Live births after bioengineering-assisted in utero embryo culture

Mock P[1-3]

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Abstract

With the advancement of in vitro culture systems and by mimicking the embryo's physiological environment, we are now able to support proper preimplantation embryo development. However, regardless of how closely we try to mimic the environment of the mother's uterus, we are still working with artificial conditions far from the in vivo complexity which are often linked to the failure of ART cycles. This study demonstrates that a transitory and more natural in vivo culture environment supported by the in utero culture system (IUCS) device is possible despite its location position which is identical to the contraceptive intrauterine device (IUD) without a negative impact on implantation rate and may positively impact embryo preimplantation development and lead to healthy pregnancies. Specifically, a total of 46 MII oocytes after insemination via intracytoplasmic sperm injection (ICSI) were loaded on the IUCS device and transferred to the uterus to allow a more "natural" development. The IUCS device was then retrieved from the uterus to select the most promising embryos for single or double embryo transfer (sET - dET, respectively). Following the overnight in utero incubation, n=23 fertilized zygotes and n=3 early cleavage-stage embryos were retrieved, for a total fertilization rate of 55% and recovery rate of 80%. Moreover, the total time of in vitro culture was significantly reduced to 1.5-2 hours before performing definitive uterine transfer. The use of the IUCS device led to two pregnancies with normal obstetrical outcomes, which showed good health after a 12-year follow-up. Overall, this work shows that the use of the IUCS device allows for a significant reduction in the time of in vitro culture period that enabled in utero fertilization and early development resulting in development of viable and good quality embryos that following transfer culminated in normal live births.

Disclaimer: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
J Reprod Biotechnol Fertil 13:10-18
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Compliance acknowledgement: This article was edited by the Australian Editorial Services (www.nativeenglisheditor.com)
Keywords: Culture, device, embryo, fertilization, implantation, in utero culture
Notification: The statements and claims in this manuscript are that of the author alone.

Introduction

Over the last three decades, numerous efforts have been made in the assisted reproductive technology (ART) field, which led to a significant improvement in vitro embryo culture conditions (Menezo et al., 2022; Lawitts and Biggers, 1992; Ménézo and Elder, 2020). The very first days of preimplantation development for both gametes and embryos still occur in the artificial environment of the in vitro culture system (Wrenzycki, 2018; Sciorio and Rinaudo, 2023). It is well known that the quality of embryos obtained from in vivo conception is significantly higher compared to those generated in vitro in terms of gene expression, cryotolerance, and metabolism (Wrenzycki et al., 2005; Duranthon et al., 2008; Lonergan et al., 2006).

Accordingly, suboptimal in vitro culture conditions result in cell senescence and activation of the apoptotic mechanism in bovine embryos (Ramos-Ibeas et al., 2020). Moreover, such conditions likely play a crucial role in increasing epigenetic alterations and chromosomal mosaicism in human embryos during in-vitro fertilization (IVF) (Munné et al., 1995). Salilew-Wondim et al (2015),

demonstrated that suboptimal culture microenvironment during preimplantation development led to changes in the DNA methylation landscape, which appeared to be linked to the in vitro culture time duration. Embryo mosaicism is particularly common in preimplantation human blastocysts produced by ART procedures. This phenomenon is primarily caused by mitotic errors during cell division, which then leads to chromosomal segregation anomalies in human embryos (Regin et al., 2022). Moreover, shortly after fertilization, important epigenetic remodeling processes occur, such as global DNA demethylation, chromatin organization, genome spatial reorganization, and substantial transcriptional changes. This event, referred to as "maternal-to-zygotic transition - MZT or embryonic genome activation" (Eckersley-Maslin et al., 2018) is an essential stage during embryo development and the failure of proper MZT is reported as one of the main causes of unsuccessful ART procedures (Tesarik, 2022).

In addition, oxidative stress related to gamete and embryo handling during in vitro culture conditions can also extensively contribute to embryo mosaicism (Al-Saleh et al., 2021). Therefore, despite best efforts to closely mimic the optimal and natural in vivo conditions for embryo development, we are still far from the ideal environment where embryos can grow. Currently, we are still unable to reproduce the physiological and fundamental in vivo embryo-endometrium cross-talk, supported by embryo-secreted extracellular vesicles (EVs) (Idelevich and Vilella, 2020). EVs are paramount in intercellular communication (Godakumara et al., 2022; Gonzalez Fernandez et al., 2023), along with the endometrium itself, as they induce the expression of genes involved in embryo implantation (Vilella et al., 2021) and maintenance of embryo ATP production (Recuero et al., 2020).

