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(54) **DEVICE FOR DIAGNOSIS OF PHYSIOLOGIC STATUS AND/OR SELECTION OF THE BEST SPERMATOZOA OF A SEMEN SAMPLE BASED ON CHEMOTAXIS, AND PROCEDURE OF USE THEREOF**

VORRICHTUNG ZUR DIAGNOSE DES PHYSIOLOGISCHEN STATUS UND/ODER SELEKTION DER BESTEN SPERMATOZOEN EINER SAMENPROBE AUF CHEMOTAKTISCHER BASIS SOWIE VERWENDUNGSVERFAHREN DAFÜR

DISPOSITIF POUR LE DIAGNOSTIC D'UN ÉTAT PHYSIOLOGIQUE ET/OU SÉLECTION DES MEILLEURS SPERMATOZOÏDES D'UN ÉCHANTILLON DE SPERME SUR LA BASE D'UNE CHIMIOTAXIE, ET PROCÉDURE D'UTILISATION DE CELUI-CI

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Description

TECHNICAL FIELD OF THE INVENTION

[0001] The present invention refers to a device for diagnosis of physiologic status and/or selection of the best spermatozoa of a semen sample based on chemotaxis, and the procedure of use thereof, enabling through a simple and inexpensive device reach a diagnosis and select the best spermatozoa in one step. Only needed is this device, a regular light microscope, and personnel with elementary knowledge of laboratory handling.

STATE OF THE ART

[0002] Sperm cell chemotaxis is a cell transport mechanism guiding spermatozoa to an attractant concentration gradient. In the last few years, efforts have been made to differentiate chemotaxis from a cell aggregation process mediated by other mechanisms.

[0003] The chemotaxis process has been identified in different mammal species, for example humans, mice, rabbits, and others.

[0004] On the other hand, it is known that progesterone is the main egg microenvironment steroid at the ovulation time, and it has been demonstrated to be a spermatozoa attractant.

[0005] Patents PCT WO 02/090373, WO 00/09648, and WO 99/66331 disclose methods and devices to assess spermatozoa chemotaxis, basically comprising two filter-separated vertical compartments, where the attractant is place in the lower and spermatozoa in the top one. Secondary usefulness of the device should be chemotaxis assessment, based on spermatozoa aggregation obtained after some time in the compartment containing the attractant. This methodology does not allow for differentiation of chemotaxis from other cell aggregation provoking processes.

Patent PCT WO 2005/027634 suggests another method to isolate capable spermatozoa. Sperm selection is performed based on sperm cell capability to respond to a temperature gradient, that is, swimming from a colder to a warmer place. The device has an external differential warming system for the fluid of each compartment. Each compartment requires temperature regulation with a difference of only 2°C between both, placed at a 1 mm distance. This physical feature is obtained by additional thermal equipment, notably making this device manipulation. Enrichment efficiency is 10%.

Patent WO 2005/009222 suggests a method for diagnosis only of the physiologic status of a semen sample based on detection the level of capable spermatozoa. The invention proposes a protocol involving cell death, only serving to diagnose a sample, since capable spermatozoa cannot be recovered for later use in assisted fecundation.

[0006] US 5,849,713 relates to chemotactic factors for human spermatozoa. The chemotactic activity is determined by a capillary assay described therein.

[0007] The article "Progesterone at the picomolar range is a chemoattractant for mammalian spermatozoa" by Teves et al. published in "Fertility and Sterility", Vol. 86, No. 3 in 2006 discloses, by means of a videomicroscopy system and a computer image analysis, chemotaxis assays to detect true chemotaxis in human spermatozoa, in parallel to immunohistochemistry detection of progtesterone inside the cumulus cell.

[0008] The article "Applications of a microfabricated device for evaluating sperm function" by Kricka et al. published in "Clinical Chemistry", Vol. 39, No. 9 in 1993 discloses inter alia the simultaneous assessment of the potency of different spermicides and spermicide concentrations using structures comprising chambers containing spermicide connected via channels to a central chamber into which semen is introduced.

[0009] The article "Progesterone from the cumulus cells is the sperm chemoattractant secreted by the rabbit oocyte cumulus complex" by Guidobaldi et al. published in "PLOS ONE", Vol. 3, No. 8 in 2008 discloses the identification of sperm chemotaxis in mammals towards several female sources as follicular fluid, oviduct fluid, and conditioned medium from the cumulus oophorus and the oocyte.

[0010] The article "Chemotaxis assays of mouse sperm on microfluidic devices" by Koyama et al. published in "Analytical Chemistry", Vol. 78, No. 10 in 2006 discloses a microfluidic device to measure sperm chemotaxis.

[0011] The article "Chemotaxis of capacitated rabbit spermatozoa to follicular fluid revealed by a novel directionality-based assay" by Fabro et al. published in "Biology of Reproduction", Vol. 67, No. 5 in 2002 discloses sperm chemotaxis towards factor(s) in follicular fluid in humans and mice.

BRIEF DESCRIPTION OF THE INVENTION

[0012] The present invention shows a device for physiologic status diagnosis and/or spermatozoa selection from a semen sample based on chemotaxis, of the type having two communicated compartments (1a, 1b), wherein said compartment communication (1a, 1b) occurs through a duct or bridge (2) located at the bottom, and above the lower level of the mentioned compartments (1a, 1b); locating on the entrances of said compartments (1a, 1b) appropriate closing means (3, 4) and appropriate air output ducts (5) communicating the compartment top ends (1a, 1b) with the exterior, wherein said closing means (3, 4) comprises a plug with a projection (3) and an elastic ring (4) and wherein the lower end of compartments (1a, 1b) have a conical base. Preferably, the elastic ring is of rubber, and said compartments (1a, 1b), bridge (2) and air output ducts (5) are formed in a transparent body of biocompatible material. The present invention shows a procedure for physiologic status diagnosis and/or selection of the best spermatozoa of a se-

men sample based on chemotaxis using the device according to the invention, comprising the performance of the following stages:

a) place a closing means (3) in one of the compartments (1a);
b) fill the bridge (2) pouring culture medium through the remaining compartment (1b);
c) place the spermatozoa suspension in compartment (1b) without closing means and placing the closing means (3);
d) extract the closing means (3) from the first mentioned compartment (1a) and filling with culture medium or attractant medium;
e) incubate the device;
f) recover the compartment solution (1a);

[0013] In an embodiment variation of the former procedure, it is possible to add the following stages: g) place a plug in said compartment (1a); h) unplug compartment (1b);and i) recover the compartment solution (1b).

