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Original Article

Embryology

Day after rescue ICSI: eliminating total fertilization failure after conventional IVF with high live birth rates following cryopreserved blastocyst transfer

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ABSTRACT

STUDY QUESTION: What is the impact of day after rescue ICSI (r-ICSI) on success of fresh and frozen embryo transfers?

SUMMARY ANSWER: The use of r-ICSI can virtually allay fears of total fertilization failure (TFF) after conventional IVF (C-IVF) and achieve high live birth rates after frozen blastocyst transfer.

WHAT IS KNOWN ALREADY: More infertility clinics have resorted to the use of ICSI in place of C-IVF in IVF treatment owing to fear of TFF or a low fertilization rate. r-ICSI has been attempted either on the day of IVF or the day after. Day after r-ICSI has proved unsuccessful in the past.

STUDY DESIGN, SIZE, DURATION: A retrospective data analysis was performed of 16 608 qualifying cases between April 2010 and July 2021 conducted at a single private academically affiliated fertility clinic.

PARTICIPANTS/MATERIALS, SETTING, METHODS: r-ICSI was performed principally on patients with >4 metaphase II oocytes, showing no signs of fertilization 18 h after C-IVF. C-IVF was performed on patients who had >4 million total motile sperm after preparation. r-ICSI was then performed 18–24 h after insemination, using the sperm sample from the previous day. r-ICSI fertilization rates, cryopreservation of cleavage and blastocysts embryos, and pregnancy rates after fresh or frozen transfer were then assessed.

MAIN RESULTS AND THE ROLE OF CHANCE: r-ICSI was performed on 377 patients (2.3% of eligible retrieval cycles) who had a mean (±SD) female and male age of 35.9 ± 4.5 and 38.1 ± 9.1 years, respectively. A total of 5459 oocytes were initially retrieved. Of the oocytes undergoing r-ICSI, 2389 (49.5%) fertilized normally, and 205 (54.4%) patients underwent a fresh embryo transfer. The live birth rates were 23/186 (12.3%) for fresh cleavage and 5/19 (26.3%) for fresh blastocyst stage transfers. In 145 cycles a blastocyst was frozen, and 137 transfers were performed with a 64/137 (46.7%) live birth rate. Of the 377 cycles receiving r-ICSI only, 25 of the qualifying cases failed to have any fertilization, reducing TFF to 25/16 608 (0.15%).

LIMITATIONS, REASONS FOR CAUTION: This was a single-center retrospective study on a specific subset of patients, which may limit its generalizability to other clinics.

WIDER IMPLICATIONS OF THE FINDINGS: r-ICSI allows a second opportunity to fertilize oocytes despite poor initial outcomes. Patients who had a frozen blastocyst transfer achieved high live birth rates, indicating that a resynchronization of the embryo with the endometrium can optimize r-ICSI cases. r-ICSI allays fears of TFF when using C-IVF, providing evidence that the overuse of ICSI in patients without male factor may not be warranted.

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Keywords: rescue ICSI / failed fertilization / frozen blastocyst transfer / fertilization / conventional insemination / delayed fertilization / uterine synchrony / day after ICSI / total fertilization failure

Introduction

Despite improvements in ART success one of the most frustrating events for patients and clinics is failed fertilization. The event of total fertilization failure (TFF) can be devastating for the patient as they invest an intense emotional and financial commitment to an IVF cycle. This risk has led to many centers worldwide adopting routine use of ICSI, with the perception that it can drastically

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reduce the risk of fertilization failure. However, to this day, the question of whether ICSI can prevent fertilization failure (Flaherty et al., 1998; Bhattacharya et al., 2001; Mahutte and Arici, 2003) and improve outcomes remains controversial (van der Westerlaken et al., 2006; Bosch et al., 2020; Geng et al., 2020; Tremellen et al., 2021). Consequently, we are still searching for both oocyte and sperm markers that may aid in predicting whether failed fertilization may occur. The only clear possibilities we have in sperm are mutations of the phospholipase C zeta protein (Yelumalai et al., 2015; Torra-Massana et al., 2019). The complexities of both sperm and oocyte biology have so far hindered progress in finding diagnostics to predict failed fertilization.

