Pregnancy Outcomes, Miscarriage and Live Birth Rates Following the Transfer of Thawed Vitrified Embryos Derived from IVF vs. ICSI, A Retrospective Analysis

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Article

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Abstract: The present study was conducted to compare embryo survival rate and treatment outcomes including pregnancy, miscarriage and live birth rates of IVF and ICSI vitrified-thawed embryo transfers. Authors suggested to compare treatment outcomes [clinical pregnancy, miscarriage, ectopic pregnancy, and live birth rates] after transfer of IVF versus ICSI vitrified-thawed embryos. Cohort retrospective study in the IVF unit at a university affiliated Medical Center which included 845 frozen thawed embryo transfer cycles of vitrified embryos from treatment cycles between the years 2013-2019. Out of 845 FET cycles of vitrified thawed embryos, 223 clinical pregnancies were achieved [26.4%]. Of these 64 [28.4%] following IVF, 131 [25.6%] after ICSI and 28 [25.9%] in the sibling oocytes group [P =0.631]. Live birth rate did not differ between the three groups: 23.6%, 22.3% and 21.5% for groups IVF, ICSI and IVF+ICSI, respectively [p=0.899]. Sub analysis by age below 35 and above 35 years old revealed no statistical difference between IVF and ICSI cycles in terms of clinical pregnancy, miscarriage, ectopic pregnancy, and live birth rates between the groups. The present study suggests that the technique of insemination has no adverse effect related to vitrification.

Keywords: embryo cryopreservation; ICSI; IVF; pregnancy; vitrification

1. Introduction

In recent years, and secondary to some changes in the practice of assisted reproductive technology [ART] the number of in vitro fertilization [IVF]/ intracytoplasmic sperm injection [ICSI] cycles in which embryos are cryopreserved for future transfer has increased dramatically [1]. Freeze all cycles, to reduce rates of ovarian hyper stimulation syndrome, for fertility preservation or for other medical and social reasons have gained popularity. Increased confidence in the cryopreservation process, secondary to improvements in embryo culture conditions and the refinement of the embryo freezing methods, have also promoted the use of cryopreservation and the increase in available post thaw embryos. Moreover, in order to reduce the rates of multiple pregnancies and their associated perinatal complications, many international fertility societies, including the Israeli Association of Obstetrics and Gynecology, ASRM and ESHRE published official guidelines and regulations restricting the number of embryos approved for transfer [2], thus yielding more embryos for cryopreservation.

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Vitrification of embryos, first introduced in Australia in 2006, has been a breakthrough in ART, resulting in a complete transformation of how we manage treatment for IVF patients. Vitrification literally means turn to glass. Vitrified solutions do not go through a phase shift from liquid to solid; the solutions are technically still in a liquid phase, but they cannot flow and appear solid. This allows for full maintenance/restoration of the physiological processes in the cells [3,4]. Compared to the previous freezing method, slow freezing, vitrification is an ultra-fast freezing method, which prevents ice formation inside the embryo, prevents damage to the embryo and allows higher survival rates after thawing [5–7]. Vitrification is relatively simple, inexpensive, and faster compared to the slow freezing technique [7]. Vitrification has also opened new options for patients, most notably fertility preservation [through oocyte cryopreservation], and donor egg banking.

There are different mechanisms by which cryopreservation can damage the cells [8]. The most significant source of damage at the time of cryopreservation is ice crystal formation, extracellular, and especially intracellular, which might thrust or burst apart the cells, destroying them by direct mechanical action. Another potential source of cellular damage is the use of cryoprotectants. To minimize cryo-damage, one must use various chemicals, typically a mix of permeable and non-permeable cryoprotectants. Though they are required for successful vitrification, cryoprotectants themselves may also induce cellular damage, which includes both direct toxicity and osmotic injury. Slow freezing [also called equilibrium freezing] is partly automated and uses low initial concentrations of cryoprotectants. On the other hand vitrification uses much higher starting concentrations of cryoprotectants and requires a very high rate of cooling which can be achieved using minimal volumes of cryo-solutions [9]. While vitrification [also called non-equilibrium cryopreservation], when performed correctly, avoids ice crystal formation there still is the risk of toxicity from the cryoprotectants.

Technical expertise is also necessary to thaw vitrified oocytes or embryos and in fact, adequate warming rates are as important [or perhaps even more important] as cooling rates for optimal results [10]. Vitrification is currently a fully hands-on procedure, however, there are attempts to automate it, at least partially [11,12].

