

1 469 813

PATENT SPECIFICATION

(11) 1 469 816

- (21) Application No. 15975/74 (22) Filed 10 April 1974
 (31) Convention Application No. 2321000 (32) Filed 19 April 1973 in (19)
 (33) Federal Republic of Germany (DT)
 (44) Complete Specification published 6 April 1977
 (51) INT CL² C12K 1/00//B03D 1/00 3/00
 (52) Index at acceptance:
 C6F 3 4



(54) PROCESS FOR THE EXTRACTION OF SPERMS HAVING A DESIRED MOTILITY

(71) We, SCHERING AKTIENGESellschaft, a Body Corporate organised according to the laws of Germany, of Berlin and Bergkamen, Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with a process for the extraction of sperms from the semen of animals according to the motility of the sperms, and especially with a process for the extraction of sperms having a Y-chromosome from the semen of animals and the use of such extracted sperms for predetermining the sex of animal offspring in favour of the male sex.

By the "Y-sperm" there is to be understood herein a sperm having a Y-chromosome and by the term "X-sperm" there is to be understood herein a sperm having an X-chromosome.

In the case of mammals their sex is determined by two different types of sperms, which have either an X- or Y-chromosome. Fertilization with Y-sperms leads to male offspring, and fertilization with X-sperms leads to female descendants. The assumption that the two kinds of sperms must differ in size and weight has lead to a series of investigations, in which the separation of the X- and Y-sperms has been attempted by sedimentation, centrifuging, electrophoresis, variation in pH-value or flotation. The desired separation has, however, not been achieved or achieved only to a minor extent by these methods.

The importance of the desired separation of Y-sperms of mammals is obvious to an expert. It need be illustrated only by a few examples. If a method of separation were available, then it would be possible, for example, for an animal breeder to predetermine in the desired manner the sex of the expected offspring by inseminating the animals with X- or Y-sperms. In breeding cattle, when meat production is of importance, insemination with Y-sperms would be effected. As wethers, after a given breeding period (for example, up to weaning or ablactation), are generally heavier than ewe lambs and meat production is of primary importance to sheep breeders, insemination would be effected preponderantly with Y-sperms in sheep breeding.

Such a method is also of great importance for human beings. The desire for a child of a particular sex is in many families, *inter alia*, one of the reasons for their having a large number of children. A method of separating X- and Y-sperms would considerably facilitate family planning, and it is believed that as a result the size of the population and the problems connected therewith would be considerably reduced.

It is an object of the present invention to provide a new process for helping to meet this widely spread need. A further object is to provide a new process for the extraction of sperms having a desired motility, which finds application in the treatment of sub-fertility.

The present invention provides a process for the extraction of sperms having a desired motility from a dispersion of a physiologically tolerable liquid of mammalian sperms some of which have the desired motility and others have a motility lower than the desired motility, wherein the dispersion is brought into interfacial contact with a physiologically tolerable fluid medium of higher density than the liquid in which the sperms are dispersed, the contact between the dispersion and the fluid medium being maintained for a period sufficient to allow sperms having the desired motility to pass into the medium.

The invention also provides a process for increasing the proportion of Y-

sperms in mammalian sperms from a dispersion of Y-sperms and X-sperms in a physiologically tolerable liquid, wherein the dispersion is brought into interfacial contact with a physiologically tolerable fluid medium of higher density than the liquid in which the sperms are dispersed, the contact between the dispersion and the fluid medium being maintained for a period sufficient to allow Y-sperms to pass into the medium.

The sperm-containing dispersion may be semen, either as such or diluted with a physiologically tolerable liquid. As however, semen is viscous even when diluted it is preferred to separate the sperm from the seminal fluid by centrifugation, preferably by first mixing it with a Tyrode solution, and re-suspend the sperm in a fresh physiologically tolerable liquid, for example, a Tyrode solution, before contacting it with the fluid medium.

The fluid medium may be entirely different from the liquid in which the sperms are dispersed. Preferably, however, they are essentially the same, for example, a Tyrode solution, with the fluid medium containing an additive which increases its density. The liquid in which the sperms are dispersed and the fluid medium may, and generally will be, miscible but in carrying out the process of the invention it is important that they form discrete layers.

We believe that the difference in density and viscosity between the liquid in which the sperms are dispersed and the fluid medium provides a barrier at their interface, which barrier has the effect of tending to separate motile sperms from immotile sperms, morphologically normal (that is healthy) sperms from abnormal (less-vigorous) sperms and Y-sperms from X-sperms. It is possible, because the progressive motility (that is active swimming movement) of the Y-sperms is greater than that of the X-sperms, to extend the swimming distance and isolation time to a point where the already more prevalent Y-sperms can continue to outdistance their remaining counterparts. Therefore, motile Y-sperms can be said to have greater ability to penetrate an interface between fluids and between discontinuous gradients of an extraction medium. Y-sperms must also swim faster than X-sperms under the handicap of swimming in a fluid with, in relative terms, high density and viscosity.

