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Elective Cryopreservation of all 2PN Oocytes after in-vitro Maturation: a Prospective, Clinical Pilot Study

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Abstract **Purpose:** To evaluate the effectiveness of temporally splitting in-vitro maturation and embryo transfer by elective cryopreserving of all 2PN oocytes after in–vitro maturation (IVM). **Methods:** This one armed clinical cohort study was prospectively performed. Twenty eight patients fulfilled the inclusion criteria after AFC assessment by transvaginal sonography. Patients were retrospectively divided into (primed) group which received a low dose of gonadotropin stimulation, and (non-primed) group which received no medication. IVM in combination with cryopreservation by vitrification of all 2PN oocytes was investigated for all included patients. **Results:** Mean number of cumulus-oocyte complex (COC) was found to be 9 ±8.4 and the average number of matured oocytes was 4.1 ±4.8. After vitrification and thawing, the overall survival rate was 92.9%. The overall clinical pregnancy rate per WHAT was 17.9%. The cumulative clinical and ongoing pregnancy rate per patient with at least one embryo transfer (ET) performed was 23.8% and 12.1-41.6% for 95% confidence interval (CI). No differences was found for in outcome between primed and non-primed patients in positive hCG results. **Conclusions:** The addition of electively cryopreservation by vitrification of all 2PN stage embryos to IVM technique applied for high AFC in patients undergoing IVF treatment in both primed and natural cycles results in an acceptable chance of ongoing pregnancy.

Keywords: *cryopreservation, PCO, IVM*

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1. Purpose

In-vitro maturation (IVM) is a technique involving the collection of immature oocytes from the ovaries, which are then subjected to in-vitro culture till they reach maturity, a prerequisite for successful fertilization [1]. This method has been proposed as an alternative treatment for women having high antral follicle count (AFC) undergoing In-vitro fertilization (IVF) [2]. The main proposed benefit of IVM is the reduced exposure to gonadotropins and therefore the prevention of risks and burdens associated with high ovarian response to stimulation, including ovarian hyperstimulation syndrome (OHSS) [3]. OHSS is considered a complication of IVF treatment, more precisely, when controlled ovarian stimulation (COS) is used, especially in the presence of polycystic ovaries syndrome (PCOS) [4] or when AFC is ten or more [5]. Applying the cut-off value of AFC as ten was adopted because the effectiveness of it varied between authors. Although some demonstrated it through ten or more follicles [6], others investigated it through less than ten antral follicles [7]. AFC importance and influence on the ovarian response and reserve was demonstrated by many [8]. Due to the big concern of OHSS especially for PCOS patients, many strategies were adopted for its prevention [9]. AFC is considered one of the useful tests for predicting ovarian response after COS [10]. Moreover, IVM represents an attractive option for cases indicated for fertility preservation, like cancer patients, because of the short time necessary to reach oocyte retrieval [11]. IVM could be done after minimal priming with gonadotropins and hCG triggering or simply without giving any medications. It's feasibility to be performed with minimal or without utilization of hormonal stimulation, and the relatively, less appointments for folliculometry make it potentially convenient due to it's low cost [12]. Additionally, it is a suitable strategy for many hormone-sensitive tumor patients undergoing fertility preservation in both follicular and luteal cycle phase [13].

However, the available evidence on the clinical efficacy of IVM suggests that there is still room for improvement of the procedure [14]. The raised concern was about an impaired oocyte developmental competence, as folliculogenesis adopts oocyte capacitation which is believed to be a follicle dependent process. Nevertheless, this process was suggested to be inducible by cytoplasmic activation in-vitro too [15], though this technique is still experimental. Alternatively, an impaired endometrial

receptivity potentially accounts for the relatively poor clinical outcome after IVM due to a too short follicular phase and due to an asynchronized hCG administration before oocyte retrieval [16]. A highly efficacious cryopreservation technique eventually allows distinguishing the consequences of follicular phase events (e.g. oocyte maturation) from luteal phase events (e.g. embryo implantation). Cryopreservation of oocytes or embryos after vitrification ends up with better results compared to the results obtained after conventional slow freezing [17]. In this clinical trial, the feasibility of combining in-vitro maturation with vitrification of all 2PN oocytes for later transfer in frozen-thawed replacement cycle has been investigated.