In recent years, ICSI, a process where a sperm is injected directly into an oocyte to fertilize it, has been shown to be an effective solution for male infertility. The ICSI procedure may theoretically harm the integrity of the zona pellucida and interfere with mechanisms operating during freezing. Only a small number of studies [2,13,14] have compared the efficacy and safety of freezing ICSI-embryos and conventional IVF embryos in the same clinical setting. However, the effect was compared with embryos that were cryopreserved by slow freezing [2]. No study has compared reproductive outcomes of IVF and ICSI vitrified-thawed embryos.

Outcomes in terms of miscarriage and live birth rates have not yet been studied with the current freezing technology, that is, in thawed embryos that have undergone vitrification.

The present study was conducted to compare embryo survival rate and treatment outcomes including pregnancy, miscarriage and live birth rates of IVF and ICSI vitrified-thawed embryo transfers.

2. Materials and Methods

This is a retrospective study based on the IVF unit database from Carmel Medical Center, a university affiliated hospital, Haifa, Israel. The study population included frozen embryo transfer [FET] cycles in women who underwent either IVF and/or ICSI and had embryos frozen by vitrification. The majority of embryos were cryopreserved at the blastocyst stage. Over 90% of the embryos were cultured and observed in a time lapse incubator [Embryoscope - EmbryoScope™ Time-lapse System, Vitrolife, Sweden]. Included were women who had undergone in vitro fertilization treatments by IVF or ICSI methods [including sperm obtained after testicular sperm extraction [TESE] treatments] and had undergone embryo freezing by vitrification with subsequent thawing and transfer of embryos. A separate third group consisted of women treated for unexplained infertility and in the same treatment cycle part of the oocytes were fertilized by IVF and others by ICSI before being vitrified. Women with missing information in databases on pregnancy, miscarriage

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and/or live birth rates after thawing of embryos frozen by vitrification after IVF or ICSI were excluded from data analysis. Also excluded were cases of embryos frozen by different methods i.e. slow freezing or slow freezing and vitrification in the same cycle.

Records of all patients who underwent frozen— thawed embryo transfer [ET] between January 2013 until September 2019 were retrospectively analyzed and divided into three groups. Our cohort size including 853 patients enables detection of a 10% difference between the IVF and ICSI groups with 95% significance level and a power of 80%. Group A included cycles of conventional IVF and subsequently cycles of frozen— thawed ET. Group B consisted of cycles of ICSI frozen—thawed ET. Group C included cycles of patients with a diagnosis of unexplained infertility in their first ART cycle in which sibling oocytes were divided and fertilized by conventional IVF or ICSI.

For both conventional IVF and ICSI treatment cycles, patients underwent a similar treatment of ovulation induction with recombinant gonadotropins, with the use of a GnRH antagonist protocol. Transvaginal oocyte retrieval was performed using ultrasound guidance, 34 to 36 hours after human chorionic gonadotropin [hCG] administration. For ICSI cycles, the ICSI procedure was performed as described previously [15], and ET was carried out on day 2 day 3 or day 5 after ovum pickup. Supernumerary embryos not used for fresh transfer were cryopreserved.

Vitrification kites by SAGE™ were used. There were two kinds of solution –the first, an equilibration solution [ART-8025-A], which is a 3- [N-morpholino] propanesulfonic acid [MOPS] buffered solution of modified Human Tubal Fluid [HTF] containing non-essential amino acids and essential, gentamicin sulfate [10 mg\L]. The number of embryos to be vitrified was carefully determined, after that, each sterile petri dish and carrier /storage device was labeled. The vitrified sample was transferred into an appropriately labeled cryotube or goblet attached to a cryocane with another goblet inverted over the top to act as a cap. The cryocane was then transferred to a storage tank of liquid nitrogen.

The warming and dilution procedure was performed at 35-37° C. A heated microscope was used and exposure to light during incubation was minimized. The liquid nitrogen reservoir was filled with liquid nitrogen to a sufficient depth to completely submerge a cryotube or goblet containing the carrier device and the liquid nitrogen freezer containing the vitrified samples was warmed. The cryocanes with the goblets containing the carrier device with vitrified embryos were removed and quickly transferred to the reservoir containing liquid nitrogen, keeping the carrier device under liquid nitrogen at all times. Finally, the embryos were transferred to a dish of pre-equilibrated appropriate culture medium and incubated in CO_2 incubator at 37° C for 3-4 hours to allow to further recovery prior to further manipulations and/or transfer

FET was carried out using endometrial preparation by estrogen and progesterone replacement or at the natural cycle.

