Optimal embryo transfer strategy in poor response may include freeze-all

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Abstract

Purpose In this retrospective cohort study, we investigated the best embryo transfer strategy in ICSI cycles with ≤ 4 oocytes collected at oocyte retrieval.

Methods Women who underwent antagonist co-treatment COS for ICSI treatment between January 2010 and December 2015 at a private ART clinic ($N=2263$). Eight hundred seventy-nine women (group 1) had ≤ 4 oocytes collected at oocyte retrieval, of whom 645 (group A) had cleavage stage embryo transfer (ET), and 234 (group B) had blastocyst ET. One thousand three hundred eighty-four women (group 2) had 10–15 oocytes collected at oocyte retrieval, of whom 676 (group C) had cleavage stage ET, and 708 women (group D) had blastocyst ET. Blastocyst vitrification was performed using the Cryotop method and FET using artificial cycles.

Results In group 1, the cancellation rate was significantly lower in group A (25.2 vs 38 %). The pregnancy rate (PR), clinical PR, implantation rate (IR), and live birth rate (LBR) per ET and per oocyte retrieval were all lower in group A. The clinical PR, IR, and LBR per ET of vitrified-warmed blastocyst ET were significantly the highest. In group 2, the cycle cancellation rate was significantly lower in group C (3.5 vs 13.4 %). The PR, clinical PR, and IR per ET and per oocyte retrieval were all lower in group C. The LBR per ET was significantly lower,

but the LBR per oocyte retrieval was not significantly lower in group C. Again, the PR, clinical PR, and IR per ET of vitrified-warmed blastocyst ET were significantly the highest.

Conclusions Day 5 ET strategy has been reserved for normal or high responders. The improved pregnancy outcomes from blastocyst culture and cryopreservation may challenge ART to extend this benefit to poor responders.

Keywords Freeze-all · Poor response · Diminished ovarian reserve · Blastocyst culture

Introduction

In 1983, the first description of a poor responder was done [1]. In 2011, a consensus definition of poor ovarian response was established during the congress of the European Society of Human Reproduction and Embryology (ESHRE). The Bologna criteria [2] allow us to better assess the incidence of poor ovarian response (POR) and to compare groups of more homogenous patients. The collection of less than four oocytes in response to ovarian stimulation protocol is one of the features listed in the Bologna criteria to define POR; however, the parameter of oocyte maturity is not included in this definition.

During the last two decades of assisted reproduction technology (ART), blastocyst culture has gained increasing popularity. The reasons for this adoption have been improved blastocyst formation rates, because of improved culture conditions, and increased implantation rates, because of the higher euploidy in normal blastocysts. Implantation rates of IVF with cleavage stage embryos (day 2 and day 3) were always deemed to be frustratingly low, especially as ART progressed to single embryo transfer (SET) to eliminate the iatrogenesis of multi-embryo transfers. Although a large retrospective study found a higher pregnancy rate with vitrified embryos

Capsule In a retrospective cohort study, day 5 blastocyst transfers were found to provide superior pregnancy rates compared to cleavage stage embryo transfers with further increases in pregnancy achieved through the transfer of vitrified-warmed blastocysts in frozen embryo transfers.

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than with blastocysts, the patients in the cleavage stage group not only had more embryos transferred per cycle but were also younger than those in the blastocyst transfer group [3]. On the other hand, a number of recent studies have shown significantly higher pregnancy and implantation rates in blastocysts compared to cleavage stage embryo transfers [4–8]. The Cochrane review of 12 randomized controlled trials demonstrated that live birth rates can be optimized by performing blastocyst transfer compared to cleavage stage embryo transfers [9]. In addition, a recent study found chromosome abnormalities were reduced at the blastocyst stage compared to the cleavage embryo stage [10].

Many authors have alluded to the fact that transfer cancellation was higher in blastocyst compared to cleavage stage embryo transfer programs [11]. In unselected IVF patients, it was shown that the risk of cycle cancellation in a systematic blastocyst transfer policy may be as high as 27 % [12]. If a threshold of four good embryos on the third day of embryo culture was used, no cycles were cancelled in patients ≤ 38 years of age [13]. An intermediate policy of having a minimum of four fertilized eggs had a cancellation rate of 10.1 % for blastocyst transfer [14].

