Original Article

How Could One Sperm and One Oocyte Proceed To Molar Pregnancy? Prevalence of Molar Pregnancy during ICSI Procedure in Over 25,000 Fresh Embryo Transfers: A Retrospective Cross-Sectional Study

Maryam Hafezi, M.D.[1*], Zahra Chekini, M.Sc.[1], Firouzeh Ghaffari, M.D.[1], Mehri Mashayekhi, M.D.[1], Nadia Zameni, M.Sc.[2]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Background: Gestational trophoblastic disease (GTD) is a heterogeneous group of diseases characterized by excessive proliferating trophoblastic tissue. The prevalence of GTD has a varied geographical distribution. However, its frequency following intracytoplasmic sperm injection (ICSI) cycles has not yet been reported. This study aimed to estimate GTD frequency and prevalence after ICSI cycles.

Materials and Methods: This retrospective cross-sectional study included all patients diagnosed with GTD subsequent to ICSI and segmental embryo transfer procedure during 2011-2019 at Royan Institute. GTD diagnosis was established for patients who met all three criteria: beta-human chorionic gonadotropin (β-hCG) levels greater than 100,000 mIU/mL, vesicular ultrasonographic pattern, and presence of pathologic features of hydatidiform mole. Although we assessed the GTD frequency in all ICSI cycles, GTD cases were only observed following fresh embryo transfer ICSI procedures.

Results: We evaluated 25,667 fresh embryo transfer ICSI procedures out of 41,540 ICSI cycles. This study identified a total of 10 GTDs confirmed by all criteria which were mentioned previously. Of these 10 GTDs, nine cases had hydatidiform mole, and one had gestational trophoblastic neoplasia. The frequency of GTD was calculated 10 cases in 41,540 (0.240 per 1000) ICSI procedures and 10 in 25,667 (0.389 per 1000) fresh embryo transfers following ICSI cycles. Also, we detected 10 GTD cases in 8,196 (1.220 per 1000) clinical pregnancies.

Conclusion: We discuss that the possibility of GTD after ICSI procedure is not as low as expected. Thus, the previous theses are insufficient to explain all aspects of molar pregnancy, and more research is required.

Keywords: Frequency, Gestational Trophoblastic Disease, Intracytoplasmic Sperm Injection, Molar Pregnancy, Prevalence

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Introduction

Gestational trophoblastic disease (GTD) consists of benign and malignant cellular proliferations arising from the placental villi's trophoblastic epithelium. Distinct forms of GTD include gestational trophoblastic neoplasms (GTN), hydatidiform mole (HM), placental site nodule (PSN), and exaggerated placental site (EPS) (1, 2). Malignant GTN could be invasive and metastatic (1). Trophoblastic proliferation and hydropic swelling of the placental villi characterize HM, categorized as either complete or partial hydatidiform mole (1, 3). It is important to note that 15% of molar pregnancies will become choriocarcinoma and might invade other organs (4). The initial diagnosis of GTD is based on human chorionic gonadotropin (β-hCG) titers and ultrasonography, thereafter, GTD will be confirmed by histopathologic features (1, 4).

Epidemiological studies showed that the prevalence of GTD has a wide geographical variation (5), ranging from 0.5-1.84 per 1000 spontaneous pregnancies in Europe and North America, and more recently, 1.67 per 1000 deliveries per year in the Netherlands. The highest rates were reported from Southeast Asian regions, with 12 per 1000 spontaneous pregnancies in Indonesia, 5 per 1000 in China, and 1.9-4.9 per 1000 in Japan (4). GTD prevalence following spontaneous pregnancy appears to be the highest in Southeast Asia (4, 5) and the lowest in North America and Europe (4).

However, the literature lacks a comprehensive report

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*Correspondence address: P.O.Box: 16635-148, Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: maryamhafezi90@gmail.com

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of GTD frequency following assisted reproductive technology (ART), and only sporadic cases have been reported (6-8). Genetically, a complete HM (CHM) arises from fertilization of an empty ovum with a duplicate genome or double sperm fertilization (9) (Fig.1). *In vitro* fertilization (IVF) appears to have the potential of predisposing to multispermia fertilization and a higher chance of HM (10); however, ICSI could ensure fertilization by a single sperm (7), which can prevent the occurrence of HM that predisposes GTDs. This study aimed to estimate the frequency and prevalence of GTD after ICSI cycles and introduce potential predisposing factors for GTD after ICSI cycles.

