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Original article

Elective frozen-thawed embryo transfer (FET) in women at risk for ovarian hyperstimulation syndrome

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ABSTRACT

Elective cryopreservation of cultured embryos has become a treatment option for women at risk for ovarian hyperstimulation syndrome (OHSS). The aim of our study was to investigate the outcome of elective cryopreservation and consecutive frozen-thawed embryo transfer (FET) in a large IVF clinic in Austria. A total of 6104 controlled ovarian hyperstimulation cycles (COH) were performed on 2998 patients including 200 patients (6.7%) who were undergoing elective cryopreservation and FET due to high risk of OHSS. We estimated the cumulative live birth rate using the Kaplan-Meier method and evaluated independent predictors for successful live births with a Cox model. A total of 270 frozen-thawed embryo transfers were performed on 200 patients with up to 4 transfers per patient. The first embryo transfer showed a live birth rate of 42.0%, the second transfer showed a cumulative rate of 58.5%. After a total of 4 FETs from the same COH cycle, a cumulative live birth rate of 61.0% per COH cycle could be achieved. Four cases of OHSS occurred amongst these patients (2.0%), all of them of moderate severity. Multivariate analysis identified maternal age, the use of assisted hatching and the number of embryos transferred at the blastocyst stage as independent predictors for cumulative live birth. Our study clearly suggests that elective FET is safe and shows excellent cumulative live birth rates. This concept can, therefore, be used to avoid the severe adverse events caused by COH and the inefficient use of cultured embryos.

1. Introduction

Ovarian hyperstimulation syndrome (OHSS) is a known iatrogenic complication of ovarian stimulation during assisted reproductive technology (ART). The relatively low incidence of OHSS among patients undergoing ovarian stimulation often leads to an underestimation of the importance of this syndrome and the impact it can have.

Previous reports have shown a statistically significant increase in pregnancy–related complications in women who suffer from OHSS compared with in vitro fertilization (IVF) controls [1,2]. As a result, several strategies have been implemented clinically in order to reduce the risk of OHSS through controlled ovarian hyperstimulation (COH). Although the incidence of severe OHSS is now low (1–2%), several reports indicate an increase in this form of the syndrome and in the percentage of patients requiring hospitalization [1,3–5]. The most worrying consequence of OHSS is that it poses a serious threat to patient welfare, as it remains a significant source of morbidity and mortality in ART [6–9]. Several treatment options have focused on overcoming the risk of OHSS but full prevention has never been achieved. Cancelling a cycle before human chorionic gonadotropin (hCG) administration or early oocyte retrieval still remains the most widely applied method of prevention but this can be frustrating and costly for the patient concerned [5,10].

It has been suggested that triggering final oocyte maturation using Gonadotropin releasing hormone (GnRH) agonist administration may lower the risk of OHSS in patients without previous down-regulation of the pituitary gland. However, several studies and a meta-analysis have shown GnRH agonists to be associated with significantly lower clinical pregnancies and higher rates of miscarriage compared to standard hCG treatment if the ET is performed during the COH cycle [11–13]. Furthermore, as pituitary desensitizing GnRH agonist protocols have become the most widely used standard in COH, GnRH agonist induction of ovulation has attained little clinical interest.

Elective cryopreservation of embryos after ART is another effective method of preventing conception within an overstimulated cycle and thus reducing the risk of severe OHSS. Several studies have reported the outcome of elective FET in patients at risk of OHSS [14,15].

However, a recent and updated evaluation of embryo freezing by

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the Cochrane collaboration group identified a lack of reliable data and studies and therefore concluded insufficient evidence to support routine cryopreservation in women at risk of OHSS [16]. Moreover, another study including 117 treatment cycles showed that elective cryopreservation does not reliably protect against the development of OHSS but that it is associated with a significantly lower clinical pregnancy and live birth rate compared to fresh embryo transfer [17].

These studies, reported in the Cochrane publication, were performed at the beginning of the 1990s and thus lacked modern cryopreservation methods such as vitrification, blastocyst transfer, assisted hatching and endometrial preparation in hormone replacement cycles [18–21]. In addition, these studies were of limited sample size and reported pregnancy rates rather than live birth rates.

The aim of our recent study was to investigate cumulative live birth rates among patients at high risk of OHSS who were treated with elective frozen-thawed embryo transfer (FET) and to evaluate this strategy in a modern era of embryo cryopreservation.

