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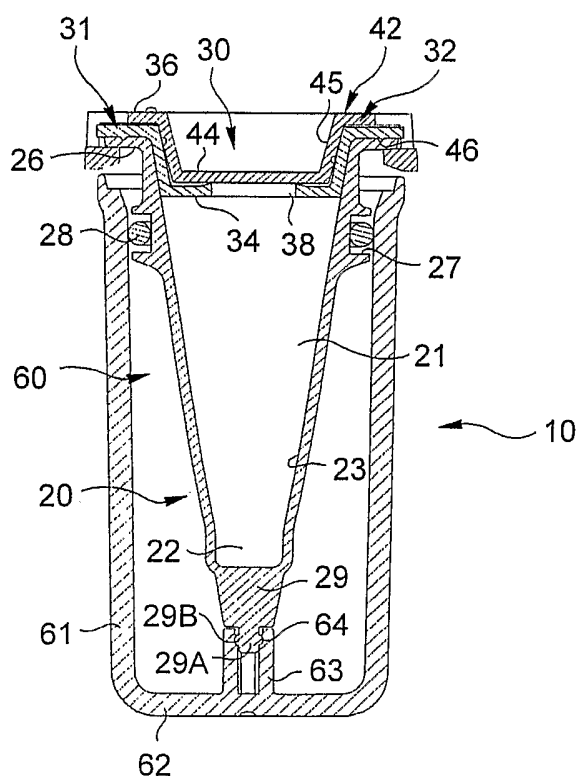
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(54) Title: INCUBATION AND/OR STORAGE CONTAINER SYSTEM AND METHOD



(57) Abstract: A container assembly (10) comprises a vessel (21) for containing a biological medium, gametes and/or one or more embryo(s). The vessel (21) has a CO₂ permeable seal (28) and a closure device (30) for selective access. A buffer chamber (60) for a CO₂ enriched atmosphere at least partially surrounds the vessel and is defined by a shell (61). The buffer chamber is in communication with the CO₂ permeable wall. Such a container assembly is particularly adapted for IVC in which case the permeable seal (28) prevents ingress of vaginal secretions. The buffer chamber (60) mediates the aqueous pH in the vessel after the container assembly is removed from a CO₂ enriched environment.

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Incubation and/or Storage Container System and Method

Background of the Invention

1. Field of the Invention

The present invention relates to an incubation and/or storage container
5 assembly for gametes and/or at least one embryo and in particular for such a
container assembly adapted for use in intravaginal incubation and culture for
humans or other mammals.

2. Description of Prior Art

Conventional in-vitro fertilization (IVF) techniques are notoriously complex.
10 They involve aerobic and sterile culture of embryos in Petri dishes at 37°C in a 5
% CO₂ enriched atmosphere which requires cumbersome and expensive
equipment such as a CO₂ incubator operating 24 hours a day during the two or
three days required for the fertilization and culture. It also involves delicate
manipulations requiring the skills and dexterity of a laboratory biologist.

15 Intravaginal culture (IVC) has been developed and comprises maturation
of gametes, fertilization of oocytes and embryo development in a sealed
container filled with a suitable culture medium which is then placed in the vaginal
cavity which serves as an incubator. This technology is disclosed in Ranoux U.S.
Patents Nos. 4,902,286 and 5,135,865. It is designed and utilized by assisted
20 procreation specialists in their offices or clinics.

To date, IVC procedures have been performed with a polypropylene
Cryotube manufactured by Nunc of Kamptrup, Denmark, which is closed after
loading the gametes and sealed in a polypropylene Cryoflex envelope also
manufactured by Nunc. IVC procedures using such a container assembly have
25 numerous drawbacks. Many of these drawbacks are overcome with the
container assembly disclosed in Ranoux et al U.S. Patent No. 6,050,935. That
patent describes a IVC container assembly comprising a container body and
resealable closure means for selectively opening and closing a container body
orifice. The container body has a main chamber with a cylindrical sidewall and a
30 microchamber in communication with each other which permits the movement of
one or more embryo(s) into and out of the microchamber. The microchamber has
sidewalls of optical quality permitting microscopic inspection of embryos. The
microchamber also facilitates the retrieval of one or more embryo(s) by means of

a catheter without endangering the embryo(s). The container body is equipped with various valve designs which are either bulky or complex construction and/or uneasy to operate. A two-piece capsule of soft flexible material envelopes the container for lodgment in the posterior fornix.

5 When such a IVC container is taken out of the posterior fornix of the vagina, the outer capsule is removed and the embryos in the microchamber may be inspected under a microscope. One or more embryos is then retrieved from the microchamber by a catheter for transfer to the uterus. This is done while the patient is being prepared for the transfer of the embryo(s). The entire procedure
10 is also designed to be carried out in an obstetrician or other assisted procreation specialist's office with a minimum of equipment.

One of the advantages of the IVC procedure is that fertilization and culture are carried out intravaginally where the atmosphere is naturally CO₂ enriched and the amount of oxygen is much lower than of the ambient environment. Both
15 properties are acknowledged as being beneficial, see Alan O. Trounson et al., Handbook of In-vitro Fertilization, CRC Press, Inc., 1993, p. 97 and Misao Fukuda et al., "Unexpected Low Oxygen Tension of Intravaginal Culture", Human Reproduction, vol. 11, no. 6, pp. 1996, 1293-9. Likewise, the temperature is that of the natural environment of the vagina. Once the IVC container is
20 removed from the vagina, it no longer benefits from this ideal natural environment. It is also known that the intravaginally CO₂ enriched environment ensures the pH in the container is relatively constant and about 7.3 and that a lower level of CO₂ in the container will cause a drop in the pH of the biological medium in which the embryo(s) reside. A relatively small change in the pH (say
25 0.5) may have drastic consequences over a long period of culture on the embryo(s).

An object of the present invention is to mitigate such drawbacks of known IVC containers and to provide an improved incubation and/or storage container assembly system and container system components and an improved method
30 for incubation and/or storage of gametes and/or one or more embryos.

According to one aspect of the invention, a buffer chamber for CO₂ enriched atmosphere is provided and cooperable with the vessel containing the biological medium gametes and/or one or more embryo(s) and is in

communication with a CO₂ permeable wall of the vessel. With such an arrangement, the vessel will remain in a CO₂ enriched environment even after it is removed from the CO₂ enriched incubation environment in particular a vagina. Thereafter, the CO₂ enriched air in the buffer chamber will be able to enter the
5 vessel and compensate for any fall in the CO₂ level inside the vessel and thereby mediate the pH in the biological medium. Indeed, it has been found if such a buffer chamber is provided on the incubation or storage vessel, the pH level of the biological medium in the vessel will fall only slightly over the period of about one or two hours after the removal of the container assembly from the CO₂
10 enriched environment. Such a small dip in the pH level does not have any significant effect on the embryo(s) in the biological medium.

According to another aspect of the invention, a buffer chamber is provided, comprising a shell mounted on the vessel with a CO₂ permeable seal disposed between the vessel and the shell to prevent the ingress of liquids or
15 other viscous fluids, in particular vaginal secretions while allowing the inflow of the CO₂ enriched air from the surroundings and in the case of intravaginal incubation, from the vagina. In practice, the CO₂ inflow rate of the permeable seal will be greater than the inflow rate of CO₂ through the permeable wall of the vessel and very much greater than the CO₂ outflow rate through the shell wall.

20 According to another aspect of the invention, the shell is mounted for movement on the vessel between open and closed positions. The shell will be in its open position when the container assembly is introduced into a CO₂ enriched air environment, such as a vagina in the case of intravaginal use, and is closed as soon as the container assembly is removed from the CO₂ enriched air
25 environment. In such an embodiment, the CO₂ enriched air outflow may be virtually nil during the period between the removal of the container assembly from the CO₂ enriched environment and the retrieval of the embryos from the vessel for transfer to a recipient, thereby ensuring CO₂ equilibration in the biological medium.

30 In the course of residence in the CO₂ enriched intravaginal environment, the level of oxygen in the buffer chamber will reach the favorably depleted O₂ level which prevails in the vagina. Thus, after the container assembly is

removed, not only is the air inside the buffer chamber advantageously enriched in CO₂ but also reduced in O₂.

According to an embodiment of the invention, the vessel is provided with a closure device including overlying disc-shaped valve members, each with an orifice, mounted for relative angular movement between an open position for
5 access to the interior of the vessel and a closed position for sealing off access to close the vessel.

According to an embodiment, the peripheral flange of the outer disc-shaped valve member has a peripheral sidewall radially beyond the peripheral
10 flange of the inner disc-shaped valve member. One of the peripheral flanges has protrusions selectively cooperable with cutouts in the peripheral sidewall in the other peripheral flange when the valve is in its closed position. Preferably, the peripheral sidewall of the outer disc-shaped valve member has one or more
hooking members for snap fitting axial retention of the outer disc-shaped valve
15 member on the inner disc-shaped valve member and/or a peripheral flange of the vessel.

According to a preferred embodiment, sealing material is affixed to one of the disc-shaped valve members for fluidtight or rubbing contact with the other of the disc-shaped valve members. Where required, an additional sealing cap for
20 impeding the ingress of vaginal fluids overlies the closure device and is in sealing engagement with the closure device and an upper portion of the outside wall of the shell.

One or both of a pair of opposed sidewalls of the microchamber has an abutment for docking a catheter at the desired location. A portion of the
25 associated recess may define a lens face for viewing one or more embryo(s) in the catheter during or after retrieval from the microchamber.

The inner wall surface of the main chamber of the vessel tapers towards the microchamber. Thus, when the container assembly is received in the posterior fornix, that is in a substantially horizontal position, except when the
30 recipient lies on her side, the inner wall surface slopes to a small zone, where gametes will tend to congregate, thereby enhancing the probability of contact between sperm and oocytes.

According to another aspect of the invention, there is provided a shell for surrounding the vessel and defining therebetween a buffer chamber for a CO₂ enriched atmosphere. According to a preferred embodiment, there are at least two shell parts and a gas flow passage between respective ones of the shell parts. Preferably, there is a CO₂ permeable seal located in the gas flow passage for allowing the inflow of CO₂ enriched air and impeding the ingress of fluid, in particular vaginal fluids into the buffer chamber. Such a shell may enclose various kinds of IVC vessels and in particular IVC vessels with closure devices for selective access to the interior of the vessel. The shell is preferably made of a smooth, rigid transparent medical grade material and sized and configured for accommodation in the posterior fornix. With such a shell no separate container sleeve or carrier is necessary.

These and another objects and advantages of the invention will be brought out in the description of embodiments given by way of example with reference to the accompanying drawings.

Brief Description of Drawings

Fig. 1 is a longitudinal sectional view of a first embodiment of the container assembly with its closure device in an open position.

Fig. 1A is an enlarged longitudinal sectional view of the lower end of the vessel of the container assembly to illustrate the catheter docking abutment in the vessel wall.

Fig. 2 is a view similar to that of Fig. 1 with the closure device in a closed position.

Fig. 3 is a perspective view, from above, of the fixed inner disc-shaped valve member of the closure device for the vessel of Fig. 1.

Fig. 4 is a top plan view of the fixed lower disc of Fig. 3.

