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REVIEW ARTICLE

The association between IVF and chromosomal abnormalities compared to spontaneous conception

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ABSTRACT

In vitro fertilization (IVF) is a process by which an egg is extracted by needle aspiration and then combined with a sperm so that fertilization can occur outside the body. Genetic defects, such as chromosomal abnormalities, are considered rare among the general population; however, even though their incidence among IVF-conceived children is uncommon, several alarming studies were published on the increased risk of chromosomal abnormalities IVF/intracytoplasmic sperm injection (ICSI)-conceived children compared to universal rates. This study aimed to review the literature and present data to answer whether IVF or ICSI is associated with an increased risk of chromosomal abnormalities inborn after IVF/ICSI treatment compared to spontaneously conceived children. Relevant published scientific articles were searched in the Medline database, using combinations of the following key terms: "IVF," "*in vitro* fertilization," "ICSI," "intracytoplasmic sperm injection," "natural conception," "spontaneous conception," along with "chromosomal abnormalities," "chromosomal defects," "sex chromosome aneuploidy," and "trisomy." The eligible studies were considered as studies exploring the association of IVF/ICSI with chromosomal abnormalities compared to spontaneous conception. The search included studies published from 1992 to 2018. The results for the association of chromosomal abnormalities and IVF remain unclear. As many studies proved a significant increase in chromosomal abnormalities and syndromes among the IVF population, other studies were contradicting and contributed the abnormalities to several environmental and technical factors.

Keywords: IVF, ICSI, spontaneous conception, chromosomal abnormalities, sex chromosome aneuploidy, trisomy.

Introduction

Infertility is defined per the Practice Committee of the American Society for Reproductive Medicine as "Failure to achieve a successful pregnancy after 12 months or more of appropriate, timed of unprotected intercourse or therapeutic donor insemination" (1). Today, it has become possible for subfertile and infertile couples to conceive a child with assisted reproduction techniques (ART). *In vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are considered as effective ART. IVF is a process by which an egg is extracted by needle aspiration and then combined with a sperm so that fertilization can occur *in vitro* or outside the body. In IVF, multiple eggs are produced at one time by stimulating a woman's ovulatory process by medications. A typically developing embryo(s) is then transferred back to the woman's uterus to achieve pregnancy. Sometimes, to increase the IVF process's success, a procedure known as ICSI is used. In ICSI, a single sperm is injected into the body of the egg (2). Genetic diseases are classified into chromosomal disorders, single-gene disorders, multifactorial disorder, and mitochondrial disorders (3). Several studies have been conducted to determine the association of these genetic defects with IVF. Despite the known benefits of IVF and ICSI techniques in infertility treatment, their rapid spread has raised scientific concerns about the potential association of these techniques with genetic

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defects. Therefore, we opted to summarize the literature and current evidence to answer whether IVF or ICSI is associated with an increased risk of chromosomal abnormalities in children born after IVF/ICSI treatment compared to spontaneously conceived children. Relevant published scientific articles were sought in Medline database, using combinations of the following key terms: "IVF", "*in vitro* fertilization", "ICSI", "intracytoplasmic sperm injection", "natural conception", "spontaneous conception", along with "chromosomal abnormalities", "chromosomal defects", "sex chromosome aneuploidy", and "trisomy". The eligible studies were considered studies exploring the association of IVF/ICSI with chromosomal abnormalities compared to spontaneous conception. The search included studies published from 1992 to 2018.

Chromosomal abnormalities and in vitro fertilization compared to spontaneous conception

