A "freeze-all" embryo strategy after in vitro maturation: a novel approach in women with polycystic ovary syndrome?

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Objective: To assess the efficiency of a "freeze-all" embryo strategy after immature oocyte retrieval (OR) and in vitro maturation (IVM) in patients with polycystic ovary syndrome (PCOS).
Design: Retrospective case series.
Settings: University-based tertiary referral center.
Patient(s): Seventy-nine consecutive PCOS patients undergoing IVM followed by vitrified-warmed embryo transfer (ET) over a 2-year period.
Intervention(s): All patients received 150 IU/d highly purified hMG for 3 consecutive days. There was no hCG trigger given before OR. All day-3 embryos of good morphologic quality were vitrified. Single or double ET was performed in 114 consecutive artificial cycles. The cumulative live birth rate (LBR) per patient was calculated, as well as the projected cumulative LBR.
Main Outcome Measure(s): LBR per patient and per retrieved immature oocyte.
Result(s): Mean age, body mass index, and antimüllerian hormone were 28.5 ± 3.5 years, 27.8 ± 7.1 kg/m^2, and 10.3 ± 5.5 μg/L, respectively. In total, 1,526 cumulus-oocyte complexes were retrieved. IVM yielded 800 metaphase II oocytes (52.4%), and 291 day-3 embryos were cryopreserved. Of these, 224 (76.9%) embryos were warmed. One hundred seventy-one survived (76.3%), and 105 ETs were performed. LBR per ET was 16.2% and the cumulative LBR per patient was 21.8%. LBR per retrieved immature oocyte was 1.1%. The projected LBR per patient was 24.2%.
Conclusion(s): IVM followed by a "freeze-all" embryo strategy is a novel approach for women with PCOS. Patients who undergo IVM should be advised that each immature oocyte retrieved yields a 1.1% chance to achieve a live birth. (Fertil Steril® 2013;100:1002–7. ©2013 by American Society for Reproductive Medicine.)
Key Words: In vitro maturation, embryo cryopreservation, PCOS, OHSS

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Oocyte in vitro maturation (IVM) is an assisted reproductive technology (ART) in which oocytes are collected from the antral follicles of unstimulated or minimally stimulated ovaries (1). IVM has been applied mainly in patients with an increased risk of ovarian hyperstimulation syndrome (OHSS), particularly in patients with polycystic ovary syndrome (PCOS) or ultrasound-only polycystic ovaries (PCO), but has not yet been introduced in routine clinical practice, because clinical outcomes are currently inferior compared with conventional hormone-driven ART (2) and because of concerns about potential epigenetic consequences of IVM (3, 4). Recent years have seen the emergence of promising strategies to reduce the risk of OHSS in patients with PCOS, including ovarian stimulation with a GnRH antagonist protocol followed by a GnRH agonist

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ovulation trigger (5) and treatment segmentation with elective freezing of all oocytes or embryos (6). Although these strategies can reduce OHSS incidence significantly, they do not completely eliminate the occurrence of OHSS in high-risk patients (7); in these patients, IVM is a potential viable method to prevent OHSS (8). Apart from its role as reproductive treatment, IVM has also emerged as a promising tool for emergency fertility preservation (9–13). With the use of IVM, cryopreservation of oocytes or embryos can be achieved with little or no hormonal pretreatment, which is highly relevant for patients with hormone-sensitive tumors, and can be performed flexibly and at short notice, after immature oocyte retrieval in either the follicular phase or the luteal phase (14, 15).

Since the introduction of IVM in our center, as a mild-approach ART for predicted high responders to gonadotropins, a non–hCG-triggered IVM system has been used to avoid the aspiration of a mixture of immature and mature oocytes at the time of oocyte retrieval. No large prospective studies exist that have compared clinical outcomes of non–hCG-triggered IVM and hCG-triggered IVM in patients with PCOS. Using this protocol we observed very poor clinical outcomes when fresh embryos were transferred, with a clinical pregnancy rate (CPR) of <10% per cycle. However, clinical outcomes were significantly improved when vitrified-warmed embryos were transferred in artificial cycles [CPR 31.8% (P=.033) (16)]. This observation led us to suggest that endometrial quality in non–hCG-triggered IVM cycles may be suboptimal and urged us to adopt a strategy in which patients are offered elective vitrification of all day-3 embryos of good morphologic quality instead of fresh embryo transfer, followed by transfer of warmed embryos in a later artificial cycle.

