 doi:10.20944/preprints202012.0261.v1

Research Article

Pre-Implantation Gender Selection: Family Balancing in Jordan

Amer Mahmoud Sindiani,[1,*] Faheem Zayed,[2] Eman Hussein Alshdaifat,[3] Hasan M. Rawashdeh,[1] Wesam Al-Woshah,[4] , Nada Zayed,[5] Yousef Khader[6]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan

[2]Irbid Speciality Hospital, Irbid, Jordan

[3]Department of Obstetrics and Gynaecology, Yarmouk University, Irbid, Jordan

[4]IBN Al-Nafis Hospital, Irbid, Jordan

[5]Department of Dermatology, Albalqa Applied University , Jordan

[6]Department of Community Medicine, Public Health and Family Medicine, Jordan University of Science and Technology, Irbid, Jordan

[*]**Corresponding author:** Amer Mahmoud Sindiani, Department of Obstetrics and Gynecology, Faculty of Medicine, Jordan University of Science and Technology, Irbid, P.O. Box: 3030, Postal code: 22110, Jordan, Tel: 00962796025538; E-mail: amsindiani0@just.edu.jo; Orcid: orcid.org/0000-0001-5115-7372

Abstract

Purpose*:* In this study, the Pre-implantation embryonic sex ratio in 125 couples who had three or more female children and underwent pre-implantation genetic diagnosis (PGD) for non-medical reason was included. Besides, we have aimed to find out whether these couples had more chances of getting a girl child once again. ***Methods****:* 125 couples who had three or more female offspring and those who underwent PGD for non-medical sex selection (XY) between 2015 and 2019 were included. Nuclear DNA was analyzed by Fluorescent *in situ* Hybridization (FISH). 2-chromosome (X, Y), 3-chromosome (21, X, Y), 5-chromosome (13, 18, 21, X, Y) probes were used for FISH. The standard protocol was followed for sperm processing and embryo culture for IVF and PGD. ***Result****:* Independent sample t-test showed that there is no significant difference between equal and unequal embryonic groups in patients' age, husbands' age, sperm count, sperm motility, total male embryos, total female embryos, normal male embryos, and normal female embryos. For patients with positive pregnancy outcome, 84.6% had unequal embryonic ratio while 15.4% had equal embryonic ratio. Similarly, patients those who were treated by short protocol had 85% of unequal embryonic ratio and 15% had equal ratio. ***Conclusion****:* Greater variability in the female to male embryos was found in these couples, confirms the fact that couples previously having girl offspring may predominantly not produce embryo of the same sex every time.

Keywords: Infertility, PGD, IVF, Embryos

 doi:10.20944/preprints202012.0261.v1

1. Introduction

The first human live births which used pre-implantation genetic diagnosis (PGD) during embryonic development to identify the presence of lethal genetic diseases in cycles of assisted reproduction were introduced in 1990 [1]. This treatment has gained momentum in assisted reproductive technology (ART). The DNA-based treatment strategy has opened a new avenue in the treatment of infertility and embryonic sex-selection for medical and non-medical reasons. One of the most trusted DNA-based diagnostic procedures in ART is PGD. This technique helps physicians to select the unaffected embryos for uterine transfer in case of patients who are carriers of single gene disorders or patients with structural chromosomal abnormalities [2]. In addition to single gene defect detections, technical advances in single-cell genetic analysis, including single nucleotide polymorphism (SNP) array, comparative genomic hybridization (CGH), and whole genome amplification (WGA), may improve diagnostic precision and permit useful pre-implantation genetic screening (PGS) in patients with recurrent pregnancy loss and unexplained *in vitro* fertilization (IVF) treatment failure, where chromosomal errors are the result of potentially de novo mutations or meiotic and mitotic aberrations [3-7]. Besides, this technique is being used for non-medical reasons, enabling the selection of embryos of the desired sex. While there is appreciation for the use of PGD for medical reason, the use of this technique for non-medical reason has raised serious ethical concerns because, non-medical sex selection can cause gender imbalance in communities and may cause destruction of unwanted normal embryos [8-10]. Gender variety or "family balancing" as it is sometimes known, is a form of PGD that is undertaken in families in which all offspring are of the same gender. Patients pursuing this option are interested in the unique experience of raising a child of the unrepresented gender [11, 12]. The ethics committees of both the American Congress of Obstetricians and Gynecologists (ACOG) and the American Society for Reproductive Medicine (ASRM) have established acceptable medical justifications for sex selection. However, the committees differ on the ethics of gender selection for non-medical indications [13].