Many types of chromosomal abnormalities exist and could be classified as either structural or numerical. Structural chromosomal abnormalities mean that part of the chromosome is either deleted, extra, or switched to another chromosome. Numerical abnormalities are when the whole chromosome is either missing or extra to the normal pair (4). The effects of chromosomal abnormalities depend on the specific abnormality. The most common chromosomal abnormality type is aneuploidy. It occurs with an abnormal chromosomal number where the chromosome could be extra or missing. A trisomy with three copies of a chromosome is more common than a monosomy, where there is only one copy of the chromosome. A common example of trisomy is Down syndrome, where an extra copy of chromosome 21 exists, and hence it is known as trisomy 21 (4). The earliest stages of human development are more susceptible to chromosomal errors during meiosis, fertilization, and early cleavage state (5). It is noted that the increased rate of chromosomal abnormalities may be due to an artifact of the procedure of the IVF or the embryonic physiological development (5). Earlier, a review of the outcomes of IVF/ICSI on children was published in volume 14 of *Human Reproduction* in 1999. It stated that the rate of chromosomal abnormalities, which seems to be ranging from 2% to 2.5%, with standard IVF, is similar to that of the general population. No significant difference was found (6). The newer volume of *Human Reproduction*, Volume 17 published 3 years later in 2002, included a study that showed similar results, and no significant increase in chromosomal defects was found in a sample of 4,224 children conceived after IVF compared to 314,605 naturally conceived children. This study included more than 85% of all Dutch-born between 1995 and 1996 (7). According to the European Registry of Congenital Anomalies, the prevalence of chromosomal abnormalities in the general population is about 0.15% from 1996 to 2009. A study was published in 2016 to compare the percentage of congenital and chromosomal defects to that of the general population published by European Registry of Congenital Anomalies and Twins. It included all pregnancies with IVF or ICSI techniques at Humanitas Fertility Center (Milan, Italy) from January 1996 to May 2009. The incidence of chromosomal abnormalities was about 0.2% from 2,351 babies born after IVF\ICSI (8). A systematic review was carried out with the end date parameter in June 2017, which included 16 cohort studies with 129,648 IVF/ICSI and 5,491,949 spontaneously conceived singleton births. Overall, the study showed an increased risk of chromosomal defects, about 23%, among the IVF/ICSI population [odds ratio = 1.23; 95% confidence interval (CI): 1.07-1.40] (9). Another systematic review published in 2018 studied the risk of multiple pregnancies with IVF/ICSI and chromosomal defects compared to normal pregnancy. It yielded 21 cohort studies to be analyzed. The risk was significantly higher among the IVF\ICSI multiple pregnancy group with a 36% increase (relative risk [RR] = 1.36; 95% CI: 1.04-1.77) (10) (Table 1).

Trisomy and IVF

In Turkey, a retrospective cohort study published in 2011 compared cytogenetic data of first-trimester abortions in ICSI and spontaneous pregnancies. The karyotype analysis revealed that 9.9% (7/71) of the ICSI group had trisomy 21 compared to 7.4% (6/81) in the control group. The rate of trisomy 13, trisomy 15, and trisomy 18 was 4.2%, 5.6%, 4.2%, respectively, in the ICSI group compared to 4.9%, 4.9%, and 1.2% in the control group. There was no difference in the rate of trisomy between the ICSI group (19/71, 26.8%) and the control group (23/81, 28.4%) (RR, 0.96; CI, 0.65-1.41). The authors concluded that the rate of aneuploidy after ICSI is similar to the spontaneous conception (15). In 2016, a study that was published in the United States, which included all live births in three states (Florida, Massachusetts, and Michigan) between 2000 and 2010, found that for women 35 years or older, there was a negative association (*p*-value, 0.001) between IVF/ICSI and Down syndrome (74/64,861 with a prevalence of 21.27 per 10,000 for ART births *vs.* 2,603/4,553,215 with a prevalence of 37.73 per 100,000; aRR, 0.63; CI, 0.49-0.80). The investigators suggested that the cause of the negative association is probably due to preimplantation genetic screening primarily for aneuploidy among older women undergoing IVF/ICSI. The prevalence of Down syndrome for women younger than 35 years was higher in the IVF/ICSI (11.64 per 10,000) compared to non-ART births (8.12 per 10,000) (aRR, 0.6; 95% CI, 0.98-1.96). However, the association was not significant (*p*-value, 0.51). The reason for the increased prevalence is unknown. Still, it could be caused by different attitudes toward the termination of pregnancy in women undergoing IVF/ICSI compared with women with natural conception. A potential explanation suggested by the authors for the negative association in older women and the non-significant positive association in younger women is that younger women conceiving with ART were less willing to undergo amniocentesis or chorionic villus sampling because of concerns about risks to the embryo; thus, fewer rates of prenatal diagnoses are made and less consequent terminations. Another possible explanation is that younger women undergoing IVF/ICSI have more severe health issues leading to poorer quality fetuses (8).

