Item Five

HFEA

Authorisation of novel process

Intrauterine culture of gametes/embryos (including insertion and removal of device, followed by transfer of embryo(s) to the same woman) (Avecova AneVivo device)

Statutory Approvals Committee

Paper Title	Authorisation of a novel process – Intrauterine culture of gametes/embryos (including insertion and removal of device, followed by transfer of embryo(s) to the same woman) (Avecova AneVivo device)
Agenda Item	
Paper Number	
Meeting Date	27 August 2015
Author	[redacted], Scientific and Clinical Policy Manager
For information or decision?	Decision
Recommendation	
Resource Implications	None
Implementation	As soon as amendments have been made to the authorised processes list on HFEA website
Communication	None
Organisational Risk	Medium
Evaluation	
Annexes	**Annex A** – Completed application for authorisation of a novel process **Annex B** – Supporting information **Annex C** – Blockeel, C et al. (2009). An in vivo culture system for human embryos using an encapsulation technology: A pilot study. Human Reproduction, 24(4), 790–796 **Annex D** – Additional information (CE marking, etc.) **Annex E** – Additional safety information **Annex F** – Retrieval success rate data **Annex G** – Decision tree for authorization of a novel process

1. Background

1.1. The Authority publishes a list of authorised processes on its website (http://www.hfea.gov.uk/139.html), with the processes arranged under each of the licensable activities permitted by the Act. If a centre wishes to carry out a process which does not appear on the list, it must apply to the Authority for permission to perform the novel process. If approved, the novel process is placed on the approved process list so that it can be performed by all centres. The Authority has delegated the authorisation of novel processes to the Statutory Approvals Committee (SAC), who are advised on the matter by the Scientific and Clinical Advances Advisory Committee (SCAAC) (Annex F).

1.2. The purpose of SCAAC in the novel process applications process is to:

- consider whether the process is novel;
- provide a view on which licensed activity/activities the process should fall under;
- consider whether there is evidence to suggest that the process is not effective;
- consider whether there is any evidence to indicate that the process is unsafe (either to patients or embryos); and
- make a recommendation to SAC.

1.3. This paper sets out SCAAC's consideration of the novel process application for authorization of intrauterine culture of gametes and embryos, and in particular using the Anecova AneVivo intrauterine device.

2. Executive summary

2.1. The Anecova AneVivo intrauterine device is an *in vivo* embryo culture device for use during IVF treatment that allows fertilisation and initial embryo development to occur in the patient's uterus within the natural uterine fluids, rather than in an incubator and artificial medium.

2.2. The intended use of the device is the placement and retrieval of gametes or embryos into and from the uterine cavity, with the objective of their culture within the device while inside the uterine cavity. This enables fertilization and early embryo development to take place *in-vivo*, reducing the exposure of embryos to synthetic in-vitro conditions during this crucial early phase of the development, but also exposing the endometrium to biochemicals produced by the developing embryos.

2.3. Information in support of the safety of the device is presented in Blockeel et al's 2009 paper, 'An in vivo culture system for human embryos using an encapsulation technology: A pilot study', which can be found in Annex C. Information in support of the efficacy and safety of the device can be found in the same publication (Blockeel et al., 2009) and also in the supplementary information found in Annex B and D. This information includes CE mark certification for the device (Annex D). The Executive has also received additional safety data from Anecova (Annex E)

3. Summary of SCAAC's discussions

Is the process novel?

3.1. SCAAC felt that use of intrauterine culture devices, such as the Anecova AneVivo, constitute a novel process. This is both because it exposes the embryo to a novel environment (the device itself, which contains a number of components, and the uterine fluids, whereas the early embryo would usually develop in the fallopian tube) and because the embryos are placed in the woman (in the device), removed again and then a suitable embryo(s) transferred.

Which licensed activity/activities does the process fall within?

3.2. The consensus of SCAAC was that intrauterine culture of gametes and embryos addresses multiple categories of licensable activities which include processing gametes, processing embryos, keeping gametes and keeping embryos.

3.3. Further to this the Executive has sought legal advice which has clarified that the process falls within two licensable activities: processing gametes and processing embryos. This is because the Act states, "This Act, so far as it governs the keeping or use of an embryo, applies only to keeping or using an embryo outside the human body."

Is there evidence to suggest the process is not safe?

3.4. Upon initial consideration of the evidence of safety provided at the SCAAC meeting of 10th June 2015 (Annex A, B, C and D), the Committee felt that they had not seen sufficient data to determine the whether intrauterine culture of gametes and embryos in a device such as the Anecova AneVivo intrauterine device was safe.

3.5. The Committee requested additional data, such as that submitted for CE marking of Conformity on the Anecova AneVivo intrauterine device, be made available to the Committee. In particular the Committee felt that information regarding the effect of all the components of the device on embryos, perhaps in an animal system would be useful to aid their decision to determine the impact of the device on embryos.

3.6. The Executive was provided with additional data on the safety of the device and this was discussed by the Committee via teleconference on 28th July 2015 (Annex E).

3.7. It was the view of the Committee that the mouse embryo assay and bovine embryo assay both indicated that the device did not negatively impact embryo development to the blastocyst stage indicating the device's safety, and the Committee also felt that the toxicity data provided was good, also supporting the safety of the device.

3.8. Members of the Committee noted that while the evidence provided asserted that testing of the device demonstrated the 'capability of the device to be safely and quantitatively loaded and unloaded', no evidence to support this was provided. The Committee felt that it was important to see data demonstrating that the retrieval rate was sufficiently high before concluding that the device was safe and asked for this further information to be provided.

3.9. The Executive was provided with additional data on the retrieval rate of the device and this was circulated to the Committee via email on 29th July 2015 (Annex F).

3.10. The Committee noted that the retrieval rates gave no cause for concern and therefore felt satisfied that the evidence provided gave no indication to suggest that the process is unsafe.

Is there evidence to suggest that the process is not effective?

3.11. SCAAC considered the evidence provided on the efficacy of the process (Annex A, B, C and D) at their Committee meeting and gave further consideration to the same data during the teleconference.

3.12. The Committee noted that sample size of people included in each clinical study was very small and that because of this it was not possible to make an objective assessment of efficacy of the process, nor to say whether it is more or less effective that current IVF techniques.

3.13. However, the Committee noted that Anecova AneVivo intrauterine device has been used in for treatment in three European countries resulting in a number of live births, suggesting that it sufficiently effective to give successful IVF outcomes some of the time. As the data on efficacy is so limited the Committee felt that it would be best to offer this treatment only as part of a clinical trial.

3.14. NOTE: The applying clinic intended to use the device has part of a clinical trial. However, they have recently heard that their funding application to the NIHR was not successful. As such, while it is still the clinic's intention to use the device as part of a clinical trial, this is dependent on securing funding. In the meantime, the clinic wishes to offer intrauterine culture of gametes/embryos to its patients in a clinical context.

3.15. The Committee also noted that it was somewhat misleading to describe the experience of the embryo in the device as more 'natural' in comparison to the laboratory environment. This is due to the fact early embryos normally develop in the fallopian tube and are exposed to fallopian fluids, which some culture media are designed to imitate, whereas in the device embryos will be exposed to uterine fluids.

3.16. Some members of the Committee felt that because of this and given this lack of evidence to support the efficacy of the device, they could not see merit of introducing this process into clinical practice. However others felt that the device had sufficient potential and was part of the process of innovation which might lead to improvements in IVF success, particularly if used as part of a clinical trial.

3.17. The Committee noted that as the device might offer no improvement and add an unnecessary cost to patients, any patient information provided by clinics should highlight this. In addition, information on the HFEA website should draw attention to the fact that the process has not yet been subject to a clinical trial, and its efficacy is therefore not known.

Further issues identified (beyond the remit of SCAAC's role)

3.18. The Committee expressed concerns that intrauterine culture systems such and the Anecova AneVivo device could allow the transportation of embryos between clinics, countries and even from one woman to another and felt that these issues should be highlighted to any clinics wishing to use the device.

3.19. It is the view of the Executive that it would be for individual clinics to consider any transportation or biosecurity risks before implementing use of the device, and that current HFEA guidance on import and export of embryos, transportation arrangements and donor screening were already sufficient and that authorisation of the device would not require any additions to the Code or Practice or General Directions.

4. SCAAC's recommendation to the committee

4.1. The Committee did not see any evidence to suggest that intrauterine culture of gametes/embryos using a device such as the Anecova AneVivo would not be effective. However they did not feel that there was sufficient clinical data to say whether the process has a greater or lesser efficacy than that of traditional IVF methods.

4.2. The Committee did not feel that there was any evidence to indicate that the process was not safe.

Actions:

4.3. SAC is asked to give consideration to SCAAC's discussion and recommendations and decide whether they feel the process of intrauterine culture of gametes is suitable to carry out a licenced activity. To this end SAC must answer the following questions:

- Is the process safe?
- Is the process effective?

4.4. Depending on the answers to these questions, SAC can either:

- refuse authorisation, with reasons;
- adjourn, and ask for further information;
- authorise the process for use at all centres;
- authorise the process for named centres only; or
- refer to the Authority for final decision.

5. Next steps

5.1. If SAC authorises the process, "Intrauterine culture of gametes and embryos (including insertion and removal of device, followed by transfer of embryo(s) to the same woman)" will be added to the authorised processes list on the HFEA website

(http://www.hfea.gov.uk/139.html) with any limitations the Committee has imposed included, and the applying centre will be informed of the Committee's decision.

5.2. If SAC refuses authorisation of the process "Intrauterine culture of gametes and embryos (including insertion and removal of device, followed by transfer of embryo(s) to the same woman)" will be added to the list of processes which are prohibited for use in clinical practice on the HFEA website (http://www.hfea.gov.uk/139.html), and the applying centre will be informed of the Committee's decision, with reasons given.

ANNEX A – Application for authorisation of a novel process

Application to carry out a licensed activity using a new process

This application from should be used by centres which wish to carry out a licensed activity using a process has not previously been authorised by the Authority

It is important that the language used in this application from is clear and understandable to non-specialist lay members and staff. All abbreviations should be explained.

Centre details

Centre Name	Complete Fertility Centre
Centre Number	0307
Person Responsible	Prof. N.S. Macklon

New/Novel process

What is the new/novel process?

Name of the process	In vivo fertilisation of oocytes as part of ART using the Anecova AneVivo intra-uterine medical device.
Description of the cells to which this preparation process is applied.	Sperm, oocytes, inseminated / injected oocytes
Please provide a brief description or a flowchart of the process	1) Patient follows the traditional course of ART treatment up to OPU, including ovarian stimulation with exogenous gonadotrophins, avoidance of premature luteinisation by either GnRH agonist or antagonist, and triggering of final oocyte maturation by hCG or GnRH agonist. 2) Egg retrieval and fertilisation preparation a. Oocyte retrieval using standard procedures b. Standard sperm preparation for fertilisation c. Standard oocyte preparation for fertilisation d. Fertilisation with a co-incubation of oocytes and spermatozoa in vitro to initiate fertilisation (2 hours) or with ICSI e. Loading of the Anecova device with cells 3) Anecova device placement in utero under ultrasound guidance. 4) Anecova device retrieval after 18 hours. 5) Fertilisation assessment (PN), embryos placed into in

	vitro culture according to standard procedures 6) Embryo selection, transfer and selection for cryo preservationaccording to standard criteria and procedures. 7) Luteal phase support according to standard procedures

Licensed Activity

Please indicate which licensed activity the new/novel process will be used to carry out

Activity	State YES if the novel process will be used to carry out the activity
Procuring gametes	
Keeping gametes	
Processing gametes	
Distribution of gametes	
Use of gametes	YES
Storage of gametes	
Storage of embryos	
Creation of embryos in vitro	
Procuring embryos	
Keeping embryos	
Embryo Testing	
Processing embryos	
Distribution of embryos	
Placing any permitted embryo in a woman	

Evidence to support application

1) Please explain why the process is necessary or desirable for carrying out the licensed activity.

In-vivo fertilisation reduces exposure of gametes to the synthetic environment provided by in-vitro culture conditions during this crucial phase of early development.
The use of this intra-uterine device for fertilization will also increase direct involvement of women in their ART treatment.

2) Please provide evidence (e.g. copies of available published studies), that the new process is safe – e.g. from animal studies or research on human embryos

An in vivo culture system for human embryos using an encapsulation technology: a pilot study. Blockeel et al, Hum Reprod, 2009:24;790-796. (see attached pdf)

Please see Appendix 1

3) Please list all reagents and materials used in the new process that come into contact with patients, gametes or embryos, providing details of the supplier and quality/safety specification. Please expand this table as necessary. If authorised, this process may be used by other licensed centres and it is acknowledged that there may be variations in the reagents used however any clinic using the process will be expected to show that they are using reagents of similar specification to those referenced below.

Reagent/material	Manufacturer or supplier	Product code	Specification e.g. CE marked, clinical grade, reagent grade, etc.
Titanium grade 2	Pierval	Titanium Grade 2	USP – Class 6 - Certified
Stainless steel	Heraeus	AISI 302 FPRO-00037	316 LVM – Class 6 - Certified
Human grade silicone	Speciality Silicone	SSF-METN-750 USP CLASS VI 29407	USP class 6 certified
Polycarbonate vessel	IT4IP / Dow Chemicals	Calibre 201-6	USP class 6 certified
Polyamide monofilament	G.KRAHMER GmbH	Polyamide monofilament	CE mark, USP and EU conformity

		12H0140R	

4) Please provide evidence (e.g. copies of available published studies), that the new process is effective.

See attached Appendix 1.

The use of the medical device for the proposed purpose has been approved by a number of European Competent Authorities, including:
Denmark (Sundhedsstyrelsen)
Czech Republic (Ministerstvo Zdravotnictví České Republiky)
The Danish Competent Authorities have considered that the use of this medical Device should not be regarded as a new treatment methodology and that as the intended use of the healthcare product was similar to established technologies of the ART sector there was no further need for limitations or obligations by healthcare personnel.

The device has already been introduced into clinical practice in these countries and is currently undergoing regulatory approval prior to clinical introduction in Spain, Hungary and Finland.

