ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 7, 2024

Online - 2455-3891
Print - 0974-2441

Research Article

PREDICTORS OF INTRA CYTOPLASMIC SPERM INJECTION SUCCESS IN COUPLES WITH UNEXPLAINED SUB-FERTILITY

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Received: 19 April 2024, Revised and Accepted: 04 June 2024

ABSTRACT

Objective: About 10-15% of sub-fertility cases facing the clinicians in the reproductive clinics remains unexplained. Opinions regarding the optimal treatment plan for them exhibit a wide differences among fertility specialists. This study aims to assess the outcome of ICSI and predictors success in couples with unexplained sub-fertility.

Methods: Sixty six sub-fertile couples with unexplained infertility were included. ICSI was done for all. They divided into 2 groups pregnant and non-pregnant. They were followed up retrospectively to evaluate the predictors of ICSI treatment success and results were compared between them.

Results: Chemical pregnancy was rate 42.2%. Pregnant females were significantly : younger with a mean age of 26.7±4.2 vs 28.6±3.6, lower BMI of 27.6± 4.9 vs 30.2± 4.9, lower cycle day 2 LH level 2.3 ±0.99 vs 2.9±0.94 and had a slightly thinner endometirum 3.3± 0.68 vs 3.9 ±1.1 than non-pregnant ones. Females of both groups exhibited no significant difference regarding the response to controlled ovarian stimulation, the mean total number of retrieved and mature oocytes were comparable, the only exception is the mean total number of immature oocytes which was significantly lower in the pregnant females 0.85± 1.1 vs 30.2± 4.9 in non-pregnant with no significant difference regarding fertilization rate, total number of embryos, cleavage rate and embryos' quality.

Conclusion: Intra cytoplasmic sperm injection has the ability, within certain limits, to bypass the obstacles of natural pregnancy failure in couples with unexplained sub-fertility and should be considered for those couples as early as possible before female partner get older.

Keywords: Unexplained sub-fertility, Body mass index, Immature oocytes, Basal luteinizing hormone level and intracytoplasmic sperm injection.

 DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i7.51169. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Unexplained sub-fertility is usually diagnosed if a couple fails to conceive after 1 year of regular, unprotected sexual intercourse when tests of ovarian function, tubal patency, and semen analysis are completely normal [1,2]. About 10–15% of couples experiencing sub-fertility, their diagnosis remains unexplained [3,4]. Subtle undetectable factors may be considered, however, the majority of those couples have no recognized abnormality. When a specific medical cause of decreased fertility is absent, a specific treatment is lacking [5]. At present, there is controversy about the selection of appropriate treatment options for such couples [6]. Meanwhile, couples with unexplained sub-fertility are exposed to several treatments; expectant management with a high rate of spontaneous conception within the following few years, medical treatment in the form of ovarian stimulation (OS), and assisted reproductive techniques [7].

With the advances in assisted reproductive techniques, *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) has emerged, being a safe and successful option for conception and giving us the opportunity to assess gametes and embryo quality, respectively. However, there is some debates about the use of ICSI as a first treatment option for couples with unexplained sub-fertility after conventional treatment failure (timed intercourse, ovulation induction and intrauterine insemination (IUI)). Five to 25% of cases of unexplained sub-fertility reported no fertilization following the ICSI procedure [8], which might be attributed to occult abnormalities in male/female gametes, sperms, and oocytes, respectively, or endometrial receptivity [9,10]. Therefore, an understanding of the available evidence for the management of unexplained sub-fertility is essential.

METHODS

The study is retrospective, the cohort, was conducted on sub-fertile couples whom were diagnosed with unexplained infertility. Sixty-six couples were collected from the fertility and IVF clinic/fertility center/Al-Sadr Medical City/Al-Najaf Al-Ashraf/Iraq throughout the period March 2021 to January 2022. Informed consent was taken from all to be included in such a study. Initial evaluations and fertility investigations for them were done, seminal fluid analysis for male partners and body mass index (BMI), cycle day 2 hormones, transvaginal ultrasound (TVUS) for endometrial thickness (ET) and antral follicle count, hysterosalpingography (HSG), and hysteroscopy for female partners.

Only couples with unexplained subfertility were included (whom their females failed to get pregnant following expectant management and following at least 2–3 cycles of intrauterine insemination (IUI) failure for more than 2 years), the age of female partners ranged between 19 and 36 years old, with a mean duration of sub-fertility of 8 years. They have normal ovulation with patent uterine tubes by HSG, no uterine pathology by hysteroscope, no history of thyroid disorders, chronic medical diseases, chronic drug intake, and previous genital surgery. While male partners have normal semen parameters, normal hormonal profile, no varicocele, no ejaculatory dysfunction, no chronic medical diseases, no chronic drug intake, and no previous surgery in genital tract. Women with polycystic ovary syndrome, endometriosis, uterine fibroid immunological disorders and tubal obstruction, males with severe impairment in semen quality, and frozen sperm were excluded.