A Dutch study published in 2002, which collected data from the national perinatal database between 1995 and

Table 1. *Descriptive characteristics of selected studies on the association between IVF and ICSI with chromosomal abnormalities.*

Study	Comparison time	Chromosomal abnormalities	IVF/ICSI group	%	Spontaneous conception group	%
Anthony (7)	Postnatal	Trisomy 21	2/4,224	0.04%	362/314 605	0.1%
		Unspecified chromosomal defects	6/4,224	0.14%	222/314 605	0.07%
Levi Setti PE (8)	Postnatal	Unspecified chromosomal defects	7/2,351	0.3%	2/ 449	0.4%
Bingol (11)	Prenatal	Sex chromosomal defects	7/34	21%	9/41	22%
		Trisomy 21	7/71	9.9%	6/81	7.4%
		Trisomy 15	4/71	5.6%	4/81	4.9%
		Trisomy 18	3/71	4.2%	1/81	1.2%
		Trisomy 8	1/71	1.4%	0/81	0%
		Trisomy 10	1/71	1.4%	0/81	0%
Westergaard (12)	Postnatal	Trisomy 21	5/2,245	0.2%	3/2,245	0.1%
		Trisomy 18	3/2,245	0.1%	0/2,245	0%
Yang (13)	Postnatal	Trisomy 21	0/134	0%	1/286	0.3%
Ghahiri (16)	Postnatal	Trisomy 21	4/225	1.8%	7/225	3.1%
Van Steirteghem (17)	Prenatal	Sex chromosomal aneuploidies	-	0.6%	-	0.2%
		Structural Chromosomal abnormalities	-	0.4%	-	0.07%
Kim (18)	Prenatal	Sex chromosome aneuploidy	9/77 ICSI	11.69%	2/62	3.23%
			4/62 IVF	6.45%		
		Trisomy 22	24/99	24.24%	10/50	20%
		Trisomy 16	18/99	18.18%	10/50	20%
		Trisomy 15	10/99	10.1%	6/50	12%
		Trisomy 21	5/99	5.05%	-	-
		Trisomy 20	4/99	4.04%	2/50	4%
		Trisomy 13	3/99	3.03%	4/50	8%
		Trisomy 18	2/99	2.02%	-	-
Bonduelle (19)	Prenatal	Trisomies	5/1,082	0.46%	-	0.3% chromosomal aberrations at the time of prenatal diagnosis (11)
		Autosomal	9/1,082	0.83%		
		Structural	4/1,082	0.36% = 1.66%		
		Sex chromosomal defects	9/1,082	0.83%		0.19% (12), 0.2% (13) and 0.23% (14)
Mazzilli (22)	Prenatal	47, XXY karyotype	17/1,794	0.9%	General population	Prenatal-postnatal (0.1%-0.2%)
		45,x	40/1,744	2.3%	General population	Prenatal rate (0.2%-0.4%)