It is possible, therefore, by the process of the present invention to extract a population of sperms that are predominantly Y-sperms, almost entirely progressively motile and physically fit.

By the process of the present invention Y-sperms can be extracted from the semen of all mammals. Of special interest, on the one hand, is the extraction from the semen of mammals, which are used in agriculture or in breeding for other economic purposes, for example primates other than man, horses, cattle, pigs, sheep, goats and rabbits, and, on the other hand, the extraction from the semen of human beings.

The present invention also provides a method for the predetermination of sex of offspring in favour of the male sex, which comprises fertilization of a female mammal with Y-sperms that have been obtained by the aforesaid preferred embodiment of the process of the present invention. By this method, for example, a human female, cow or ewe may be fertilized by artificial insemination with such extracted Y-sperms.

The present invention further provides a preparation for predetermining the sex of a mammal in favour of the male sex, which comprises an extract containing Y-sperms that has been obtained by the process of the present invention in admixture or conjunction with a physiologically tolerable carrier.

The liquid in which the sperms are dispersed (either by diluting semen with the liquid or re-suspending the separated sperms in it) must not weaken the fertility of the sperms to be subjected to extraction, that is to say, it must not damage or destroy the sperms. On the other hand, the liquid should preferably contain nutrients, which keep the sperms in the semen alive, if desired for a long period. The liquid may have a pH-value within the range of from 7 to 8, and should also have an osmotic pressure at which the sperms are not compressed or disintegrated. As such liquid media there come into consideration, for example, physiologically tolerable solution, for example Tyrode solution, Ringer solution, Hank's solution, physiological sodium chloride solution, Medium 199 and Eagle's Medium which may be adapted to the desired conditions by additives known to those skilled in the art, for example salts, low molecular weight organic compounds, for example mono- or oligo-saccharides, amino-acids, peptides, proteins or proteids, or polymers, for example polypyrrolidone. If the extracted Y-sperms are first to be stored under appropriate conditions, for example when they are intended for sperm banks or depots, it is preferable to start from semen dispersed in solutions; in other cases, however, it is also possible to start from undiluted semen.

With regard to the fluid medium used for the contacting, in general the same

(12)

preconditions apply as those mentioned above for the first liquid in which the sperms are dispersed. Thus, there come into consideration all physiologically tolerable solutions, for example those mentioned above which are adapted by means of additives known to pharmacologists and physiologists to the desired conditions, especially to the higher density. The fluid medium used in accordance with the present invention may thus consist of a further quantity of the liquid used in the sperm-containing dispersion and at least one additive. Among the additives there may be mentioned, for example, salts, low molecular weight or high molecular weight organic compounds, for example mono- or oligo-saccharides, amino-acids, peptides, proteins, for example bovine serum albumin, ovalbumin or bovine α -globulin, or proteids, or polymers, for example poly-pyrrolidone.

If some of the additives used for producing the desired density are not completely soluble in the starting solutions, there may be used as contacting fluid media suspensions, sols or gels. The fluid medium used for contacting the sperm-containing dispersion should have a higher density than the liquid in which the sperms are dispersed, because the density gradient has a decisive influence on the rates at which the sperms enter the contacting medium and hence the degree of separation of the sperms. An increase in the density gradient causes an increase in the degree of separation of, for example, the Y-sperms from the X-sperms. At a very low density gradient it is possible for the semen to lower the efficacy of the separation of Y-sperms from X-sperms to such an extent that only motile sperms are separated from immotile sperms without a substantial separation of Y-sperms from X-sperms.

The present invention further provides a method of fertilization, which comprises fertilization of a female mammal, with motile sperms that have been obtained by the motile sperm-extraction process of the present invention. By this method, for example, a human female, cow or ewe may be fertilized by artificial insemination with such extracted motile sperms.

A logical clinical application of the "cleaned up" sperm fraction from which non-motile and many morphologically abnormal forms have been excluded would be for artificial insemination using sperms from sub-fertile men.

The density gradient between the liquid in which the sperms are dispersed and the fluid medium which may be used for separating the motile sperms from immotile sperms or for separating Y-sperms from X-sperms depends on the nature and quality of, for example, the mammalian semen used. In general, the density gradient required between the liquid and the fluid medium of higher density is 1 to 50%, and a density gradient lower than 3% results preponderantly in the separation of, for example motile sperms from immotile sperms, for the separation of, for example human sperms the density gradient required to separate motile sperms from immotile sperms is approximately 1% to 5%, preferably 3% to 5%, and for the separation of Y-sperms from X-sperms it is approximately 3% to 50%, preferably 10% to 25%.

