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(54) **PROCESS FOR INTRA-UTERINE FERTILIZATION IN MAMMALS AND DEVICE FOR IMPLEMENTATION THEREOF**

(75) Inventor: **Claude Ranoux**, Winchester, MA (US)

(73) Assignee: **Biofertec Ltd.**, Cambridge, MA (US)

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128/898, 899; 604/890.1-892.1, 42, 55, 19,
604/279, 403, 906, 288

See application file for complete search history.

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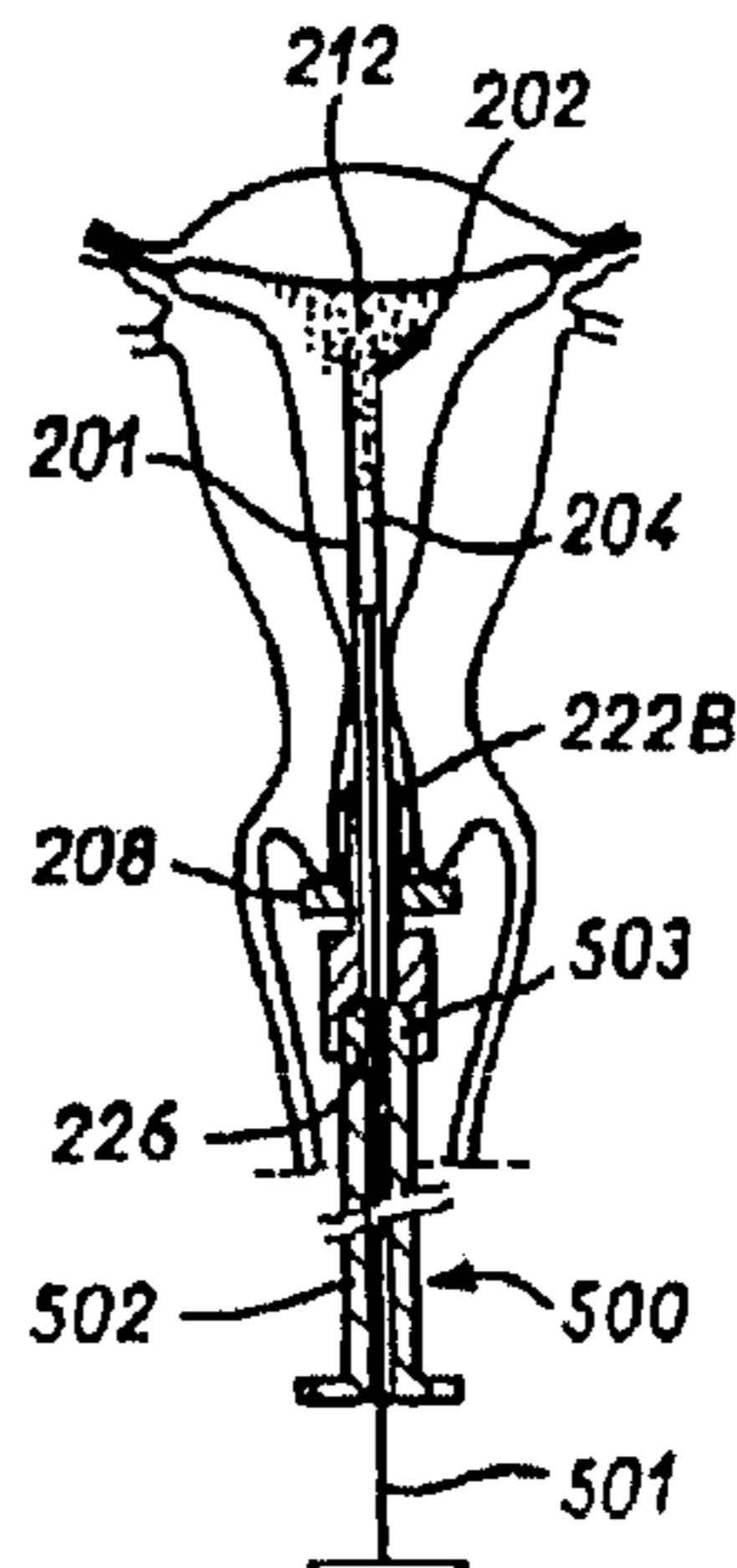
Primary Examiner—Karin M. Reichle

(74) *Attorney, Agent, or Firm*—Young & Thompson

(57) **ABSTRACT**

[The] A fertilization process [comprises] includes the following steps: a) a container (15) which can be introduced into, and lodged in, the uterine cavity of the mammal is provided; b) the container (15) is filled with a culture medium, at least one ovocyte of the mammal, and spermatozoa; c) the filled container (15) is inserted into the uterine cavity; d) the container (15) is left for a given length of time to allow fertilization of the ovocyte(s) to take place; e) the contents of the container (15) are released into the uterine cavity. The container may be made from a tube of biodegradable or non-biodegradable material. In the latter case, it is associated with a stiffening device. A biodegradable container (15) filled with gametes and a culture medium is placed in the uterine cavity using an implantation device (20) which is then withdrawn from the mammal's genital tract.