BRIEF DESCRIPTION OF THE FIGURES

[0014] For a better understand of the object of the present invention, it has been schematically illustrated, in its preferred embodiment, assuming a characteristic of demonstrative example, where:

Figures la, 1b and 1c illustrate respective top, front, and lateral view of the device of the present invention;
Figure 2 shows a cut according to line A-A of Figure 1c;
Figure 3 shows a cut according to line A-A of Figure 1c incorporating a closing means;
Figure 4 shows a graph of optimum spermatozoa concentration (S) used in sperm separation. Values depict Mean ± SEM of percentage of spermatozoa aggregated in compartment la by chemotaxis (n= 3). * p<0.05; ***p<0.001 vs. 6 million S/ml.
Figure 5 shows a graph of Progesterone concentration used in sperm separation. Values depict Mean ± SEM of percentage of spermatozoa aggregated in compartment 1a by chemotaxis (n= 3). *p<0.01 vs. 10 pM of progesterone.
Figure 6 shows a graph of incubation times employed for sperm separation. Values depict Mean ± SEM of percentage of spermatozoa aggregated in compartment la by chemotaxis (n= 3). *p<0.05, **p<0.01, ***p<0.001 vs. 20 min.
Figure 7 shows a graph of sperm separation using the device designed by us in conditions to obtain a maximal response (6 million S/ml, 10 pM progesterone, and 20 minutes incubation), described above, and illustrated in Figures 4 to 6, respectively. Values depict Mean ± SEM, of percentage of aggregated spermatozoa in compartment la by chemotaxis. Ratio of aggregated spermatozoa by chemotaxis in the compartment with progesterone is of about 10% (Δ; n= 5). ** p<0.01 vs. Control.

[0015] Additional tests were performed to verify that sperm separation occurs by chemotaxis and not by other sperm mechanisms (for example, hyperactivation, chemokinesis, etc.).

[0016] Figure 8 shows the percentage of aggregated spermatozoa (Mean ± SEM; n=3) in compartment la by chemotaxis when cells were previously treated with the inhibitor (ddAdo) of an enzyme (mAC) participating in the chemotactic signal. In the presence of the inhibitor, no sperm aggregation due to chemotaxis is seen (* p<0.05).

[0017] Figure 9 shows percentage of aggregated spermatozoa (Mean ± SEM; n=3) in compartment la by chemotaxis when cells were previously treated with antibody "c262" against progesterone receptor (anti-PR Ab). In the presence of antibody, spermatozoa do not aggregate in compartment 1a (*p<0.05), while treatment with non-specific antibody (anti-IgG Ab) does not prevent sperm aggregation.

[0018] In Figure 10, percentage of aggregated spermatozoa in the presence of an increasing gradient of progesterone was similar to one found in a decreasing gradient of attractant, while in the absence of gradient of progesterone (equal concentration of hormone in both compartments 1a and 1b) sperm aggregation is reduced (* p<0.05).

[0019] Figure 11 compares proportion of aggregated spermatozoa by chemotaxis with the inventive method, with percentage of chemotactic spermatozoa determined by video-microscopy and image computer analysis. A high and significant correlation is found between both methods. (r=0.794; p<0.05; n=6).

[0020] Figure 12 shows correlation between proportion of spermatozoa with induced acrosome reaction (AR) and proportion of aggregated spermatozoa by chemotaxis with the inventive method. Values depict Mean ± SEM (n= 5). (r=0.947; p<0.05; n=5).

[0021] Results of Figures 8 to 12 confirm that spermatozoa aggregation by the inventive invention is caused by chemotaxis.

[0022] One application of the invention is using the method for diagnosis of physiologic status and/or selection of the best spermatozoa of a semen sample.

[0023] Figure 13 shows Enrichment Rate in capable spermatozoa before and after sperm separation. Values depict Mean ± SEM (n= 9), where the value before sperm separation represents 100%, from which the times capable spermatozoa proportion increased is estimated after sperm separation by chemotaxis. In semen samples defined as normal, ratio of capable spermatozoa obtained after sperm separation by the invention is increased in an average of 200% (*p<0.05 vs. before separation). When the invention is applied to sub-fertile patients samples (T: terato-zoospermic, A-T: asteno - terato-zoospermic, O: oligo-zoospermic, ESCA: sterility of

no apparent cause), after sperm separation it is possible to obtain a capable spermatozoa enrichment rate similar to that found in normal samples. That is, independently of the semen sample pathology, the invention use allows quality improvement of sub-fertile samples.

[0024] Figure 14 shows a range of maximum and minimum enrichment rate values of capable spermatozoa reached after sperm separation ("After"), performed with the invention in normal (n=8) and sub-fertile patients semen samples (T: terato-zoospermic, n=29; A-T: asteno - terato-zoospermic, n=15; O: oligo-zoospermic, n=2; ESCA: sterility of no apparent cause, n=3). In normal samples, it is found that they all improve the enrichment rate at least in 100%, compared to before separation, even exceeding values before separation in up to 600%. It is also found that although all pathological samples achieved a capable spermatozoa enrichment exceeding the one found before ("Before") sperm separation, in about 30% of these samples the enrichment level was below 100%. This observation allows the use of the invention for diagnosis, specially in ESCA-type samples, where conventional diagnosis tests do not allow their identification as sub-fertile.

DETAILED DESCRIPTION OF THE INVENTION

[0025] The device suggested here (two compartments separated by a space where an attractant gradient is formed) has been designed as a function of the physical-chemical features of the attractant, where the length of the bridge (2 mm) and the incubation time of the set-up system (20 min) are adapted to use chemotactic concentration of progesterone (10pM), enabling formation of a concentration gradient of this attractant.

[0026] The present device prevents mass flow of spermatozoa from one compartment to the other due to a physical effect of communicating vessels, thus assuring that the process is solely due to chemical action of the attractant. In other words, the mere connection of two compartments, where spermatozoa are placed in one of them, and the attractant in the other, in order to form a concentration gradient between both, does in no way guarantee assessment of the chemotaxis process. This is therefore guaranteed by a device having the feature of containing a hermetic closure comprising an elastic ring (4) seated on the top part of compartments Ia and 1b, a pair of plugs (3) seated on the elastic ring (4), a pair of openings (5) located above the elastic ring (4), which allow the air pushed by inserting the plug to exit to the exterior instead of pushing the fluid of 1b towards 1a. These invention adaptations are complemented by the device filling sequence.

