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Dr. Deepak Patil
Command Hospital Air Force
Bangalore, Karnataka, India

Dr. Shahla Yazdani Abraham
Command Hospital Air Force
Bangalore, Karnataka, India

Corresponding Author:
Dr. Deepak Patil
Command Hospital Air Force
Bangalore, Karnataka, India

Retrospective study of rescue intra cytoplasmic sperm injection (ICSI) as an emergency procedure aimed at salvaging the IVF ET cycle

Dr. Deepak Patil and Dr. Shahla Yazdani Abraham

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Abstract

Background: Rescue Intra Cytoplasmic Sperm Injection (ICSI) is an emergency micromanipulation endeavour aimed at salvaging the IVF ET cycle. It is aimed to save the situation in which the ART team and the couple has invested so much.

Aim: The aim of this study was to assess the option of recue ICSI in cases of Fertilisation Failure (FF) in order to salvage the IVF ET.

Material and Methods: This retrospective study was done in a tertiary care Assisted reproductive techniques centre of Armed Forces. Records were assessed to study the cases of fertilization failure. All those cases which had an obvious etiology for probable FF were excluded. Remaining cases where rescue ICSI was done were studied to assess its role.

Study design: Retrospective Observational study

Study location: A tertiary care ART centre in Armed Forces Hospitals

Study duration: Dec 2018 to Dec 2020

Subjects and selection method: All couples going IVF ET for various etiologies were studied for fertilization failure. Only cases with unexplained FF were studied to assess the role of rescue ICSI.

Inclusion criteria

1. Normal BMI (in the range of 18.5 to 24.5 kg/m2)
2. Normal ovarian reserves (AMH above 1.0 ng/ml)
3. Conventional IVF used for fertilization

Exclusion criteria

1. Male factor infertility
2. Prior fertilisation failures
3. Advanced maternal/paternal age (Below 40 yrs)

Procedure methodology: Records of all patients meeting the inclusion and exclusion criteria were studied

Stimulation protocol: Antagonist protocol was used universally for stimulation in our centre. Patients were started on Inj Recombinant Follitropin Alpha (Merck Pharmaceuticals) 1050 IU/1.75 ml powder with solvent for stimulation. Personalised Stimulation protocol was started based on Age, BMI, Ovarian volume, AMH and previous stimulation data if any.

Inj Ovurelix containing Citrorelix 0.25 mg from Sun Pharmaceuticals was started as antagonist on evidence of sufficient endogenous Estrogen production. Namely ultrasounds follow up showing follicular size reaching 12mm or endometrial thickness more than 6mm.

On adequate stimulation with a cohort of at least 4 follicles of size 18mm, trigger was given with inj Ovitrelle 250 mcg (Contains Recombinant Choriogonadotropin Alpha,250 mcg, Merck Ltd)) and ovum pickup was done after 36-40h.

Conventional Insemination was done only if post wash specimen shows a sperm concentration of more than 20 million/ml with more than 50% grade 4 motility. After denudation metaphase 2 mature oocyte without two Pronuclei stage were reassessed after 2h to rule out delayed fertilization. Those m2 oocytes which failed to fertilize were provided rescue.

ICSI was done using micromanipulation disposable Injecting and holding needles from Vitromed, with bend angle of 30 degree. The holding needle had an inner diameter of 20 micro meters and injecting needle had an inner diameter of 5 micrometer. The freshly prepared semen sample was taken in PVP media under oil overlay and ICSI performed in the standard way.

Results: The incidence of fertilization failure in our clientele was about 11%. Out of 415 cases, 45 patients had complete fertilization failure. Patients meeting our inclusion and exclusion criteria for FF were 23 cases. Most common cause of FF was unexplained infertility with mean duration of marriage as 9y 7mo.Overall 15% cases were due to poor ovarian reserve and with poor yield on OPU. Male factor infertility in spite of ICSI failed to fertilize in 6 cases.

We obtained a fertilization rate of 41% with rescue ICSI and on further growth 56% reached 4 cell stages. Off these 21 had minimal fragmentation. Those embryos which had minimal fragmentation and equal blastomere were allowed to grow and 6 of 73 fertilized oocyte reached 8 cell stage.