36 Claims, 2 Drawing Sheets



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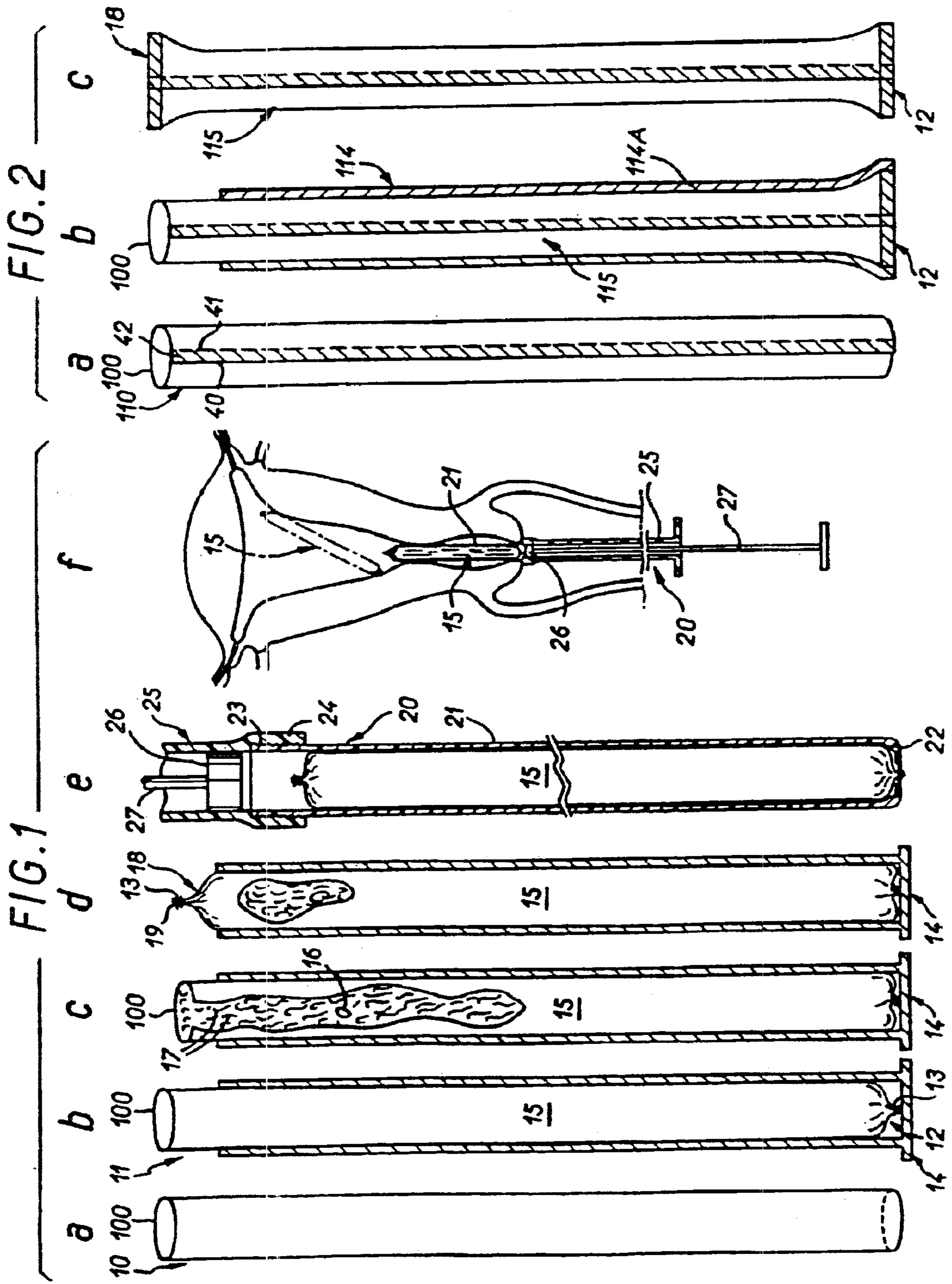
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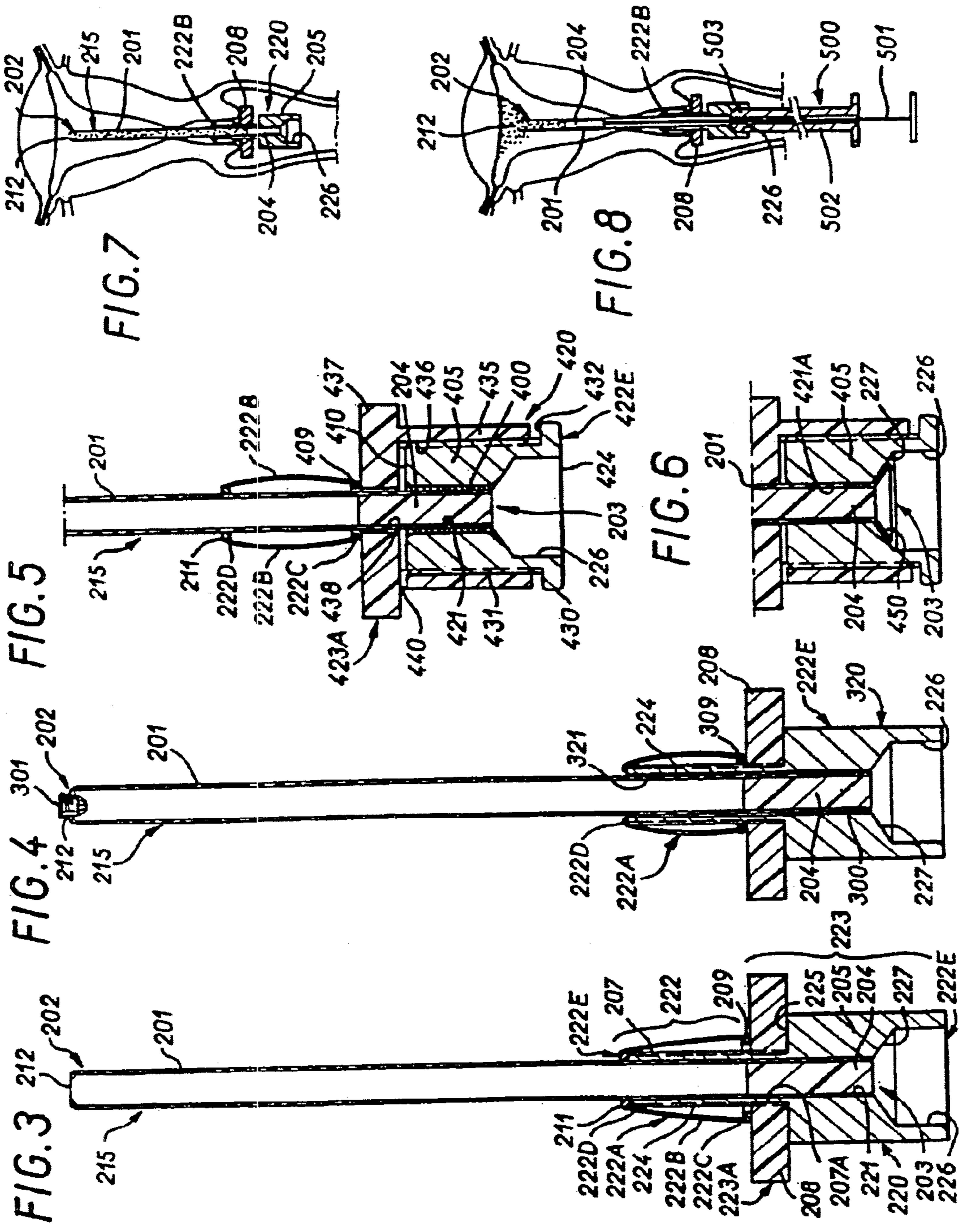
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**PROCESS FOR INTRA-UTERINE
FERTILIZATION IN MAMMALS AND
DEVICE FOR IMPLEMENTATION THEREOF**