Fig. 5 is a perspective view from above of the rotatable upper disc-shaped valve member of the closure device taken on its own.

Fig. 6 is a perspective view from below of the rotatable upper disc-shaped valve member of Fig. 5.

Fig. 7 is a perspective view from above of the upper part of the container assembly with the closure device in its closed position.

Fig. 8 is a longitudinal sectional view of the container assembly including the container sleeve or carrier for lodging the container assembly in the posterior fornix.

Fig. 8A is an enlarged detail of the vessel wall and lower valve member to illustrate the congregating of oocytes when the container assembly is lodged in the posterior fornix.

Fig. 9 is a longitudinal sectional view of a second embodiment of the container assembly in the open position of the buffer chamber, the closure device being in its closed position.

Fig. 10 is a longitudinal sectional view similar to Fig. 9 in the closed position of the buffer chamber.

Fig. 11 is a perspective view, partially cut away, of the container assembly of Fig. 1 received in an isothermal holding block for maintaining the temperature of the vessel and its contents and inspecting the embryo(s).

Fig. 12 is a longitudinal sectional view of the upper part of the container assembly according to a third embodiment which is a variant of the embodiment of Figs. 1-8.

Fig. 13 is a perspective view from above of the sealing cap, illustrated on its own, and part of the third embodiment of Fig. 12.

Fig. 14 is a longitudinal sectional view of the modified rotatable upper disc-shaped valve member, taken on its own, according to the third embodiment of Fig. 12.

Fig. 15 is an enlarged cross sectional detail illustrating the angular abutment between the upper rotating disc-shaped valve member and the lower fixed disc-shaped valve member of the third embodiment of Fig. 12.

Fig. 16 is an exploded perspective view of the entire container assembly according to a fourth embodiment of the invention.

Fig. 17 is a longitudinal cross sectional view of the entire container assembly of the fourth embodiment in its assembled and closed position.

Fig. 18 is a longitudinal sectional view of an upper part of the shell of the fourth embodiment.

Fig. 19 is an enlarged scale cross sectional detail illustrating the label holder on the inside wall of the lower part of the shell of the fourth embodiment.

Detailed Description of Embodiments of the Invention

The first embodiment of the container assembly 10 for incubating and/or storing gametes and/or one or more embryos is illustrated in Figs. 1-8. Such a container assembly is suitable for intravaginal incubation or culture (IVC) of human or mammalian embryos, and for use as a storage and transport container for gametes and/or one or more human or other mammalian embryos.

The terms such as "upper" and "lower" are used by convention in the specification and claims, in respect to all embodiments, to refer to relative positions in the container assembly as oriented for example in Figs. 1 and 2. It goes without saying that such terms are not intended to be in any way limiting as to orientation or location of the container assembly which in actual practice will vary depending on the stage of the procedure in which it is employed.

The container assembly 10 comprises an inner vessel 20, also referred to as the vessel, the vessel having a closure device 30 for opening and closing access to the interior thereof. The inner vessel 20 is at least partly surrounded and preferably substantially entirely surrounded by a buffer chamber 60 comprising in the illustrated embodiment a shell 61 cooperating with the inner vessel 20.

The inner vessel 20 comprises an upper, main chamber 21 and a lower, microchamber 22 in communication with each other. The inner wall surface 23 of the main chamber tapers towards the generally parallelepipedic microchamber 22. As the upper end of the main chamber in this environment is circular and the lower end is substantially rectangular, the contour of the inner wall surface varies from a circle to a rectangle. The overall shape of the inner wall surface 23 is generally frustoconical with transverse sections that are somewhat flattened oval shapes. The portions of the inner wall surface 23 which lead into wider sidewalls 24 of the microchamber 22 are generally flatter than the portions of inner sidewall which lead into the narrower end walls 25 of the microchamber. At least one of the opposed walls, here sidewalls 24, are of sufficient optical quality to permit inspection under microscope or other magnification instrumentation. In practice, the microchamber 22 and in fact the entire vessel will be made of a medical grade material of good optical quality, such as polycarbonate. A polycarbonate which may be suitable is Makrolon RX.2530 45 1118 available

from Bayer Chemicals. This polycarbonate has a CO₂ permeability of the order of about 43.0 cm³ x cm/m² x 24 hr x atm. at standard temperature and pressure. Preferably, however, the vessel body is made of a crystal polystyrene, such as Nova High Heat Crystal Polystyrene, ref. 1204. Regardless of the constituent
5 material, the vessel has a peripheral flange 26 extending radially outwardly from the upper end thereof.

The closure device 30 is provided at the open upper end of the vessel body and comprises in a preferred embodiment a valve 31 including two overlying disc-shaped valve members 32, 42. One of the valve members is fixed
10 and the other is mounted for relative angular movement. In practice, the lower valve member 32 is fixed by ultrasonic welding to the upper end of the vessel in practice, the peripheral flange thereof. Each of the valve members comprises a central panel 34, 44 having a port or orifice 38, 48, adapted to be brought into registration in the fully open position of the closure device and out of
15 communication in the fully closed position of the closure device. Each of these orifices 38, 48, is of the same D-shaped contour in the illustrated embodiment. Such a D-shaped contour may limit the access area to permit the entry of only the thinnest of catheters or the largest of pipettes. Obviously, other contours are possible, in particular circular, such as disclosed in the third embodiment. The
20 contour edge of one of the orifices 38, 48 and preferably the orifice 38 in the lower valve member 32 has a raised lip or bead 39 for enhanced sealing engagement with the underside of the central panel 44 of the upper valve member. The upper surface of the central panel 44 of the lower valve member has another, second, raised lip or bead 40 spaced from the first raised lip or
25 bead 39 and of C-shape as shown, which extends proximate to the outer periphery of the solid portion of central panel 34. The second raised lip or bead 40 ensures that the central panels 34, 44 of the valve members remain parallel to each other to avoid leaking.

Each of the central panels 34, 44 is respectively surrounded by an
30 upwardly or outwardly flaring frustoconical sidewall 35, 45, from the upper end of which extends a radially outwardly extending peripheral flange 36, 46. The respective central panels 34, 44, flaring sidewalls 35, 45 and the peripheral flanges 36, 46 are respectively parallel to each other. One of the mutually

contacting surfaces of the sidewalls has a grooved screwthread 47 and the other of the mutually contacting surfaces of the sidewalls has a slider 37 adapted to be received and guided in the grooved screwthread 47. The screwthread 47 and slider 37 have a dual function. One function is to guide angular movement of one disc relative to the other disc and the other function is to separate one disc relative to another disc to break contact between the protruding lip 39 and the central panel 44 of the facing valve member. Other guiding means may be provided instead of the screwthread groove and slider permitting both of these functions. Alternatively, the axial displacement function can be eliminated and a circular groove used in which case there is simply rubbing contact between the raised lips or beads 39, 40 and the facing central panel of the other valve member when the valve member is rotative. In fact, both of these functions may be eliminated, such as disclosed in the third embodiment described below and illustrated in Fig. 12.

A peripheral flange 46 extends downwardly from the peripheral flange 46 of the upper valve member 42 and has a radially inwardly projecting hooking member 49 cooperable with the undersurface of at least one of the peripheral flanges of the vessel and fixed valve member and as shown under the undersurface of peripheral flange 26 of the vessel 20. The peripheral flange 46 and the adjoining peripheral sidewall 46A have a plurality of spaced cutouts 50, a first portion 50A of each cutout having radially inwardly flaring sides 50B being located in the peripheral flange and a second portion 50C extending downwardly along the peripheral sidewall 46A and defined by leading and lagging parallel edges 50D, 50E generally in alignment with the respective hooking members 49.

The outer peripheral edge 36A of the peripheral flange 36 of the lower valve member has one or more protrusions 36B defined by a generally radial edge and generally circumferential or tangent edge and two such protrusions 36B diametrically opposed and mirror images of each other, as shown. The protrusions are adapted to clickingly clear the respective leading edges of the second portions 50D of the cutouts 50 to provide an audible signal that the closed position of the closure member has been reached (see Fig. 7).

The lower and upper disc-shaped valve members 32, 42, may be assembled in the following manner. The upper valve member 42 is positioned on

top of the lower valve member 32 previously ultrasonically welded to the vessel, and pressed downwardly. The edge 36A of the peripheral flange 36 will ride along and clear the oblique undersurfaces 49A of the hooking members 49 and snap into the space 49C between the upper end surface of the hooking member 49 and the underside of peripheral sidewall 46A of the upper valve member 42. The outer diameter of the peripheral flange 36 of the lower valve member and the peripheral flange 26 of the vessel is slightly greater than the diametrical distance between the radially inner ends 49B of the hooking members 49 thereby preventing the escape of the outer valve member off of the peripheral flange of the vessel.

The lower valve member 32 may be made of the same polycarbonate or better polystyrene used for the vessel body or some other medical grade material compatible for ultrasonic welding with the peripheral flange of the vessel. The upper valve member is preferably made of a softer material than the material used for the lower valve member in order to enhance the sealing action of the contour lip or bead. For example, a polypropylene available from Huntsman Corp. under reference 13G9A is suitable. Such a polypropylene has a permeability of about $60 \text{ cm}^3 \times \text{cm/m}^2 \times 24 \text{ hr} \times \text{atm.}$ at standard temperature and pressure.

The outer surface of the vessel body has a radially outwardly opening annular groove 27 for accommodating a sealing member 28 which may be a O-ring, as illustrated in Figs. 1 and 2. When the vessel is received in the shell 61, the sealing member 28 is in sealing engagement with the intermediate, bight portion of the groove 27 and the inner wall surface 67 of the shell 61 in alignment therewith. The sealing member in the illustrated embodiment has various features, the most important of which is its high CO_2 permeability and CO_2 flow rates permitting the inflow of CO_2 enriched air from a surrounding CO_2 enriched environment. The CO_2 inflow rate should enable the CO_2 level in the buffer chamber to reach the level in the surrounding CO_2 environment in less than about eight hours and preferably in less than about three hours. The flow rate should not be too high so as to cause a significant outflow of the CO_2 enriched gas from the buffer chamber in less than two hours. Another advantageous feature of the sealing member is its permeability to O_2 to enable the depleted

levels of O₂ in the CO₂ enriched environment to replace the normal level of O₂ in the ambient air after the container assembly is placed in the CO₂ enriched and O₂ lean environment. In practice, the sealing member will be air permeable and therefore allows the in- and outflow of all gases in the ambient air, especially N₂,
5 CO₂ and O₂. Another advantageous feature of the sealing member is to define a barrier to liquids or viscous substances and in particular vaginal secretions when the container assembly is intended for intravaginal use. Another advantageous feature of the sealing member is to define a barrier against the entry of bacteria and even viruses present in a vagina when the container assembly is to be used
10 intravaginally. Such a sealing member effective against the ingress of vaginal secretions, bacteria and viruses will prevent their entry into the buffer chamber and avoid possible contamination of the contents of the vessel via the vessel walls. A suitable material having all foregoing features is a medical grade silicone which has a very high permeability of the order of 30,500 cm³ x cm/m² x 24 hr x
15 atm. at standard temperature and pressure. Such an example is, however, not intended to be limiting. The CO₂ permeability of the seal may be very much less than that of medical grade silicone and even low as about 0.45 cm³ x cm/m² x 24 hr x atm. at standard temperature and pressure in the case of a nylon 6.6 gasket. Whatever the seal material is selected, it should enable equilibration between
20 CO₂ level in the CO₂ enriched environment of the vagina or other incubator and that of the buffer chamber in less than about eight hours and preferably in about three hours.