In one retrospective study, 122 IVF/PGD cases from the year 2004 to 2009 were studied where PGD success rate for gender selection was found to be significantly less compared to conventional IVF [14]. In another study, PGD reports of 276 patients of US national were reviewed retrospectively. This study found to have no biological significance to the fact that couples previously having children of a particular gender are more likely to produce embryos of that same gender [15]. In a recent study, pre-implantation embryonic sex ratio was studied retrospectively in nine Israeli women who underwent PGD for nonmedical sex selection. The results revealed a lower percentage of the desired embryos obtained in PGD compared to IVF. Hence, it was believed that the mode of fertilization may improve the result of sex selection in non-medical reason. However, the result of this study cannot represent the rest of the population due to very low sample size. Hence, in the present study, we aimed at finding the embryonic sex ratio in 125 couples who had three or more female offspring and underwent PGD for non-medical reason. We also have checked whether these types of couples have more chances of getting a girl child again.

2. Methods

This study was approved by the Institutional Review Board of Jordan University of Science and Technology(JUST) / King Abdullah University Hospital(KAUH) (36/121/2019). Patient consent

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 December 2020 doi:10.20944/preprints202012.0261.v1

was waived as this retrospective study involves electronic medical records review and analysis was performed on de-identified data. Patient data privacy and confidentiality are maintained as this study was conducted in compliance with the ethical standards per Helsinki declaration. In this retrospective study, 125 couples who had three or more female offspring and those who underwent PGD for non-medical sex selection (XY) between 2015 and 2019 were included. The patients' details were collected from the repositories at Ibn AlNafis Hospital and King Abdulla University Hospital / Jordan University of Science and Technology.

All patients were counseled by their medical providers and then provided informed consent to participate in IVF/PGD. Patients underwent ovarian stimulation with gonadotropins using GnRH-agonist, few antagonist cases; luteal-phase down regulation using short or long protocol to prevent premature luteinization of follicles. Serial monitoring by a physician was performed for controlled ovarian stimulation by hormone and ultrasound analysis. When at least 2-3 follicles measured 18 mm in diameter, Human Chorionic Gonadotropin (HCG) (5000-10,000 IU intramuscularly) was administered by injection and transvaginal ultrasound-guided oocyte retrieval was performed 36 hours later. In all cases, Intra-Cytoplasmic Sperm Injection (ICSI) was performed. All patients had embryo biopsy performed on Day 3 after oocyte retrieval, by direct aspiration of a single blastomere through an opening created by laser degradation of the zona pellucida. The biopsied blastomere was fixed to a glass microscope slide and the cytoplasm was removed before PGD analysis.

Nuclear DNA was analyzed by Fluorescent *in situ* Hybridization (FISH). 2-chromosome (X, Y), 3-chromosome (21, X, Y), 5-chromosome (13, 18, 21, X, Y) probes were used for FISH. PGD results were evaluated by the geneticists, embryologists and the physician responsible for embryo transfer on Day 4 or Day 5 of embryo development. Patients were counseled about the FISH results prior to embryos were available for transfer.

The patients who could reach ovum pick-up stage and at least had complete molecular diagnosis of one embryo followed by Day 3 blastomere biopsy were included in the study. The standard protocol was followed for sperm processing and embryo culture for IVF and PGD.

Statistical analysis was performed using IBM SPSS version 21. Descriptive statistics were calculated to describe the participant demographic characteristics. Ratio statistics was performed to find out whether greater variability in the sex-ratio exists in the study population or not. Independent sample t-test and chi-squire test were performed for continuous and categorical variables respectively. The level of significance was set at $p<0.05$.