The aim of the present study was to assess the efficiency of immature oocyte retrieval followed by IVM and elective embryo cryopreservation in women with PCOS and to calculate the potential live birth rate per patient and per retrieved immature oocyte.

MATERIALS AND METHODS

Patient Population

This study is a retrospective analysis of the clinical outcomes in patients with PCOS who underwent IVM and an elective "freeze-all" embryo strategy followed by vitrified-warmed embryo transfer (ET) in an artificial endometrial priming cycle. Data extraction was performed from records of all PCOS patients who had not previously undergone IVM and who were offered a IVM cycle with the intention to freeze all embryos of good morphologic quality on day 3 after intracytoplasmic sperm injection (ICSI). The study was performed from January 2010 to December 2012. The diagnosis of PCOS was established according to the 2003 Rotterdam criteria (17, 18).

The elective "freeze-all" embryo strategy was approved by the local Ethical Committee, and each of the patients provided written informed consent for data analysis.

Ovarian Stimulation and Oocyte Retrieval

Ovarian stimulation in IVM cycles was performed as previously described (16). Briefly, on day 3 after spontaneous menses or progestin-induced withdrawal bleeding, all patients received 150 IU highly purified hMG (hp-hMG; Menopur; Ferring Pharmaceuticals) daily for 3 days. No hCG trigger was administered before oocyte retrieval. Oocyte retrieval was performed 42 hours after the last injection of hp-hMG and before the development of a dominant follicle. Immature oocytes were retrieved with a 17-gauge single-lumen needle (K-OPS-1230-VUB; Cook Medical) at an aspiration pressure of −70 mm Hg. Follicular aspirates were filtered (Falcon 1060; 70 mm mesh size) and cumulus-oocyte complexes (COCs) were collected from the culture dish. Collected COCs were matured for 40 hours in the Medicult IVM System (Origio) supplemented with 75 mIU/mL recombinant FSH (rFSH), 100 mIU/mL hCG, and 10% human albumin solution (Vitrolife). After IVM, mature oocytes were inseminated by ICSI as previously described (19). Oocytes and embryos were cultured individually in 25-mL medium droplets covered with mineral oil. Embryos were cultured until day 3 after ICSI in sequential Sage cleavage media (Cooper Surgical).

Embryo Freezing

Embryos with good morphology in the morning of day 3 after ICSI were selected for vitrification. Embryos were vitrified if at least six blastomeres with ≤20% fragmentation were present, and embryos with a fragmentation rate of 20%–50% were vitrified if they had reached the 8-cell stage. Occasionally, embryos with multinucleated blastomeres or irregular cell size were vitrified and scored as moderate-quality embryos. Embryos with >50% multinucleated blastomeres were not vitrified.

Vitrification and Warming Protocols

Embryo vitrification was performed with the use of closed CBS-VIT High Security (HS) straws (Cryo Bio system) in combination with DMSO-EG-S as cryoprotectant (Irvine Scientific Freeze and Thaw Kit). The vitrification and warming procedure were carried out as described previously (20). Embryos were vitrified individually. The straw was heat sealed and plunged into liquid nitrogen. The total time needed to vitrify the embryo starting from vitrification droplet I to the loading of the straw and plunging into liquid nitrogen did not exceed 90 seconds. The embryos with best morphologic quality were warmed one by one until one or two embryos of good quality were suitable for transfer. Warming of IVM embryos occurred 1 day before ET. After warming, morphologic survival was assessed. Only embryos with ≥50% of blastomeres intact were cultured overnight. If the embryo did not survive, an extra embryo was warmed until one or two embryos were available for overnight culture.

