The results of pregnancies after gender selection by pre implantation genetic diagnosis and its relation with couple's age

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ABSTRACT

Background: Non-medical utilization of pre-implantation genetic diagnosis (PGD), like sex selection, is increasing, therefore it is necessary to follow-up the health and outcome of fertilization and newborn's birth followed PGD. The aim of this study was to evaluate the outcome of fertilization after sex selection by PGD and the relation between the age of parents and the outcome of fertilization.

Materials and Methods: This was a retrospective descriptive correlative study conducted on 218 couples in Isfahan. Samples were selected through convenience sampling. The rate of chemical and clinical pregnancy and abortion, the frequency of success in achieving the desired sex, and the mean of gestational age and weight of newborns were gathered through reviewing medical files and phone interviews. Data was analyzed using independent *t* test and Pearson correlation test.

Results: The rate of chemical and clinical pregnancy was 30.7% and 30.3% respectively, the rate of abortion was 26.9%, the frequency of success in achieving the desired sex was 100%, and the mean of gestational age and weight of newborns was 3260 (616) kg and 37.7 (2.07) weeks respectively. There was no significant relation between the age of parents and the rate of abortion, the rate of chemical and clinical pregnancy and newborn's gestational weight. But there was a significant relation between the age of men and gestational age of newborns (P = 0.04).

Conclusions: PGD method was 100% successful in achieving the desired sex, but relatively high rate of abortion could indicate the effect of PGD on the embryo development process.

Key words: Abortion, age, chemical pregnancy, Iran, PGD, pregnancy, pre implantation, sex selection

INTRODUCTION

The desire to select child's gender is rooted in ancient cultures. Nowadays also international studies about general interest in sex selection are increasing.[1] Sex selection could be categorized in two groups: Medical and non-medical. Medical sex selection would be performed to prevent a sex related disease but non-medical selection is merely performed to satisfy the parent's desire to breed a specific sex.[2] Mostly negative critics of sex selection were aimed for non-medical type.[3] Different methods of sex selection are as follows: Pre-implantation methods

such as pre-implantation genetic diagnosis (PGD), the Ericsson method or albumin gradient in intrauterine insemination, the scheduling method (having intercourse at a specific time related to ovulation would affect child's sex), following a specific diet and finally the microsort method that uses flow cytometry device to separate sperms but its use has been criticized in human cases due to consumption of fluorescent colors and laser beam.[4] Post implantation method usually means aborting the embryo with undesired sex and after birth methods usually contain killing or abandoning the child with undesired sex.[5] PGD was first invented for those couples with genetically risky pregnancies to prevent the transmission of genetic diseases. The ability to determine the sex of embryo with PGD is

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a challenging matter, especially when in a society that one sex is more preferable. Low success rate, complicate techniques and high cost without insurance coverage are other problems of this method. Also it doesn't seem ethical to make a woman go through PGD merely for sex selection.[6] Using PGD for non-medical purposes including sex selection is increasing.[7] There are controversies about usefulness and practicality of this fast growing technology.[6] Conflicting results have been reported in different studies for pregnancies following this method. Thomaidis *et al.* in their study concluded that PGD mostly resulted in C section, preterm labor and low birth weight of the infant. Furthermore most of the children conceived through pre-implantation genetic diagnosis have normal levels of cognitive skills but one fifth of them show a low to moderate level of cognitive skills.[8] Hardarson *et al.* in their study revealed that not only pre-implantation screening does not have any useful results in older women, but also it would decrease their chance of pregnancy.[9] Mastenbroek *et al.* in their study compared two groups of older women, who were impregnated through IVF method with or without PGD, and concluded that PGD would significantly decrease the rate of pregnancy progress and live births after IVF. They proposed different mechanisms for the failure of PGD to enhance the results of IVF including that blastomere biopsy at the third day could be a potential danger to implantation.[10] Many other studies have also reported bad pre-natal results. Middleburg *et al.* found no relation between PGD and mental, psycho-motor and behavioral disorders in children at age 2.[11] Also the results of Desmyttere *et al.* showed no significant difference between premature birth, the average birth weight, low birth weight, pre-natal death, major disorders and neonate's hospitalization in singleton and twin births following PGD and intra cytoplasmic sperm injection (ICSI).[12] Regarding the existing controversies, the aim of this study was to assess the outcomes o reproduction after sex selection by PGD in fertile couples and since age is an effective factor in assisted reproductive treatments, the relation between the outcomes and the age of couples was also evaluated.