5) Please note that clinics using this process will be expected to be able to show that they have:

- provided suitable information to patients about the nature of the treatment including any consequences and risks arising as a result of the use of this process;
- that staff have been suitably trained in the application of the new process and can provide evidence of the assessment of their competence;
- that the process and any equipment used in the process has been fully validated;
- there are mechanisms in place for monitoring the effectiveness of the process through regular audit

Can you provide brief details of your plans, with timelines, to ensure that these requirements are met.

The clinical team at Complete Fertility Centre has undergone preliminary training in the use of the device, and this will be completed and certified prior to commencing clinical use.

The following information will be provided to patients prior to start the treatment of patients:
Patient Information Leaflet, Patient Consent form. The option of using the device, and details regarding its use, pros and cons will also be explained during patient information evenings.
The device has already been presented to our patient support group and feedback from this will inform the preparation of patient information resources.

Instruction for the use of the AneVivo Device:
Detailed Standard Operation Procedures for a step-by-step guidance of the use of the AneVivo Device will be provided by the company and adapted to fit those of the Complete Fertility Centre Southampton.

When completed, please submit this application form and any associated papers and information to your centre's inspector.

ANNEX B – Supporting information

Introduction

Since the pioneering days of IVF, the procedure for gamete fertilization and pre-implantation development has by necessity taken place in synthetic in-vitro culture conditions. While these conditions have increased in sophistication, there is growing evidence that periconceptional developmental conditions can alter embryo programming, the epigenetic process which determine gene expression, growth and birthweight of the offspring and long term cardiovascular health. There remains an unmet need to minimize exposure of human gametes and embryos to synthetic conditions in this crucial and vulnerable phase.

Over a period of some 10 years, Anecova (Lausanne, Switzerland) has designed, tested and introduced into clinical practice the first *in vivo* embryo culture device that substitutes the use of incubator and artificial medium by the maternal environment of the patient's uterus and the natural tubal and uterine fluids for embryo fertilization and development. Extensive testing of the device has been performed through 3 iterations. before A pilot study demonstrated not only the feasibility of the technology on humans, but also trends of higher implantation potential and higher proportion of euploidy for the embryos cultured *in vivo.* Two recent studies using the resulting version (Anecova-d5) have confirmed the observed trend to higher developmental competency with larger numbers, after only 18h of *in vivo* culture. In the case of the NCVd12H study reported in this white paper, the implantation rate per transferred embryo was high as 45% in the *in vivo* arm versus 25% *in vitro*.

Two further studies have shown that the Anecova device and its insertion method is a safe and efficient approach with minimal measurable effects on the uterine cavity.

Anecova-d device & use of Anecova in ART procedure

Intent of use of the Anecova-d device

The intended use of the Anecova-d5 device lies within the current practice of assisted reproductive technology. The intended use of the device is placement and retrieval of embryos (or gametes), into and from the uterine cavity with the objective of their culture inside the uterine cavity for the purpose of enabling fertilization take place in-vivo. This reduces exposure to synthetic in-vitro conditions during this crucial early phase of the development.

The Anecova-d device, manufactured by Anecova SA (Lausanne, Switzerland), is CE Marked and belongs to the class IIa medical device (non active, non implantable, with a short term residence in the uterine cavity after insertion through a natural body orifice). The device is composed of a capsule attached to a stabilizing system (fig.1).

The capsule is a small micro-perforated polycarbonate vessel covered by a perforated silicon tube that simply retains the embryo in the uterine cavity. The capsule has been designed to allow a bi-directional fluidic and molecular diffusion with a rapid rate of equilibration. The capsule is approximately 1 mm in diameter and 1 cm long. The proximal part of the tube composed by titanium plugs can be opened/closed to load/retrieve oocytes/embryos.

The stabilizing system, assembly of stainless steel and silicon, is approximately 2.2 cm long and sits in the lower part of the womb. A small portion of the stabilizer will stay in the cervix and a flexible blue polyamide string extends out into the vagina to enable the easy retrieval of the device.

The device is placed in the uterine cavity under ultrasound guided trans-cervical insertion through commercially available embryo transfer catheters.

Injected or inseminated oocytes are placed in the device for in vivo culture during the desired development period. Upon retrieval of the device, the zygotes or embryos will undergo the standard selection process and subsequently embryo transfer and cryopreservation of non- transferred embryos.

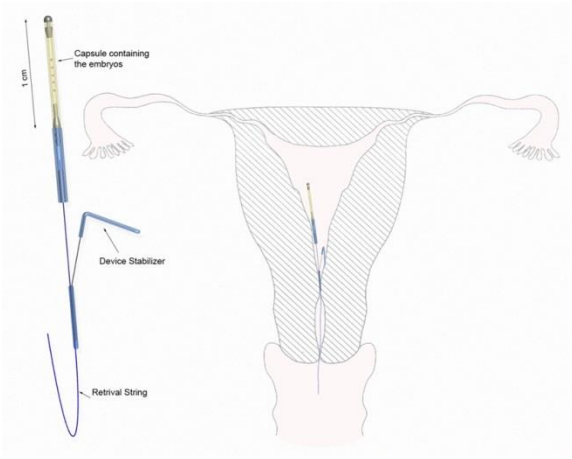

fig1. The Anecova device

Use of Anecova-d device in ART procedure for in vivo culture of embryos

The “Anecova procedure” (fig. 2) is intermingled within the standard ART treatment procedure (fig. 3) and is also comprised of several standard fertility centre (user) routine procedures.

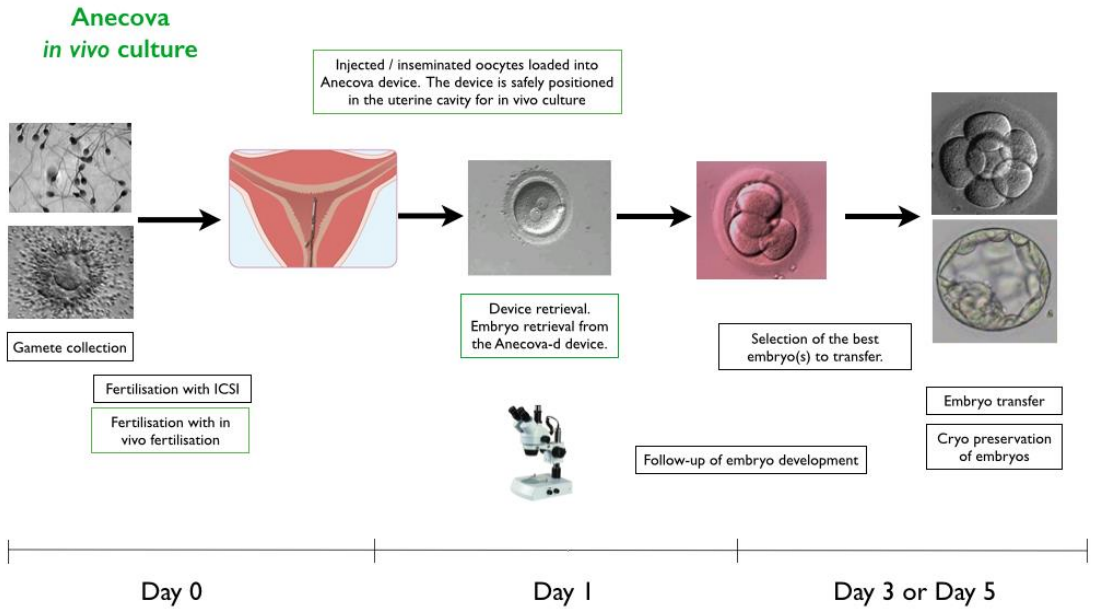

Fig.2. Anecova in vivo culture method

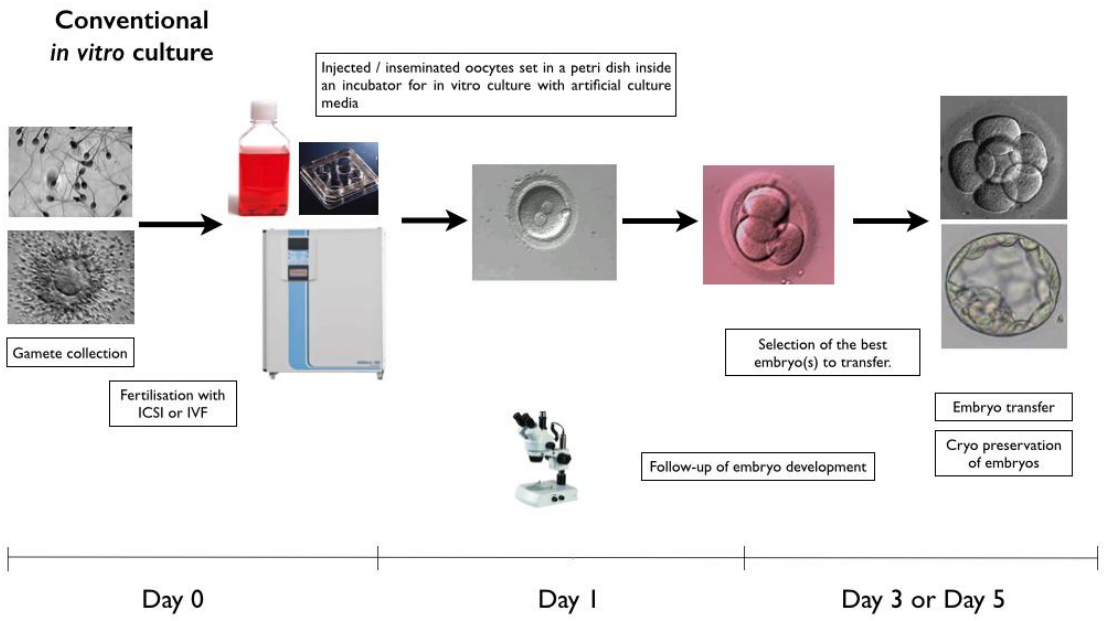

Fig.3. Conventional in vitro culture method

Anticipated benefits of the use of Anecova-d device

The design concept enables placement of gametes in the maternal uterine cavity for the process of fertilization, thus offering the benefits of a natural and adapted environment for this phase and for early embryo culture.

After the device placement in the uterine cavity and thanks to the porous membrane, the fluids (loading media and fluids present in the uterine cavity) rapidly equilibrate to provide a physiological and dynamic environment to the inseminated or injected oocytes during in vivo culture. This natural culture media, consisting of uterine and tubal derived fluid components gathers the optimized biophysical conditions (temperature, pH, gas dissolution, osmolarity) and the full range of the molecules needed for the embryo development and for the regulation of the development (gene expression regulation, imprinting, epigenetic). Moreover, with the presence of the embryo in the uterine cavity, the endometrium can respond and adapt to the presence of the embryo through paracrine interactions, as it is the case in natural conception.

This in vivo culture method has been developed with the goal to obtain the simultaneous production of a fully receptive endometrium and of high developmental competency embryos.

These anticipated benefits are directly linked with the early molecular cross talk that exists naturally in vivo between the fertilized oocyte, zygote or embryo and the maternal intra-uterine cavity environment which contains a combination of uterine and tubal fluid components.

Clinical Research and Development Program

A program of Clinical & Research and Development studies has been executed with the goal to measure the safety, the feasibility, and the performance of our in vivo culture method as well as the possible effects on the uterine cavity of the use of the Anecova-d device. The following data have been generated by Anecova, SA and by a number of fertility centres in Europe. Except for the pilot study, none of these results have been published.

Pilot Study (NCVPM2B)

A pilot study was performed in Brussels by the group directed by Prof. Devroey and published in 2009 (Blockeel, Mock, Verheyen, et al., Human Reprod., 24, 4, 2009). The study included 19 patients undergoing ICSI and 167 metaphase II oocytes in a sibling oocytes randomization distribution between an *in vivo* arm and an *in vitro* arm.

A trend towards a higher proportion of good quality embryo was obtained for in vivo produced embryos at day 3 and at day 5 for all tested sub-groups (day 3 G1:65% vs 43%, G2:62% vs36%, G3:74% vs63%; and day 5 G1:43% vs 33%, G2:38% vs 21%, G3:58% vs31%).

The assessment of the euploidy of the sibling oocytes on a small number of embryos cultured from day 0 to day 3 *in vivo* resulted in a higher proportion of euploid embryos for *in vivo* cultured embryos (88.2% *in vivo* vs. 46.7% *in vitro*). The study also resulted in pregnancies with a trend of a higher pregnancy rate for *in vivo* produced embryos (50% *in vivo* vs. 33% *in vitro*). Two clinical pregnancies and healthy live births were obtained from the *in vivo* arm.

The results of this study showed that the use of the Anecova device for *intra-uterine* culture of embryos is safe and feasible. The data also strongly suggests that the uterine environment meets all the requirements for normal fertilization, normal embryo development with a very high proportion of euploid embryos within a sibling cohort.

NCVd6H

This study randomized oocytes from16 patients equally into two groups, *in vivo* and *in vitro*. The *in vivo* oocytes were introduced into the Anecova device after fertilization from day 0 to day 1. Upon retrieval of the device, fertilization and zygote score was assessed. 2 *in vivo* embryos were then cultured to day 2 or day 3 and transferred back to the patient.

This study was exploratory by design and therefore not powered to obtain statistically significant differences. The aim was to obtain reasonable estimates for the selected primary endpoint (zygote score) and for the secondary endpoint (fertilization rate). The study results

show a trend for a similar fertilization rate between *in vivo* and *in vitro* cultured embryos (64% in vivo vs. 72% in vitro) and a similar global zygote score (12.5 in vivo vs.13 in vitro; analyzed parameters were centering, proximity and orientation of the pronuclei, number and polarization of the nucleolar precursor bodies and cytoplasmic halo). 7 babies were born from *in vivo* cultured embryos and the proportion of twin pregnancies when transferring 2 embryos was very high (19%) compared to the generally observed percentage (10%). This suggests that even with similar morphological score the embryos cultured *in vivo* demonstrated higher developmental competency.

These trends indicate that the production of in vivo embryos was positively influenced by the uterine environment and supports the safety of Anecova device as well as the feasibility of in vivo culture.