Regrettably, the fear of TFF leads us to an overreliance on ICSI as a go-to technique for fertilization. Despite the dearth of evidence demonstrating a clear benefit when using ICSI versus conventional IVF (C-IVF) in patients with non-male factor infertility (Li et al., 2018), the worldwide rates of ICSI use are now approaching 70% (Boulet et al., 2015; Chambers et al., 2021). With the belief that ICSI reduces the occurrence of TFF, it has become the first choice over C-IVF. Perplexingly, although ICSI is a more laborious process and evidence exists of improved results when using C-IVF (Bhattacharya et al., 2001; Boulet et al., 2015), the use of C-IVF is still declining. More important is the fact that the arbitrary sperm selection by an ICSI embryologist using light microscopy does not compare with the hurdles put in place by years of evolutionary pressure, which include the cumulus/granulosa cells, zona pellucida, and oolemma.

Despite the overuse of ICSI, clinics still perform C-IVF. To aid patients who fail or have poor outcomes with C-IVF, 'rescue ICSI' (r-ICSI) is sometimes performed on oocytes that fail to fertilize, allowing patients to salvage their cycle. r-ICSI has been reported in numerous guises. Initially, when ICSI was first adopted in the early 1990s as a backup plan for cycles that failed C-IVF (Yuzpe et al., 2000; Kuczyński et al., 2002), it was performed the day following oocyte retrieval and embryos were usually transferred fresh at the cleavage stage. Overall, this approach is not very successful (Amarin et al., 2005; Beck-Fruchter et al., 2014), most likely as the delay in fertilization led to a detrimental asynchrony between the embryo and endometrium. Subsequently, many clinics stopped pursuing it as a mode of treatment. A second approach has been to assess inseminated oocytes in the subsequent hours after placing them with sperm, and if a second polar body is not observed then ICSI is performed (Chen and Kattera, 2003; Jin et al., 2014; Zeng et al., 2022). This technique has been reported more recently with varying success (Zeng et al., 2022), however, the risk of performing ICSI on an already fertilized oocyte exists.

In the current study, we revisit the earlier approach whereby, after failed C-IVF, r-ICSI was performed the following day. The objective of the present study is to evaluate r-ICSI fertilization rates, blastulation rates and, primarily, to investigate live birth rates in patients that had r-ICSI performed. Secondarily, we sought to compare the success rates of fresh embryo transfers versus frozen embryo transfers and how implementation of r-ICSI alters the overall TFF rates following C-IVF.

Materials and methods

This was a retrospective data analysis of patient cases between 1 April 2010 and 31 July 2021 conducted at a single academically affiliated fertility clinic, Boston IVF. r-ICSI is performed when oocytes fail to fertilize after C-IVF, allowing patients to salvage their current cycle. This data analysis was conducted under an institutional review board approval (#2019P000560) that determined the study to be non-human research. Written informed consent was not required from patients for the analysis and to perform r-ICSI.

Patient population

There was a total of 16 608 eligible (see below) retrieval cycles, utilizing C-IVF with greater than four oocytes retrieved, at Boston IVF during the study period. Frozen embryo transfers occurring up to 31 December 2021 were included. All r-ICSI cases were examined using a specifically validated IVF database from a single large urban IVF center in Massachusetts, USA, an insurance mandated state.

Insemination for IVF

IVF, using C-IVF, was performed in cases where the male had at least 4 million total motile sperm per milliliter post-processing. A total of five oocytes per 100 μl droplet of 5% human serum albumin in CSC media (Irvine Scientific, Santa Ana, CA, USA) were inseminated with 30 000 motile sperm per cumulus–oocyte complex. Patients with <4 million total motile sperm per milliliter post-processing undergo ICSI routinely in our institution. Over the course of the study ∼45–50% of retrievals in our clinic underwent C-IVF.