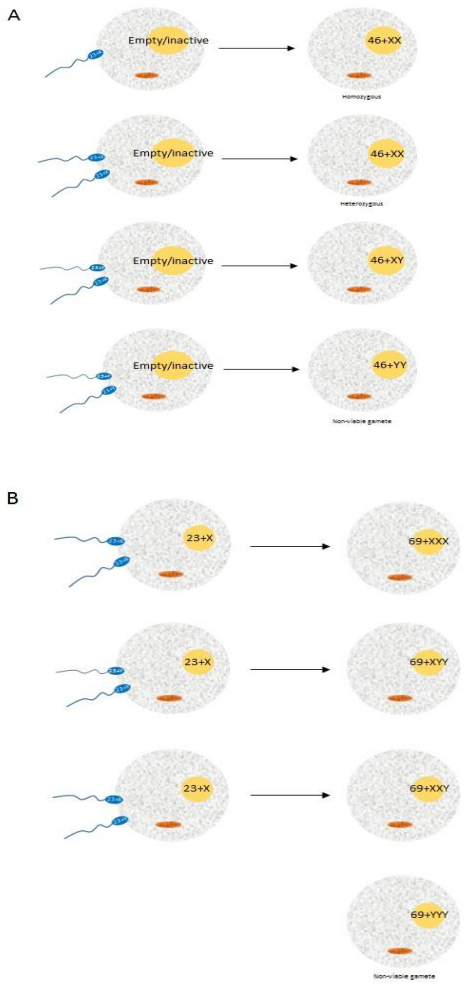

Fig.1: Genetically origin of hydatidiform mole. **A.** Complete hydatidiform mole and **B.** Partial hydatidiform mole.

Materials and Methods

This cross-sectional study was approved by the Reproductive Biomedicine Research Center Ethics Committee at Royan Institute (IR.ACECR.ROYAN.REC.1396.126). All participants completed the informed consent form. The current study evaluated the pregnancy outcomes in couples who underwent fresh or frozen embryo transfer after ICSI procedure from 2011 to August 2019 in Royan Institute.

Inclusion criteria are as follows: i. Serial high levels of serum β-hCG (>100,000 mIU/mL); ii. Ultrasonography findings such as enlarged uterine, mixed echogenic vascular mass, diffuse hydropic chorionic villi in the presence of complete molar pregnancy, and gestational sac with or without fetus in the presence partial mole; iii. trophoblastic hyperplasia confirmed by histological assessment of the evacuated uterine contents. Patients, who did not satisfy one or more of the above-noted criteria, would be excluded from the study.

The overall evaluated ICSI cycles were 41,540 cases. But GTD cases were only limited to fresh embryo transfers, accounting for 25,667 of the total ICSI procedures.

For estimating GTD frequency in clinical pregnancy, a total of 8,196 clinical pregnancies were assessed during this study period.

Results were categorized, according to the world health organization (WHO) classification of GTD (11), as HM (complete and partial) and malignant GTN. GTD frequency was reported for ICSI cycles, clinical pregnancy (CP), and fresh embryo transfer after ICSI procedures.

Statistical analysis

A data collection sheet was used to obtain information about the patients and their potentially related risk factors, namely maternal age, blood group, history of miscarriage and previous GTD, and familial history. Statistical analysis of median (interquartile range, IQR), mean ± SD, and variables' frequency were done using the Statistical Package for the Social Sciences (Release 6.0, SPSS Inc., Chicago).

Results

In the beginning, 15 patients were diagnosed with GTD based on increased serum β-hCG levels (>100,000 mIU/mL), and mixed echogenic vascular mass and diffuse hydropic chorionic villi on ultrasonography. However, the pathology specimens of four patients were not consistent with standard GTD pathologic findings, and another case had a twin pregnancy with one of the gestational sacs being a complete mole and the other a normal fetus leading to live birth. As a result, 10 GTD cases were confirmed by three inclusion criteria, out of which 9 HM (8 complete and 1 partial HM) and 1 GTN, which had pulmonary metastases, were detected. GTN was detected by high hCG levels after normal miscarriage and confirmed by lung metastases shown by chest x-ray and histopathological findings.