The shell is made of a medical grade material having good clarity for inspection of the contents in the microchamber through the wall of the shell. To
25 this end, it preferably has diametrically opposed planar zones of optical quality adapted to be in alignment with the sidewalls of the microchamber (this feature not being shown in the embodiment of Figs. 1-8 but designated 65 in the embodiment of Figs. 9 and 10). A suitable material for the shell is PETG such as Eastar MN058 available from Eastman Chemical Co. having a permeability of
30 about 83 cm³ x cm/m² x 24 hr x atm. at standard temperature and pressure. Alternatively, polycarbonate but preferably crystal polystyrene may be used for the shell wall. When polycarbonate or crystal polystyrene is also used for the vessel wall, the thickness of the shell wall should be at least about twice the

thickness of the vessel wall to ensure that the CO₂ flow rate through the vessel wall will be substantially greater than the CO₂ flow rate through the shell. The shell may alternatively be made of a material having a substantially nil CO₂ permeability such as, for example, glass having suitable mechanical properties.

- 5 When a shell of nil or very low permeability is employed, obviously essentially all CO₂ and/or O₂ flow will be through the seal between the vessel wall and the shell wall.

According to an embodiment, the CO₂ permeability of the seal is selected to be, say, one or two orders of magnitude greater than the permeability of the vessel wall and at least two orders of magnitude greater than the CO₂ permeability of the shell wall. An example of such an embodiment is a silicone seal having a CO₂ permeability of the order of about 30,500 cm³ x cm/m² x 24 hr x atm. at standard temperature and pressure, a vessel made of Makrolon polycarbonate having a CO₂ permeability of about 43.0 cm³ x cm/m² x 24 hr x atm. at standard temperature and pressure and a shell made of Eastar PETG having a permeability of about 83 cm³ x cm/m² x 24 hr x atm. at standard temperature and pressure. Preferably, however, the shell is made of medical grade polystyrene having a permeability of about 69 cm³ x cm/m² x 24 hr x atm. at standard temperature and pressure.

- 20 Preferably, the CO₂ permeability of the constituent materials is selected so that the CO₂ permeable seal is between about 10,000 and about 40,000 cm³ x cm/m² x 24 hr x atm. at standard temperature and pressure whereas the CO₂ permeability of the vessel is between about 50 to about 500 cm³ x cm/m² x 24 hr x atm. and the CO₂ permeability of the shell is between 0 (corresponding to glass) and 200 cm³ x cm/m² x 24 hr x atm.

The vessel and/or the seal material may be also chosen in order to slightly delay the entry of the CO₂ enriched gas into the vessel to counter the initial generation of acidic metabolic products during which the CO₂ in the vessel which should be allowed to permeate through the vessel wall into the buffer chamber maintaining the desired equilibration level, while thereafter allowing the CO₂ enriched environment to flow into the vessel in order to maintain a pH of about 7.4 once acidic metabolic products cease to be produced.

When the container assembly is not intended for intravaginal use, there may be no need to prevent the ingress of liquids or other viscous fluids.

Sealing member configurations other than O-rings may be useful and in particular annular gaskets having a rectangular cross section and therefore the same gas flow rate through the entire radial extent of the cross section.

In practice, the sealing member will have an inner diameter in its rest configuration which is slightly less than the corresponding outer diameter of the complementary bight portion of the groove and an outer diameter which is slightly greater than the inner surface of the shell in contact to cause elastic deformation and thereby ensure a snug fit and satisfactory tightness .

The lower end 29 of the vessel 20 that is the trapezoidal shaped portion (as shown) of the vessel situated below the microchamber 22 will in practice be solid and not hollow. The lower end 29 of the vessel has a locating member 29A cooperable with a complementary locating member 63 of hollow cylindrical configuration and upstanding from the bottom 62 of the shell 61 in the illustrated embodiment. The locating member 29A has at least one protruding bead or boss 29B which is cooperable with a complementary groove or recess 64, so as to define a stable position of the vessel when the vessel is fully inserted into the buffer chamber. Alternatively, or in combination with the aforesaid locating members 29A, 63, the abutting surfaces of the top edge of the locating member 63 and the downwardly facing annular shoulder of the lower end 29 may define the fully inserted position of the vessel relative to the shell 61.

Guiding members (not illustrated in this embodiment) may be provided to guide the movement of the vessel to ensure the locating member 29A at the lower end 29 is correctly engaged into the complementary locating member 63. Such guiding members may for example comprise two or more fin-like elements integral with the outer wall of the vessel or the inner wall of the shell and cooperable with the other of the outer wall of the vessel or the inner wall of the shell. Such guiding members are described and illustrated below in connection with the fourth embodiment.

Such a container assembly as illustrated in Figs. 1 and 2 may be filled with a suitable biological medium, such as INRA Menoza B2 medium available from Laboratoire CCD in Paris, or Complete P1® Medium with SSS™, ref. 9926,

available from Irvine Scientific, Santa Ana, California or any other suitable biological medium for sustaining fertilization of gametes and/or embryo development for up to about three days, whereupon the gametes, namely sperm and oocytes may be introduced in that order through the orifices at least partly in registry to enable the insertion of a catheter or pipette into the main chamber of the vessel while minimizing the size of the open access area. Thereafter, the catheter or pipette is taken out and the closure device is immediately closed, sealing off the interior of the vessel from the environment. The shell 61 is preferably positioned with respect to the vessel prior to filling and loading of gametes. It is then suitable for incubation at about 37°C in a conventional incubator with a CO₂ enriched environment in which case the main function of the sealing member will be to ensure the build-up of CO₂ enriched environment in the buffer chamber and which after removal of the container assembly from the incubator will serve as a reservoir for CO₂ enriched air to mediate the aqueous pH level inside the vessel.

This assembly, however, is especially designed for use in intravaginal incubation. To this end, it will be preferably enveloped in a container sleeve or carrier 70 for facilitating intravaginal residence in the posterior fornix. The container sleeve 70 is made of a soft smooth elastic biocompatible material. In the illustrated embodiment, the sleeve 70 is of one-piece construction with an apertured sidewall 71 extending between opposed rounded ends 72, 73 suitable for cooperation with the vaginal vault. The lower rounded end 73 has on its outside surface a plurality of circumferentially spaced dimples 76 for facilitating the removal of the entire container assembly by means of forceps cooperating with dimples. The upper portion of the lower rounded end converges inwardly (in the rest condition) in order to enhance the elastic engagement with the bottom end of the shell 61. The sidewall 71 comprises in practice a plurality, here two, circumferentially spaced longitudinal straps 74 defining apertures 75 therebetween. At least one of the apertures 75 is suitable for the introduction of the container assembly into the internal space 76 of the container sleeve 70. In the embodiment illustrated, the upper rounded end 72 is larger than the lower rounded end 73 and comprises a plug portion 77 complementary in shape and adapted to be received in the recess defined by the sidewalls 45 and central

panel 44 of the upper valve member 42. One or both of the straps 74 may have one or more radially inwardly protruding lip 79 cooperable with the outer edge of the lower valve member and/or peripheral flange 26 of the vessel. Similarly, the inner surface of the bottom rounded end 73 is generally complementary to the bottom wall of the shell 61. In the relaxed position of the container sleeve 70, that is before it is fitted on the container assembly 10, the distance between the inner face of the plug portion 77 of the upper rounded end and the inner or the lower face of the lower rounded end of the container sleeve is less than the distance between the outer surface of the bottom wall 62 of the shell and the outer surface of the central panel 44 of the upper valve member, so that an axial biasing force is exerted by the container sleeve 70 in order to urge the inner and outer valve members into contact and define a second tier sealing between the interior of the vessel and the surrounding environment. In practice, the total length of the entire container assembly with the container sleeve will be about 4-5 cm for a woman or about 5-15cm for a cow. The container sleeve may be made of any medical grade thermoplastic elastomer, such as AES Santoprene 8281-35 W237 having a hardness of 35 Shore A and good cushioning properties. Santoprene has a CO₂ permeability of about 30-300 cm³ x cm/m² x 24 hr x atm. at standard temperature and pressure.

After the container assembly 10 is closed with the sleeve fitted thereon, it may be introduced into the vaginal vault and positioned in the posterior fornix for about 48 to about 72 hours according to current procedure. Prior to introduction into the vaginal vault, the container assembly may undergo pre-incubation at 37°C with or without the sleeve for less than about two hours, safely in a conventional incubator without a CO₂ enriched environment. Alternatively, the whole incubation period may be carried out in an artificial CO₂ enriched environment.

When the container assembly is lodged in the posterior fornix, the longitudinal axis of the vessel will be generally horizontal. As the inner wall surface slopes away from the microchamber and towards the closure device, gametes and in particular oocytes will tend to congregate in the vicinity of the zone where the undersurface of the central panel of the lower valve member meets the inner wall surface of the vessel, as illustrated in Fig. 8A, as this will be

the lowest level of any part of the combined main and micro chambers when the container assembly is lodged in the posterior fornix. This arrangement is advantageous for enhancing the potential of contact between sperm and oocytes. In a variant (not illustrated), the inner wall surface of the vessel may have its largest dimension intermediate the upper and lower ends of the main chamber, for example by adopting a double frustoconical the sidewall surface joined at their large bases. This variant arrangement, as well as other possible arrangements may assist the congregating of the gametes in a limited zone of the main chamber to enhance the potential for fertilization of oocytes.

10 After intravaginal residence, the container assembly is removed. For this purpose, a monofilament string (not shown) of biocompatible material may be attached, bonded to, or integrally formed with, one of the ends or the straps of the container sleeve.

The container assembly is then taken out of the container sleeve. The contents of the microchamber where the embryo(s) will settle by gravity (in the Fig. 1 position) may then be inspected through one of the opposed sidewalls 24 of the microchamber in a recumbent or upright position. The shell 61 has corresponding aligned parallel surfaces 65 of optical quality aligned with the opposed sidewalls 24, in order not to interfere with the inspection of the embryo(s) which will normally be carried out with a laboratory microscope.

Once the desired embryo(s) have been selected, an implantation catheter such as Frydman or Wallace catheter is introduced after slightly opening the closure device by turning the upper valve member. The catheter is then snaked through the main chamber to a location proximate the junction of the main chamber and the microchamber which is equipped with an abutment 22A in a wall of the microchamber, and in practice a pair of abutments in the opposed sidewalls for docking the end of the catheter at a sufficient height above the floor 22B of the microchamber to prevent the catheter from coming into direct contact and thereby possibly crushing or otherwise injuring the embryo(s) in the microchamber (see Fig. 1A) As illustrated, the docking abutment(s) is located midway across the opposed sidewalls 24 of the microchamber so that the microchamber is aspirated to either side. Alternatively, the docking abutment may be located to one side or the other of the microchamber as disclosed in

Ranoux et al. U.S. Patent 6,050,935. The desired embryo(s) may then be aspirated into the catheter and inspected as it or they are drawn upwardly. Indeed, for that purpose, a portion of the recess 22C defining the abutment 22A also defines an interior lens face 22D. The outer surface of the vessel proximate to the junction of the main chamber and microchamber has an exterior lens face 22E in optical alignment with the interior lens face 22D. The lens on one or both sides of the microchamber may be used for viewing the one or more embryo(s) in the catheter during or after the retrieval from the microchamber.