2. Methods

A single-center, prospective, one-armed, cohort study was performed at the Department of Gynecological Endocrinology and Reproductive Medicine at the University Hospital of Schleswig-Holstein, Campus Lübeck, from 1/2007 until 1/2008.

Screening criteria for eligibility were: female age less than 37 years, indication for ICSI, both ovaries present, no endometriosis AFS III/IV, and no previous poor response to ovarian stimulation. On cycle days 2-5 of the following cycle, the AFC was assessed and only those patients with an AFC of 10 or more were recruited into the study after appropriate counseling and signing the written informed consent sheet. In the course of the study, a first group of patients (primed) received low dose gonadotropin stimulation (150 IU per day) and 5000 IU hCG as soon as the leading follicle reached 10-12 mm size. A second group of patients (non-primed) received no medication at all. In the latter case, OPU was scheduled for the following day that the leading follicle reached 10-12 mm. Sage medium (Comp. Cledone-Germany) was used for IVM. All 2PN oocytes were cryopreserved by the cryotopvitrification method. Embryo transfer (ET) was performed in cycles programmed with trans-dermal estrogen and vaginal progesterone. The primary outcome measure was the cumulative clinical and ongoing pregnancy rate at 10 weeks of gestation per intention-to-treat (ITT). Results are presented as means and standard deviation, or rates with 95% confidence intervals (CI).

2.1. Stimulation Protocol and Ovum Pick up (OPU)

All patients included in the study had spontaneous menstruation or progestin induced withdrawal bleeding, after which patients in the first group received stimulation by gonadotropins (rFSH or menotropin) 150 IU for 4 days starting on menstrual cycle day 2. Hormonal assessment by measuring progesterone, LH, and E2 values and endometrial thickness U/S measurement were done on menstrual cycle days 5 or 6 and thereafter as deemed necessary until the leading follicles reached 10-12 mm. Triggering by hCG was performed by 5000 IU. Oocyte pick-up (OPU) was performed 34-40 hours following hCG injection. Cumulus-oocyte-complexes were retrieved from each follicle larger than 4 mm by puncture with a 17 G double-lumen needle and manual flushing with flushing medium. After ICSI according to standard protocol, oocytes at the pronuclear stage were cryopreserved by vitrification as previously described by Kuwayama [18].

2.2. Artificial Cycles and Endometrial Priming

At first menses day, the endometrial priming started and continued through 14 to 15 days using trans-dermal E2 patches (Estraderm TTS 100) in increasing doses. Hormonal assessment and endometrial thickness measurement were done on day 14 to 15. If endometrial thickness had reached minimally 7 mm, secretory transformation of the endometrium was induced by vaginal progesterone (Crinone 8% once daily). Once embryo availability had been confirmed, ET took place on day 2 of embryonic development, e.g. day three of progesterone action [19].

2.3. Vitrification and Thawing Protocols

All cases underwent the same protocol of vitrification and thawing. At stage of 2PN zygote, open protocol of vitrification took place according to the methods described previously [18,20]. All zygotes were incubated in equilibration solution comprising 7.5% ethylene glycol (EG) (Sigma-Aldrich, Steinheim, Germany), and 7.5% dimethyl sulphoxide (DMSO) (Sigma-Aldrich). Media used was Ham's F-10, with 20% patient serum supplement. Vitrification solution having 15% EG, 15% DMSO, and 0.5 M sucrose (Merck, Darmstadt, Germany), till cellular shrinkage noticed, after which, placing of no more than two zygotes on each Cryotop (Kitazato, Japan) took place. Storage of the Cryotops was in liquid nitrogen.

Thawing solution was (1 M sucrose), it had the zygotes placed in it for 50-60 seconds. After warming, the zygotes were washed and embryo morphological assessment and scoring was done in preparation for ET.