Based on 'patients' demographic data, females had a median (IQR) age of 27 (20-33) years, and their partners had a median (IQR) age of 32 (29-45) years. Basal hormone levels in serum on the third cycle day were within the normal range. Of note, 50% of participants had blood type O, which was the most common blood type. None of the patients had a history of GTD; however, one case reported spontaneous abortion (Table 1).

Table 1: Demographic and clinical characteristics of GTDs patients

Characteristics	Mean ± SD	Median (IQR)	Frequency n (%)
Partner age (Y)	34.3 ± 5.25	32 (29-45)	-
Maternal age (Y)	26.3 ± 3.71	27 (20-33)	-
BMI (kg/m^2)	25.95 ± 4.09	26.44 (20.57-34.05)	-
FSH (IU/ml)	6.17 ± 3.81	5.3 (1.7-14.9)	-
LH (IU/ml)	4.94 ± 2.45	4.25 (1.9-8.7)	-
TSH (IU/ml)	1.31 ± 1.14	1.15 (0.04-3.89)	-
AMH (IU/ml)	3.4 ± 3.51	2.2 (1.9-8.7)	-
Previous GTD history	0	0	-
Miscarriage history	0	0	-
Blood groups			
A	-	-	3(30)
B	-	-	2 (20)
O	-	-	5(50)

IQR; Interquartile range, GTD; Gestational trophoblastic disease, BMI; Body mass index, FSH; Follicle-stimulating hormone, LH; Luteinizing hormone, TSH; Thyroid-stimulating hormone, and AMH; Anti-mullerian hormone.

The overall estimated frequency of GTD was 10 in 41,540 cases (0.240 per 1000) in ICSI procedures, 10 in 25,667 cases (0.389 per 1000) following fresh embryo transfer after ICSI cycles, and 10 in 8,196 GTD cases (1.220 per 1000) clinical pregnancies.

Table 2 lists the patients' characteristics during the standard stimulation cycles. Semen characterization was analyzed according to Kruger criteria which showed the median (IQR) number of 27.5 (.01-70)×10^6/ml for sperm count with 22.5 (.01-63) motility.

The median (IQR) number of oocytes retrieved was 9 (6-11). The achieved MII oocytes were 8 (5-11), which resulted in 4 (1-10) two-pronucleus (2PN) embryos. The number of embryos transferred per each cycle was 2 (2, 3), and most of them were transferred at a good stage (47.82%) and 30.44% at the blastocyst stage (Table 3).

Table 2: Ovarian stimulation characterizations, and spermogram in GTDs

Variables	Number	Median (IQR)	Frequency n (%)
Type of protocol			
Standard long agonist	9	-	90
Standard antagonist	1	-	10
Total gonadotropins dose	1755 ± 839.04	1500 (900-3525)	-
Sperm count (10^6/ml)	28.8 ± 22.27	22.5 (0.01-63)	-
Motility	27.1 ± 17.64	28.5 (0-50)	-
Morphology	5.3 ± 6.23	2 (0-15)	-

Data are presents as mean ± SD. IQR; Interquartile range and GTD; Gestational trophoblastic disease.

Table 3: Oocyte and embryo characterizations in GTDs

Variables	Number	Median (IQR)	Frequency n (%)
No. of oocytes retrieved	8.4 ± 2.01	9 (6-11)	-
No. of MII	7.7 ± 2.16	8 (5-11)	-
No. of 2PN embryo	4.6 ± 2.59	4 (1-10)	-
No. of ET per cycle	2.3 ± 0.48	2 (2-3)	-
Total No. of achieved ET	23	-	-
Embryo grade at cleavage stage	16	-	69.56
Embryo grade at blastocyst stage	7	-	30.44
Excellent	10	-	43.5
Good	11	-	47.82
Faire	1	-	4.34
Poor	1	-	4.34

Data are presents as mean ± SD or n (%). IQR; Interquartile range, GTD; Gestational trophoblastic disease, MII; Metaphase II, 2PN; Two pronuclei, and ET; Embryo transfer.

Discussion

Genetically CHM presents excess paternal genome (dispermia) and maternal chromosome loss, most commonly resulting in a 46,XX embryo (90%) with genomic content of androgenic chromosome and maternal mitochondrial DNA (mtDNA) (4, 12). However, partial HM results from the ovum fertilized by two spermatozoa leading to triploid conceptus (13). Based on the etiology of moles, the occurrence of this event following ICSI, where one sperm is injected into one oocyte, is unlikely.