Frozen embryo transfers (FET) have increasingly being used as the primary transfer strategy, as they are deemed more physiological, with potentially better implantation, pregnancy, and perinatal outcomes. Although cryopreservation may entail some risks to the embryo, FET may provide an endometrium with increased receptivity, as compared to that after ovarian stimulation. The timing of the “window of implantation” (WOI) in stimulated cycles is complicated by the fact that it may be advanced or delayed, and therefore easily mistimed [15–22]. Shapiro et al. [23] showed that the clinical pregnancy rate per transfer is significantly greater in the cryopreservation group than in the fresh group in normal responders and concluded that the difference was due to impaired endometrial receptivity in fresh embryo transfer. Similarly, endometrial receptivity was shown to be adversely affected by ovarian stimulation in high responders [24].

Although numerous studies have been published on POR, most have focused on strategies to obtain the most oocytes possible [25], with no consideration for the best transfer strategy in patients with poor response to ovarian stimulation. Transfer strategy studies have understandably been confined to normal or high responder patients. In this study, we investigate for the first time which transfer strategies, i.e., cleavage stage, blastocyst stage, fresh, or frozen, provide the best pregnancy outcomes in poor response.

Materials and methods

This retrospective cohort study was conducted on ICSI cycles performed between January 2010 and January 2015, at a

private ART center. The study period spanned two distinct policy periods, (1) January 2010 to December 2012 in which the policy was to perform cleavage stage embryo transfers and (2) January 2013 to December 2014 in which the policy was to perform blastocyst stage embryo transfers. In total, 2263 cycles were extracted for analysis from the ART database. Only a single cycle per patient was included (i.e., chronologically the patients first cycle), with cycles excluded in which female patient age was ≥ 43 years. Institutional ethics committee approval (reference number 530/2014) was obtained to perform the study, with patients also providing consent for the use of their anonymized data in research.

Patients

Two groups of patient cycles were extracted from both the time periods, those with ≤ 4 oocytes (poor responders) collected at oocyte retrieval and those with 10–15 oocytes (normal responders) collected. In group 1, 879 poor response cycles were extracted, 645 from period 1 (group A, cleavage stage), and 234 from period 2 (group B, blastocyst stage). In group 2, 1384 normal response cycles were extracted, 676 from period 1 (group C, cleavage stage), and 708 from period 2 (blastocyst stage). In subgroup analyses, group A was divided into subgroup A1 (day 2) and subgroup A2 (day 3), group B was divided into subgroup B1 (fresh day 5 ET) and subgroup B2 (frozen-thawed day 5 FET), group C was divided into subgroup C1 (day 2) and subgroup C2 (day 3), and group D was divided into subgroup D1 (fresh day 5 ET) and subgroup D2 (frozen-thawed day 5 FET).

Stimulation protocol

Controlled ovarian stimulation (COS) was performed using a GnRH antagonist (Cetrotide, 0.25 mg, Merck Serono, Turkey) co-treatment protocol with a combination of rFSH (Gonal-F, Merck Serono, Turkey) and hMG (Menopur, Ferring Pharmaceuticals Ltd, India) and ovulation induction with either hCG (250 mcg, Ovitrelle®, Merck Serono, Turkey), GnRH agonist (0.2 mg, Gonapeptyl®, Ferring Pharmaceuticals Ltd, India) or a combination of the two.

Oocyte pick-up and embryo transfer

Oocyte collection and embryo culture was performed using the Sydney IVF (IVF media, COOK Medical, USA) media, with incubation conditions set at 6 % CO₂, 5 % O₂, and 37.0 °C (K-Systems, Kivex Biotech Ltd, Denmark).

Embryos were assessed according to blastomere number, blastomere size and regularity, and the percentage of fragmentation. Good quality embryos were those with equal sized, spherical blastomeres with <10 % fragmentation, and scored as 1 on a scale of 1–5. Blastocysts were scored according to

the three-part grading system, blastocyst expansion on a scale of 1 to 6; the inner cell mass (ICM) on a scale of A to C according to the number and degree of compaction of the cells; and the trophectoderm (TE) on a scale of A to C according to the number, size, and contiguous arrangement of the trophectoderm cells.

