THE ANTIGENICITY OF BOVINE SPERMATOZOA

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That spermatozoa exhibit antigenic characteristics has been previously well described by Henle (5) and Henle et al. (6) in their work with the parenteral injections into rabbits of whole sperm and supersonically separated heads and tails of sperm. Landsteiner and Levine (7) also have demonstrated the antigenic activity of spermatozoa by showing that the sperm cells of humans of the appropriate blood type would adsorb specifically and almost completely the immune antibodies (from rabbits) to the A and B antigens of human erythrocytes. Cooper (2) reported from studies of the common leopard frog that antigens closely related to or identical with those of adult sperm were present in the eggs, embryos and larvae. The work of Burke et al. (1) established that demonstrable amounts of antigens exist in chick embryos which resemble the antigens of the adult sex organs.

The results reported in this paper deal with the development of techniques by which the antigens recognized in bovine blood cells by Ferguson (3), Ferguson et al. (4) and Stormont (8) can be recognized in the spermatozoa of bulls.

METHODS

Semen samples were obtained at intervals during this study from certain bulls through the cooperation of The Central Ohio Breeding Association. The semen was centrifuged and the fluid discarded, after which the spermatozoa were washed three times in 0.9 per cent saline solution. Following the last centrifugation, the supernatant was discarded and the packed spermatozoa were resuspended in saline solution to make a 5 per cent suspension.

Blood samples from the bulls, and from other animals used in certain of the tests to be described, were collected from the jugular vein in 3.5 per cent sodium citrate solution. The erythrocyte suspensions used in the tests were prepared by washing the cells three times in 0.9 per cent saline solution, after which the cells were resuspended in saline to make a 3 per cent suspension.

The lytic test, which was used to determine the antigens present in the blood, was that described by Ferguson (3) and used routinely in this laboratory in the blood typing of cattle. This technique consists of mixing 0.1 ml. of serum reagent for a particular antigen, 0.05 ml. of the erythrocyte suspension to be tested and 0.05 ml. of fresh rabbit serum as a source of complement. Thirty-two of the serum reagents, each prepared from isoimmune serum and each containing antibodies for a single antigenic component of the bovine cell, were used in this study.

Preliminary trials using the methods of Henle et al. (6) indicated that the

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spermatozoa were agglutinated specifically by the serum reagents but that the reactions were apparent only upon careful microscopic examination. In an attempt to obtain a more critical method of demonstrating the specific antigen-antibody reaction, an indirect method was developed using the lytic test as an indicator system.

The technique finally adopted was as follows: 0.05 ml. of the sperm suspension, diluted approximately 1:64 in 0.9 per cent saline, was added to 0.1 ml. of the serum reagent. This mixture was incubated 30 min. at room temperature (25 to 28° C). To this mixture was added 0.05 ml. of suspension of red blood cells, known from previous blood typing to be reactive with this particular serum reagent, and 0.05 ml. of fresh rabbit serum as complement. A parallel tube containing the serum reagent, the same red blood cell suspension and complement served as a control. The amount of lysis was recorded after 0.5, 1.5 and 3 hr. Controls on the complement and saline with each blood cell suspension were included in each test.

Repeatable results required a careful standardization of all materials used in the test. A dilution of each serum reagent was used which contained just enough antibody to produce complete or nearly complete lysis of the cells in a lytic test. Because the spermatozoa were anticomplementary in the more concentrated suspensions, it was necessary to determine by titration, prior to each test, the lowest dilution of spermatozoa which exerted no inhibitory effect on the complement.

Young, healthy sheep were used in a study to determine the antigenicity of bull spermatozoa. Ten ml. of a 5 per cent suspension of washed bull spermatozoa were injected intravenously in a ewe at weekly intervals for eight consecutive injections. The immune serum was collected from the ewe 3 days following the last injection and was stored at -20° C.

RESULTS AND DISCUSSION

Three bulls were chosen for these studies on the basis of the regular availability of semen for the detailed studies to be described. The antigens present in the blood of each of these bulls were as follows:


The spermatozoa of each of these bulls were examined by means of the inhibition test and the following antigens were demonstrated:

Bull 1—A, B, F, H, O (Antigens beyond O were not determined since additional semen was not available.)
Bull 2—A, B, G, P, Y, Z, C', E', M', 2, 3, 6, 10, and 12

A comparison of the antigens demonstrated in the blood and in the spermatozoa of each bull reveals a close correlation. Although the tests were not completed on bull 1, there was agreement for each antigen, except that variable results were
obtained with reagent for antigen H. These results can be explained by the fact that this reagent uniformly produces weak, incomplete serological reactions.

Although antigens F, H and W were demonstrated in the blood of bull 2, they were not found in the spermatozoa. The failure to demonstrate antigen H may have resulted from the weak nature of the serum reagent. An explanation of the absence of antigens F and W in the spermatozoa is not apparent.

Antigen Y was not found in the spermatozoa of bull 3, even though it was present in the blood cells. Except for this apparent discrepancy there was agreement for all the other antigens.

A comparison of the blood test results and the results of the spermatozoa inhibition tests shows that there were some antigens on the red blood cells which could not be demonstrated on the spermatozoa. However, no antigens could be demonstrated on the spermatozoa which were not present, also, on the red blood cells.

Several of the reactions were checked, particularly some of the systems which gave variable results, by means of antibody-adsorption tests. The serum reagent was mixed with washed spermatozoa and after 30 min. the spermatozoa were removed by centrifugation. The adsorbed serum reagent then was tested in a routine lytic test with a blood known to have the antigen in question. The results of these tests showed that bovine spermatozoa adsorbed only those antibodies which were inactivated in the inhibition test. In no instance did the spermatozoa adsorb a reagent which it did not inhibit. These results add additional weight to the validity of the inhibition test.

The antigenicity of bull spermatozoa was demonstrated by immunizing sheep with the washed spermatozoa of bull 2. There are certain antigenic components present in the erythrocytes of sheep which are similar to or identical with those in bovine blood. The blood cells of the sheep used in this immunization were typed and a comparison of the components of the sheep cells and those of bull 2 indicated that antibodies for antigens B, F, G, P, W, Z, C', E', M', 3, 6, and 12 might be expected. Antibodies were demonstrated in the sheep serum by a microscopic agglutination test with bull spermatozoa. Also, antibodies were demonstrated in a hemolytic test using the sheep serum, bovine red blood cells and rabbit serum as complement. Further, the sheep serum, following adsorption with selected bovine erythrocytes, remained weakly reactive with certain bovine bloods. The results suggested that antibodies remained, in very low concentration, for antigens W and 3.

There have been reasons to suspect that the bovine cellular antigens might be present in other tissues of the body, just as antigens A and B of the human blood groups can be demonstrated in tissues other than the erythrocytes. This work shows that most of the bovine cellular antigens which were present on the red blood cells also were present on the spermatozoa.

**SUMMARY**

Iso-immune serum, containing antibodies for bovine erythrocytes, also reacted specifically with bovine spermatozoa.
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Antibodies produced in sheep against bovine spermatozoa caused agglutination of bovine spermatozoa and also produced specific lysis of erythrocytes of certain cattle.

This evidence supports the theory that the antigens previously recognized in bovine erythrocytes have similar or identical counterparts in the spermatozoa.

REFERENCES