[0027] Likewise, the proposed device use sequence or method in addition to diagnose physiologic status of a given semen sample dada, allows to select and isolate the best spermatozoa in the same procedure. The present device allows removal of the content or solution of compartment Ia and diagnosis of the physiologic status of the semen sample. To the effects of the present application, diagnose physiologic status of a sample means assessment of the chemotactic response capacity spermatozoa show in the presence of the physiologic attractant progesterone. Thus, net ratio of spermatozoa aggregated in the compartment containing progesterone (1a) is determined. Although said type of assay enables assessment of all types of sub-fertile samples, the invention shall be particularly useful to assess physiologic status of ESCA-type samples, since routine diagnosis assays do not allow identification their pathology.

[0028] The method is based on the feature of mammal spermatozoa of orienting their movement towards the production source of an attractant. This chemotactic capacity can also be exerted only by spermatozoa that have completed the "capability" process, a condition enabling them to fertilize the ovule. Therefore, the content of compartment Ia may also be used to select and/or separate spermatozoa capable of fertilizing ovules, where spermatozoa thus selected may be employed in the assisted fertilization techniques.

[0029] It is essential that the device consists of two compartments, one where spermatozoa are placed and the other for the attractant, in order to create an attractants concentration gradient.

[0030] After some time, capable spermatozoa go chemotactically towards the attractant source and aggregate in the compartment containing the attractant (1a), that is, in the latter an enrichment of capable spermatozoa shall be found, compared to the original semen sample. Thus, selection and aggregation of capable spermatozoa is performed physiologically by chemotaxis-mediated recruitment.

[0031] With this device, an enrichment of capable spermatozoa of up to 600% superior to the original semen sample can be obtained. Said enrichment was verified determining the ratio of spermatozoa performing the pharmacologically induced acrosome reaction, a procedure known as capacitation indicator (Figures 13 and 14).

[0032] The present device combines a disposition in two compartments with an attractant gradient, and dimensions adapted for an attractant effective at low concentrations, for example values between 1 and 100 pM progesterone, thus obtaining high efficiency in selection of the best spermatozoa, those capacitated.

[0033] In a preferred embodiment, the device may have particular dimensions adapted to progesterone features (its capacity to diffuse and form a concentration gradient, and its effectiveness at low concentrations). For example, the size of the connecting bridge between both compartments may be of about 2 mm diameter by about 2 mm long.

[0034] Since it is a communicating vessel system device, at the time of placing a liquid in one of the compartments, the fluid tends to pass to the other compartment through the bridge until the volume heights are equal. This phenomenon is not desirable, since it breaks the attractant gradient formation, and thus conveys mechan-

ically, that is non-physiologically, spermatozoa towards the attractant-containing compartment.

[0035] To prevent the latter problem, the device is provided with a hermetic closing system. Optionally, both device compartments have a conical base, where the bridge connector insertion between both compartments is above said cone. The conical base allows dead or low movement spermatozoa decant to the bottom of the tube due to simple gravity, preventing them from being drawn to the bridge and the other compartment by the faster chemotactic spermatozoa.

[0036] The present device confers the necessary hermetic conditions to prevent fluid movement is such a small system, and also prevent external contamination.

[0037] Summarizing, the device suggested herein allows diagnosing and selecting the best spermatozoa in only one step, in a simple, low cost, and highly efficient way that can be done in less than one hour by personnel with elemental laboratory knowledge.

[0038] In relation to the applications of the suggested device, its use for diagnosis of physiologic status of given semen sample is provided, at the same time recruiting the best spermatozoa for later use to improve fertilization rates.

[0039] It must be noted that the suggested method checking tests were performed using a methodology to determine a very sophisticated chemotactic response by spermatozoa, that requires a costly equipment and highly trained personnel.

[0040] In Figures 1 to 3 it is noted that the device, preferably manufactured in acrylic, of biocompatible material and necessarily transparent for an efficient use of the, consists of two cylindrical vertical compartments "la" and "1b", of a preferable length of 12 mm by 4 mm diameter (equivalent capacity of 130 μl), connected by a tube or bridge "2", 2 mm long by 2 mm diameter (equivalent capacity of 20 μl).

[0041] In one of the compartments, for example "1b", the suspension containing spermatozoa is placed, and in the, "1a", the attractant is placed. Along the bridge 2, an increasing attractant concentration gradient is formed, allowing the selection of spermatozoa by chemotaxis, which aggregates in the attractant compartment 1a.

[0042] In preferred embodiment, the present device dimensions are adapted to the use of progesterone as attractant, and to minimize the volume placed in each compartment. It shall be evident to an expert in the art that the attractant may be any chemoattractant effective at low concentrations, for example concentrations in the order of picomoles.

[0043] Since it is a communicating vessel system, an important aspect is to prevent fluid flow through the bridge 2 while filling or emptying compartments "la" and "1b". This unwanted effect may break the attractant gradient, thus loosing the device function principle, or else mechanically convey the content of a compartment to the other, which also notably diminishes the efficiency of the device.

[0044] In order to prevent said inconveniences, means are provided to hermetically close compartments, thus combining adaptation of the present device with a particular closing sequence.

[0045] In each compartment, "1a" or "1b", said closing means comprise a plug with a projection "3" seated on an elastic rubber ring "4", on which the hermetic closing occurs. In addition, an opening "5" in the top part of each compartment "1a" and "1b" is provided between the plug "3" and the rubber ring "4", which allows constant outflow of the contained air therein while placing plug "3", in order to hermetically close compartment "la" or "1b".

[0046] Another detail of the device is the conical finishing "6" of each compartment base, located under the bridge connection, where the gradient where swimming spermatozoa migrate is formed. This adaptation allows dead or low mobility spermatozoa decant by gravity to the bottom of the compartment, thus preventing them from being drawn by the mobile spermatozoa into the bridge.

