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A real-world retrospective cohort study examined the role of split IVF with ICSI and early rescue ICSI in preventing low fertilization rates during the first ART cycle

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Abstract: In ART while we are performing conventional in vitro fertilization procedures, sometimes it ends with the cancellation of the current treatment cycle due fertilization failure or lack of fertilization. Modern diagnostic methods such as intracytoplasmic sperm injection (ICSI) are limited in their ability to predict and prevent such failures. Intra cytoplasmic sperm injection (ICSI), carried out 16 to 18 hours after fertilization, has been shown to slightly improve fertilization and cleavage rates then conventional in vitro fertilization. Co-culture of short gametes with IVF (ECO-S) along with early rescue ICSI (R-ICSI) have been employed as two solutions to this issue.

OBJECTIVES: In vitro fertilization with short-term gamete co-culture (IVF-C) and early intracytoplasmic sperm injection (R-ICSI) in combination with fractionated IVF-ICSI were the two antihypertensive assays investigated in this study. retrospective cohort. It aims to assess the effectiveness of various methodologies. Fertility rate by assisted reproductive technology (ART) cycles.

METHODS. One of two treatments (fractional IVF-ICSI or IVF-C with R-ICSI) was used in high-risk couples with reduced fertility during the first cycle of ART. This study assessed fertility, Clinical findings and embryonic quality.

RESULTS. The study included 720 couples in the Splitting IVF-ICSI group (Group 1). and 188 couples to the IVF-C & R-ICSI group (Group 2) after comparing propensity scores. The two groups' average fertility rates were comparable. Group 1 outperformed the other groups in terms of both the proportion of numerous pronuclei (10.42% compared 4.50%, p=0.001) and the percentage of embryo utilization (59.84% versus 53.60%, p=0.001). High-quality embryos, embryo transfers, clinical pregnancies, and live deliveries were equally prevalent in both groups. In vitro fertilization rates between groups 1 and 2 were 4.79% and 9.03%, respectively, but no observable differences in fertility or embryonic development were found.

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In conclusion, fractional IVF-ICSI and early R-ICSI have proven to be effective methods to prevent infertility. Given the advantages of high embryo availability, fewer required ICSI procedures, and equal rates of clinical pregnancy and live birth, IVF with early R-ICSI appears to be the best procedure.

Keyword: Low fertilization, insufficient gamete co-cultivation time, early salvage IVF-ICSI, and ART in the first cycle.

1. Introduction

Traditional IVF (in vitro fertilization) procedures sometimes end in cycle cancellation due to an entire lack of fertilization. Modern diagnostic methods are limited in their ability to predict and prevent such failures. The rates of subsequent embryonic development and pregnancy are significantly lower than those of those oocytes, but remain suboptimal due to aging. Late intracytoplasmic sperm injection (ICSI), carried out 16 to 18 hours after fertilization, has been shown to slightly improve fertilization and cleavage rates. Co-culture of short gametes with IVF (ECO-S) along with early rescue ICSI (R-ICSI) have been employed as two solutions to this issue.

According to observations, oocytes typically become fertilized between two and six hours after being exposed to sperm, and 90% of fertilized oocytes release their second polar body within six hours. Time-lapse video is used to identify coculture fertilization of short gametes early, allowing for quick R-ICSI intervention before oocyte quality degrades with age. Under a microscope, the granulosa cells that surround the oocyte must be removed using this technique. IVF has a variable effect on fertilization and embryo quality, according to earlier research. Numerous studies demonstrate that by minimizing the negative effects of sperm and its metabolites on the embryo, IVF-C can enhance embryo quality and clinical pregnancy rates. Besides, elimination of granulosa cells after a short co-culture of gametes for 6 hours reduces the incidence of aberrant fertilization compared to elimination after 20 hours of fertilization. However, early removal of granulosa cells has been reported to increase the rate of double fertilization. However, most researchers believe that removal of granulosa cells after a short 6-hour co-culture of gametes allows early detection of reduced or absent fertilization without harming embryo quality. that the early elimination of granulosa cells increases the rate of double fertilization. However, most researchers believe that removal of granulosa cells after a short 6-hour co-culture of gametes allows early detection of reduced or absent fertilization without harming embryo quality. that the early elimination of granulosa cells increases the rate of double fertilization. However, most researchers believe that removal of granulosa cells after a short 6-hour co-culture of gametes allows early detection of reduced or absent fertilization without harming embryo quality. Early elimination of granulosa cells increases the rate of double fertilization. However, most researchers believe that removal of granulosa cells after a short 6-hour co-culture of gametes allows early detection of reduced or absent fertilization without harming embryo quality. Early elimination of granulosa cells increases the rate of double fertilization. However, most researchers believe that removal of granulosa cells after a short 6-

hour co-culture of gametes allows early detection of reduced or absent fertilization without harming embryo quality.