NCVd12H

This study randomized 35 patients into two groups: *in vivo* and *in vitro* embryo culture. For the patients randomized to the in vivo culture group, all the injected oocytes were introduced shortly after ICSI into the Anecova device and into the uterine cavity from day 0 to day 1. All recovered fertilized zygotes were then further cultured in vitro to day 5. For the patients randomized to the in vitro culture group, all injected oocytes were cultured *in vitro*. Embryos were assessed by morphology on day 3 and 5.

This study was exploratory by design and therefore not powered to obtain statistically significant differences. The aim was to obtain reasonable estimates for the selected primary endpoint (embryo quality at day 3) and for the secondary endpoints (fertilization rate, embryo quality at day 5, implantation rate, clinical pregnancy rate).

The study results show a trend for a similar fertilization rate and a similar morphological embryo quality at day 3 (50% in vivo vs. 47% in vitro) and day 5 (29% in vivo vs. 33% in vitro) for both study arms. However the study also shows a trend toward significance for the improvement of the implantation (45% versus 25%) and pregnancy rates (50% versus 36%) for embryos cultured *in vivo* versus *in vitro*.

In addition to these trends, a higher number of twin pregnancies have been reported following the transfer of two embryos from the in vivo arm of the study (27% *in vivo* vs. 9% *in vitro*). These results repeat the trends observed during the Pilot Study and the NCVd6H Study and strengthen the hypothesis of a positive effect of *in vivo* culture on the developmental competency of the embryo. The safety of Anecova-d device and the feasibility of in vivo embryo development were further confirmed by this study.

Endometrial study NCVd3H

To study the possible effects of the presence of the Anecova device on the endometrium and endometrial receptivity, a further study was carried out in oocyte donors. The study compared the histology, hormonal profile and genomic profile (microarray) of endometrial tissue samples obtained from 20 oocyte-donors at hCG+7 days. Subjects were evenly distributed in 4 groups: insertion of the Anecova-d device for 1, 3, 5 days after OPU or no insertion (control). All patients underwent a routine stimulation protocol. The histopathological study did not show any preponderance of signs of endometritis nor did it find endometritis among any of the tested 4 groups. No obvious difference was found in the global genomic profiles among specimens of any of the Anecova groups when compared to control group.
The study concluded that the use of the Anecova device for up to 5 days does not seem to affect the endometrium at a histological level or genomic level (Anecova: Data on file).

Endometrial study NCVCD003H

The aim of the study was the Analysis of the uterine environment for the evaluation of the incidence of the device deposit in the uterine cavity. The study has been performed on 14 eligible volunteers.
The evaluation of the incidence of the device deposit in the uterine cavity was assessed by cytokines, chemokines and growth factors profile (multiplex analysis of the uterine fluids contained in the Anecova device at retrieval) and by the Histo-cyto analysis of the cells contained in the Anecova device after in vivo residence in women uterine cavity. The profile of molecules and the cellular content of the device were used to assess the inflammatory and immune response status after transfer and removal of an Anecova device. The study showed that the currently employed system device and delivery method is a safe and efficient approach with minimal measurable effects on the uterine cavity when compared to control patients in whom no device was inserted. (Anecova: Data on file)

CONCLUSIONS on PERFORMANCE:

The studies carried out on the Anecova intra-uterine embryo culture system have demonstrated the safety, the feasibility and the performance of the Anecova-d device.
22 healthy babies have been born to date from embryos cultured *in vivo*.
The Anecova-d studies show that the uterine environment positively influences the production of in vivo embryos. Studies are showing similar trends for embryos cultured *in vivo*:

- An increase in the implantation rate per embryo transferred with a slightly higher or similar morphological score;
- A high proportion of twin pregnancies when 2 embryos have been transferred, which further suggests a higher developmental competency of the *in vivo* cultured embryo;
- The production of a higher proportion of euploid embryos, normal chromosome embryos, within a sibling cohort after in vivo culture.

The recurrent repetition of the same tendencies means that the probability for a non-random effect of the in vivo culture on embryo development is high.

CONCLUSIONS on SAFETY:

During the reported clinical studies, one Adverse Event has been reported. After the analysis of the event, this AE was considered not serious, probably not related to the Anecova-d device. This AE has been considered as resolved and closed.
To date, the use of the Anecova-d device is safe. As far as we have been able to appreciate, demonstrate or conclude, with the primary medical reports and with the follow-up reports, no incident (AE & SAE) directly pointing on the Anecova-d5 device or on the procedure using the Anecova-d5 device has been reported.

GENERAL CONCLUSIONS:

For the proposed clinical indication of the use of Anecova-d device in the treatment of the infertility:

- The clinical evidence demonstrates conformity with relevant regulatory requirements
- The intended performance and the safety of the device as claimed have been evaluated
- The anticipated benefits have been evaluated
- The risks associated with the use of the device are acceptable when weighed against the benefits to the patient

Considering the already available data on the Anecova-d device, it is concluded that the Anecova-d device can be expected to exhibit the performance claimed for the intended use. Potential undesirable clinical effects and risks seem to be acceptable and comparable to those seen in the standard ART procedure.

Human Reproduction, Vol.24, No.4 pp. 790–796, 2009
Advanced Access publication on March 10, 2009 doi:10.1093/humrep/dep005

human reproduction

ORIGINAL ARTICLE *Embryology*

An *in vivo* culture system for human embryos using an encapsulation technology: a pilot study

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BACKGROUND: Animal studies have demonstrated better embryo development *in vivo* than *in vitro*. This pilot study tested the feasibility of using a novel *in utero* culture system (IUCS) to obtain normal human fertilization and embryo development.

METHODS: The IUCS device comprised a perforated silicone hollow tube. The study included 13 patients (<36 years) undergoing a first intracytoplasmic sperm injection (ICSI) treatment and 167 metaphase II oocytes in three groups. In Group 1, 1–2 h after ICSI, sibling oocytes were assigned to IUCS or conventional *in vitro* culture. The device was retrieved on Day 1, and all zygotes were cultured *in vitro* till Day 5. In Group 2, fertilized oocytes were assigned on Day 1, embryos retrieved on Day 3 and all embryos cultured till Day 5. In Group 3, after Day 0 assignment, embryos were retrieved on Day 3 for blastomere biopsy and fluorescence *in situ* hybridization (FISH) and cultured until Day 5. The highest quality blastocysts were transferred on Day 5.

RESULTS: Fertilization and embryo development were comparable in the *in vitro* and IUCS arms, with a tendency towards better embryo quality in the IUCS. FISH analysis in Group 3 revealed more normal embryos using the IUCS ($P = 0.049$). Three clinical pregnancies and live births were obtained: two from the IUCS arm and one from the *in vitro* arm.

CONCLUSIONS: Our pilot study shows that this new IUCS appears to be feasible and safe, supporting normal fertilization, embryo development and normal chromosomal segregation. Furthermore, live births are possible after the transient presence of a silicone device in the uterus. Clinicaltrials.gov: NCT00480103.

Key words: *in vivo* culture / *in utero* culture system / ICSI / embryos / oocytes

Introduction

Between 1 and 4% of children are born through assisted reproduction techniques (ARTs) (Hansen *et al.*, 2005). These techniques imply that fertilization occurs outside the fallopian tube, and the preimplantation embryos are cultured *in vitro* until transferred into the uterus. Between patients and within one patient, embryo quality may differ considerably. On the one hand, about 50% of human embryos arrested during the first week as a result of chromosomal abnormalities (Munné *et al.*, 1995). On the other hand, suboptimal *in vitro* culture conditions may be associated with apoptotic events in human embryos (Hardy *et al.*, 2001).

†The first three authors have contributed equally.

In the bovine model, it has been demonstrated that the post-fertilization environment affects embryo quality in terms of gene expression, cryotolerance and metabolism (Wrenzycki *et al.*, 2005, 2007; Lonergan *et al.*, 2006; Duranthon *et al.*, 2008). In humans, however, there is no similar evidence from the literature.

In the current ARTs, a tendency to optimize the cost/benefit ratio exists by providing more 'physiological' treatment conditions, including mild ovarian stimulation and single embryo transfer. Mimicking physiological culture conditions could provide us with new insights into early embryonic development.

The present pilot study was designed to explore the safety and feasibility of a novel *in utero* encapsulation technology (Lysaght and Aebischer, 1999) for human embryos and aimed to compare the characteristics of the resulting embryos with their *in vitro* developed counterparts, in patients younger than 36 years of age undergoing a first intracytoplasmic sperm injection (ICSI) treatment. The technique involves the introduction of microinjected human oocytes into a retrievable and permeable tubing system that allows optimal exchange between the uterine maternal environment and the developing embryo.

Materials and Methods

Study design

The pilot study was carried out at the Centre for Reproductive Medicine, UZ Brussel. The *in vitro* fertilization laboratory is certified according to ISO15 189. All patients included in the study gave written informed consent.

Individual oocytes/embryos from patients of <36 years old undergoing a first ICSI treatment were randomly assigned to either the *in vivo*, *in utero* culture system (IUCS) or the *in vitro* culture system. If less than eight metaphase II (MII) oocytes were obtained on the day of oocyte retrieval, then the patient was excluded from the study. Other exclusion criteria were endometriosis, polycystic ovary syndrome and severe male-factor infertility. A total of 13 patients were included and numbered consecutively according to the order of enrolment. Overall, 167 MII oocytes were analysed. Three distinct groups were used in order to compare fertilization, embryo development and chromosomal constitution obtained *in vivo* and *in vitro*. In Group 1, half of the oocytes were inserted into the device shortly after microinjection (Day 0) and retrieved on Day 1 in order to observe fertilization. In Group 2, half of the *in vitro* fertilized oocytes were inserted on Day 1 and retrieved on Day 3 in order to compare embryo development. In Group 3, half of the injected oocytes were inserted into the device shortly after microinjection (Day 0) and retrieved on Day 3 in order to compare embryo development and chromosomal constitution (proof of normal fertilization). The best morphologically graded (euploid) blastocysts were transferred into the uterus on Day 5.

The *in vivo* culture device

The encapsulation system (Fig. 1) is composed of a 1-cm long microperforated hollow silicone elastomer tubing with an outer diameter of 0.75 mm and an inner diameter of 0.43 mm. Eight longitudinal lines of

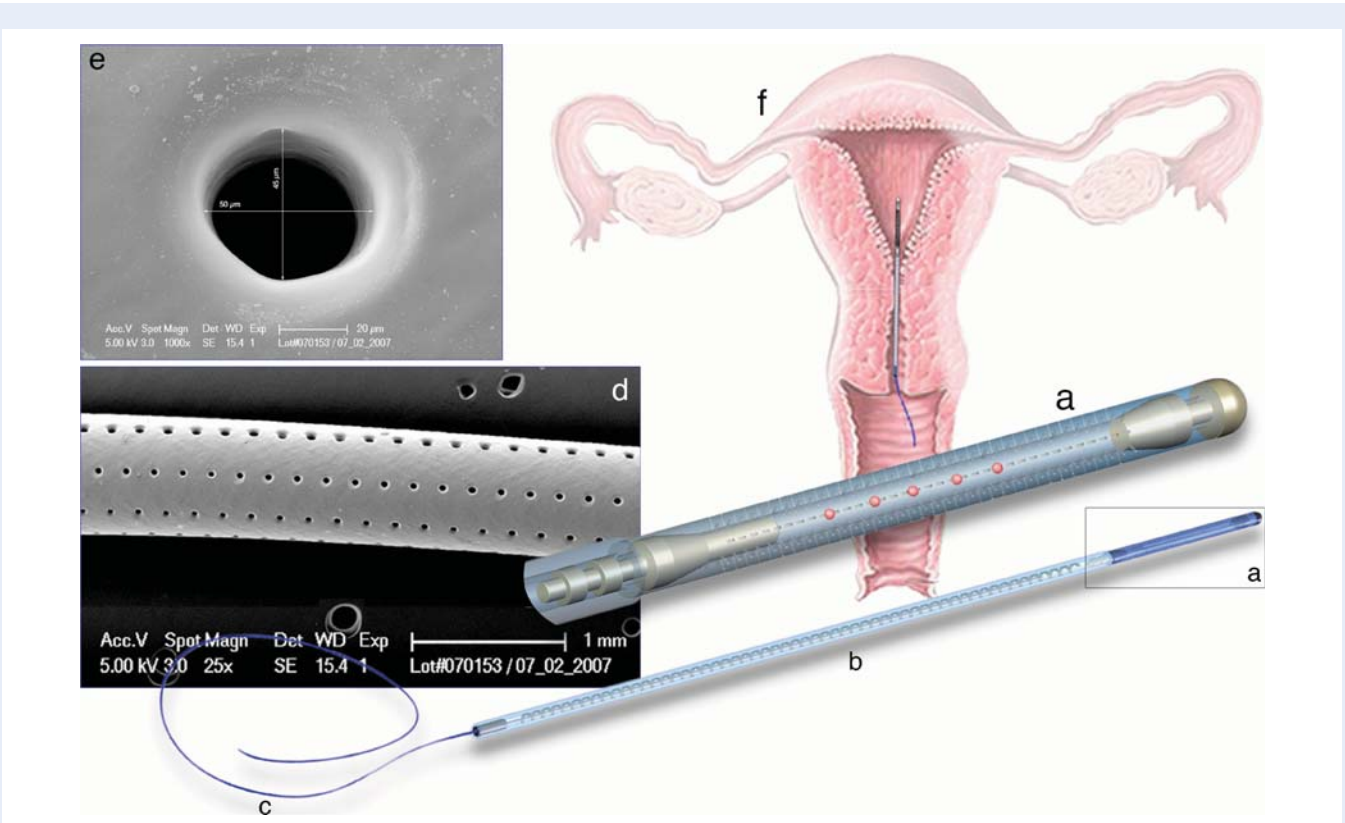

Figure 1 Diagram of the IUCS used in women undergoing ICSI: (a) microdrilled silicone segment containing the oocytes/embryos; (b) stabilization segment; (c) extraction segment; (d) scanning electron microscopy of the drilled silicone segment (magnification ×25); (e) high magnification of a laser-drilled hole (scanning electron microscopy, magnification ×1000); (f) schematic representation of the device position in the uterine cavity.