The sperm-containing dispersion may be brought into interfacial contact with the fluid medium in any desired manner. Care must be taken, however, to ensure that there is no mixing of the dispersion with the fluid medium. One method of contacting the dispersion with the fluid medium is to underlay the dispersion with the fluid medium. It is, however, preferred to overlay cautiously the fluid medium with the dispersion. When the sperm-containing dispersion is brought into contact with the fluid medium, a single contacting, for example an overlaying, leads to a distinct separating effect. This can be increased by repeating the process. The repeated process may be carried out, on the one hand, by contacting not only with one fluid medium, but with a plurality of, for example two or three, physiologically tolerable fluid media of progressively higher density. This may be achieved, for example, by layering the dispersion of sperms over a fluid medium of higher density, which fluid medium has been layered over a second fluid medium still higher in density. Such a repeated contacting carried out as a one step procedure may be carried out by layering discontinuous gradients of, for example, bovine serum albumin or ovalbumin within a single column. On the other hand, the repeated contacting may be carried out by separating the fluid medium, after the contacting, from the already largely separated dispersion of sperms, recovering the sperms contained in the fluid medium, dispersing these sperms in a suitable liquid and contacting this dispersion in accordance with the present invention with a further fluid medium having a higher density. A further possibility is to separate the fluid medium which contains the sperms extracted from the starting dispersion, and then to contact it with a second fluid medium of still higher density.

Sperm recovery (that is the number of sperms that enter the medium or media of higher density) is dependent on sperm motility and the density of the medium(s) of higher density, plus the added factor of time. The distance sperms must travel is

also a factor, but of course the sperms are under gravity and when the medium(s) of higher density is/are below the medium of lower density, non-motile sperms will with time move in the same direction. Sperm recovery is, therefore, kept to the required value by regulating sperm numbers, time and density of the medium(s) of higher density.

The contacting of the dispersion of sperms with the fluid medium may be carried out in vessels of any form, for example, those that are customarily used by those skilled in the art for working with small quantities of liquids, and there may be mentioned, for example, burettes, pipettes, separating tubes and columns having a suitable closing device. As the form of the vessel has little or no influence on this type, of contacting, there are available to a person skilled in the art many forms of construction which he may modify as desired. The time required for carrying out the isolation (or extraction) depends on the species of, for example, mammal, the nature of the liquids employed and the type of isolation or extraction used. When the dispersion of sperms is overlaid with the fluid medium periods within the range of from 0.5 to 10 hours, and preferably within the range of from 1 to 3 hours, are generally sufficient.

The necessary working materials are advantageously made up into the form of a pack suitable for use by a practitioner intending to extract, for example, Y-sperms and employ them for insemination purposes.

The present invention accordingly further provides a pack for carrying out the process of the present invention, which comprises:

- (a) a vessel for receiving small quantities of liquids as discrete layers, for example a Pasteur pipette,
- (b) a physiologically tolerable liquid for mammalian sperms, for example, one of the liquids described above, for example, a Tyrode solution,
- (c) a physiologically tolerable fluid medium for mammalian sperms which is of higher density than physiologically tolerable liquid, for example, a Tyrode solution containing bovine serum albumin,
- (d) instructions for carrying out the process, and, if desired,
- (e) an insemination syringe, for example, one generally known to those skilled in the art.

Since the fluid medium can generally be prepared from the liquid used for the dispersion of sperms, the present invention includes within its scope a modification of the pack, as defined above, in which the component (c) is replaced by at least one additive which when added to the liquid used for the dispersion increases its density. Component (c) may therefore consist of, for example, bovine serum albumin, ovalbumin or bovine α -globulin.

The following Examples illustrate the invention. Before the extraction described in each Example the number and motility of the sperms was determined [cf. *Praktische Andrologie*, C. Schirren, Verlag Bruder Hartmann, Berlin 1971]; numbers of sperm were determined with a haemocytometer. The subsequent isolation (or extraction) was controlled by determining the X- and Y-sperms according to the quinacridine technique [cf. *Acta biol. med. germ.* 28 (1972) 189-92]. When it was desired to recover the sperms this was carried out by centrifuging. Each of the centrifuging operations described in the Examples was carried out at room temperature for 15 minutes at 4,000 r.p.m.. Each of the sealed Pasteur pipettes used in the Examples was a Pasteur pipette heat-sealed at the point of tapering.

Unless otherwise stated, all parts and percentages are by weight, each of the Tyrode solutions had a pH-value of 7.6 and each of the Tyrode solutions containing bovine serum albumin, ovalbumin or bovine α -globulin had a pH-value within the range of from 7.4 to 7.6.