2. Materials and methods

2.1. Patients

We performed a retrospective cohort study evaluating 2998 patients who underwent a total of 6104 COH cycles with autologous oocyte retrieval between March 2000 and December 2012. Due to increased risk of OHSS, elective cryopreservation of all good quality embryos was conducted at the Private Kinderwunsch-Clinic Dr. J. Zech GmbH in Innsbruck, Austria. During the same period, a total of 1685 FET cycles were also performed, the majority of which were hormonally induced. Within the reported 6104 COH cycles, 61 episodes of OHSS requiring subsequent hospital admission were observed (1%).

A total of 200 patients analyzed in this study were at risk of OHSS, defined as elevated peak estradiol (E2) levels of more than 3500 pg/ml and/or more than 25 follicles (greater than 17 mm) evaluated by ultrasound assessment. Patients at high risk of OHSS or those displaying symptoms indicative of OHSS before embryo transfer were informed about the option of bypassing a fresh embryo transfer, cryopreserving all embryos, if possible at the blastocyst stage (day 5 or 6), and doing an elective FET in a later cycle than oocyte retrieval.

Patients were followed for at least 1 year, until treatment was either discontinued or a live infant was delivered. In addition, a questionnaire was returned to our clinic 3 months after delivery to assess obstetric complications during pregnancy, preterm delivery, caesarean delivery, birth defects, small for gestational age (SGA) and perinatal mortality.

All patients gave informed consent. Institutional review board (IRB) was not obtained. In Austria, there is no legal obligation to obtain IRB approval for retrospective, non-interventional studies.

2.2. Controlled ovarian hyperstimulation and elective cryopreservation policy

According to pre-existing pathology, most patients received COH in long or short protocols with GnRH agonists (Decapeptyl®, Ferring, Germany) and recombinant follicle-stimulating hormone (FSH) (Puregon®, Organon, Netherlands). FSH dosage was adjusted according to ovarian response as assessed by frequently performed ultrasound assessment. For final maturation of oocytes and induction of ovulation, 5000 to 10,000 units of human chorionic gonadotropin (hCG, Pregnyl®, Saint-Prex, Switzerland) or GnRH analogon *(Decapeptyl®, Ferring, Germany)* were administered. Transvaginal, ultrasound-guided follicular aspiration was performed 35–36 h after induction of ovulation. Oocytes were identified, washed in culture medium and incubated in groups with 800 μL of culture medium per well in humidified benchtop incubators (K-MINC-1000 Mini-incubator, COOK, Australia) set at 37 ° Celsius, 6.6% carbon dioxide, 5% oxygen and 88.4% nitrogen. Fertilization of oocytes was performed either with conventional IVF, intra cytoplasmatic sperm injection (ICSI) or intracytoplasmic morphologically-selected sperm injection (IMSI). The choice of fertilization method was dependent on the quality of oocytes and the presence, or absence, of substantial male factor infertility. Fertilization status was assessed at day 1 (16–18 h after insemination) and embryo imaging was applied during culture. Embryonic development was evaluated on day 3 using a scoring system that focusses on the number and regularity of blastomeres, as well as the percentage of fragmentation [22]. Embryos cultured until day 5 or 6, the blastocyst stage, were scored according to a numerical morphology grading system similar to the criteria established by Gardner and Schoolcraft.

Day 3 embryos with eight cells and < 15% fragmentation were considered good. Depending on the patient's medical history, top quality embryos were either frozen on day 2 or 3 (Cryopreservation/Thawing Kit, COOK®, Sydney, Australia) or cultured until day 5/6 for blastocyst cryopreservation. Expanded blastocysts with an inner cell mass of at least grade B and trophectoderm of at least grade C were considered suitable for cryopreservation (Blastocyst cryopreservation/Thawing Kit, Sydney, Australia). Embryos from day 3 to 6 were vitrified using the VitriFreeze™ Media Kit and warmed with VitriThaw™ Media Kit (FertiPro, Belgium). All samples were stored in liquid nitrogen. The loss of embryos during freezing and thawing processes was not documented.