Vitrification and warming of blastocysts were performed using the Cryotop method, as described by the manufacturer (Kitazato, BioPharma Co. Ltd, Japan).

All FET procedures were performed as artificial cycles, with a step-up regimen of estrogen (Estrofem, Novo Nordisk, Istanbul, Turkey; 2 mg/day from day 1 to 6, 4 mg/day from day 7 to 10, and 8 mg/day from day 11 to 14). Progesterone supplementation was started on day 15 (twice a day, Crinone 8 %, Merck Serono, Turkey), and estrogen supplementation continued at 6 mg/day, with the vitrified-warmed blastocyst transfer performed on the sixth day of progesterone.

All transfer procedures were performed using a Hamilton syringe (50 µL, Hamilton Company, USA) attached to an embryo replacement catheter (Wallace, Smiths Medical International, UK) and trans-abdominal ultrasound guidance. Luteal phase support consisted of daily estrogen (6 mg/day) and progesterone (8 % BD) supplementation, with supplementation continuing for at least 12 weeks of gestation.

Statistical analysis

The primary outcome measures used to compare the outcomes between the two transfer groups were the following: oocyte maturation, fertilization rate, pregnancy rate (i.e., day 14 βhCG concentration of ≥ 30 IU/L), clinical pregnancy rate (i.e., fetal with cardiac activity on ultrasound), implantation rate (i.e., the ratio number of fetal hearts to number of embryos transferred), and live birth rate.

SigmaPlot version 12.5 was used for statistical analysis. The *t* test was used to compare the continuous variables (means), and the chi-square test was used to compare

categorical variables (percentages). $p < 0.05$ was considered to be significant.

Results

During the study period, a total of 6261 oocyte retrievals were performed for women receiving ICSI treatment for infertility at a single ART center. In 879 cycles, ≤ 4 oocytes were collected at oocyte retrieval, resulting in a poor response incidence of 14.04 %.

In group 1 (poor response), the patient characteristics were non-significantly different (i.e., age, infertility duration, and BMI) between group A and B (Table 1). The mean number of oocytes (2.68 vs 2.61, $p = 0.200$), oocyte maturity rates (74.6 vs 76.2 %, $p = 0.374$), and fertilization rates (73.1 vs 77.0 %, $p = 0.061$) were non-significantly different between group A and group B. The number of embryos per transfer was also similar between the two groups (1.54 vs 1.63, $p = 0.072$). In group A, 482 patients (74.7 %) and in group B, 146 patients (62.3 %) had treatment cycles that included ET (Table 2), with the cancellation rate in group A significantly lower than in group B (25.2 vs 38.0 %, $p < 0.001$).

In the poor responder groups (Table 3), the total pregnancy rate (PR) per ET, clinical PR per ET, and live birth rate (LBR) per ET, as well as per oocyte retrieval were all significantly lower in group A (cleavage stage) than in group B (blastocyst stage), with LBR per oocyte retrieval of 16.1 and 25.6 %, respectively. The total implantation rate (IR) in group A (18.2 %) was significantly lower than in group B (36.6 %). In the subgroups, the PR, clinical PR, and LBR increased from the lowest in subgroup A1 (day 2 cleavage stage) to the highest in subgroup B2 (blastocyst FET). In subgroup B2, the IR was 47.0 % and the LBR per ET was 48.2 %. The LBR for subgroup B2 was significantly higher than for any other of the subgroups. In subgroup B2 one FET cycle (0.01 %) cancelled as the result of blastocyst degeneration.