Rescue ICSI criteria, embryo culture, and pregnancy outcomes

Not all patients undergo r-ICSI if they fail to have fertilization using C-IVF. Prior to November 2016, criteria were based on a case-by-case assessment in relation to either TFF or low fertilization rates. Since November 2016, different criteria were adopted. Firstly, the patient must have > 4 metaphase II (MII) oocytes, showing no signs of fertilization 18 h post insemination and/or a fertilization rate of <15% per mature oocyte. Secondly, if the patient has four or more normally fertilized oocytes, regardless of total number of oocytes, the r-ICSI procedure is not performed. The current r-ICSI criteria at our institution are shown in Supplementary Table S1. r-ICSI is performed 18–24h after insemination; typically between 8:00 a.m. and noon. r-ICSI is also performed using the previous day's sperm sample, which is maintained at room temperature overnight. On the day following r-ICSI, the embryos are assessed for the presence of pronuclei and, if present, the embryos are placed in culture. The patient may then undergo either a cleavage or blastocyst fresh transfer or have blastocysts vitrified in accordance with clinical and patient choice. Briefly, for patients with four or more fertilized embryos, they will be cultured directly to the blastocyst stage, otherwise they will undergo a cleavage stage (Day 3) transfer (Sakkas and Gardner, 2017). If the patient identifies a preference for either fresh cleavage or blastocyst transfer the patient's choice is respected. A small number of patients pre-October 2012 had cleavage embryos frozen using a slow protocol. After October 2012, all patients' embryos were cultured out to the blastocyst stage to be vitrified. Protocols for embryo culture, vitrification, and embryo transfer have been previously published (Shear et al., 2020; Esiso et al., 2021). Briefly, blastocysts showing a Gardner grade greater than or equal to Stage 3 and inner cell mass and trophectoderm greater than or equal to B grade are cryopreserved. The timeline of embryo development and vitrification after r-ICSI is therefore clearly delayed by up to 24 h when compared to C-IVF.

A small number of patients underwent preimplantation genetic testing for aneuploidies (PGT-A) prior to vitrification. Briefly, a trophectoderm biopsy was performed on all good

quality embryos (defined as an embryo between Stages 3 and 6 with Grade A or B inner cell mass or trophectoderm). Embryos were deemed chromosomally normal according to the predetermined copy number cutoffs established by the commercial genetics testing laboratory. In accordance with Boston IVF protocol, embryo mosaicism data were not reported, hence any embryos falling under the cutoff for normal were not transferred.

Clinical pregnancy was defined as confirmation of a fetal sac at ultrasound, while miscarriage was defined as a loss of pregnancy after confirmation of a clinical pregnancy. Implantation rate was calculated as the number of fetal sacs per embryo transferred.

Statistical analysis

All statistical analyses and calculations of descriptives were performed using SPSS software version 22.0 (SPSS, Armonk, NY, USA) or MS Excel (Microsoft Corporation, Redmond, WA, USA). The χ^2 test was used to compare the fertilization, pregnancy, miscarriage, ongoing, and live birth rates between different clinical strategies. Student's *t*-tests were used to compare means when appropriate. A *P*-value of <0.05 was set as statistically significant.

Results

Patient demographics

The study population comprised 377 patients (2.3% of eligible retrieval cycles) who had r-ICSI performed because of either failed or very low fertilization rates (<15%) after C-IVF. The mean (±SD) female age of the 377 r-ICSI cases was 35.9 ± 4.5 years. The mean age of the male partner in the r-ICSI cases was 38.1 ± 9.1 years. Briefly, the patient demographics reflected women with an average BMI of 27.1 ± 6.7 kg/m^2, basal Day 3 FSH of 9.1 + 12.2 (IU/l) and anti-Müllerian hormone of 3.4 ± 3.6 (ng/ml). For 288 (76.4%) of the 377 patients this was their first retrieval, with the majority of the patients (328/377, 87%) nulligravid.

