United States Patent

Bhattacharya et al.

[15] **3,692,897**

[45] **Sept. 19, 1972**

[54] **IMMUNOLOGICAL METHOD AND COMPOSITION FOR CONTROLLING THE SEX OF MAMMALIAN OFFSPRING**

[72] Inventors: **Bhairab Chandra Bhattacharya,** Omaha, Nebr.; **Gustaaf J. van den Bovenkamp,** Mill Valley, Calif.

[73] Assignee: **Bio-Controls, Inc.,** by said van den Bovenkamp

[22] Filed: **Nov. 4, 1969**

[21] Appl. No.: **873,795**

[52] **U.S. Cl.** **424/85**

[51] **Int. Cl.** **A61k 27/00**

[58] **Field of Search** 424/85

Primary Examiner—Richard L. Huff

Attorney—Flehr, Hohbach, Test, Albritton & Herbert

[57] **ABSTRACT**

An immunological method for controlling the sex of mammalian offspring, making use of spermatozoa which has been previously separated into fractions having the desired sex characteristics an antigens. A substantially pure sperm fraction containing the sex chromosomes of a single type (i.e., X chromosomes or Y chromosomes) is introduced into the body of a mammal in sufficient quantity to produce antibodies in the blood stream. A blood sample is then taken from the mammal, the blood coagulated and the blood serum containing the antibodies isolated. Fresh mammalian sperm is inoculated with the blood serum to inactivate and destroy sperm reactive with the antibodies in the blood serum and the treated sperm used to artificially inseminate the female, thereby inducing conception and offspring of desired sex as determined by the remaining unreacted sperm.

In one application of the invention, antibodies reactive with either the X or Y chromosomes may be added to a dose of semen to cause death to sperm containing that type of chromosome before insemination. Alternatively, the antibodies may be introduced into the female either prior or subsequent to copulation (e.g., in a vaginal jelly or as a vaccine) to provide the possibility of sex selection at conception or possible embryonic death to a fetus of undesired sex.