Tyrode solution contains, per 1000 ml of water, the following compounds:—

NaCl	8.0 grams
KCl	0.2 gram
CaCl ₂ · 1H ₂ O	0.2 gram
MgCl ₂ · 6H ₂ O	0.1 gram
NaHCO ₃	1.0 gram
NaH ₂ PO ₄ · 2H ₂ O	0.05 gram
Glucose	1.0 gram

Some data regarding solutions used in the Examples is as follows:—

14

1,469,816

5

Densities of solutions (expressed in g/ml at 24°C)

Distilled water	0.998
Tyroides	1.005

BSA	Ovalbumin
25% 1.075	15% 1.041
20% 1.062	10% 1.028
15% 1.047	6% 1.018
10% 1.033	
6% 1.021	Alpha Bovine Globulin
3% 1.012	10% 1.028

Viscosities of BSA
(pH 7.6; at 20°C)

dynamic viscosity in centipoise	Time
6% 0.68016708	9.4 sec
10% 0.83140448	11.6 sec
15% 1.0923913	15.4 sec
20% 1.4449581	20.6 sec
25% 2.0334804	29.4 sec

Example 1

a) Fresh human semen was mixed in the ratio by volume of 1:1 with a Tyrode solution, and in order to separate the sperms from the other constituents of the semen the mixture was centrifuged. After determining the motility and number of sperms, 50×10^6 sperms were re-suspended in 0.5 ml of a Tyrode solution. Simultaneously a separate Tyrode solution having a pH-value of 7.6 was adjusted with bovine serum albumin (hereinafter in the Examples abbreviated to BSA) to a BSA-concentration of 6% of BSA. 0.9 ml of the BSA-solution was overlaid in a sealed Pasteur pipette with the sperm-containing Tyrode solution. After one hour the supernatant Tyrode solution and the BSA-solution were separated from each other. The sperms in the BSA-solution were recovered, and their number and motility and also the contents of X- and Y-sperms were determined. The results are summarised below:

Tyrode solution: IM=70, FM=25;
6% BSA-Tyrode solution: R=30, M=90-95, Y=63-67.

In this and the subsequent Examples the symbols used have the following meanings:

IM: Initial motility (that is percentage of the sperms initially suspended in the additive free-Tyrode solution that were motile).
R: % of the sperms initially present in the additive free-Tyrode solution that were recovered from the appropriate additive containing-Tyrode solution.
FM: Final motility in the additive free-Tyrode solution (that is percentage of the sperms finally present in the additive free-Tyrode that were motile).
M: Motility of the recovered sperms (that is percentage of the recovered sperms that were motile).
Y: % of recovered sperms that were Y-sperms.

b) 50×10^6 sperms obtained as described in Example 1(a) and containing 63-67% of Y-sperms were suspended in 0.5 ml of a Tyrode solution. 0.9 ml of a Tyrode solution containing BSA in a concentration of 10% was overlaid in a sealed Pasteur pipette with the sperm-containing Tyrode solution. After one hour the supernatant Tyrode solution and the BSA-solution were separated from each other. The sperms in the BSA-solution were recovered, and their number and motility and also the contents of X- and Y-sperms were determined. The results were as follows:

Tyrode solution: IM=90, FM=40-60;
10% BSA-Tyrode solution: R=40, M=95, Y=73-78.

Example 2

Fresh human semen was mixed with a Tyrode solution in the ratio by volume of 1:1, and the mixture was centrifuged to separate the sperms. After determining the motility and number of the sperms, 50×10^6 sperms were re-suspended in 0.5 ml of Tyrode solution. 0.4 ml of a Tyrode solution containing BSA in a concentration of 15% was overlaid in a sealed Pasteur pipette with 0.8 ml of a Tyrode solution containing BSA in a concentration of 6%. The BSA-solution of 6% strength was overlaid with the sperm-containing Tyrode solution. After one hour the uppermost layer was separated off. After a further 30 minutes the remaining solutions were separated from each other. The sperms in the BSA-solutions were recovered, and their number and motility and also the contents of X- and Y-sperms were determined. The results are summarized below:

Tyrode solution:	IM=70, FM=25;
6% BSA-solution:	R=20, M=90-95, Y=60-65;
15% BSA-solution:	R=10, M=95, Y=78-80.

Example 3

a) 50×10^6 sperms obtained as described in Example 1(a) and containing 63-67% of Y-sperms were suspended in 0.5 ml of a Tyrode solution. 0.9 ml of a Tyrode solution containing BSA in a concentration of 10% was overlaid in a sealed Pasteur pipette with the sperm-containing Tyrode solution. After one hour the supernatant Tyrode solution and the BSA-solution were separated from each other. The sperms in the BSA-solution were recovered, and their number and motility and also the contents of X- and Y-sperms were determined. The results are summarized below:—

Tyrode solution:	IM=90, FM=45-60;
10% BSA-solution:	R=45-50, M=95-98, Y=74-78.

b) 50×10^6 sperms obtained as described in Example 3(a) and containing 74-78% of Y-sperms were suspended in 0.5 ml of a Tyrode solution. 0.9 ml of a Tyrode solution containing BSA in a concentration of 20% was overlaid in a sealed Pasteur pipette with the sperm-containing Tyrode solution. After one hour the supernatant Tyrode solution and the BSA-solution were separated from each other. The sperms in the BSA-solution were recovered, and their number and motility and also the contents of X- and Y-sperms were determined. The results are summarized below:—

Tyrode solution:	IM=95, FM=92;
20% BSA-solution:	R=43, M=97, Y=83.