r-ICSI cycle outcomes

A total of 377 patients was analyzed with a total of 5459 oocytes retrieved. A synopsis of patient outcomes is shown in Table 1. Of the 5459 oocytes initially undergoing C-IVF a small percentage (152, 2.7%) did achieve fertilization (Table 1). Of the other 97.3% which failed to fertilize, r-ICSI was performed on 4015 (73.5%) mature oocytes the morning after C-IVF. The 152 normally fertilized oocytes were removed from calculations of r-ICSI blastocyst development and pregnancy assessment. Of the oocytes undergoing r-ICSI, 2389 (59.5%) fertilized normally with two pronuclei (2PN) visible. In addition to the 2PN embryos, a further 271/4015 (6.7%) showed three or more pronuclei, while 12/4015 (0.3%) had one pronucleus (Table 1). The routine ICSI fertilization rate for the past 5 years at our clinic is considerably higher, at 73.3% of 2PN embryos, with 1.8% and 1.7% having three or more pronuclei and one pronucleus embryos, respectively (data not shown).

The number of cycles receiving a fresh transfer and freezing blastocysts is shown in Table 1. Of the patients undergoing blastocyst culture after r-ICSI, 73 had preimplantation genetic testing for aneuploidy (PGT-A) performed on 105 blastocysts. Of these embryos, 40 (54.8%) patients had 47 (44.8%) blastocysts diagnosed as euploid.

Of the r-ICSI patients, 205 (54.4%) underwent a fresh embryo transfer, the majority of which were transferred at the cleavage stage (Table 2).

Blastocyst utilization rate

Since October 2012, 316 cycles had embryos cultured for blastocyst freezing. Many embryos were transferred fresh at the cleavage or blastocyst stage, however of those remaining 709/1932 (36.7%) reached the blastocyst stage while only 369 (19.1%) were of high enough quality to be cryopreserved. Of the 316 cycles which had embryos cultured for blastocyst freezing, 145 (45.8%) of the cycles had a blastocyst frozen including 50 patients with one frozen blastocyst, 40 with at least two, and 55 with greater than two. Of the 369 blastocysts frozen, 159 (43.0%), 205 (55.6%), and five (1.4%) were frozen on Days 6, 7, and 8 post-retrieval, respectively.

Pregnancy outcomes of r-ICSI cases

The pregnancy outcomes per transfer originating from r-ICSI cases are shown in Tables 2 and 3. A total of 92 live births were achieved. There was a significant (Chi square = 39.6: $P < 0.001$) difference in successful live birth outcomes, with only 28/205 (13%) from fresh transfers (Table 2) and 64/147 (41%) live births from frozen transfers (Table 3). The data indicate that having a frozen r-ICSI blastocyst transfer will give patients a higher chance of achieving a live birth, compared to a fresh transfer (Tables 2 and 3). The implantation rate was also significantly greater (Chi square = 84.7: $P < 0.001$) in frozen (48.8%) versus fresh transfers (12.3%) (Tables 2 and 3). The greatest benefit of r-ICSI was obtained for those that were able to achieve a frozen blastocyst transfer (Tables 2 and 3). Four cases of frozen blastocyst transfer led to twin pregnancies, all after the transfer of two blastocysts. Of the fresh transfers, only one cleavage stage transfer of two embryos led to a twin birth.

Of note, miscarriage rates were not elevated compared to our usual non r-ICSI cases (Tables 2 and 3). A small number of cases

Table 1. Conventional IVF cycle outcomes of the 377 rescue ICSI cycles performed.