The standard estrogen replacement treatment included estradiol 6 mg/day, in three divided doses [Estrofem; Novo Industry A/S, Copenhagen, Denmark] starting on day 3 of the menstrual cycle for 9-10 days. At that time the patient's endometrial thickness was evaluated by trans vaginal ultrasound. Dose adjustments and monitoring were, if needed, individualized according to the measured endometrial thickness. When endometrial thickness reached at least 7- 8 mm, micronized progesterone [P] tablets [Utrogestan; Besins, Iscovesco, France] were concomitantly added. Utrogestan was introduced vaginally at a dose of 400 mg twice times a day, for a total dose of 800 mg/day. The transfer of the thawed embryos took place 2-5 days after Utrogestan initiation, depending on the stage at which they were frozen. Embryos of two to four cells, six to eight cells and blastocysts were transferred at 2,3 or 5 days after Utrogestan initiation, respectively. Patients were instructed to continue luteal phase support and perform pregnancy test 14 days following ET. If the pregnancy test was positive, the patient's treatment was continued, and patients underwent transvaginal ultrasound to confirm fetal viability at 7-8 weeks.

Clinical pregnancy was defined as the presence of a gestational sac on transvaginal ultrasound. In the presence of positive fetal heart beats, hormonal treatment was continued until 10 weeks of gestation. Missed abortion was defined as a nonviable intrauterine pregnancy in the presence of a

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closed cervix and minimal abdominal cramping or vaginal bleeding. Ectopic pregnancy was defined as a pregnancy outside of the uterus and live birth was defined as delivery of a viable infant.

Embryo survival rate and laboratory and clinical outcome of FET cycles were compared between the groups. Quantitative data was presented using mean and standard deviation, qualitative data was presented using frequencies and percentages. Chi-square tests, Fisher's exact test, Student's t-test and multivariate logistic regression analysis were used according to the data. A P value < 0.05 was considered statistically significant.

Statistical analyses were performed using IBM SPSS Statistics 27.0.1.

3. Results

There were 853 FET cycles from treatment cycles using the antagonist protocol between the years 2013-2019. Eight cycles had incomplete data and the final analysis was done on 845 FET cycles. Group A included 225 cycles of conventional IVF and subsequently cycles of frozen— thawed ET. Group B consisted of 513 cycles of ICSI frozen—thawed ET. Group C included 108 cycles of patients with a diagnosis of unexplained infertility in their first ART cycle in which sibling oocytes were divided and fertilized by conventional IVF or ICSI.

The median number of vitrified-thawed embryos transferred per cycle in both IVF, ICSI and IVF+ ICSI groups was 2. The average age of the patients was 32 years in all groups. Pregnancy rate was higher in ET with two or more embryos transferred per cycle. The median number of vitrified-thawed embryos transferred per cycle in both IVF, ICSI and IVF+ ICSI groups was 2 [1.5-2.9] p-value 0.526. The average age of embryo at freezing was 3 days. Over 88% of the embryos were cultured and observed in the embryoscope time lapse incubator. No difference was observed in survival rate in IVF, ICSI and sibling oocytes group [P=0.541]. Out of 845 FET cycles of vitrified thawed embryos, 223 clinical pregnancies were achieved [26.3%]. Of these 64 cases [28.4%] following IVF, 131 [25.6%] after ICSI and 28 [25.9%] in the sibling oocytes group [P =0.631]. The live birth rate did not differ between the three groups: 23.6%, 22.3% and 21.5% for groups IVF, ICSI and IVF+ICSI, respectively [p=0.899]. Sub analysis by age groups, below 35 and above 35 years old; revealed no difference between IVF and ICSI cycles in terms of clinical pregnancy and live birth rates. The miscarriage and ectopic pregnancy rates also did not differ between groups.

Another sub analysis was performed according to the infertility indication for treatment. We observed significantly higher clinical pregnancy rates in groups with PCOS, but not in groups single with donor sperm, unexplained infertility, mechanical factor, endometriosis, male factor, secondary infertility as well as low responders.

However, in a multi-logistic regression test only the number of embryos transferred was found significant.