Artificial Endometrial Priming and Vitrified-Warmed Embryo Transfer

Vitrified-warmed embryos were transferred in an artificial endometrial priming cycle after priming of the endometrium with transdermal E_2 gel (2 mg administered three times a day) (Oestrogel; Besins Healthcare). When an endometrial

thickness of >6 mm was reached, luteal phase support was started with the use of intravaginal micronized progesterone tablets (200 mg three times a day; Utrogestan; Besins Healthcare). Transfer of one or two embryos was scheduled 5 days later. As a rule, one or two embryos were warmed for transfer unless only one cryopreserved embryo was available. In case of nonsurvival, further embryos were warmed until one embryo was available for transfer. ET was performed under ultrasound guidance with the use of a soft catheter (K-soft 5100; Cook).

Outcome Parameters

Live birth rate per patient after transfer of embryos electively cryopreserved after IVM was the main outcome of this study. We additionally calculated the projected live birth rate per patient. Embryologic and clinical outcomes (including clinical pregnancy rate and ongoing pregnancy rate) were secondary outcomes.

Statistical Analysis

Data were analyzed with the use of SPSS 20.0 statistical software.

RESULTS

From January 2010 to December 2012, 79 consecutive patients with PCOS underwent 79 IVM treatment cycles according to an elective "freeze-all" embryo strategy. Twenty-five patients had oligomenorrhea and 54 amenorrhea. Sixty-three patients had primary infertility and 16 secondary infertility, with a mean duration of infertility of 3.1 ± 3.0 years. Additionally, three patients had a partner with severe oligoasthenoteratospermia requiring testicular sperm extraction for ICSI. Seven patients had a history of smoking. Mean age, body mass index (BMI), and antimüllerian hormone were 28.5 ± 3.5 years, 27.8 ± 7.1 kg/m^2, and 10.3 ± 5.5 μg/L, respectively. Patient baseline characteristics are presented in Table 1.

Laboratory Outcome

In total, 1,526 oocytes were retrieved, of which 800 oocytes reached metaphase II (MII) after IVM (52.4% maturation rate), and eventually 508 oocytes were fertilized (63.5% fertilization rate). Two hundred ninety-one embryos were cryopreserved, including 150 embryos (51.5%) of excellent morphologic quality, 114 embryos (39.2%) of good quality, and 27 embryos (9.3%) of moderate quality. Overall, the average number of oocytes retrieved per patient was 19.6 ± 12.5, the average number of vitrified embryos per patient was 3.7 ± 2.6, and the average number of cryopreserved embryos of excellent quality per patient was 1.9 ± 1.9 (Table 2).

Clinical Outcome after a "Freeze-All" Embryo Strategy

At the time of writing, 78 out of 79 patients had undergone 114 artificial endometrial priming cycles and 224 cryopreserved embryos had been warmed. Of those, 117 (52%) had an excellent morphology before freezing, 84 (38%) had been graded as good-quality embryos before freezing and 23 (10%) had been classified as moderate-quality embryos. Of these, 171 vitrified embryos survived the warming process (an embryo survival rate of 76.3%). A mean of 1.5 embryos were transferred. In 7.9% (9/114) of artificial cycles, no ET was performed owing to poor embryo survival after warming. Twenty-six patients (26/78) underwent at least two consecutive artificial cycles, either because no pregnancy was achieved or no ET was performed in the preceding artificial cycle.

Overall, vitrified-warmed ET resulted in a clinical pregnancy rate, ongoing pregnancy rate, and live birth rate of 35.9% (28/78), 24.4% (19/78), and 21.8% (17/78) per patient, respectively; 1.8% (28/1,526), 1.2% (19/1,526), and 1.1% (17/1,526) per retrieved oocyte, respectively; 9.6% (28/291), 6.5% (19/291), and 5.8% (17/291) per vitrified embryo, respectively; and 26.7% (28/105), 18.1% (19/105), and 16.2% (17/105) per ET, respectively. The implantation and miscarriage rates were 16.3% (28/171) and 32.1% (9/28), respectively

TABLE 1

Baseline patient characteristics.