Nowadays, several bioengineering studies are focused on reproductive medicine and fertility-restoring strategies (Francés-Herrero et al., 2022). Specifically, Ferraz et al. demonstrated that by recreating a more natural microenvironment, using an artificial oviduct-on-a-chip model, it is possible to obtain zygotes that more closely resemble in vivo ones compared to conventional in vitro produced zygotes in terms of global DNA methylation levels and transcriptome (Ferraz et al., 2018).

Subjects and methods

Patient selection

This was a longitudinal, non-randomized, pilot study carried out at the InVivo-BabyImpulse IVF Clinic at the Clinique des Grangettes, Hirslanden Hospital in Switzerland. All patients participating provided their written informed consent. Overall, 6 patients (mean age ± SD: 33±3.4 years), were included in the study and a total of 46 metaphase two (MII) oocytes were obtained and loaded into the IUCS, following insemination via ICSI. The IUCSs were then transferred inside the uterus for an overnight in utero culture before withdrawal. Overall, a total in vitro culture time duration of 1.5-2 hours before definitive uterine transfer of the selected zygote/early cleavage stage embryo. The IUCS devices enabled fertilization and early embryo development in the natural environment of the woman's uterus, with ideal temperature, oxygen tension, and as well as bidirectional exchange of a wide variety of molecules present in the uterus. The device was transferred inside the uterine cavity using an embryo transfer catheter.

Ovarian stimulation protocol

Highly purified menopausal gonadotropin (hMG FSH/Menopur, Ferring Pharmaceuticals) and gonadotropin-releasing hormone (GnRH)-antagonist Ganirelix (Orgalutran®, Organon) were used for ovarian stimulation (Blockeel et al., 2009)22. In brief, a low-dose, monophasic-combined oral contraceptive pill (OCP) containing 150µg of desogestrel and 30µg of ethinylestradiol (Microgynon®) was administered for 2 weeks starting on Day 1 of the pre-ART cycle. Recombinant follicle-stimulating hormone (rFSH) at 200 IU per day was administered 5 days after discontinuing the OCP. Ganirelix was initiated at a daily dose of 0.25mg on Day 6 of the rFSH stimulation. LH surge and ovulation were induced by administering 10.000 IU of hCG (Ovitrelle 250µg) when at least three follicles ≥17mm diameter were detected by ultrasound. Oocyte retrieval was carried out 36 hours after Ovitrelle injection using a vaginal ultrasound-guided puncture of ovarian follicles.

Intracytoplasmic sperm injection and assisted in utero culture system

After ultrasound-guided oocyte retrieval, cumulus-oocyte complexes (COCs) were denuded from the surrounding cumulus cells. Only competent MII oocytes were inseminated with motile sperm cells by ICSI. Shortly after ICSI, injected oocytes were loaded into the IUCS and then transferred into the uterus for overnight culture. Around 18-21 hours post in utero transfer, patients returned to the clinic for the retrieval of the IUCS device. Viable zygotes and early cleavage-stage embryos were obtained. Definitive uterine transfer was then performed following the zygote/embryo. A rise in serum human Chorionic Gonadotropin (hCG) levels, suggesting biochemical pregnancy (BP) occurred in two out of six patients (33% of biochemical pregnancy rate - BPR). Around 7 weeks after, clinical pregnancies (CP) were confirmed by the presence of at least one gestational sac, along with fetal heartbeat found by ultrasonography.

In utero culture system (IUCS)

The IUCS device ***[Patent application granted on 2012-09-04; International patent n° PCT-IB02/03363 (22/7/2002); Priority provisional US 60/309,274]*** consisted of a 1cm long micro-perforated hollow silicone elastomer tube with 0.75mm outer diameter and 0.43mm inner diameter (Figure 1). Eight longitudinal lines composed of 45 holes, each with a diameter of 0.4mm, were made using a laser-based system. The specific structure of the IUCS allowed proper exchange of molecules present in the uterine environment, without enabling zygotes or embryos to escape. Moreover, the IUCS device could be opened or closed to load and retrieve oocytes/zygotes/embryos. The proximal part of the IUCS device was secured by a titanium head attached to a silicone tube, which was reinforced by a stainless steel spiral wire. A polypropylene string was fixed to the silicone tube distal end, thus permitting the extraction of the device. The IUCS could be loaded into a standard embryo transfer catheter (Prince Medical, Ercuis, France) to be placed into the uterine cavity.