[0047] As related the way of using the present device, operation is described below:

[0048] Separation of mobile spermatozoa (S) of seminal plasma is done by the Percoll technique. A volume of about 2 ml semen is used, which is deposited on 1 ml Percoll gradient (lower layer: 500 μl Percoll 95%, upper layer: 500 μl of Percoll 47.5%, in HAM-F10). After centrifuging 20 minutes at 1800 rpm, the cell pellet is recovered and washed twice by centrifugation for 7 min at 1000 rpm, with HAM-F10 medium. Finally, the recovered pellet is suspended in 1 ml of HAM-F10 medium, cell count is performed in a cell count chamber, and volume is adjusted in order to obtain a concentration of 8 million S/ml. S capability is increased in HAM-F10 medium supplemented with 1% human serum albumin (HSA) for 4 hours a 37°C, under 5 % CO_2 atmosphere in air.

[0049] When capacitation time is finished, S concentration is adjusted in order to obtain 6 million mobile S/ml, and two devices are set up as described in the preceding section (Figures 1-3), one without attractant as negative control, and the other with progesterone as attractant.

[0050] To set up the device control, a device as described in Figures 1 to 3, proceed as follows: 1) the plug is placed in compartment 1a, where culture medium (HAM-F10/1%HSA) is placed, 2) the bridge linking both compartments is filled with culture medium, 3) S suspension is placed in compartment 1b (130 μl of the solution of 6 million mobile S/ml) and this compartment is closed with the plug, 4) compartment la is unplugged and filled with culture medium (130 μl).

[0051] For device set up with the attractant, another device is prepared as described in Figures 1 to 3, like the device control but replacing culture medium with a 10 pM Progesterone solution diluted in culture medium. Once both devices are set up ("Control" and "Attractant"), they are incubated for 20 minutes at 37°C, under 5 % CO_2 atmosphere in air.

[0052] To take the device apart and recover the best

spermatozoa, the compartment solution (1a) containing culture medium or attractant is removed. On the spermatozoa suspension removed from compartment la of both devices (control and attractant) cell count is performed in a cell count chamber. Then the difference in percentage of S found in each of these two compartments is determined, which enables to value net percentage of S migrating to the attractant-containing compartment ("Δ").

[0053] Once the device design stage is finished, a series of experiments then proceeded in order to define experimental conditions of the use thereof, and then to proceed with method validation.

[0054] The best experimental conditions to obtain optimal sperm separation, (assessed as the difference "Δ" between the ratio of S aggregating in the compartment with culture medium and with attractant), were: 1) a suspension with a concentration of about 6 million S/ml (Fig. 4), 2) a concentration of about 1 to 100 pM progesterone (Fig. 5), and 3) a device incubation of about 20 minutes (Fig. 6).

[0055] Thereafter, method validation for diagnosis (based on chemotactical response of S) was done in three ways: 1) by inhibition of chemotactical response, inhibiting the mAC enzyme, which participates in the chemotactical signaling, 2) by inhibition of chemotactical response, blocking the progesterone receptor located on the sperm cell membrane, 3) by comparison with a decreasing progesterone gradient and exposure to the absence of progesterone gradient, 4) by comparison with determination of chemotaxis by video-microscopy, and 5) by comparison with induced acrosome reaction level (AR). Sperm chemotaxis-mediated separation towards progesterone enabled the observation of a spermatozoa subpopulation significantly migrating towards Progesterone ($9 \pm 0.4\%$; $p<0.01$; Fig. 7 "Δ"). Such cell population correlated with the percentage of chemotactical spermatozoa assessed by video-microscopy ($r=0.79$, $p<0.01$; Fig. 11 and of induced AR ($r=0.94$, $p<0.05$; Fig. 12). Ratio of aggregated spermatozoa by an increasing progesterone gradient was similar to the observed with a decreasing progesterone gradient, with no sperm aggregation observed in the absence of progesterone gradient ($p<0.001$; Fig. 10). Sperm aggregation by chemotaxis was prevented by inhibition of an enzyme participating in the cell process, and also by blocking progesterone receptor activity ($p<0.05$; Fig. 8 and 9) .

[0056] Method validation to select the best spermatozoa was done by calculating percentage of increase in the induced acrosome reaction level value, in population of obtained spermatozoa after sperm separation, and in comparison with the value obtained in the spermatozoa sample before separation, considering the latter as 100%. Results showed that the enrichment level in capable spermatozoa after sperm separation may reach up to 600% (Fig. 13 and 14).

[0057] In summary, the device design involving adaptation of dimensions to the used attractant, a hermetic closure, and the definition of the precise filling and emptying sequence, which provide unique features to this device and the procedure, results in a substantial improvement in diagnosis efficiency and selection or separation of the best spermatozoa.

Claims

1. A device for diagnosis of physiologic status and/or selection of spermatozoa of a semen sample based on chemotaxis, of the type having two communicated compartments (1a, 1b), **characterized in that** said compartments (1a, 1b) communication occurs through a duct or bridge (2) located on the bottom, and above the lower level of the mentioned compartments (1a, 1b); by placing on the entrance of said compartments (1a, 1b) the appropriate closing means (3, 4) and appropriate air output ducts (5) communicating the upper end of compartments (1a, 1b) with the exterior, wherein said closing means (3,4) comprises a plug with a projection (3) and an elastic ring (4) and wherein the lower end of compartments (1a, 1b) have a conical base (6).

2. The device of claim 1 **characterized in that** said compartments (1a, 1b), bridge (2) and air output ducts (5) are formed in a transparent body of biocompatible material.

3. The device of claim 1 **characterized in that** said compartments (1a, 1b) are 12 mm in length and 4 mm in diameter with conical base ends and have a capacity equivalent to 130 μl, and the duct or bridge (2) is 2 mm in length and 2 mm in diameter with a capacity equivalent to 20 μl.

4. The device of claim 1 **characterized by** being manufactured in acrylic, biocompatible and necessarily transparent material, for the efficient use of the device.

5. A procedure for diagnosis of physiologic status and/or selection of spermatozoa of a semen sample based on chemotaxis using the device according to any one of claims 1 to 4, **characterized by** comprising the following stages:

    a) place a closing means (3) in one of the compartments (1a);
    b) fill the bridge (2) by pouring culture medium or attractant medium through the remaining compartment (1b);
    c) place the suspension spermatozoa in the compartment (1b) without closing means and place the closing means (3);
    d) remove the closing means (3) of the first mentioned compartment (1a) and fill with culture medium or attractant medium;

e) incubate the device.
f) recover the compartment (1a) solution;

6. The procedure of claim 5 **characterized by** performing the following stages after stage "f"':

g) place the plug in said compartment (1a);
h) unplug compartment (1b);
i) recover compartment (1b) solution.