45 holes with a diameter of 40 μm are made with a laser-based system. The size of the holes does not allow the embryos to escape the capsule, but allows exchange of nutrients, endometrial cells and other cellular and non-cellular components. The proximal part of the tube can be opened/closed to load/retrieve oocytes/embryos using a titanium hooking device attached to an unperforated silicone tube with an outer diameter similar to the capsule. The plain silicone tubing is reinforced by a stainless steel spiral wire. A polypropylene filament is fixed to the distal end of the silicone tubing to allow the extraction of the device. This IUCS can be loaded into a standard embryo transfer catheter (Prince Medical, Ercuis, France) for introduction into the uterine cavity. A sterile tampon is placed into the vagina in order to keep the device in place. Biocompatibility and toxicity tests have been performed in an *in vitro* bovine model (unpublished data). Materials used for the manufacture of the IUCS device are FDA USP class VI and ISO 13 485 approved for medical applications.

Ovarian stimulation protocol

Recombinant FSH (rFSH) (Puregon®, Organon) and GnRH antagonist Ganirelix (Orgalutran®, Organon) were used for ovarian stimulation. In summary, a low-dose, monophasic-combined oral contraceptive pill (OCP) containing 150 μg of desogestrel and 30 μg of ethinylestradiol (Marvelon®, Organon) was administered for 2 weeks starting on Day 1 of the pre-ARTs cycle. rFSH at a dose of 200 IU per day was started 5 days after discontinuation of the OCP. Ganirelix was initiated at a daily dose of 0.25 mg on Day 6 of the rFSH stimulation. Oocyte maturation was induced by the administration of 10 000 IU of hCG (Pregnyl®, NV Organon) when at least three follicles ≥17 mm diameter were present on ultrasound scan. Oocyte retrieval was carried out 36 h after Pregnyl® injection by vaginal ultrasound-guided puncture of ovarian follicles.

ICSI, fertilization and embryo culture

Pooled cumulus–oocyte complexes were denuded of cumulus cells. MII oocytes were injected by a qualified laboratory technician with a single fresh motile spermatozoon using ICSI (Van Landuyt *et al.*, 2005). Injected oocytes were placed in individual 25 μl droplets of cleavage medium (Medicult®) under paraffin oil (Irvine Scientific®).

Group 1

Group 1 was designed to test the ability to obtain normal fertilization after 18 h *in vivo* incubation using the IUCS (Fig. 2). Eighty-one cumulus–oocyte complexes were retrieved from seven patients (mean of 11.6 per patient). A total of 79 MII oocytes were microinjected with ejaculated sperm from the partner. Half of the survived oocytes were subjected to *in vivo* culture 1 to 2 h post-injection. Randomization for allocation to *in vivo* or *in vitro* culture was performed as follows. After ICSI, oocytes were randomly allocated to individual culture droplets. In the odd-numbered patients, the first half of the culture droplets with injected oocytes was allocated to the *in vitro* arm and the other half was allocated to the IUCS arm. In the even-numbered patients, the opposite allocation procedure was performed, i.e. with the first half being allocated to the IUCS and the second half being further cultured *in vitro*. In case of an uneven oocyte number, an

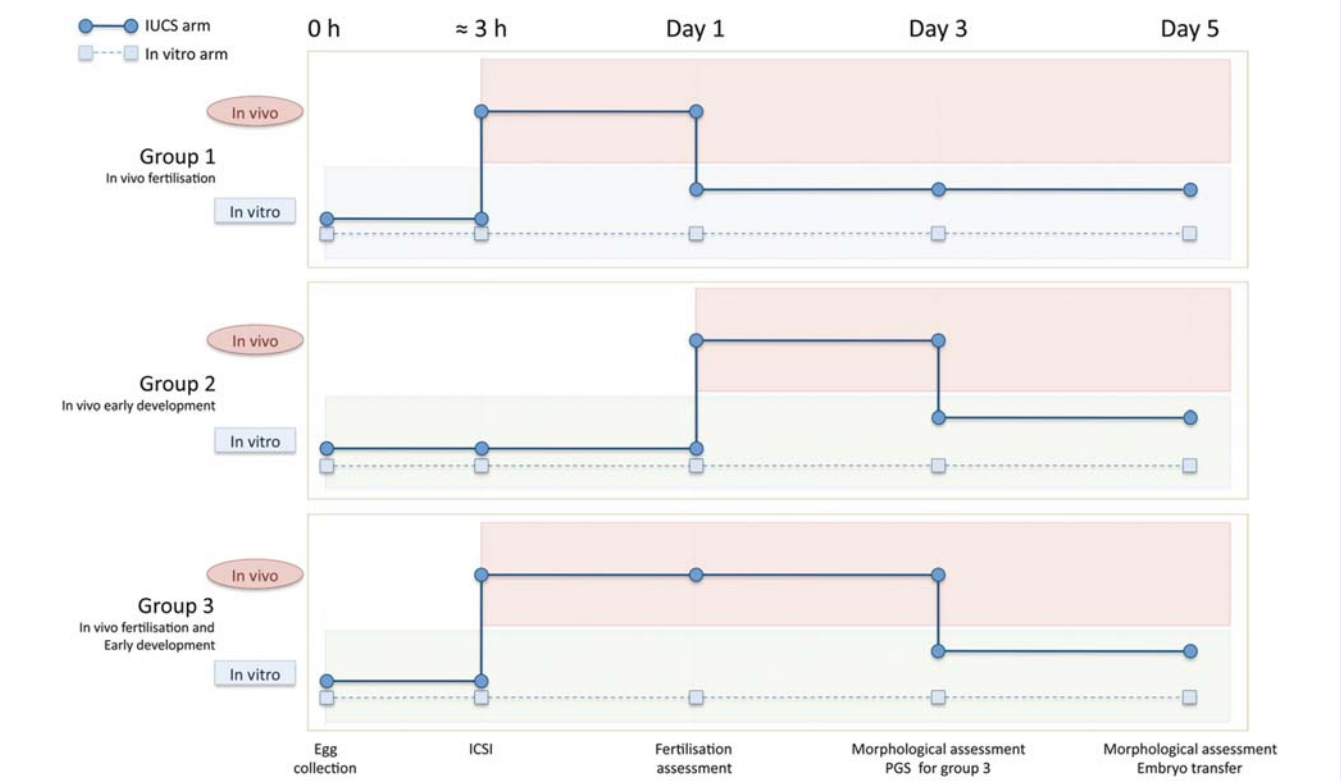

Figure 2 Timeline for the pilot study, designed to test the utility of the IUCS in supporting normal human fertilization and embryo development. For each patient, after ICSI, half of the injected oocytes were allocated to the IUCS arm (*in vivo*) and the other half to the *in vitro* arm. The times were selected to assess the IUCS for the following aspects of early development—Group 1: Day 0 to 1, *in vivo* fertilization; Group 2: Day 1 to 3, *in vivo* early embryo development and Group 3: Day 0 to 3, *in vivo* fertilization and early embryo development.

extra oocyte was allocated to *in vitro* culture. The microinjected oocytes assigned to the IUCS arm were loaded into the capsule (Fig. 1) under microscopic control at ×5 to ×10 magnification. Loading was performed in a dish filled with HEPES-buffered medium kept at 37°C. The device was inserted into a catheter and transferred to the uterus. After *in vivo* culture for 18 h, the IUCS was removed from the uterine cavity and immediately placed into HEPES-buffered medium at 37°C. The silicone capsule was opened at both ends, and the zygotes were recovered by aspirating the content of the capsule with a glass pipette or by flushing the contents with a blunt needle. Zygotes were individually placed into 25 μl droplets of cleavage medium under oil. Fertilization obtained *in vivo* or *in vitro* was evaluated by the appearance of two pronuclei at 18 to 20 h after injection, and all zygotes were further cultured *in vitro*. Embryo development was evaluated daily by the assessment of number of cells, fragmentation rate, cell size, symmetry, granulation, vacuolization and multinucleation. The different parameters were combined in an embryo score, ranging from Q1 (excellent) to Q4 (poor).

In the morning of Day 3, all embryos were transferred to blastocyst medium (Medicult®). Embryos were evaluated until transfer (Day 5) or until freezing (Day 5 or 6). For blastocyst evaluation, the scoring system of Gardner and Schoolcraft (1999) was used, and a combined embryo score (Q1 to Q4) was given to the blastocysts, considering blastocyst grade, fragmentation, completeness of compaction and estimation of the number of cells in the inner cell mass and trophectoderm. The morphologically best embryos were transferred into the uterus. Only supernumerary blastocysts of Q1 and Q2 (excellent and good, respectively) were cryopreserved with glycerol as cryoprotectant.

Group 2

Group 2 was designed to compare the effect of both culture systems on early embryo development at 66 h post-injection (Fig. 2). Only normally fertilized oocytes (two pronuclei) were randomized for *in vivo* or *in vitro* culture. Forty-six cumulus–oocyte complexes were retrieved from three patients (mean of 15.3 per patient). A total of 41 MII oocytes were microinjected. On Day 1, half of the normally fertilized oocytes were cultured *in vivo* and the other half *in vitro*. Random allocation to the *in vitro* or the *in vivo* arm was similar to the procedure already described for Group 1. Embryos were retrieved from the capsule on Day 3 and further cultured *in vitro* up to Day 5 or 6.

Group 3

Group 3 was designed to examine the feasibility of obtaining both normal fertilization and normal early embryo development using the IUCS. From three patients, 57 cumulus–oocyte complexes were retrieved. A total of 47 MII oocytes were injected and the surviving oocytes were randomly allocated to *in vivo* or *in vitro* culture (as described earlier) 1 to 2 h after microinjection. Embryos were retrieved from the capsule on Day 3. In order to assess normal fertilization and euploidy, *in vivo* and *in vitro* embryos were biopsied and analysed by fluorescence *in situ* hybridization (FISH). Embryos were further cultured *in vitro* up to Day 5 or 6.

Embryo biopsy and FISH protocol

Embryo biopsy was performed on Day 3 embryos with ≥5 blastomeres and ≤50% fragmentation.

Before the biopsy, embryos were placed in a droplet containing Ca^{2+} and Mg^{2+} free medium (G-PGD, Vitrolife, Kungsbacka, Sweden). Laser technology was used to perforate the zona pellucida. One blastomere was withdrawn with a bevelled aspiration pipette and spread on a slide according to the HCl/Tween-20 method (Staessen *et al*., 2003).

In all blastomeres, five chromosomes were analysed by FISH in a one-step procedure. Centromeric probes for chromosomes X, Y and 18, and locus-specific probes for chromosomes 13 and 21 were used for hybridization (Vysis Inc., Downers Grove, IL, USA).

The percentage of normal embryos in each arm was defined as the number of euploid embryos upon the number of embryos for biopsy.

Embryo transfer and pregnancy test

In each group, the best blastocysts, regardless of culture condition (*in vivo* or *in vitro*), were transferred to the uterine cavity on Day 5. In case of similar quality, preference was given to the *in vitro* embryo. A rise in serum hCG on two consecutive occasions from 11 days after embryo transfer indicated pregnancy. A clinical pregnancy was defined by the presence of a gestational sac with fetal heart beat at ultrasonography after ~7 weeks of pregnancy.

Statistical evaluation

Categorical data are presented as number of cases and percentages for each culture arm. For each group, the *in vivo* and *in vitro* culture arms were compared by using Fisher's exact probability test, with a value of 0.05 as the limit of significance. The Statistical Package for the Social Sciences version 16.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Registration

This study was registered with number NCT00480103 in the Clinical Trial web (www.clinicaltrials.gov), and it received institutional review board approval by the Ethics Committee of the Centre for Reproductive Medicine, UZ Brussel, Brussels, Belgium.

Results

Group 1

In Group 1, 74 out of 79 MII oocytes were successfully injected and randomly assigned to *in vivo* or *in vitro* culture. Five injected oocytes degenerated shortly after ICSI, before randomization was performed. In patients with an odd number of successfully injected oocytes (six cases), the extra oocyte was allocated to *in vitro* culture. Overall, 40 injected oocytes were cultured *in vitro*, while 34 injected oocytes were loaded into the capsule and cultured within the IUCS inside the maternal uterus for 18 h (mean of 4.9 per capsule, range 3–8). From the IUCS, 32 out of 34 oocytes were successfully retrieved and 23 showed normal fertilization (2PN, 67.6%) (Table I). *In vitro*, 30 out of 40 oocytes showed 2PN (75%). On Day 3, 15 out of 23 embryos from the *in vivo* arm (65.2%) were good-quality embryos (Q1 + Q2) versus 14 of the 30 *in vitro* cultured siblings (46.7%). A similar trend was observed on Day 5, the day of embryo transfer for all seven patients. Three embryos were selected for transfer from the *in vitro* arm, and four embryos were chosen from the IUCS arm. A similar number of embryos was cryopreserved in both arms. Three of the seven patients in Group 1 showed a clinical pregnancy: two of them with a blastocyst of the IUCS arm and one with a blastocyst of the *in vitro* arm. Three healthy children were born: two from IUCS and one from *in vitro* culture.

Group 2

In Group 2, a total of 41 MII oocytes were microinjected, 32 survived and 29 normally fertilized zygotes were randomly assigned to *in vivo* or *in vitro* culture (Table II). Fifteen zygotes were cultured *in*

Table I Results of fertilization and embryo development *in vivo* (using IUCS) and *in vitro* (Group 1)

	7 patients 81 COC[1] 79 MII[2] oocytes 74 successfully injected MII oocytes			
	***in vitro* culture**		***in vivo* culture**	
Day 0				
Allocated injected oocytes	40		34	
Day 1*				
2PN	30/40	75.0%	23/34	67.6%
1PN	0/40	0.0%	2/34	5.9%
≥3PN	7/40	17.5%	4/34	11.8%
Zero PN	3/40	7.5%	3/34	8.8%
Not retrieved			2/34	5.9%
Day 3**				
Q1[3]	5/30	16.7%	8/23	34.8%
Q2[4]	9/30	30.0%	7/23	30.4%
Q3[5]	10/30	33.3%	4/23	17.4%
Q4[6]	6/30	20.0%	4/23	17.4%
Day 5***				
Q1	4/30	13.3%	4/23	17.4%
Q2	6/30	20.0%	6/23	26.1%
Q3	5/30	16.7%	4/23	17.4%
Q4	15/30	50.0%	9/23	39.1%
Embryos for ET	3		4	
Embryos frozen	9		8	

[1]Cumulus–oocyte complexes, PN: pronuclei, ET: embryo transfer.
[2]Metaphase II.
[3]Q1: excellent quality.
[4]Q2: good quality.
[5]Q3: fair quality.
[6]Q4: poor quality.
Using Fisher's exact probability test:
*$P = 0.29$, for fertilization in the *in vitro* versus *in vivo* culture arms.
**$P = 0.41$, for distribution of embryo quality on Day 3 in *in vitro* versus *in vivo* culture arms.
***$P = 0.86$, for distribution of embryo quality in *in vitro* versus *in vivo* culture arms.