Another method to prevent unsuccessful fertilization is separate IVF-ICSI insemination. Studies have shown that the failure of in vitro fertilization occurs in a significant proportion of couples with unexplained infertility, but in parallel with conventional in vitro fertilization, the formation of fraternal cumulus-oocyte complexes occurs, which does not did not occur during ICSI on the body (COC). . Use of ICSI without male infertility is associated with higher fertility but does not improve live birth outcomes. Patients with moderate male infertility and polycystic ovarian syndrome (PCOS) have been shown to benefit from separate insemination IVF-ICSI, which has been shown to boost fertility rates and decrease overall loss of fertility. However, compared to IVF, ICSI had a decreased rate of blastocyst development.

It's critical to create strategies to stop fertility loss because there are currently no clinical recommendations for the standard IVF insemination of high-risk couples. There are presently no studies that directly compare the outcomes of fractional IVF-ICSI and IVF-C with initial R-ICSI. We thus aimed to evaluate the efficacy of these two strategies in preventing a potentially unanticipated drop in fertility in this real-world retrospective cohort research.

2. Materials and methods

2.1 Participants

At Sun Yat Sen Memorial Hospital, a retrospective cohort research was carried out with a focus on patients who had in vitro fertilization by sperm injection (IVF-C) using two distinct techniques. IVF-ICSI (fractional IVF with intracytoplasmic sperm injection) was used on Group 2. The research was conducted between January 2017 and July 2019. All subjects had four or more oocytes harvested, were symptomatic, and were on their first round of assisted reproductive technology (ART). At least one high risk factor, such as unexplained infertility, borderline sperm parameters, or infertility lasting more than five years. Diagnostic standards for unexplained infertility include regular ovulatory cycles, a healthy uterus, and the patency of the fallopian tubes. The World Health Organization's recommended normal levels for semen values are met, and endometriosis is not visible clinically or on ultrasound. The analysis excluded patients whose oocyte maturation was impaired. The ethics committee of Sun Yat Sen Memorial Hospital approved this experiment. To protect patient privacy, the data was anonymized, and no informed consent from any particular person was needed. The ethics committee of Sun Yat Sen Memorial Hospital approved this experiment. The data was anonymised to safeguard patient privacy, and no individual informed permission was required. The Sun Yat Sen Memorial Hospital's ethics committee gave its approval for this project. The data was anonymised to safeguard patient privacy, and no individual informed permission was required.

Table 1 - Demographics of patient populations in two study groups

	Before SME		**p-value**	**After PSM**		**p-value**
	Group 1 (N=191)	Group 2 (N=775)		Group 1 (N=188)	Group 2 (N=720)	
age (g)	31.37±3.85	32.34±3.96	0.002	31.46±3.79	31.94±3.72	0.112
Years without pregnancy (y)	4.89±2.66	5.96±3.20	0.000	4.93±2.66	5.89±3.15	0.000
Infertility factors (%), primary infertility (%) (n)	78.53 (150/191)	67.48 (523/775)	0.003	78.72 (148/188)	69.58 (501/720)	0.013
Ovulation disorder	9.42 (18/191)	10.71 (83/775)	0.131	9.57 (18/188)	10.98 (79/720)	0.089
fallopian tube factor	21.99 (42/191)	26.58 (206/775)		21.28 (40/188)	27.5 (198/720)	
endometriosis	4.71 (9/191)	3.23 (25/775)		4.79 (9/188)	3.06 (22/720)	
light male element	16.75 (32/191)	15.23 (118/775)		16.49 (31/188)	14.58 (105/720)	