MATERIALS AND METHODS

This was a retrospective descriptive correlative study. Sampling method was convenience sampling and the studied population included all the couples who referred to Isfahan Infertility Clinic from 4/4/2011 to 20/6/2013 for sex selection by PGD. This center takes informed consent from everybody undergoing this procedure, to use their own and their child's information for educational and research purposes and also to keep in touch for phone follow-ups, confidentially. Also the study has been confirmed by the Ethical Committee of Isfahan University of Medical Sciences. Data was gathered through reviewing patient files and recording information by the researcher through phone interviews. Inclusion criteria were being impregnated with fresh embryo (those with frozen embryos were excluded from the study), not having a history of infertility, not having a history of obstetric and medical complication in previous pregnancies (including repeated abortion, premature labor, low birth weight, stillbirth, gestational hypertension, gestational diabetes and uterine abnormalities) and mother's lack of systemic diseases (cardiovascular, renal, endocrine diseases, cancer and rheumatologic diseases) that have undesired effects on pregnancy (information was gathered from patients' files or through phone interviews). Through the process of PGD at this clinic, based on the standard protocol, all of the patients went through IVF or ICSI and then at the third day (6 to 8 celled stage) with a biopsy from the embryo one blastometer was separated; using fluorescence *in situ* hybridization (FISH) the sex of the blastometer was determined and at the fifth day of development one or mostly two embryos with the desired sex was transferred to the mother. The main evaluated variables included the rate of chemical and clinical pregnancy, the frequency of success in achieving the desired sex, the rate of spontaneous abortion among those with chemical pregnancy and infant's average gestational weight and age. Chemical pregnancy was considered as positive serum human chorionic gonadotropin (βHCG) 14 days after the transmission, clinical pregnancy was to find gestational sac 5 weeks after the transfer of embryo and infant's heartbeat and spontaneous abortion was defined as termination of the pregnancy before 20 weeks. Data was analyzed using SPSS 16. Descriptive statistics (mean, frequency, standard deviation, percent and frequency distribution) and independent *t* test and Pearson correlation test were used for analysis. *P* value of 0.05 was set as the significance level.

RESULTS

From 400 couple 218 had the inclusion criteria. Results showed that the mean age of women (4/11) was 34.5 years (23 to 42 years old) and the mean age of men (4/8) was 40.33 years (29 to 65 years old). The result of participants personal and social characteristis are shown in Table 1. The results of the rate of chemical and clinical pregnancy, the rate of abortion and the mean of infants' gestational weight and age are shown in Table 2. Also results showed that all of the born infants had the selected sex and success rate in achieving the desired sex was 100%. There was no significant relation between the age of women (*P* = 0.12) and the age of men (*P* = 0.68) with the rate of chemical pregnancy. Also there was no significant relation between the age of women (*P* = 0.07) and the age of men (*P* = 0.56) with the rate of clinical pregnancy [Table 3].

Also no significant relation was found between the rate of abortion and the age of women ($P = 0.26$) and the age of men ($P = 0.6$) [Table 4]. Pearson correlation test showed no significant relation between the age of women and men ($P = 0.15$, r = -0.15) with infant's gestational age. But there was a significant and reverse relation between the age of men ($P = 0.04$, r = -0.24) and infant's gestation age.

Table 1: Participants' personal and social characteristics

Variable	N=218	
	Number	Percent
Men's employment status		
Unemployed	2	0.9
Employee	17	7.8
Labor	91	41.7
Sel-employed	108	49.5
Women's employment status		
Housewife	153	70.2
Employed	65	29.8
Economic status		
Well	64	29.4
Moderate	135	61.9
Weak	19	8.7
Educational status		

Education level	Women		Men	
	Number	Percent	Number	Percent
Less than diploma	43	19.7	44	20.2
Diploma	64	29.4	52	23.9
Associate degree	24	11	19	8.7
Bs	68	31.2	75	34.4
MS or higher	19	8.7	28	12.8

BS: Bachelor of science, MS: Master of science

Table 2: The outcomes o reproduction after sex selection by PGD

Variable	Number (%)			
Clinical pregnancy	66 (30.3)			
Positive βHCG	67 (30.7)			
Abortion	18 (26.9)			
Infant's gestational weight	Minimum	Maximum	Mean	SD
	1700	5000	3260	616.5
Infant's gestational age	32	40	37.35	2.05

