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Case Report

The first report of pregnancies following blastocyst automated vitrification in Europe

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ABSTRACT

Embryo cryopreservation is a valuable technique in assisted reproductive technology (ART) that increases cumulative pregnancy rates and allows postponement of embryo transfer in patients with undesirable uterine or clinical conditions. Although vitrification has been considered the most efficient method to freeze oocytes and embryos, it is time-consuming and highly operator-dependent. Gavi® is the first semi-automated machine for vitrification capable of controlling crucial variables such as temperature, volume, concentration and exposure time during the vitrification process. We report the first two pregnancies obtained with blastocysts cryopreserved with the Gavi® semi-automated vitrification system in Europe. These outcomes suggest that the utilization of semi-automated vitrification may contribute to improve the outcomes and laboratory logistics of fertility clinics.

Introduction

During the last two decades, in vitro fertilization (IVF) related techniques have undergone substantial innovation and improvement. However, most IVF related methods are still manual and operator-dependent, which constantly challenges the repeatability of the results [1–3]. Embryo cryopreservation has been largely adopted as a strategy to increase cumulative pregnancy rates [4]. In addition, it is also important for allowing postponement of embryo transfer in the face of inadequate uterine or clinical conditions, such as risk of ovarian hyperstimulation [5]. Vitrification has been recognized as the most efficient method to freeze embryos and oocytes regardless their developmental stage. Over the last ten years, vitrification has nearly completely replaced slow freezing since it has consistently provided superior clinical outcomes [6,7].

Nevertheless, the utilization of vitrification has been troubled by its technical complexity. There are several alternative protocols for vitrification of oocytes and embryos utilizing different solutions, devices, volumes, freezing and thawing rates [1,2]. Furthermore, vitrification relies on high technical accuracy, which requires manual skills and intensive training from the embryologist. Consequently, the consistency of vitrification outcomes at the clinic level is highly dependent upon the overall expertise and homogeneity of the laboratory staff.

Gavi® has been developed by Genea (Sydney, Australia) and Planet Innovation (Melbourne, Australia), being the first semi-automated system for vitrification. The instrument uses a closed device named “Pod”, which is inserted in the “Cassette” that can load up to four Pod units. Embryo position is maintained in Pod’s microfluidic wells throughout the vitrification process, while the robotic unit of Gavi®, utilizing automated pipettes and single-use pipette tips, replaces automatically and sequentially the vitrification media. Subsequently, the heat sealing unit of Gavi® seals each Pod and the Cassette is inserted in the “Gavi Storage Unit”, previously placed into the liquid nitrogen vats. The protocol is very simple for the embryologist to conduct, being only necessary to place the embryo into the Pod, press the start button, and at the end of Gavi® protocol, transfer the Cassette containing the Pod units into liquid nitrogen [8].

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Automated vitrification allows better control of all important variables during the vitrification process including temperature, embryo position, media volume, media concentration and exposure times to different vitrification media. These variables are crucial to maximize embryo developmental competence after cryopreservation, and depend upon technical accuracy of the embryologist when vitrification is performed manually. Therefore, automated vitrification reduces intra-operator and inter-operator variability, contributing for the optimization and repeatability of the results. Additionally, Gavi® is capable of vitrifying several embryos simultaneously (up to 4 blastocysts or 8 cleavage stage embryos per round) within a short time (15 min for cleavage stage embryos and 17 min for blastocysts), which can contribute for the logistics of the laboratory [8].

Here, we report the first two pregnancies obtained in Europe after embryo automated vitrification.

Case reports

We report herein two ongoing pregnancies resulting from the transfer of blastocysts cryopreserved with Gavi®.

The first patient (Patient 1) is 32 years old and was diagnosed with polycystic ovarian syndrome. She had tried to conceive naturally from 2016 to 2017, after which the couple tried four unsuccessful intrauterine inseminations. Subsequently, the couple experienced an unsuccessful ICSI cycle, in which two day-three embryos were transferred.