unexplained factors	27.74 (53/191)	22.19 (172/775)		28.19 (53/188)	22.50 (162/720)	
multiple female factors	10.99 (21/191)	8.13 (63/775)		11.17 (21/188)	7.92 (57/720)	
women and men	8.38 (16/191)	13.94 (108/775)		8.51 (16/188)	13.47 (97/720)	
Basic FSH (u/l)	8.05±3.33	7.88±3.91	0.164	8.06±3.35	7.83±3.90	
AMH (ng/ml)	5.03±3.85	5.40±3.83	0.172	4.98±3.79	5.54±3.87	
COS protocol, % (n)						
GnRH agonist	73.82 (141/191)	65.16 (505/775)	0.023	73.94 (139/188)	64.86 (467/720)	0.019
GnRH antagonist	26.18 (50/191)	34.84 (270/775)		26.06 (49/188)	35.14 (253/720)	
excitement time	11.38±2.47	11.01±2.87	0.102	11.40±2.48	11.01±2.93	0.097
Gun (UI)	2109±865	2003±774	0.097	2122±864	1982±768	0.03
Sperm concentration (M/ml)	63.07±36.39	56.63±35.04	0.02	61.73±33.97	57.74±33.34	0.145

Sperm motility (%)	55.47±12.15	53.87± 12.80	0.11 8	55.61±11.74	53.88±12. 84	0.093
Total sperm motility (M)	94.48±74.07	85.75± 81.40	0.17 7	93.78±73.35	87.44±81. 23	0.332

Note: Data is presented as the mean plus or minus standard deviation or as a percentage. Significant results ($p < 0.05$) are in bold. Group 1 includes short-term co-culture of gametes with intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF) for early resuscitation. Group 2 refers to ICSI and partial IVF.

Basal FSH means for "basal follicle-stimulating hormone," AMH stands for "anti-mullerian hormone," COS stands for "controlled ovarian hyperstimulation," Gn stands for "gonadotropin," M is for "million," and PSM stands for "predisposition score scale." These and other abbreviations are regularly used.

2.2 Clinical procedure

Both a GnRH agonists and an antagonist were given to each patient. Age, ovarian reserve, blood estrogen focus, and ultrasound-guided follicle development all have to be taken into account while changing the amount taken of recombinant or extremely pure follicle-stimulating hormone. Whenever at least three oocytes have grown to a median size of sixteen millimeters or when a minimum of two follicles have grown to a mean diameter of 18 mm or more, inject hCG (human chorionic gonadotropin) or a Fsh agonist to guarantee oocyte maturation. A full 36 hours after launch, ova were gathered.

Masturbation was used to obtain sperm samples, which were then kept at 37°C for 30 minutes. According to guidelines published by the World Health Organization, the samples were evaluated for sperm concentration and motility after liquefaction. Selected sperm were isolated by gradient centrifugation, then washed for fertilization and resuspended in IVF solution.

Three to five hours after oocyte retrieval, the quality of progressive spermatids was assessed before gamete pooling, then the oocytes were fertilized with progressive spermatids at 1.0 x 10 5 /ml. Experienced embryologists selected mature oocytes from groups with loose layers of cumulus cells. Oocyte polar bodies were found after elimination of cumulus cells. Fertilized oocytes were then cultured using standard in vitro fertilization techniques and unfertilized oocytes were subjected to the first R-ICSI together with the remaining sperm. In another group, the method of insemination (ICSI or IVF) was randomly assigned to the cumulus-oocyte complex.

Embryo quality was assessed based on morphology using established guidelines. Embryos were transferred 3 days after cleavage or in the blastocyst state. Progesterone support was provided to

all patients from the day of egg collection. Pregnancy was confirmed by measurement of serum β-hCG levels at 6-7 weeks' gestation followed by ultrasound. Luteal phase support continued until pregnancy was confirmed at 10 weeks gestation. After the birth, the parents were contacted by telephone to receive information on the future of the newborn.

2.3 Statistical analysis

2.3.1 Case Matching

After implementing specific inclusion and exclusion criteria, age and sperm concentration at recruitment varied among participant groups. To eliminate the potential influence of these early discrepancies as confounding variables, we employed a 1:4 propensity score matching (PSM) technique that included the woman's age and sperm concentration. The objective of this strategy was to mitigate the effects of initial deviations in the actual studies.