3 Claims, 1 Drawing Figure

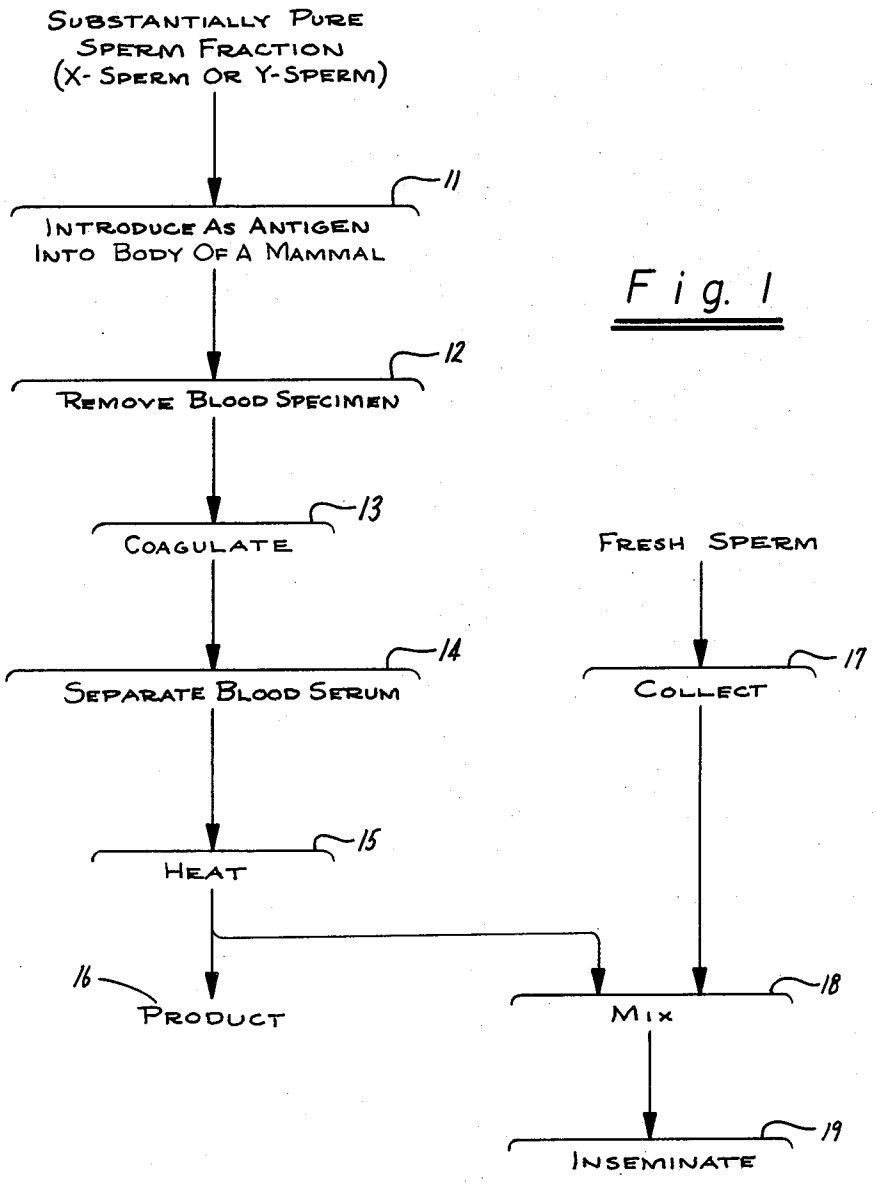


Fig. 1

INVENTORS
Bhairab Chandra Bhattacharya
Gustaaf J. Van Den Bovenkamp
BY
Flehr, Hohbach, Test, Albritton & Herbert
Attorneys

IMMUNOLOGICAL METHOD AND COMPOSITION FOR CONTROLLING THE SEX OF MAMMALIAN OFFSPRING

CROSS REFERENCE TO RELATED APPLICATION

Reference is made to the co-pending application of Bhairab Chandra Bhattacharya, Ser. No. 443,473, filed Mar. 29, 1965, wherein a method and means for separating sperm into fractions containing substantially all X chromosomes, or all Y chromosomes, is disclosed.

BACKGROUND OF THE INVENTION

As pointed out in the aforementioned co-pending application, the sex of offspring is controlled by the chromosomes of the particular spermatozoon or sperm cell which fertilizes the egg. More specifically, some spermatozoa (hereinafter called "sperm") are genetypically known to contain X chromosomes which carry female producing genes, while others contain Y chromosomes which carry male producing genes. As further disclosed therein, the sperm containing X chromosomes (hereinafter called X-sperm) are somewhat more dense than the sperm containing the Y chromosomes (hereinafter called Y-sperm). This difference in density makes possible the separation of sperm in the ejaculation of a mammalian male to obtain substantially pure sperm fractions containing either X-sperm or Y-sperm. The disclosed separation technique is suitable for use with all mammals, including human beings and other primates, cattle, swine (i.e., hogs and pigs), sheep, rabbits, cats, goats, horses, donkeys and buffalo. In general the method of separation is to apply a buoyant force to the sperm to cause the more buoyant sperm to attain a different level in a separation medium than the less buoyant sperm, the buoyant force being either positive or negative. In the technique specifically described, the sperm is separated by sedimentation in a system wherein gravity acts as a negative buoyant force.

In the method disclosed in the above identified co-pending application, the sex of offspring is controlled by artificially inseminating the female with a separated sperm fraction, containing either X-sperm or Y-sperm, to thereby obtain offspring of the desired sex.

SUMMARY OF THE INVENTION AND OBJECTS

This invention relates generally to an immunological method for controlling sex of mammalian offspring, and to compositions useful in providing offspring of one sex. More particularly, the invention relates to an immunological method for isolating antibody compositions reactive with sperm containing sex chromosomes of only one type, and to the use of such antibody compositions to permit conception by sperm containing chromosomes of the opposite type.