The embryo(s) may then be implanted in accordance with current IVC practice.

Another embodiment is illustrated in Figs. 9 and 10. This second embodiment is suitable for the same purposes as the first embodiment and is of particular interest when the container assembly with its gamete(s) and/or embryo(s) are to be stored for a prolonged period, for example to enable the contents to be shipped prior to implantation. Indeed, in this embodiment, a closure seal is provided between the vessel and the shell and in series with the CO₂ permeable sealing member to prevent the egress of the CO₂ and/or O₂ out of and/or the ingress of gas into the buffer chamber when the container assembly is removed from the vagina or a CO₂ enriched incubator.

Features of the second embodiment corresponding to features of the first embodiment are identified by the same references augmented by "100" and will not again be described.

In the second embodiment, the upper or outer disc-shaped valve member terminates in the peripheral flange 146 which comprises opposed pairs of radial projections 147 alternating with and separated by concave zones. The radial projections 147 alternating and separated by and/or the concave zones facilitate the grasping of the upper disc-shaped valve member for facilitating turning between open and closed positions of the valve. As in the first embodiment, a slider on the upper or outer valve member 142 may ride along the screwthread groove in the lower valve member between a position in which the orifices 138, 148 are out of communication with each other and the solid portions of the central panels 134, 144 overlying each other and are in mating contact with the contour edges of the orifices. The materials employed in the second embodiment

are preferably the same as noted above in connection with the embodiment of Figs. 1-8.

Instead of a single position of the vessel relative to the shell disclosed in the first embodiment, the vessel 120 and the shell 161 have two stable positions, namely an open position or condition for use when the container assembly is placed in a CO₂ enriched environment for incubating the contents and a closed position or condition for sealing the buffer chamber and preventing the escape of the CO₂ enriched and O₂ depleted contents or the entry of ambient air from the surroundings after the container assembly has been removed from the incubating environment.

The first position or condition is illustrated in Fig. 9 and the second position or condition illustrated in Fig. 10. The Fig. 9 position corresponds substantially to the Fig. 2 position of the first embodiment. The lower end portion 129 has a downwardly protruding locating member 129A selectively cooperable with a complementary corresponding locating member 163 of hollow cylindrical configuration, as illustrated, and upstanding from the bottom wall 162 of the shell 161. The locating member 129A has a pair of axially spaced protruding beads or bosses 129B, 129C, selectively cooperable with corresponding complementary groove or recess 164. The protruding beads 129B, 129C are located approximately at 90° from each other relative to the general longitudinal axis of the vessel 120. Thus, in the first position, the protruding beads or bosses 129B come into engagement with the groove or recess 164 and in the second position, the protruding beads or bosses 129C come into engagement with the complementary groove or recess 164. To change positions, the vessel 120 must be rotated 90° and depressed (or raised) until it reaches the other position.

In the lower position, a closure seal 180 is defined by the annular notch 169 at the upper end of the shell 161 which is cooperable with a peripheral portion 181 of the undersurface of the peripheral flange 126 of the vessel and the free edge 182 of the peripheral flange of the vessel and possibly the free edge of the peripheral flange of the lower valve member 132. The closure seal 180 is essentially defined by the contact between the notch and the portions of the peripheral flange of the vessel. In accordance with a variant, not illustrated, an additional sealing member or gasket may be provided either at the upper end of

the shell or at the peripheral flange of the vessel and/or lower valve member. Such an additional sealing member or gasket will be of very low gas permeability to prevent the escape of the atmosphere contained in the buffer chamber or the entry of the ambient atmosphere into the buffer chamber. Such an embodiment
5 is therefore suitable for prolonged storage of many hours, or even days or transit or shipment.

For such a purpose, the container assembly may be loaded into a preheated isothermal holding block for maintaining the contents of the vessel substantially constant at about 37°C. An embodiment of such a holding block
10 100 is illustrated in Figure 11. The holding block is preferably made of steel, but alternatively may be made of any material having a relatively high level of thermal inertia. As illustrated, the block is parallelepipedic with a lateral bore 101 extending from one side of the block to a point beyond the middle thereof where it is in communication with a vertical bore 102. The vertical bore 102 extends
15 from the top to the bottom of the block, the lower portion of the bore being of smaller cross section than the upper portion of the bore. Such a preheated isothermal holding block may also be used for temporary storage of a container assembly containing one or more embryos. And to this end, heating block 100 may have one or more additional bores 103.

20 Before the holding block is to be used, it is heated to the desired temperature of about 37°C. When the connecting assembly is fully inserted in the lateral bore, the microchamber and the corresponding surface 65 of optical quality on the shell 61 will be aligned with the vertical bore 102 for viewing the embryo(s) or other contents of the microchamber with a microscope. The part of
25 the container assembly and in particular the microchamber located at the intersection of the lateral and vertical bores is lit from below through a light shaft defined by the lower portion of the vertical bore 102.

Alternatively, the container assembly without the shell may be introduced into the lateral bore for viewing the contents of the microchamber in which case
30 there is no need for the surface(s) 65 of optical quality. According to another embodiment (not shown), the block is equipped with a heating element for maintaining the temperature of the block substantially constant at about 37°C and may be of particular interest for use when the container is to be shipped or

transported to another location for inspection of the embryo(s). The top surface of the block also has one or more vertical aligned bores 103 for receiving in a substantial vertical position one or more container assemblies prior to inspection or smaller tubes for containing sperm or oocytes.

5 Another, third embodiment is illustrated in Figs. 12-15 which is a variant of the embodiment of Figs. 1-8. The features of this third embodiment corresponding to features of the first embodiment are identified by the same references augmented by "200" and will not be described except where necessary to distinguish the third embodiment from the first.

10 In the third embodiment, the sealing tightness of the closure device is improved over that of the first embodiment. The third embodiment also includes an optional sealing cap for better impeding the ingress of vaginal fluids in the course of vaginal residence.

 The modified closure device 230 comprises a valve 231 including two
15 overlying disc-shaped valve members 232, 242. One of the disc-shaped valve members is mounted for relative angular movement. As in the Fig. 1 embodiment, the lower disc-shaped valve member 232 is fixed by ultrasonic welding to the upper end of the vessel body and in practice the peripheral flange thereof. As in the Figs. 1-8, the disc-shaped valve members include a central
20 panel 234, 244 having a port or orifice 238, 248 adapted to be brought into registration in the fully open position of the closure device 230 and out of communication in the fully closed position of the closure device. In this embodiment, orifices 238, 248 are both preferably of circular contour, as illustrated, though a D-shape member contour may be adapted as in the Figs. 1-
25 8 embodiment. Preferably, one of the disc-shaped valve members 232, 242 has a sealing material affixed to the side of the central panel 234, 244 facing the central panel of the other of the disc-shaped valve members. Preferably, in practice, it is the upper, rotatable disc-shaped valve member 242 that has a liner or layer 239 of sealing material on at least the lower surface thereof facing the
30 central panel 244 of the lower, fixed disc-shaped valve member. Preferably, both the upper and lower surfaces of the central panel of the upper, rotatable disc-shaped valve member 242 have respective liners or layers 239, 240 affixed thereto. These liners or layers are advantageously overmolded on the central

panel of the disc-shaped valve member 242, but obviously could be bonded or secured with an adhesive. Of course, the orifice 248 also extends through the or each of the liners or layers of sealing material. The lower surface of the lower liner or layer 239 is in fluidtight or rubbing contact with the adjacent upper
5 surface of the lower, fixed disc-shaped valve member, thereby enhancing the sealing capability of the closure member in the closed position thereof where the orifices are out of registration of each other. Unlike the Figs. 1-8 embodiment, the sidewalls between the central panel and the peripheral flanges of the disc-shaped valve members do not have the screwthread groove and complementary
10 slider. Instead, the inner surface of the sidewall of the lower, fixed, rotatable disc-shaped valve members is only frustoconical to a position two thirds the way of the sidewall surface. The uppermost portion of that surface is slightly axially outwardly convergent, so that when the upper disc-shaped valve member must be pressed downwardly upon assembly and is held snugly axially in place by the
15 outwardly tapering upper portion of the sidewall surface of the lower disc-shaped valve member.

The third embodiment also includes an additional sealing cap 280 which has a central panel 281 which overlies the upper disc-shaped valve member, here rotatable disc-shaped valve member 242, and more particularly the upper
20 layer or liner 240 thereon, for sealing engagement thereof. Central panel 281 is recessed with an adjoining generally cylindrical sidewall 282, adjoining an upper annular flange 283 which overlies and is in sealing engagement with the corresponding annular flange of the upper disc-shaped valve member 242 and has a peripheral sidewall 284 which extends downwardly overlying the peripheral
25 sidewall 246A of the upper disc-shaped valve member and in sealing contact therewith. The sidewall 284 of the sealing cap then extends obliquely (zone 285), that is downwardly and radially inwardly towards the shell 261 where the cylindrical lower part 285 of the sidewall 284 of the sealing cap comes into sealing engagement with the outer surface of the sidewall of the shell 261. In
30 practice, the sealing cap is made of a soft and pliable sealing material, such as medical grade silicone; After the closure device has been brought to its closed position, the sealing cap 280 can be pushed or pulled down over the closure device 230 and the outer surface of the upper part of the shell sidewall. The

elasticity and slightly smaller dimensions of the souple sealing cap compared with the corresponding dimensions of the closure device and shell sidewall ensure fluidtightness once the sealing cap is in place on the closure device and on the upper part of the shell sidewall.

5 The container sleeve 270 is fitted over the sealing cap and the shell before introduction into the vagina, substantially as described above, in connection with Figs. 1-8 embodiment.

As illustrated in Fig. 15, abutment means are provided to limit the angular movement of the rotatable disc-shaped valve member 242 relative to the fixed
10 disc-shaped valve member 232. In practice, a pair of diametrically opposed protuberances or abutments 248 (only one of which is illustrated) are provided at the periphery of the flange of the fixed disc-shaped valve member 232. The inner surface of the longitudinally extending tabs 249B for at least one of the hooking members 249 has a protruding rib or complementary abutment 249C
15 longitudinally extending along the full height of the tab 249B to the hooking portion which projects obliquely and inwardly to be received under the peripheral flange of the vessel. One of the opposed protuberances 248 is in contact with the protruding rib 249C to define the fully open position of the closure device and the other opposed protuberances (not shown) is in contact to define the fully closed
20 position.