2.3.2 Statistical methods

In New Zealand, PSM was developed using R 3.5.3 at the University of Auckland. IBM SPSS Statistics for Windows, Edition 24.0 was used to analyze the data, which was developed by IBM Corp. To determine the necessary quantity, we conducted a "power study" with the help of PASS (Power Estimation and Sample Size) 15.0.5. The frequency with which the two bands perform was used to determine the sample sizes. To examine the differences in the groups' continuous outcomes, t-tests were conducted randomly. To accommodate for continuous variables, we utilized the mean standard deviation. Numbers of participants in each group were compared using either the Fisher's exact test or the chi-square test. The estimates were expressed as percentages.

3. Results

According to previously established inclusion and exclusion criteria, 191 patients were divided into group 1 and 775 patients into group 2. As indicated in Table 1, group 1 comprised 188 outbreaks and group 2 contained 720 outbreaks based on the propensity score comparison (PSM). The length of infertility was prolonged in the second group, albeit to a smaller degree, as evidenced by the findings in Table 1. Furthermore, more patients in group 1 than in group 2 received GnRH agonist treatment. After SCM, there were no discernible changes between the two groups' sperm count and motility on the day of egg collection, average patient age, infertility reasons, or early blood FSH levels.

Nine IVF failures and five complete IVF failures were excluded from the reference group using early R-ICSI. Group 2 failures included 65 moderate IVF failures and 32 overall IVF failures. Table 2 shows that Group 1 and Group 2 had the same IVF failure rate (2.66% versus 4.44%) and a lower IVF rate (4.79% versus 9.03%). Each group also showed normal cleavage and fertilization during in vitro fertilization, as well as overall normal cleavage and fertilization. They also produced high-quality embryos on day three, formed blastocysts, transplanted high-quality blastocysts and embryos, and had clinical pregnancies, miscarriages lost in subsequent pregnancies, and live births.

Table 2: Clinical results, embryonic quality and fertility in the study groups.

	1st group (n = 188)	**Second group (n = 720)**	**x2 value**	**p-value**
Total IVF failures, % (n)	2.66 (5/188)	4.44 (32/720)	1.215	0.270
Low FIV, % (n)	4.79 (9/188)	9.03 (65/720)	3.581	0.058
p (oocyte)	2333	10035		
IVF frequency (2PN), % (n)	57.31 (1337/2333)	58.00 (2814/4852)	0.306	0.580
IVF frequency >2PN, % (n)	10.42 (243/2333)	8.41 (408/4852)	7.701	**0.006**
Normal IVF fracture rate, % (n)	97.16 (1299/1337)	97.58 (2746/2814)	0.660	0.417
Fertility rate (2PN), % (n)	58.81 (1372/2333)	59.12 (5933/10035)	0.078	0.781
Percentage >2PN, % (n)	10.42 (243/2333)	4.50 (452/10035)	124.728	**0.000**
Normal amputation rate, % (n)	97.23 (1334/1372)	97.30 (5773/5933)	0.022	0.881
D3 embryos with high quality cleavage, % (n)	32.08 (428/1334)	30.63 (1768/5773)	1.080	0.299

Rate of blastocyst formation, % (n)	60.03 (479/798)	59.33 (1886/3179)	0.129	0.719
Percentage of quality blastocysts, % (n)	18.79 (90/479)	January 16 (302/1886)	2.129	0.144
n (embryo transfer cycle)	142	503		
n (embryo transfer)	276	907		
Embryo implantation stage, % (n)				
D3 cleavage	95.77 (136/142)	90.85 (457/503)	3.616	0.057
blastocysts	4.23 (6/142)	9.15 (46/503)		
Implantation rate, % (n)	41.30 (114/276)	38.15 (346/907)	0.887	0.346
Clinical pregnancy rate, % (n)	56.33 (80/142)	53.28 (268/503)	0.417	0.519
Abortion rate, % (n)	10.00 (8/80)	11.94 (32/268)	0.228	0.633
Frequency of early miscarriage, % (n)	3.75 (3/80)	8.21 (22/268)	1.837	0.175
Loss to Track Rate, % (n)	2.50 (2/80)	1.49 (4/268)	0.369	0.544
Birth rate, % (n)	49.30 (70/142)	43.94 (221/503)	1.285	0.257

Note: Data included as a percentage are bolded if they have a statistically significant result ($p < 0.05$). In vitro fertilization (IVF) and intracytoplasmic sperm injection for early support (ICSI) are under group 2, whereas short-term co-culturing of gametes during IVF with ICSI falls under group 1. In this research, we focus on the pronucleus (PN).