Table II Results of embryo development *in vivo* and *in vitro* (Group 2)

	3 patients 46 COC 41 MII oocytes 32 successfully injected MII oocytes 29 2PN oocytes			
	***in vitro* culture**		***in vivo* culture**	
Day 1				
Allocated 2PN oocytes	15		14	
Day 3*				
Q1	3/15	20.0%	4/14	28.6%
Q2	2/15	13.3%	4/14	28.6%
Q3	4/15	26.7%	3/14	21.4%
Q4	6/15	40.0%	2/14	14.3%
Not retrieved			1/14	7.1%
Day 5**				
Q1	2/15	13.3%	2/14	14.3%
Q2	1/15	6.7%	3/14	21.4%
Q3	2/15	13.3%	3/14	21.4%
Q4	10/15	66.7%	5/14	35.7%
Not retrieved			1/14	7.1%
Embryos for ET	1		3	
Embryos frozen	3		2	

Using Fisher's exact probability test:
*$P = 0.45$, comparing the distribution of embryo quality on Day 3 in *in vitro* and *in vivo* culture.
**$P = 0.39$, comparing the distribution of embryo quality on Day 5 in *in vitro* and *in vivo* culture.

vitro, while 14 were loaded into the capsule and cultured within the IUCS inside the uterus for 48 h (mean of 4.7 per capsule, range 3–8). On Day 3, 13 out of 14 *in vivo* embryos were successfully retrieved, while one was damaged. The morphological scores revealed that 8 out of 14 embryos showed good quality (Q1 + Q2) (57.1%) in the IUCS arm, while 5 out of 15 good embryos were observed (33.3%) in the *in vitro* arm. The morphological scores on Day 5 showed a similar trend. Two patients received one embryo for transfer and one patient exceptionally received two embryos. Three embryos were selected from the IUCS, and one embryo was chosen from the *in vitro* arm. One biochemical pregnancy was obtained in the patient who received one IUCS and one *in vitro*-derived embryo. No fetal sac was observed on ultrasound.

Group 3

In Group 3, 57 cumulus–oocyte complexes were retrieved from three patients (mean of 19.0 oocytes per patient). A total of 47 MII oocytes were microinjected, 3 degenerated before randomization, resulting in 44 surviving oocytes of which 22 were cultured *in vitro* and 22 in the IUCS (Table III). On Day 3, 19 out of 22 embryos were successfully retrieved from IUCS. As it is not possible to evaluate the euploidy of the *in vivo* arm embryos at the time of morphological qualification, the proportion of embryo quality has to be calculated on allocated oocytes instead of fertilized oocytes (Table III). The morphological scores on Days 3 and 5 showed a non-significant trend in favour of the *in vivo* culture (63.6% versus 45.5% good quality on Day 3 and 50.0% versus 22.7% on Day 5). On Day 3, biopsy was performed in 13 *in vitro* embryos and 17 *in vivo* embryos. FISH analysis revealed euploidy in 15 out of 17 biopsied IUCS embryos and in 7 out of 13 biopsied *in vitro* embryos ($P = 0.049$, Table III). For two *in vitro* embryos, no FISH diagnosis was obtained. Embryo transfer was performed in two out of three patients: one patient with an embryo of the *in vivo* arm and one patient with an embryo of the *in vitro* arm. The embryos of the third patient, although genetically normal,

Table III Results of embryo development and fluorescence *in situ* hybridization (chromosomes X, Y, 13, 18, 21) *in vivo* and *in vitro* (Group 3)

	3 patients 57 COC 47 MII oocytes 44 successfully injected MII oocytes			
	***in vitro* culture**		***in vivo* culture**	
Day 0				
Allocated injected oocytes	22		22	
Day 1				
2PN oocytes	16/22	72.7%		
Day 3*				
Q1	6/22	27.3%	3/22	13.6%
Q2	4/22	18.2%	11/22	50.0%
Q3	4/22	18.2%	5/22	22.8%
Q4	2/22	9.1%	0/22	0.0%
Not retrieved			3/22	13.6%
Day 5**				
Q1	2/22	9.1%	3/22	13.6%
Q2	3/22	13.6%	8/22	36.4%
Q3	4/22	18.2%	1/22	4.5%
Q4	7/22	31.8%	7/22	31.8%
Not retrieved			3/22	13.6%
Embryos for ET	1		1	
Embryos frozen	1		9	
FISH results***				
Embryos biopsied	13/22	59.0%	17/22	77.3%
Normal[1]/embryos biopsied	7/13	53.8%	15/17	88.2%
Abnormal/embryos biopsied	4/13	30.8%	2/17	11.8%
No FISH result	2/13	15.4%	0	0.0%
Normal/allocated injected oocytes	7/22	31.8%	15/22	68.2%

[1]For chromosomes X, Y, 13, 18, 21.
Using Fisher's exact probability test:
*$P = 0.13$, comparing the distribution of embryo quality on Day 3 in *in vitro* and *in vivo* culture.
**$P = 0.28$, comparing the distribution of embryo quality on Day 5 (Q1 to Q4) in *in vitro* and *in vivo* culture.
***$P = 0.049$, comparing normal fluorescence *in situ* hybridization findings in *in vitro* and *in vivo* culture.

showed insufficient morphological quality for transfer. No pregnancy was obtained.

Technical issues

Initially, a total of 19 patients were included in the present pilot study. However, only 13 patients were included in the comparative embryo quality trial because of technical problems in five cases. The first technical issue (with two devices in the first four patients) was related to the introduction of the IUCS into the transfer catheter. To solve this problem, the catheter was adapted and no more difficulties occurred later on. The second problem was linked to the intrauterine stabilization of the device, as 3 out of 19 were found in the vagina on Day 3. The stabilization system was modified in order to decrease the risk of IUCS migration. In one patient, all the oocytes cultured in the IUCS were arrested in the one-cell stage at retrieval on Day 3 and the zona pellucida had disappeared. The cause of this observation could not have been elucidated.

Flushing technique and tools were improved during this pilot study and consequently the number of embryos damaged by technical procedures decreased progressively (8 embryos damaged out of 24 loaded in the first six patients, and 1 damaged out of 80 loaded in the last seven patients). No sterility problems were observed for all of the embryos cultured *in utero*.

Discussion

Although in animal models better embryo development has been observed *in vivo* than *in vitro*, no comparative data in the human are available from the literature so far. The present work is the first report of an IUCS allowing communication between the embryos and the endometrium and supporting the formation of normal zygotes and good-quality embryos after microinjection, as well as normal chromosomal segregation. The capsule can be inserted transiently into the uterine cavity up to 3 days without bleeding and without evidence of clinical impact on endometrial receptivity. Three clinical pregnancies leading to healthy births have been achieved, two after partial *in vivo* and one after *in vitro* culture, but all three after the transient presence of a foreign object in the uterus.

During the last three decades, many researchers have tried to optimize the *in vitro* environment for the developing embryo, either by mimicking the *in vivo* composition of genital tract secretions—the 'back-to-nature' strategy (Menezo *et al.*, 1984; Gardner *et al.*, 1996; Leese *et al.*, 1998)—or by the 'let the embryo choose' strategy (Lawitts and Biggers, 1992; Biggers and McGinnis, 2001). In the 1990s, embryos were cultured in the presence of other cell types named co-culture. The most relevant systems were the use of heterologous human oviductal (Yeung *et al.*, 1992) or endometrial cells (Plachot *et al.*, 1994), and more recently autologous endometrium epithelial cells (Simon *et al.*, 1999). Finally, emerging technologies, such as microfluidic systems, have tried to provide a dynamic microenvironment, in contrast to the static microdrop culture technology (Beebe *et al.*, 2000; Glasgow *et al.*, 2001). All these strategies are dealing with similar remaining problems inherent to the *in vitro* system: an absence of the complexity of cross-talk between the embryo and surrounding tissues and fluid. In addition, physicochemical conditions are far from physiological conditions, considering exposure to environmental light outside the incubators, fluctuations in temperature and pH, and elevated oxygen levels.

Our novel IUCS was designed to circumvent many of the shortcomings inherent to the *in vitro* systems and appears to provide a microenvironment that supports equal or superior preimplantation embryo development, as suggested by the present data. The perforated silicone membrane might allow specific and complex elements in the uterine fluid to migrate and interact with the developing embryo, and perhaps protect the embryo against harmful reactive oxygen species and heavy metal toxicity. It should, however, be recognized that culture of the oocyte and placement of the early cleavage-stage embryo into the

uterus is a non-physiological condition. Nevertheless, the present data suggest that the uterine environment of the human meets the nutritional requirements for normal fertilization and embryo development, resulting in a higher proportion of euploid embryos compared with *in vitro* culture.

While initially some technical problems led to damage or loss of oocytes/embryos, these problems were largely solved during the course of the trial. With the numbers available, we observed a non-significant tendency to improved embryo quality in the *in vivo* arm of all three groups, even after short-term encapsulation for 18 h after ICSI. Although the number of oocytes/embryos in this pilot study was limited, a significant difference in the proportion of euploid embryos (chromosomes X, Y, 13, 18, 21) was observed in favour of the *in vivo* arm. These findings open again the discussion on *in vitro* culture conditions and their impact on epigenetic modifications, abnormal chromosome segregation and gene expression.

In conclusion, this new *in vivo* culture system, which allows communication between the embryo and the endometrium, appears to be feasible and safe, as regards fertilization and embryo development. The present pilot study further suggests superior embryo development and a higher proportion of euploid embryos after *in utero* culture than after conventional *in vitro* culture. It also demonstrates the possibility of obtaining pregnancies after transient presence of this device in the uterus. These interesting observations, however, need to be confirmed in a large RCT.

Author contribution

P.M. is the inventor of the IUCS concept (US patent No. 20042617994A1). N.B. and P.A. developed the IUC device at the Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland in collaboration with P.M., M.V. and P.L.G. from ANECOVA S.A., Geneva, Switzerland. M.J.L.S., M.F.-S. and C.S. initiated the study of the stability and safety of the intrauterine capsule at the Instituto Valenciano de Infertilidad, Valencia, Spain. Y.H., K.H., C.W. and H.N. performed the embryo toxicity study and the loading training using bovine embryos at the FLI, Neustadt, Germany, and INRA, Jouy en Josas, France. G.V., C.B. and P.D. performed the present clinical pilot study at the Centre for Reproductive Medicine at the UZ Brussel. P.H. provided useful statistical advice.

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Submitted on June 2, 2008; resubmitted on December 16, 2008; accepted on December 28, 2008

CERTIFICATE

Number: 2124763CE01

CE MARKING OF CONFORMITY
MEDICAL DEVICES

Issued to:

Anecova S.A.
EPFL PSE-D
1015 Lausanne
Switzerland

For the product category:

Anecova-d family devices and accessories for encapsulation of gametes and / or embryos in Assisted Reproductive Technology ART

DEKRA grants the right to use the EC Notified Body Identification Number illustrated below to accompany the CE Marking of Conformity on the products concerned conforming to the required Technical Documentation and meeting the provisions of the EC-Directive which apply to them:

0344

Documents, that form the basis of this certificate:

Certification Notice 2124763CN, initially dated 1 October 2009
Addendum, initially dated 1 October 2009

DEKRA hereby declares that the above mentioned manufacturer fulfils the relevant provisions of 'Besluit Medische Hulpmiddelen', the Dutch transposition of the Directive 93/42/EEC of June 14, 1993 concerning Medical devices, including all subsequent amendments, and that for the above mentioned product category the Conformity Assessment Procedure Annex II for class IIa products, is executed by the Manufacturer in accordance with the provisions of the Council Directive 93/42/EEC of June 14, 1993. For placing on the market of Class III devices an additional EC design examination certificate according to Annex II, section 4 is mandatory. The necessary information and the reference to the relevant documentation, of the products concerned and the assessments performed, are stated in the Certification Notice which forms an integrative part of this certificate.

This certificate is valid until: 1 October 2015
Issued for the first time: 1 October 2009
Reissued: 11 October 2012

DEKRA Certification B.V.

drs. G.J. Zoetbrood
Managing Director

ing. A.A.M. Laan
Certification Manager

All testing, inspection, auditing and certification activities of the former KEMA Quality are an integral part of the DEKRA Certification Group.

DEKRA Certification B.V. Utrechtseweg 310, 6812 AR Arnhem P.O. Box 5185, 6802 ED Arnhem, The Netherlands
T +31 26 356 2000 F +31 26 352 5800 www.dekra-certification.com Company registration 09085396

CERTIFICATE

Number: 2124763

The management system of:

Anecova S.A.

EPFL PSE-D
1015 Lausanne
Switzerland

including the implementation meets the requirements of the standard:

ISO 13485:2003
ISO 9001:2008

Scope:
Design, development, manufacturing and distribution of encapsulation technology based medical devices including assisted reproductive technology ART

Certificate expiry date: 1 October 2015
Certified for the first time: 1 October 2009
Certificate effective date: 11 October 2012

DEKRA Certification B.V.

drs. G.J. Zoetbrood
Managing Director

ing. A.A.M. Laan
Certification Manager

MGMT. SYS. RvA C 509

IAF

All testing, inspection, auditing and certification activities of the former KEMA Quality are an integral part of the DEKRA Certification Group.