1996, found that the rate of Down syndrome in neonates conceived by IVF was 0.05% (2/4,224) and 0.1% (362/314,605) in naturally conceived children (7). They concluded that the small increase in the IVF group resulted from differences in maternal characteristics and not any aspect of the IVF procedure. A Danish study published in 1999, which reported data from the IVF registry from 1994 to 1995, found the rate of Down syndrome in the study group to be 0.2% (5/2,245) and 0.1% (3/2,245) in the control group. Also, Edward's syndrome (Trisomy 13) was increased in the IVF group 3/2245 compared to no cases reported in the control group (16). A retrospective study was conducted in Korea to compare the obstetric and perinatal outcomes of dichorionic twin pregnancies after IVF and spontaneous conception. One case of Down syndrome was found in 286 spontaneously conceived infants, and no case was found among 134 IVF infants (17). A nation-wide Swedish study was published in 2001 to identify congenital malformation in an infant born after IVF. The study included all IVF infants born between 1982 and 1997, a total of 9,175 IVF children, and a 1,690,577 population-based control group. Eighteen cases of Down syndrome were observed among 9,175 IVF/ICSI infants, while the expected cases were 20.2 (RR, 0.9; 95% CI, 0.5-1.4) (18). In 1992, a study reported the outcome of 82 amniocenteses of IVF embryos from 1985 to 1989. The main indication for undergoing amniocentesis was women's age (35 years and above). There were two cases (3.5%) of Down syndrome among 56 amniocenteses in patients older than 35, which is only slightly higher than the incidence in natural conception fetuses. Based on the small number of patients, the investigators did not believe that the results represent an increased Down syndrome incidence after IVF treatment (19). Moreover, a cohort study that was conducted in Iran and published in 2014 found the rate of Down syndrome after IVF treatment to be 1.8% (1/57) and 1.7% (1/58) after ICSI compared to 3.1% (7/225) in the control group. However, the authors could not propose a precise result due to the limitation of the study. For instance, the chromosomal analysis was only carried out for clinically suspicious infants (20) (Table 1).

Sex chromosome aneuploidy

In 2002, a study reported 2,139 fetal karyotypes from seven different studies. Based on the karyotype analyses, there was a slight but significant increase in *de-novo* sex chromosomal aneuploidy compared with the general population (0.6% instead of 0.2%). However, the investigators noted that the ART population might be different from the general population in terms of women's age and factors related to infertility (21). A retrospective Korean study, published in 2010, evaluated cytogenetic results after first-trimester abortion in the ART group compared with a control group. Sex chromosome aneuploidy was observed in nine (11.69%) patients in the ICSI compared to four (6.45%) in the IVF group and two (3.23%) in the control group. The authors suggested that the increasing number of sex chromosomal abnormalities in the ICSI group with male infertility is possibly due to the underlying parental risk of abnormalities and not due to the procedure itself (22). In 1999, a prospective study conducted in Belgium found nine (0.83%) cases of *de-novo* sex chromosomal aneuploidy in the prenatal karyotype analyses of 1,082 ICSI infants. The incidence of prenatal sex chromosomal aberrations is comparable with postnatal incidence. Thus, it was compared with the total newborn population and was four times higher, and the difference was statistically significant. The mean age of the mothers was 32.5 years, which is not explaining the higher observed rate. The investigators concluded that a higher rate is due to a higher frequency of chromosomal aberrations in spermatozoa from men with fertility problems rather than the ICSI procedure itself (23).

One of the common sex chromosome abnormalities is Turner's syndrome and Klinefelter's syndrome. Turner's syndrome is characterized by short stature, gonadal dysgenesis, and other physical characteristics, such as the webbed neck, cubitus valgus, and congenital heart disease, commonly coarctation of the aorta. The prevalence is estimated to be 1 in 2,000 females (24). Klinefelter syndrome is the most common sex chromosome disorder in males, occurring in 0.1%-0.2% of newborn males. It is characterized by tall stature, thin body habitus, small testes, gynecomastia azoospermia, and infertility (25).A cohort study conducted in Italy and published in 2018 found the prevalence of a 47, XXY karyotype among male blastocysts without autosomal aneuploidies to be 0.9% (17/1,794). When compared with the prevalence reported in the prenatal and postnatal periods (0.1%-0.2%), the authors suggested different possible scenarios: the latter prevalence is underestimated, 47, XXY blastocysts result in a lower implantation rate and higher miscarriage rate than euploid embryos, and the prevalence of 47, XXY blastocyst is higher in infertile patients of advanced maternal age undergoing IVF. The prevalence of 45, X karyotype among female blastocysts without autosomal aneuploidies was 2.3% (40/1,744). Compared with the reported prenatal diagnosis rate (0.2%-0.4%) and the conception rate 3%-4% product, the investigators supposed that 45, X blastocysts result in a higher miscarriage rate and lower implantation rate (26). Another retrospective cohort study, published in 2011 and conducted in Turkey, compared the cytogenetic data of first-trimester abortions in ICSI for non-male factor and spontaneous pregnancies. The karyotype analyses revealed that the rate of turner syndrome was similar in the ICSI and control group (7% (5/71) and 9.9% (8/81), respectively). (11).The risk of chromosomal abnormalities with IVF\ICSI conceived children remains controversial. As many studies proved a significant increase for chromosomal abnormalities and syndromes among the IVF population, other studies were contradicting and contributed the abnormalities to several environmental and technical factors (Table 1).