We separated the patients into two distinct categories based on their post-IVF fertility for a more thorough investigation of the research population. At the 30% cutoff, low fertility (n = 74) and normal fertility (n = 834) were contrasted. Age, the length of infertility, the frequency of primary infertility, the underlying cause of infertility, baseline levels of FSH and AMH, the controlled ovarian stimulation (COS) protocol, and spermatozoa are only a few of the baseline characteristics of the two groups that are compared in Table 3. This is brought on by the sperm's high concentration and mobility.

Table 3 Baseline characteristics of low and normal IVF groups

	low rate of in vitro fertilization	**Normal IVF Fertilization Rate**	**p-value**
No	74	834	
age (g)	31.86±3.37	31.84±3.77	0.959
Years of infertility (g)	5.49±2.87	5.71±3.09	0.567
Primary infertility (%), infertility factors, % (n)	77.03 (57/74)	70.98 (592/834)	0.270
Ovulation disorder	6.76 (5/74)	11.03 (92/834)	0.634
fallopian tube factor	25.68 (19/74)	26.26 (219/834)	
endometriosis	4.05 (3/74)	3.36 (28/834)	
light male element	14.86 (11/74)	14.99 (125/834)	

unexplained factors	22.97 (17/74)	23.74 (198/834)	
multiple female factors	6.76 (5/74)	8.75 (73/834)	
women and men	18.92 (14/74)	11.87 (99/834)	
Basic FSH (u/l)	7.47±2.34	7.92±3.89	0.332
AMH (ng/ml)	6.33±4.32	5.33±3.80	0.079
COS protocol, % (n)			
GnRH agonist	59.46 (44/74)	67.39 (562/834)	0.165
GnRH antagonist	40.54 (30/74)	32.61 (272/834)	
excitement time	10.88±2.56	11.11±2.87	0.499
Gun (UI)	1956±811	±789 in 2016	0.53
Sperm concentration (M/ml)	53.61±37.80	59.00±33.08	0.238
Sperm motility (%)	52.50±12.96	54.39±12.60	0.218
Total sperm motility (M)	72.07±61.91	90.24±80.92	0.060

Note: The information in the table is presented as percentages, means, and standard deviations. The abbreviation "basal FSH" is used to refer to basal follicle-stimulating hormone.

Nine cases in the first group and 65 cases in the second group showed reduced fertility after in vitro fertilization. The remaining 47 MII oocytes underwent R-ICSI, but only 7 of the 92 IVF-C group 1 oocytes were fertilized. In group 2, IVF was used to fertilize 51 of 417 oocytes and ICSI was used to fertilize 231 of 331 MII oocytes. According to Table 4, the two categories are comparable in terms of age, duration of infertility, incidence of primary infertility, causes of infertility and sperm motility. However, sperm concentration was slightly lower in couples in group 1 compared to couples in group 2. Subgroups included cleavage, day 3 high-quality embryos, insemination after in vitro fertilization, normal insemination by ICSI, high quality of blastocyst development and availability of high quality blastocysts and embryos. Group 1 couples transferred a total of 14 embryos over 7 cycles compared to group 2 couples who transferred 74 embryos over 44 cycles. According to Table 4, there were no differences between the two groups in terms of canceled embryo transfers, implantations, clinical pregnancies and live births. Group 1 couples transferred a total of 14 embryos over 7 cycles, while group 2 couples transferred 74 embryos over 44 cycles. According to Table 4, there were no differences between the two groups in terms of canceled embryo transfers, implantations, clinical pregnancies and live births. Group 1 couples deposited aggregates of 14 embryos over 7 cycles. On the other hand, the couples of group 2 were transferred 74 embryos in 44 cycles. According to Table 4,

Table 4 Baseline characteristics of early R-ICSI, fertility, embryonic development and clinical outcomes for couples with low levels of IVF in groups 1 and 2.