The fourth embodiment illustrated in Figs. 16-19 will now be described. It relates to a shell 361 which defines a buffer chamber 360 for CO₂ enriched air surrounding the vessel including its closure device. The vessel and closure device illustrated in this embodiment are those of the third embodiment
25 illustrated in Figs. 12, 14 and 15 but does not include a sealing cap for preventing any ingress of vaginal fluids which is unnecessary in the fourth embodiment as will be understood hereinafter. The shell 361 of the fourth embodiment can be used with other designs of vessels for accommodating biological medium, gametes and/or one or more embryo(s). Regardless of the
30 design, such vessels must have a CO₂ permeable wall or walls.

The novel shell 361 of fourth embodiment comprises at least two shell parts 363, 364. A gas flow passage 362 is defined between an outer upper wall of the lower shell part 364 and an inner lower wall of the upper shell part 363, the

inner lower wall of upper shell part 363 having a diameter slightly greater than the diameter of the outer upper wall of the lower shell 364. As illustrated, this gas flow passage is annular. It is understood that other forms may be adopted including a plurality of distinct longitudinal grooves. The gas flow passage 362
5 has a so-called downstream end in communication with the buffer chamber 360 which, in this embodiment, virtually surrounds the entire vessel including its closure device, unlike the Fig. 1 embodiment where the buffer chamber does not also extend around the uppermost end of the vessel body and the closure device. The so-called upstream end of the gas flow passage is defined between
10 an upwardly facing annular shoulder 366 on the lower shell part 364 and a downwardly facing free edge 367 of the upper shell part 363. A CO₂ permeable seal or gasket, and preferably an O-ring 328 made for example of silicone is located between the inner wall surface of the shell and the outer wall of the vessel. The constituent material has the same properties and is selected for the
15 same reasons as the CO₂ permeable O-ring discussed above in connection with the first embodiment. Thus, the CO₂ permeable seal 365 is preferably not only of relatively high CO₂ permeability but also prevents the ingress of vaginal fluids including vaginal secretions into the buffer chamber and into contact with the vessel or its closure device. Similarly, the features and properties of the other
20 main components, and in particular the CO₂ permeability of the vessel and the shell are the same and are selected for the same reasons as discussed above.

The shell parts have coupling means 370 comprising a groove 370A in the inner wall surface of the upper shell part 363 including a longitudinal portion 371 extending from the free edge 367 of the upper shell part to a circumferential
25 portion 372 extending in the counterclockwise as illustrated. Short of the endwall 375 of the circumferential portion of the groove 370A is a longitudinally extending bump 374. In practice, there are at least two, and preferably three, such grooves. The circumferential portion defines a central angle of about 30°. A corresponding number of radial projections 377 are provided proximate to the
30 free upper edge of the lower shell part 364 (see Fig. 16).

In the illustrated embodiment, the CO₂ permeable seal is located at the interface between the upper and lower shell parts. According to an alternative embodiment which is not illustrated, the upper and/or lower shell parts may be

provided with one or more CO₂ permeable members located for example along part of the circumference of the necked or smaller diameter cylindrical portion of the lower shell member or in the central area of the domed portion of the upper shell member. These portions will be in sealing engagement with the surrounding portions of the lower or upper shell members but allow CO₂ to permeate into the buffer chamber when the shell is in communication with a CO₂ enriched atmosphere. Similarly, the upper or lower shell parts may have rigid transparent or non-transparent zones of plastic materials having different CO₂ permeabilities. In this case, the portion or portions of the lower CO₂ permeable material may be overmolded around the round portions of higher CO₂ permeability.

After the vessel is loaded with a biological medium, ovocytes and sperm, and/or one or more embryos when used for storage purposes, the closure device is brought to the fully closed position, thus sealing the vessel. The lower end of the vessel has a locating member 229 which is adapted to be received in a locating socket 380 on the bottom wall of the lower shell part 364. In this embodiment, the locating member 229 is received with clearance in the locating socket 380. If the clearance is sufficiently ample, the locating socket 380 does not ensure an stable upright position of the vessel on their own. In this case, the coaxial position of the vessel relative to the lower shell part 364 is ensured by guiding means on the vessel or a part appurtenant thereto and inner wall of the lower shell part. To this end in the illustrated embodiment the longitudinally extending guiding members 381 are provided on the inside wall of the lower part of the shell which are cooperable with a ring, and in particular an O-ring 338 as illustrated, received in an outwardly opening groove 227 on the side wall of the vessel. It will be understood that the function of O-ring 338 is not the same of that of the O-ring 238. Indeed, the O-ring 338 needs not to have a particular permeability or be able to impede the ingress of vaginal fluids, for example. The outer diameter of the ring is preferably slightly greater than the diameter defined by the guiding members 381 at the same location thereby ensuring in cooperation with the complementary locating member and locating socket a stable coaxial position. Thanks the resilience of the O-ring and the loose fit of the complementary locating members; some movement of the vessel relative to the shell is possible. Alternatively, a more rigid coaxial positioning of the vessel

relative to the shell is possible in which case the O-ring may be either of less resilient material or replaced by a openable rigid ring or even a fixed or integrally molded with the vessel itself. The guiding members 381 are circumferentially spaced from each other and are preferably L-shaped in cross section for
5 receiving a label (not shown) for identifying the person to whom the oocytes or embryo(s) belong.

For assembling the shell parts 363, 364 they are moved towards each other initially longitudinally, guided by the cooperation of the radial projections 377 and the longitudinal portions 371 of the grooves 370A. Additional
10 longitudinal force is exerted to compress the CO₂ seal gasket slightly whereupon the radial projections 377 may enter the respective circumferential portions, and the shell parts may then be turned relative to one another until the radial projections 372 move beyond the bumps 374 in the circumferential portions. The circumferential outer surface of the radial projections 377 ride onto the bumps
15 374, as the radial projections reach the endwalls 375 of the circumferential portions 372 of the groove 370A, thus tightening the engagement between the shell parts and thereby resisting inadvertent relative angular movement once the shell is closed, and also in the course of vaginal residence.

Each of the shell parts 363, 364, is made of molded rigid, transparent
20 medical grade biocompatible material such as a crystal polystyrene and in particular Nova High Heat Crystal Polystyrene, ref. 1204, available from Nova Chemicals, Moon Township, Pennsylvania though polycarbonate may also be suitable. The polystyrene will have a highly smooth or "polished", surface finish which has been found to be highly suitable for the about 48-72 hours contact
25 with vaginal tissue of the posterior fornix with a reduced risk of irritation than with the Santoprene container sleeve or carrier of the type illustrated in Fig. 8 or 12 and/or the silicone sealing cap.

The sidewall of the lower shell part 364 has a substantially cylindrical wall portion between upwardly and downwardly flaring portions. The cylindrical wall
30 portion of reduced diameter facilitates the manual or mechanical gripping of the shell, for example, with a tenaculum. The total length of the shell is preferably 40-50 mm and the transverse dimension of the cylindrical wall of smaller

diameter is preferably 20-25 mm in the case of a shell intended for a woman's vagina.

The sidewall of the lower shell part 364 may be provided with a portion or portions of optical quality (not shown) permitting the viewing of embryos settled
5 in the microchamber of the vessel.

According to a non-illustrated feature, once the container assembly is removed from the vagina, the CO₂ permeable seal is inhibited or overridden, for example by positioning or placing over the CO₂ permeable seal, a complementary sealing ring of low CO₂ permeability, e.g. of low permeability
10 nylon, over the CO₂ permeable seal, or simply at the upstream end of the gas flow passage between the upper and lower shell parts, so as to seal off or substantially seal off the gas flow passage connecting the buffer chamber to the surroundings, and thereby reduce or eliminate the loss of the CO₂ enriched air and/or O₂ depleted atmosphere from the buffer chamber. With such a sealing
15 ring in place, the shell can be used for storage or transit of the embryo(s) prior to retrieval and transfer. Alternatively, other kinds of seal may be provided at the gas flow passage, for example a high seal CO₂ permeable tape with a suitable adhesive affixing it to the outer surface of the upper and lower shell parts. In the latter case, the annular shoulder 366 of the lower shell part may be followed by a
20 cylindrical portion substantially of the same diameter as the outer surface of the lower portion or skirt of the upper shell part. Similarly, an adhesive tape can carry on its adhesive face a low CO₂ permeability sealing ring adapted to close off or substantially close off the gas flow passage.

In any event after incubation the vessel with or without the shell may be
25 transferred to an isothermal insulating block illustrated in Fig. 11 for examination and selection of the embryos before the transfer via catheter as described above

It would be appreciated that these and other modifications and variants may be adopted without departing from the spirit and scope of the invention
30 defined by the appended claims.

THERE IS CLAIMED

1. A container assembly comprising (i) a vessel for containing a biological medium, gametes and/or one or more embryo(s), the vessel having a CO₂ permeable wall; (ii) a closure device for selective access to the interior of the vessel, and (iii) a buffer chamber for a CO₂ enriched atmosphere cooperable with the vessel and in communication with the CO₂ permeable wall.

2. A container assembly according to claim 1, wherein the buffer chamber has an open condition for communication with a CO₂ enriched environment and a closed condition for closing off the chamber from the surroundings.

3. A container assembly according to claim 1 or claim 2, further comprising a CO₂ permeable seal for impeding the ingress of liquids into the buffer chamber while allowing the entry of CO₂.

4. A container assembly according to any of the preceding claims, wherein the buffer chamber is defined by a shell disposed at least partly around the vessel.

5. A container assembly according to claim 4, wherein the buffer chamber is defined by a shell disposed at least partly around the vessel and wherein said shell is mounted relative to the vessel for movement between an open position and a closed position corresponding to the respective open and closed conditions.

6. A container assembly according to claim 5, further comprising a fluidtight seal operatively disposed between the vessel and the shell to prevent ingress and egress of fluids to and from the buffer chamber in the closed position.

7. A container assembly according to any one of the preceding claims, wherein the vessel comprises a main chamber and a microchamber for communication of the biological medium, gametes and/or one or more embryo(s) therebetween, the microchamber and at least part of the main chamber being surrounded by the buffer chamber and the CO₂ permeable wall including a wall defining the microchamber.

8. A container assembly according to any one of the preceding claims, wherein substantially the entire vessel wall is CO₂ permeable.

9. A container assembly according to any one of the preceding claims, wherein the buffer chamber has a lower CO₂ outflow rate than the CO₂ inflow rate of the vessel.

10. A container assembly according to any one of the preceding claims,
5 wherein the buffer chamber ensures CO₂ equilibration after removal of the container assembly from a CO₂ enriched environment.

11. A container assembly according to any one of claims 3 to 10, for use in intravaginal fertilization and culture, wherein the CO₂ permeable seal is operatively disposed between the buffer chamber and the surroundings for
10 impeding the ingress of vaginal fluids into the buffer chamber while allowing the entry of the CO₂ enriched gas.

12. The container assembly according to any one claims 3 to 11, wherein the CO₂ permeable seal is readily replaceable with another seal having a different CO₂ permeable inflow rate.

15 13. The container assembly according to any one of the preceding claims, wherein the closure device comprises a valve including disc-shaped members in overlying relationship mounted for relative angular movement.

14 .The container assembly according to claim 13, wherein an inner one of the disc-shaped members is fixed relative to the vessel and an outer one of
20 the disc-shaped members is mounted for angular movement.