7. The procedure of claim 5 **characterized in that** the attractant is progesterone.

8. The procedure of claim 5 **characterized in that** the semen is semen of a mammal.

9. The procedure of claim 5 **characterized in that** the semen is semen from other zoological group species.

10. The procedure of claim 5 **characterized in that** the attractant medium comprises progesterone at a concentration between 1 and 100 pM.

11. The procedure of claim 5 **characterized in that** stage e) of incubating is carried out for a period of time of 10 to 30 minutes at a temperature of 37°C and in a 5% CO_2 environment in air.

12. The procedure of claim 5 **characterized in that** the spermatozoa suspension stage c) has a spermatozoa concentration of between $1x10^6$ and $9,9x10^6$ mobile spermatozoa /ml.

Patentansprüche

1. Vorrichtung zur Diagnose eines physiologischen Status und/oder Selektion von Spermatozoen aus einer Samenprobe basierend auf Chemotaxis, von der Bauart aufweisend zwei kommunizierende Kammern (1a, 1b), **dadurch gekennzeichnet, dass** bei den Kammern (1a, 1b) Kommunikation stattfindet zwischen einem Kanal oder Brücke (2), der/die sich an der Unterseite befindet, und oberhalb der niedrigeren Ebene der erwähnten Kammern (1a, 1b); durch das Platzieren des passende Schließmittels (3, 4) auf den Eintritt dieser Kammern (1a, 1b) und passende Luft-Auslasskanäle (5), die das obere Ende der Kammern (1a, 1b) mit der Außenseite kommunizieren lassen, wobei das Schließmittel (3, 4) einen Stopfen mit einem Vorsprung (3) und einem elastischen Ring (4) umfasst, und wobei das untere Ende der Kammern (1a, 1b) einen konischen Boden (6) aufweist.

2. Vorrichtung nach Anspruch 1, **dadurch gekennzeichnet, dass** die Kammern (1a, 1b), Brücke (2) und Luft-Auslasskanäle (5) aus einem transparenten Körper biokompatiblen Materials geformt sind.

3. Vorrichtung nach Anspruch 1, **dadurch gekennzeichnet, dass** die Kammern (1a, 1b) 12 mm in der Länge und 4 mm im Durchmesser sind, mit konischen Bodenenden und eine Kapazität äquivalent zu 130 μl aufweisen, und der Kanal oder die Brücke (2) 2 mm in Länge und 2 mm im Durchmesser ist, mit einer Kapazität äquivalent zu 20 μl.

4. Vorrichtung nach Anspruch 1, **dadurch gekennzeichnet, dass** es in acrylischem, biokompatiblen und notwendigerweise transparentem Material hergestellt ist, zur effizienten Verwendung der Vorrichtung.

5. Verfahren zur Diagnose eines physiologischen Status und/oder Selektion von Spermatozoen aus einer Samenprobe basierend auf Chemotaxis unter Verwendung einer Vorrichtung gemäß irgendeinem der Ansprüche 1 bis 4, **dadurch gekennzeichnet, dass** es die folgenden Stufen umfasst:

a) Platziere ein Schließmittel (3) in einer der Kammern (1a);
b) Fülle die Brücke (2) durch das Gießen von Kulturmedium oder Lockstoffmedium durch die verbleibende Kammer (1b);
c) Platziere die Spermatozoen-Suspension in die Kammer (1b) ohne Schließmittel und Platziere das Schließmittel (3);
d) Entferne das Schließmittel (3) der zuerst erwähnten Kammer (1a) und fülle mit Kulturmedium oder Lockstoffmedium;
e) Inkubiere die Vorrichtung;
f) Gewinne die Kammer (1a) -Lösung.

6. Verfahren nach Anspruch 5, **gekennzeichnet durch** das Durchführen der folgenden Stufen nach Stufe "f":

g) Platziere den Stopfen in die Kammer (1a);
h) Entferne Stopfen von Kammer (1b);
i) Gewinne Kammer (1b) -Lösung.

7. Verfahren nach Anspruch 5, **dadurch gekennzeichnet, dass** der Lockstoff Progesteron ist.

8. Verfahren nach Anspruch 5, **dadurch gekennzeichnet, dass** der Samen Samen eines Säugetiers ist.

9. Verfahren nach Anspruch 5, **dadurch gekennzeichnet, dass** der Samen Samen einer Spezies einer anderen zoologischen Gruppe ist.

10. Verfahren nach Anspruch 5, **dadurch gekenn-**

zeichnet, dass das Lockstoff - Medium Progesteron in einer Konzentration zwischen 1 und 100 pM umfasst.

11. Verfahren nach Anspruch 5, **dadurch gekennzeichnet, dass** Stufe e) des Inkubierens durchgeführt wird für einen Zeitraum von 10 bis 30 Minuten bei einer Temperatur von 37 °C und in einer 5 % CO_2-Umgebungsluft.

12. Verfahren nach Anspruch 5, **dadurch gekennzeichnet, dass** die Spermatozoen-Suspension bei Stufe c) eine Spermatozoen-Konzentration von zwischen 1 x 10^6 und 9,9 x 10^6 mobile Spermatozoen / ml aufweist.

Revendications

1. Dispositif pour le diagnostic de l'état physiologique et/ou la sélection de spermatozoïdes d'un échantillon de sperme, se fondant sur la chimiotaxie, du type ayant deux compartiments communicants (Ia, Ib), **caractérisé en ce que** ladite communication entre les compartiments (Ia, Ib) s'effectue par une conduite ou un pont (2) situé sur le fond, et au-dessus du niveau inférieur des compartiments (Ia, Ib) mentionnés, par mise en place, sur l'entrée desdits compartiments (Ia, Ib), d'un moyen de fermeture approprié (3, 4) et des conduites appropriées de sortie d'air (5) faisant communiquer l'extrémité supérieure des compartiments (Ia, Ib) avec l'extérieur, ledit moyen de fermeture (3, 4) comprenant un bouchon comportant une saillie (3) et une bague élastique (4), et l'extrémité inférieure des compartiments (Ia, Ib) ayant une base conique (6).