	Number of cycles	Number	Mean	SD	Min	Max
CONVENTIONAL IVF OUTCOMES						
Oocytes retrieved/inseminated	377	5459	14.5	9.0	1	45
Fertilizations occurring by conventional IVF	80	152 (2.8%)	0.4	0.9	0	5
RESCUE ICSI OUTCOMES						
Mature oocytes undergoing r-ICSI	377	4015	10.6	6.7	1	36
Fertilized r-ICSI (2 pronuclei)	377	2389 (59.5%)	6.3	5.0	0	33
≥3 pronuclei		271 (6.7%)	0.7	1.0	0	6
1 pronucleus		12 (0.3%)	0.0	0.2	0	2
Fresh cleavage stage transfers	186	332	1.8	1.0	1	5
Fresh blastocyst transfers**	19	26	1.4	0.6	1	3
Total cleavage embryos frozen	14	35	2.5	1.8	0	6
Total blastocysts frozen	145	369	1.0	1.7	0	10

** Four cases of morula transfer.
r-ICSI, rescue ICSI.

Table 2. Pregnancy outcomes after fresh cleavage or blastocyst transfer of rescue ICSI generated embryos.

Fresh transfers	Number of transfers	Mean number of embryos transferred (±SD)	Pregnant [+ve HCG] n (%)	Clinical pregnancy# rate n (%)	Miscarriage rate n (%)	Implantation rate (%)	Live birth rate n (%)
Cleavage	186	1.8 (±1.0)	45 (24.2)	30 (16.1)	6 (3.2)	39/332 (11.7)	23 (12.4)
Blastocyst	19	1.4 (±0.6)	6 (31.6)	5 (26.3)	0 (0.0)	5/26 (19.2)	5 (26.3)
Total	205	1.7 (±1.0)	51 (24.8)	35 (17.1)	6 (2.9)	44/358 (12.3)	28 (13.7)

Clinical pregnancy was defined as confirmation of a gestational sac by ultrasound.

Table 3. Pregnancy outcomes after transfer of frozen rescue ICSI generated embryos.

Frozen transfers	Number of transfers	Mean number of embryos transferred (±SD)	Pregnant [+ve HCG] (%)	Clinical pregancy# rate n (%)	Miscarriage rate n (%)	Implantation rate N (%)	Live birth rate n (%)
Cleavage	10	1.8 (±0.6)	3 (30.0)	0 (0.0)	0 (0.0)	0/18 (0.0)	0 (0.0)
All blastocyst	137	1.1 (±0.3)	94 (68.6)	75 (54.7)*	11 (8.0)	84/154 (54.5)*	64 (46.7)*
Non-PGT-A	*122*	*1.1 (±0.3)*	*83 (68.0)*	*67 (54.0)*	*11 (9.0)*	*75/139 (53.9)*	*56 (45.9)*
PGT-A	*15*	*1.0 (±0.0)*	*11 (73.3)*	*8 (53.3)*	0 (0.0)	*9/15 (60.0)*	*8 (53.3)*
Total	147	1.2 (±0.4)	97 (66.0)	75 (51.0)	11 (7.5)	84/172 (48.8)	64 (43.5)

Clinical pregnancy was defined as confirmation of a gestational sac by ultrasound.
* Clinical pregnancy rate and live birth rate for frozen blastocyst transfers are significantly different ($P < 0.05$) compared to the fresh cleavage and blastocyst transfers shown in Table 2.
PGT-A, preimplantation genetic testing for aneuploidies.

(n = 15) had a frozen blastocyst transfer of PGT-A tested embryos (Table 3) achieving a live birth rate of 53.3% with no miscarriages. The mean (±SD) birthweight of the fresh transfer live births was 3342 ± 581 g. The mean (±SD) birthweight of the frozen transfer live births was 3357 ± 677 g. Fresh versus frozen live birthweights did not differ significantly.

Total fertilization failure

Of the 377 cycles receiving r-ICSI, only 25 failed to undergo fertilization. When examining all ICSI cycles with four or more MII injected, the TFF rate was 1.2% (168/13 819) during the same period. The TFF for C-IVF was higher in our study 377/16 608 (2.3%), however, the utilization of r-ICSI lowered TFF to only 25 cycles which did not have any fertilization, thereby reducing the TFF rate to 25/16 608 (0.15%).