Conclusion The emergency rescue ICSI, in window of 18-20 hours can help salvage a cycle faced with complete fertilisation failure. It will reduce the physical and financial burden of IVF ET cycle to some extent. We conclude it is a viable option in a perplexing situation of complete fertilisation failure

Keywords: IVF ET, fertilisation failure, rescue ICSI, reinsemination

Introduction

Infertility is a global problem. Worldwide it affects around 20-30% of couples. Since introduction by Patrick Steptoe and Robert Edwards in the 1970s there have been rapid advances in all aspects of IVF-ET cycle. More patient friendly soft stimulation protocol has lessened the burden and side effects of super ovulation. Improvement in media formulation and less batch to

batch variation has made culturing gametes more optimal. Better regulated quality of disposable has, improved culture conditions.
The situation of fertilisation failure (FF) is challenging and perplexing, both for the couple and treating team at ART Centre. Options available include reassessing stimulation protocol and auditing preparation and handling of gametes. Rescue Intra Cytoplasmic Sperm Injection (ICSI) is an emergency micromanipulation endeavour aimed at salvaging the IVF ET cycle. It is aimed to save the situation in which the ART team and the couple has invested so much.

Material and Methods

This retrospective study was done in a tertiary care Assisted reproductive techniques centre of armed forces. Records were assessed to study the cases of fertilization failure. All those cases which had an obvious etiology for probable FF were excluded. Remaining cases where rescue ICSI was done were studied to assess its role.

Study design: Retrospective observational study.

Study location: A tertiary care ART centre at Guwahati.

Study duration: Dec 2018 to Dec 2020.

Subjects and selection method

All couples going IVF ET for various etiologies were studied for fertilization failure. Only cases with unexplained FF were studied to assess the role of rescue ICSI.

Inclusion criteria

1. Normal BMI (in the range of 18.5 to 24.5 kg/m2).
2. Normal ovarian reserves (AMH above 1.0 ng/ml).
3. Conventional IVF used for fertilization.

Exclusion criteria

1. Male factor infertility
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Procedure methodology

Records of all patients meeting the inclusion and exclusion criteria were studied.

Stimulation protocol

Antagonist protocol was used universally for stimulation in our centre. Patients were started on Inj Recombinant Follitropin Alpha (Merck Pharmaceuticals) 1050 IU/1.75 ml powder with solvent for stimulation. Personalised Stimulation protocol was started based on Age, BMI, Ovarian volume, AMH and previous stimulation data if any.
Inj Ovurelix containing Citrorelix 0.25 mg from Sun Pharmaceuticals was started as antagonist on evidence of sufficient endogenous Estrogen production. Namely ultrasounds follow up showing follicular size reaching 12mm or endometrial thickness more than 6mm.
On adequate stimulation with a cohort of at least 4 follicles of size 18mm, trigger was given with inj Ovitrelle 250 mcg (Contains Recombinant Choriogonadotropin Alpha, 250 mcg, Merck Ltd)) and ovum pickup was done after 36-40h.

Ovum pick up and IVF-ET

The aspirated oocyte Cumulus complexes (OCC) were double washed in HEPES based media. They were dissected free of extra cumulus and blood clots under magnification and transferred to bicarbonate-based media in micro drop (50 microlitre) culture system with overlay of mineral oil.
All the media required for the OPU were aliquoted on the previous evening for overnight equilibration. Bicarbonate media was taken out in loose cap tubes and HEPES Based in tight screw cap type tubes. Plates were prepared under oil the previous evening based on expected oocyte yield.
Semen preparation was done using double density gradient method. The liquefied semen sample was overlaid, over a double density gradient of ORIGIO Gradient 40/80 from cooper surgical. It contained silane coated colloidal silica particles with HEPES as buffering agent. The conical tube is centrifuged at 1700 rpm for 15 min. The resultant pellet was resuspended in 5 ml of HEPES based media and centrifuged at 800 rpm for 5 min. After removing the supernatant, the pellet was overlaid with 1 ml of Bicarbonate based media for 30 min to harvest the swim up sperms. For swim up Universal IVF Medium from Cooper Surgical was used. Post wash was assessed to see suitability for conventional IVF. Conventional Insemination was done only if post wash specimen shows a sperm concentration of more than 20 million/ml with more than 50% grade 4 motility.
After 3-4 hour of culture in 6% Carbon Dioxide and at temperature of 37degree Celsius the droplets were inseminated with prepared semen sample. For culturing the embryos and insemination, micro drops of 50 microliter of Universal Fertilisation Media under mineral oil were used. Five such micro drops were made in one 60mm human IVF grade Nunc Petri Dish. Each such micro drop contained not more than 4 oocytes.
Fertilisation was assessed after denudation after 16-18 hrs of insemination. After denudation metaphase 2 mature oocyte without two Pronuclei stage were reassessed after 2h to rule out delayed fertilization. Those m2 oocytes which failed to fertilize were provided rescue.
ICSI was done using micromanipulation disposable Injecting and holding needles from Vitromed, with bend angle of 30 degree. The holding needle had an inner diameter of 20 micro meters and injecting needle had an inner diameter of 5 micrometer. The freshly prepared semen sample was taken in PVP media under oil overlay and ICSI performed in the standard way.
The injected oocytes were transferred to fresh Bicarbonate based media in micro drop culture and incubated again for 16-18 hrs to assess the fertilization. Findings were recorded.
Data was analyzed with chi square test.