Table 1 Patient and cycle characteristics of poor and normal ovarian responders

		Poor response group A	Poor response group B	<i>p</i> value	Normal response group C	Normal response group D	<i>p</i> value
Age (years)	Mean $\pm$ SD	35.9 $\pm$ 5.04	36.7 $\pm$ 4.64	0.383	31.8 $\pm$ 5.05	33.04 $\pm$ 5.25	0.074
Infertility duration (years)	Mean $\pm$ SD	5.84 $\pm$ 4.78	5.73 $\pm$ 4.94	0.383	5.29 $\pm$ 4.55	5.67 $\pm$ 4.32	0.056
BMI (kg/m ²)	Mean $\pm$ SD	26.01 $\pm$ 5.06	25.8 $\pm$ 5.08	0.294	26.2 $\pm$ 4.8	25.88 $\pm$ 5.31	0.120
Number oocyte per OPU	Mean $\pm$ SD	2.68 $\pm$ 1.09	2.61 $\pm$ 1.08	0.200	12.3 $\pm$ 1.66	12.32 $\pm$ 1.67	0.412
Number MII oocytes per OPU	Mean $\pm$ SD	2.00 $\pm$ 1.18	1.99 $\pm$ 1.15	0.456	8.8 $\pm$ 2.44	9 $\pm$ 2.51	0.067
Maturation rate	%	74.6	76.2	0.374	71.8	72.8	0.115
Fertilization rate	%	73.1	77	0.061	71.8	73.3	0.06
Number embryos per transfer	Mean $\pm$ SD	1.54 $\pm$ 0.51	1.63 $\pm$ 0.49	0.072	1.96 $\pm$ 0.35	1.93 $\pm$ 0.35	0.064

$p < 0.05$ was considered to be significant

Table 2 Cycle outcomes leading to embryo transfer

		Poor response group A	Poor response group B	<i>p</i> value	Normal response group C	Normal response group D	<i>p</i> value
Number OPU	N	645	234	NA	676	708	NA
Number cycles of failed embryo development ^a	<i>n</i> (%)	141 (21.8)	53 (22.6)	0.875	15 (2.22)	12 (1.7)	0.610
Arrested development	<i>n</i> (%)	22 (3.4)	34 (14.5)	<0.001	9 (1.3)	83 (11.7)	<0.001
Number cycles cancelled ^b	<i>n</i> (%)	163 (25.2)	89 (38.0)	<0.001	24 (3.5)	95 (13.4)	<0.001
Day 2 ET	<i>n</i>	344	NA	NA	591	NA	NA
Day 3 ET	<i>n</i>	138	NA	NA	61	NA	NA
Day 5 ET	<i>n</i>	NA	59	NA	NA	375	NA
Day 5 FET	<i>n</i>	NA	87	NA	NA	238	NA
Failed FET (degeneration)	<i>n</i>	NA	1	NA	NA	0	NA
Total ET	<i>n</i> (%)	482 (74.7)	146 (62.3)	<0.001	652 (96.4)	613 (86.5)	<0.001

$p < 0.05$ was considered to be significant

NA not applicable

^a Cycles with failed embryo development include those with zero MII oocytes, zero fertilization, and zero embryo cleavage

^b Cycles cancelled include cycles with failed embryo development and embryo development arrest

Table 3 Pregnancy rates, clinical pregnancy rates, implantation rates, and live birth rates of poor responders according to the chosen embryo transfer strategy

		Poor response group A ≤4 oocytes	Poor response group B ≤4 oocytes	<i>p</i> value
Number of OPU		645	234	
Total ET		482 (74.7 %)	146 (62.3 %)	<0.001
Total PR per ET		138 (28.6 %)	85 (58.2 %)	<0.001
Day 2 PR per ET	A1	98 (28.4 %)	NA	
Day 3 PR per ET	A2	40 (28.8 %)	NA	A1 vs A2 $p = 0.998$
Day 5 PR per ET		NA	B1 31 (52.5 %)	
Day 5 FET PR per ET		NA	B2 54 (62 %)	B1 vs B2 $p = 0.330$
Total PR per OPU		21.3 %	36.3 %	<0.001
Total clinical PR per ET		116 (24 %)	67 (45.8 %)	<0.001
Day 2 clinical PR per ET	A1	82 (23.8 %)	NA	
Day 3 clinical PR per ET	A2	34 (24.6 %)	NA	A1 vs A2 $p = 0.946$
Day 5 clinical PR per ET		NA	B1 20 (33.8 %)	
Day 5 FET clinical PR per ET		NA	B2 47 (54 %)	B1 vs B2 $p = 0.026$
Total clinical PR per OPU		17.9 %	28.6 %	<0.001
Total IR		18.2 %	36.6 %	<0.001
Day 2 IR	A1	17.8 %	NA	
Day 3 IR	A2	19.1 %	NA	A1 vs A2 $p = 0.804$
Day 5 IR		NA	B1 24.1 %	
Day 5 FET IR		NA	B2 47 %	B1 vs B2 $p = 0.002$
Total LBR per ET		104 (21.5 %)	60 (41.1 %)	<0.001
Day 2 LBR per ET	A1	74 (21.5 %)	NA	
Day 3 LBR per ET	A2	30 (21.7 %)	NA	A1 vs A2 $p = 0.946$
Day 5 LBR per ET		NA	B1 18 (30.5 %)	
Day 5 FET LBR per ET		NA	B2 42 (48.2 %)	B1 vs B2 $p = 0.049$
Total LBR per OPU		16.1 %	25.6 %	0.002