During their second ICSI attempt, the patient was subjected to an ovarian stimulation protocol with recombinant FSH and GnRH antagonist. The protocol began on the third day (Day 3) of the cycle with recombinant FSH (Puregon® 225 IU; MSD ITALIA Srl) once a day. On Day 7, daily injections of recombinant LH (Luveris® 75 IU; Merck Serono) and GnRH antagonist (Fyremadel® 025 mg/0,5 ml; RANBAXY ITALIA SpA) were included in the hormonal treatment. On Day 11, when the leading follicle reached 18 mm in diameter, due to the risk of ovarian hyperstimulation syndrome, Puregon, Luveris and Fyremadel were discontinued and a GnRH agonist (Fertipeptil® 0,2 mg; Ferring Spa) was administered to trigger oocyte maturation. Estradiol plasma concentration on the day of oocyte pick-up was 5620 pg/ml. Oocyte pick-up was performed 36,5 h after the administration of Fertipeptil. Seventeen oocytes were retrieved and cumulus cell-oocyte complexes were cultured in fertilization medium (Sequential Fert™; Origio®). After 2 h from collection, cumulus cells were removed by brief exposure to culture medium containing hyaluronidase (80 IU/ml; HYASE™; Vitrolife), followed by mechanical action. Twelve mature oocytes were injected and after 5 days four good blastocysts were obtained. Due to the risk of ovarian hyperstimulation syndrome, all embryos were frozen. One blastocyst was cryopreserved using Gavi® (Gardner grading score: BL-5AA; Fig. 1) according to the manufacturer's instructions [9], while the other blastocysts were frozen using the Kitazato Vitrification Cryotop Kit (Cryotop®; Kitazato, Japan). After one month, the patient returned for embryo transfer. From the Day 2 of the cycle her endometrium was prepared with 2 mg oestradiol valerate (Progynova®; BAYER Spa) three times per day (total dose: 6 mg). From the 14th day of the cycle, luteal phase was supported with progesterone (Progeffik® 200 mg; EFFIK) three times per day (total dose: 600 mg). On day 19 of the cycle, the blastocyst cryopreserved with Gavi® was thawed according to the Gavi® protocol [9] and transferred (Gardner grading score: BL-5AA; Fig. 2). Twelve days later, serum βhCG levels were measured revealing the value of 596 IU/l and thus pregnancy. A monochorionic pregnancy was diagnosed during an ultrasound examination performed at the fourth week of gestation, which was confirmed by a second ultrasound examination performed at the sixth week of gestation.

Fig. 1. Illustrative image of the blastocyst obtained from Patient 1 before being subjected to automated vitrification (Gardner grading score: BL-5AA).

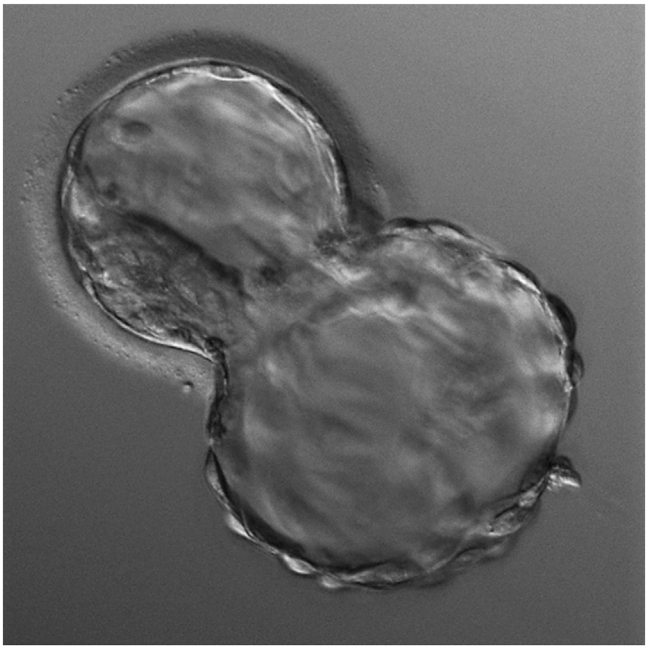

Fig. 2. Illustrative image of the blastocyst obtained from Patient 1 after being subjected to automated vitrification and thawed (Gardner grading score: BL-5AA).