Matter enclosed in heavy brackets [] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

This application is a continuation of application Ser. No. 07/898,407, filed Jun. 15, 1992, now abandoned, which is a reissue of U.S. Pat. No. 5,084,004 issued Jan. 28, 1992.

FIELD OF THE INVENTION

The present invention concerns a fertilisation process for mammals and a device for implementation thereof.

THE KNOWN PRIOR ART

The applicant has previously described a human fertilisation process using a container in patent applications FR-85 16558 and PCT/FR 86 00378.

In this process, ovocytes are removed from a patient in the normal manner, in general using echographic monitoring. The patient initially undergoes ovarian stimulation using Clomid-HMG or LH-RH agonists such as busereline and DTR-P-6.

Follicular liquid is taken into a syringe and immediately examined in the laboratory.

Up to eight ovocytes are then placed in a container constituted by a tube which is open at one end and completely filled with culture medium, for example MENEZZO culture medium B2, containing 10,000 to 20,000 mobile human spermatozoa per milliliter. The tube is then hermetically sealed without the interposition of air, i.e. with no layer of air above the liquid phase.

The sealed tube is then placed in the patient's vaginal cavity for 44 to 50 hours. The tube is then retrieved, opened and the ovocytes are examined in the laboratory.

Fertilised ovocytes which have already started to divide are embryos in their first states of development.

Up to four of these embryos are then replaced in the patient's uterine cavity using a Frydman catheter.

This CIVETE (intravaginal culture and embryonic transplantation) technique has shown cleavage rates, transfer rates and surgical birth rates at least equal to those obtained using the FIVETE (in vitro fertilisation and embryonic transplantation) technique.

In the CIVETE technique, up to eight ovocytes are placed in a container containing 3.2 ml liquid constituted by medium B2 containing 10,000 to 20,000 mobile spermatozoa per milliliter. The mean volume of liquid per ovocyte is thus 400 microliters, containing 4,000 to 8,000 mobile spermatozoa.

Whilst the CIVETE technique has a number of advantages over the FIVETE technique as it does not require an incubator with an atmosphere of CO₂-enriched air, there are a few disadvantages. The number of stages of manipulation of male and female gametes is still high and in particular the technique necessitates replacement of one or more embryos in the uterus. Such a number of stages leads to toxicity or even to embryo loss.

SUMMARY OF THE INVENTION

In order to overcome the disadvantages briefly mentioned above the Applicant proposes a process of fertilisation in

mammals which is characterised in that it comprises the following steps:

- a) providing a container which can be introduced into and lodged in the uterine cavity of the mammal;
- b) filling the container with a culture medium, at least one ovocyte of the mammal and spermatozoa;
- c) inserting the filled container into the uterine cavity;
- d) leaving the container for a specific period of time to allow fertilisation of the ovocyte(s) therein;
- e) allowing the contents of the container to escape into the uterine cavity.

[This method avoids in particular the final step of embryo transfer from outside the mammal to the interior of the uterine cavity.]

[According to a preferred feature of the process of the invention, said escape is effected by ejecting the container contents into the back of the uterine cavity in order to increase the chances of implantation of fertilised ovocytes or embryos.]

[For the same reasons, if the container is of biodegradable-material it is still preferable to insert it towards the back of the uterus.]

This process requires a container of a size which is suitable for introduction into and lodging in the uterine cavity of a mammal. It must be much smaller than a container for the FIVETE technique, particularly for human females, as the neck of the human uterus has an opening of only a few millimeters.

[The method described above has been developed by the Applicant in work conducted since December 1986 in the form of preliminary studies involving a process comprising the following steps:

1. providing a fine tube having a capacity of about 250 microliters;
2. filling the tube with culture medium, at least one human ovocyte and mobile human spermatozoa;
3. closing at least one end of the tube and immersing the other end in a receptacle containing culture medium;
4. placing the filled tube and its receptacle in an oven at 37° C. for 20 to 30 hours;
5. collecting the embryos and placing them in culture medium in the incubator at 37° C. until the following day;
6. replacing one or more embryos in the uterine cavity of the patient using a Frydman catheter.]

[The embryo(s) are replaced 44 to 50 hours following filling the tube with the mixture of culture medium, ovocyte(s) and spermatozoa.]

[These preliminary studies showed that in tests covering 57 ovocytes 33 were fertilised to give embryos for replacement (a cleavage rate of about 58%), whilst of 88 ovocytes removed in similar manner followed by in vitro fertilisation (IVF) or intravaginal culture (IVC), 44 embryos for replacement were produced, i.e. a cleavage rate of 50%. Two pregnancies can certainly be attributed to replacement of fine tube fertilised ovocytes.]

[These preliminary studies showed that up to four human ovocytes can be fertilised in a fine tube of this kind. It has been shown that fine tube fertilisation of this kind requires an average volume of culture medium per ovocyte of only about 60 microliters, containing 600 to 1,200 mobile spermatozoa. The culture medium used in these studies was MENEZZO culture medium B2.]

The present invention also proposes a container for carrying out the intra-uterine fertilisation process according to the invention, characterised in that it is of a size which enables it to be inserted into and lodged in the uterine cavity

of a mammal and which also enables it to receive a culture medium, at least one ovocyte and spermatozoa and in that it comprises means for defining an exit area for at least one fertilised ovocyte into the uterine cavity distanced from the neck of the uterus.