2. Dispositif selon la revendication 1, **caractérisé en ce que** lesdits compartiments (Ia, Ib), ledit pont (2) et lesdites conduites de sortie d'air (5), sont formés dans un corps transparent en un matériau biocompatible.

3. Dispositif selon la revendication 1, **caractérisé en ce que** lesdits compartiments (Ia, Ib) ont une longueur de 12 mm et un diamètre de 4 mm, avec des extrémités de base coniques, et ont une capacité équivalente à 130 μl, et la conduite ou le pont (2) a une longueur de 2 mm et un diamètre de 2 mm, avec une capacité équivalente à 20 μl.

4. Dispositif selon la revendication 1, **caractérisé en ce qu'**il est fabriqué en un matériau acrylique, biocompatible et nécessairement transparent, pour une utilisation efficace du dispositif.

5. Mode opératoire pour le diagnostic de l'état physiologique et/ou la sélection de spermatozoïdes d'un échantillon de sperme sur la base d'une chimiotaxie, par utilisation du dispositif selon l'une quelconque des revendications 1 à 4, **caractérisé en ce qu'**il comprend les étapes suivantes :

a) mise en place d'un moyen de fermeture (3) dans l'un des compartiments (Ia) ;
b) remplissage du pont (2) par versement d'un milieu de culture ou d'un milieu attracteur par le compartiment restant (Ib) ;
c) mise en place des spermatozoïdes en suspension dans le compartiment (Ib) sans le moyen de fermeture, et mise en place du moyen de fermeture (3) ;
d) enlèvement du moyen de fermeture (3) du premier compartiment mentionné (Ia), et remplissage avec le milieu de culture ou le milieu attracteur ;
e) incuber le dispositif ;
f) récupérer la solution du compartiment (Ia).

6. Mode opératoire selon la revendication 5, **caractérisé par** la mise en oeuvre des étapes suivantes après l'étape f) :

g) mise en place du bouchon dans ledit compartiment (Ia) ;
h) enlever le bouchon du compartiment (Ib) ;
i) récupérer la solution du compartiment (Ib).

7. Mode opératoire selon la revendication 5, **caractérisé en ce que** l'attracteur est la progestérone.

8. Mode opératoire selon la revendication 5, **caractérisé en ce que** le sperme est un sperme de mammifère.

9. Mode opératoire selon la revendication 5, **caractérisé en ce que** le sperme est un sperme provenant d'autres espèces de groupes zoologiques.

10. Mode opératoire selon la revendication 5, **caractérisé en ce que** le milieu attracteur comprend de la progestérone à une concentration comprise entre 1 et 100 pM.

11. Mode opératoire selon la revendication 5, **caractérisé en ce que** l'étape e) d'incubation est mise en oeuvre pendant un laps de temps de 10 à 30 minutes à une température de 37°C et dans un environnement à 5 % de CO_2 dans l'air.

12. Mode opératoire selon la revendication 5, **caractérisé en ce que** l'étape c), suspension des spermatozoïdes, correspond à une concentration des spermatozoïdes comprise entre 1.10^6 et $9{,}9.10^6$ spermatozoïdes mobiles par ml.

