Case Report: First Tunisian successful delivery using vitrified human oocytes

Merdassi G[1]*, Bouricha M[1], Wahbi D[1], Meftah MB[2], Ferchiou M[2], Zhioua F[2]

Merdassi G, Bouricha M, Wahbi D, et al. Case Report: First Tunisian successful delivery using vitrified human oocytes. J Reprod Biol Endocrino 2020;4(2):1-2.

This paper is a case report on the result of the first documented baby born achieved in Tunisia with frozen-warmed oocytes in the IVF center of Aziza Othmana Hospital. A patient was setted off by ovitrelle at j11. On oocyte pick-up (OPU), her husband was unable to ejaculate for psychological reasons. OPUwas done and we collected 12 MII-phase oocytes. The failure of ejaculation leads us to vitrify 12 oocytes in four pailletes with three oocytes/PHS. After that, we cryopreserved the husband sperm in five pailletes. Oocytes were microinjected with frozen sperm and we obtained four embryos in day 2. Two embryos, which one of them was top, were transferred. The pregnancy was lead until 38 weeks of amenorrhea. After inducing lung maturity, a cesarean section was performed and one healthy fetus was delivered with a weight of 3.300 kg.

Key Words: *vitrification; warming; failure of ejaculation; pregnancy*

INTRODUCTION

Oocyte vitrification has been created in 1990 in order to preserve a woman's eggs [1]. This technique has been used to enable women to postpone pregnancy to a later date. Many indications are proposed essentially fertility preservation in case of radiotherapy or chemotherapy, premature ovarian failure and endometriosis [2], but also in case of ejaculation failure the day of oocyte pick up inorder to avoid emotional and financial burden on patients. Many freezing protocols have been developed in order to maximize oocyte survival after devitrification [1]. Despite the first success obtained in the 1990s with frozen oocytes by slow freezing, the advent of vitrification allowed a breakthrough of this fine procedure.

Vitrification has started on 1999 in the world [3] and probably on in Tunisia. Some pregnancies were reported after oocyte vitrification but without a published live birth. We will present the first baby born achieved in Tunisia with frozen-warmed oocytes.

CASE PRESENTATION

The clinical follow-up of a 35-year-old female applying to our ART center.

The woman attempted our centre for her first ART trial.

Biological analysis showed in day 3: AMH 3.05 ng/ul, FSH 4.24m UI/ml, LH 5.67m UI/ml estrogen 40.5 pg/ml and prolactin 35 ng/ml (Table 1).

Her husband has 36 years old with normal sperm parameters.

Table 1
Normal sperm parameters Infectious balance of the couple was negative.

Parameters	Values
Volume	2.5ml
Numeration	51 millions/ml
PR Mobility	74%
Vitality	90%
Typical Shapes	17%

The woman underwent a standard luteal phase antagonist protocol, with controlled ovarian hyper stimulation. She took a daily 225 IU recombinant gonadotropin for ten days and cetrotid 0.25 for five days. She was setted off by ovitrelle at j11. Oocytes retrieval was performed after 36 hours. On oocyte pick-up (OPU), her husband was unable to ejaculate for psychological reasons. We were therefore placed in the position of having to make an emergency decision. OPU was done and we collected 12 MII-phase oocytes.

The failure of ejaculation leads us to vitrify 12 oocytes in four pailletes with three oocytes/PHS using vitrification kit: Vitrolife Irvine Scientific. After that, we cryopreserved the husband sperm in five pailletes.The couple consult 6 months later, in November 2018 for oocytes devitrification. We found only six oocytes with a positive survival (>50%) and good morphology. The six others degenerated.

Oocytes were microinjected with frozen sperm and we obtained four embryos in day 2. Two embryos, which one of them was top, were transferred. The pregnancy was lead until 38 weeks of amenorrhea, when pre-term delivery started. After inducing lung maturity, a cesarean section was performed and one healthy fetus was delivered with a weight of 3. 300 kg.