$p < 0.05$ was considered to be significant. A1 and A2, subgroups of group A; B1 and B2, subgroups of group B

OPU oocyte pickup, ET embryo transfer, PR pregnancy rate, FET frozen embryo transfer, IR implantation rate, LBR live birth rate, NA not applicable

In group 2 (normal responder), the patient characteristics were non-significantly different (i.e., age, infertility duration, and BMI) between group C and D (Table 1). The mean number of oocytes (12.32 vs 12.3, $p = 0.412$), oocyte maturity rates (71.8 vs 72.8 %, $p = 0.115$), and fertilization rates (71.8 vs 73.3 %, $p = 0.06$) was non-significantly different between group C and group D. The number of embryos per transfer was also similar between the two groups (1.96 vs 1.93, $p = 0.064$). In group C, 652 patients (96.4 %) and in group D, 613 patients (86.5 %) had treatment cycles that included ET (Table 2), with the cycle cancellation rate in group C significantly lower than in group D (3.5 vs 13.4 %, $p < 0.001$).

In the normal responders (Table 4), similar to that in the poor responders, the total PR per ET, clinical PR per ET, and LBR per ET, as well as the PR per oocyte retrieval and clinical PR per oocyte retrieval were all significantly lower in group C (cleavage stage) than in group D (blastocyst stage). Moreover, in the normal responders, the LBR per oocyte retrieval was non-significantly different (41.1 vs 44.9 % $p = 0.171$). The

total IR, however, was again significantly lower in group C than in group D (26.2 vs 46.3 % $p < 0.001$). In the subgroups, the PR, clinical PR, and LBR again increased from the lowest in subgroup C1 (day 2 cleavage stage) to the highest in subgroup D2 (blastocyst FET). In subgroup D2, the IR was 55.5 % and the LBR per ET was 57.9 %. In subgroup D2, there were no FET cycles cancelled as the result of blastocyst degeneration.

On comparing the outcomes of group 1 (poor response) with group 2 (normal response), the differences in cycle cancellation rates were most apparent (Table 2). The mean ages in poor responders were higher than in normal responders. Moreover, the lower number of oocytes collected at oocyte retrieval in the poor responders increased the chances of adverse outcomes (i.e., zero MII oocytes, zero fertilization, zygote cleavage arrest) resulting in cycle cancellation (Table 2). These early adverse outcomes contributed most to cycle cancellation (group A vs group C; 21.8 vs 2.22 % and group B vs group D; 22.6 vs 1.7 %), while embryo developmental arrest

Table 4 Pregnancy rates, clinical pregnancy rates, implantation rates, and live birth rates of normal responders according to the chosen embryo transfer strategy