Biocompatibility and toxicity tests were previously performed using an in vitro bovine model (unpublished data). Materials used for manufacturing the IUCS device were all FDA USP Class VI and ISO 13485 approved for medical applications.

The in utero culture system (IUCS) device reported in this study represents an emerging alternative to currently used ART procedures that can efficiently sustain oocyte fertilization and early embryo development directly in the natural environment of the woman's uterus by significantly minimizing the in vitro culture time duration. The IUCS device consists of a small silicone tube secured by a titanium head and a polypropylene string fixed on the opposite side to allow safe extraction of the device. ICSI-inseminated oocytes can safely be loaded into the IUCS device, which can then be placed inside the uterine cavity with a standard embryo transfer catheter.

Results

A total of 46 post-ICSI MII oocytes were loaded on the IUCS device. After 18-21 hours of in utero incubation, 23 viable zygotes and 3 early cleavage-stage embryos were retrieved (Table 1). The fertilization rate was 55%, and the recovery rate from IUCS was 80%.

It is possible to speculate that the lost oocytes were possibly digested by macrophages that were found inside the device. The total duration time of in vitro culture ranged between 1.5 and 2 hours before the definitive uterine transfer of the selected zygotes or embryos. Two pregnancies were obtained following the in utero culture via IUCS. Specifically, a healthy boy was born as a result of a double zygote transfer, and a girl from a triple cleavage-stage embryo transfer. Both pregnancies and obstetrical outcomes were normal with normal-term vaginal deliveries and birth weights (3400g and 2800g, respectively), and no adverse effects were observed on the offspring after a 12-year follow-up.

Overall, this work demonstrates that it is possible to achieve healthy pregnancies following in utero culture of ICSI-inseminated MII oocytes. Specifically, this study opens new perspectives in the reproductive medicine field by combining ART procedures and a new in utero culture system (IUCS), which allows fertilization and early preimplantation embryo development directly in the mother's uterus.

Figure 1: Intra Uterine Culture System (IUCS) device

Table 1: In utero culture of ICSI-inseminated oocytes loaded on the IUCS device

No. patient	Age	PIO loaded into IUCS (n)	In utero over-night culture (h.mn)	Type and number of cells retrieved from IUCS					% Ferti-lization	Trans-ferred zygote embryos (n)	Pregnancy / % Clinical Preg-nancy
				DZ	UO	DO	LO	EC			
1	34	4	20	2	2	0	0	0	50	2	NP
2	31	11	21	2	0	0	6	3	45	3	LB
3	29	12	21.3	7	2	1	2	0	58	3	NP
4	27	5	18.45	2	3	0	0	0	40	2	LB
5	36	8	20.3	7	0	0	1	0	87.5	3	NP
6	38	6	20.3	3	3	0	0	0	50	3	NP
Tot/Ave	33	46	20.2	23	10	1	9	3	55	16	33

PIO = post ICSI oocyte, IUCS = in utero culture system, DZ = Diploid zygote, UO, unfertilized oocyte, DO = degenerated oocyte, LO= lost oocyte, EC = early cleavage-stage embryo; NP = not pregnant, LB = Live birth.

Case n°1

The first couple involved in this study was diagnosed with stage 1 endometriosis and oligoasthenozoospermia with increased fragmentation rates. The ovarian stimulation protocol was initiated by daily administration of 225 IU of gonadotropins (Menopur) until at least three follicles with diameter ≥17mm were detected by ultrasound. Ovulation was then triggered by injection of hCG and oocyte retrieval was performed 36 hours later, with a total of 11 oocytes collected.

Following cumulus cell removal, the 11 metaphase II (MII) oocytes were successfully inseminated by ICSI. Immediately after insemination, the injected oocytes were loaded into the IUCS device and then transferred to the uterus using an embryo transfer catheter. After an overnight in utero incubation of 20 hours, the IUCS device was extracted and a total of 2 zygotes and 3 early cleavage-stage embryos were recovered. Briefly, after the IUCS withdrawal and zygote-embryo collection, the 3 cleavage-stage embryos were transferred into the uterine cavity, using an ultrasound-guided transfer catheter. The total time after the embryos were recovered from the IUCS device the embryos were placed in in vitro culture for 2 hours before transfer. Two weeks later, a clinical pregnancy was confirmed by a human chorionic gonadotropin blood (β-hCG) test. At the end of the gestation period, the woman gave birth to a healthy girl (birth weight: 2800g).

