Will She Calve? Test May Detect Pregnancy Early

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Agricultural Communications

A dairyman takes a drop of blood from the tail vein of a cow bred just 2 weeks earlier. He mixes the drop into a specially prepared sampling vial.

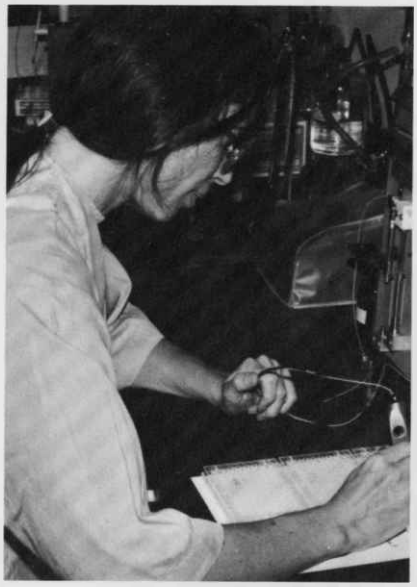
If the mixture turns color, this cow is pregnant. If not, she needs to be bred again to conceive a calf, and subsequently start producing milk.

This scenario is fiction now. Dairymen can rarely tell if a cow is pregnant until 2 months or more after conception.

However, research by UA animal scientist Dr. Gerald H. Stott and colleagues makes early detection of pregnancy look possible within a few years. By knowing whether cows are pregnant within 2 weeks of breeding, dairymen could re-breed the non-pregnant ones during their next heat cycle. Compared to current methods, that would reduce the non-milking period for those cows by a month or two, boosting overall productivity. Beef producers could benefit, too. Similar kits for early pregnancy detection might be developed for other species, even humans.

The UA scientists caution that their work might not pan out quickly or at all. However, Stott said, "We're very optimistic with the results so far." He retired in February, but his co-workers are continuing the

Photograph: Dr. Gerald Stott takes a small blood sample from a cow at the UA dairy farm in Tucson. (Photos by Ted Bundy.)



Research assistant Stephanie Moore examines multi-chambered test plates to see which samples produced antibody reactions.

research. They are immunologists Dr. William A. Fleenor of the Animal Sciences Department and Dr. David O. Lucas of the UA College of Medicine.

The key to reliable early detection of pregnancy is finding an antibody that reacts with a factor that is discernably more abundant in pregnant cows' blood than in blood from non-pregnant cows. No one has identified this pregnancy factor, but many scientists suspect its existence as a signal in the bloodstream that stimulates changes in the cow when pregnancy begins.

The next two steps for making detection practical would be to produce the right antibody in large, purified amounts, and to devise a simple method to check for the reaction between the antibody and the pregnancy factor. Stott's group has tentatively solved these two problems for rapid pregnancy detection. They have adapted a new, genetic engineering tool of cell cultures that produce monoclonal antibodies – that is, a single type of antibody produced by each culture of cells. Also, they have developed a new enzymatic method for rapidly measuring extremely low concentrations of antibodies.

The scientists have used these techniques already to produce specific monoclonal antibodies useful for measuring the immunity levels that individual newborn calves have against several costly diseases of cattle.

Recently, Stott, Fleenor and Lucas found 8 "presumptive positive" antibodies that reacted with blood serum from pregnant cows but not with serum from non-pregnant ones. Some of the antibodies also reacted with a crude serum extract from pregnant cows that was prepared by Dr. Janice Barr of the University of Illinois. She sent the extract to Stott and Fleenor to see if a pregnancy factor is detectable by their new measuring techniques.

The samples for the recent tests came from just a few cows, so the difference in their reactions with specific antibodies could be due to factors other than pregnancy.

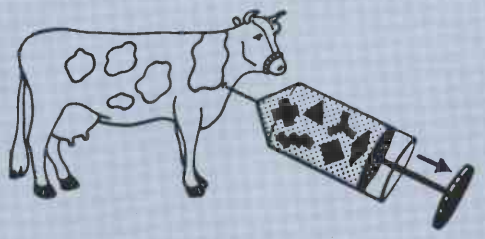
"The next step is to test these antibodies with serum samples from several hundred head of cows – different types of cows in different stages of pregnancy and non-pregnant ones at different stages of their estrus cycles," said Stott. That testing is planned as soon as the antibody-producing cell cultures have grown out enough to give the quantities needed. Meanwhile, the researchers are collecting and freezing serum samples from cows in the university's dairy herd.