Example 4

Fresh human semen was mixed with a Tyrode solution in the ratio by volume of 1:1, and the sperms were separated by centrifuging. After determining the motility and number of sperms, 50×10^6 sperms were re-suspended in 0.5 ml of a Tyrode solution. 0.9 ml of a Tyrode solution containing BSA in a concentration of 3% was overlaid in a sealed Pasteur pipette with the sperm-containing Tyrode solution. After one hour the supernatant Tyrode solution and the BSA-solution were separated from each other. The sperms in the BSA-solution were recovered, and their number and motility and also the contents of X- and Y-sperms were determined. The results are summarized below. There was achieved preponderantly an extraction of motile from immotile sperms.

Tyrode solution:	IM=65, FM=25;
3% BSA-solution:	R=30-45, M=85-90, Y=57.

Example 5

Fresh human semen was mixed with a Tyrode solution in the ratio by volume of 1:1, and the sperms were separated by centrifuging. After determining the motility and number of sperms, 50×10^6 sperms were re-suspended in 0.5 ml of a Tyrode solution. 0.3 ml of a Tyrode solution containing BSA in a concentration of 25% was overlaid in a sealed Pasteur pipette with 0.3 ml of a Tyrode solution containing BSA in a concentration of 15%. The BSA-solution of 15% strength was overlaid with 0.3 ml of a Tyrode solution containing BSA in a concentration of 10%. The BSA-solution of 10% strength was overlaid with the sperm-containing solution. After

one hour the uppermost layer was separated. After a further 30 minutes the next uppermost layer was separated. After a still further 30 minutes the remaining solutions were separated from each other. The sperms in the BSA-solutions were recovered, and their number and motility and also the contents of X- and Y-sperms were determined. The results are summarized below:

Tyrode solution:	IM=65, FM=20;		
10% BSA-solution:	R=10—15, M=50—60, Y=55—60;		
15% BSA-solution:	R=10, M=75—85, Y=60—70;		
25% BSA-solution:	R=10, M=95, Y=75.		

Example 6

Fresh rabbit semen was mixed with a Tyrode solution in the ratio by volume of 1:1, and the mixture was centrifuged to separate the sperms. After determining the motility and number of sperms, 100×10^6 sperms were re-suspended in 0.5 ml of a Tyrode solution. 0.4 ml of a Tyrode solution containing BSA in a concentration of 15% was overlaid in a sealed Pasteur pipette with 0.8 ml of a Tyrode solution containing BSA in a concentration of 6%. The BSA-solution of 6% strength was overlaid with the sperm-containing Tyrode solution. After one hour the uppermost layer was separated off. After a further 30 minutes the remaining solutions were separated from each other. The sperms in the BSA-solutions were recovered, and their number and motility were determined. The results are given below: —

Tyrode solution:	IM=75, FM=70;
6% BSA-solution:	R=10, M=90;
15% BSA-solution:	R=5, M=98.

Example 7

Fresh human semen was mixed in the ratio by volume of 1:1 with a Tyrode solution, and the mixture was centrifuged to separate the sperms from the other constituents of the semen. After determining the motility and number of sperms, 50×10^6 sperms were re-suspended in 0.5 ml of Tyrode solution having a pH-value of 7.6. Simultaneously, a separate Tyrode solution was adjusted with ovalbumin to an ovalbumin-concentration of 10% of ovalbumin and a pH-value of 7.3—7.4. 0.9 ml of the ovalbumin-solution was overlaid in a sealed Pasteur pipette with the sperm-containing Tyrode solution. After one hour the supernatant Tyrode solution and the ovalbumin-solution were separated from each other. The sperms in the ovalbumin-solution were recovered, and their number and motility and also the contents of X- and Y-sperms were determined. The results are summarized below:

Tyrode solution:	IM=75—80, FM=25—30;
10% Ovalbumin solution:	R=25—30, M=85—90, Y=62—66.

Example 8

Fresh human semen was mixed in the ratio by volume of 1:1 with a Tyrode solution, and the mixture was centrifuged to separate the sperms. After determining the motility and number of sperms, 50×10^6 sperms were re-suspended in 0.5 ml of a Tyrode solution. 0.4 ml of a Tyrode solution containing ovalbumin in a concentration of 15% was overlaid in a sealed Pasteur pipette with 0.8 ml of a Tyrode solution containing ovalbumin in a concentration of 6%. The ovalbumin-solution of 6% strength was overlaid with the sperm-containing Tyrode solution. After one hour the uppermost layer was separated off. After a further 30 minutes the remaining solutions were separated from each other. The sperms of the ovalbumin-solutions were recovered, and their number and motility and also the contents of X- and Y-sperms were determined. The results are summarized below:

Tyrode solution:	IM=65, FM=20;		
6% Ovalbumin solution:	R=30, M=75, Y=65—73;		
15% Ovalbumin solution:	R=5, M=80—85, Y=70—75.		