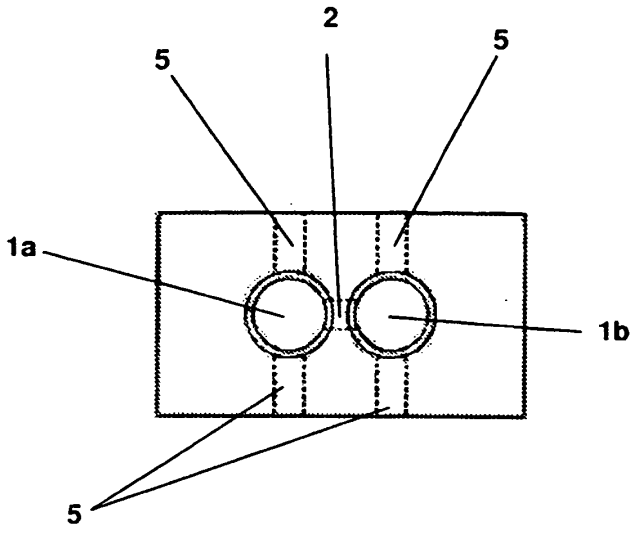

FIG. 1a

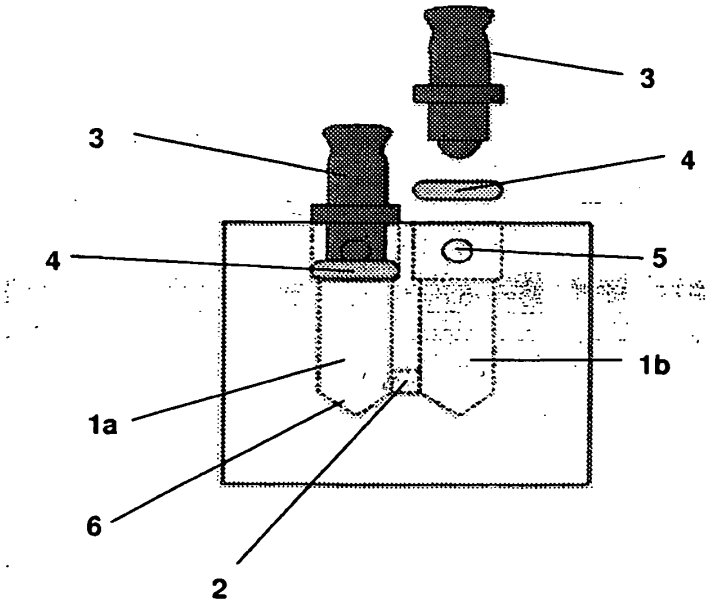

FIG. 1b

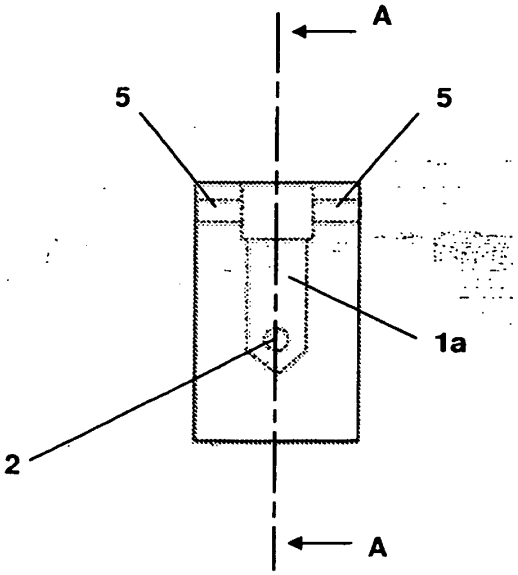

FIG. 1c

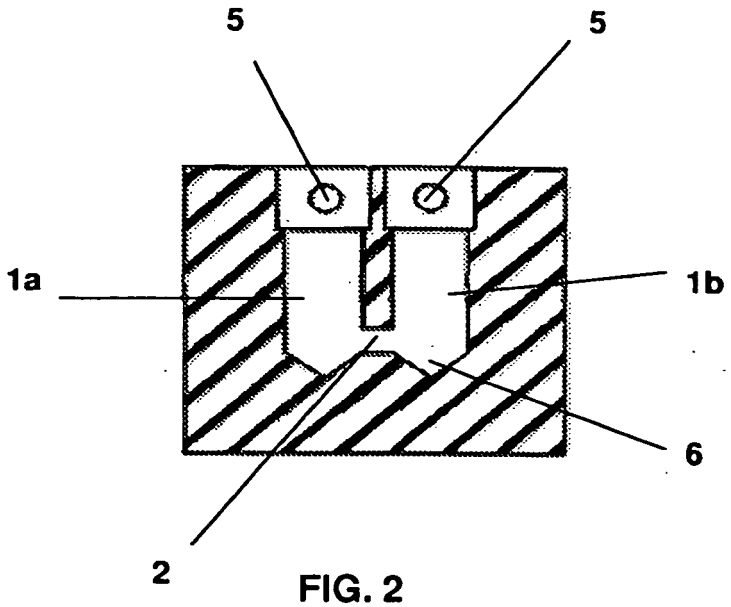

FIG. 2

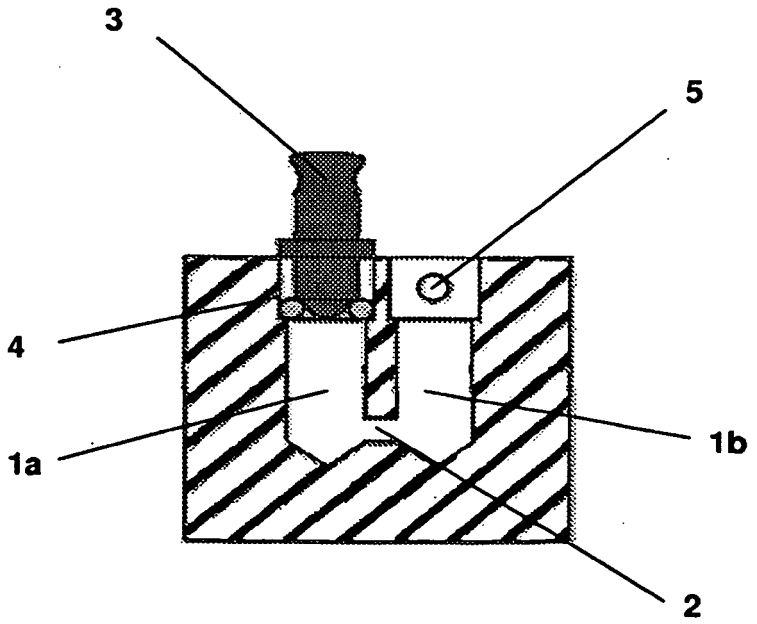

FIG. 3

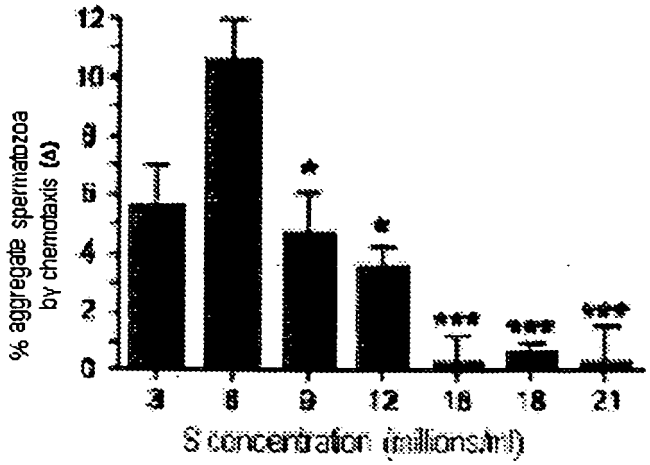

Figure 4

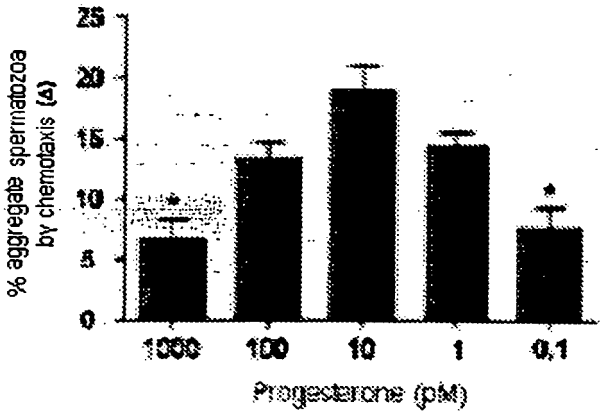

Figure 5

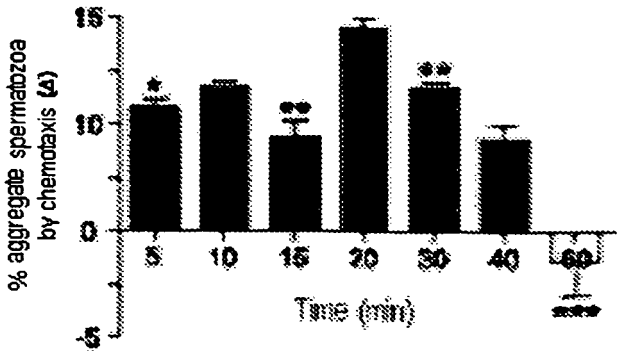

Figure 6

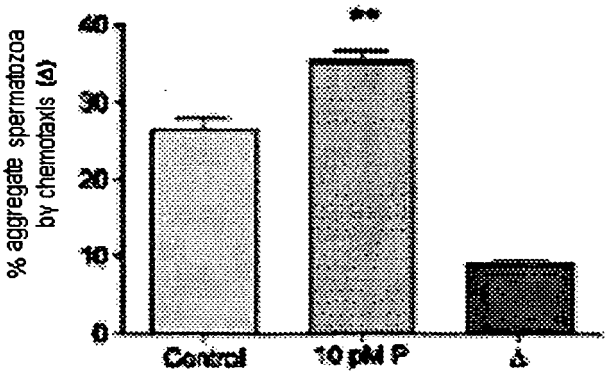

Figure 7