Results

1. **Basic data:** In 415 cycles we retrieved 3320 oocytes. Fertilization failure occurred in 45 cases. Incidence of OHSS was about 2.6%. 2 of 11 patient needed paracentesis. On average 8 oocytes were retrieved per ovum pickup. No follicle could be retrieved in 7 patients on the day of OPU. Most of these turned out to be error in dispensing and timing of trigger. Embryo transfer was not done in 9 cases. Four had poor endometrial thickness. Three had fluid in endometrial cavity on day of transfer. In remaining three transfers was deferred due to OHSS. In all cases of non-severe male factor infertility, we did conventional insemination. Our fertilization rate has been optimal with good embryo quality. ICSI was done in 93 cases of severe male factor infertility; with a fertilization rate of 94%. 6 cases had fertilization failure in spite of primarily ICSI. Most of these had surgically retrieved spermatozoa. Vitrification was decided based on number of embryos, fragmentation percentage, rate

of division and patient profile. Vitrification was done on Day2 for 25%, Day 3 for 60% and in 15% cases we did Day 5 Vitrification. (Table 1)

2. **Patient characteristics:** In our study population we saw that as the 18 of 43 patients were in 38-40year age group. This amounted to 40 percent of cases of fertilization failure. While assessing the duration of infertility, the maximum distribution of cases of FF occurred in couples married and infertile for more than 12 years. Cases of FF were equally distributed along various AFC cut-offs.[Figure 1(a), (b) & (c)].
3. **Etiology fertilization failures:** The incidence of fertilization failure in our clientele was about 11%. Out of 415 cases, 45 patients had complete fertilization failure. Patients meeting our inclusion and exclusion criteria for FF were 23 cases. Most common cause of FF was unexplained infertility with mean duration of marriage as 9y 7mo.Overall 15% cases were due to poor ovarian reserve and with poor yield on OPU. Male factor infertility in spite of ICSI failed to fertilize in 6 cases. (Table 2)
4. **Lab data:** The choice of media did not affect occurrence of FF. There were total 178 oocytes which failed to fertilize. 70% were mature metaphase 2 ooctes.41 were immature germinal vesicle stage oocyte. 13 were found to be grossly abnormal in shape and size. (Table 3 & 4).

We obtained a fertilization rate of 41%. On further growth 56% reached 4 cell stages. Off these 21 had minimal fragmentation. Those embryos which had minimal fragmentation and equal blastomere were allowed to grow and 6 of 73 fertilized oocyte reached 8 cell stage. (Table 5& 6)

Table 1: Basic Data (IVF Cycle and Embryology)

S. No.	Entity	Number
1.	Total IVF ET Cycles	415
2.	Total oocyte retrieved	3320
3.	Fertilisation failure	45
4.	Mod to severe OHSS	11
5.	Empty Follicle Cases	07
6.	ET abandoned	9
7.	Primarily conventional IVF	314
8.	Primarily ICSI	93
9.	RESCUE ICSI	23
10.	Verification on D2 (n=363)	91
11.	Verification on D3 (n=363)	217
12.	Verification on D5 (n=363)	55

Fig 1(a): Age

Table 2: Etiology (Distribution of cases with Fertilisation Failure)

S. No.	Cause of Infertility	Cases
1.	Endometriosis	5
2.	Unexplained Infertility	11
3.	Poor ovarian reserve	7
4.	Excessive BMI	5
5.	Recurrent FF	4
6.	Male factor infertility	6
7.	Bilateral Tubal block	3
8.	Genital Tuberculosis	4