According to one aspect of the invention the container is made entirely of biodegradable material. This has the advantage that the container filled with culture medium and gametes can be introduced into the uterine cavity with no further intervention for implantation of the embryo(s).

According to another aspect of the invention a non-biodegradable container is associated with a holding device which can be removably fixed to the neck of the uterus of the mammal.

This avoids the possibility of losing the non-biodegradable container in the uterine cavity and also enables positioning of the exit area of the container towards the back of the uterus to improve the chances of implantation.

According to a further aspect of the invention, the container is a tube closed at one, so-called lower end by a plug acting as a watertight piston, the tube being fastened to the holding device towards its lower end by means of a longitudinal bore through the latter.

These features allow an operator to eject the container contents at the appropriate moment by manipulating the plug acting as a piston, as will be described below.

Since the holding device extends out of the uterus, the plug is readily accessible through the vagina.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be better understood by the following description with reference to the appended drawings wherein:

FIGS. 1a, b, c, d, e, f show enlarged cross-sectional views of a first embodiment of a container according to the invention formed from a tube of biodegradable material and insertion of the filled container in the uterine cavity of a patient;

FIGS. 2a, b, c show a second embodiment of a container according to the invention formed from a thin sheet of biodegradable material;

FIG. 3 shows a schematic longitudinal cross-section through a third embodiment of a container associated with a holding device for retaining it in the neck of the uterus of the mammal;

FIG. 4 shows a longitudinal cross-section of a variant of the container embodiment associated with the holding device from FIG. 3, with a biodegradable plug;

FIG. 5 shows a longitudinal cross-section of a further embodiment of a container associated with a holding device for retaining it in the neck of the uterus of the mammal;

FIG. 6 shows a variant embodiment of the container and its associated holding device from FIG. 5;

FIG. 7 shows schematically the container and the holding device from FIG. 3, the container being lodged in the uterus and held in place by the holding device fixed in the neck of the uterus;

FIG. 8 shows schematically the step of the process according to the invention wherein the container from FIG. 3 is emptied into the uterine cavity.

DETAILED DESCRIPTION OF THE [DRAWINGS] PREFERRED EMBODIMENTS

In order to overcome the disadvantages of the prior art, the steps of the present invention include:

a) providing a container which can be introduced into and lodged in the uterine cavity of the mammal;

b) filling the container with a culture medium, at least one ovocyte of the mammal and spermatozoa;

c) inserting the filled container into the uterine cavity;

d) leaving the container for a specific period of time to allow fertilisation of the ovocyte(s) therein;

e) allowing the contents of the container to escape into the uterine cavity.

This method avoids in particular the final step of embryo transfer from outside the mammal to the interior of the uterine cavity.

According to a preferred feature of the process of the invention, said escape is effected by ejecting the container contents into the back of the uterine cavity in order to increase the chances of implantation of fertilise ovocytes or embryos.

For the same reasons, if the container is of biodegradable-material it is still preferable to insert it towards the back of the uterus.

This process requires a container of a size which is suitable for introduction into and lodging in the uterine cavity of a mammal. It must be much smaller than a container for the FIVETE technique, particularly for human females, as the neck of the human uterus has an opening of only a few millimeters.

The method described above has been developed by the Applicant in work conducted since December 1986 in the form of preliminary studies involving a process comprising the following steps:

1. providing a fine tube having a capacity of about 250 microliters;

2. filling the tube with a culture medium, at least one human ovocyte and mobile human spermatozoa;

3. closing at least one end of the tube and immersing the other end in a receptacle containing culture medium;

4. placing the filled tube and its receptacle in an oven at 37.degree. C. for 20 to 30 hours;

5. collecting the embryos and placing them in culture medium in the incubator at 37.degree. C. until the following day;

6. replacing one or more embryos in the uterine cavity of the patient using a Frydman catheter.

The embryo(s) are replaced 44 to 50 hours following filling the tube with the mixture of culture medium, ovocyte(s) and spermatozoa.

These preliminary studies showed that in tests covering 57 ovocytes 33 were fertilised to give embryos for replacement (a cleavage rate of about 58%), whilst of 88 ovocytes removed in similar manner followed by in vitro fertilisation (IVF) or intravaginal culture (IVC), 44 embryos for replacement were produced, i.e. a cleavage rate of 50%. Two pregnancies can certainly be attributed to replacement of fine tube fertilised ovocytes.

These preliminary studies showed that up to four human ovocytes can be fertilised in a fine tube of this kind. It has been shown that fine tube fertilisation of this kind requires an average volume of culture medium per ovocyte of only about 60 microliters, containing 600 to 1,200 mobile spermatozoa. The culture medium used in these studies was MENEZZO culture medium B2.

FIG. 1a shows, enlarged a cylindrical tube 10 with a length less than the depth of the uterus. The tube is produced by extrusion and has a wall 100 of constant thickness.

It is made from animal or vegetable polymer, particularly a hydrophilic polymer, for example collagen, fibrinogen or a polymeric sugar.

FIG. 1b shows a tube **11** produced from the open tube **10** by closing its lower end **12** with a knot of suture **13**. Suture **13** is preferably biodegradable or even resorbable by the mammal's body. Such sutures are used in surgery as resorbable stitches. The knot turns the tube **11** into a container **15**, its lower end **12** resembling the end of a sausage. The upper and lower ends of tube **10** may also be closed by welding or by gluing with a biodegradable adhesive or simply by pressure. The exit area is defined here by a wall **100** of material which will be biodegradable over a known period by the contents (culture medium, spermatozoa, ovocyte(s)) and the intra-uterine environment. Container **15** is entirely formed of material which will biodegrade over a known period.

Tube **11** is slid into a footed test tube **14** which has an internal diameter substantially equal to the external diameter of tube **11** and which acts as a support.

The footed test tube may be made of a low friction material, for example polytetrafluorethylene or polyethylene, and is slightly shorter than closed tube **11**.

FIG. 1c shows, partially cut away, container **15** completely filled with culture medium, in this case MENEZZO culture medium B2, and containing an ovocyte **16** and spermatozoa **17**. Test tube **14** counter-balances the hydrostatic pressure of the liquid filling container **15** and thus avoids premature rupture of the very thin container wall. The filled container closed at only one end **12** may be left as it is, the other end remaining open for insertion in the uterine cavity, provided that the contents cannot spontaneously empty out when the tube is upended and its open end directed downwards.