Assisted hatching through a three-dimensional partial zona dissection was introduced at our center in March 2001 *and* was applied according to the thickness of the zona pellucida [23]. In November 2005, laser assisted hatching was implemented (Octax, MTG, Germany); this procedure was conducted shortly after warming according to a similar method used by Mantoudis [24]. From June 2007, embryos or blastocysts were moved from culture medium to EmbryoGlue (Vitrolife, Gothenburg, Sweden) approximately 30 min–3 h prior to embryo transfer.

In later exogenous hormone replacement cycles, estrogen (Progynova mite® 1 mg, Bayer, Vienna, Austria) and progesterone (Utrogestan® 100 mg, Meda Pharma, Vienna, Austria) were administered orally, transdermally and vaginally, in order to achieve exact synchronization between endometrial maturation and embryo development. During hormonal substitution, frequent ultrasound scans were performed to monitor endometrial thickness and secretory state and to determine the optimal time for FET. Embryo transfers were performed with a full bladder and under transabdominal ultrasound guidance. The embryo(s) were placed 1 cm from the most distal fundal region of the uterine cavity using a catheter attached to a microinjector (Narishige, Tokyo, Japan). Emptying of the bladder and then 20–30 min of bed rest followed. In the case of pregnancy, luteal support was continued until the 8th–12th week of gestation.

2.3. Statistical analysis

Systematic and routine documentation at the IVF clinic provided the data basis for this retrospective study. Sample size was not pre-specified. Baseline information included patient characteristics, details of IVF techniques and treatment outcomes. The primary outcome of this study was defined as the delivery of one or more live infants following elective FET. The secondary outcome was the occurrence of OHSS. The cumulative probability of the first live birth per woman was estimated using the Kaplan-Meier method, according to the number of FET attempts. Analyses were stratified by maternal age and performed for both an optimistic and a conservative scenario as reported previously in the setting of ART [25]. The optimistic scenario assumed that patients who did not return for a subsequent FET would have the same chance of a pregnancy resulting in a live birth as patients who continued treatment. The conservative scenario assumed no live births among patients who did not return for further treatment.

A Cox proportional hazards model was used to evaluate independent predictors for a successful live birth. Hazard ratios (HR) and their 95% confidence intervals (95%CI) were assigned to indicate the prognostic relevance of covariates such as age, the use of reproductive

Table 1
Patient characteristics.

Number of patients	200
Age (mean ± SD)	32.9 ± 4.4 years
Age groups	
< 30	48 (24.0%)
30–35	91 (45.5%)
35–40	50 (25.0%)
40 +	11 (5.5%)
Body mass index (mean ± SD)	22.1 ± 3.6 kg/m^2
Indication for assisted reproduction	
Polycystic ovary syndrome (PCOS)	132 (66%)
Unknown (Sterilitas matrimonii)	27 (13.5%)
Endometriosis	12 (6%)
Antiphospholipid antibody syndrome (APAK)	16 (8%)
Tubal factor infertility	7 (3.5%)
Uterine factor infertility	2 (1%)
Hyperprolactinemia	4 (2%)
Male factor infertility	76 (38.0%)
Previous spontaneous abortion	30 (15.0%)
Previous failed in vitro fertilization attempts (other IVF centers)	34 (17.0%)
Previous laparoscopy	61 (30.5%)
Previous extrauterine gravidity	2 (1.0%)
Stimulation protocol	
Short	153 (76.5%)
Long	47 (23.5%)
Mean number of embryos transferred	1.48 ± 0.5
Single embryo transfer	128 (47.5%)
2 embryos transferred	137 (50.7%)
3 embryos transferred	5 (1.9%)
Embryos transferred in the blastocyst stage	230 (85%)
Mean number of:	
Follicles	21.8 ± 15.6
Oocytes *eligible for further culture* (6–43)	13.7 ± 9.5
Injected oocytes	10.2 ± 7.5
Pronucleus stage embryos	8.6 ± 5.5
Day 3 embryos	7.1 ± 6.1
Blastocysts	4.7 ± 4.2
Assisted hatching	184 (68%)
Vitrification	168 (62.2%)
EmbryoGlue	49 (18.0%)
Endometrial thickness at transfer (mm)	9.5 ± 0.8
Ovarian hyperstimulation syndrome (OHSS)	4 (2.0%)
Multiple pregnancy rate	15/122 (12.3%)

techniques or pre-existing causes of infertility. Covariate selection for the multivariable Cox model was based on clinical relevance and statistical significance in univariate comparisons. Univariate comparisons for categorical variables were performed with the log-rank test, for continuous variables a univariate Cox model was applied. P-values < .05 were considered to indicate statistical significance.