Choice of media in cases of fertilisation failure

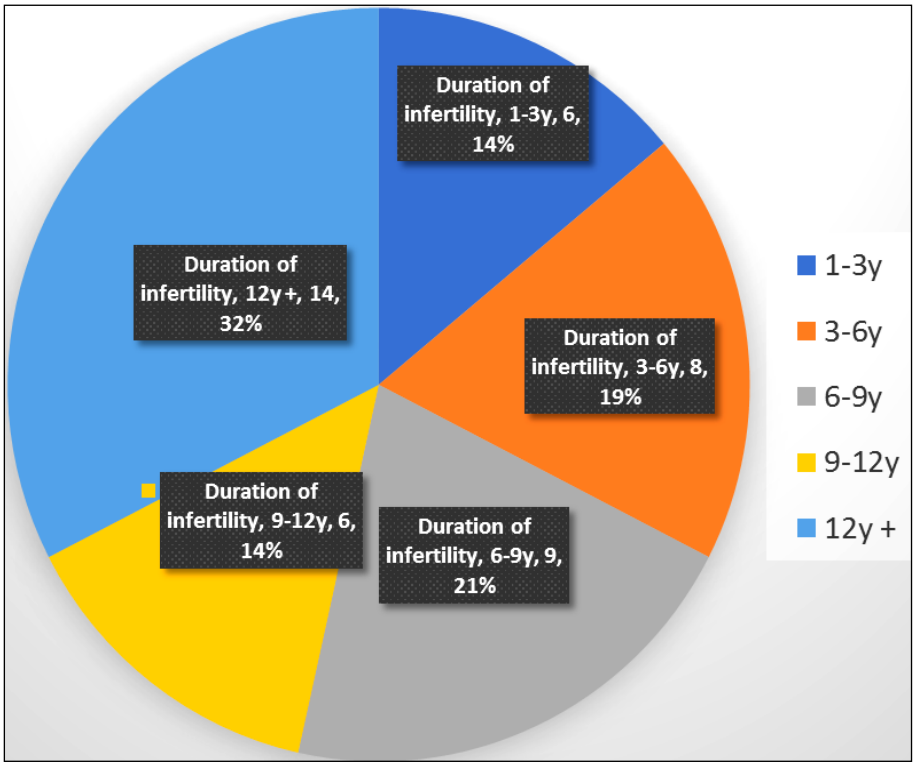

Fig 1(b): Durability of infertility

Table 3: Media for Embryo Growth

S. No.	Single step media	Sequential media
1	26 (57.7%)	29 (64.4%)

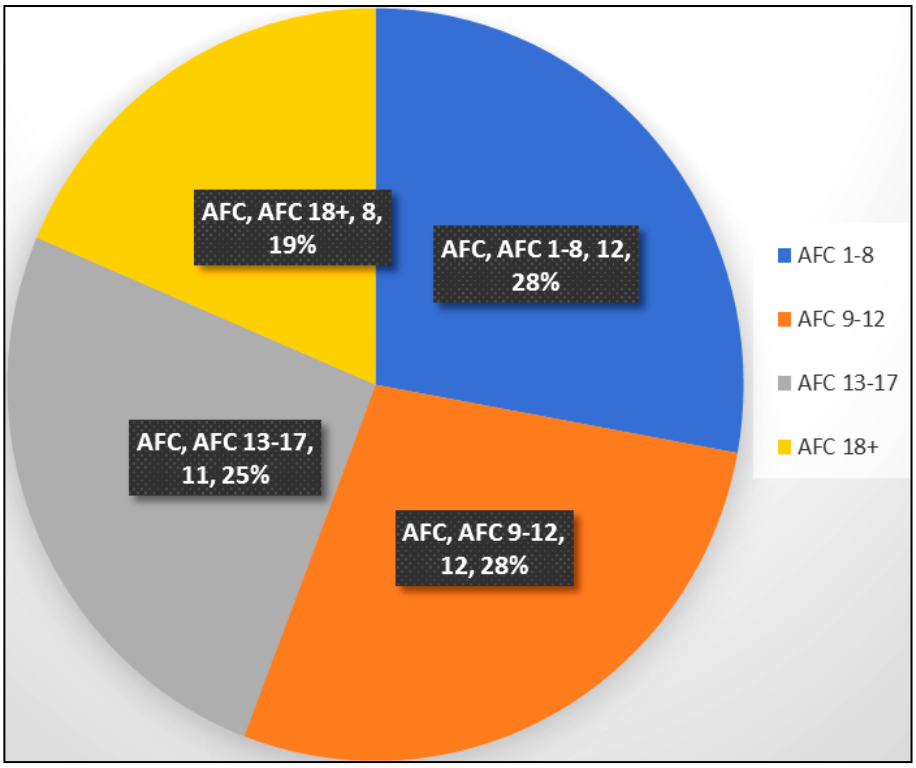

Fig 1(c): AFC

Table 4: Distribution of Oocyte Quality

S. No.	Oocyte grading	Number
1.	M2 Mature	124
2.	M1 Immature	41
3.	Morphologically abnormal	13

Result rescue ICSI

Table 5: Outcome of Rescue ICSI

S. No.	Fertilised	Unfertilised	Degeneration
1	73 (n=178, 41%)	76 (n=178, 42%)	29 (n=178, 16%)

Embryo quality post rescue ICSI

Table 6: Embryo growth Pattern Post Rescue ICSI

S. No.	Cell Stage	Number of embryos	Fragmentation mild (Below 10%)	Moderate (10-25%)	Severe (More than 25%)
1.	2PN arrest	9	0	0	0
2.	2c arrest	17	4	9	4
3.	4c	41	21	13	7
4.	8c	6	4	2	1

Discussion

Fertilization is the hallmark event of coming together of male and female gamete. It's a complex and robust mechanism leading to formation of a zygote. Healthy fertilization requires optimal health of the oocyte and spermatozoa [1].

Fertilization failure is a unique complication of IVF ET Cycle. Incidence is about 5-25% following conventional IVF and about 5% with ICSI. More importantly there is a high recurrence rate of Fertilization failure [2, 3]. After undergoing all the stimulation protocol and surgical procedure of oocyte aspiration the couple and clinician are faced with a unique challenge. The treatment is both mentally, physically and financially taxing.

In our study the incidence of Empty follicle syndrome (EFS) was 1.6%.Most of these were false EFS.

After initial success of IVF ET and live births it was felt that male factor is the limiting factor. As safer ways to retrieve oocytes advanced from laparoscopy to vaginal, it became evident that a method to bypass the shortcoming of male gamete was required. Over time from Zona drilling (Gorden *et al.*, 1988), Subzonal Insemination (Ng *et al.*, 1988), Zona dissection (Malter and Cohen, 1989) and then placement of sperm directly in the ooplasm (ICSI, Palermo *et al.*, 1992) developed. Various innovations in the techniques, disposables and media finally lead Gianpiero Palermo to accidently performs a successful and reproducible procedure of ICSI [4].