The container may be filled in the normal manner using a syringe where the internal diameter of the needle is greater than that of the ovocyte.

Tube **10** may also be filled by aspiration to produce the filled tube of FIG. 1c as follows:

Tube **10**, open at both ends, is inserted in an elongate hollow support cylinder having an internal diameter equal to that of test tube **14** from FIG. 1b but open at both ends so that tube **10** projects from both ends of the hollow cylinder. A suction connector is then fixed to the top of the cylinder so that it is airtight and does not bend the upper end of tube **10**. The lower end of tube **10** and that of the support cylinder are then immersed in culture medium B2 containing spermatozoa and one or more ovocytes. These gametes and the culture medium are sucked up to fill the tube **10** open at both ends. The lower ends are removed from the liquid and the lower end of tube **10** is closed by tying, welding or gluing before removing the suction connector from the top of the support cylinder. A filled tube is thus obtained analogous to that shown in FIG. 1c except that test tube **14** is replaced by a hollow cylinder open at both ends.

FIG. 1d shows a partially cut away view of container **15** from the preceding FIG. closed at its upper end **18** by a knot using the same resorbable suture **13**. Container **15**, closed at both ends **12**, **18**, can therefore contain a liquid without the interposition of air, if required.

When knot **18** is tied some excess fluid remains in the frustoconical section **19** of tube **10** above the upper knot.

The thickness of wall **10** of biodegradable material tube **10** of course determines the time lapse before the contents of the container can escape into the uterine cavity. In the

process according to the invention, this wall must disintegrate a certain time after introduction of the sausage-like container **15** from FIG. 1d into the uterine cavity of the mammal in order that the contents may spontaneously escape into the uterine cavity after a predetermined period to enable one or more embryos to implant. This escape can be facilitated if one end of tube **11** remains open, i.e. if only one end of tube **10** is closed.

The container material must be resorbable and non-immunogenic. In this instance the container has a wall of a single thickness but it is also possible to employ a biodegradable container having walls of different thickness.

The mammalian uterus provides a warm humid environment in the uterine cavity, but without any high enzymatic activity which will provoke degradation of a biodegradable material.

On the other hand, mobile mammalian spermatozoa are highly active enzymatically and may attack the inside of wall **100** of container **15** sufficiently to degrade and burst it.

In order to determine the wall thickness required for escape of the contents after a specified period of between 15 and 55 hours, for example, the following in vitro test is conducted.

A container having wall thickness E is produced and filled as shown in FIG. 1d with a culture medium containing 10,000 to 20,000 spermatozoa per milliliter, but no ovocytes.

This container when filled in this way is placed in an incubator saturated with water vapour at about the internal temperature of the mammal. The state of the container is observed regularly and the time at which the contents spontaneously escape from the container is noted. If this time is greater than 55 hours a further container is produced having walls thinner than E, for example 0.5E, and the above in vitro test carried out on this container. Using an iterative method a thickness of wall **100** can be obtained which produces the desired in vitro degradation time, for example 15 to 55 hours.

Wall **100** of biodegradable material tube **10**, **11** may have a thickness of between 0.01 and 1 mm.

Tests have shown that MENEZZO culture medium B2 containing 10,000 to 20,000 spermatozoa per milliliter degrades in this in vitro test a cured collagen wall 0.1 mm thick in over 72 hours and a gelose wall 0.1 mm thick, as used in gastroresistant capsules, in 15 minutes.

FIG. 1e shows container **15** filled as in FIG. 1d separated from its supporting test tube **14** before introduction into an implantation device **20** comprising a cylindrical tube **21** having an internal diameter substantially equal to that of test tube **14**. Cylindrical tube **21** is longer than the container **15** and its length may for example be close to or greater than the depth of the mammalian uterine cavity.

At its lower end cylindrical tube **21** comprises a rounded edge **22** which slightly reduces the diameter of the orifice in order to retain tube **15** and prevent it from sliding out. Towards its upper end tube **21** has an external screwthread **23** onto which end **24** of a cylindrical tube **25** is screwed, tube **25** having inside it a piston **26** which slides with low friction and is fixed to shaft **27**. The internal diameter of cylinder **25** is equal to that of tube **21** to allow continuity of sliding movement of piston **26** in cylindrical tube **21**.

FIG. 1f shows a schematic view of the introduction into and lodging of container **15** in the uterine cavity of a mammal by entry of tube **21** into the uterine cavity through the neck of the uterus.

Tube **21** is introduced into the uterine cavity so as to leave sufficient depth for filled container **15** to lodge therein, and

piston **26** then is pushed using shaft **27** which protrudes from the body of the mammal, as does cylindrical tube **25**. This deposits container **15** near the back of the uterus. The implantation device **20** is then withdrawn from the uterus and vagina of the mammal and the contents of filled container **15** escape after a period determined by biodegradation of at least one part of the container.

FIGS. **2a, b, c** show another embodiment of a biodegradable material container.

FIG. **2a** shows how a hollow elongate tube **110** is formed from a thin strip of biodegradable material. This strip may, for example, be rolled around a solid cylindrical mandrel (not shown) and edges **40** and **41** may overlap slightly to produce a common contact area **42** which can be bonded using a biodegradable adhesive or pressure or heat welded.

The tube **110** has a thin wall **100**, common area **42** being thicker.

Like tube **10**, tube **110** may be filled by aspiration of a culture medium containing spermatozoa and at least one ovocyte.

FIG. **2b** shows the lower end of tube **110** closed by clamping or bonding to form a container **115** which can be held in a support **114** which is flared and open at the base.

Support **114** is slightly shorter than container **115**.

In addition, support **114** can be separated into two shells **114A** to release container **115**.

FIG. **2c** shows container **115** filled, closed by clamping or bonding its upper end **18** and ready for insertion into the uterine cavity of the mammal using an implanting device similar to implanting device **20**.

FIG. **3** shows a container **215** associated with a support [decice] device **220** which can be removably fixed in the neck of the mammal's uterus. This container is a tube **201** formed from a synthetic material which cannot be biodegraded by spermatozoa, for example polyethylene or polypropylene which has no toxic effects on the spermatozoa, the ovocytes or the mammal itself.