		Normal response group C 10–15 oocytes	Normal response group D 10–15 oocytes	<i>p</i> value
Number of OPU		676	708	
Total ET		652 (96.4 %)	613 (86.5 %)	<0.001
Total PR per ET		334 (51.3 %)	409 (66.7 %)	<0.001
Day 2 PR per ET	C1	299 (50.6 %)	NA	
Day 3 PR per ET	C2	35 (57.3 %)	NA	C1 vs C2 $p = 0.382$
Day 5 PR per ET		NA	D1 233 (62.1 %)	
Day 5 FET PR per ET		NA	D2 176 (73.9 %)	D1 vs D2 $p = 0.003$
Total PR per OPU		49.4 %	57.7 %	<0.001
Total clinical PR per ET		292 (44.7 %)	372 (60.6 %)	<0.001
Day 2 clinical PR per ET	C1	262 (44.3 %)	NA	
Day 3 clinical PR per ET	C2	31 (50.8 %)	NA	C1 vs C2 $p = 0.404$
Day 5 clinical PR per ET		NA	D1 212 (56.5 %)	
Day 5 FET clinical PR per ET		NA	D2 160 (67.2 %)	D1 vs D2 $p = 0.011$
Total clinical PR per OPU		43.1 %	52.5 %	<0.001
Total IR		26.2 %	46.3 %	<0.001
Day 2 IR	C1	26.2 %	NA	
Day 3 IR	C2	26.3 %	NA	C1 vs C2 $p = 0.913$
Day 5 IR		NA	D1 41.2 %	
Day 5 FET IR		NA	D2 55.5 %	D1 vs D2 $p < 0.001$
Total LBR per ET		278 (42.6 %)	318 (51.8 %)	$p = 0.001$
Day 2 LBR per ET	C1	249 (42.1 %)	NA	
Day 3 LBR per ET	C2	29 (47.5)	NA	C1 vs C2 $p = 0.498$
Day 5 LBR per ET		NA	D1 180 (48 %)	
Day 5 FET LBR per ET		NA	D2 138 (57.9 %)	D1 vs D2 $p = 0.020$
Total LBR per OPU		41.1 %	44.9 %	0.171

$p < 0.05$ was considered to be significant. C1 and C2, subgroups of group C; D1 and D2, subgroups of group D

OPU oocyte pickup, ET embryo transfer, PR pregnancy rate, FET frozen embryo transfer, IR implantation rate, LBR; live birth rate, NA not applicable

contributed less, were remarkably similar, but dependent on the length of culture (group A vs group C; 3.4 vs 1.3 % and group B vs group D; 14.5 vs 11.7 %).

Discussion

In this study, we investigated the most optimal embryo transfer strategy in poor ovarian response. Even though the cancellation rate in the blastocyst (day 5) strategy was significantly higher (38 vs 25.2 %) than in the cleavage stage (day2/3) strategy, the PR, clinical PR, and even LBR per oocyte retrieval still remained significantly higher in the blastocyst strategy. The highest PR, clinical PR, IR, and LBR per ET were obtained in the cycles with blastocyst FET. The extremely low blastocyst loss rate (0.01 %) observed with vitrification warming and the similar post-fertilization embryo developmental arrest rate observed for poor and normal responders are strong motivational factors in the push for extended culture and blastocyst FET in poor ovarian responders. To the best of our knowledge, this is the first study to address the issue of poor pregnancy outcome in poor responders by investigating the benefits of different transfer strategies.

Numerous alternate ovarian stimulation protocols have been investigated for use in poor responder patients, i.e., high gonadotropin dose, alternate gonadotropin type, alternate pituitary suppression regimens, GnRH agonist flare-up, and even natural cycles, with no protocol demonstrating consistent superiority [26–29]. In addition, a number of strategies to augment ovarian stimulation with modifiers have also been considered, i.e., DHEA, T, and LH; however, here too no conclusive benefit has been established [30, 31]. GnRH antagonist co-treatment protocols continues to be the most often used protocol in poor responder cycles, mostly because ovarian reserve can be assessed by ultrasound on day 2–3 of the cycle, and there is no pituitary suppression in the follicular recruitment phase. The real focus in patients with poor ovarian response should therefore be on strategies to obtain oocytes with genetic and cytoplasmic competence, to eliminate the high pre-first mitosis loss rate, rather than on strategies to obtain more than three oocytes.

The reported evidence on the pregnancy outcomes of patients that respond poorly to ovarian stimulation has been that they generally have poor outcomes, therefore equating low oocyte number cycles with low pregnancy outcomes. From a registry-based retrospective analysis of 658,519 SART reported (2004–2012) fresh autologous IVF cycles, the analysis showed that poor responders (i.e., 1–5 oocytes), whether from low (<1000 IU FSH) or high (>5000 IU FSH) total FSH dose cycles, had reduced pregnancy compared normal responders (i.e., 11–15 oocytes) with similar ovarian stimulation, 21.7 versus 46.5 % and 12.5 versus 26.2 %, respectively [32]. The results of this study, however, exposes the notion held

by many that pregnancy outcomes in poor ovarian responders are necessarily always severely reduced, with pregnancy rates of >50 % achieved in cycles with successful blastocyst culture and transfer, especially if transferred in FET. The results from this poor prognosis study corroborate the results of previous studies that showed that the reproductive outcomes of FET were better than fresh ET in good prognosis patients [33, 34]. Other than pregnancy, all other reproductive outcomes (i.e., clinical pregnancy, implantation, and live birth) were significantly higher in FET.