We performed the procedure of rescue ICSI by around 20 hrs post insemination. As the time from insemination to reinsemination by ICSI increases, there is a fear of oocyte ageing, chances of infection and cytogenetic abnormalities in the embryo. To avoid these, Lixia Zhu *et al.* proposed early rescue ICSI as early as 4-6 h after insemination[5].Proponents of early rescue recommends rescue as early as 6h after primary insemination. It is supposed to give better fertilization, pregnancy and implantation rate [6].

Early rescue can lead to high incidence of 3 Pronuclei formation, thus polyploidy due to polyspermy and attendant cytogenetic aberration [7].

We got a fertilization rate of 41%. Other studies also recommend a rescue procedure of, 19-22 h post initial insemination [8].

We saw that incidence of fertilization Failure in our clientele was about 11% of 415 cycles done for various indications. A quarter of our couples with FF were unexplained infertility. This was the largest contributor in our study group. Numerous other studies also found that in unexplained infertility as duration increases chances of FF increases [9, 10].

Ladies with poor ovarian reserve with low oocyte yield were also significant part of FF cohort. A healthy oocyte is a major prerequisite for fertilization. Mere presence of polar body may not be an indication of adequate oocyte maturity. Oocyte maturity is sum of Nuclear and cytoplasmic maturity [11, 12]. Thus

apparently metaphase 2 oocyte may not be competent to participate in a complex fertilization process leading to FF.

Miaomiao Jing *et al.* in a retrospective study of 1638 individuals in long agonist cycle and 621 patients of antagonist cycle concluded that Antagonist cycle are more economical. This was because of lower doses of gonadotropin were required as well as less hospitalization due to OHSS [13].

In our study stimulation protocol did not affect incidence of FF. There was even distribution between the two protocols being widely followed. It is usually hypothesized that antagonist protocol are better. They lead to better oocyte quality. In our study population it was not seem to affect the incidence of fertilization failure.

