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Evaluation of the effect of methods of insemination (ICSI versus conventional IVF) in the IVFPGD cycle on the incidence of chromosomal abnormalities

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ABSTRACT— Despite the development in fertility treatments, the prevalence of chromosomal abnormalities remains a crucial indicator of implantation and fertilization rates. Recently, the application of PGD following IVF or ICSI has been widely debated on the incidence of aneuploidy; however, the effect of these methods on chronic abnormalities is yet unknown. Hence, this study aimed to evaluate and identify the effect of insemination methods (ICSI versus IVF) on the incidence of chromosomal abnormalities. In this retrospective cohort study, a total number of 202 women attending Al Manar IVF center and undergoing fertility treatment between 2016 and 2021 were enrolled. The overall number of participants was allocated to 74 women who had performed IVF, and 155 women who had undergone ICSI. Following IVF or ICSI, the preimplantation genetic diagnosis was performed via trophectoderm biopsy. A total of 589 embryos were yielded, out of which n= 165 were obtained from IVF and n= 424 from ICSI. The occurrence of aneuploid embryos was higher in IVF embryos in comparison to ICSI embryos (74% versus 71%). The percentage of different chromosomal abnormalities that involved trisomy 18 was higher in IVF versus ICSI (23.1% vs 18.1%). However, the incidence of trisomy 13 was similar between both groups (28.2% vs 28.3%). Sex chromosome abnormalities were lower in the IVF group as opposed to ICSI (18.7% vs 20.9%). The rate of aneuploid embryos was higher in ICSI compared to conventional IVF. Based on the findings of this study, conventional IVF is the preferred insemination method in PGD cycles.

KEYWORDS: Preimplantation genetic diagnosis, intracytoplasmic sperm injection, in-vitro fertilization, chromosomal abnormalities, aneuploid, euploid.

1. INTRODUCTION

The transmission of genetic material to offspring during embryogenesis might be contrived by several chromosomal abnormalities [1]. Such inherited genetic errors are crucial to the survival pattern of the embryo during its development, and their severity might result in miscarriage [1]. Commonly, up to 70% of conceptions with chromosomal anomalies tend to end during the first trimester [2]. According to research studies, aneuploidy is a major cause of miscarriages as a result of the development of autosomal trisomies or sex chromosome anomalies [3]. The likelihood of complicated conception has adhered to various parental factors [4- 6]. While the quality of sperm and fertilized oocytes are crucial indicators for genetic abnormalities, outside factors such as high BMI and maternal age contribute equivalently [7], [8]. Along with the development of clinical research, the origination of assisted reproductive techniques (ART) has permitted the detection and identification of innumerable genetic abnormalities [9]. Over the years, the utilization of infertility treatment techniques such as conventional insemination and intracytoplasmic sperm injection (ICS) has been accompanied by the use of preimplantation genetic testing (PGD) [9]. The latter

has been implemented in IVF and ICSI to eliminate any risk of passing genetic anomalies of parental origin to the offspring [9]. Studies have revealed that PGD has limited the rate of fertilization failure and miscarriages [10]. The application of PGD in in-vitro fertilization (IVF) has been widely adopted for aneuploid embryos detection [11]. However, previous research has emphasized the high incidence of aneuploid embryos obtained by IVF [12]. Contrary, it has been reported that the prevalence of aneuploid embryos is increased in ICSI as opposed to IVF [13]. Furthermore, a higher incidence of sex chromosome abnormalities following ICSI was reported in the literature [2], [5]. In contrast, other studies have revealed that the rate of sex chromosomal abnormalities was similar between ICSI and natural pregnancy [14]. In a study conducted by [15], aneuploid embryos produced by intracytoplasmic sperm injection (ICSI) was observed to be higher than those produced by conventional insemination (IVF). Furthermore, it has been reported that the significantly higher aneuploidy rate in ICSI- conceived embryos was associated with the quality of sperm or the methodological aspects of the technique [16]. [17], on the other hand, has found similar rates of aneuploid embryos between IVF and ICSI. However, the identification of sub-types of chromosomal abnormalities has rarely been studies directly. Thus, to investigate the repercussions of infertility treatments on the prevalence of aneuploidy, this study aimed to evaluate the effect of IVF and ICSI methods on the incidence of chromosomal abnormalities.