3. Results

Forty patients were prospectively registered and enrolled. Of those, 28 fulfilled all the inclusion criteria on the day of AFC assessment in the planned treatment cycle and proceeded to ovum pick up.

As presented in Table 1, the mean number of COC was 9 ±8.4 of which on average 40% matured to form a mean number of 4.1 ±4.8 mature oocytes. After ICSI, 3.5 ±2.8 2PN oocytes were on average available for vitrification. The average number of embryos after vitrification/thawing technique post IVM and ICSI for transfer was 1.3 ± 0.6 embryos per patient.

Table 1.

	Mean ±SD
Number of COC's	9.0 ±8.4
Number of mature oocytes	4.1 ±4.8
Number of 2PN	3.5 ±2.8
ET/patient	1.3 ±0.6

SD = Standard Deviation; COC's = cumulus oocyte complex's
PN = Pronuclei; ET = Embryo Transfer

Table 2 shows that after vitrification and thawing the embryos, the survival rate was 92.9%. Two patients had not undergone a frozen-thawed cycle yet. The overall clinical pregnancy rate was 5 pregnancies out of 28,

resulting in 17.9% (95% CI: 9.0-32.5%). The cumulative clinical and ongoing pregnancy rate per patient with at least one ET performed was 5/21, showing 23.8% and 12.1-41.6% for 95% CI. The ongoing pregnancy rate per first frozen-thawed cycle was 2/21, giving 9.5% and 3.2-25.1% for 95% CI. The number of cases experienced fertilization or maturation failure was in five patients out of 28. No differences in the outcome of both primed and non-primed group in positive hCG results although the primed group had slightly higher figure. The ongoing pregnancy per first FET was in favour of the primed group as the non-primed group didn't have any. Of the five clinical pregnancies, two (16.66) belonged to the non-primed group and three (18.75%) to the primed group as shown in Table 3.

Table 2.

Survival rate after vitrification and thawing	92.9%	
Clinical pregnancy rate	17.9%	95% CI 9.0-32.5%
Clinical and ongoing pregnancy/patient with at least one ET	23.8%	95% CI 12.1-41.6%
Ongoing pregnancy/first FET cycle	9.5%	95% CI 3.2-25.1%

CI = Confidence Interval; ET = Embryo Transfer; FET = Frozen Embryo Transfer

Table 3.

Results	Primed group (16)	Non primed group (12)
Positive hCG	3/16 (18.75%)	2/12 (16.66%)
Ongoing pregnancy/first FET cycle	2/13 (15.4%)	0/8 (0%)

hCG= human Chorionic Gonadotropin; FET = Frozen Embryo Transfer

4. Conclusions

This study is proof of the concept that IVM combined with elective cryopreservation will result in an acceptable chance of ongoing pregnancy in patients intended to be treated with IVM, who present with an AFC of ≥10. It is however necessary to obtain a more precise estimate of the true treatment effect by conducting larger sized studies. Nevertheless, the present results compare favourably with our historical controls (von Otte et al., GeburtshFrauenheilk 2007; 67: 1009–1017) of 57 patients with fresh embryo transfer after IVM. We suggest that this concept warrants further clinical studies.

According to the German law at this study writing time, all embryos were vitrified at 2PN stage, and without selection or grading. AFC was introduced in the IVF treatment as a potential predictive marker for ovarian response to fit the patient in the optimal treatment protocol [21]. Being performed lately for lower and higher responders prediction, made AFC a reliable test to be adopted in our study. However, after measuring AFC and espousing the value of 10 or more follicles to be the cut-off, this trial presents the first model illustrating the importance of transferring the cryopreserved embryos after performing the IVM technique. All that was done prospectively, in groups of primed and natural cycles for IVF.

Cryopreservation after IVM showed success long time back by the delivery of baby product of their association [22]. Cryopreservation through vitrification became recently adopted as the golden method for many centres. Vitrification method gained popularity after the successful results when compared to slow freezing and in achieving good clinical pregnancy rate (CPR) [23]. Moreover, cryopreservation by vitrification survival results overweighed the slow cryopreservation ones in cycles preceded by IVM [24].