DEKRA Certification B.V. Utrechtseweg 310, 6812 AR Arnhem P.O. Box 5185, 6802 ED Arnhem, The Netherlands
T +31 26 356 2000 F +31 26 352 5800 www.dekra-certification.com Company registration 09085396

ADDENDUM

Belonging to certificate: 2124763CE01

CE MARKING OF CONFORMITY
MEDICAL DEVICES

Anecova-d family devices and accessories for encapsulation of gametes and / or embryos in Assisted Reproductive Technology ART

Issued to:

Anecova S.A.
EPFL PSE-D
1015 Lausanne
Switzerland

This certificate covers the following product(s):

Anecova-d1
Anecova-d2
Anecova-d3
Anecova-d4
Anecova-d5

Accessories:
- Insertion kit
- Flushing needle
- Tweezers

Initial date: 1 October 2009
Revision date: 18 May 2012

DEKRA Certification B.V.

drs. G.J. Zoetbrood
Managing Director

ing. A.A.M. Laan
Certification Manager

All testing, inspection, auditing and certification activities of the former KEMA Quality are an integral part of the DEKRA Certification Group.

DEKRA Certification B.V. Utrechtseweg 310, 6812 AR Arnhem P.O. Box 5185, 6802 ED Arnhem, The Netherlands
T +31 26 356 2000 F +31 26 352 5800 www.dekra-certification.com Company registration 09085396

EUSKO JAURLARITZA GOBIERNO VASCO

OSASUN SAILA
Osasun Sailburuordetza
Plangintza, Antolamendu eta Ebaluazio Sanitarioko Zuzendaritza

DEPARTAMENTO DE SALUD
Viceconsejería de Salud
Dirección de Planificación, Ordenación y Evaluación Sanitaria

EUSKO JAURLARITZA GOBIERNO VASCO
OSASUN SAILA
DEPARTAMENTO DE SALUD

2015 API. ABR. 14

SARRERA	IRTEERA
Zk.	Zk. 134112

IVI
Paseo Landabarri, 1
48940 Leioa (Bizkaia)

Acusamos recibo de su notificación de intención de uso de un tipo de incubador que se introduce en el útero de la mujer para el desarrollo de embriones de la empresa ANECOVA.

Esta notificación se incorpora a su expediente, no precisando de nueva autorización dado que no supone modificación de la oferta asistencial.

Atentamente

Vitoria-Gasteiz, a 14 de abril de 2015

EUSKO JAURLARITZA GOBIERNO VASCO
OSASUN SAILA
DEPARTAMENTO DE SALUD

Fdo.: Jaime Muñiz Saitua
Jefe del Servicio de Ordenación y Acreditación Sanitaria

Donostia - San Sebastian, 1 – 01010 VITORIA-GASTEIZ
Tfno. 945 01 93 08 – Fax 945 01 92 80 - E-mail: secplan-san@ej-gv.es

GOBIERNO DE ESPAÑA · MINISTERIO DE SANIDAD, SERVICIOS SOCIALES E IGUALDAD · agencia española de medicamentos y productos sanitarios

Comercialización de productos sanitar

1.3.6 - PRODUCCION

ANECOVA_PMPS [Portal de Productos Sanitarios - Usuario externo] Christian Lowe [C1816944]

Desconectar

Nueva Comunicación
- Diagnóstico In Vitro
- Producto Sanitario Implantable Activo

Modificaciones

Buzones/Estados
- AEMPS
  - Nueva Com. Pdte de Registro
  - Nueva Com. Registrada
  - Modif Pdte de Registro
  - Modif registrada
  - Usuario pendientes
  - Borrador de Nueva Com.
  - Nueva Com. Incidencia
  - Modif incidencia
  - Traspasos
- Pdtes de confirmar
  - Actualizar documentación
- Usuario confirmadas
  - Anotadas
  - No anotadas
  - Dadas de baja
  - Suspendidas

Búsqueda de Comunicaciones

Desconexión

Tramitar Comunicación

Comunicaciones o modificaciones de comunicaciones en las que se ha incluído el mismo documento de pago de tasas

Un registro encontrado. [1]

Nro. Comunicación
PS/2013/0977

Datos Generales

Nro. Comunicación:	PS/2013/0977	**Fecha Comunicación:**	02/04/2013
Nro. Entrada en Registro:	21897/RG73036	**Fecha Entrada en Registro:**	02/04/2013
Ref. de Abono:	Orden de Transferencia 7913170275936	**Documento de pago de tasas:**	Abono_20130402.pdf

Datos específicos de la comunicación

En calidad de:	Fabricante	**Otro especificar:**	

Datos de la persona que efectúa la comunicación

Nombre:	Christian Lowe	**NIF/Pasaporte:**	C1816944
País nacionalidad:	Suiza	**Domicilio:**	Parc Scientific EPFL, PSE-D
Localidad:	Lausanne	**País:**	Suiza
CP:	1015	**Teléfono:**	+41216938449
Fax:	+41216938455	**E-mail:**	info@anecova.com

Datos de la empresa comunicante

Nombre Empresa:	ANECOVA, S.A.	**NIF/CIF:**	CHE110.586.6
Domicilio:	Parc Scientific EPFL, PSE-D	**Localidad:**	Lausanne
CP:	1015	**País:**	Suiza
Fax:	+41216938455	**Teléfono:**	+41216938449
		E-mail:	info@anecova.com

Datos del producto

Este producto ha sido comunicado con anterioridad a la fecha 15 de Marzo de 2010 a la AEMPS ☐

Nombre Comercial:	Anecova-d		
Nomenclatura:	**Clase Prod.:** Clase IIa		Se
Categoría Prod.:	10 - Productos de un solo uso	**Genérico Prod.:**	Otro
		Otro Especificar:	Dispositivo para la encapsulación de gametos y/o embriones
Tipo de producto:	Otro	**Otro especificar:**	Productos para almacenamiento prolongado de sustancias biológicas

Descripción breve: trata de un dispositivo para la encapsulación de gametos y/o embriones para la tecnología de reproducción asistida. Consta de una cápsula, un conector e hilo de extracción, catéter de inserción y dos agujas de lavado.

Finalidad Prevista:	Encapsulación de gametos y/o embriones para la tecnología de reproducción asistida.	**Fecha de comercialización y/o puesta en servicio en España:**	01/04/2013

Otros nombres comerciales

-

Nombre Productos Incluidos

Nombre
Anecova-d
Aguja de lavado
Pinzas
Kit de inserción

Documento de productos incluídos

Nom_Modelos_20130401.pdf

-

Documento de modelos

Agrupación

Datos del fabricante

Nombre Empresa:	ANECOVA, S.A.		
NIF/CIF:	CHE110.586.6	**Licencia:**	
Domicilio:	Parc Scientific EPFL, PSE-D	**Localidad:**	Lausanne
		País:	Suiza
CP:	1015	**Teléfono:**	+41216938449
Fax:	+41216938455	**E-mail:**	info@anecova.com

Datos de los Distribuidores en España

Nombre Empresa	Localidad	Domicilio	NIF/CIF	Teléfono	Fax	E-mail
ANECOVA, S.A.	Lausanne	Parc Scientific EPFL, PSE-D	CHE110.586.6	+41216938449	+41216938455	info@anecova.com

Datos de los procedimientos de Evaluación

Clase	Procedimiento	Número ON	Número Certificado	Fecha Caducidad	Documento	Producto
Clase IIa	Anexo II punto 3	0344	2124763CE01	01/10/2015	Certificado_20130401_044009.pdf	

Etiquetado e instrucciones de uso

	Etiquetado e Instrucciones con las que se comercializa en España	Etiquetado e Instrucciones presentados a/certificados por el Organismo Notificado
Han sido sometidas al Organismo Notificado quedando incluida la versión española		EtiquettesExternesBoite_Etiq_ON_20130401_044149.pdf
Han sido sometidas al Organismo Notificado quedando incluida la versión española		IFU_d5_V03_final_booklet_print_Inst_ON_20130401_044318.pdf

Observaciones de la Comunicación y Documentación Asociada

Observaciones:	**Documentación asociada:**

Volver **Dar De Baja** **Subsanar**

Para cualquier duda consulte el manual de usuario en la sección ayuda (parte superior izquierda, con el símbolo ?)

Parque Empresarial "Las Mercedes", Edif 8, C/ Campezo 1 - 28022 MADRID Email: pmps@aemps.es
pmps_incidencias@aemps.es

Subject: SV: SV: Sagen er sendt videre
From: Copenhagen Fertility Center <info@copenhagenfertilitycenter.com>
Date: 09/01/15 14:09
To: [redacted]

Dear [redacted]

Thank You for this information. I will be happy for an answer next week.

Med venlig hilsen, best regards

COPENHAGEN FERTILITY CENTER

Lygten 2C, 4th Floor
DK-2400 Denmark
Phone: +4533257000
Fax: +4533257005
E-mail: info@copenhagenfertilitycenter.com

ISIVF COPENHAGEN DENMARK 27 - 30 september 2015 ISIVF 2015 18th WORLDCONGRESS OF IN VITRO FERTILIZATION

Fra: [redacted]
Sendt: 9. januar 2015 10:00
Til: Copenhagen Fertility Center
Cc: [redacted]
Emne: SV: SV: Sagen er sendt videre
Prioritet: Høj

Kære [redacted],

Tak for dine mails og beklager den lange svartid, der delvis skyldes misforståelser og sygdom.

Da vi talte sammen lovede jeg at jeg ville sørge for at, din efterfølgende mail, ville blive sendt videre til den der skulle håndtere sagen. Det gjorde jeg også. Min kollega Bo Kjellmann Bruun, det ville varetage sagen for afdelingen oplyser at han efterfølgende har vendt det med Michael Cox. De blev enige om at Michael overtog og oprettede sagen. Jeg sende derfor din mail videre til Michael Cox.

Med venlig hilsen/ kind regards,

[redacted]
Akademisk sagsbehandler
Scientific Officer
T (dir.) +45 44 88 97 52
[redacted]

Sundhedsstyrelsen
Enheden for lægemiddelovervågning og medicinsk udstyr
Danish Health and Medicines Authority
Pharmacovigilance and Medical Devices
T +45 72 22 74 00
sst@sst.dk

Sundhedsstyrelsen
Danish Health and Medicines Authority

Fra: Copenhagen Fertility Center [mailto:info@copenhagenfertilitycenter.com]
Sendt: 23. december 2014 12:17
Til: [redacted]
Emne: SV:

Til

[redacted]

Jeg tillader mig at bringe nedenstående korrespondance til din opmærksomhed igen, da vi ikke har modtaget noget svar.

Med venlig hilsen

[redacted]
Copenhagen Fertility Center

Fra: Copenhagen Fertility Center

Sendt: 3. december 2014 13:58
Til: [redacted]
Cc: [redacted]
Emne:

[redacted]

Akademisk medarbejder

Sundhedsstyrelsen

Re: vores telefonsamtale i sidste uge vedrørende behandling med Anacova dyrkningskammer i uterus ved IVF behandling.

E-mail: [redacted]

Baggrund:

Vi ønsker at behandle patienter i IVF behandling med ovenstående medicinske utensilie, der er CE mærket ,og som jeg opfatter det (se e-mail fra M. Cox hos jer, vedlagt), er det ikke et problem at anvende jf. EU-vævsdirektivet.

Behandlingen er ikke en ny ide, og man opnår med denne behandlingsform at komme tættere til det naturlige miljø i uterus, ligesom denne teknologi reducerer det menneskelige æg og sædcellers påvirkning af dyrkningsmedier og andre forhold i laboratoriet. Man må sige, vi forsøger at gå til bage til de naturlige steder for ægget og sædcellerne.

Vore spørgsmål er således, om dette er lovligt for vores klinik at tilbyde denne form for behandling af patienterne i IVF behandling.

Jeg har nedenfor vedhæftet

1. Michael Cox svar fra Sundhedsstyrelsen , se vedlagte bilag 1.
2. CE-certifikat med tilbehør, se attachment med samme navn
3. Note for competent authorities, se attachment med sammen navn

Med venlig hilsen

[redacted]

Professor dr. med.

Copenhagen Fertility Center

Bilag 1.

De: [redacted]

Objet: Rép : IVF Medical Device - Encapsulation of gametes/embryos in ART - Medidee/Switzerland.

Date: 13 novembre 2014 12:44:48 UTC+1

À: '[redacted]

Cc: [redacted]

Dear Sir,

Thank you very much for your answer. As the discussed device is indeed CE marked according to EC 93/42 for an intended use similar to established Assisted Reproductive Technologies, I understand there is no futher obligation for the healthcare professional and they may proceed with the provided device.

I wish you a good day,

Best regards,

[redacted]

[redacted]

Senior Partner

Le Communeau 43 - 1474 Châbles

Switzerland - +41 79 214 24 09

www.medidee.com - [redacted]

Le 13 nov. 2014 à 12:08, [redacted] a écrit :

Dear [redacted]

There are a range of materials/reagents (e.g. media kits/processing materials) which are used in the preparation/processing or storage of reproductive cells/tissues. In the regulatory framework of human tissues/cells for therapeutic application any critical materials and reagents (e.g. used in a fertility clinic) shall meet documented requirements and specifications, and where applicable the requirements of Directive 93/42/EEC. Further text can be found in Directive 2006/86/EC, Annex IC. Where the intended use of the healthcare product (i.e. the reagent) is similar to established technologies of the ART sector there is no further need for limitations or obligations by healthcare personnel.

Venlig hilsen/ Best Regards

[redacted]

Lægemiddelinspektør, Inspektion.

T (dir) +45 4488 9632, T (m) +45 4124 9823

[redacted]

Fra: [redacted]
Sendt: 6. november 2014 14:27
Til: [redacted]

Emne: BBR Question on IVF Medical Device

Dear Sir,

Following our call of today, please find hereafter my inquiry:

I'm currently evaluating the conditions for the placement of an IVF/ART Medical Device on the Danish market. The intened use of the device is *Encapsulation of gametes and/or embryos in Assisted Reproductive Technology ART*. The device is a class IIa medical device and it is CE Marked.