3. Results

Of the couples with three or more female children, undergoing PGD for sex selection, the total numbers of male embryos were 289 and total female embryos were found to be 296. The mean age of the patients (females with 3 or more girl children) and their husbands was 35 year and 41 years respectively. The youngest patient was 25 years old and the oldest patient was 47 years old. Similarly, the age of the youngest husband was 28 and oldest husband was 57 years. Out of 125 patients, 83.2% were with unequal embryonic ratio and 16.8% had equal embryonic ratio. The median embryonic ratio was found to be 1. Besides, within 20% of median inclusion only 24.2%

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 December 2020 doi:10.20944/preprints202012.0261.v1

patients were there. It indicates larger variability in the ratio of female to male embryos exist in the study population. Hence, the probability of getting again a girl child with PGD is not certain.

The patients were divided into two groups based on the ratio of total male to total female embryos. Independent sample t-test showed that there is no significant difference between equal and unequal embryonic groups in patients' age, husbands' age, sperm count, sperm motility (%), number of eggs, number of embryos, total male embryos, total female embryos, normal male embryos, normal female embryos (Table 1). Chi square test was performed to check the relationship between the type of protocol, embryo ratio and pregnancy outcome (for total embryos). For patients with positive pregnancy outcome, 84.6% had unequal embryonic ratio while 15.4% had equal embryonic ratio. Similarly, patients who were treated by short protocol had 85% of unequal embryonic ratio and 15% had equal ratio (Table 2). Similarly, independent sample t-test and chi-square test were performed for normal embryos to check whether any significant relationship exist between variables (Table 3) (Supplementary Tables 1 and 2).

4. Discussion

We found larger variability in the ratio of female to male embryos in couples who had three or more girl children and underwent PGD for opposite sex selection. The ratio statistics performed in this population confirmed the fact that couples previously having girl children may not predominately produce the embryo of same gender every time. Consistent with the finding of this study, performed a retrospective study on a large series of PGD procedures for gender selection in a wide geographical region in the USA [15]. A significant deviation towards male sex preference was found in patients of Chinese, Indian and middle-eastern ethnicity. In another study, the embryonic sex ratio was found to be 1 [16]. This reported that the sex ratio at both fertilization and implantation is between 1.29, 1.50 and 1.07 for PGD, IVF and ICSI cycles respectively [17].

In another study, the effect of male age on the sperm sex ratio was studied [18]. They observed a significant difference between live birth and sperm-sex ratio ($P<0.0001$). However, the finding of this study did not support this finding.

Panahi and Fahami in the year 2015 studied the result of pre-implantation genetic diagnosis in relation with couple's age [19]. Their result suggested no significant relationship between the age of the patient with the rate of chemical and clinical pregnancy and gestational weight of newborn. However, PGD method was 100% successful in achieving the desired sex.

Knowledge of the proportion of one gender in couples who have offspring of the other gender can help the physician during counseling the couples who look for PGD sex selection for probability of having the desired embryos. This study revealed the fact that PGD do not ascertain 100% predictability of the gender of the desired embryo in couples undergoing sex selection for non-medical reasons.

5. Conclusion

 doi:10.20944/preprints202012.0261.v1

This is the first study in Jordan where embryonic sex ratio was observed in a larger population (couples with three or more female offspring) who underwent PGD for sex selection. A larger variability in the female to male embryonic ratio in the studied population was found.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Funding Statement

The authors declare that they have not received any funds from any source.

 doi:10.20944/preprints202012.0261.v1

References

[1] A. H. Handyside, E. H. Konotogianni, K. Hardy, and R. M. Winston, "Pregnancies from biopsied human pre-implantation embryos sexed by Y-specific DNA amplify-cation," *Nature*, vol. 334, no. 6268, pp. 768-770, 1990.

[2] M. Kirshenbaum, B. Feldman, A. Aizer, J. Haas, and R. Orvieto, "Pre-implantation embryos sex ratios in couples with four or more children of same sex, what should be expected from a pre-implantation genetic diagnosis cycle?," *Gynecological Endocrinology*, vol. 35, no. 6, pp. 515-517, 2019.