Most of the reported studies included could not separate the IVF/ICSI procedures and calculated the risk equivalently. The two procedures sound similar, but results might change when each is taken independently, as in some studies. However, whether the magnitude of change when each is accounted for alone is significant or not is not the focus of our review.

Conclusion

In conclusion, precise results could not be established. Studies on sex chromosome aneuploidy showed a slightly significant increase in the IVF\ICSI population. However, the studies also suggested that the results might be flawed, and the cause is not the procedure itself but other ill-factors. The review of Down syndrome studies was conflicting and fluctuated between positive association and no association. A possible explanation is while preimplantation genetic screening before ART reduces the risk of having a child with Down syndrome, maternal old age has a positive association with Down syndrome with or without ART. Our understanding of IVF's potential effects is underdeveloped, and further comprehensive research is needed to distinguish the risks related to parental factors from those exclusively resulting from IVF procedures.

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References

1. Practice Committee of the American Society for Reproductive Medicine. Electronic address aao. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertil Steril 2020;113(3):533–5. https://doi.org/10.1016/j.fertnstert.2019.11.025
2. Gottlieb S. Assisted reproduction increases the risk of congenital disabilities, study says. BMJ. 2002;346:725–30 [cited 2020 Jul 1].
3. Human Genomics in Global Health. Genes and human diseases. Geneva, Switzerland: World Health Organization. [cited 2020 July 20]. Available from: https://www.who.int/genomics/public/geneticdiseases/en/
4. Genetic Alliance; The New York-Mid-Atlantic Consortium for Genetic and Newborn Screening Services. Understanding genetics: a New York, mid-atlantic guide for patients and health professionals. Washington, DC: Genetic Alliance; 2009. pp7–8.
5. Maurer M, Ebner T, Puchner M, Mayer RB, Shebl O, Oppelt P, et al. Chromosomal aneuploidies and early embryonic developmental arrest. Int J Fertil Steril 2015;9(3):346–53.
6. Tarlatzis B, Grimbizis G. Pregnancy and child outcome after assisted reproduction techniques. Hum Reprod 1999;14(1):231–42. https://doi.org/10.1093/humrep/14.suppl_1.231
7. Anthony S, Buitendijk SE, Dorrepaal CA, Lindner K, Braat DDM, den Ouden AL. Congenital malformations in 4224 children conceived after IVF. Hum Reprod 2002;17(8):2089–95. https://doi.org/10.1093/humrep/17.8.2089
8. Levi Setti PE, Moioli M, Smeraldi A, Cesaratto E, Menduni F. Obstetric outcome and incidence of congenital anomalies in 2351 IVF/ICSI babies. J Assist Reprod Genet 2016;33(6):711–7. https://doi.org/10.1007/s10815-016-0714-4
9. Liang Y, Chen L, Yu H, Wang H, Li Q, Yu R. Which type of congenital malformations is significantly increased in singleton pregnancies following after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. Oncotarget 2017;9(3):4267–78. https://doi.org/10.18632/oncotarget.23689
10. Zheng Z, Chen L, Yang T, Yu H, Wang H, Qin J et al. Multiple pregnancies achieved with IVF/ICSI and risk of specific congenital malformations: a meta-analysis of cohort studies. Reprod Biomed Online 2018;36(4):472–82. https://doi.org/10.1016/j.rbmo.2018.01.009
11. Ferguson-Smith MA. Prenatal chromosome analysis and its impact on the birth incidence of chromosome disorders. Br Med Bull. 1983;39(4):355–64. https:doi.org/10.1093/oxfordjournals.bmb.a071848
12. Jacobs PA, Browne C, Gregson N, Joyce C, White H. Estimates of the frequency of chromosome abnormalities detectable in unselected newborns using moderate levels of banding. J Med Genet. 1992;29(2):103–8. https:doi.org/10.1136/jmg.29.2.103
13. Hook ED, Hamerton JL. The frequency of chromosome abnormalities detected in consecutive newborn studies, eds. Population cytogenetics studies in humans. New York: Academic Press Inc.; 1977. pp 63–79.
14. Nielsen J, Wohlert M. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Hum Genet. 1991;87(1):81–3. https:doi.org/10.1007/BF01213097
15. Bingol B, Abike F, Gedikbasi A, Tapisiz OL, Gunenc Z. Comparison of chromosomal abnormality rates in ICSI for non-male factor and spontaneous conception. J Assist Reprod Genet 2012;29(1):25–30. https://doi.org/10.1007/s10815-011-9646-1
16. Westergaard H, Johansen A, Erb K, Andersen AN. Danish national *in-vitro* fertilization registry 1994 and 1995: a controlled study of births, malformations and cytogenetic findings. Hum Reprod 1999;14(7):1896–902. https://doi.org/10.1093/humrep/14.7.1896
17. Yang H, Choi YS, Nam KH, Kwon JY, Park YW, Kim YH. Obstetric and perinatal outcomes of dichorionic twin pregnancies according to methods of conception: spontaneous versus *in-vitro* fertilization. Twin Res Hum Genet 2011;14(1):98–103. https://doi.org/10.1375/twin.14.1.98