According to the Directive EC 93/42, this device is allowed to be placed on the Danish market with no specific restriction. According to your website, I understand that national requirements have to be taken into accounts which are proper use of danish language in the IFU.

I also understand that Medically Assisted Reproductive technologies are also regulated under specific national laws (Lov om krav til kvalitet og sikkerhed ved håndtering of humane væv of celler). Theses laws may cover the requirements of EC 2004/23 where specific provisions are defined to ensure high quality and safety standards for the use of blood, organs and other substances of human origin.

With regards to the use of this device, can you please inform me if bearing the CE Mark is sufficient to allow the health professionals (clinics, embryologists, gynecologists, etc.) to use the device in their procedure or the procedure in itself has to be registered or announced or approved any any way by your authority?

As this device is CE Marked, it already address the essential requirements of the EC 93/42 but I would like to ensure that there are no specific limitations, restrictions or obligations for the healthcare professionals related to use the device.

Thank you in advance for your kind response

Best regards,

[redacted]

[redacted]

Senior Partner

Le Communeau 43 - 1474 Châbles

Switzerland - +41 79 214 24 09

www.medidee.com

Subject: IVF - Anecova udstyr - dyrkning af ægceller i klinikken.
From: [redacted]
Date: 14/01/15 18:16
To: Copenhagen Fertility Center <info@copenhagenfertilitycenter.com>, [redacted]

Dear [redacted]

Thank you for your e-mail dated 3rd December 2014, which has latterly been referred to Tissue & Cell colleagues for a response.

As you are aware the Anecova device is a CE marked product, which is part of a system (i.e. an in-vivo culture process) in a fully receptive endometrium for the optimized developmental competency of embryos. Supplementary technical information has also been considered in the response below.

We have duly considered the national guidance on “Assisteret Reproduktion, Sundhedsstyrelsen 2012” and take the initial view the use of the Anecova device should not be regarded as a new treatment methodology, as similar techniques in the ART sector have been published earlier (1).

However, as a departure from my earlier response (dated 13th November 2014), it should be noted there are several parts of the process, for example egg retrieval, fertilization, in-vivo culture, morphological quality assessment, cryopreservation, (2), which fall within the scope of the Tissue Establishment Directives: in particular Part B, Annex II of the sister Directive 2006/86/EC (Bekendtgørelse 984; Bilag 2B). A tissue establishment (i.e. fertility clinic) is required to document and suitably validate the different stages of the process. It has also been noted the cited publication is a pilot study (3) and other comprehensive clinical data, as well as routine monitoring of clinical outcomes, is beneficial.

As you are aware tissue establishments in Denmark are subject to periodic site inspections, and the review of technical processes, as an integral part of the inspection practice, and linked to the site authorisation certificate for the tissue establishment.

We hope this information is useful.

Venlig hilsen/ Best Regards

[redacted]
Lægemiddelinspektør, Inspektion.
T (dir) +45 4488 9632, T (m) +45 4124 9823
[redacted]

(1) Ariff Bongso et al, Fertilization and early embryology: Isolationa and culture of inner cell mass cells from human blastocysts, Oxford journals Human Reproduction, Vol 9, Issue 11, p. 2110-2117.
(2) Note for Competent Authorities (Page 4).
(3) An in-vitro culture system for human embryos using an encapsulation technology: a pilot study. Blockeel C et al, Human Reproduction, Vol 24, N0. 4, p. 790 - 796, 2009.

Subject: SV: IVF - Anecova udstyr - dyrkning af ægceller i klinikken.
From: Copenhagen Fertility Center <info@copenhagenfertilitycenter.com>
Date: 15/01/15 09:13
To: [redacted]
CC: [redacted]

Dear [redacted]

Thank You for this information. It is very helpful.

I understand the answers as follows:

1) Yes we can start treatment using this device in our Fertility Clinic.
2) The preliminary evaluation in our clinic in the clinical trial with Anacova, which has been evaluated during the clinical study is sufficient to start the treatment.
3) Our GLP/GCP/ISO standard and accreditation i.a. EU Tissue directive for implementing a treatment is in place according to the EU tissue directive.

Yours sincerely

[redacted]
Professor dr. med.

Copenhagen Fertility Center

-----Oprindelig meddelelse-----
Fra: [redacted]
Sendt: 14. januar 2015 18:16
Til: Copenhagen Fertility Center; [redacted]
Cc: [redacted]; [redacted]
Emne: IVF - Anecova udstyr - dyrkning af ægceller i klinikken.

Dear [redacted],

Thank you for your e-mail dated 3rd December 2014, which has latterly been referred to Tissue & Cell colleagues for a response.

As you are aware the Anecova device is a CE marked product, which is part of a system (i.e. an in-vivo culture process) in a fully receptive endometrium for the optimized developmental competency of embryos. Supplementary technical information has also been considered in the response below.

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However, as a departure from my earlier response (dated 13th November 2014), it should

be noted there are several parts of the process, for example egg retrieval, fertilization, in-vivo culture, morphological quality assessment, cryopreservation, (2), which fall within the scope of the Tissue Establishment Directives: in particular Part B, Annex II of the sister Directive 2006/86/EC (Bekendtgørelse 984; Bilag 2B). A tissue establishment (i.e. fertility clinic) is required to document and suitably validate the different stages of the process. It has also been noted the cited publication is a pilot study (3) and other comprehensive clinical data, as well as routine monitoring of clinical outcomes, is beneficial.

As you are aware tissue establishments in Denmark are subject to periodic site inspections, and the review of technical processes, as an integral part of the inspection practice, and linked to the site authorisation certificate for the tissue establishment.

We hope this information is useful.

Venlig hilsen/ Best Regards

[redacted]
Lægemiddelinspektør, Inspektion.
T (dir) +45 4488 9632, T (m) +45 4124 9823 [redacted]

(1) Ariff Bongso et al, Fertilization and early embryology: Isolationa and culture of inner cell mass cells from human blastocysts, Oxford journals Human Reproduction, Vol 9, Issue 11, p. 2110-2117.
(2) Note for Competent Authorities (Page 4).
(3) An in-vitro culture system for human embryos using an encapsulation technology: a pilot study. Blockeel C et al, Human Reproduction, Vol 24, N0. 4, p. 790 - 796, 2009.

Subject: IVF - Anecova udstyr - dyrkning af ægceller in-vivo.
From: "[redacted]
Date: 22/01/15 11:02
To: 'Copenhagen Fertility Center' <info@copenhagenfertilitycenter.com>
CC: [redacted]

Dear [redacted],

Thank you for the supplementary queries (in email dated 15th January 2015) related to the use of the Anecova medical device in a fertility clinic setting. For convenience we have copied your queries, and set in our response, in the text below.

1) Yes we can start treatment using this device in our Fertility Clinic.

In this regard, for your consideration, is the summary of the published article (1) in which they conclude " ..... this new in vivo culture system, which allows communication between the embryo and the endometrium, appears to be feasible and safe, as regards fertilization and embryo development. The present pilot study further suggests superior embryo development and a higher proportion of euploid embryos after in utero culture than after conventional in vitro culture. It also demonstrates the possibility of obtaining pregnancies after transient presence of this device in the uterus .....". The evaluation, suitability and final choice of any specific treatment is the responsibility of the fertility clinic and does not fall within the remit of our office (for Tissues/Cells).

2) The preliminary evaluation in our clinic in the clinical trial with Anecova, which has been evaluated during the clinical study is sufficient to start the treatment.

The quality, safety and performance standards of this medical device are indicated by the CE mark, via the conformity assessment procedures in the Medical Devices Directive (93/42/EC), where thereafter it has been placed on the market for use. In addition, the pilot study, in the published article (1), indicates proof of concept and some of the conclusions are summarized (in point 1 of this email) for information. The routine use of any specific treatment is the responsibility of the fertility clinic, and does not fall within the remit of our office (for Tissues/Cells).

3) Our GLP/GCP/ISO standard and accreditation i.e. EU Tissue directive for implementing a treatment is in place according to the EU tissue directive.

As stated in our earlier e-mail there are several parts of the process, for example egg retrieval, fertilization, in-vivo culture, morphological quality assessment, cryopreservation, in the scope of the Tissue Establishment Directives: in particular Part B, Annex II of the sister Directive 2006/86/EC (Bekendtgørelse 984; Bilag 2B). A tissue establishment (i.e. the fertility clinic) shall have documented and suitably validated the different stages of the process in the quality system, which is then subject to periodical review during our inspection programme. We recognize and support the principles of other relevant standards (e.g. GCP, GLP, ISO 9001 Quality Management Systems) which are not a direct part of our responsibilities in the Tissues & Cells Directives. With regard to the latter, we are able to confirm the Copenhagen Fertility Centre is the current holder of a valid license related to the national transpositions of the Tissue Establishment Directives.

If you should wish to discuss further by telephone you are welcome to contact us. We hope the supplementary information is useful.

(1) An in-vitro culture system for human embryos using an encapsulation technology: a pilot study. Blockeel C et al, Human Reproduction, Vol 24, N0. 4, p. 790 – 796, 2009.

Venlig hilsen/ Best Regards

[redacted]
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T (dir) +45 4488 9632, T (m) +45 4124 9823
[redacted]

ANNEX E – Additional safety information

Anecova Answers to HFEA request from July 8th 2015

HFEA:
As a side point the Committee highlighted that any centre using the Anecova AneVivo intrauterine device, or similar, should take into account the fact the device allows for transport of gametes/embryos. They also expressed a desire to see any patient information which might be provide and were interested to know whether any studies on patient experience have been conducted, though recognised that this information was beyond the remit of their considerations.

Anecova:
We are currently starting commercialization in a progressive controlled market release in selected Fertility centres in countries where authorisations from Competent authorities have been obtained (Czech Republic, Spain, Denmark). To date, the first healthy pregnancies are being reported in clinical use in Spain.

Other Fertility centres are in the pipeline to start commercialization. The Fertility centres are currently in the process of Product/ Procedure validation by their local Competent Authorities in (UK, Finland, Hungary).

HFEA:
On the day, the Committee did not feel they had been provide with sufficient evidence to make an assessment of the safety of the intrauterine culture of gametes and embryos in a device such as the Anecova AneVivo intrauterine device and asked for additional evidence to be provided. In particular the Committee felt that information regarding the effect of all the components of the device on embryos, perhaps in an animal system (e.g. a mouse embryo assay), would be useful to aid their decision to determine the impact of the device on embryo safety. Subsequently, the Committee has been made aware that that the CE mark that the device has been given is that of a Class IIa device and that this classification of medical device would require conformity assessment by a notified body, which includes the requirement for a clinical evaluation conducted in accordance with Annex X to Directive 93/42/EEC or with Annex 7 to Directive 90/385/EEC. It's a slightly tricky situation. While the purpose of the Committee is to assess the safety and efficacy of the process and not the device (which has already had its safety assessed in the CE marking process), in this case the members feel that the two things are so inextricably linked that they cannot make an assessment of one and not the other. Do you think you would be able to get any information from AneCova on the effect of the device on embryos?

Anecova:
Please find below an extract from the CE mark Tech file for the AneVivo medical device (Class IIa). Please note that the tech File and all other applicable documents are assessed and validated regularly by our Notified Body (DEKRA).
This extract contains:

- **Bovine Embryo Assay**: Bovine Embryo Toxicity Testing on AneVivo device residence from fertilisation to blastocyst stage.

- **ISO 10993-5 and ISO 10993-10 tests of raw materials**.

In addition you will find the results of our **Routine Mouse Embryos Assay / embryo toxicity test** from fertilisation to blastocyst stage.

Bovine Embryo Assay:

Early bovine embryo development and bovine oocyte fertilization were chosen for the compatibility trials. The bovine model was chosen for the high sensitivity of bovine zygotes
Experiments on Bovine model with the **AneVivo** device were performed at the following institutions:
Mariensee (FAL – Institut für Tierzucht – Germany); monitored by Dr. C. Wrenzycki. Jouy-en-Josas (INRA – France); monitored by Dr. Y. Heyman.
The bovine model was chosen for the high sensitivity of bovine zygotes to the culture environment.

For the early embryo development, 315 zygotes (5 distinct experiments) were divided in 3 groups.
Group 1, the control group, (n=125 zygotes) zygotes were cultivated in 400 µl of SOF culture medium for 7 days, 5 zygotes per dish. The embryos were analyzed at the seventh day.
Group 2 (n=99 zygotes), the same protocol was applied with the addition in each dish of one **AneVivo** device.
Group 3 (n=91 zygotes) zygotes were loaded inside the **AneVivo** device (5 oocytes per capsule) and then cultivated with the same culture conditions.
The seventh day the embryos were flushed out of the device and were analyzed.
For the fertilization, n=99 partially denuded oocytes (3 distinct experiments) were divided in 2 groups. For both groups, the oocytes were placed in fertilization medium (Fert-Talp plus HHE) with the addition of capacitated spermatozoids (5 oocytes and 10000 spermatozoids per dish). Fertilization was assessed after 24 hours. In the group two 2, an **AneVivo** device was added in each dish.

Early development results.
After seven days in culture, the number of healthy embryos was similar for the 3 groups (Table 1). The ratios of healthy embryos as well as the speed of development were not affected by the presence of the **AneVivo** device in the culture dish. The same observation was made for the embryos loaded in the **AneVivo** device.

Fertilization results.
No fertilization rate differences were observed between the control group and the group including the **AneVivo** device in the dish (Table 2).

Interpretation.
These results demonstrated not only the non-toxicity of the material composing the **AneVivo** device, but also the device biological neutrality towards the embryos and the oocyte fertilization.

Table 1, Embryo development results

	Average [%]	SD
Blastocyst & morula at day 7		
Inside **AneVivo** device	26	7
AneVivo device in the medium	27	15
Control	31	15

Table 2, Oocyte fertilization results

	% of fertilization	SD
AneVivo device in the medium	42	22
Control	57	21

This test provided evidence that components and materials used in the device are not toxic for bovine embryos.
This test also provided evidence that capsulated bovine embryos can be fed by culture medium through capsule wall porosity.
This test also provided evidence on the capability of the device to be safely and quantitatively loaded and unloaded with bovine embryos that have an equivalent size to human embryos at the same development stage.