[3] L. Werlin, I. Rodi, A. DeCherney, E. Marello, D. Hill, and S. Munne, "Pre-implantation genetic diagnosis (PGD) as both a therapeutic and diagnostic tool in assisted reproductive technology," *Fertility and Sterility*, vol. 80, no. 2, pp. 467-468, 2003.

[4] Pre-implantation Genetic Diagnosis International Society, "Guidelines for good practice in PGD: programme requirements and laboratory quality assurance," *Reproductive Biomedicine Online*, vol. 16, no. 1, pp. 134-147, 2008.

[5] L. Wilton, A. Thornhill, J. Traeger-Synodinos, K. D. Sermon, and J. C. Harper, "The causes of misdiagnosis and adverse outcomes in PGD," *Human Reproduction*, vol. 24, no. 5, pp. 1221-1228, 2009.

[6] C. M. Lewis, T. Pinel, J. C. Whittaker, and A. H. Handyside, "Controlling misdiagnosis errors in pre-implantation genetic diagnosis: a comprehensive model encompassing extrinsic and intrinsic sources of error," *Human Reproduction*, vol. 16, no. 1, pp. 43-50, 2001.

[7] W. Piyamongkol, M. G. Bermúdez, J. C. Harper, and D. Wells, "Detailed investigation of factors influencing amplification efficiency and allele drop-out in single cell PCR: implications for pre-implantation genetic diagnosis," *Molecular Human Reproduction*, vol. 9, no. 7, pp. 411-420, 2003.

[8] G. DeWert and W. Dondorp, "Preconception sex selection for non-medical and intermediate reasons: ethical reflections," *Facts, Views & Vision in Obgyn*, vol. 2, no. 4, pp. 267-277, 2010.

[9] H. Strange, "Non-medical sex selection: ethical issues," *British Medical Bulletin*, vol. 94, no. 1, pp. 7-20, 2010.

[10] K. J. Abbate, R. Klitzman, W. K. Chung, R. Ottman, C. S. Leu and P. S. Appelbaum, "Views of pre-implantation genetic diagnosis among psychiatrists and neurologists," *The Journal of Reproductive Medicine*, vol. 59, no. 7-8, pp. 385-392, 2014.

[11] C. F. Westoff and R. R. Rindfuss, "Sex preselection in the United States: some implications," *Science*, vol. 184, no. 4137, pp. 633-666, 1974.

[12] R. Steinbacher and F. D. Gilroy, "Preference for sex of child among primiparous women," *The Journal of Psychology*, vol. 119, no. 6, pp. 541-547, 1985.

[13] ACOG Committee Opinion No. 360, "Sex selection," *Obstetrics and Gynecology*, vol. 109, no. 2, pp. 475-478, 2007.

[14] T. Mukherjee, E. Flisser, A. B. Copperman, L. Grunfeld, B. Sandler, and J. Barritt, "Pre-implantation genetic diagnosis for gender selection: you don't always get what you want." *Open Journal of Obstetrics and Gynecology*, vol. 2, no. 3, pp. 291-297, 2012.

[15] J. Hsu, H. Zeng, K. Lemke, et al., "Pre-implantation genetic diagnosis for gender selection in the USA," *Reproductive Biomedicine Online*, vol. 19, no. 2, pp. 16-22, 2009.

[16] B. Feldman, A. Aizer, M. Brengauz, et al., "Pre-implantation genetic diagnosis should we use ICSI for all?," *Journal of Assisted Reproduction and Genetics*, vol. 34, no. 9, pp. 1179-1183, 2017.

[17] J. Tarın, M. A. Garcıa-Perez, C. Hermenegildo, and A. Cano, "Changes in sex ratio from fertilization to birth in assisted-reproductive-treatment cycles," *Reproductive Biology and Endocrinology*, vol. 12, no. 1, pp. 56, 2014.

[18] J. Graffelman, E. F. Fugger, K. Keyvanfar, and J. D. Schulman, "Human live birth and sperm-sex ratios compared," *Human Reproduction*, vol. 14, no. 11, pp. 2917-2920, 1999.