The prevalence rates of GTD have a wide geographic variation (13). However, in Iran, Javey and Sajadi (14) showed that the prevalence of HM among spontaneous pregnancies was 3.1 per 1000 conceptions (1 in 314 pregnancies) in Southern Iran. Almasi et al. (15) found 61 HM following 8614 spontaneous pregnancies (0.7% or 7 per 1000 pregnancies) in the prenatal clinics of Iran University of Medical Sciences, Iran. They reported 32% blood group O distribution in HM patients. This global variation possibly reflects differences in genetic factors, ethnic groups, socioeconomic status, diet, and epidemiologic data (2). Reports on the frequency of GTD after ART are limited to some case reports (6-8, 16). Makhseed et al. (17) reported 1 HM per 203 (0.4%) clinical pregnancies as the outcome of multiple pregnancies following IVF/ICSI.

GTD frequency after intrauterine insemination (IUI) was determined 4 cases in 1482 pregnancies (2.69:1000) (18). GTD occurrence is possible after IUI, because it is a stimulated spontaneous pregnancy, and one oocyte is encountered with multiple spermatozoa. Still, GTD frequency following ICSI cycles is not as justifiable. Moreover, to author's knowledge, previous studies thus far have only estimated the prevalence of GTD in spontaneous pregnancies.

Here, the authors described the frequency and prevalence of GTD in ICSI cycles, fresh embryo transfers

and clinical pregnancies. The overall frequency of GTD in this study was 0.240 per 1000 ICSI cycles, 0.389 per 1000 fresh embryo transfers, and 1.220 per 1000 clinical pregnancies.

Based on the present study, the proportion of GTDs per CP was 2-3 times less than that reported from Asian regions after spontaneous pregnancies but similar to that of Europe and the North American populations.

The most commonly accepted etiology for complete moles considers an androgenic cause with lost or inactive maternal chromosomal content (4). In the current study, all GTD cases had undergone ICSI. It is assumed that ICSI process can prevent polyploidy by delivery of a single spermatozoa to the oocyte (19). However, recent studies have suggested that biparental diploids (9), oocyte defects, and maternal mtDNA (20) are involved in the etiology of GTDs and cannot be prevented by ICSI process. Oocytes might have a critical role in GTD occurrence following ICSI (21). However, various methods like the ICSI and frozen embryo transfer procedure are the recommended treatment method. Nevertheless, GTD remains a rare complication of ICSI (19).

The risk factor most associated with GTD is maternal age, with an increased risk for older ages. It has been indicated that paternal age, blood group, gravidity, and increased parity also predispose to GTD (2). The risk of GTD is increased two- to three folds in women with previous histories of miscarriage. In women who previously had a GTD, the risk for subsequent GTD increased to approximately 1% (22). In patients included in the present study, no one had a history or familial background of GTD, and only one case had spontaneous abortions. This study revealed that blood type O (50%) was the most common blood group in these patients, possibly representing the general population status. As mentioned before, Almasi et al. (15) showed that 32% of patients with HM had blood group O compared to non-molar pregnancies (40.1%), which was not significant. In contrast, previous studies found that maternal A and AB blood groups were associated with GTD (23). Also, Jagtap et al. (24) found the highest incidence of GTD in patients with blood group A followed by blood group O, and the least was noted in blood group B.

Conclusion

The current study described the frequency and prevalence of GTD following ICSI cycles. This result was comparable with that observed after spontaneous pregnancies. The proportion of GTD after ICSI, estimated by the present study, was less than that for normal pregnancies reported by previous studies. Concerning the accepted GTD etiology, it is concluded that GTD should not occur after ICSI procedure as two spermatozoa fertilize one oocyte. Therefore, it is preferable to consider dinucleic spermatozoa, biparental diploids, and oocyte defects as additional factors that play a role in GTD occurrence, especially after ICSI procedure. Thus more research is essential to deeper understand the mechanisms of GTD.

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Authors' Contribution

M.H.; Contributed to conception and design, administrative support, and manuscript revision. Z.Ch.; Contributed to the collection, assembly, analysis of data, manuscript drafting, and revision. F.Gh., M.M.; Contributed to literature review, interpretation of data, and manuscript revision. N.Z.; Contributed to collection of data and manuscript revision. All authors read and approved the final version of the manuscript.

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