2. Method

In this monocentric retrospective cohort study, data were collected from records of 202 women who underwent ICSI or IVF cycles at the Al Manar IVF center between 2016 and 2021. Out of the 202 women, about 47 have had in-vitro fertilization (IVF), while 155 underwent intracytoplasmic sperm injection (ICSI). Demographic data collected from pre- existing IVF and ICSI records included the participants' age, weight, and medical history. Preimplantation genetic diagnosis (PGD) test outcomes were noted for aneuploid and euploid embryos. Patients with high BMI or any medical complications were excluded from this study. Women of high maternal age were not included. Patients have received the short protocol gonadotrophin-releasing hormones (GnRH) antagonist stimulation. GnRH antagonist was administered on day 6 of the stimulated cycle along with gonadotropin (FSH) being given from the second day of the cycle. Ovarian stimulation was monitored for 3 to 4 days using vaginal sonography until follicular maturity criteria converged (follicles of size 18mm). Consequently, a single dose of human chorionic gonadotrophin (hCG) (Pregny, 10,000 IU) was then administered to trigger ovulation. About 36 hours following hCG administration, transvaginal follicular aspiration was performed to retrieve oocytes. In the case of conventional IVF, retrieved oocytes were placed in culture media and incubated. On the other hand, sperm was cultured either using swim-up or density gradient. Following oocyte incubation, mature oocyte (MII) and cultured sperm are incubated together in a fertilizing media at a ratio of 50,000-75,000:1 for 1-4 hours or 16-24 hours. For intra- cytoplasmic injection (ICSI), oocytes were denuded from their cumulus cells enzymatically using hyaluronidase and mechanically using gentle pipetting in order to observe their nuclear maturity. Oocytes were then injected using immobilized normal sperm and incubated. The fertilized oocytes were checked after approximately 20 hours to monitor their fertilization status. If two distinct pronuclei were observed, the oocytes were said to be normally fertilized. After 24 hours following fertilization, the embryos were monitored for cleavage using an inverted microscope. Embryos were then evaluated and graded based on their cleavage and fragmentation during days 3 until 6. The cleavage-stage was considered between days 3 and 4, while the blastocyst stage was days 5 and 6 following con-incubation or fertilization. A trophectoderm biopsy was performed on day 5 blastocyst stage embryo using laser-based zona pellucida breaching. All biopsy samples were evaluated using PGD at a single laboratory. Outcomes of the diagnosis were rated either as abnormal (aneuploid) or normal (euploid) embryos. Furthermore, rates of aneuploid were evaluated as trisomy 13, 18, 21, and XY abnormalities.

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Ethical Approval Patients included in this study had previously consented to PGD application. The study was approved by the Arabic Board of Obstetrics and Gynecology, and confidentiality was applied by coding and depersonalizing the collected data.

Statistical Analysis: Data presented were expressed as percentages (%) and frequency (n) values. Chi-squared test was used for categorical variables to compare sample characteristics between conventional IVF and ICSI groups. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

A total of 202 couples have undergone fertility treatments, out of which 155 have had ICSI and 47 underwent IVF. Women enrolled in this study were mainly of age less than 37 years old. However, 3.3% of ICSI patients (n=5) were between 38 and 40, while 1.7% (n=1) for IVF patients. A significant difference in the maternal age between IVF and ICSI patients has been observed ($p< 0.05$). In addition, about 32.3% of ICSI patients (n=50) have had male- related infertility, while 52.1% (n= 24) of IVF patients had ovulatory-related factors. Interestingly, 34% (n=16) of patients undergoing IVF have had mixed infertility factors in comparison to 28.3% (n= 44) of patients who have undergone ICSI treatment. Nonetheless, a significant difference of $p< 0.05$ has been shown between the causes of infertility in IVF and ICSI. The maternal BMI of patients was majorly below 25 (IVF= 70.2%; ICSI= 71.7%), whereas only 6 to 10% of patients had a BMI higher than 29 (Table 1).

Table 1: Characteristics of patients with IVF or ICSI.

	IVF n (%) n=47	**ICSI n (%) n=155**
Maternal age*		
<30	17 (37.1)	60 (38.9)
30-34	23 (49.3)	68 (43.9)
35-37	6 (11.9)	22 (13.9)
38-40	1 (1.7)	5 (3.3)
Cause of infertility*		
Male	1 (2.1)	50 (32.3)
Endometriosis	1 (2.1)	2 (1.3)
Tubal	24 (52.1)	32 (21)
Ovulatory	1 (2.1)	5 (3.2)
Unknown	4 (7.6)	22 (14)
Mix	16 (34)	44 (28.3)
Maternal BMI kg/m^2		
<25	33 (70.2)	111 (71.7)

25-29	11 (23.4)	34 (21.9)
>29	3 (6.4)	10 (6.4)

BMI: Body Mass Index, IVF: In-vitro Fertilization, ICSI: Intracytoplasmic Sperm Injection, n: frequency, %: percentage.

*p-value <0.05 Out of the 47 IVF cycles, 165 embryos underwent genetic analysis, and from the 155 ICSI cycles, 424 were subjected to genetic testing. The prevalence of abnormal embryos was found to be higher in ICSI compared to the conventional IVF (74% versus 71%), while the number of normal embryos was higher in conventional IVF in comparison to ICSI (29% versus 26%) (Table 2).