Case n° 2

The second couple was diagnosed with idiopathic infertility after trying to naturally conceive for 18 months. Following two unsuccessful intrauterine inseminations (IUI), an IVF cycle was recommended. Similarly to the precedent case, the ovarian stimulation protocol was initiated by daily administration of 225 IU of gonadotropins (Menopur) and ovulation was then triggered by hCG injection. Oocyte retrieval was performed 36 hours after hCG stimulation, with a total of 5 oocytes collected. After denuding, the 5 MII oocytes were injected with motile sperm cells by ICSI. Immediately after insemination, the injected oocytes were loaded into the IUCS device and transferred inside the uterus as described above. After an overnight in utero incubation, 2 viable zygotes and 3 unfertilized oocytes were collected from the IUCS. Briefly, the 2 zygotes were transferred into the uterine cavity, using an ultrasound-guided transfer catheter. The total in vitro culture time prior to transfer was 1.5 hours. Two weeks later, a clinical pregnancy was confirmed by a human chorionic gonadotropin blood (β-hCG) test. After 39 weeks of gestation, the woman gave birth to a healthy boy (birth weight: 3400g).

Discussion

This study provides evidence that the IUCS device effectively supports fertilization and preimplantation embryo development, offering a more "natural" environment for the development of the embryo, almost identical to the uterine milieu, that led to healthy live births (Blockeel et al., 2009).

The main goal of this work was to significantly decrease the in vitro culture time for ICSI-inseminated oocytes to a maximum of 2 hours of in vitro culture before performing uterine transfer. Interestingly, viable zygotes and good-quality cleavage-stage embryos were retrieved from the IUCS device after an overnight in utero culture, and uterine transfer of selected embryos was performed after a minimal in vitro culture time duration compared to standard in vitro culture (2 hours versus 48 hours, respectively).

Although in vitro fertilization and ART are nowadays highly effective with over 2.6 million cycles and about 500,000 babies born worldwide each year (de Mouzon et al., 2020), we are still far from the ideal embryo culture condition that recapitulates the in vivo preimplantation development conditions. It is well known that suboptimal in vitro culture conditions during ART may significantly increase stress responses in gametes/embryos and can negatively affect the epigenetic remodeling that occurs between fertilization and embryo preimplantation development, resulting in a significant decrease in embryo quality and implantation competence, as well as in important side effects for fetal development and future offspring (Cannarella et al., 2022; Håberg et al., 2022).

Furthermore, it has been recently demonstrated that epigenetic/imprinting errors

can arise during different steps of the ART cycles, such as controlled ovarian hyperstimulation, in vitro preimplantation embryo culture, and, ultimately as the effect of abnormal intake of folic acid (FA) supplementation in women undergoing ART pregnancies (Ménézo et al., 2022). The fact that failure of ART cycles is linked to the in vitro artificial environment. The advent of time-lapse microscopy (TLM) in embryo culture procedures has led to a significant reduction in handling of oocytes, zygotes, and embryos and, therefore has resulted in less stressful culture conditions. However, due to their high costs, TLM are not yet widely available to all IVF centers worldwide; consequently, oocytes, zygotes, and embryos are still subjected to in vitro handling during the delicate stages of fertilization and preimplantation embryo development, thus enhancing important stress responses (Ramos-Ibeas et al., 2019).

During embryo preimplantation development, essential epigenetic reprogramming occurs in a specific time window that is particularly sensitive to environmental changes (Chason et al., 2011). The use of IUCS devices would decrease possible stressful conditions for the embryo, which in turn may lead to short-term (implantation competence) and long-term (healthy adulthood) positive effects.

The emergence of bioengineering studies focused on reproductive medicine and fertility-restoring strategies, such as the development of biohybrid microrobots, are now becoming very popular. Specifically, sperm-driven magnetic tubes (Magdanz et al., 2013), magnetized synthetic sperm flagella (Khalil et al., 2018), sperm hybrid micromotor for drug delivery (Xu et al., 2018), 3D bioprinted helical spermbots for immotile sperm cells and many other applications are now emerging in the reproductive medicine field (Singh et al., 2020). Interestingly, poor-motility sperm cells carried within the spermbot robots can be specifically guided to reach specific parts of the human body, such as oocyte location and, thus, allowing fertilization in the natural environment of the woman's body (Magdanz et al., 2014).