The second patient (Patient 2) is 37 years old and had been previously diagnosed as nulliparous with unexplained infertility. This was the second ICSI attempt of the couple, in which the patient was subjected to ovarian stimulation with recombinant FHS in a GnRH agonist protocol. The hormonal treatment began on the second day (Day 2) of the cycle with daily injections of a GnRH agonist (Fertipeptil® 0,1 mg; Ferring Spa) and recombinant FSH (Puregon® 225 IU; MSD ITALIA Srl). On Day 13, when the leading follicle reached 18 mm in diameter, Fertipeptil and Puregon were discontinued and oocyte maturation was induced with 10,000 IU of hCG (Gonasi® HP 5000 IU; IBSA Farmaceutici Italia Srl). Estradiol

plasma concentration on the day of oocyte pick-up was 2190 pg/mL. Oocyte pick-up was performed 36,5 h after the administration of hCG. Eighteen cumulus-oocyte complexes were retrieved and cultured in fertilization medium (Sequential Fert™; Origio®). After 2 h from pick-up, cumulus cells were removed by exposure to culture medium containing hyaluronidase (80 U/ml; HYASE™; Vitrolife), followed by mechanical action. Fourteen mature oocytes were injected and eleven of them were fertilized. The patient did not undergo fresh transfer due to the risk of ovarian hyperstimulation syndrome. Three good blastocysts were obtained, one of which was cryopreserved using Gavi® (Gardner grading score: BL-4BB; Fig. 3) according to the manufacturer's instructions [9], while the others were cryopreserved with the Kitazato Vitrification Cryotop Kit (Cryotop®; Kitazato, Japan). One month later, the patient was prepared for transfer from the first day (Day 1) of her cycle with 2 mg oestradiol valerate (Progynova®; BAYER Spa) three times per day (total dose: 6 mg). From Day 12, the luteal phase was supported with progesterone (Prometrium® 200 mg; Rottapharm) three times per day (total dose: 600 mg). On Day 17, the blastocyst cryopreserved with Gavi® was thawed according to the Gavi® protocol [9] and then transferred (Gardner grading score: BL-4BB; Fig. 4). Pregnancy was diagnosed 12 days later by serum βhCG measurement (84 IU/l), and then confirmed at its sixth week by ultrasound examination.

These two patients are the first to conceive following transfer of vitrified embryos with Gavi® in our Reproductive Medicine Centre.

At the time this case report was written, pregnancies were at the 30th and 28th weeks and progressing normally.

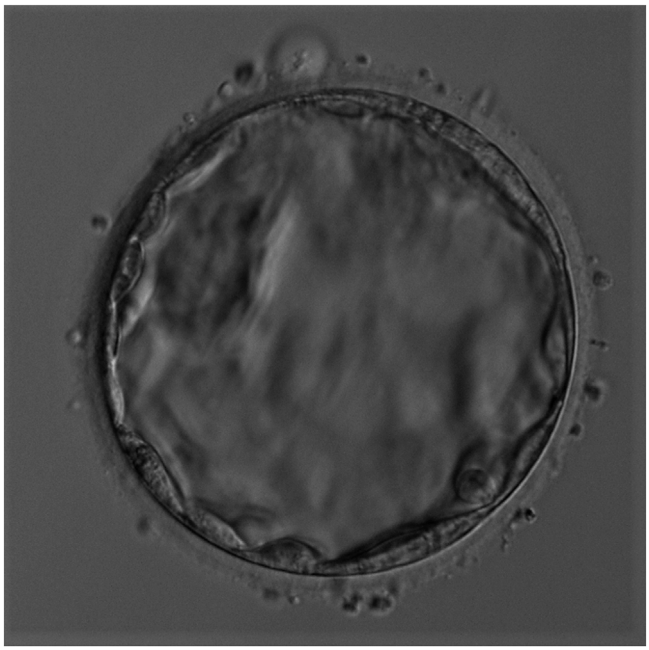

Fig. 4. Illustrative image of the blastocyst obtained from Patient 2 after being subjected to automated vitrification and thawed (Gardner grading score: BL-4BB).