18. Ericson A, Källén B. Congenital malformations in infants born after IVF: a population-based study. Hum Reprod 2001;16(3):504–9. https://doi.org/10.1093/humrep/16.3.504

19. Würfel W, Haas-Andela H, Krüsmann G, Rothenaicher M, Hirsch P, Kwapisz HK, et al. Prenatal diagnosis by amniocentesis in 82 pregnancies after in vitro fertilization. Eur J Obstet Gynecol Reprod Biol 1992;44(1):47–52. https://doi.org/10.1016/0028-2243(92)90312-M

20. Ghahiri A, Firozmand A, Ghasemi M, Nasiri F, Sharifi M, Abdollahi M. A comparative cohort study for detecting the incidence of trisomy 21 in ART and non-ART neonates. Iran J Reprod Med 2014;12(6):435–8.

21. Van Steirteghem A, Bonduelle M, Devroey P, Liebaers I. Follow-up of children born after ICSI. Hum Reprod Update 2002;8(2):111–6. https://doi.org/10.1093/humupd/8.2.111

22. Kim JW, Lee WS, Yoon TK, Seok HH, Cho JH, Kim YS, et al. Chromosomal abnormalities in spontaneous abortion after assisted reproductive treatment. BMC Med Genet 2010;11:153. https://doi.org/10.1186/1471-2350-11-153

23. Bonduelle M, Camus M, De Vos A, Staessen C, Tournaye H, Van Assche E, et al. Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. Hum Reprod 1999;14(1):243–64. https://doi.org/10.1093/humrep/14.suppl_1.243

24. Ranke M, Saenger P. Turner's syndrome. Lancet 2001;358(9278):309–14. https://doi.org/10.1016/S0140-6736(01)05487-3

25. Bird RJ, Hurren BJ. Review Anatomical and clinical aspects of Klinefelter's syndrome. Clin Anat 2016;29(5):606–19. https://doi.org/10.1002/ca.22695

26. Mazzilli R, Cimadomo D, Rienzi L, Capalbo A, Setti PEL, Livi C, et al. Prevalence of XXY karyotypes in human blastocysts: multicentre data from 7549 trophectoderm biopsies obtained during preimplantation genetic testing cycles in IVF. Hum Reprod 2018;33(7):1355–63. https://doi.org/10.1093/humrep/dey110