PGD: Pre-implantation genetic diagnosis, SD: Standard deviation

DISCUSSION

This study evaluated the outcomes o reproduction after sex selection by PGD and its relation with the age of couples. The rate of chemical and clinical pregnancy was 30.7% and 30.3%, respectively. In the study of Mukherjee et al. titled "PGD for sex selection" the rate of chemical pregnancy was reported 45.9%.[13] DeVos et al. reported that the rate of chemical pregnancy among patients with one cell biopsy was 46.7%, among patients with two cell biopsy was 36.3% and among those underwent ICSI only with no biopsy (without PGD) was 48.6%. Since the rate of chemical pregnancy among patients with two cell biopsy was significantly lower than the two other groups ($P = 0.028$), therefore they assumed that one cell biopsy was in favor of the outcomes of reproduction by PGD.[14] It must be mentioned that in the present study one cell biopsy was performed on the patients. In the study of Mastenbroek et al. the rate of chemical pregnancy among women with PGD was 39% which was significantly lower ($P = 0.008$) than this rate among women who only had IVF without PGD (52%). One of the reasons they proposed for this reduction was that the biopsy that was performed during PGD process could have been potentially harmful to the development and implantation of embryo.[10] Embryo biopsy is a necessary yet aggressive procedure for performing PGD.[12] The effect of one or two cell biopsy at the cleavage level on development and implantation of the embryo has been discussed many times before.[14] Based on the results of different studies it seems that the more cells biopsied during PGD, the more damage would be caused. Therefore most of the Fertility Infertility centers would try to only biopsy one cell as much as it's possible. Hardarson et al. in a clinical trial concluded that the rate of chemical pregnancy among older women (older than 38 years) who had PGD for screening and having eupliod embryos was 8.9% which is lower than the present study. Based on the achieved results and due to the significantly higher rate o clinical pregnancy in the control group who had IVF without PGD, they suggested that PGD should not be used as a routine for screening older women.[9] Results showed that the rate of abortion was 26.9%. DeVos et al. in their study reported that the rate of abortion among patients with one 8-cell biopsy was 17% and among those with two 8-cell biopsy was 22%.[14] In Aflatyoon et al. study

Table 3: The mean age of women and men in two groups with positive and negative βHCG and positive and negative clinical pregnancy

Group variable	Positive βHCG		Negative βHCG		Independent t test		Positive clinical pregnancy		Negative clinical pregnancy		Independent t- test	
	Mean	SD	Mean	SD	P	t	Mean	SD	Mean	SD	P	t
Women's age	33.8	4.5	34.8	3.8	0.12	1.52	33.8	4.42	35	4.9	0.07	1.76
Men's age	40.13	4.4	40.42	4.4	0.68	0.4	40.04	4.4	40.46	5.01	0.56	0.57

SD: Standard deviation, βHCG: Beta-human chorionic gonadotropin

Group variable	With abortion (18)		Without abortion (49)		Independent t-test	
	Mean	SD	Mean	SD	P	t
Women's age	34.9	4.6	33.5	4.5	1.14	0.26
Men's age	40.6	4.4	39	4.5	0.51	0.6

SD: Standard deviation

the rate of abortion among patients who underwent just ICSI or IVF and had fresh embryo was 9%; this rate among those who received frozen embryo was 14.5% which was significantly higher than those received fresh embryo (P = 0.04).[15] In the present study all the studied cases received fresh embryo. Also to prevent the effect of confounding factors, women with repeated abortions and diseases that have unwanted effects on pregnancy were excluded from the study. To explain the high rate of abortion and low rate of chemical pregnancy in the present study it must be said that the participants in this study, unlike most of the previous studies, do not have a history of infertility and also their average age was lower so theoretically better outcomes was expected; but it was not so. It could be due to loss of large number of samples (about 50%) for different reasons including not having the inclusion criteria, unwillingness to answer the phone and not providing the right phone number in their files that led to a significant decrease in the number of samples; this could be a confounding factor that led to high rates of abortion and low rates of chemical pregnancy in the present study. The frequency of success in achieving the desired sex among those who gave birth was 100%. With PGD technology the chance to have a child with undesired sex is almost zero.[2] In comparison to usual traditional methods, PGD is more effective.[13] The rate of achieving the desired sex with other methods is varied in different studies. One of these methods is following a diet which is a simple, low risk and low cost method and it has a more generalizability socially and culturally especially in Muslim societies. Ganjloo et al. in their study showed that most of the participants (76.9%) were successful in achieving the desired sex; the success rate of food regimen in achieving the desired sex among those who wanted a boy was 75% and among those desired a girl was 83.3%. So there was no significant difference between both groups and food regimen could be used for both of these groups.[16] Another sex selection method is using intrauterine insemination (IUI). In the study of Khalili et al. that used albumin gradient in IUI for sex selection, the success rate in achieving male gender was 85%. In another study conducted by Silverman et al. the rate of having a girl with the same method was 74%. It seems that Ericsson method to separate the sperm using albumin gradient and IUI is an appropriate non-aggressive way for fertile couples.[4]