The tube is flexible and unbreakable to enable it to deform during its difficult passage through the neck of the uterus and to adapt to the internal shape of the uterine cavity of the mammal. Tube **201** has a so-called upper open end **202** which may be closed by a small plug (not shown) of biodegradable material, for example collagen, fibrinogen or a polymeric sugar.

Open end **202** has a restricted orifice **212** as compared with the internal diameter of tube **201**.

The other, so-called lower end **203** of the tube is closed by a plug **204** which forms a watertight piston inside tube **201**.

Tube **201** has a length about equal to the depth of the uterine cavity of the mammal.

For a patient tube **201** may be between 5 and 7 cm long and have an exterior diameter of up to 3 mm to enable it to be slid into the uterine cavity through the neck of the uterus without causing any trauma.

Tube **201** is fixed to holding device **220** over a portion of its length near its lower end **203** by means of a longitudinal bore **221** passing through the holding device.

Holding device **220** comprises, in longitudinal succession, a first section **222** which can be introduced into the neck of the uterus and a second section **223** which can be located in the vagina external to and abutting the cervix. The first section comprises a radially expansible element **222A** having a rest position for insertion into the cervix and a second position in which it presses against the internal walls of the neck of the uterus.

Expansible element **222A** is formed of thin walled flexible material which can expand radially into the second position.

The expansible element comprises at least two longitudinal tangs **222B** each having a movable lower end **222C** and an upper end **222D** which is fixed longitudinally relative to tube **201**.

Holding device **220** further comprises a mobile portion **223A** for controlling the longitudinal position of the lower end **222C** of expansible element **222A** and a portion **222E** which is fixed relative to tube **201** and on which mobile portion **223A** can move.

In more detail, fixed portion **222E** comprises a longitudinal cylindrical extension **224** forming part of said first section **222** of holding device **220**. Screwthread **207** is carried on the external surface of extension **224**.

Cylindrical extension **224** is formed in one piece with a cylindrical sleeve **205** of greater diameter. Where they join, a shoulder **225** is formed on the upper external surface of sleeve **205**.

Extension **224** and sleeve **205** are formed with a first bore **221** having a slightly smaller diameter than the external diameter of tube **201** so that the latter can be forced into the bore and retained therein.

Sleeve **205** comprises a second bore **226** having a larger diameter than the outside diameter of tube **201**, the first bore opening into the second through a frustoconical cavity **227**.

Second bore **226** has a depth and diameter sufficient to receive a pusher device for displacing plug **204** in tube **201**.

Mobile portion **223A** comprises a knurled disc **208** which can screw onto screwthread **207** because it has a complementary screwthread **207A**.

Thus fixed section **222B** and mobile section **223A** comprise complementary screwthreads **207, 207A** to allow the mobile section to turn and to move longitudinally relative to the fixed section.

FIG. **3** shows that knurled disc **208** has a diameter sufficient to abut the neck of the uterus. In its lowest position on the complementary screwthread **207**, one of the faces of the knurled disc is in contact with shoulder **225**. A so-called lower ring **209** around cylindrical extension **224** rests on the other, broadly circular side of the disc.

Tube **201** comprises a welded collar **211** which substantially abuts the top of cylindrical extension **224**.

Each tang **222B** is fixed at its lower end **222C** lower ring **209** and at its upper end to welded collar **211**. The upper end of expansible element **222A** is therefore fixed to collar **211** welded to tube **201**. Each tang is very slightly curved with its concave side oriented towards cylindrical extension **224**.

Thus if ring **209** and collar **211** are brought closer together the curvature of the tangs **222B**, their radial extension and flexion increase.

FIG. **4** shows a variant embodiment of tube **201** associated with holding device **320**.

The upper end **222D** of expansible element **222A** is attached to the upper end of cylindrical extension **224** of fixed portion **222E**. This eliminates the need for the welded collar of the preceding embodiment.

Longitudinal bore **321** through holding device **320** has a diameter which is slightly greater than the external diameter of tube **201** so that the latter can be joined to fixed part **222E** by adhesive **300**.

Lower ring **309** which rests on knurled disc **208** has a slightly smaller diameter to that of ring **209** from the preceding figure, but it is still slidable longitudinally on cylindrical extension **224**.

Orifice 212 at the upper end 202 of tube 201 is closed by a plug 301 of biodegradable material the same as that described above. The shape of the plug is such that it can be readily expelled when the liquid contents of the container are ejected on upward displacement of piston 204 inside the tube towards the upper end 202 of tube 201.

FIG. 5 shows another embodiment of tube 201 and holding device 420.

Tube 201 comprises a welded collar 211 attached to the upper end 222D of each tang 222B, the lower ends 222C of which are fixed to a ring 409 which is slidable on tube 201.

In this preferred embodiment, the cylindrical extension of the preceding embodiments is dispensed with.

Holding device 420 comprises a cylindrical sleeve 405 comprising, starting from its upper circular surface 410, an axial bore 421 having a depth of about half the longitudinal height of sleeve 405.

The diameter of bore 421 of length 1 is about the same as the external diameter of tube 201 and enables sleeve 405 to be fixed to the corresponding lower end of tube 201 over a length 1 by glue 400.

Bore 421 opens into a longitudinal bore 226 through a frustoconical portion 227. Bore 226 opens into the lower circular surface 424 of sleeve 405. As in the embodiments of FIGS. 3 and 4, bore 226 and frustoconical portion 227 which prolongs it can removably receive a suction nozzle if plug 204 which closes the lower end 203 of tube 201 is tripartite (plug as used in CASSOU tubes in France). Alternatively, bore 226 and frustoconical portion 227 can receive the end of a sliding shaft pusher device which can displace piston plug 204 inside and towards the upper end 202 of tube 201.

Aligned with and extending from the lower surface 424 of sleeve 405 is a flange 430 to facilitate handling.

A screwthread 431 is provided over the full height of the outer cylindrical surface of sleeve 405. It stops near the base of shoulder 432 formed by flange 430.

