for 13 years. Mark's parents raised goats because Mark and his sister were lactose intolerant and naturally Mark raised goats for his FFA projects and has been involved with goats ever since. The focus of Bethel Farm is high production with a 2009 rolling herd average of 2933 pounds. As a result of this study, Mark commented, "I decided that blood testing for pregnancy is an important tool to use for reproductive efficiency."

Mark first used BioPRYN on 15 does in March 2006. He had used the test on a selected number of does between 2006 and 2009. In early fall 2008, Doug Pals, Director of Marketing for BioTracking, was talking on the phone with Mark about BioPRYN testing. He shared that an ultrasound technician was scheduled to transabdominal ultrasound 180 does. It was at this time that the idea to draw blood for a pregnancy test on those does the same day the ultrasound was done would be a great comparative study between ultrasound and BioPRYN on goats. Mark's wife, Gwen, is a nurse so it worked out that the blood sample could be drawn without too much disruption with the ultrasound process.

Blood samples were drawn on 162 does on November 15, 2008. Samples were shipped to BioTracking via 2-day FedEx and the report was sent to Mark via email. The BioPRYN report included an optical density reading for each goat reflecting the amount of PSPB protein in the blood sample. Based on the optical density readings, each doe was placed in one of four categories: pregnant, pregnant recheck, open or open recheck.

After all of the does had kidded Mark sent the ultrasound data (open, pregnant and number of kids), demographic information on the goats, and kidding data which included the date of kidding and the number of kids delivered, to BioTracking.

The 162 goats included the Alpine (60%) and Saanen (40%) breeds. Mark classified 37 of the goats as small, 31 as large and 86 as medium size. There were 8 goats not included in this size classification because the doe died or her embryo died. Out of the 162 does 14 were not tested with the ultrasound because they were bred 30 or less days at the time of testing. Those 14 were tested using BioPRYN to determine of the blood-based test was accurate less than 30 days post breeding. From the BioPRYN report, and the ultrasound and kidding data the following summary was developed:

The results show that BioPRYN and ultrasound were in agreement on 146 does and there were four animals in which the tests did not agree. Ultrasound called one goat open that BioPRYN called pregnant. The doe was injected with prostaglandin (Lutalyse) and aborted two kids. The other three disagreements were minor. Ultrasound called one "weird" and BioPRYN

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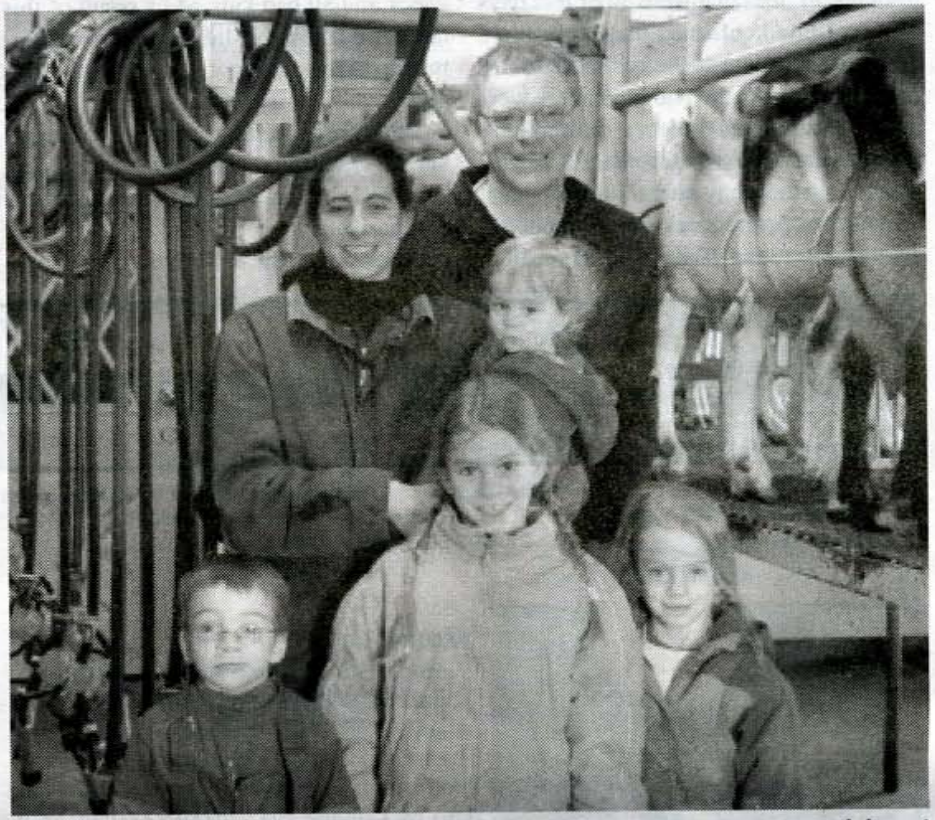

The Mark and Gwen Hostetler family of Parnell, Iowa operate a commercial goat dairy and helped provide data for the study.