Individual, family and social characteristics are effective on gestational weight and preterm labor, but regardless of these characteristics, assisted reproductive treatments by themselves could have negative effect reproduction outcomes, gestational weight and preterm labor.[17-19] In the present study not having a history of infertility could be one of the reasons that, unlike many other studies about assisted reproductive treatments, infants' mean gestational weight and age were within normal range. Desmytter et al. in their study reported that infants' gestational weight in the PGD group was 3262.8 ± 543.5 gr, which is similar to our results. Also they reported that the gestational weight of the control groups was 3236.5 ± 583.2 gr; there was no significant difference between both groups. Also they reported that the gestational age in the PGD group was 38.7 ± 1.9 weeks which is one week more than our study. This number for their control group was 38.7 ± 2.2 weeks. Based on the results, they concluded that embryo biopsy in the PGD process does not have an extra risk for adverse neonatal outcomes or disorders.[12] In the study by Bahrami the mean of gestational weight for 3076 births was 3248.18 ± 458 gr[20] which is in accommodation with the results of our study. Results showed no significant relation between women's age with chemical and clinical pregnancy, abortion and gestational weight and age. Women's age is an important actor in successfulness of assisted reproductive treatments. Women over 40 years old in comparison to younger women are atrisk of not getting pregnant 4 times more. Higher age o mother has a significant effect on decrease in harvested eggs, fertilization and pregnancy. Women over 40 years old are 20 time more likely to have stillbirths. By going over 35 years in women, increased rate of abortion and decreased rate of live births have been observed.[21] There was a significant relation between the age of mother and the rate of abortion in Keshavarz et al. study, which is in contrast with our results.[22] But Yeh et al. in their study revealed that women using donated oocytes have appropriate and acceptable reproduction outcomes including implantation and clinical pregnancy until age of 45, and they have no significance difference with women younger than 45 years old; but after the age of 45 these rates would be reduced constantly and significantly.[23] These results are almost in accommodation with the results of the present study because in our study the mean age of women was 34 years old and according to the center's routine PGD would not be performed for women older than 42 years old. Different studies have reported different results about the effect of mother's age on gestational weight and age; some of them are in accordance with our results. In the study of Tabatabaei et al. there was a significant relation between mother's age and low birth weight.[21] But in a study conducted by Suzuki the increase in mother's age did not increase preterm labor.[24] Bahrami et al. in their study found

no significant relation between the increase in mother's age and low birth weight.[20] Also the results of the present study showed that there was no significant relation between the age of men with chemical and clinical pregnancy, abortion and gestational weight and there was only a significant and reverse relation between men's age and infants' gestational age (*P* = 0.04). There are controversial results about the effect of father's age on pregnancy. The number of studies that has evaluated the effect of mother's age on pregnancy is way much more than those evaluated the effect of father's age. Some large studies showed no significant relation between increased father's age and decreased pregnancy outcomes.[23] Dain *et al.* in their study concluded that there was no clear relation between the increase in father's age and fertilization, implantation and pregnancy. Regarding sperm's parameters, except for semen's volume, its other characteristics including movement, thickness and morphology would not be decreased by aging[25] which is in accordance with our results. But De La Rochebrochard *et al.* in their study resulted that father's higher age (over 40 years) is related with abortion and neonatal death.[26] Studies about the effect of father's age on low birth weight and preterm labor have different results. In a review study Prakesh concluded that father's higher age had a relatively low effect on low birth weight[17] which confirms our results. The limitation of the present study was low number of samples that would not allow us to generalize the results. It is suggested that in case of increase in using PGD method and availability to more samples, reproduction outcomes of PGD should be assessed again.

CONCLUSIONS

In the end, although results showed that PGD was 100% successful in achieving the desired sex and also the mean of infants' gestational weight and age were within normal ranges, but considering the low rate of chemical and clinical pregnancy and relatively high rate of abortion that could be due to PGD, and also considering important ethical issues and challenges regarding PGD and its high costs, it is recommended that all of the applicants for sex selection should go through realistic consultations to help them make the right decision.

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