In patients with previous IVF failure, Seylit Temel Ceyhan *et al.* found similar fertilization rate and clinical pregnancy rate using either Antagonist or agonist cycle in the subsequent cycle. This study was done over 10 years in patients with previous IVF failure due to any reason [14].

In our study population choice of media did not affect the incidence of FF. It is usually felt that continuous single step media improves the rate of blastocyst formation[15]. Because of less handling and less exposure to fluctuation in incubation conditions, continuous media is likely to provide better culture condition. Martin Stimpfel *et al.* in a retrospective study of 127 patients found that with continuous media can be equivalent to sequential media. With stringent quality control and robust supply chain, the embryo growth in both these culture condition are similar [16].

In conventional IVF, it follows the rule of natural selection. The best suited sperm reach the oocyte leading to cortical reaction. This heralds the onset of flux in cytoplasmic calcium and cascade of fertilization. Numerous authors support use of conventional IVF to improve embryo quality and quantity in non-severe male factor [17]. Implantation rate, clinical and live delivered pregnancy rate were significantly higher in conventional IVF group. We included only cycles with conventional IVF in our study. This also ruled out male factor as cause of FF. Table 6 shows fragmentation rate in rescue ICSI cohort of embryos. Best quality embryos with maximum implantation potential are embryos with minimal or below 10% fragments in the cytoplasm. Fragments are defined as anuclear cytoplasmic granules of blastomeric origin. As the fragments increase during the process of mitosis, the daughter blastomere inherits reduced

Cytoplasmic component [18]. This lead to reduced potential to grow and implant. For 2 cell and 4 cell embryos, 58 and 79% respectively were best embryos at freezing. For 8 cell embryos fragmentation increased on day 3.Only 31% had best embryo quality on day 3. This may be due to oocyte aging and deterioration of cytoskeleton framework of mitotic spindle.

Some authors have given a very poor implantation and clinical pregnancy rate in rescue ICSI embryos [19]. These may be due to poor oocyte quality and cytogenetic abnormality of embryos. Other reason may be due to dyssynchrony between embryo development and endometrial growth. But till further research in reproductive medicine Rescue ICSI remains the only modality to face FF.

Timing of rescue ICSI remains a controversial issue. We used a window of 18-20 hrs and got a fertilization rate of 41%. Yuxia He *et al.* and Shun Xiong recommended that in patients with high risk of FF, a short insemination period of 6h is adequate. Removal of cumulus cells and followed by a rescue ICSI if needed is suggested [20, 21].

Linjun Chen *et al.* studied 233 children born following rescue ICSI with a control group of 906 conceived following ICSI with ejaculated sperm. This is one of the largest studies of rescue ICSI and attests to the safety of the procedure. There was no increased risk of birth defects, perinatal death or stillbirths. The two groups did not differ in gender rate, birth weight and gestational age [22].

Limitation of our study

1. Further evaluation of vitrified embryos and their outcome was beyond the scope of this study.
2. Increased study sample size could give more statistical significance to the findings.

Conclusion

The emergency rescue ICSI, in window of 18-20 hours can help salvage a cycle faced with complete fertilisation failure. It will reduce the physical and financial burden of IVF ET cycle to some extent. We conclude it is a viable option in a perplexing situation of complete fertilisation failure.

The ART Centre should be ready to undertake a rescue procedure on day of fertilisation check. This is essential because the situation of Fertilisation Failure is usually unexpected and very traumatic of all the stakeholders.

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Declarations

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Conflict of interest: None declared.

Ethical approval: The study was approved by the Institutional Ethics Committee.