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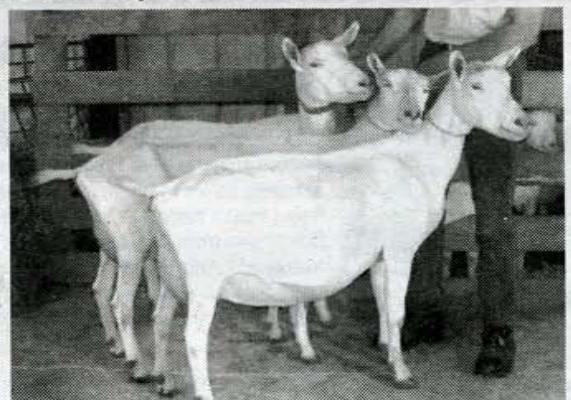

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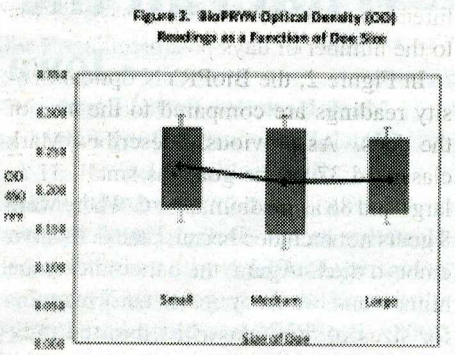
called her open (no kid). BioPRYN called one doe pregnant recheck and another open recheck. These two does did not have a follow-up test, and both does kidded. Data from the 14 does that were 30 or less days post breeding confirmed that 30 or more days post-breeding is the best time to test does with BioPRYN. Although there were only 14 does in this category, the data supported observations that the BioPRYN test was calling too many goats either pregnant recheck or open recheck when blood was drawn earlier than 30 days post breeding. Out of the 14 does only the BioPRYN tests for three does matched their kidding data. Three others were called open and actually had their kids on time. The remaining 8 were either pregnant recheck or open recheck and all had kids. As a result of this data BioTracking did raise the recommended days to draw a sample to 30 or greater days post breeding.

Days post-breeding, doe size and number of kids, were compared to the does' BioPRYN optical density readings. These comparisons were in response to many questions over the last several years asking if the optical density reading were correlated with the age of the embryo or fetus; the breed or size of the doe; or with the doe carrying twins or triplets. See Figure 1 for comparisons of BioPRYN optical density as a function of embryo age.

The bars in Figure 1 depict the maximum and minimum optical density reading (which reflects the amount of PSPB in the serum) for each of the three days post breeding ranges: 26-58; 60-79; and 80+ days. The range in OD values as indicated by the bar plots is related to the individual differences in reproductive physiology of the does. The diamonds within each of the bars are the means for those ranges. There



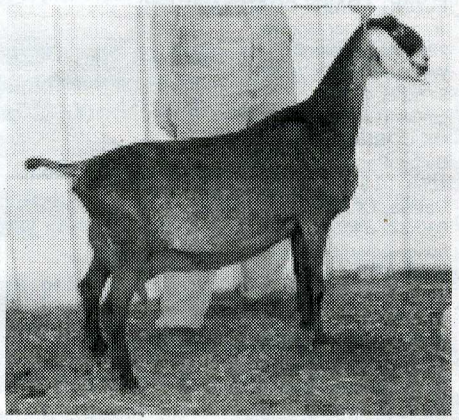
were no significant differences among the three ranges in optical densities in relation to the number of days post breeding.

In Figure 2, the BioPRYN optical density readings are compared to the size of the does. As previously described Mark classified 37 of the goats as small, 31 as large and 86 as medium in size. There were 8 goats not included because the doe or her embryo died. Again, the bars indicate the highest and lowest optical density readings for sizes of does classified into the three groups: small, medium and large. The diamonds in each of the size classifications represents the mean of the optical density values for that doe size category. There were no differences detected in the optical density readings when compared to doe size.

The data in Figure 3 compares the number of kids the doe was carrying when she was pregnancy tested to the optical density reading for the BioPRYN pregnant test. The does were placed into four categories: open, one kid, two kids and 3 kids. The bars represent the maximum and minimum optical density readings for each of the four categories. The diamonds within each category represents the means of the optical density readings for that category. There is a significant difference in open does because PSPB is produced only in response to a viable embryo. Although the size of the embryo, or the presence of multiple embryos probably contributes to the amount of PSPB in the serum, the results presented in Fig. 3 indicate that the current format of goat BioPRYN assay cannot determine any difference in the number of embryos/kids the does are carrying.

When analyzing the data from this study, although optical density readings (i.e. PSPB levels) can vary depending on the individual does, it is apparent that the optical density readings have no relationship to the number of kids, breed or size of the doe. The data, however, do indicate that the BioPRYN blood-based test is an accurate method of pregnancy detection in goats 30 or more days post breeding. BioPRYN is a useful tool for goat producers and veterinarians to keep in their tool box.

Dr. Chuck Passavant, Senior Research Scientist at BioTracking, commented that the study was important in developing future research for improving the BioPRYN pregnancy test for goats. Goats are known to produce several different types of pregnancy specific proteins, and it is believed that these different forms may be produced in different amounts and at different times during gestation. One of the current research projects at BioTracking is directed at isolating and identifying antibodies that react specifically with these different protein forms. If other forms or different amounts of pregnancy proteins are produced at specific times during gestation, these antibodies could prove useful in reducing the number of days post breeding required for an accurate pregnancy determination, and possibly for determining embryo age or number in a future, improved BioPRYN assay.



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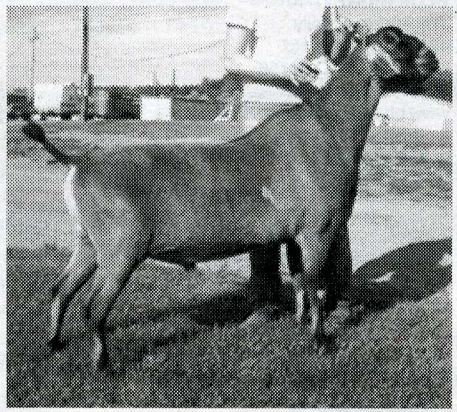

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