3. Results

A total of 200 patients (6.7%) undergoing ART at our clinic fulfilled the inclusion criteria for this study. The mean age of patients was 32.9 ± 4.4 years. A major indication for ART and elective cryopreservation of all cultured embryos was PCOS (66.0%). Prevalence of male factor infertility was 38.0%, either independent of or in addition to female factor infertility. Previous spontaneous abortion was reported in 15.0% of patients and 17.5% underwent previous ART cycles at another IVF center. Table 1 shows baseline characteristics.

A total of 270 FETs were performed on the 200 patients with up to 4 transfers per patient with embryos derived from the same COH cycle. The median delay from oocyte retrieval to the first FET was 93 days (range from 29 to 603 days). Approximately half of all transfers (47.5%) were single embryo transfers and 85.0% of embryos transferred were in the blastocyst stage. Assisted hatching of the zona pellucida was performed on 67.8% of all embryos transferred.

Despite cryopreservation, 4 cases of OHSS (2.0%) were reported, all of them of moderate severity. Hospital admission was necessary in the case of 2 patients but invasive treatment, such as ascites fluid aspiration, was not necessary for any patients with OHSS.

There were 122 live births with 15 twin births, resulting in a total of 137 live infant deliveries. Fig. 1 shows the cumulative live birth rate per FET attempt estimated both optimistically and conservatively. Initial embryo transfers showed a success rate of 42.0% with 84 deliveries of live infants. A second FET was performed in 57 cases and resulted in a cumulative live birth rate of 58.5%. A third transfer was performed on 11 patients and resulted in a cumulative live birth rate of 61.0%. After a total of 4 FETs, the cumulative live birth rate per COH cycle was 61.0%. Fig. 2 shows the cumulative live birth rate stratified according to maternal age with 35 years as the cut-point.

As live birth rate served as the primary outcome measure of our study, miscarriage was defined as a negative outcome irrespective of whether the criterion defined by the Austrian government IVF fund [26] was fulfilled (positive heartbeat at week 8). The overall miscarriage rate was 9.3% (25 miscarriages including 1 extrauterine gravidity).

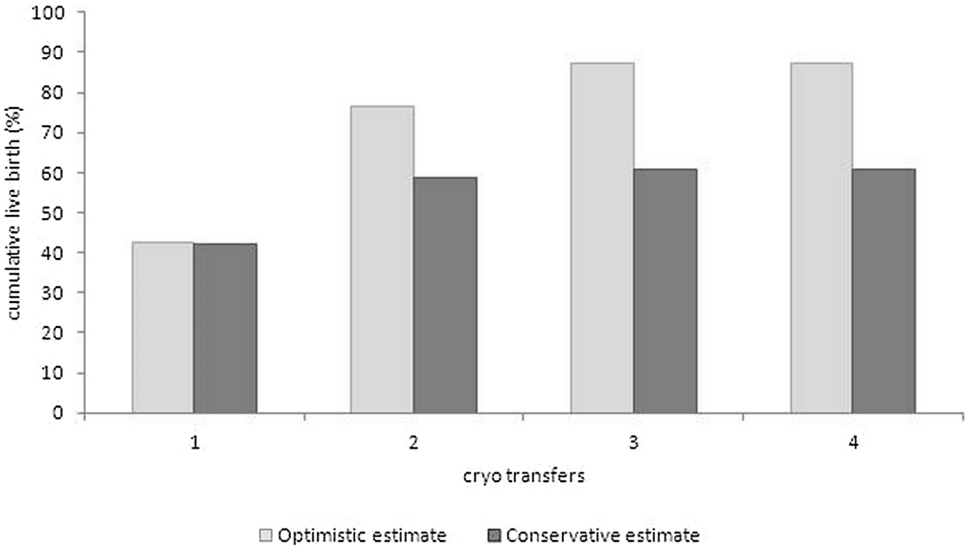

Fig. 1. Cumulative live birth rates (%) after elective FET due to high risk of OHSS shown as optimistic versus conservative estimates.