Tables

Table 1. Cycle characteristics after transfer of frozen-thawed embryos obtained from conventional IVF and ICSI in all groups.

	IVF	ICSI	IVF+ ICSI	p
Age of patients	31.5±5.3	32.2±5.5	32.0±5.5	0.076
Number of thawed embryos median [IQR]	2 [1; 2]	2 [1; 2]	2 [1; 2]	0.164
Number of thawed embryos N [%]				0.059
1	102 [45.3]	193 [37.6]	36 [33.3]	
>=2	123 [54.7]	320 [62.4]	72 [66.7]	
Number of transferred embryos median [IQR]	2 [1; 2]	2 [1; 2]	2 [1; 2]	0.526
Number of transferred embryos N [%]				0.294
1	102 [45.3]	201 [39.2]	44 [40.7]	
>=2	123 [54.7]	312 [60.8]	64 [59.3]	
Age of embryo at moment freezing median [IQR]	3 [3; 5]	4 [3; 5]	3 [3; 5]	0.639
Presence in embryoscope N [%]	205 [91.1]	447 [87.1]	97 [90.7]	0.229
Survival of embryos N [%]	225 [92.3]	513 [93.8]	108 [92.7]	0.541

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Table 2. Reproductive outcomes after transfer of frozen thawed embryos obtained from conventional In Vitro Fertilization [IVF] and Intracytoplasmic Sperm Injection [ICSI] in all groups.

	IVF	ICSI	IVF+ ICSI	*p-value*
No of gestational sacs N [%]				
0 [%]	156 [69.3]	364 [71.1]	78 [72.2]	0.835
1 [%]	69 [30.7]	148 [28.9]	30 [27.8]	
Clinical pregnancy N [%]	64 [28.4]	131 [25.6]	28 [25.9]	0.631
Miscarriage N [%]	15 [6.7]	28 [5.5]	7 [6.5]	0.789
Ectopic pregnancy N [%]	3 [1.3]	5 [1.0]	0	0.524
Live birth N [%]	53 [23.6]	113 [22.3]	23 [21.5]	0.899
No of viable fetuses [%]				0.843
0 [%]	172 [76.4]	394 [77.9]	84 [78.5]	
1[%]	45 [20.0]	89 [17.6]	20 [18.7]	
2 [%]	8 [3.6]	23 [4.5]	3 [2.8]	

4. Discussion

In the present study, we sought to investigate the possible differences in post vitrification embryo survival rate and treatment outcome between two fertilization methods- conventional IVF and ICSI.

To define appropriately the possibility of an ICSI related injury after a vitrification and thaw process a comparison of the outcome of ICSI derived thawed embryos vs those of conventional IVF derived embryos during the same period and under identical clinical conditions should be done. Only a small number of studies directly address this question.

Simon et al. [2] compared the success rate after transferring frozen – thawed embryos generated from either ICSI or conventional IVF after slow freezing technique. They found that a higher proportion of the ICSI derived embryos survived the freeze and thaw process [92.5% vs 85.6% respectively]. The pregnancy rate, on the other hand, was significantly higher [32.5%] in the conventional IVF group compared to ICSI the group [20%]. They assumed that this difference resulted from the fact that the IVF group included frozen embryos of a higher quality than those of ICSI group. The abortion rate did not differ between the two groups [22% vs 26.8%, respectively]. They concluded that embryos originating from ICSI were not vulnerable to cryopreservation and when implanted resulted in comparable abortion rate to thawed embryos of IVF.

A prospective, controlled clinical study by Hu Y et al. [16] analyzed the effect of freezing early-stage embryos derived from intracytoplasmic sperm injection [ICSI] or from IVF. No significant difference in embryo survival rate [88% and 81% respectively] and implantation rate [18% and 11% respectively] was found between the two groups. However, the clinical pregnancy rate was significantly higher for the ICSI derived embryos [52% vs 25%]. They concluded that early-stage embryos [either zygote or 2-4 cells] derived from ICSI can be frozen with confidence and higher pregnancy rates can be achieved when compared with those from conventional IVF.

Ours is the first study comparing clinical outcomes of the two fertilization methods after transfer of an embryo frozen using the vitrification technique. Our results show that reproductive outcomes including clinical pregnancy, live birth rate and miscarriage rates, are comparable. Regarding live birth rate, we have demonstrated a 22% success rate achieved in either technique.