Screwed on sleeve 405 is a bush 435 whose internal cylindrical surface carries a screwthread 436 complementary to screwthread 431 on sleeve 405.

The longitudinal depth of bush 435 is substantially equal to the height of the sleeve less the height of flange 430.

The top of bush 435 is in one piece with and closed by a knurled disc 437. The disc diameter is greater than that of the bush to facilitate manipulation of the knurled disc. Disc 437 comprises a cylindrical axial passage 438 whose diameter is slightly greater than the external diameter of tube 201 to permit sliding contact. To facilitate equilibrium of the pressure in the cavity defined between circular surface 410 of sleeve 405 and the base of bush 435 defined by disc 437, a hole 440 is provided in the cylindrical wall of bush 437 in the immediate vicinity of the base.

Thus in this embodiment, fixed section 422E and mobile section 423A comprise complementary screwthreads 431, 436 so that the mobile section can turn and move longitudinally relative to the fixed section.

Screwthreads 431, 436 are respectively on an external cylindrical surface of fixed section 422E and on an internal cylindrical surface of a bush 435 on mobile section 423A.

FIG. 6 shows a variant of the preceding embodiment wherein the lower end 203 of tube 201 comprises a flange 450 which matches the shape of a part of the conical surface of the frustoconical cavity portion joining bore 421A to bore 226 for the connector.

This flange 450 is intended to increase the integrity of tube 201 with sleeve 405 by preventing longitudinal dis-

placement of the tube from bore 226 into bore 421A. In this case the tube is simply crimped into bore 421A.

FIGS. 7 and 8 show container 215 and holding device 220 from FIG. 3 holding the container in position in the neck of the uterus.

The fertilisation process using the device of FIG. 3 is described below. The skilled person will readily be able to adapt the process for the other devices described with reference to FIGS. 4, 5 and 6.

If plug 204 is a tripartite plug of a type used in French CASSOU tubes, a syringe connector is inserted in bore 226 of sleeve 205 and culture medium, ovocyte(s) and spermatozoa are sucked into container 215 consisting of tube 201, via its upper end 202.

If the plug is not a tripartite plug the same contents can be introduced into tube 201 using a syringe and needle.

If required, the tube is then plugged at its upper end 202 using a plug of biodegradable material (not shown in FIGS. 7 and 8).

The device constituted by container 215 and its holding device 220 is introduced into the vagina of the mammal.

End 202 of tube 201 is introduced into the neck of the uterus and the device is pushed in until knurled disc 208 abuts the neck of the uterus.

Sleeve 205 is then held against rotation using forceps (not shown) and knurled disc 208 is moved away from shoulder 225 by turning it on screwthread 207, thus increasing the flexion of the two tangs 222B which then press against the internal walls of the neck of the uterus, thus removably fixing the holding device 220 of container 215 in the neck of uterus. FIG. 7 shows that the length of tube 201 is preferably selected so that its upper end 202 is situated near the back of the uterus. After removing the forceps the mammal is allowed to rest for 15 to 55 hours to obtain one or more embryos in tube 201.

FIG. 7 shows this rest stage.

FIG. 8 shows the contents of container 215 being ejected after this period into the back of the uterine cavity, after fertilisation of one or more ovocytes, by pushing plug 204 towards the upper end 202 of tube 201 to place the contents of container 215 directly in contact with the endometrium to implant one or more embryos. The plug is pushed using a pusher device 500 comprising a flexible shaft 501 which can move in a sheath 502. Connector 503 of sheath 502 fits closely into bore 226.

Shaft 501 has a smaller diameter than the internal diameter of tube 201 and its length is such, in relation to the lengths of casing 502, plug 204 and tube 201, that plug 204 cannot pass out of tube 201 through its end 202. The restricted orifice 212 also acts as a security measure to keep the plug in the tube.

Knurled disc 208 is then unscrewed to release tangs 222B and free holding device 220. The apparatus is then removed from the genital tract of the mammal.

The holding devices have been described with reference to FIGS. 3 and 6 as comprising two tangs 222A. It is possible to use three or more tangs regularly distributed about tube 201.

I claim:

1. [Process] *A process* of intra-uterine fertilization in mammals comprising the following steps:

- a) providing a container of a size suitable for introduction and lodging in the uterine cavity of a mammal and having an exit area;
- b) filling the container with culture medium, at least one ovocyte of the mammal and spermatozoa;

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- c) introducing and lodging the filled container in the uterine cavity;
- d) leaving the container lodged in the uterine cavity for a specified period of time to allow fertilization of the at least one ovocyte therein; and
- e) accessing the uterine cavity through the exit area of the container [without removal of the container from] *lodged in* in the uterine cavity to enable transfer of the at least one fertilized ovocyte *directly from the container* through the exit area into the uterine cavity [without removal of the at least one fertilized ovocyte from the uterine cavity between fertilization and transfer].

2. [Process] *The process* according to claim 1, wherein the uterine cavity is accessed upon completion of the introduction and lodging steps.

3. [Process] *The process* according to claim 1, further comprising temporarily closing off the exit area with a plug before the container is lodged in the uterine cavity, wherein the accessing step comprises expelling the plug from the exit area.

4. [Process] *The process* according to claim 1, further comprising, upon accessing the uterine cavity through the container, ejecting the contents of the container into the back of the uterine cavity.

5. [Process] *The process* according to claim 1, further including the exit area being defined by a biodegradable portion of the container, wherein the accessing step comprises biodegradation of the biodegradable portion.

6. [Process] *The process* according to claim 5, wherein the container is lodged towards the back of the uterine cavity.

7. [Process] *The process* according to claim 1, further comprising holding the container lodged at the neck of the [uterine cavity] *uterus* during steps c) and e).

8. [Process] *The process* according to claim 1, wherein the container is lodged in the uterine cavity so that the exit area is disposed relatively adjacent the back of the uterine cavity.

9. [Device] *A device* for intra-uterine fertilization in mammals comprising a container [means] sized for introduction and lodging in the uterine cavity of a mammal and for containing a culture medium and at least one ovocyte of the mammal and spermatozoa while introduced into and lodged in the uterine cavity of the mammal for a time period sufficient to allow fertilization of the at least one ovocyte, said container [means] including [means for defining] an exit area to allow access to the uterine cavity from the container [means without removal thereof from the uterine cavity] and enabling transfer of the at least one fertilized ovocyte from the container [means] into the uterine cavity [without removal thereof from the uterine cavity between fertilization and transfer].