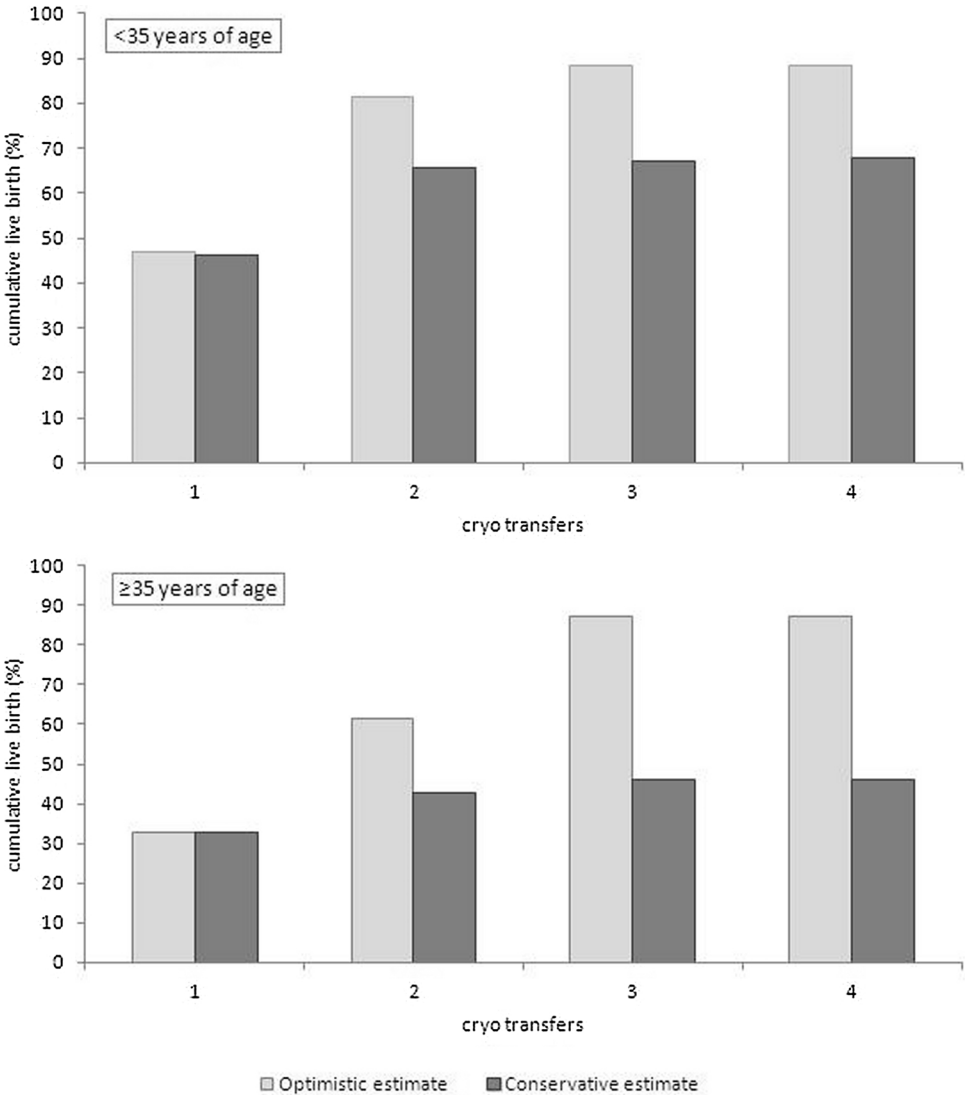

Fig. 2. Cumulative live birth rates (%) according to age group after elective FET due to high risk of OHSS shown as optimistic versus conservative estimates.

Kaplan-Meier analysis and log-rank testing were performed to identify parameters associated with a successful outcome following FET by means of cumulative live birth rate. Indications for ART (PCOS or other indications, p = .53), metabolic factors such as obesity (p = .77) and smoking (p = .78) were not associated with inferior cumulative live birth rates. Advanced maternal age at oocyte retrieval was a strong factor for predicting inferior outcomes (p = .027).

Assisted hatching (p < .001) and the transfer of at least 1 blastocyst stage embryo (p < .001) were associated with significantly improved outcomes of FET. The fertilization method (IVF, ICSI or IMSI, p = .5), vitrification (p = .73), the use of EmbryoGlue (p = .20), the stimulation protocol used (long or short protocol, p = .15) and the use of GnRH-agonists instead of hCG for oocyte maturation (p = .29) had no impact on FET success. Regarding male factors, age of the male partner (p = .47), male factor infertility (p = .31) and the need for invasive sperm retrieval (p = .48) were not associated with inferior outcomes in univariate analysis.