In addition, the use of these biorobots may also support science-exploratory investigations that could lead to a better understanding of the natural route of sperm cells inside the female reproductive system as well as improving our knowledge of infertility (Magdanz et al, 2014). Likewise, the IUCS technology may represent an accessible and clinically effective alternative to improve the success of ART cycles. Specifically, IUCS device permits in utero incubation of ICSI-inseminated oocytes directly in the natural environment of the uterus with a significant reduction of the in vitro culture time in laboratories.

As a result, the most sensitive stages of preimplantation embryo development, such as fertilization and early embryo cleavage, both occur inside the uterus. Consequently, the highly complex intra-uterine microenvironment in offered by the IUCS device probably facilitates the communication between endometrium and embryo, regulated by endocrine, paracrine, and autocrine factors, as well as by several signals that are sent from the embryo to the mother in the form of EVs (Idelevich and Vilella, 2020).

Moreover, it is important to highlight the psychological advantage of this innovative procedure. The couples that decided to use the IUCS device felt more involved during the whole ART process and, generally, more content knowing that early fertilization and embryo development were going to occur inside the uterus and, therefore, in a more "natural" way.

To better understand the beneficial effects of this newly proposed in utero embryo culture system, which allows "natural" fertilization and embryo preimplantation development, leading to a healthy pregnancy, requires further studies using the IUCS device. Specifically, further analysis of the impact of IUCS on embryo epigenetic alterations, chromosomal abnormalities, and implantation non-competence that generally occur as a result of the in vitro culture conditions, need be addressed on an urgent basis. In addition, the possibility of extending the in utero culture from the current overnight culture duration, reported in this study, to a complete in utero preimplantation development up to the blastocyst stage need also be explored.

Overall, it is well established that ART procedures can negatively impact essential processes that happen during preimplantation

embryo development as well as during fetal life, which are important risks that should be investigated in order to ensure disease-free future offspring. Therefore, further investigations into the development of IUCS devices and reproductive bioengineering research in order to offer a more “natural” microenvironment during preimplantation embryo development are now an urgent matter , which may lead to ART cycles with higher embryo implantation potential and, thus, to healthy pregnancies in couples struggling with infertility.

As a result of the in utero-assisted culture system, supported by the use of the IUCS device, two healthy pregnancies were achieved. The findings offers a framework for the future use of IUCS devices in the reproductive bioengineering medicine field and opens avenues for further clinical applications and advances in the development of new in utero culture systems that may become a more optimized fertility option for couples undergoing ART treatments.

Conclusion

This work described an innovative IUCS device that allows better early mother/embryo interaction, which in turn leads to high levels of fertilization and good quality zygotes and cleavage-stage embryos that when transferred to the uterus can implant and lead to healthy pregnancies and live births.

Acknowledgements

The author would like to thank Mario Biondo and Mary Gojon, both embryologists and shareholders in Anecova, for their active contribution; the Anecova's team, Martin Velasco (CEO, Chairman, and cofounder), Stéphane Rivière (CSO) and Christian Lowe for their valuable support; Professor Patrick Aebischer and Professor Nicolas Bouche from the Swiss Federal Institute of Technology Lausanne (EPFL) for their contribution at the level of engineering and for their institutional support; Denny Sakkas, PhD, embryologist, Professor René Frydman, Professor Carlos Simon, and Professor Umberto Simeoni who supported the author during the in vivo investigation for many years; the team of the reproductive biology laboratory, Babylmpulse, Hirslanden, including cofounders Sidi el Matribi, Didier Chardonnens MD, and, Grace Bianchi-Movarekhi MD *with Privat-Docents*, and Blaise Bourrit, MD; his wife and Assistant Aurélie Mock, and Martina Balli, PhD, embryologist, who helped draft and submit this manuscript. Finally, the author thanks his father Claude Mock for his assistance in drawing the prototype of the in utero culture system device.

Financial support

The author declares that the study was supported by Anecova and that he has only an affiliation with the company as the inventor of this new in utero culture system and as a shareholder without any other positions or financial support from the company..

Ethical standards

The current study is the result of data concerning only two case reports. By the Swiss ethics guidelines, this case report study is not considered to be generalizable scientific results according to the Human Research Act (HRA) in Switzerland and, therefore, does not require submission to the Geneva Cantonal of the Research Ethics Commission (CCCER).

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