10. [Device] *The device* according to claim 9, wherein said [means for defining an] exit area [comprising] comprises a permanent opening in said container [means].

11. [Device] *The device* according to claim 9, wherein said [means for defining an] exit area comprises a biodegradable [means defining a] portion of the container [means] which is biodegradable through contact with its contents and/or the intra-uterine environment for providing access to the uterine cavity after fertilization of the at least one ovocyte.

12. [Device] *The device* according to claim 11, wherein said biodegradable [means] portion comprises a temporary [sealing means] seal at the exit area.

13. [Device] *The device* according to claim 11, wherein said biodegradable [means] portion is a wall portion of the container [means].

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14. [Device] *The device* according to claim 13, wherein the biodegradable [means] portion has a biodegradable period of 15 to 55 hours.

15. [Device] *The device* according to claim 13, wherein [the] a biodegradable thickness of the biodegradable [means] portion is between 0.01 and 1 mm.

16. [Device] *The device* according to claim 11, wherein the container [means] is substantially entirely formed of biodegradable material and the biodegradable [means] portion is a part thereof.

17. [Device] *The device* according to claim 11, wherein the biodegradable [material] portion is a natural animal or vegetable polymer.

18. [Device] *The device* according to claim 17, wherein the natural polymer is selected from a group consisting of collagen, fibrinogen and a polymeric sugar.

19. [Device] *The device* according to claim 9, wherein said [means defining an] exit area is temporarily closed by a biodegradable plug adapted to be expelled from the container [means].

20. [Device] *The device* according to claim 9, further comprising [means for ejecting] an ejector constructed and arranged so as to be able to eject the contents of the container [means] including the at least one fertilized ovocyte into the uterine cavity.

21. [Device] *The device* according to claim 20, further comprising [means for holding] a holder to hold the device in the neck of the [uterine cavity] *uterus* when the container [means] is lodged in the uterine cavity and the contents are ejected into the uterine cavity.

22. [Device] *The device* according to claim 9, further comprising [means for] a cylinder and piston arranged so as to allow introducing the container [means] into the neck of the [uterine cavities] *uterus* and transferring [it] the container to the uterine cavity.

23. [Device] *A device* for intra-uterine fertilization in mammals comprising a tube [means] sized for introduction through the neck of the [uterine cavity] *uterus* of the mammal into the uterine cavity and for accommodating culture medium, at least one ovocyte and spermatozoa of the mammal while lodged in the uterine cavity for a time sufficient to allow for fertilization of the at least one ovocyte therein, a piston [means] disposed proximate one end of the tube [means], said piston [means] being mounted for sliding movement towards the other end of the tube [means], [holding means for] a tube holder to removably [holding] hold the device in the neck of the [uterine cavity] *uterus* and for lodging the tube [means] in the uterine cavity, [and means] an exit area proximate the other end of the tube [means] adapted to face the back of the uterine cavity, said [for defining an exit area to allow access to the uterine cavity without removing the] tube [means from the uterine cavity and enabling transfer of the] being adapted to accommodate culture medium, at least one [fertilized] ovocyte, and spermatozoa for intra-uterine fertilization therein, [from the tube means into the uterine cavity without removal thereof from the uterine cavity between fertilization and transfer,] said piston [means] being movable toward the other end to expel the at least one fertilized ovocyte from said tube [means].

24. [Device] *The device* according to claim 23, wherein a flange is disposed proximate to the one end of said tube [means].

25. [Device] *The device* according to claim 23, wherein a plug of biodegradable material is disposed at the exit area for temporarily closing off the tube [means], said plug being adapted to be [injected] ejected in response to the movement of the piston [means] towards said other end of said tube [means] and incipient expulsion of the contents thereof.

26. [Device] *The device* according to claim 23, wherein said tube [means] is received in a longitudinal bore formed in said [holding means] *tube holder* and fastened thereto.

27. [Device] *The device* according to claim 23, wherein said [holding means] *tube holder* comprises a first section 5 adapted to be received in the neck of the [uterine cavity] *uterus* and a second section adapted to abut against the cervix, the first section comprising a flexible expansible element having a rest position for insertion and an operating position for bearing against the internal walls of the neck of 10 the [uterine cavity] *uterus*.

28. [Device] *The device* according to claim 27, wherein the flexible expansible element expands radially from the rest position to the operating position.

29. [Device] *The device* according to claim 27, wherein 15 the expansible element comprises at least two substantially longitudinal tangs, first ones of the ends of said longitudinal tangs being disposed relatively adjacent said [holding means] *tube holder* and being movable relative to said tube [means], and second ones of the ends being fixed longitudinally 20 relative to said tube [means].

30. [Device] *The device* according to claim 29, wherein the second *ones of the* ends of the tangs are fixed to a collar secured to said tube [means].

31. [Device] *The device* according to claim 29, wherein 25 said [holding means] *tube holder* further comprises a mobile section to control the longitudinal position of the first *ones*

of the ends of the expansible element and a section fixed relative to said tube [means] on which the mobile section is movable.

32. [Device] *The device* according to claim 31, wherein the fixed section and the mobile section comprise complementary screwthreads which allow the mobile section to turn and move longitudinally relative to the fixed section.

33. [Device] *The device* according to claim 32, wherein the screwthreads are on an external surface of the fixed section and on an internal surface of a bush on the mobile section.

34. [Device] *The device* according to claim 32, wherein said mobile section comprises a knurled disc on a longitudinal extension of the fixed section, the extension comprising 15 on an external surface [having] a complementary screwthread cooperable with that of the knurled disc.

35. [Device] *The device* according to claim 34, wherein the second *ones of the* ends of the tangs of the flexible expansible element are attached to a corresponding end of 20 the extension of the fixed section.

36. [Device] *The device* according to claim 31, wherein the fixed section comprises a bore of greater diameter than the tube [means] for receiving a pusher device for displacing the piston [means] towards the other end of said tube 25 [means].

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