No. of IVM patients	79
No. of IVM oocyte retrievals	79
Age (y)	28.5 ± 3.5
BMI (kg/m^2)	27.8 ± 7.1
AMH (μg/L)	10.3 ± 5.5
Testosterone	0.43 ± 0.19
FTc	6.7 ± 4.8
AFC	46.2 ± 15.6
Basal serum FSH (mIU/mL)	5.2 ± 1.6
Basal serum E_2 (ng/L)	46.3 ± 17.2
Basal serum P (μg/L)	0.70 ± 0.58
Basal serum LH (mIU/mL)	7.9 ± 5.2

Note: Values presented as mean ± SD. AFC = antral follicle count; AMH = antimüllerian hormone; BMI = body mass index; FTc = calculated free testosterone; IVM = In vitro maturation.

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TABLE 2

Laboratory outcome: embryo cryopreservation after in vitro maturation.

Total COC retrieved	1,526	
Mean COC retrieved	19.6 ± 12.5	
Total MII oocytes, n (%)	800 (52.4)	
Mean MII oocytes	10.2 ± 6.9	
Total 2PN, n (%)	508 (63.5)	
Mean 2PN	6.5 ± 4.9	
Cryopreserved embryos	Total	Mean
Total	291	3.7 ± 2.6
Excellent quality, n (%)	150 (51.5)	1.9 ± 1.9
Good quality, n (%)	114 (39.2)	1.4 ± 1.5
Moderate quality, n (%)	27 (9.3)	0.3 ± 0.6

Note: Values presented as mean ± SD. COC = cumulus oocyte complex; MII = metaphase II; 2PN = two pronuclei; Excellent quality = at least seven blastomeres and ≤10% fragmentation; Good quality = at least six blastomeres and ≤20% fragmentation or at least eight blastomeres with 20%–50% fragmentation; Moderate quality = at least six blastomeres and ≤20% fragmentation with irregular cell size or multinucleated blastomeres in <50% of cells.

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(Table 3). When analyzing miscarriage rates within different BMI groups [according to the World Health Organization classification (21)] no statistically significant differences were observed (P=.458; Supplemental Table 1, available online at www.fertstert.org), although the number of patients in each BMI group was too low to make strong conclusions.

Clinical Outcome after Single-Embryo Transfer

Thirty-nine SET procedures were performed in 31 patients. In 15 of these transfer cycles, elective single-embryo transfer (SET) was performed (22), in 10 cycles SET was performed after warming two or more embryos (and only one embryo eventually survived the warming process), and in 14 cycles SET was performed because only one embryo was cryopreserved.

Overall, SET resulted in a clinical pregnancy rate, an ongoing pregnancy rate, and a live birth rate of 25.8% (8/31), 16.1% (5/31), and 16.1% (5/31) per patient, respectively, and 20.5% (8/39), 12.8% (5/39), and 12.8% (5/39) per ET, respectively. The implantation and miscarriage rates were 20.5% (8/39) and 37.5% (3/8), respectively (Table 3).

Projected Cumulative Live Birth Rates

The projected cumulative live birth rate per patient was calculated by extrapolating the outcome of the remaining vitrified embryos in women without a live birth. In total, 67 embryos remained cryopreserved, of which 24 belonged to patients without a live birth. Therefore, the projected live birth rate per patient after a "freeze-all" embryo strategy after IVM would increase to 24.2% (Table 4).

TABLE 3

Clinical outcome: frozen embryo transfer cycles, % (n).