[19] S. Panahi and F. Fahami, "The results of pregnancies after gender selection by pre implantation genetic diagnosis and its relation with couple's age," *Iranian Journal of Nursing and Midwifery Research*, vol. 20, no. 6, pp. 670-675, 2015.

Table 1. The participants'' characteristics.

Total Female Embryo	296
Total Male Embryo	289
Patients with Equal embryonic ratio (%) F:M	16.8
Patients with Unequal Embryonic Ratio (%) F:M	83.2
Median Embryonic ratio F:M (N=95, Missing data=30): Median (minimum, maximum)	1.00 (0, 8)
Co-efficient of concentration (Within 20% of median inclusion)	24.2%

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 December 2020 doi:10.20944/preprints202012.0261.v1

Table 2. Independent sample t-test (for total embryos).

Variable	Equal ratio Mean ± SD	Unequal Ratio Mean ± SD	P-value
Patient Age	33.94 ± 3.39	35.19 ± 4.48	0.273
Male partner Age	40.37 ± 4.89	41.45 ± 5.66	0.472
Sperm Count (Million)	89.35 ± 45.5	78.62 ± 50.87	0.417
Motility (%)	58.64 ± 9.65	52.10 ± 15.28	0.091
Eggs (number)	11.70 ± 4.76	12.77 ± 7.07	0.550
Embryos (Number)	6.35 ± 2.34	6.82 ± 3.11	0.552
Total male embryos (Number)	2.64 ± 1.16	2.84 ± 1.84	0.672
Total Female Embryos (Number)	2.64 ± 1.16	2.83 ± 2.03	0.715
Normal Male Embryos (Number)	2.25 ± 1.18	2.60 ± 1.55	0.380
Normal Female Embryos (Number)	2.31 ± 1.13	2.91 ± 1.66	0.171

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 December 2020 doi:10.20944/preprints202012.0261.v1

Table 3. Chi-square test (for total embryos).

			Embryo Ratio		P-value
			Unequal Ratio	Equal Ratio	
Pregnancy outcome	Negative	N	43	7	.843528
		% within Pregnancy outcome	86.0%	14.0%	
	Positive	N	44	8	
		% within Pregnancy outcome	84.6%	15.4%	
Type of protocol	Short	N	96	17	1.0
		% within Type of protocol	85.0%	15.0%	
	Long	N	2	0	
		% within Type of protocol	100.0%	0.0%	

 doi:10.20944/preprints202012.0261.v1

Supplementary Table 1. Independent sample t-test (for normal embryos)

Variable	Equal ratio Mean ± SD	Unequal Ratio Mean ± SD	P-value
Patient Age	33.66 ± 4.69	35.29 ± 4.26	.119
Male partner Age	40.95 ± 5.32	41.38 ± 5.62	.753
Sperm Count (Million)	71.54 ± 41.27	82.00 ± 51.73	.398
Motility (%)	49.25 ± 14.97	53.85 ± 14.64	.205
Eggs (number)	11.30 ± 5.14	12.88 ± 7.06	.342
Embryos (Number)	6.71 ± 2.74	6.76 ± 3.08	.940
Total male embryos (Number)	2.71 ± 1.18	2.83 ± 1.85	.773
Total Female Embryos (Number)	2.95 ± 1.59	2.77 ± 2	.710
Normal Male Embryos (Number)	2.28 ± 1.23	2.61 ± 1.56	.362
Normal Female Embryos (Number)	2.28 ± 1.23	2.95 ± 1.66	.089

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 December 2020 doi:10.20944/preprints202012.0261.v1

Supplementary Table 2. Chi square test (for normal embryos)

			Embryo Ratio		
			Unequal Ratio	Equal ratio	P-value
Pregnancy out come	Negative	N	45	5	0.68
		% within Pregnancy out come	90.0%	10.0%	
	Positive	N	39	13	
		% within Pregnancy out come	75.0%	25.0%	
Type of protocol	Short	N	93	20	1
		% within Type of protocol	82.3%	17.7%	
	Long	N	2	0	
		% within Type of protocol	100.0%	0.0%	