The multivariate Cox model identified maternal age, the use of assisted hatching and the number of embryos transferred at the blastocyst stage as independent predictors for cumulative live birth success rates. Assisted hatching increased the chances of a live birth by 1.75 (95%CI 1.1–2.8), transfer of a blastocyst instead of a cleavage stage embryo by 1.67 (95%CI 0.67–3.9) and two transferred blastocysts by 2.4 (95%CI 1.1–5.6). See Table 2 for details.

Table 2
Results of a multivariate Cox regression analysis investigating independent predictors for cumulative live birth success rates with FET.

	Wald	Sig.	HR (95% CI)
Age groups (female, years):			
< 30	reference		1
30–35	0.098	0.75	1.1 (0.71–1.6)
35–40	2.5	0.11	0.65 (0.38–1.1)
40 +	2.1	0.15	0.46 (0.17–1.3)
Assisted hatching	5.2	0.022	1.75 (1.1–2.8)
Number of blastocysts transferred			
0 (cleavage stage embryos)	reference		1
1	1.1	0.29	1.6 (0.67–3.9)
2	4.0	0.045	2.4 (1.1–5.6)

4. Discussion

The results of our study clearly demonstrate that elective cryopreservation of cultured embryos with consecutive FET is associated with an exceptionally high cumulative live birth rate of 61.0% per COH cycle in patients at high risk of OHSS.

This concept also raises considerations as to whether elective FET should be introduced into daily ART routine in order to prevent patients from developing OHSS. This strategy not only prevents ART related complications but also results in optimum utilization of cultured

embryos within a single COH cycle instead of additional COH cycles that consume more resources and burden patients.

There may be several factors responsible for the exceptionally high live birth rate seen in patients at high risk of OHSS when applying elective FET. In our study, 85.0% of frozen-thawed embryo transfers occurred at the blastocyst stage and only 15.0% were cleavage stage embryo transfers. This may have contributed to the high live birth rate, even considering the fact that almost 50.0% of patients did single embryo transfers. These results are in concordance with other studies, which demonstrate similar clinical pregnancy rates but show significantly higher cumulative live birth rates than with cleavage stage fresh embryo transfers [27]. In the Cochrane review of 50 trials, including 23 randomized clinical studies, the authors concluded that future studies should aim to report miscarriage and live birth rates instead of clinical pregnancy alone in order to enable ART consumers and service providers to make well informed decisions about the best treatment option available.

The high success rates seen in patients undergoing FET with a day 5 or 6 blastocyst has enabled us to increase the rate of elective single embryo transfer to almost 50% of transfer cycles and thus prevent multiple pregnancies and the associated obstetrical complications [28].

Conversely, national reports released by the Swedish, US and Canadian ART registry have reported an increased risk of preterm birth (< 37 weeks) but not of very preterm birth (< 32 weeks) in singleton pregnancies resulting from a blastocyst stage transfer when compared to those from a cleavage stage transfer [29–31]. This observation was still valid even after adjustment for significant confounding factors. However, these large reports included fresh IVF/ICSI transfers only. Therefore, bypassing a fresh embryo transfer in a COH cycle may overcome the risk of adverse outcomes, such as SGA infants and preeclampsia, in patients at high risk of OHSS. In a study by Imudia and colleagues, elective cryopreservation followed by a later FET in a more physiologic hormonal replacement cycle was seen to significantly reduce adverse obstetric complications and SGA deliveries [32].

The creation of ideal near physiologic endometrial conditions for embryo implantation in a delayed cycle may further explain the excellent outcomes of FET in patients at risk of OHSS.