Example 9

Fresh human semen was mixed with a Tyrode solution in the ratio by volume of 1:1, and the mixture was centrifuged to separate the sperms from the other constituents of the semen. After determining the motility and number of sperms, 50×10^6 sperms were re-suspended in 0.5 ml of a Tyrode solution having a pH-value of 7.6. At

the same time a separate Tyrode solution having a pH-value of 7.6 was adjusted with bovine α -globulin (hereinafter abbreviated to BG) to a BG-concentration of 10%. 0.9 ml of the BG-solution was overlaid in a sealed Pasteur pipette with the sperm-containing Tyrode solution. After one hour the supernatant Tyrode solution and the BG-solution were separated from each other. The sperms in the BG-solution were recovered, and their number and motility and also the contents of X- and Y- sperms were determined. The results are summarized below:

Tyrode solution:	IM=50, FM=25;
10% BG solution:	R=24, M=80, Y=60.

Example 10

Fresh rabbit semen was mixed with a Tyrode solution in the ratio by volume of 1:1 and was centrifuged to separate the sperms. After determining the motility and number of the sperms, 50×10^6 sperms were re-suspended in 0.5 ml of a Tyrode solution. 0.4 ml of a Tyrode solution containing BSA in a concentration of 15% was overlaid in a sealed Pasteur pipette with 0.8 ml of a Tyrode solution containing BSA in a concentration of 10%. The BSA-solution of 10% strength was overlaid with the sperm-containing Tyrode solution. After one hour the uppermost layer was separated off. After a further 30 minutes the remaining solutions were separated from each other. The sperms in the BSA-solutions were recovered, and their number and motility were determined. The results are summarized below:

Tyrode solution:	IM=68, FM=45;
10% BSA-solution:	R=25, M=60-75;
15% BSA-solution:	R=8-10, M=70-80.

In Examples 1 to 8 above it is possible to obtain identical results by carefully underlaying the sperm-containing Tyrode solution with the particular additive-containing Tyrode solution, instead of overlaying the latter with the sperm-containing solution. Similarly, in Examples 2, 5, 6 and 8 the Tyrode solutions containing a lower concentration of additive may be underlaid with the appropriate Tyrode solutions containing a higher concentration of additive.

WHAT WE CLAIM IS:—

1. A process for increasing the proportion of Y-sperms in mammalian sperms from a dispersion of Y-sperms and X-sperms in a physiologically tolerable liquid, wherein the dispersion is brought into interfacial contact with a physiologically tolerable fluid medium of higher density than the liquid in which the sperms are dispersed, the contact between the dispersion and the fluid medium being maintained for a period sufficient to allow Y-sperms to pass into the medium.
2. A process for the extraction of sperms having a desired motility from a dispersion in a physiologically tolerable liquid of mammalian sperms some of which have the desired motility and others have a motility lower than the desired motility, wherein the dispersion is brought into interfacial contact with a physiologically tolerable fluid medium of higher density than the liquid in which the sperms are dispersed, the contact between the dispersion and the fluid medium being maintained for a period sufficient to allow sperms having the desired motility to pass into the medium.
3. A process as claimed in claim 1 or claim 2, wherein the sperm-containing dispersion is brought into interfacial contact with the fluid medium by overlaying the fluid medium with the dispersion.
4. A process as claimed in claim 1 or claim 2, wherein the sperm-containing dispersion is brought into interfacial contact with the fluid medium by underlaying the dispersion with the fluid medium.
5. A process as claimed in any one of claims 1 to 4, wherein the sperms are human sperms.
6. A process as claimed in any one of claims 1 to 4, wherein the sperms are cattle sperms.
7. A process as claimed in any one of claims 1 to 4, wherein the sperms are sheep sperms.
8. A process as claimed in any one of claims 1 to 7, wherein the dispersion is semen.
9. A process as claimed in any one of claims 1 to 8, wherein the physiologically tolerable liquid in which the sperms are dispersed is a Tyrode solution.
10. A process as claimed in claim 9, wherein the dispersion has been prepared by

(17)



5 the same time a separate Tyrode solution having a pH-value of 7.6 was adjusted with bovine α -globulin (hereinafter abbreviated to BG) to a BG-concentration of 10%. 0.9 ml of the BG-solution was overlaid in a sealed Pasteur pipette with the sperm-containing Tyrode solution. After one hour the supernatant Tyrode solution and the BG-solution were separated from each other. The sperms in the BG-solution were recovered, and their number and motility and also the contents of X- and Y- sperms were determined. The results are summarized below:

Tyrode solution: IM=50, FM=25;
10% BG solution: R=24, M=80, Y=60.