All females were included in controlled OS (COS) by gonadotropin-releasing hormone agonist short protocol; Decapeptyl 0.1 mg*1 S.C followed by gonadotropin stimulation, recombinant follicle-stimulating hormone; Follitrope 75IU*2 S.C for 10–14 days as a step for ICSI. When both ovaries show 7–14 follicles of a size more than 17 mm, ovulation trigger was done by human chorionic gonadotropin (HCG), Pregnyl 5000 IU*2 I.M followed by oocyte retrieval under general anesthesia and TVUS. A luteal phase progesterone suppositories 3 times daily started in the following day and lasted for 14 days. Concomitantly, semen was prepared by centrifugation and direct swim up from the pellet (according to the World Health Organization, 2010). A microscopic assessment of oocytes' maturity was done. Only mature oocytes (metaphase II [MII]) were injected [11]. An assessment of fertilization and embryo quality was done [12]. Fresh embryo transfer was done on the 3rd day of injection when 3 good quality embryos were returned to the uterus. The fertilization rate was calculated by dividing the number of zygotes (2PN)/the number of injected mature MII oocytes *100%. The cleavage rate was calculated by dividing the number of embryos/by the number of zygotes*100%. Pregnancy is assessed chemically by a positive pregnancy test (b-HCG) 14 days after embryo transfer. The couples were divided into 2 groups: pregnant (n=28) and non-pregnant (n=38). Data analysis by the Statistical Package for the Social Sciences (V.4), mean±standard deviations/independent sample student *t*-test for continuous data or a total number and percentage/Chi-square test for categorical data. A significant p value is ≤0.05.

RESULTS

Demographic data of the couples in both groups were best illustrated in Table 1. Only the age of female partners exhibited a significant variance between both groups in such a way that the pregnant females were significantly younger at a p-value of 0.057.

While Tble 2 illustrates the hormonal profile, ET at cycle day 2 and body mass index of female partners in both groups. BMI, serum LH and ET showed significant variance between both groups at a p-value ≤0.05.

While the response to ovarian stimulation and ICSI outcome were represented in Tables 3 and 4. Only total number of immature oocytes exhibited a significant difference between both groups being significantly less in pregnant group at a p-value 0.05.

DISCUSSION

Despite the great advances in ARTs, a gap between success rate and couples' expectations is still present. In this study, couples with unexplained sub-fertility showed a good response to COS, produced an acceptable number of mature oocytes with a higher fertilization rate and good quality embryos. The overall success rate of ICSI in couples with unexplained sub-fertility (42.4%) is somewhat similar worldwide and recognized by some studies [13-15]. Thus, ICSI has the ability, within certain limits, to bypass the obstacles of natural pregnancy failure in couples with unexplained sub-fertility. Hence, this treatment modality should be considered and discussed with such couples to increase their chance of having a baby. Several factors are shown to adversely affect ICSI outcomes in unexplained sub-fertility. Our results showed that maternal age is an important predictor for having a successful ICSI outcome. As the age of female partners increases, there is an exponential decline in the total number and quality of stored oocytes [16]. This decline is associated with an increase in the embryonic aneuploidy rate, implantation failure, and sub-fertility [17,18]. This result is in agreement with studies that confirmed that the pregnancy rate reduced when the woman's age increased [19,20]. The current study found a significant difference between the two groups regarding the BMI, and a BMI >27 kg/m^2 is associated with failed conception. It was suggested that obesity can impair fertility despite female age by altering the follicular environment leads to the development of fewer, incompetent oocytes with a lower fertilization potential and sub-optimal embryo quality. High BMI also impairs embryo implantation by negatively influencing the endometrium [21-23]. Regarding the hormonal profile of females with unexplained sub-fertility, the study exhibited no significant hormonal differences between the pregnant and non-pregnant females; the only exception is serum luteinizing hormone (LH) levels at the start of OS as shown in Table 2. Despite of being within normal limits in the females of both groups (2–15 IU/L) [24], pregnant females tend to have significantly a lower value than non-pregnant ones. Whether could be responsible to affect ICSI outcomes by increasing the rate of developing immature oocytes (Table 3) and implantation failure is

Table 1: The demographic data of the couples in both groups including the age of both partners, type and duration of sub-fertility