15. The container assembly according to claim 14, wherein each of the disc-shaped members has an orifice in a central panel for introducing a catheter or pipette for gametes and/or one or more embryo(s), an upstanding sidewall around the central panel and a peripheral flange extending radially outwardly
25 from the sidewall.

16. The container assembly according to any one of the preceding claims, wherein the vessel comprises a main chamber and a microchamber for the flow of the biological medium and movement of gametes and/or embryos therebetween, the inner wall surface of the main chamber member tapering from
30 an end fitted with the closure device, towards the microchamber.

17. The container assembly according to claim 16, wherein an upper part of the vessel has an upwardly flaring sidewall and a peripheral flange extending outwardly therefrom, the sidewalls of the disc-shaped members being nested in

the upwardly flaring sidewall of the vessel, and the peripheral flanges of disc-shaped members extending parallel to the peripheral flange of the vessel.

18. The container assembly according to any one of claims 15 to 17, wherein a liner of sealing material is affixed to the central panel of one of the
5 disc-shaped members.

19. The container assembly according to any one of claims 15 to 17, wherein one of the disc-shaped members has a protruding lip along an edge defining an orifice therein, the lip facing the other of the disc-shaped members of the valve and sealingly engageable therewith.

10 20. The container assembly according to claim 19, wherein said one of the disc-shaped members has a raised portion of substantially the same height of the protruding lip and spaced therefrom for maintaining the central panels parallel to each other.

21. The container assembly according to any one of claims 15 to 17,
15 wherein the peripheral flange of upper one of the disc-shaped members has a peripheral sidewall radially outwardly beyond the peripheral flange of a lower one of the disc-shaped members and the peripheral flange of the lower disc-shaped member, the peripheral flange of the lower disc-shaped members having protrusions selectively cooperable with cutouts in the peripheral sidewall in a
20 closed position of the closure device.

22. The container assembly according to any one of claims 15 to 17, wherein the peripheral sidewall of the upper disc-shaped member has one or more hooking members for snap-fitting axial retention of the upper disc-shaped member on the lower disc-shaped member.

25 23 The container assembly according to any one of claims 5 to 22, wherein an upper edge of the shell is in sealing engagement with a radially outwardly extending flange at an upper end of the vessel in the closed position of the buffer chamber.

24. The container assembly according to any one of claims 5 to 22,
30 wherein said shell has an upstanding locating member on a bottom wall thereof and the vessel has a protruding complementary locating member at the lower end thereof.

25. The container assembly according to claim 24, wherein the vessel in the open condition of the buffer chamber is angularly offset from the vessel in the closed condition of the buffer chamber.

26. The container assembly according to any one of claims 5 to 25,
5 wherein a locating member and complementary locating member define two locating positions corresponding respectively to the open and closed positions of the shell, a portion of a lower end part of the vessel being in engagement with the complementary locating member in a first locating position, a portion of the lower end part of the vessel extending beyond the upper part of the
10 complementary locating member in the second locating position.

27. The container assembly according to claim 26, wherein the locating member and complementary locating member have respective cooperable detent means for defining said first and second locating positions.

28. The container according to any one of the preceding claims, wherein
15 the buffer chamber comprises a shell having a marking surface on an external wall for patient identification.

29. The container assembly according to any one of the preceding claims, wherein the vessel and closure device define an intravaginal container for intravaginal incubation, and further comprising a container sleeve with opposed
20 rounded ends suitable for cooperation with a vaginal vault, the rounded ends having inner faces cooperable with opposed ends of the container, and an elastic sidewall connecting the rounded ends and urging the inner faces towards each other when the container is received in the sleeve.

30. The intravaginal incubation container assembly according to claim 29,
25 wherein the elastic sidewall has one or more openings for introduction and removal of the intravaginal container.

31. The intravaginal incubation container assembly according to claim 29 or to claim 30, wherein an inner face of one of the sleeve ends has a plug engageable in and mating with a central recess defined by the closure member
30 for urging elements of a closure member towards each store.

32. The container assembly according to any one of claims 7 to 31, wherein the microchamber has opposed walls of suitable quality for viewing the contents of the microchamber under magnification, an abutment being provided

on an inner surface of one or both of the opposed walls for docking a catheter, substantially at the middle of the opposed walls.

33. The container assembly according to claim 32, wherein the abutment is a part of a recess in the one of the opposed walls of the microchamber.

5 34. The container assembly according to claim 33, wherein a portion of the recess in the one of the opposed walls of the microchamber defines an interior lens face and the outer surface of the vessel proximate to a junction of the main chamber and the microchamber and in viewing alignment with the interior lens face comprising an exterior lens face, a lens thus defined by the lens
10 faces being located for viewing one or more embryos in a catheter during or after retrieval from the microchamber.

35. The container assembly according to any one of claims 32 to 34, wherein a portion of the opposed walls of the microchamber and a portion of the main chamber proximate to a junction of the main chamber and the
15 microchamber define an interior lens face in viewing alignment with an exterior lens face, the lens thus defined by the lens faces being located for viewing one or more embryos in the catheter during or after retrieval from the microchamber.

36. The container assembly according to claim 35, wherein the zones adjoining internal walls and a floor of the microchamber include an inclined
20 portion for opposing the formation of fluid vortexes.

37. The container assembly according to any one of claims 7 to 36, wherein, the inner wall surface of the main chamber is generally frustoconical and includes a small end section adjoining and merging into the microchamber, the microchamber being of generally rectangular cross section, the configuration
25 of the inner wall surface of the main chamber favoring the flow of biological medium therebetween.

38. The container assembly according to any one of the preceding claims, wherein said buffer chamber mitigates temperature changes inside the vessel when the container assembly is removed from a CO₂ enriched environment at a
30 temperature of about 37°C.

39. The container assembly according to any one of claims 6 to 38, wherein the fluidtight seal operatively disposed between the vessel and the shell in a closed position of the shell is adapted to prevent inflow of the atmosphere of

the surroundings after removal from a temperature controlled CO₂ enriched environment and/or outflow of CO₂ enriched atmosphere from the buffer chamber.

40. The container assembly according to any one of claims 6 to 38,
5 wherein the fluidtight seal operatively disposed between the vessel and the shell in a closed position of the shell is adapted to prevent inflow of O₂ into the buffer chamber after removal from a temperature controlled O₂ depleted atmosphere.

41. The container assembly according to any one of claims 4 to 40,
10 wherein the shell defining the buffer chamber impedes the loss of gas therefrom, and the shell has a much lower CO₂ outflow than the CO₂ inflow rate of the vessel wall.

42. The container assembly according to any one of the preceding claims, wherein the CO₂ permeable seal has a CO₂ permeability at least an order of magnitude greater than that of the vessel wall.

15 43. The container assembly according to any one of the preceding claims, wherein the CO₂ permeable seal has a CO₂ permeability at least two orders of magnitude greater than that of the shell.

44. The container assembly according to any one of claims 7 to 43,
20 wherein the volume of the main chamber is between about 10 and about 100 ml, and the volume of the microchamber is between about 0.4 and about 1.5 ml.

45. The container assembly according to any one of claims 21 to 44, wherein the at least radial protrusion is adapted to audibly clear an edge of at least one of the cutouts, as the closure device reaches the closed position.

46. The container assembly according to any one of the preceding claims
25 1 to -3, 7 to 22, 26 to 45, wherein the buffer chamber is defined by a shell surrounding the vessel.

47. The container assembly according to claim 46, wherein said shell has at least two parts, the shell parts having coupling means for coupling the shell parts in the closed position.

30 48. The container assembly according to claim 47, wherein a gas flow passage is defined between respective ones of said at least two shell parts.

49. The container assembly according to claim 48, wherein a CO₂ permeable seal is located in the gas flow passage for allowing the flow of CO₂ enriched air from a source of CO₂ enriched air into the buffer chamber.

50. The container assembly according to claim 49, wherein said CO₂ permeable seal is adapted to prevent the ingress of vaginal fluids into the buffer chamber.

51. The container assembly according to any one of claims 47 to 50, wherein the CO₂ permeable seal is compressed between respective shell parts in the closed position.

52. The container assembly according to any one of claims 46 to 51, wherein the shell has rounded ends and is sized and configured for residence in the posterior fornix.

53. The container assembly according to any one of claims 46 to 52, wherein the shell is made of rigid and transparent biocompatible material.

54. The container assembly according to any one of the claims 46 to 53, wherein the shell has a transverse dimension of about 20 mm to about 25 mm and a longitudinal dimension of about 40 mm to about 50 mm.

55. The container assembly according to any one of claims 47 to 54, wherein said coupling means holds the respective shell parts against angular and longitudinal movement in the closed position of the shell.

56. The container assembly according to claim 55, wherein said coupling means comprises a groove in one of said respective shell parts having a first longitudinal portion followed by a circumferential portion, and the other one of the respective shell parts having a boss shaped for restrained movement in the groove.

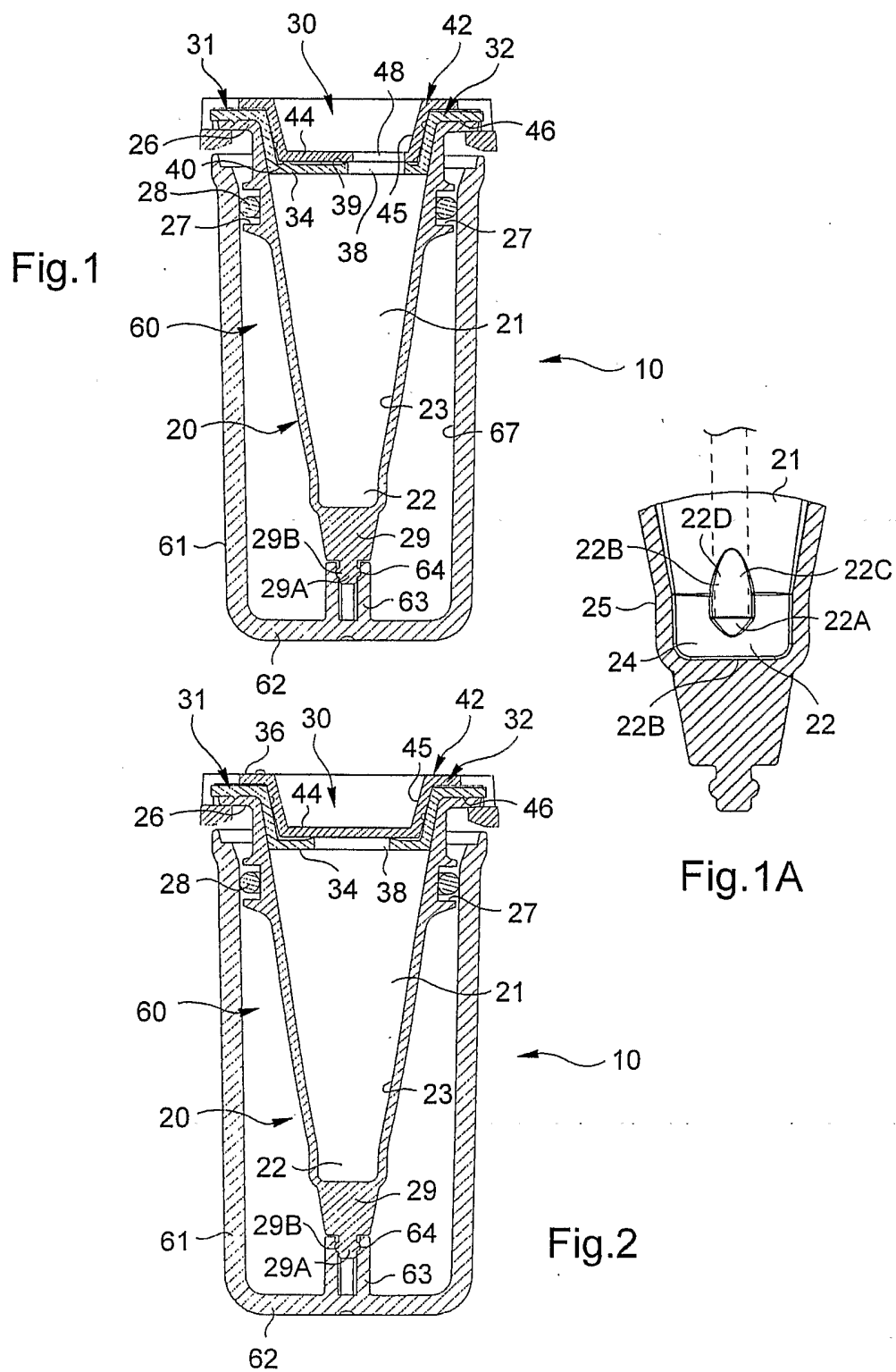
57. The container assembly according to claim 56, wherein the circumferential portion has an endwall cooperable with the boss for defining the closed position of the shell and a bump located in the circumferential portion spaced from the endwall to limit inadvertent relative angular movement of the shell parts from the closed position.