According to the given results of survival rate after vitrification and thawing, we find performing them in association with IVM is reasonable method to be implemented. The given survival rate result of >92% after IVM, could be comparable with the conventional IVF survival results post vitrification and thawing. We state that as such, because the developmental capabilities of our embryos was not tested, as they were vitrified at zygote stage before grading. Nevertheless, we understand that our study group is not as big as others, but still the results figures are promising compared to their's [25].

The poor outcome as yet held by IVM due to impaired endometrial receptivity was noted by some [16, 26]. PCO patients might be featuring that challenge by their endometrial improper thickness. In contrast, others noted IVM as the helping method for understanding the folliculogenesis complex [27]. In our trial, we tried to circumvent this difficulty featured by follicular developmental impairment by freezing all zygotes, to give room for synchronisation between embryo at certain stage and endometrium by priming.

The measurers of our outcome are acceptable but still not comparable to other studies done for the combination of IVM and freeze all strategy [28].

Conclusion: This study is proof of the concept that IVM combined with elective cryopreservation will result in an acceptable chance of ongoing pregnancy in patients intended to be treated with IVM, who present with an AFC of ≥10. The results given irrespectively, whether treatment cycle was primed or natural. It is necessary to obtain a more precise estimate of the true treatment effect by conducting larger sized studies. We suggest that this concept warrants further clinical studies.

References

[1] Hardy K, Wright CS, Franks S, Winston RM. In vitro maturation of oocytes. Br Med Bull. 2000; 56 (3): 588-602.

[2] Wiser A, Shehata F, Holzer H, Hyman JH, Shalom-Paz E, Son WY, Tulandi T. Effect of high LH/FSH ratio on women with polycystic ovary syndrome undergoing in vitro maturation treatment. J Reprod Med. 2013; 58 (5-6): 219-23.

[3] De Vos M, Ortega Hrepich C, Albuz FK, Guzman L Polyzos NP, Smitz J,Devroey P. Clinical outcome of non-hCG-primed oocyte in vitro maturation treatment in patients with polycystic ovaries and polycystic ovary syndrome. FertilSteril 2011; 96: 860-4.

[4] Jacobs HS, Agrawal R.Complications of ovarian stimulation. Baillieres Clin Obstet Gynaecol. 1998; 4: 565-79.

[5] Broer SL, Dólleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. Human Reprod Update 2011; 17 (1): 46-54.

[6] Kwee J, Schats R, McDonnell J, ThemmenA, de Jong F, Lambalk C. Evaluation of anti-Müllerian hormone as a test for the prediction of ovarian reserve. FertilSteril 2008; 90 (3): 737-743.

[7] Pelin O, Sezai S, Meral C, Tulay I, Onur G, and Ismail C. Serum anti-Müllerian hormone and antral follicle count as predictive markers of OHSS in ART cycles. J Assist Reprod Genet. 2011; 28 (12): 1197-1203.

[8] Wiser A, Shalom-Paz E, Hyman JH, Sokal-Arnon T, Bantan N, Holzer H, Tulandi T. Age-related normogram for antral follicle

count in women with polycystic ovary syndrome. Reprod Biomed Online. 2013; 27 (4): 414-8.

[9] Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. Human Reprod 2011; 26 (10): 2593-7.

[10] Himabindu Y, Sriharibabu M, Gopinathan K, Satish U, Louis TF, Gopinath P. Anti-mullerian hormone and antral follicle count as predictors of ovarian response in assisted reproduction. J Hum Reprod Sci. 2013; 6 (1): 27-31.

[11] Diedrich K, Fauser BC, Devroey P; Evian Annual Reproduction (EVAR) Workshop Group 2009. Cancer and fertility: strategies to preserve fertility. Reprod Biomed Online. 2011;22(3):232-48.

[12] Roesner S, Von Wolff M, Eberhardt I, Beuter-Winkler P, Toth B, Strowitzki T. In vitro maturation: a five-year experience. Acta Obstet Gynecol Scand. 2012; 91 (1): 22-7.