Discussion

The present study supports the idea that r-ICSI coupled with frozen blastocyst transfer is the best mode of action for patients who have TFF or low fertilization rates when using C-IVF. Nearly 50% of r-ICSI cycles resulted in a frozen blastocyst and live birth rates of over 45% were achieved in couples who had blastocysts arising from r-ICSI vitrified and transferred at a later date. Our data support the recent meta-analysis by Paffoni *et al.* (2021) that concluded the transfer of cryopreserved r-ICSI embryos offered a substantial improvement in success rates, with a pregnancy rate per embryo transfer and implantation rate of 36% and 18%, respectively. They suggested that coupling r-ICSI with frozen embryo transfer may ameliorate the clinical pregnancy rate for embryo transfer, with an odds ratio of 4.7 (95% CI: 2.6–8.6), compared to fresh transfer. Our study differs from the meta-analysis as it presents data largely on vitrified blastocysts, which appears to convey an extra advantage to these cases as shown by the beneficial live birth rates. Furthermore, we present evidence that the use of r-ICSI largely eliminates the risk of TFF in C-IVF cases.

The different strategies that can be adopted to perform r-lCSl have pros and cons. Firstly, the wide spread use of r-ICSI after several hours (early r-ICSI) by investigating polar body extrusion (Jin *et al.*, 2014; Shibahara *et al.*, 2022; Zeng *et al.*, 2022) allows respectable pregnancy rates but the risk of performing ICSI on already fertilized oocytes remains. This was also problematic in our own study, with over double the rates of polyspermy observed in the r-ICSI group, even though we waited 18–24 h prior to performing ICSI. The timing of pronuclear formation in these r-ICSI cases remains challenging and may be better served by use of timelapse imaging. These fears have been slightly allayed, as some studies have reported low (3%) (Shibahara *et al.*, 2022) 3PN rates. In addition, the use of spindle imaging combined with early r-ICSI was shown to effectively prevent fertilization failure and decreased the polyspermy rate (Guo *et al.*, 2017). A further benefit of early r-ICSI is that it appears to maintain the ability to perform fresh transfers. Most of the early r-ICSI studies have been performed on cleavage stage transfers. In contrast to early r-ICSI attempts, the use of day after r-ICSI has the obvious drawback in that it creates a desynchrony between the embryo and endometrium. This is best exemplified by the poor rates of ongoing pregnancy after fresh transfer described in the meta-analysis (Paffoni *et al.*, 2021) and other individual studies (Kuczyński *et al.*, 2002; Shalom-Paz *et al.*, 2011; Ming *et al.*, 2012). In the present study, both fresh cleavage and blastocyst transfers had low live birth rates per transfer, at 12.4% and 26.3%, respectively. Although small in number, the use of fresh blastocyst transfers may confer an advantage, however, this needs to be confirmed. Moreover, the success of PGT-A on r-ICSI blastocysts (53% live birth) shows that this strategy is also very successful.

It has become clear that resynchronization of Day 7 vitrified blastocysts into the uterus has clear advantages (Hernandez-Nieto *et al.*, 2019; Tiegs *et al.*, 2019) over previous attempts to transfer delayed blastocysts (Shoukir *et al.*, 1998). The combination of improvements in blastocyst culture technique and vitrification in particular has provided definite advantages for the possibility of day after r-ICSI. The lack of pregnancies using slow frozen cleavage stage r-ICSI embryos also exemplifies the benefits of blastocyst culture and vitrification. In our study, at least one blastocyst was cryopreserved in 45.8% of r-ICSI cycles. Given

that these cycles commenced with poor outcomes on the initial day of fertilization assessment, the success of using r-ICSI as a strategy provided a positive live birth outcome for a high percentage of patients. It also provided benefit to patients undergoing C-IVF as it nearly eliminated the unwanted outcome of TFF (0.15%). Day after r-ICSI coupled with blastocyst vitrification leads to a valid tool in treating these patients, while allaying fears of TFF when using C-IVF.