Parameter	Pregnant mean±SD	Non-pregnant mean±SD	p-value
Age of female partners (years)	26.7±4.2	28.6±3.6	0.057
Age of male partners (years)	32.2±5.0	34.2±5.9	0.17
Duration of sub-fertility (years)	7.3±3.7	8.9±4.0	0.10
Type of sub-fertility, total number			
Primary	14	26	0.13
Secondary	14	12	
Total	28	38	

SD: Standard deviation

Table 2: Initial investigations of the female partners represented by cycle day 2 hormonal analysis and BMI in both groups

Parameter	Pregnant mean±SD	Non-pregnant mean±SD	p-value
Estrogen (pg/mL)	33.0±13.4	36.0±11.6	0.42
FSH (iu/L)	4.3±1.6	4.7±1.7	0.38
LH (iu/L)	2.3±0.99	2.9±0.94	0.009
Prolactin (ng/dL)	17.4±6.8	14.0±9.1	0.09
ET (mm)	3.3±0.68	3.9±1.1	0.013
BMI (kg/m^2)	27.6±4.9	30.2±4.9	0.03

FSH: Follicle-stimulating hormone, ET: Endometrial thickness, BMI: Body mass index, SD: Standard deviation, LH: Luteinizing hormone

Table 3: The response to controlled ovarian stimulation of the female partners in both groups

Parameter	Pregnant mean±SD	Non-pregnant mean±SD	p-value
Total dose of Gn (iu/l)	1596.4±485.1	1653.9±506.8	0.64
ET on the day of the trigger (mm)	9.8±1.6	10.4±2.2	0.27
Total number of retrieved oocyte	8.7±5.3	9.4±6.8	0.64
Total number of mature oocytes	7.7±4.6	7.5±6.1	0.87
Total number of immature oocytes	0.85±1.1	1.8±2.4	0.05

ET: Endometrial thickness, SD: Standard deviation

Table 4: Total number of embryos, their quality, fertilization rate, and cleavage rate in both groups

Parameters	Pregnant mean±SD	Non- pregnant mean±SD	p-value
Total number of embryos	5.7±3.7	5.0±4.8	0.55
Total number of good quality	5.3±3.3	4.2±4.0	0.26
Total number of bad quality	0.36±0.82	0.78±1.3	0.15
Fertilization rate	75.4±19.8	73.5±27.2	0.75
Cleavage rate	98.0±4.7	96.3±15.1	0.57

SD: Standard deviation

questionable. The current results highly go with what was mentioned previously, especially the non-pregnant females who had higher LH levels produced a significantly higher number of immature oocytes. Unproved role and no significant role of high follicular phase LH on ICSI outcome had been reported [25]. Regarding embryo quality, females of both groups produced comparable numbers of good quality embryos without significant variation. Non-pregnant females despite producing good quality embryos, their embryos failed to be implanted successfully. This could be explained either by poor endometrial receptivity (absence of certain cytokines or loss of adhesive molecules), high LH levels [26], or due to genetic and chromosomal abnormalities of the embryos [27-29]. It has been recognized that good quality embryo does not mean chromosomally normal and there is a percentage of good quality embryos that exhibit chromosomal abnormalities in the form of aneuploidies [30] which might explain the natural pregnancy failure, pregnancy failure following ICSI and miscarriage rate in unexplained sub-fertility making both the American Society for Reproductive Medicine and the NICE practice committees yet do not recommend routine ICSI for couples with unexplained subfertility [30,31].

CONCLUSION

Intracytoplasmic sperm injection has the ability, within certain limits, to bypass the obstacles of natural pregnancy failure in couples with unexplained sub-fertility and should be considered for those couples as early as possible before the female partner gets older. However, several factors may decrease the chance of having a child in those couples following ICSI; the most important one is female age followed by high BMI and high serum LH level before stimulation which in turn significantly affect the maturity of oocytes. Despite a normal ET and good quality embryos, implantation failure still existed, whether related to previous factors or others; either at the level of intrinsic gametes defects, embryo chromosome or poor endometrial receptivity is a subject of wide research.

ETHICAL APPROVAL

It was approved by the Medical Ethics Committee of the Kerbala University/College of Medicine, no. 109-Date: January 26, 2021.

ACKNOWLEDGMENTS

None.

AUTHORS' CONTRIBUTION

Muhjah conceived and designed the study. Rabab collected the sample, performed parts of the statistical analysis, and helped to draft the manuscript. Ali and Zainab performed the statistical analysis, wrote the discussion, and revised the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTERESTS

According to the author's claim, there are no conflicts of interest.

FUNDING/SUPPORT

This study did not have financial support.

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