58. The container assembly according to any one of claims 47 to 57, wherein at least one of the shell parts has a label groove along the inside wall of said at least one shell part.

59. The container assembly according to claim 58, wherein the label groove protrudes inwardly from the inner sidewall of the at least one part of the shell for cooperating with the vessel or an appurtenance thereon for axially aligning the vessel relative to the shell.

- 5 60. The container assembly for storage or shipment of gametes and/or at least one embryo according to any one of the preceding claims.

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Fig.4

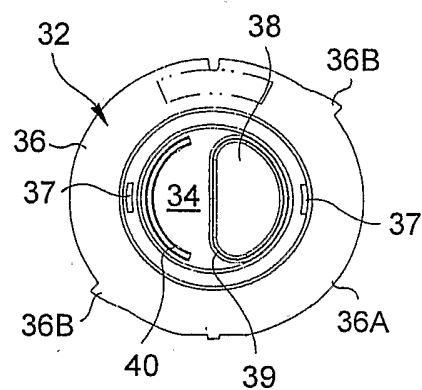


Fig.3

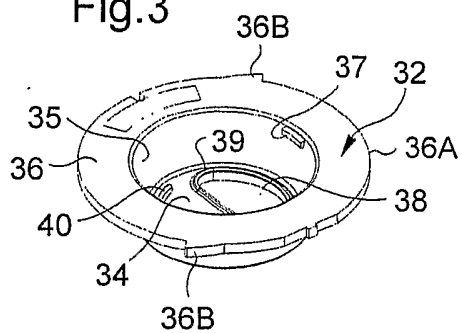


Fig.6

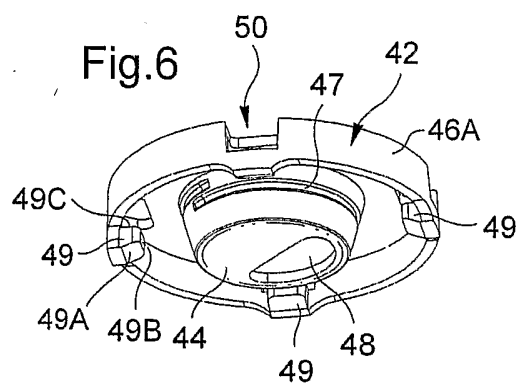


Fig.5

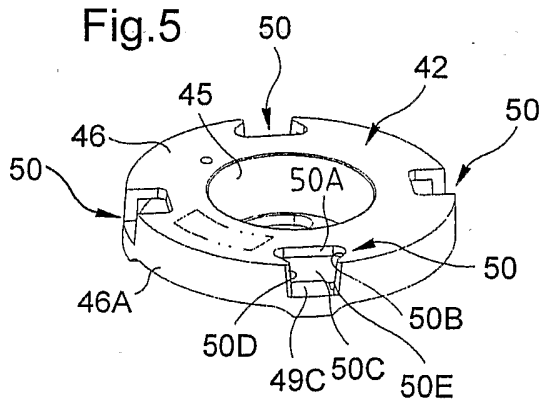
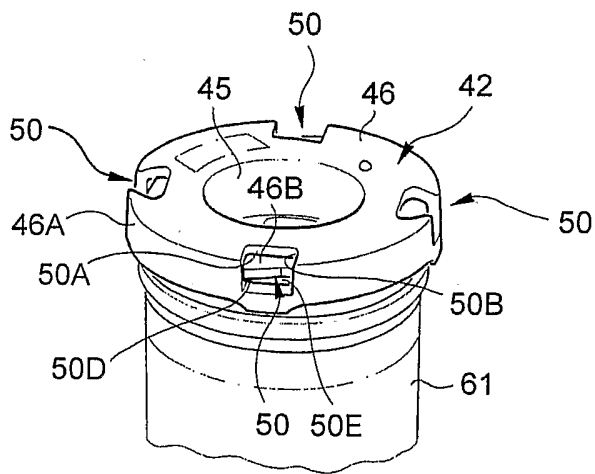
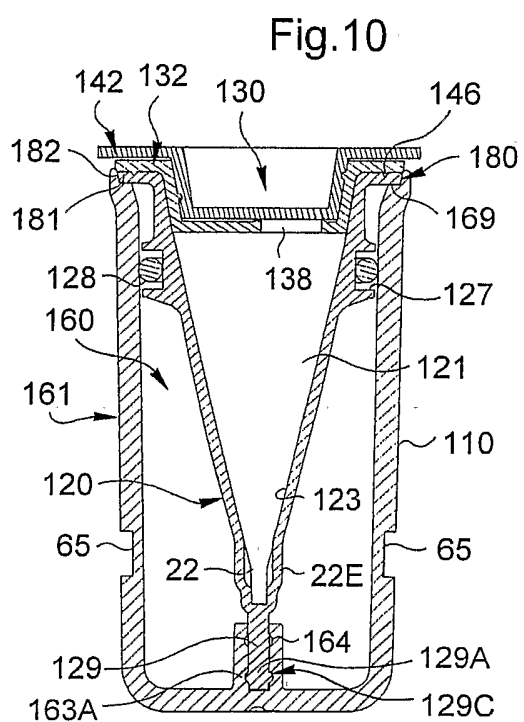
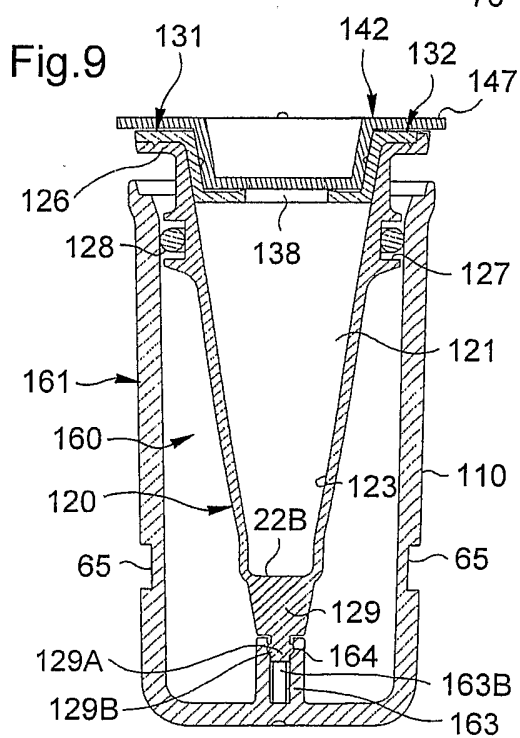
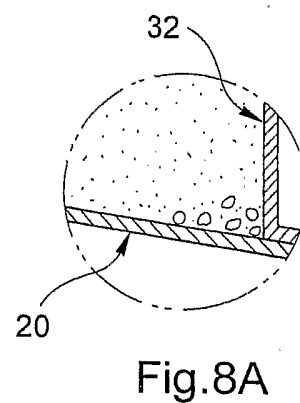
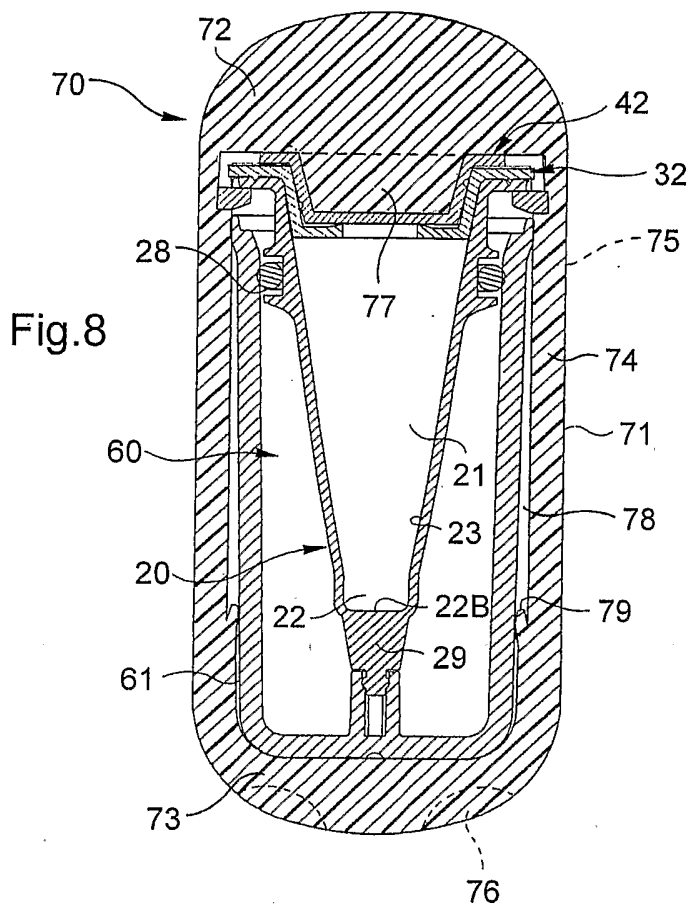