[13] Ronn R, Holzer HE. Oncofertility in Canada: cryopreservation and alternative options for future parenthood. Curr Oncol. 2014 Feb; 21 (1): e137-46.

[14] Álvarez C, García-Garrido C, Taronger R, González de Merlo G. In vitro maturation, fertilization, embryo development & clinical outcome of human metaphase-I oocytes retrieved from stimulated intracytoplasmic sperm injection cycles. Indian J Med Res. 2013; 137 (2): 331-8.

[15] Mermillod P, Oussaid B, Cognié Y. Aspects of follicular and oocyte maturation that affect the developmental potential of embryos. JReprodFertil Suppl. 1999; 54: 449-60.

[16] Buckett WM, Chian RC, Tan SL. Human chorionic gonadotropin for in vitro oocyte maturation: does it improve the endometrium or implantation? Reprod Med. 2004; 49 (2): 93-8.

[17] Levron J, Leibovitz O, Brengauz M, Gitman H, Yerushalmi GM, Katorza E, Gat I, Elizur SE. Cryopreservation of day 2-3 embryos by vitrification yields better outcome than slow freezing. Gynecol Endocrinol. 2014; 30 (3): 202-4.

[18] Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online. 2005; 11 (3): 300-8.

[19] Griesinger G, Weig M, Schroer A, Diedrich K, Kolibianakis EM. Mid-cycle serum levels of endogenous LH are not associated with the likelihood of pregnancy in artificial frozen-thawed embryo transfer cycles without pituitary suppression. Hum Reprod. 2007; 22 (10): 2589-93.

[20] Al-Hasani S, Ozmen B, Koutlaki N, Schoepper B, Diedrich K, Schultze-Mosgau A.Three years of routine vitrification of human zygotes: is it still fair to advocate slow-rate freezing? Reprod Biomed Online. 2007; 14 (3): 288-93.

[21] Kwee J, Elting ME, Schats R, McDonnell J, LambalkCB. Ovarian volume and antral follicle count for the prediction of low and hyper responders with in vitro fertilization. Reprod Biol Endocrinol. 2007; 5: 9.

[22] Tucker MJ, Wright G, Morton PC, Massey JB. Birth after cryopreservation of immature oocytes with subsequent in vitro maturation. FertilSteril. 1998; 70 (3): 578-9.

[23] Beyer DA, Amari F, Diedrich K, Al Hasani S. Embryo survival after vitrification of 2 PN oocytes. Middle East FertilSoc J. 2011; 16 (1): 45-49.

[24] Son WY, Chung JT, Gidoni Y, Holzer H, Levin D, Chian RC, Tan SL. Comparison of survival rate of cleavage stage embryos produced from in vitro maturation cycles after slow freezing and after vitrification. FertilSteril. 2009; 92 (3): 956-8.

[25] Pavone ME, Innes J, Hirshfeld-Cytron J, Kazer R, Zhang J. Comparing thaw survival, implantation and live birth rates from cryopreserved zygotes, embryos and blastocysts.J Hum Reprod Sci. 2011; 4 (1): 23-28.

[26] Lucena E, Moreno-Ortiz H. Granulocyte colony-stimulating factor (G-CSF): a mediator in endometrial receptivity for a patient with polycystic ovary (PCO) undergoing in vitro maturation (IVM). BMJ Case Rep. 2013.

[27] Xu J, Xu M, Bernuci MP, Fisher TE, Shea LD, Woodruff TK, Zelinski MB, Stouffer RL. Primate follicular development and oocyte maturation in vitro. Adv Exp Med Biol. 2013; 761: 43-67.

[28] Ortega-Hrepich C, Stoop D, Guzmán L, Van Landuyt L, Tournaye H, Smitz J, De Vos M. A ''freeze-all'' embryo strategy after in vitro maturation: a novel approach in women with polycystic ovary syndrome? FertilSteril. 2013; 100 (4): 1002-7.