The present invention is predicated on our discovery that the two sperm genotypes of mammals (X and Y) possess different antigenic properties, and that this difference in antigenic properties can be employed to create antibodies which are physico-chemically specific in their reactions with respect to either the X-sperm or Y-sperm present in mammalian sperm. The term "antibody" is used herein to identify the substance or substances produced within the body of a mammal in response to the introduction of a substantially pure sperm fraction containing either X-sperm or Y-sperm. Thus, in the practice of the invention, a substantially pure sperm fraction containing sex chromosomes of one type can be injected under the skin or in the abdomen or even in a vein of a mammal, according to normal procedures, and the mammal will produce in its blood chemical substances, viz., antibodies, which will act to inactivate and destroy sperm of the same type as injected. Since the antibodies thus produced are reactive with sperm containing sex chromosomes of only one type, they have no appreciable effect on sperm containing sex chromosomes of the opposite type. This sex selectivity of the antibodies makes possible a choice or selection of the ultimate sex of offspring through use of various techniques, for example, by inoculation of fresh sperm to cause death of one of the two types of sperm present before insemination, or by introduction of the antibody into the vaginal cavity before or after copulation by various known techniques. The present invention accordingly enables parents, breeders of animals, farmers, and others engaged in the field of animal husbandry to deliberately control or select the sex of offspring to obtain a child, calf, colt, or other animal of a particular desired sex.

In general, it is an object of the present invention to provide a truly successful, immunological method for controlling the sex of mammalian offspring.

Another object of the present invention is to provide a method for isolating antibodies in a form capable of entering into antibody reactions with sperm containing sex chromosomes of only one type.

Another object of the present invention is to provide a method for isolating antibodies capable of being used in procedures for artificial insemination of the female, to obtain offspring of the desired sex.

A further object of the invention is to provide novel compositions containing antibodies reactive to inactivate and destroy either X-sperm or Y-sperm, thereby enabling use of such compositions to cause death of one type of sperm in a mammalian ejaculate prior or subsequent to insemination.

A still further object of the invention is to provide novel compositions of the above character which can be positively tested prior to use, to ensure their utility as anti-serums with respect to sperm of particular sex characteristics.

Additional objects and advantages of the invention will appear from the following description in which the preferred embodiments have been set forth in detail in conjunction with the accompanying drawing.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a flow sheet illustrating the method of the present invention.

DESCRIPTION OF PREFERRED EMBODIMENT

As illustrated in FIG. 1, a substantially pure sperm fraction, which may be either substantially pure X-sperm or Y-sperm, is isolated by techniques known or available in the art. For example, substantially pure X or Y-sperm fractions can be isolated in accordance with the procedure of the aforementioned co-pending application, Ser. No. 443,473. While sedimentation is

specifically described therein as a procedure for separating a pure X or Y-sperm fraction, other procedures may also be used, for example, employing forces greater than gravity in a centrifuge or forces to achieve positive buoyancy as well as sedimentation in columns containing media of carefully controlled density. In general, the sperm fraction so isolated is maintained and preserved within a nutrient medium such as fresh mammalian milk or other body fluids and nutrient liquids, as herein disclosed.

Referring to the flow sheet of FIG. 1, the separated, substantially pure fraction of either X or Y-sperm is introduced into the body of a mammal, in step **11**, as an antigen, the purpose being to induce antibody production in the blood of such mammal. In accordance with known procedures, the antigen sperm fraction can be injected in successive doses to induce antigenic stimulation, leading to the highest possible levels of antibody formation. In due course a blood sample is removed from the mammal, in step **12**, and the blood allowed to coagulate, as in step **13**. The coagulation of the blood (a phenomenon related to clotting of the plasma) causes the non-coaguable blood sperm to separate from the blood cells and coagulated plasma, as represented in step **14**. The blood sperm separated in step **14** is of primary importance to the method of the present invention, for it contains the antibodies developed as a result of introduction of the sperm fraction as an antigen. While the precise mechanism by which the antibodies are formed in the blood stream of the mammal is not known or fully understood, it is evident that the antibodies are finally present in the blood serum as separated in step **14**. In a preferred practice of the invention, the blood serum separated in step **14** is inactivated by heating in step **15**, viz, at a temperature of 56°C. for a period of about ½ hour. The blood serum containing antibodies of desired type is now in form suitable for carrying out antibody reactions in accordance with the present invention. If desired, the blood serum in this form may be separated and held as an intermediate product, represented at **16**.