Discussion

Cryopreservation of embryos helps to increase cumulative pregnancy rates [4] and enables the postponement of embryo transfer for patients with inadequate clinical conditions such as elevated risk of ovarian hyperstimulation syndrome [5]. Vitrification has been recognized as the gold standard method for oocyte and embryo cryopreservation and has practically replaced slow freezing [6,7]. Since vitrification outcomes are challenged by its technical complexity and dependence on operator accuracy, automated vitrification protocols concentrate great interest. This is the first report of pregnancies following blastocyst automated vitrification in Europe.

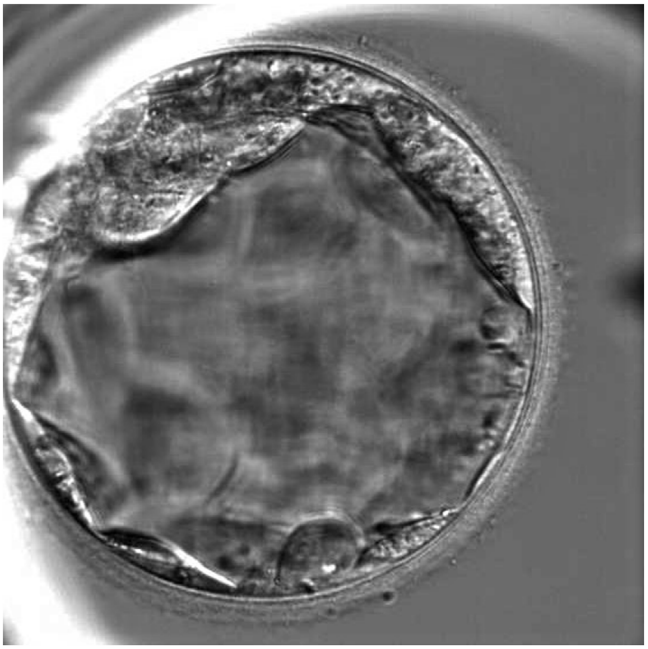

Fig. 3. Illustrative image of the blastocyst obtained from Patient 2 before being subjected to automated vitrification (Gardner grading score: BL-4BB).

There are several different vitrification protocols, the most widespread being the Cryotop method. Survival rates of embryos cryopreserved with Cryotop have been reported to be above 90%, with outcomes equivalent to those obtained from fresh transfers [10,11]. However, the Cryotop method is manual, time-consuming and operator-dependent. Technically, vitrification is quite challenging due to the use of very concentrated and viscous solutions that embed oocytes and embryos in very small volumes during short periods of time (<1 min) [12]. Consequently, the success of the procedure is highly variable between operators and IVF laboratories [13].

Automated vitrification permits full control of all vitrification variables simultaneously, minimizing errors associated with embryo and media manipulation [8]. Moreover, Gavi® can vitrify up to eight cleavage stage embryos or four blastocysts at once and quickly (15 min for cleavage stage embryos and 17 min for blastocysts) [8], which benefits technical efficiency and laboratory work flow. The cost of automated vitrification is around 75 € per embryo individually vitrified, but falls to around 62.5 € per embryo when four embryos are frozen together. Therefore, although the individual cost is higher than conventional vitrification, automated vitrification can considerably save embryologist work time and thus be economically advantageous depending on the logistics of the clinic.

We report herein the first two pregnancies in Europe obtained with blastocysts cryopreserved with an automated vitrification system. These were the first two patients subjected to embryo transfer following blastocyst vitrification with an automated system in the Biogenesi Reproductive Medicine Centre. These outcomes motivated us to continue to utilize Gavi® in the clinical routine and to test its efficiency in relation to the Kitazato Vitrification Cryotop in undergoing studies. The present case reports suggest that automated vitrification may benefit the

application of ART and laboratory logistics, but specifically designed studies are required to assess its impact on live births and clinic outcomes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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