References

1. Snehil Budhwar, Vertila Singh, Priyanka Verma, Kiran Singh. Fertilisation Failure and gamete health: is there a link? Front Biosci 2017;9:395-419.
2. Neal G Mahutte, Aydin Arici. Curr Opin Obstet Gynecol 2003;15(3):211-8.
3. Ronit Beck-Fruchter, Michal Lavee, Amir Weiss, Yoel Geslevich, Eliezer Shalev. Rescue ICSI: A systematic review, Fertility and Sterility 2014;101(3):P690-698.
4. Zev Rosenwaks, Nigel Pereira. The pioneering of Intracytoplasmic sperm injection: historical perspective. Reproduction 2017;154(6):F71-F77.
5. Lixia Zhu, Qingsong Xi, Rui Nie, Wen Chen, Hanwang Zhang, Yufeng Li. Rescue intracytoplasmic sperm injection: a prospective randomised study. J Reprod Med. Sep-Oct 2011;56(9-10):410-4.
6. Jeong Hee Moon, Weon-Young Son, Sara Henderson, Alina Mahfoudh, Michael Dahan, Hananel Holzer. Spindle examination in unfertilised egg using the polarisation microscopecan assist rescue ICSI. Reprod Biomed Online 2013;26(3):280-5.
7. Christopher Chen, Suresh Kattera. Rescue ICSI of oocytes that failed to extrude the second polar body 6h post insemination in conventional IVF. Hum Reprod 2003;18(10):2118-21.
8. Yuzpe AA, Liu Z, Fluker MR. Rescue ICSI- Salvaging IVF Cycles after total or near total fertilisation failure. Fertil Steril 2000;73(6):1115-9.
9. Inci Kahyaoglu *et al*. Total Fertilisation Failure: is it end of the story. J Assist Reprod Genet 2014;31(9):1155-1160.

10. Avner Hershlag, Terry Paine, Georgina Kvapil, Huailiang Feng, Barbara Napolitano. *In vitro* fertilisation – intracytoplasmic sperm injection split: an insemination method to prevent fertilisation failure. Fertil Steril 2002;77(2):229-32.
11. Check JH, Bollendorf A, Summers-Chase D, Horwath D, Hourani W. Conventional oocyte insemination may result in a better pregnancy outcome than ICSI for unexplained infertility. Clin Exp Obstet Gynecol 2009;36(3):150-1.
12. Jason Swain E, Thomas Pool B. ART failure: oocyte contributions to unsuccessful fertilisation, Hum Reprod Update. Sep-Oct 2008;14(5):431-46.
13. Miaomiao Jing *et al*. Cost effectiveness analysis of Gnrh agonist long protocol and GnRH antagonist protocol for *in vitro* fertilisation. Sci Rep 2020;10(1):8732.
14. Seyit Temel Ceyhan, Yeşim Bayoğlu Tekin, Mehmet Sakinci, Cihangir Mutlu Ercan, Uğur Keskin. What should be the protocol selection after failure of I vitro fertilisation at normoresponder patients: Agonist or Antagonist? Turk J Obstet Gynecol 2014;11(4):198-202.
15. Jie Deng, Qianying Zhao, Cengiz Cinnioglu, Refik Kayali, Ruth B Lathi, Barry Behr. The impact of culture condition on blastocyst formation and aneuploidy rates: a comparision between single step and sequential media ina large academic practice. J Assist Reprod Genet 2020;37(1):161-169.
16. Martin Stimpfel, Lili Bacer-Kermavner, Nina Jancar, Eda Vrtacnik-Bokal. The influence of the type of embryo culture media on the outcome of IVF/ICSI cycles. Taiwan J Obstet Gynecol 2020;59(6):848-854.
17. Martin Stimpfel, Nina Jancar, Eda Vrtacnik-Bokal, Irma Virant-Klun. Conventional IVF improves blastocyst rate and quality compared to ICSI when used in patients with mild or moderate teratozoospermia. Systems Biology in Reproductive Medicine 2019;65(6):458-464.
18. Van Royen E, Mangelschots K, De Neubourg D, Laureys I, Ryckaert G, Gerris J. Calculating the implantation potential of day 3 embryos in women younger than 38years of age: a new model. Human Reprod 2001;16(2):326-32.
19. Kuczyński W, Dhont M, Grygoruk C, Pietrewicz P, Redzko S, Szamatowicz M. Rescue ICSI of unfertilised oocytes after IVF. Human Reproduction 2002;17(9):2423-2427.
20. Yuxia He, Haiying Liu, Haiyan Zheng, Li Li, Xin Fu, Jianqiao Liu. Effect of early cumulus cell removal and early rescue ICSI on pregnancy outcomes in high risk patients of fertilization Failure. Gynecol Endocrinol 2018;34(8):689-693.
21. Shun Xiong *et al*. Effect of cumulus cell removal after 6h co incubation of gametes on the outcome of human IVF. J Assist Reprod Genet 2011;28(12):1205-11.
22. Linjun Chen *et al*. Neonatal Outcome of early rescue ICSI and ICSI with ejaculated spermatozoa. J Assist Reprod Genet 2014;31(7):823-828.