	All	SET	DET
No. of patients	78	31	50
No. of started FET	114	NA	NA
No. of warming cycles with ET	105	39	66
No. of embryos transferred	171	39	132
No. of cancelled cycles, n (%)	9 (7.9)	NA	NA
hCG (+)			
/patient	42.3 (33/78)	29.0 (9/31)	48.0 (24/50)
/oocyte retrieval	41.8 (33/79)	NA	NA
/retrieved immature oocyte	2.2 (33/1,526)	NA	NA
/matured oocyte	4.1 (33/800)	NA	NA
/embryo vitrified	11.3 (33/291)	NA	NA
/started FET cycle	28.9 (33/114)	29.0 (9/39)	36.4 (24/66)
/embryo transfer	31.4 (33/105)	29.0 (9/39)	36.4 (24/66)
/embryo transferred	19.2 (33/171)	29.0 (9/39)	18.2 (24/132)
Clinical pregnancy			
/patient	35.9 (28/78)	25.8 (8/31)	40.0 (20/50)
/oocyte retrieval	35.4 (28/79)	NA	NA
/retrieved immature oocyte	1.8 (28/1,526)	NA	NA
/matured oocyte	3.5 (28/800)	NA	NA
/vitrified embryo	9.6 (28/291)	NA	NA
/started FET cycle	24.6 (28/114)	20.5 (8/39)	30.3 (20/66)
/embryo transfer	26.7 (28/105)	20.5 (8/39)	30.3 (20/66)
/embryo transferred	16.4 (28/171)	20.5 (8/39)	15.1 (20/132)
Ongoing pregnancy			
/patient	24.4 (19/78)	16.1 (5/31)	28.0 (14/50)
/oocyte retrieval	24.0 (19/79)	NA	NA
/retrieved immature oocyte	1.2 (19/1,526)	NA	NA
/matured oocyte	2.3 (19/800)	NA	NA
/vitrified embryo	6.5 (19/291)	NA	NA
/started FET cycle	16.7 (19/114)	12.8 (5/39)	21.2 (14/66)
/embryo transfer	18.1 (19/105)	12.8 (5/39)	21.2 (14/66)
/embryo transferred	11.1 (19/171)	12.8 (5/39)	10.6 (14/132)
Live births			
/patient	21.8 (17/78)	16.1 (5 /31)	24.0 (12/50)
/oocyte retrieval	21.5 (17/79)	NA	NA
/retrieved immature oocyte	1.1 (17/1,526)	NA	NA
/matured oocyte	2.1 (17/800)	NA	NA
/vitrified embryo	5.8 (17/291)	NA	NA
/started FET cycle	14.9 (17/114)	12.8 (5/39)	18.2 (12/66)
/embryo transfer	16.2 (17/105)	12.8 (5/39)	18.2 (12/66)
/embryo transferred	9.9 (17/171)	12.8 (5/39)	9.1 (12/132)
Implantation rate	16.3 (28/171)	20.5 (8/39)	15.1 (20/132)
Miscarriage rate/ET	32.1 (9/28)	37.5 (3/8)	30.0 (6/20)

Note: DET = double-embryo transfer; ET = embryo transfer; FET = frozen embryo transfer; NA = not applicable; SET = single-embryo transfer.

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TABLE 4

Overall embryo survival rate after warming.

Total cryopreserved embryos	291
Total thawed embryos	224
Total embryos transferred	171
Survival rate after thawing (%)	76.3
Remaining cryopreserved embryos	67
Remaining cryopreserved embryos in patients without LB	24
LB per embryo transferred	0.1 (17/171)
Extrapolated LB from unthawed embryos in women without LB	2.4 (24 × 0.1)
Projected LB per patient	24.2 (21.8 + 2.4)

Note: LB = live birth.

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DISCUSSION

This is the first study reporting clinical outcomes after vitrified-warmed ET following a "freeze-all" embryo strategy in infertile patients with PCOS undergoing IVM.

Embryo cryopreservation has become a well established adjuvant technique in current conventional ART. The efficiency of cryopreservation significantly improved after the introduction of vitrification, which combines extreme cooling rates (23) with a high concentration of cryoprotectants and completely eliminates the formation of ice-crystal (24). Multiple studies comparing slow freezing and vitrification after ART show a higher embryo survival rate and less deleterious effects on postwarming embryo morphology after vitrification (25–27). Similar results have been observed with cryopreserved embryos generated after oocyte IVM (28).