The strength of our study lays in the large cohort, with minimal demographic differences. Our results align with those of previously published data in slow freezing cycles, and thus help to reaffirm that insemination by ICSI is as safe as conventional IVF also in embryos frozen by the vitrification method. The limitations of the present study are the retrospective data collection methodology with its inherent biases and the relative wide time span of seven years, in which different treatment protocols might have changed, as dictated by the expanding knowledge.

5. Conclusions

In conclusion, the present study found that the type of insemination has no adverse effect on cryopreservation by vitrification. Clinical pregnancies, miscarriages, ectopic pregnancies and live births rates were similar after transfer of cryopreserved embryos originating from IVF, ICSI and both IVF and ICSI embryos derived from sibling oocytes retrieved from the same cycle.

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References

1. Rienzi LF, Iussig B, Dovere L, Fabozzi G, Cimadomo D, Ubaldi FM. Perspectives in gamete and embryo cryopreservation. Semin Reprod Med. 2018 Sep;36(5):253–64.
2. Simon A, Holzer H, Hurwitz A, Revel A, Zentner BS, Lossos F, et al. Comparison of cryopreservation outcome following intracytoplasmic sperm injection and conventional in vitro fertilization. J Assist Reprod Genet. 1998 Aug;15(7):431–7.
3. Liebermann J. Chapter 11 human embryo vitrification. Methods Mol Biol. 2017; 1568:141–59.
4. Balaban B, Urman B, Ata B, Isiklar A, Larman MG, Hamilton R, et al. A randomized controlled study of human Day 3 embryo cryopreservation by slow freezing or vitrification: vitrification is associated with higher survival, metabolism and blastocyst formation. Hum Reprod. 2008 Sep;23(9):1976–82.
5. Liebermann J, Tucker MJ. Comparison of vitrification and conventional cryopreservation of day 5 and day 6 blastocysts during clinical application. Fertil Steril. 2006 Jul;86(1):20–6.
6. Son W-Y, Tan SL. Comparison between slow freezing and vitrification for human embryos. Expert Rev Med Devices. 2009 Jan;6(1):1–7.
7. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. The international committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary on ART terminology, 2009. Hum Reprod. 2009 Nov;24(11):2683–7.
8. Pegg DE. Principles of cryopreservation. Methods Mol Biol. 2015; 1257:3–19.
9. Nagy ZP, Shapiro D, Chang C-C. Vitrification of the human embryo: a more efficient and safer in vitro fertilization treatment. Fertil Steril. 2020;113(2):241–7.
10. Mazur P, Seki S. Survival of mouse oocytes after being cooled in a vitrification solution to -196°C at 95° to 70,000°C/min and warmed at 610° to 118,000°C/min: A new paradigm for cryopreservation by vitrification. Cryobiology. 2011 Feb;62(1):1–7.
11. Roy TK, Brandi S, Peura TT. Chapter 20 Gavi-Automated Vitrification Instrument. Methods Mol Biol. 2017; 1568:261–77.
12. Arav A, Natan Y, Kalo D, Komsky-Elbaz A, Roth Z, Levi-Setti PE, et al. A new, simple, automatic vitrification device: preliminary results with murine and bovine oocytes and embryos. J Assist Reprod Genet. 2018 Jul;35(7):1161–8.
13. al-Hasani S, Ludwig M, Gagsteiger F, Küpker W, Sturm R, Yilmaz A, et al. Comparison of cryopreservation of supernumerary pronuclear human oocytes obtained after intracytoplasmic sperm injection (ICSI) and after conventional in-vitro fertilization. Hum Reprod. 1996 Mar;11(3):604–7.
14. Osmanagaoglu K, Kolibianakis E, Tournaye H, Camus M, Van Steirteghem A, Devroey P. Cumulative live birth rates after transfer of cryopreserved ICSI embryos. Reprod Biomed Online. 2004 Mar;8(3):344–8.

 doi:10.20944/preprints202401.0388.v1

15. Loutradi KE, Kolibianakis EM, Venetis CA, Papanikolaou EG, Pados G, Bontis I, et al. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. Fertil Steril. 2008 Jul;90(1):186–93.
16. Hu Y, Maxson WS, Hoffman DI, Ory SJ, Eager S. A comparison of post-thaw results between cryopreserved embryos derived from intracytoplasmic sperm injection and those from conventional IVF. Fertil Steril. 1999 Dec;72(6):1045–8.