10 Example 10
Fresh rabbit semen was mixed with a Tyrode solution in the ratio by volume of 1:1 and was centrifuged to separate the sperms. After determining the motility and number of the sperms, 50×10^6 sperms were re-suspended in 0.5 ml of a Tyrode solution. 0.4 ml of a Tyrode solution containing BSA in a concentration of 15% was overlaid in a sealed Pasteur pipette with 0.8 ml of a Tyrode solution containing BSA in a concentration of 10%. The BSA-solution of 10% strength was overlaid with the sperm-containing Tyrode solution. After one hour the uppermost layer was separated off. After a further 30 minutes the remaining solutions were separated from each other. The sperms in the BSA-solutions were recovered, and their number and motility were determined. The results are summarized below:

Tyrode solution: IM=68, FM=45;
10% BSA-solution: R=25, M=60-75;
15% BSA-solution: R=8-10, M=70-80.

25 In Examples 1 to 8 above it is possible to obtain identical results by carefully underlaying the sperm-containing Tyrode solution with the particular additive-containing Tyrode solution, instead of overlaying the latter with the sperm-containing solution. Similarly, in Examples 2, 5, 6 and 8 the Tyrode solutions containing a lower concentration of additive may be underlaid with the appropriate Tyrode solutions containing a higher concentration of additive.

30 WHAT WE CLAIM IS:—

1. A process for increasing the proportion of Y-sperms in mammalian sperms from a dispersion of Y-sperms and X-sperms in a physiologically tolerable liquid, wherein the dispersion is brought into interfacial contact with a physiologically tolerable fluid medium of higher density than the liquid in which the sperms are dispersed, the contact between the dispersion and the fluid medium being maintained for a period sufficient to allow Y-sperms to pass into the medium.
2. A process for the extraction of sperms having a desired motility from a dispersion in a physiologically tolerable liquid of mammalian sperms some of which have the desired motility and others have a motility lower than the desired motility, wherein the dispersion is brought into interfacial contact with a physiologically tolerable fluid medium of higher density than the liquid in which the sperms are dispersed, the contact between the dispersion and the fluid medium being maintained for a period sufficient to allow sperms having the desired motility to pass into the medium.
3. A process as claimed in claim 1 or claim 2, wherein the sperm-containing dispersion is brought into interfacial contact with the fluid medium by overlaying the fluid medium with the dispersion.
4. A process as claimed in claim 1 or claim 2, wherein the sperm-containing dispersion is brought into interfacial contact with the fluid medium by underlaying the dispersion with the fluid medium.
5. A process as claimed in any one of claims 1 to 4, wherein the sperms are human sperms.
6. A process as claimed in any one of claims 1 to 4, wherein the sperms are cattle sperms.
7. A process as claimed in any one of claims 1 to 4, wherein the sperms are sheep sperms.
8. A process as claimed in any one of claims 1 to 7, wherein the dispersion is semen.
9. A process as claimed in any one of claims 1 to 8, wherein the physiologically tolerable liquid in which the sperms are dispersed is a Tyrode solution.
10. A process as claimed in claim 9, wherein the dispersion has been prepared by



* C L M S *

1,469,816

9

mixing semen with a Tyrode solution, separating the sperms from the resulting mixture by centrifuging and then suspending the separated sperms in a Tyrode solution.

11. A process as claimed in claim 10, wherein the semen is mixed with the Tyrode solution in the ratio by volume of 1:1.

12. A process as claimed in any one of claims 1 to 11, wherein the fluid medium consists of a further quantity of the physiologically tolerable liquid containing at least one additive.

13. A process as claimed in any one of claims 1 to 12, wherein the fluid medium contains an ovalbumin or bovine α -globulin.

14. A process as claimed in any one of claims 1 to 12, wherein the fluid medium contains bovine serum albumin.

15. A process as claimed in claim 14, wherein the fluid medium is a 3 to 25 per cent solution by weight of bovine serum albumin in a Tyrode solution.

16. A process as claimed in any one of claims 1 to 15, wherein the dispersion is contacted with the fluid medium for a period within the range of from 0.5 to 10 hours.

17. A process as claimed in claim 16, wherein the dispersion is contacted with the fluid medium for a period within the range of from 1 to 3 hours.

18. A process as claimed in any one of claims 1 to 17, wherein the density of the fluid medium is from 3% to 50% greater than the density of the liquid in which the sperms are dispersed.

19. A process as claimed in any one of claims 1 to 18, wherein the fluid medium is brought into interfacial contact with a physiologically tolerable second fluid medium of higher density than the first fluid medium, the contact between the two fluid media being maintained for a period sufficient to allow sperms of the desired motility to pass into the second medium to give a higher proportion of such sperms (with respect to sperms of lower motility) in the second medium than in the liquid in which the sperms are dispersed or the fluid medium.

20. A process as claimed in any one of claims 1 to 18, wherein the fluid medium is brought into interfacial contact with a physiologically tolerable second fluid medium of higher density than the first fluid medium, the contact between the two fluid media being maintained for a period sufficient to allow Y-sperms to pass into the second medium to give a higher proportion of Y-sperms (with respect to X-sperms) in the third liquid than in the liquid in which the sperms are dispersed or the fluid medium.