Moreover, although the use of ICSI has increased dramatically over the years (Boulet *et al.*, 2015; Chambers *et al.*, 2021), C-IVF has been shown to provide certain benefits to non-male factor patients in comparison to the overuse of ICSI. Boulet *et al.* (2015) showed that ICSI use was associated with lower rates of implantation, live birth, and multiple live birth versus conventional IVF. Numerous studies have now shown that the overall live birth rate is not improved when using ICSI (Boulet *et al.*, 2015; Li *et al.*, 2018). In fact, a recent study by our group has demonstrated that the use of ICSI in non-male factor patients leads to 5% fewer euploid embryos in comparison to C-IVF (Patel *et al.*, 2023).

The use of r-ICSI has also exemplified how adaptive the human reproductive system is. Firstly, oocytes and sperm 18–24 h after collection can contribute effectively to viable blastocysts. The resilience of sperm is well documented (Critchlow *et al.*, 1989), but the question remains of whether further improvement could be gained by using a fresh sample. The logistics of coordinating this are complicated, as in many cases it could further delay performing ICSI as the partner may not be able to return to the clinic in a timely manner. The r-ICSI data indicate that the oocyte is also able to be delayed by up to 24 h but still maintain sufficient mRNA stores to reach embryonic genome activation and form a viable blastocyst. This indicates greater flexibility in human oocytes in the dynamics and relevance of maternal mRNA clearance than previously thought (Sha *et al.*, 2020). Postovulatory aging of mammalian oocytes has been shown to decrease their developmental capabilities (Di Nisio *et al.*, 2022). Aged oocytes display a wide range of various abnormalities, such as dysfunction of actomyosin and the microtubular cytoskeleton, decreased activity of M-phase promoting kinases, deregulation of energy metabolism and epigenetic alterations (Pickering *et al.*, 1988; Van Blerkom, 2011; Mackenzie *et al.*, 2016; Szpila *et al.*, 2019). In particular, depletion of calcium stores caused by aging have been shown to impact both fertilization and embryo development (Wakai and Fissore, 2013). Despite this deleterious effect of aging, certain oocytes appear to retain their viability even though their competence is greatly challenged by delaying fertilization and activation of the triggers that are initiated during fertilization (Zuccotti *et al.*, 2011; Innocenti *et al.*, 2022). An indicator that some loss of oocyte function could be evident is the high rate of oocytes with multiple pronuclei. Whether their oocytes were already fertilized or if these patients may have had underlying oocyte anomalies needs to be determined. Secondly, the concept of a strict synchrony needed for an embryo and the endometrium is challenged yet again by the r-ICSI model. Although the blastocyst is placed in a timed uterus, it appears that blastocysts of different ages are able to successfully implant. This goes against the central dogma of many other mammals where a tight synchrony is needed between embryo and endometrium for a successful pregnancy to be established (Paulson and Comizzoli, 2021).

The current study has a number of strengths and limitations. The study highlights how changes in protocols have improved the utilization of r-ICSI over the years. Pre-2012, there was a less stringent implementation of r-ICSI and it was coupled with a preference for fresh cleavage transfer and/or slow freezing. A strength of the study is that it delineates a patient population and protocol that clearly impacts TFF when using C-IVF coupled with blastocyst vitrification. Whether this approach is applicable to all patients, regardless of initial egg number that failed C-IVF, remains to be determined. A limitation of this study is that limited postnatal data is presented and follow up of these births is needed.

Mandates for IVF coverage are associated with lower ICSI use for non-male-factor infertility cycles (Dieke *et al.*, 2018). This indicates that there is also an economical burden on many patients who have ICSI performed. Our own data and that of others (Paffoni *et al.*, 2021) support the more widespread use of C-IVF without the of fear of fertilization failure. Given that r-ICSI coupled with blastocyst vitrification provides respectable chances of live births and the growing evidence that using ICSI does not necessarily favor non-male-factor patients over that of C-IVF, we propose that r-ICSI be reconsidered as a technique in the armory of the ART clinic.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Authors' roles

S.B., G.A., O.O., P.M., and D.S. contributed to the conceptualization of the idea of the manuscript and collection of data. S.B., G.A., and D.S. drafted the initial manuscript. All authors participated in the critical revision of subsequent drafts and approval of the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest in relation to the data published in the article.

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