Table 2: Number of embryos undergoing genetic analysis among the 47 IVF and 155 ICSI cases

	IVF n (%) n= 165 embryos	**ICSI n (%) n= 424 embryos**
Normal	114 (29)	114 (26)
Abnormal	117 (71)	310 (74)

IVF: In-vitro Fertilization, ICSI: Intracytoplasmic Sperm Injection, n: frequency, %: percentage.

The genetic testing performed on embryos has revealed different aneuploidies between the two groups. Around 28.2 % of ICSI embryos (n= 87) have had Trisomy 13 in comparison to 28.3% (n= 33) for IVF embryos. Additionally, 23.1% (n=26) of IVF embryos had trisomy 18, which is higher than that of ICSI embryos (18.1%; n= 56). However, ICSI embryos had higher percentages of trisomy 21 (32.8%; n= 102) compared to IVF embryos (29.9%; n= 36). Moreover, the percentage of XY anomalies was higher in ICSI embryos (20.9%; n= 65) as opposed to IVF embryos (18.7%; n= 22) (Table 3).

Table 3: Number of abnormal chromosomes in IVF and ICSI embryos

	IVF n (%) n= 117 embryos	**ICSI n (%) n= 310 embryos**
13	33 (28.3)	87 (28.2)
18	26 (23.1)	56 (18.1)
21	36 (29.9)	102 (32.8)
XY	22 (18.7)	65 (20.9)

4. Discussion

PGD breakthroughs have allowed the transfer of single euploid embryos, maintaining high pregnancy rates and minimising losses [18]. Increased use of ICSI for PGD has increased application dangers [19]. PGD is related with a reduced implantation rate and increased mosaicism in traditional insemination [12]. Comparing IVF with ICSI for chromosomal disorders is disputed. This research evaluated IVF and ICSI's influence on chromosomal abnormalities. Several variables cause pregnancy chromosomal abnormalities

[20]. Aneuploid embryos are more common after age 38. [21]. Maternal and paternal BMI are connected with genetic abnormalities and aneuploidy [21], [22]. Medical problems may potentially exacerbate chromosomal abnormalities, particularly if they are inherited [4]. Preimplantation genetic diagnosis was supposed to reduce these risks [11], [19]. ICSI or IVF methods are used [23]. This limits passing or transferring genetically defective embryos, lowering miscarriage rates [22]. This research doesn't support using ICSI to reduce chromosomal abnormalities. ICSI has more defective embryos than IVF (74% vs. 71%). IVF had 29% more normal embryos than ICSI (26%). These results didn't match the literature. [12] found that IVF had higher aneuploidy rates than ICSI (45% vs 43.1%). IVF and ICSI exhibited identical euploid and aneuploid embryo frequencies. 2006 research showed no difference in aneuploidy rates between ICSI and IVF embryos [24]. Literature shows that ICSI doesn't enhance aneuploidy [24]. However, chromosomal abnormalities were greater in ICSI embryos [2]. Other research supported research. [15] found that ICSI generated more aneuploid embryos than IVF. ICSI therapies may be the main cause of aneuploid embryos. The greater aneuploidy rate in ICSI-conceived embryos was also linked to sperm quality or procedure [16]. [24] found a higher incidence of trisomy in IVF embryos (32.4 vs. 11%). Other studies suggest a higher incidence of acrocentric trisomies with ICSI (19.7% vs. 15.5%) [20]. This investigation demonstrated the greater prevalence of trisomy in ICSI-conceived embryos. ICSI had a greater trisomy rate than IVF (32.8% vs. 29.9%). These findings are consistent with recent studies that suggest trisomies (21 and 18) are more common in ICSI-transferred embryos [14]. Trisomy 13 was found in 28.3% and 28.2% of both groups. XY chromosomal abnormalities were more common in the ICSI group (20.9% vs. 18.7%). This conclusion agrees with [2] who reported that ICSI embryos had more sex chromosomal abnormalities. [14] found that ICSI and spontaneous conception had equal sex chromosomal abnormality rates. IVF and ICSI cure infertility well. Retrospectively, ICSI embryos had a higher prevalence of chromosomal abnormalities. The small number of participants and the disparity in numbers between ICSI and IVF might generate bias. The monocentric investigation prevents generalisation of data. Further research employing a large, randomised sample is needed to evaluate this study's results. Also, sperm abnormalities and maternal age should be examined based on the occurrence of chromosomal abnormalities.

5. Conclusion

This retrospective cohort research examined the influence of insemination procedures (IVF/ICSI) on PGD-identified chromosomal abnormalities. ICSI was related with a greater proportion of aneuploid embryos, hence in-vitro insemination is preferred. In ICSI, sex chromosomal abnormalities were more common. The supplied data helps evaluate risk factors for aneuploidy and chromosomal abnormalities in ICSI embryos. Future research must confirm this study's results.

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