The results presented here, i.e., a maturation rate of 52.4%, a fertilization rate of 63.5%, and an embryo survival rate after warming of 76.4%, are in accordance with results reported in earlier studies (11, 29–31). However, survival rates after warming of embryos generated after conventional ART are >90% (32, 33); this leads us to conclude that the postwarming survival rate of IVM embryos is lower than that of embryos generated after conventional ART, which may reflect the intrinsically lower developmental potential of IVM embryos.

The data of the present study show that a "freeze-all" embryo strategy results in live birth rates of 21.8% per patient after one IVM cycle. Furthermore, the projected live birth rate per patient increases to 24.2% when the remaining cryopreserved embryos belonging to patients without live birth are taken into account. Overall, the live birth rate could also be expressed as 1.1% per retrieved immature oocyte and 2.1% (17/800) per mature oocyte. In comparison, the live birth rate per retrieved oocyte after conventional ovarian stimulation was 4.47% in the study by Stoop et al. (34), which is approximately twice as high.

In our study, the miscarriage rate of 32.1% was remarkably high. No correlation was found between BMI and miscarriage rates in this patient population. Whether increased miscarriage rates after IVM could be related to the procedure itself or to the PCOS condition of the patients remains a matter of controversy. Clinical miscarriage rates after IVM vary substantially among reported series but have ranged to as high as 50% (35, 36). Buckett et al. reported significantly higher miscarriage rates in patients undergoing IVM compared with IVF/ICSI (P=.0049), but found no significant differences when comparing patients with PCOS undergoing IVM or IVF (24.5% vs. 22.2%; P=.72). Consequently, the authors suggested that miscarriage rates appear to be related to the polycystic ovarian syndrome itself rather than to the IVM procedure (37). Nevertheless, oocyte IVM may have a deleterious effect on the organization of the meiotic spindle and chromosomes of immature oocytes. This could result in significantly decreased developmental competence of IVM embryos, lower implantation rates, and an increased incidence of miscarriages (38).

Although the data of the present study may need to be interpreted with caution owing to the retrospective design, they support that transfer of vitrified-warmed embryos generated after IVM is a promising method to improve low clinical outcomes after fresh ET in a non–hCG-primed IVM system. This method circumvents the potential problem of suboptimal endometrial quality in non–hCG-triggered IVM cycles and provides good clinical outcomes after frozen ET. Although recent studies have shown markedly improved clinical outcomes after fresh ET in a in non–hCG-triggered IVM system (39), further studies are necessary to improve the endometrial receptivity in this IVM system and to enhance clinical outcomes after fresh IVM ET. If IVM is to emerge as a simplified, patient-friendly, and OHSS-free alternative for conventional ART, concerted efforts are necessary to improve oocyte potential after IVM and to improve endometrium quality.

In conclusion, transfer of vitrified-warmed embryos generated after IVM and a "freeze-all" strategy has potential as an OHSS-free ART in patients with PCOS, especially when fresh ET after IVM in a non–hCG-triggered system yields poor results. Patients with PCOS who undergo IVM for embryo cryostorage should be advised that each immature oocyte retrieved yields a chance of ~1% to achieve a live birth, based on the results of this study.

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SUPPLEMENTAL TABLE 1

Body mass index (BMI) and miscarriage rate in patients with polycystic ovary syndrome after elective "freeze-all" embryo strategy following in vitro maturation.

BMI classification[a]	Miscarriage rate
Underweight (BMI <18.50 kg/m^2)	0% (0/0)
Normal weight (BMI 18.50–24.99 kg/m^2)	30% (3/10)
Overweight preobese (BMI 25.00–29.99 kg/m^2)	42.8% (3/7)
Obese class I (BMI 30.00–34.99 kg/m^2)	0% (0/5)
Obese class II (BMI 35–39.99 kg/m^2)	50% (1/2)
Obese class III (BMI ≥40 kg/m^2)	50% (2/4)

[a] Body mass index according to the World Health Organization classification (20).

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