ISO 10993-5 and ISO 10993-10 tests of raw materials

Raw materials and materials tested:
Connector retrieval string, capsule B (protective silicone), inner polycarbonate micro-porous membrane, distal cap (Ti), insertion kit (PEHD)

Conclusion

Cytotoxicity test, Intracutaneous injection tests and Kligman maximisation test meet the requirement of ISO guidelines.

Implanted material summary

<u>Inserted part (from 1 to 5 days)</u>:

- **Titanium grad 2**
- **Stainless steel**
- **Human grad silicone**
- **Polycarbonate vessel**

<u>Insertion material (from 1 to 10 minutes)</u>:

- **Polyethylene high density**

Silicone Human Grad 6	
Compatibility with relevant substances	N/A
Compatibility with tissues or body fluids	USP class 6 certified
Whether characteristics relevant to safety are known	The materials of the device were extensively experimented in embryo culture on bovine

	and mouse models and were found safe.
Polycarbonate (vessel)	
Compatibility with relevant substances	N/A
Compatibility with tissues or body fluids	USP class 6 certified
Whether characteristics relevant to safety are known	The materials of the device were extensively experimented in embryo culture on mouse models and were found safe.
Stainless steel	
Compatibility with relevant substances	N/A
Compatibility with tissues or body fluids	316 LVM – Class 6 - Certified
Whether characteristics relevant to safety are known	The materials of the device were extensively experimented in embryo culture on bovine and mouse models and were found safe.
Titanium Grad 2	
Compatibility with relevant substances	N/A
Compatibility with tissues or body fluids	USP – Class 6 - Certified
Whether characteristics relevant to safety are known	The materials of the device were extensively experimented in embryo culture on bovine and mouse models and were found safe.
Polyethylene High density	
Compatibility with relevant substances	N/A
Compatibility with tissues or body fluids	USP – Class 6 - Certified
Whether characteristics relevant to safety are known	The materials of the device were extensively experimented in embryo culture on mouse models and were found safe.
Silicone Adhesive	
Compatibility with relevant substances	N/A
Compatibility with tissues or body fluids	USP – Class 6 - Certified
Whether characteristics relevant to safety are known	Cytotoxicity testing
Retrieval String: Polyamide monofilament	
Compatibility with relevant substances	N/A
Compatibility with tissues or body fluids	CE mark
Whether characteristics relevant to safety are known	USP an EU conform

A routine Mouse Embryo Assay is performed on every batch, before releasing polycarbonate vessel and silicone.

Routine Mouse Embryo Assay / embryo toxicity test

Although all materials used for the manufacture of our device are human grade or USP class 6 certified, it is essential to ensure their non-toxicity and their biological neutrality vis-à-vis embryos viability and development.
Every new material, new raw material lot and sample from produced device batch is tested using *in vitro* mouse embryo assays.

Anecova routine procedure for mouse embryo toxicity assays:
Tested groups

- Control well: *in vitro* culture of mouse zygotes in 400µl of medium
- Control droplet: *in vitro* culture of mouse zygotes in droplet under oil
- Testing group: *in vitro* culture of mouse zygote in 400µl of medium with the presence of the tested material or loaded in device for production batch testing

Measures (when embryo are assessable on a daily basis: not loaded in a device)

- Proportion of fertilized oocytes on day 1
- Proportion of degenerated embryos reported every day until day 5
- Number of cells per embryo reported every day until morula stage
- Proportion of morula reported every day since day 4
- Proportion of blastocysts reported every day since day 5

Measures (when embryo are not assessable on a daily basis: loaded in a device)

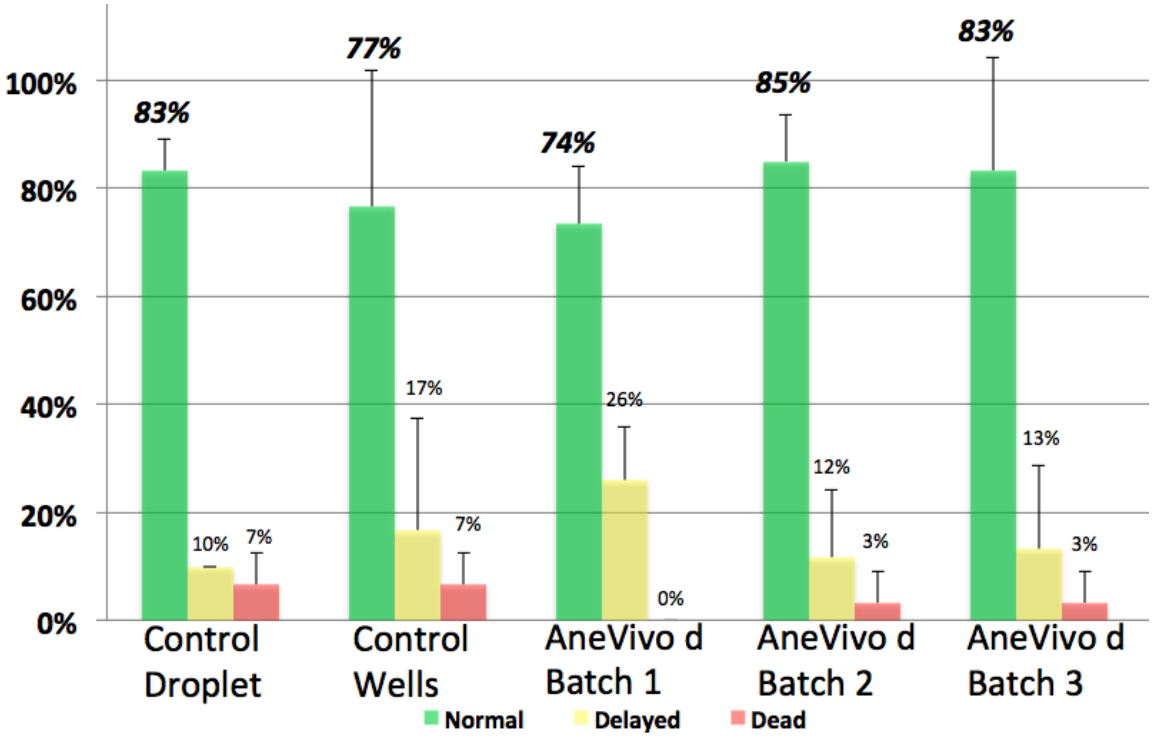
- Proportion of blastocysts reaching day 5 (**normal**)
- Proportion of **delayed** development or arrested embryos (morula, cleavage stage embryo) at day 5
- Proportion of **dead** embryos at day 5

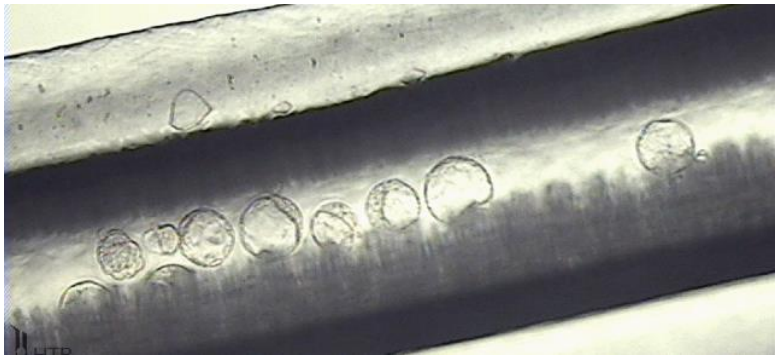
Number of embryos

- At least 3 test replicates per group
- At least 10 zygotes for the control group
- At least 10 zygotes for the testing group

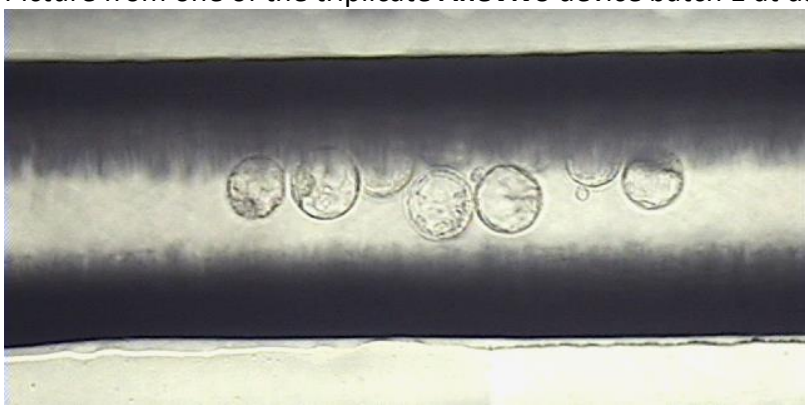
Example of mouse embryo assay performed on 3 different batch of AneVivo device
(random assay picked from our routine analysis)**.**
A grand total of 150 mouse embryos were tested (in triplicate) for a 5-day culture with the Anecova routine embryo toxicity conditions (10 oocytes per conditions):

- Control well: *in vitro* culture of mouse zygotes in 400µl of medium
- Control droplet: *in vitro* culture of mouse zygotes in droplet under oil
- Testing group: *in vitro* culture of mouse zygote loaded in device for production batch testing in 400µl of medium for each production batch (**AneVivo d**evice batch 1, **AneVivo d**evice batch 2 and **AneVivo d**evice batch 3).





Picture from one of the triplicate **AneVivo** device batch 1 at day 5



Picture from one of the triplicate **AneVivo** device batch 3 at day 5

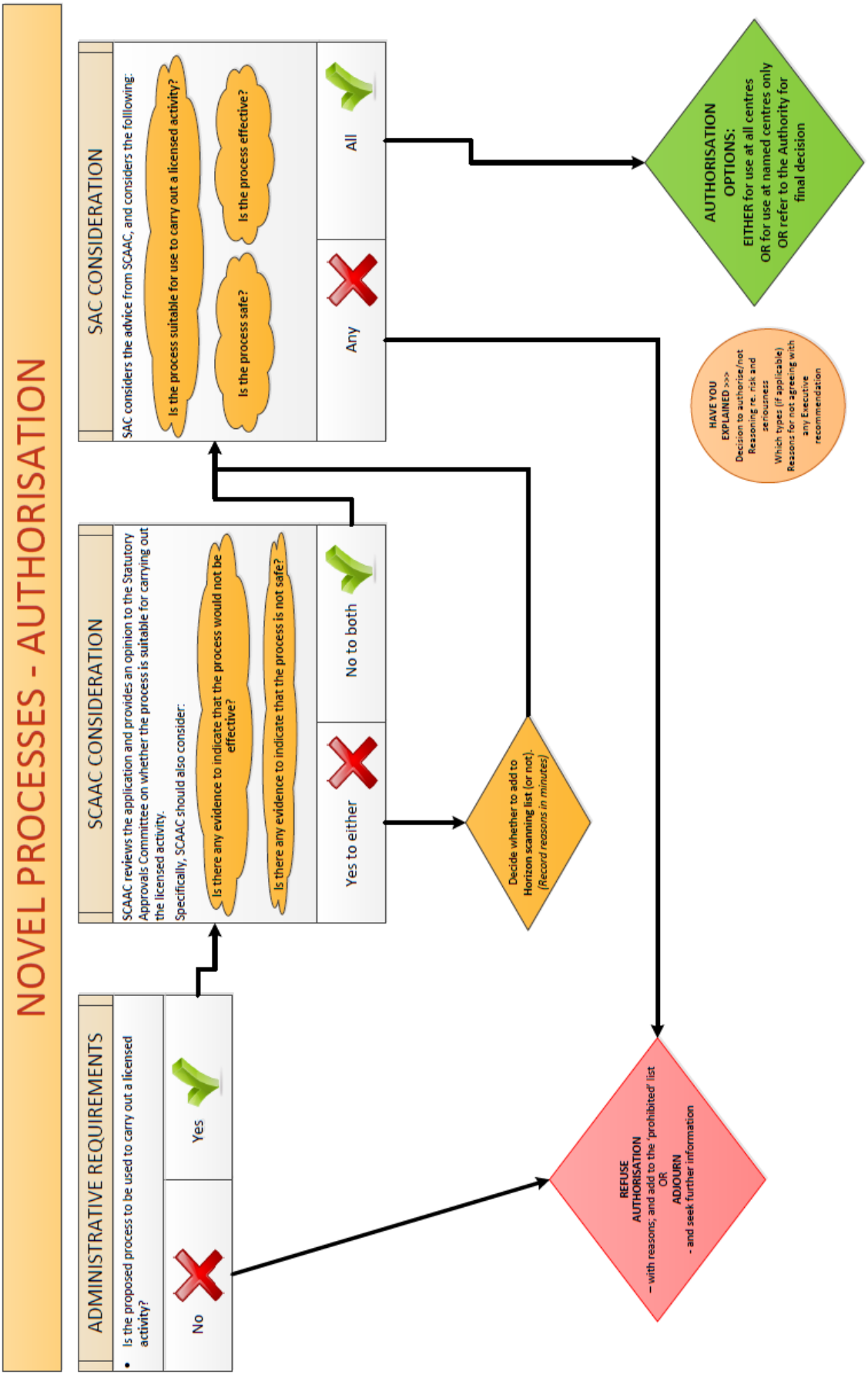
Conclusion

For this test no significant differences have been found for embryos developed in the **AneVivo** device (batch 1, batch 2, and batch 3) when compared to control groups. It has been concluded that no negative impact on the development of mouse embryos have been observed from these samplings representing 3 different **AneVivo** device batch.

ANNEX F – Retrieval success rate data

Embryos recovered after in vivo residence	Embryos not found after in vivo residence or lost after mismanipulation during procedure execution	1 PN	2 PN	≥ 3 PN	Unfertilized	Degenerated
99.15%	0.85%	0%	72.50%	10%	15%	2.50%
percentage calculated with 118 embryos from all procedures		percentage calculated with 45 embryos from short in vivo residence procedures (18 hours)				
Anecova - Strictly confidential						

ANNEX G – Decision tree for authorization of a novel process



Novel process authorisation – Intrauterine culture of gametes/embryos (Avecova AneVivo device)
25