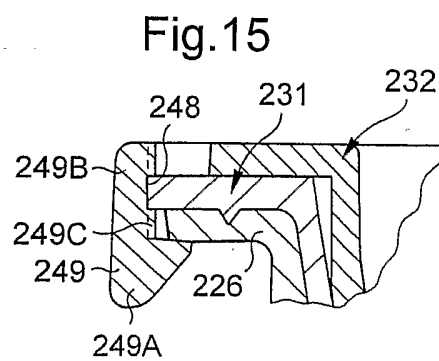
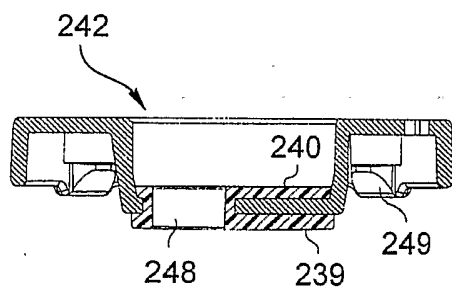
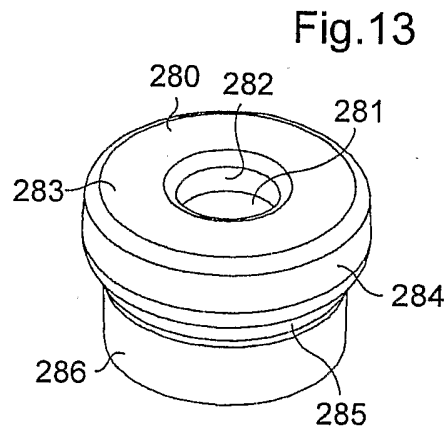
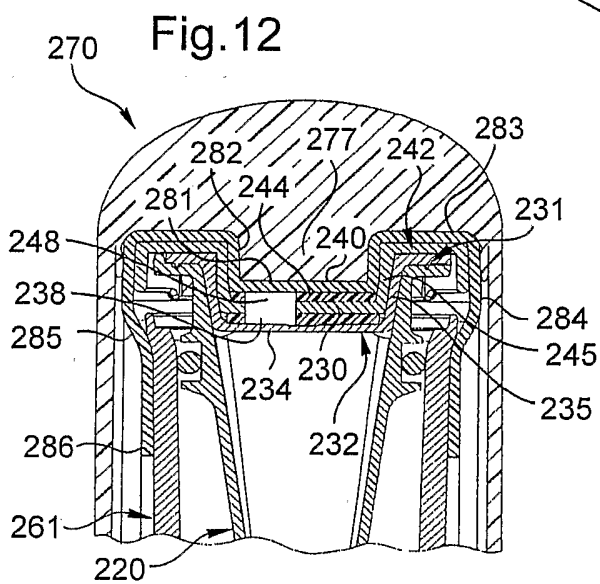
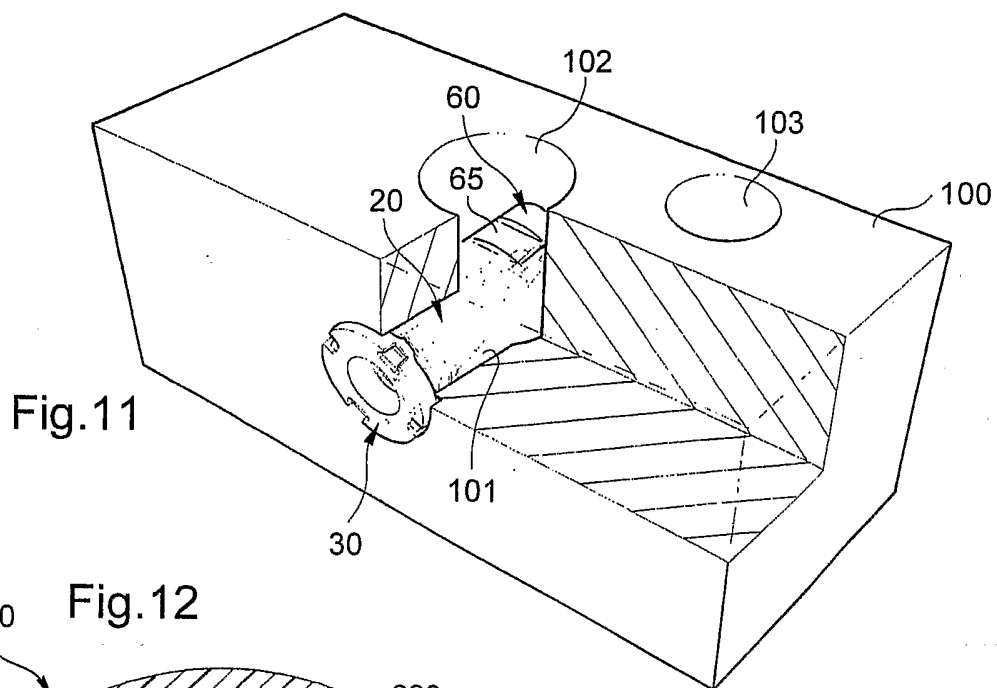
Fig.7



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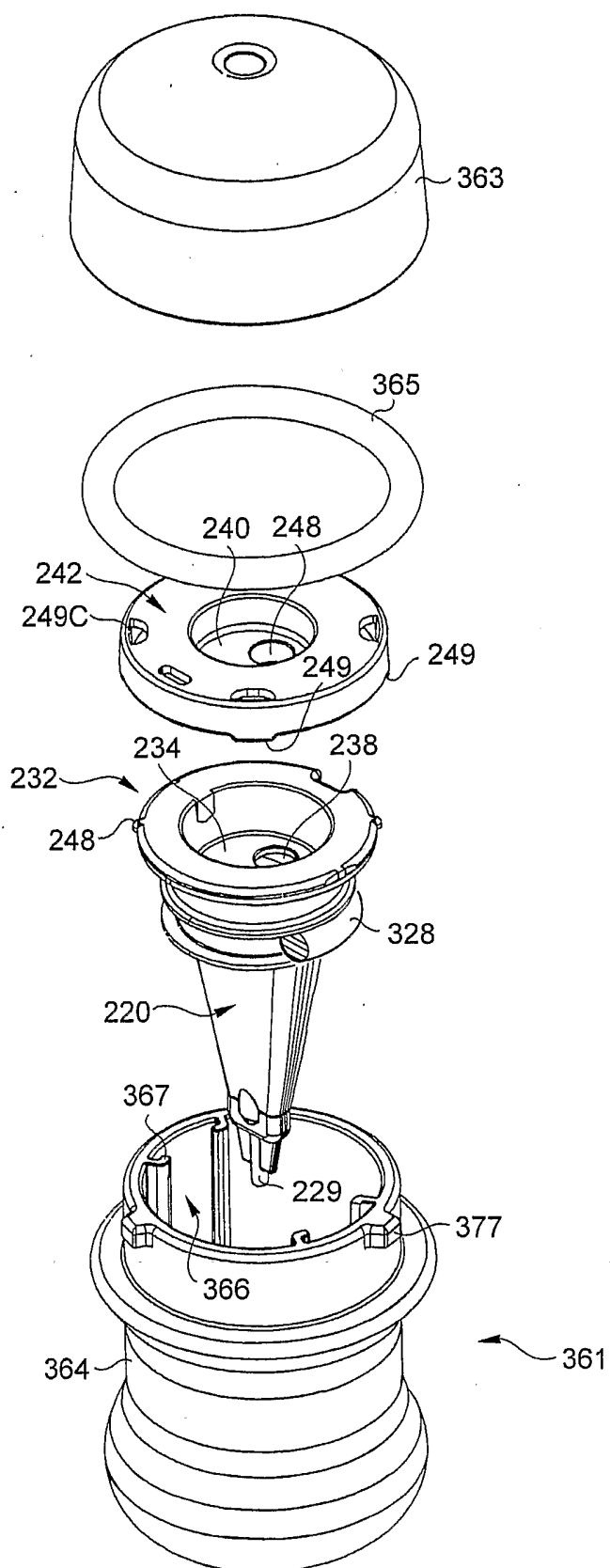


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Fig.16



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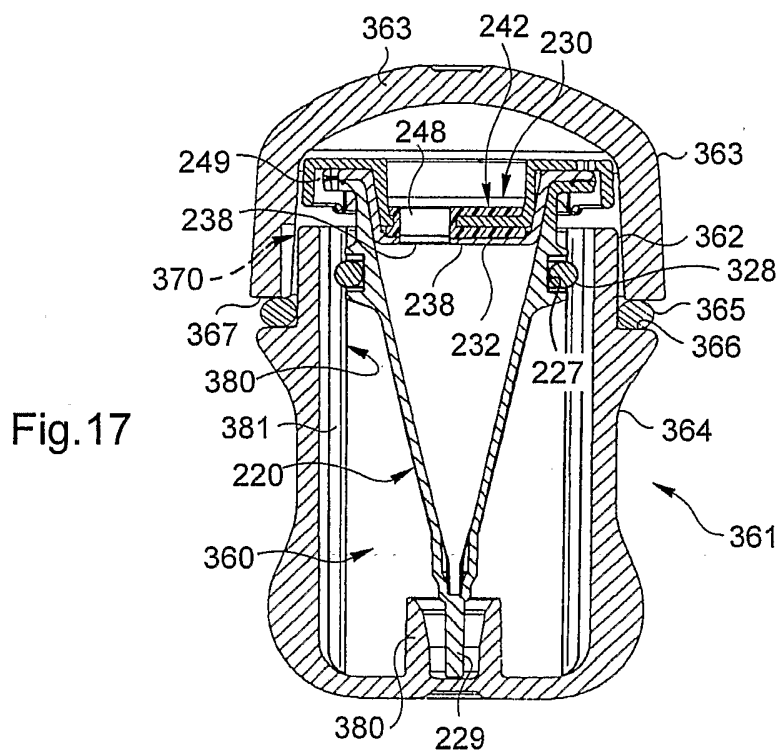


Fig.18

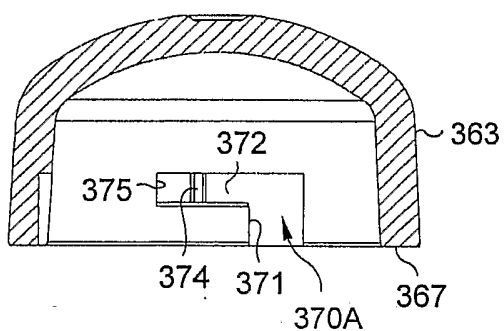
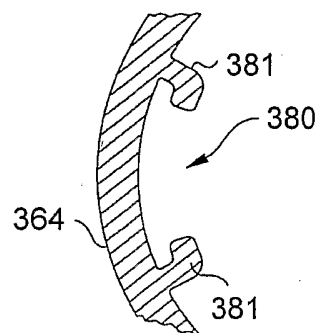


Fig.19



INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/US2004/003656

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00 A61D19/02 A61D19/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L A61D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 902 286 A (RANOUX CLAUDE) 20 February 1990 (1990-02-20) cited in the application claim 1; figure 1 ----	1
A	US 6 050 935 A (RANOUX CLAUDE ET AL) 18 April 2000 (2000-04-18) cited in the application column 2, line 52 -column 4, line 11 ----	1
A	EP 0 131 166 A (FERTILITY & GENETICS ASS) 16 January 1985 (1985-01-16) abstract; figure 2 -----	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- * & * document member of the same patent family

Date of the actual completion of the international search

7 June 2004

Date of mailing of the international search report

18/06/2004

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Tragoustis, M

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2004/003656

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