21. A process as claimed in claim 19, wherein the contacting step is repeated by bringing the second fluid medium into interfacial contact with a physiologically tolerable third fluid medium of higher density than the second fluid medium, to give a higher proportion of sperms of the desired motility (with respect to sperms of lower motility) in the third fluid medium than in the other fluid media.

22. A process as claimed in claim 20, wherein the contacting step is repeated by bringing the second fluid medium into interfacial contact with a physiologically tolerable third fluid medium of higher density than the second fluid medium, to give a higher proportion of Y-sperms (with respect to X-sperms) in the third medium than in the other media.

23. A process as claimed in claim 19 or claim 20, wherein the second fluid medium is positioned below the first fluid medium.

24. A process as claimed in any one of claims 21 to 23, wherein the third fluid medium is positioned below the second fluid medium.

25. A process as claimed in claim 1, conducted substantially as described in any one of Examples 1 to 3, 5, 7 and 8.

26. A process as claimed in claim 2, conducted substantially as described in Example 4 or 6.

27. A process as claimed in claim 1 or claim 2, conducted substantially as described in Example 10 herein.

28. A process as claimed in claim 2, conducted substantially as described in Example 11 herein.

29. Motile mammalian sperm whenever obtained by the process claimed in any one of claims 2 to 24 and 26 to 28.

30. An extract of sperms containing Y-mammalian sperms whenever obtained by the process claimed in any one of claims 1 and 3 to 25, 27 and 28.

31. A method for the biasing of sex in offspring in favour of the male sex, which comprises fertilization of a female mammal with an extract of sperms containing Y-mammalian sperms that has been obtained by the process claimed in any one of claims 1 and 3 to 18, 20, 22 to 25 and 27.

32. A method as claimed in claim 31, wherein a human female is fertilized by artificial insemination with an extract of sperms containing Y-mammalian sperms that has been obtained by the process claimed in any one of claims 1, 3 to 5, 8 to 18, 20, 22 to 25 and 27.

33. A method as claimed in claim 29, wherein a cow is fertilized by artificial insemination with an extract of sperms containing Y-sperms that have been obtained by the process claimed in any one of claims 6, 8 to 18, 20 and 22 to 24.

34. A method of fertilization, which comprises fertilization, of a female mammal with motile sperms that have been obtained by the process claimed in any one of claims 2 to 19, 21 and 23, 24, and 26 to 28.

35. A method as claimed in claim 34, wherein a human female is fertilized by artificial insemination with motile sperms that have been obtained by the process claimed in any one of claims 2 to 5, 8 to 19, 21 and 23, 24 and 27.

36. A preparation for biasing the sex of a mammal in favour of the male sex, which comprises an extract of sperms containing mammalian Y-sperms as claimed in claim 30 in admixture or conjunction with a pharmaceutically suitable carrier.

37. A preparation for biasing the sex of a human in favour of the male sex, which comprises an extract of sperms containing human Y-sperms that have been obtained by the process claimed in any one of claims 1, 3 to 5, 8 to 18, 20, 22 to 25 and 27, in admixture or conjunction with a pharmaceutically suitable carrier.

38. A preparation for biasing the sex of cattle in favour of the male sex, which comprises an extract of sperms containing cattle Y-sperms that have been obtained by the process claimed in any one of claims 6 and 8 to 18, 20 and 22 to 24, in admixture or conjunction with a pharmaceutically suitable carrier.

39. A preparation for increasing the chances of a successful insemination in the artificial insemination of a female mammal, which comprises motile mammalian sperms as claimed in claim 29, in admixture or conjunction with a pharmaceutically suitable carrier.

40. A pack for carrying out the process as claimed in any one of claims 1 to 28, which comprises:

- (a) a vessel for receiving small quantities of liquids as discrete layers;
- (b) a physiologically tolerable liquid, for mammalian sperms,
- (c) a physiologically tolerable fluid medium, for mammalian sperms, of higher density than the physiologically tolerable liquid (b), and
- (d) instructions for carrying out the process.

41. A modification of the pack as claimed in claim 40, which comprises, instead of the fluid medium (c), at least one additive (e) which, when added to the physiologically tolerable liquid (b), increases the density as compared with the liquid without the additive.

42. A pack as claimed in claim 40 or 41, which also contains an insemination syringe.

43. A pack as claimed in any one of claims 40 to 42, wherein the vessel (a) is a Pasteur pipette.

44. A pack as claimed in any one of claims 40 to 43, wherein the liquid (b) is a Tyrode solution.

45. A pack as claimed in any one of claims 40 and 42 to 44, wherein the fluid medium (c) is a Tyrode solution containing bovine serum albumin.

46. A pack as claimed in any one of claims 41 to 44, wherein the additive (e) is bovine serum albumin.

47. A pack as claimed in any one of claims 41 to 44, wherein the additive (e) is ovalbumin.

48. A pack as claimed in any one of claims 41 to 44, wherein the additive (e) is bovine α -globulin.

ABEL & IMRAY,
Chartered Patent Agents,
Northumberland House,
303-306 High Holborn,
London, WC1V 7LH.