COH is known to have a negative influence on the endometrial environment, leading to impaired endometrial receptivity, abnormal placentation and possibly also negatively triggering trophoblast development within the endometrial stroma [33–35]. These findings may be supported by previous studies that report higher rates of miscarriage and lower birth weight among children born after a fresh cycle embryo transfer than after FET [36,37]. Our concept of elective cryopreservation and FET includes a programmed hormonal replacement cycle that creates optimal and synchronal endometrial conditions necessary for successful embryo implantation, placentation and subsequent pregnancy. In addition, the low miscarriage rate of 9.3% among patients with elective FET compared to 11.4% among fresh ET within the COH cycle in our study may further support this concept. Our observations are in line with a recently published meta-analysis assessing the miscarriage rate as a secondary endpoint identifying a trend towards lower miscarriage rates among FET compared to fresh ET in three eligible publications [38]. Currently, there are no state-of-the-art criteria that determine whether a subsequent FET should be performed in a natural or hormonally induced cycle. The ANTARCTICA trial aimed to answer this question but was terminated as the minimum of patients required for adequate statistical power could not be achieved and live birth rates were lower than anticipated [39].

There are several reasons for the use of hormonal replacement cycles for subsequent elective FETs. Firstly, PCOS patients may be at highest risk of OHSS, a risk that has been shown to be highest in patients who conceive during an “overstimulated” COH cycle. Furthermore, anovulatory cycles and low ability to achieve optimal endometrial thickness and secretory conditions are frequently observed in this patient group during natural cycles [40]. As a result, programmed hormonal replacement cycles seem to be a cost-effective method to improve implantation success of frozen-thawed embryos. As an alternative, OHSS could also be avoided in these patients through in vitro maturation. However, this approach is still controversial and is often associated with diminished clinical outcomes, most likely due to dysregulation in gene transcription or post-transcriptional modification of genes in vitro matured oocytes [41]. This fact could explain the high incidence of aneuploidy [42], increased spindle abnormalities and chromosomal misalignments that have been associated with these oocytes [43,44]. Our results show that PCOS patients can indeed benefit from FET and experience less pregnancy complications.

Outcomes regarding fresh versus frozen ET in PCOS patients were investigated in a randomized controlled trial [45] showing similar results as compared to our study. FET was associated with significantly higher live birth rates and lower rates of pregnancy loss compared to fresh transfer, in line with the results of our study. However, in their study, all embryos were cultured until day 3. Moreover, FET was associated with lower rates of OHSS but paradoxically with a higher rate of preeclampsia. Their study differed also regarding the inclusion criteria taking only first IVF cycles into account which does not reflect clinical practice. Our observational study additionally included a large proportion of patients undergoing previous ART cycles at other centers, also patients who already experienced severe OHSS during previous ART cycles.

Most studies of elective cryopreservation were conducted during the early 1990s. Subsequent developments in embryonic culture, cryopreservation and the thawing process have significantly favored successful enhancement of FET outcomes. Such improvements are of utmost importance and support widespread use of FET, not only in patients at risk of OHSS.

Furthermore, a large study of 2313 vitrified-warmed transfer cycles in 1481 patients showed that assisted hatching and elective cryopreservation to prevent OHSS were significantly associated with successful clinical pregnancy when compared to FET transfers using surplus embryos after a failed fresh transfer [46]. However, this evaluation introduces bias as repeated FET cycles in the same patient were treated as independent analysis. Therefore, we evaluated cumulative live birth rates using the Kaplan-Meier method for both an optimistic and perhaps a more likely conservative scenario.

In our study, assisted hatching was shown to be the most effective method to enhance embryo implantation and improve consecutive live birth rates. The use of EmbryoGlue and vitrification (instead of slow freezing) appeared to have no impact on the outcome. Optimized endometrial conditions created by hormonal replacement cycles may have further contributed to the improvement of elective FETs.

Recent studies have shown that babies born from FET cycles showed significantly higher birth weight, less obstetric complications and lower preterm birth rates than babies delivered from fresh COH cycles. This supports the fact that non-physiologic hormonal overstimulation is responsible for abnormal placentation and trophoblast disorders and, therefore suggests a broader use of elective FET for patients undergoing ART [32,36,47].

In this retrospective study we lacked information such as details regarding the used cryopreservation kits or detailed information about the embryo imaging that could potentially confound or limit the results of the statistical analysis. Moreover, due to the retrospective character of this study, we were not able to assess specific information regarding the procedure of embryo imaging.

4.1. Conclusions

Elective cryopreservation was originally implemented to avoid substantial harm to the patient. The results in terms of live birth rates presented in this study clearly suggest a broad implementation of this concept into daily ART routine in order to prevent patients from developing OHSS. Elective cryopreservation and consecutive frozen-

thawed embryo transfer provides a successful strategy to avoid severe adverse events caused by controlled ovarian hyperstimulation.

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