As further illustrated in the flow sheet of FIG. 1, fresh sperm is collected from the male, in step **17**, and in such form contains equal amounts of X-sperm and Y-sperm. The fresh sperm can now be mixed with the blood serum or antiserum, in step **18**, to initiate the antibody reactions previously described. Assuming the use of X-sperm as an antigen in step **11**, the antibody reactions will be specific to the X-sperm present in the freshly collected sperm and will act to cause death of the X-sperm. Alternatively, where Y-sperm is used as the antigen in step **11**, the antibody reactions will cause death of the Y-sperm present in the freshly collected material. The resulting sperm, containing approximately equal amounts of inactive X-sperm (or Y-sperm) and viable Y-sperm (or X-sperm), as the case may be, is used in step **19** to artificially inseminate a female of the species from which the sperm was taken. It will be understood that prior to insemination, the sperm collected in step **17** may be mixed with an appropriate nutrient medium and gradually cooled, or otherwise preserved, in accordance with the procedures generally described in the aforementioned co-pending application, Ser. No. 443,473.

As noted previously, the immunological method of the present invention is suitable for use with all mammals. Of particular interest are human beings and other primates, cattle, swine (i.e., hogs and pigs), sheep, rabbits, cats, goats, horses, donkeys and buffalo. In general, the antigenecity of a mammalian cell is under the influence or regulated by the cell nucleus. In the case of mammalian sperm, the X-sperm and Y-sperm differ in chromosomal structure and very possibly in other properties. For example, in microscopes, the X chromosomes appear larger in size than the Y chromosomes. The probability of a difference in antigenic properties between X-sperms and Y-sperms is believed to be related to this difference in chromosomal structure and to contribute in a direct way to the capacity of a mammal to create different antibodies when injected with either X or Y-sperm. Accordingly, a sperm fraction effective to carry out the present invention should contain a sufficient proportion of either X or Y-sperm, according to the selection, to ensure some success in achieving a desired proportion of antibodies. Generally this proportion should be at least 70percent. For practical or commercial purposes, however, at least 80 to 90 percent of the sperm in the antigen sperm fraction should possess a known sex characteristic to ensure that the chance of success in producing antibodies of the desired type is statistically tolerable. Of course, a sperm fraction containing 100 percent sperm of known type is the most desirable since there then is no chance of error. Also, a high proportion of sperm of known sex characteristics in the antigen sperm fraction makes possible a simple test of the anti-serum capacity of the blood serum (obtained in step **14**), as hereinafter described.

The disclosure herein makes reference to an "immunological" method for controlling the sex of mammalian offspring. In a strict technical sense, the analogy to techniques for acquiring or imparting immunity is somewhat inaccurate, since sperm are not properly classified as pathogenic or infectious organisms. On the other hand, the present invention makes use of available techniques for preparing immune serums, or antiserums. To illustrate, procedures for artificially generating passive immunity in mammals most generally involve the introduction of the undesired microorganism into the body of an experimental animal, commonly the rabbit, horse, goat or the sheep, to thereby induce production of antibodies against the injected microorganism. Thus, in conventional immunization technology, active immunity is effected by stimulating the production of antibodies through use of the appropriate infectious microorganism as an antigen. In the present case however, the antigen is constituted by living or dead microorganisms in the form of either X-sperm or Y-sperm. Although the techniques employed in the present invention may be equated in terms to those used in conventional procedures for providing passive immunity, (e.g., an anti-serum may be obtained from a mammal which has been previously hyperimmunized to X or Y-sperm through production of antibodies) the "immunity" actually provided is not to infection or disease in the normal sense, but rather to birth of offspring of undesired sex. Moreover, while such immunity may properly, be classified as immediate or of short-term, it is absolute in the sense that

conception is prevented by destruction of the unwanted type of sperm. Consideration of the present invention should therefore take into account the foregoing distinctions as well as analogies.

As a generalized example, illustrating the practice of the invention, anti-serums containing antibodies against either X-sperm or Y-sperm can be prepared as follows:

Fresh bull sperm is subjected to processing by sedimentation or centrifugation, as described in the copending application, Ser. No. 443,473, to obtain a substantially pure sperm fraction (e.g., 20,000,000 sperm of which approximately 80 percent are X-sperm). The bull sperm is washed out of the separation medium by centrifuging in a tube at 2,000 r.p.m. for 10 minutes, the centrifugal force thus developed causing the X-sperms to move to the bottom of the centrifuge tube After decanting the supernatant, the sperm are suspended in saline (0.9 percent NaCl in distilled H_2O) and then washed two more times by an identical procedure, viz, involving centrifuging at 2,000 r.p.m. for 10 minutes, decanting the supernatant, and resuspension of the sperm in saline. After adding antibiotics (e.g., penicillin, streptomyocin, etc.) in appropriate amount, the thrice washed sperm is injected subcutaneously or intra-abdominally into rabbits in successive injections, each injection involving the introduction of approximately 18 to 20 million X-sperms. Following a procedure customarily used to achieve hyper-immunization, the rabbits are injected two times a week over a 6 week interval to thereby achieve a total injection level of approximately 200,000,000 X-sperms. It should be noted that before initiation of each of the foregoing injection series, the experimental rabbit was tested for eventual spontaneous agglutination of bull sperms, and any rabbits showing such agglutination were eliminated. At the end of the 6 week period of injections, a blood sample was taken from each test rabbit by a procedure involving opening of a suitable blood vessel (i.e., an ear vein) from which the blood was caused to flow into a container. Blood specimens typically averaged 4 to 5 cc. Blood serum was prepared in conventional manner by letting the blood stand for a sufficient period of time (e.g., overnight) to achieve coagulation. Clear non-coaguable blood serum (e.g., approximately 3 cc.) was thereafter removed from the coagulum with a Pasteur pipette, and placed in a small test tube or other suitable container.

The blood serum obtained by the foregoing procedure was tested for its capacity to act as an agglutinin or precipitin with respect to both X and Y-sperms, using the test method as generally described by S. Kibrick, et al. in Volume 3 of the Journal of Reproductive Fertility, 1962. In accordance with this procedure, the blood serum is diluted with saline (0.9 percent NaCl in distilled H_2O) to achieve dilutions of 1:10, 1:100 and 1:200. Two sets of dilutions were prepared each containing about 0.2 ml. of diluted anti-serum. To one set of the dilutions substantially pure fractions of X-sperm in saline (i.e., approximately 10,000 sperm in a total volume of 0.2 ml.) were added. The approximately equal volumes of sperm suspensions and anti-serums were mixed by sucking into a Pasteur pipette and releasing to ensure thorough intermixing. To the other set of dilutions was added a substantially pure Y-sperm fraction (i.e., approximately 10,000 sperm in saline suspension to a total volume of 0.2 ml.) using an identical procedure. The two sets of dilutions containing alternatively X and Y-sperm were then placed in an incubator at 37° C. for 20 minutes. Following this period of incubation, the two sets of dilutions were checked for agglutination.

The following table shows the degree of agglutination separately caused by adding X-sperm or Y-sperm to the different dilutions of anti-X-sperm in saline.

Saline Dilution	Degree of Agglutination X-sperm	Y-sperm
1:10	Marked	None
1ϕ	Moderate	None
1:200	Slight	None

The foregoing table shows that the blood serum obtained from rabbits injected with X-sperm contained antibodies against X-sperm but no antibodies against Y-sperm.

From the foregoing it will be apparent that the immunological method of the present invention has utility wherever it is desired to control the sex of mammalian offspring. It is of extreme practical and commercial importance in the field of animal husbandry, for example, in permitting the breeder or farmer to have a choice in selecting the sex of animal offspring. By way of illustration, the dairy framer can elect to obtain only female offspring and thereby advantageously breed only milk producing cows rather than bulls. As respects human procreation, it provides parents with a simple, easily employed means to select or control the sex of offspring to quickly satisfy the desire to have a child of a particular sex, thus providing the opportunity to reduce the total number of children desired.

We claim:

1. In a method for isolating antibodies capable of entering into antibody reactions with sperm containing sex chromosomes of only one type, said antibodies having no appreciable effect on sperm containing sex chromosomes of the opposite type, the steps of introducing a separated substantially pure sperm fraction containing sex chromosomes of a single sex type into the body of a mammal, said sperm fraction containing sperm in sufficient quantity to induce production of antibodies in the blood of said mammal, removing a portion of the blood of said mammal, coagulating the blood so removed and separating therefrom the blood serum containing antibodies reactive only to sperm of the sex type present in said sperm fraction.

2. A method as in claim 1 wherein the said sperm fraction is introduced into the body of the mammal in successive steps, each step involving the introduction of a portion of the separated sperm fraction.

3. A method as in claim 1 wherein the blood serum containing antibodies is inactivated by heating at a temperature of the order of 56° C.

* * * * *