[1]*ART Center, Aziza OthmanaHospital, Tunis, Tunisia;*

[2]*Gynecology Department, Aziza OthmanaHospital, Tunis, Tunisia.*

Correspondence: Merdassi Ghaya, ART Center, Aziza OthmanaHospital, Tunis, Tunisia

Telephone; 21644303939, e-mail: merdassighaya@yahoo.fr

Received: September 30, 2019; Accepted: March 17, 2020; Published: March 24, 2020

DISCUSSION

Ejaculation failure the day of oocyte pick up was the indication of oocyte vitrification.Usually oocyte vitrification is indicated in fertility preservation, but several publications have treated several other indications. Van der veen and al have subcategorized indications in six group: no sperm available during IVF or ICSI treatment, planned gonadotoxic therapy, ovarian surgery, risk on premature ovarian insufficiency, previous gonadotoxic therapy [2]. Vitrification is the use of a high concentration of cryprotectants which replace most of the water within the cell and inhibit the formation of ice crystals using slush nitrogen (SN2) with a temperature of-196°. The result of vitrification is a solid glass-like cell, free of ice crystal [3].

The process of devitrification consists in warming the contents of the straw, removing the cryprotectant by dilution in thawing solution [4]. Vitrification, can lead to damage during the warming of cells especially in oocyte, mainly because of oocyte large size, low surface area to volume ratio, relatively high water content and presence of the meiotic spindle. It is not plausible for each oocyte to fertilize normally and develop into an available embryo, and finally to a live birth [1]. That's why many vitrification protocols have been developed such as closed vitrification system and open one [5]. In fact, the use of open or closed vitrification system has a direct impact in oocyte survival. Sarandi and al have reported 93.2% of oocyte survival after thawing with open vitrification system compared to 64.5% with closed one. No difference were reported concerning meiosis resumption rate and maturation rate [6].

In our case, we have used closed vitrification system: Rapid-I kit, inorder to avoid nitrogen contamination even though survival rate in closed system is lower than open system. Our Oocyte survival was 50%. Our results are in agreement with Sarandi and al concerning the closed vitrification system [6]. A Retrospective analysis of Goldman and co compare the efficiency of oocyte cryopreservation (OC) and fresh IVF using the metric "live births per mature oocyte retrieved " [7]. Forty women who underwent OC with thaw attempt. There was no significant difference in live-birth rate per mature oocyte retrieved (2.7% *vs.* 4.2%, respectively) or live-birth rate per ET (45.8% *vs.* 51.9%). Significantly, more oocytes were harvested in the OC versus fresh IVF cycles (21 *vs.* 16); however, fewer blastocysts developed (3.9 *vs.* 6.3 $p<0.05$).

Thus, we noted an age-independent approximate twofold decrease in blastocyst formation in the OC group. Their study suggests that OC may be approaching fresh IVF when live birth is the primary consideration [4]. Indeed, Oocyte vitrification followed by intracytoplasmic sperm injection leads to lower embryo developmental competence compared with when fresh insemination methods are used. However, pregnancy and implantation rates are higher when embryos are transferred into a "more receptive" endometrium, free of the adverse effects of gonadotropin [7]. Another parameter influences live birth from vitrified oocytes is women's age.

In fact, for women ≤ 35 years of age, the percentage of survive of a MII oocyte after thawing is 76.5% [8]. Implantation rates per embryo transferred (43% vs. 35% with fresh oocytes) and clinical pregnancy rates per transfer (57% *vs.* 44%) were significantly higher with vitrified-warmed compared with fresh oocytes. The overall vitrified-warmed oocyte to live born child efficiency was 28 % [9]. CLBRs significantly decrease with increasing age among women ≥ 38 years of age, with the most prominent and clinically relevant decline observed at 42-43 years old, and clear evidence for futility in women aged ≥ 44 years (25.9% at 38-39 years, 16.4% at 40-41 years, 7% at 42-43 years and 1.2% from 44 years onwards). The higher the number of oocytes retrieved, the higher the CLBR; however, this is more evident up to 41 years old and no clear benefit is observed from 44 years and beyond [10]. Therefore, there is also an oocyte cohort influence in live birth percentage.Indeed, live birth probability increased up to seven oocytes retrieved to reach 15% and remained relatively unchanged (increase or decrease of ≤ 5%) between seven and 20 oocytes retrieved. Furthermore, in patients with >20 oocytes retrieved the rate was exceeding 20% [11].

CONCLUSION

There is a chance for oocytes to stay alive with acceptable embryo formation and quality rates after vitrification warming of oocytes. Oocyte survival after thawing depends on several factors such as vitrification protocol, women age and vitrified oocyte cohort. Vitrification in Tunis has started tardively comparing to the literature, and we reported here the first live birth after oocyte thawing out of the indication of fertility preservation.

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