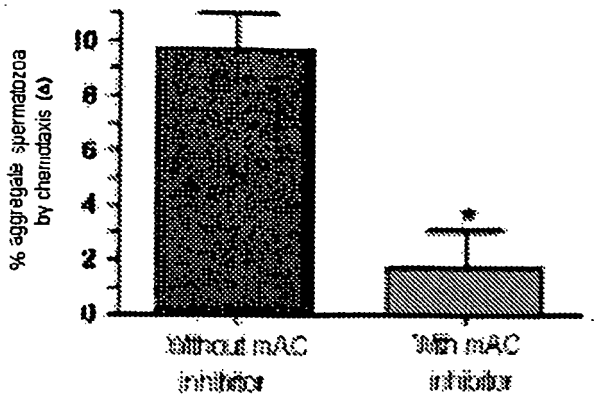

Figure 8

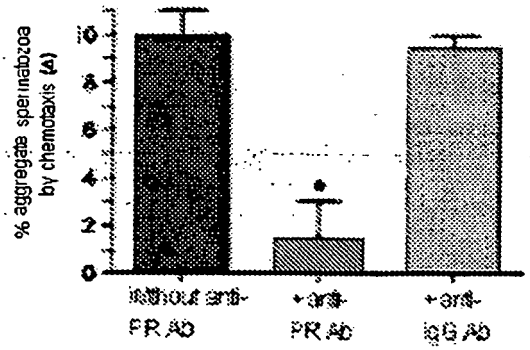

Figure 9

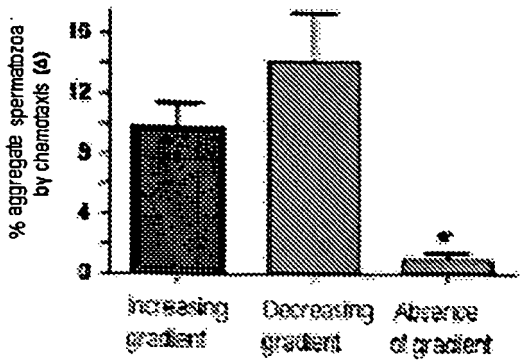

Figure 10

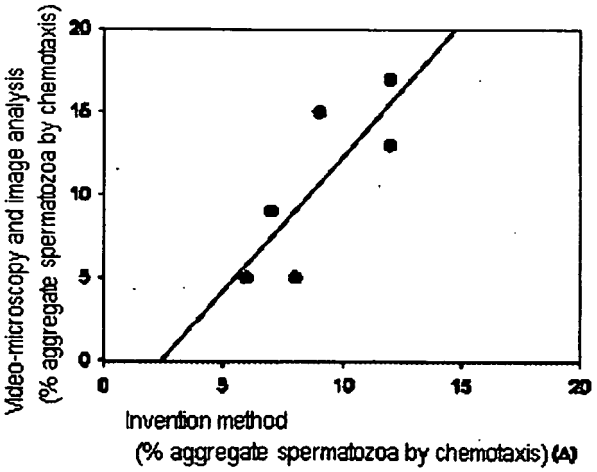

Figure 11

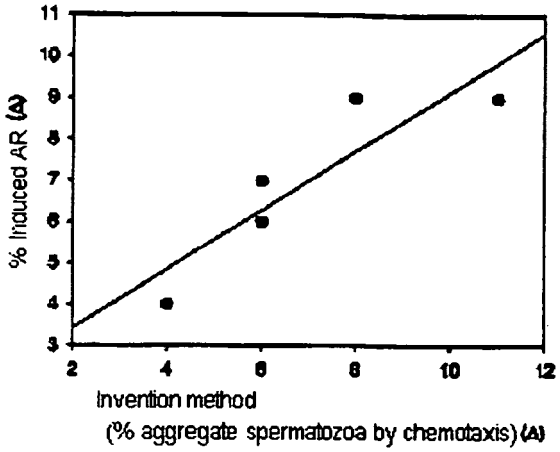

Figure 12

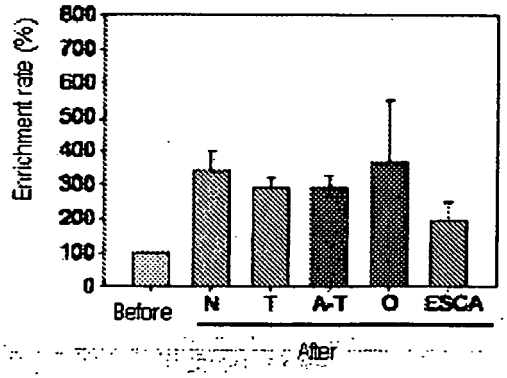

Figure 13

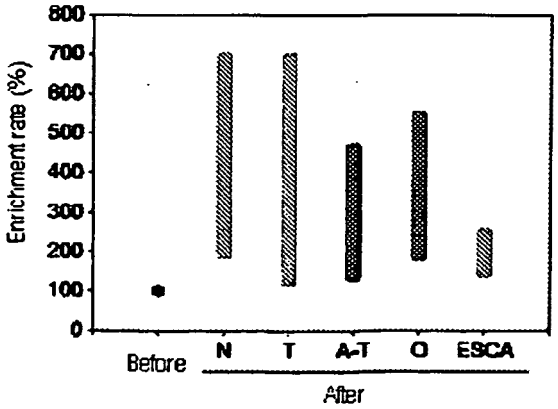

Figure 14

REFERENCES CITED IN THE DESCRIPTION

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