The most recent studies comparing fresh with frozen blastocyst ET have also shown that blastocyst FET was associated with lower risk of ectopic pregnancy, pre-eclampsia, low birth weight (LBW), small for gestational age, prematurity, preterm LBW, antepartum hemorrhage, placental abruption, and perinatal death compared to fresh transfer [34–56]. FET has increasingly being used as the primary transfer strategy, as it is deemed to be more physiologic and cryopreservation technology improvements have seen significant increases in the percentage of embryos surviving with normal competency [57]. A strategy then that may certainly also benefit the reproductive outcomes of poor responders.

The outcomes of this study are important, as they will guide the future sentiment of doctor-patient communication in cycles with reduced ovarian response to standard COS, in regard to cycle expectations and the strategy that will provide the best possible outcome. At our center, the pre-2010 advice to patients with ≤ 4 oocytes collected at oocyte retrieval was that they should have an embryo transfer early. The reason given was the anecdotal belief that in vivo culture was superior to in vitro culture, i.e., some embryos that fail to develop to the blastocyst stage in vitro may achieve this in vivo. However, transferring embryos early may expose embryos earlier than normal to intrauterine conditions i.e., in vivo embryos are only exposed to uterine conditions at the blastocyst stage. Moreover, in fresh ET cleavage stage, embryos will also be exposed to altered early luteal phase conditions [57–59] and will be so for an extended period of time i.e., time to blastocyst implantation [60]. All the mentioned factors may reduce rather than increase the chances of embryo implantation and pregnancy, as confirmed in studies using animal models [61]. Even though not without controversy, blastocyst culture and blastocyst transfer have been shown to result in superior reproductive outcomes, at least in normal responders [9, 11], therefore, why not in poor responders. Genomic studies have also provided evidence to substantiate the argument for extended culture in IVF, as blastocyst has generally been found to have reduced aneuploidy rates [62].

Cycle cancellation is a major concern in all ART programs, emotionally and economically. In poor responders, the risk for cycle cancellation is significantly higher than in normal responders, due mainly to the low number of oocytes retrieved. Importantly, current vitrification technology has been shown

to result in blastocyst survival of $\geq 95\%$ [3], with some reports proclaiming 100 % survival. The high survival rates ($\approx 99.99\%$) with vitrification were corroborated in this study, showing that the risk for cycle cancellation due to cryopreservation damage was becoming extremely low. Moreover, embryo developmental arrest risk was seen to be similar between poor and normal responders. Even though extended culture for blastocyst development results in cycle cancellation [11, 12], transferring embryos early in these cycles may only increase early pregnancy loss and non-pregnancy [63]. Oocyte developmental competence, especially in low oocyte numbers, therefore, is the primary concern and should be the focus in future poor responder investigations.

With the currently available technology in ART, i.e., culture technology and cryopreservation technology, we believe the challenge to choose extended culture and blastocyst vitrification in poor responders is not unrealistic. The statistical impact of the study may be limited by being a retrospective cohort study and the two distinct periods used in the comparison, but its strength is that it was performed at a single center with the transfer strategy guided by policy and not by personal preference of clinician or patient. We, therefore, believe that the study results clearly show that decision-making in poor responders requires reevaluation and therefore justifies the need for randomized controlled trials. Poor responders were found to have reduced, but not severely reduced, pregnancy outcomes, as compared to good prognosis patients. We believe that future COS strategies that increase the probability of competent oocytes in poor responders may result in pregnancy rates clinically equivalent to those of normal responders.

Compliance with ethical standards

Funding This study received no financial support.

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval All participants in this study signed an informed consent (institutional *Ethics committee form* included).

Study data The study data was partly presented at the 71st Annual Meeting of the ASRM, Baltimore, Maryland, USA, 17 to 21 October 2015.

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