	Initial R-ICSI for Group 1	**group 2 ICSI**	**p-value**
not	9	65	
age (g)	32.67±4.39	31.75±3.24	0.451
Years of infertility (g)	5.22±3.46	5.53±2.81	0.765
Primary infertility (%), infertility factors (%)	77.78 (7/9)	76.92 (50/65)	0.954
- ovulation disorders	11.11 (1/9)	6.15 (4/65)	1.000
- Fallopian tube factor	33.33 (3/9)	24.62 (16/65)	

- Endometriosis	0 (0/9)	4.62 (3/65)	
There are male elements	11.11 (1/9)	15.38 (10/65)	
- unexplained factors	22.22 (2/9)	23.08 (15/65)	
- some feminine elements	0 (0/9)	7.69 (5/65)	
- both women and men	22.22 (2/9)	18.46 (12/65)	
p (oocyte)	92	865	
n (ICCI MII oocyte)	47	331	
Sperm concentration (M/ml)	30.56±21.42	56.80±38.57	0.007
Sperm motility (%)	47.22±14.39	53.23±12.70	0.194
Total sperm motility (M)	37.67±27.67	76.84±63.92	0.004
IVF frequency, % (n)	7.61 (7/92)	12.23 (51/417)	0.207
Fertility ICSI (2PN), % (n)	74.47 (35/47)	69.79 (231/331)	0.511
ICSI Normal Stop Frequency, % (n)	100.00 (35/35)	97.40 (225/231)	0.335
Fertility ICSI (>2PN), % (n)	2.13 (1/47)	0.91 (3/331)	0.444

D3 embryos with high quality cleavage, % (n)	38.46 (15/39)	33.59 (88/262)	0.549
Rate of blastocyst formation, % (n)	20.00 (2/10)	54.37 (56/103)	0.081
Percentage of quality blastocysts, % (n)	0.00 (0/2)	16.07 (9/56)	1.000
n (embryo transfer cycle)	7	44	
n (embryo transfer)	14	74	
Embryo implantation stage, % (n)			
- D3 embryo cleavage	100.00 (7/7)	90.91 (40/44)	1.000
- Blastocyst	0.00 (0/7)	9.09 (4/44)	
Embryo transfer cancellation rate, % (n)	22.22 (2/9)	32.31 (21/65)	0.540
Implantation rate, % (n)	57.14 (8/14)	41.89 (31/74)	0.292
Clinical pregnancy rate, % (n)	71.43 (5/7)	52.27 (23/44)	0.344
Birth rate, % (n)	71.43 (5/7)	40.91 (18/44)	0.132

Note: Values are presented as mean and standard deviation for continuous variables and as percentage for categorical variables. The p-value displays the statistical significance of the difference between the two groups.

4. discussion

This study examined some of the safety measures used by couples undergoing in vitro fertilization (IVF) with high-risk factors to prevent impaired fertility and fertility loss. In this study, 188 couples who had partial intracytoplasmic sperm injection (IVF-ICSI) and 720 couples who underwent IVF with early intracytoplasmic sperm injection (R-ICSI) were both involved. The findings revealed that 8.15% of couples had a poor IVF success rate, while 4.07% of couples were absolutely infertile.

4.79% of patients in group 1 responded to early R-ICSI, compared to 9.03% of group 2 patients who received IVF-ICSI treatment. The terms implantation, clinical pregnancy, miscarriage, loss to follow-up, live birth, and delivery are only a few examples of the various titles given to quality. Additionally, group 1 utilized more embryos than the other groups. While 65 individuals in group 2 underwent fractional IVF-ICSI, nine patients in group 1 had early R-ICSI with a low rate of IVF. Both groups showed comparable levels of fertility, embryonic development, and clinical outcomes.

This study shows that diminished fertility or complete reproductive failure following conventional IVF are still frequent and unexpected. High risk factors for infertility include infertility that can't be explained, low-quality sperm, and infertility that lasts more than five years. It was said that early R-ICSI had a higher chance of the process of fertilization implantation, and pregnancy than day 2 rescue ICSI, and that it stopped fertilization from failing completely.

The IVF-ICSI splitting procedure, in which oocytes are at random assigned to either IVF or ICSI, helps to some extent lower the chance of infertility or fertilization failure. This study found that early R-ICSI had a higher conception rate than group 1 IVF, which had a higher conception rate, and split ICSI had a higher conception rate than split group 2 IVF. weak. In conclusion, early R-ICSI and stand-alone IVF-ICSI have been proved to be successful preventative treatments against IVF failure and decreased fertility in high-risk couples.