Stott hopes that at least one of the "presumptive positive" antibodies will consistently react with samples from pregnant cows and not with samples from other cows. A cell culture that produces such an antibody might be grown out and cloned to become the source for the key ingredient in pregnancy detection kits.

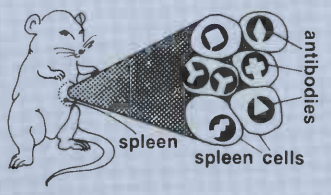
If none of the 8 selected antibodies works, the researchers could create a new field of antibody candidates by repeating their steps for making monoclonal antibodies. Figure 1 shows that process, which generally takes 4 to 6 months to complete.

The genetically engineered hybridoma technique that Stott, Fleenor and Lucas have adapted was invented in 1975 by Georges Kohler and Cesar Milstein. Researchers worldwide have seized it as a way to produce specific antibodies in large quantity.

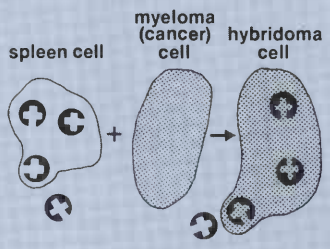
The UA team's first round with 2 injected mice resulted in 80 viable hybridoma cultures, each from a different mouse-spleen cell and each making a single type of antibody. In a screening method they



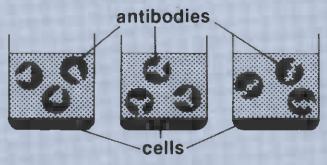
1. A blood serum sample is taken from a pregnant cow. The sample contains many types of factors (proteins and other chemicals) that can act as antigens. One factor may signal pregnancy.



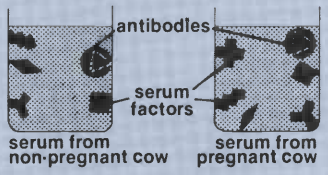
2. The serum sample is injected into a mouse. Cells in the mouse's spleen begin producing antibodies that each can bond specifically with one of the antigens in the serum.



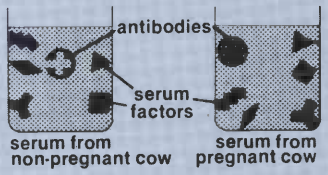
3. Individual spleen cells are artificially fused with a type of mouse cancer cell called myeloma. The result, called a hybridoma cell, carries the spleen cell's ability to produce one type of antibody and the cancer cell's ability to survive well and reproduce rapidly.



4. Hybridoma cells are grown into cultures. Each culture descends from a single fused hybridoma cell, so each culture produces only one type of antibody. That antibody becomes concentrated in the fluid in which the culture grows.



5. The antibodies from each culture are tested against samples of serum from pregnant and non-pregnant cows. Some react with factors present in both types of serum.



6. Some react with nothing in the samples. The aim is to find one type of antibody that reacts with a factor only in the pregnant cow's serum. That antibody is then a candidate for further testing in pregnancy detection.

(Illustrations by Elizabeth Wolf.)

developed for earlier hybridoma work, they checked for reactions to serum samples from pregnant and non-pregnant cows. That is how they identified the 8 "presumptive positive" antibodies. Their screening method uses an enzyme and other chemicals to turn the antibody reaction into a measurable color response. The reaction shows up even if only one out of every 100 million molecules in the sample is the type that reacts with the antibody being screened.

Fleenor, a former graduate student of Stott's, worked out most details for the color test. He described the task of finding an antibody for an unknown factor in pregnant cows' blood as "looking for a needle in a haystack." But he continued, "How do you find a needle in a haystack? You burn down the haystack and use a magnet. This technique is our magnet." It can zero in on the one antibody being sought in a complex mixture of thousands of antibodies.

First, the hybridoma technique breaks the mixture of antibodies into discrete, testable units. Then, the screening technique identifies which of those singled-out antibodies reacts with the factor characterizing pregnancy.

"People have been trying to isolate that specific factor for years, but haven't been able to do it," said Stott. "This is definitely the way we'll have to go to find it . . . I think it's going to happen. It may be a long time, but it may be right away."