The prevalence of a number of NPs was also studied in this study in relation to the impact of early granulosa cell ablation. Mixed findings came from the survey. While some research showed no difference or low or high risk scenarios in a paddy field, some have reported a greater prevalence of multiple NPs with early granulosa cell ablation. The development, maturation, and fertilization of oocytes can be impacted by the removal of granulosa cells in terms of secondary meiosis and cortical responses.

In this work, the early identification of unfertilized eggs within 6 hours after traditional insemination required the exact localisation of polar bodies. Six hours after IVF, some zygotes could still be fertilized, and R-ICSI might result in aberrant fertilization. The findings of this study suggest that more precise fertilization predictions may be made in the future by investigations using quicker incubators.

This study showed that early R-ICSI and fractional IVF-ICSI are both good ways for couples at high risk of infertility and IVF failure to lower their chances of not getting pregnant or having IVF fail. Even though there is a good chance of having a lot of PPs, early R-ICSI is better from a

business and child safety point of view. The success rate of fertilization, the rate of embryonic growth, the rate of clinical pregnancy, and the rate of live babies are all the same as with individual IVF-ICSI. This study's flaws include the use of historical methods and the fact that the sample size is so small that the statistical power is very low.

5. Conclusion

Early R-ICSI IVF or split IVF are both good ways for couples with a high risk of IVF failure to lower ovulation and prevent IVF failure. Early R-ICSI is superior than fractional IVF-ICSI because it uses more embryos, requires fewer ICSI operations, and has equivalent clinical pregnancy and delivery rates. To completely comprehend the effects of early granulosa cells ablation on several PN parameters and to solve the shortcomings of present research, more study is required.

6. Direction of future research

Possible future research directions in this area include:

Improvement of embryo selection methods. Develop and validate more sophisticated embryo selection methods to improve the efficiency of in vitro fertilization (IVF). This may include the use of time-lapse imaging, genetic screening, and other advanced techniques to select the most viable embryos for transplantation.

Personalized Fertility: Explore the potential of personalized fertility medicine based on factors such as genetic profiles, biomarkers and individual patient characteristics. This approach can lead to more individualized and effective treatment strategies.

Noninvasive Fertility Assessment: Explore and validate noninvasive methods for assessing fertility in men and women. This may include the development of new biomarkers, imaging modalities, or wearable devices to provide accurate and convenient fertility assessment.

Assisted Reproductive Technology (ART) Optimization: Constantly improving and optimizing the protocols and methods used in ART procedures such as IVF and Intracytoplasmic Sperm Injection (ICSI). This includes examining the effect of various parameters such as stimulation protocol, environment, and laboratory conditions on treatment outcomes.

Fertility preservation: expanding research into fertility preservation options for people facing fertility-threatening conditions such as cancer treatment and age-related decline. This includes not only improving methods of cryopreservation of oocytes and embryos, but also exploring alternative methods such as cryopreservation of ovarian tissue and artificial ovaries.

Male infertility: Strengthen research activities to better understand and treat the problem of male infertility. This includes learning new diagnostic tools, developing targeted treatments, and learning about lifestyle and environmental factors that can contribute to male infertility.

The Psychological Impact of Fertility Treatment: explores the psychological and emotional impact of fertility treatment on individuals and couples. This research can help develop interventions, counseling programs and resources to help patients manage their emotions during fertility treatment.

Long-term outcomes of assisted reproductive technologies: Conduct long-term follow-up studies to assess the health impact of children conceived through assisted reproductive technologies.

This research should focus on the potential risks and benefits associated with ART and assess the long-term physical and psychological health of offspring.

Cost-Effectiveness and Accessibility of Fertility Treatments: Evaluate the cost-effectiveness of various fertility treatments and evaluate strategies to improve access to fertility treatments for individuals and couples who may be facing financial difficulties. This includes consideration of alternative financing models, insurance coverage and public health policies.

Fertility and aging: studying the effect of age on fertility and developing strategies to optimize fertility in the elderly. This may include exploring new approaches to ovarian rejuvenation, optimizing preconception care for older couples, and addressing the unique challenges of managing infertility in the elderly.

These future lines of research aim to improve our understanding of fertility, improve fertility success rates, and provide better support and care for individuals and couples aspiring to fertility.

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