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Original Research Article

A COMPARATIVE STUDY BETWEEN INCIDENCE OF CHROMOSOMAL ABNORMALITIES IN SPONTANEOUSLY ABORTED FETAL SAMPLES OF ART AND NON-ART ORIGIN

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ABSTRACT

Objective: Spontaneous abortions are quite frequent among the reproductive age group of our country.With the advent of fast life,infertility has become a major issue and artificial reproductive techniques are often the resort taken. This study aims to throw some light on the strength of association of chromosomal abnormalities with artificial reproductive technique.

Design and participants: A cross sectional study was done on 50 aborted fetal samples of both first and second trimesters in a Government Medical College,Kolkata.

Settings: The samples were put to collagenase action,cultured and karyotyped to see the incidence of chromosomal abnormality.

Main Outcome Measures and results: It was seen that karyotype abnormality is present in 36.4% cases which applied ART in comparison to incidence of chromosomal abnormality being 43.6% in case of normal pregnancies.

Conclusion: So it is seen that Artificial reproductive technique did not seem to have strong association with the incidence of chromosomally abnormal fetus.

KEY WORDS: Artificial reproductive techniques, chromosomal abnormality, aborted fetal samples, karyotyping.

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INTRODUCTION

Assisted reproductive technology (ART) has been an important therapy method and a basic technique in many infertile couples to have children. Although the ART pregnancy rate of multiple Infertility treatment centres is stable at around 40%, the take home baby rate is still 20–30%, one of the important reasons being the high rate of early spontaneous abortion [1,2].

First-trimester miscarriage occurs in 10%–15% of all clinically recognized pregnancies [3] with embryonic chromosomal abnormalities being the most common cause of spontaneous miscarriage, which accounts for approximately 60% of these pregnancy losses [4]. However, the rate of early spontaneous abortion in patients after ART is ranging from 22%–63% [5,6]. The failure of ART treatment is associated with many factors

factors, out of which, genetic defects especially embryonic chromosomal abnormalities are one of the major causes of spontaneous miscarriage during the first trimester [7]. Cytogenetic analysis of Products Of Conception (POC) is essential to examine the cause of the spontaneous abortion. It hasbeen speculated that the different type of assisted reproductive technologies utilized, including In Vitro Fertilization-Embryo Transfer(IVF-ET) and Intracytoplasmic Sperm Injection(ICSI) and Frozen Embryo Transfer (F-ET) and Intrauterine Insemination (IUI) may determine the risk of cytogenetically abnormal products of conception [8,9].

A number of studies have been conducted to investigate the pregnancy outcomes after ART, and the relationship between risk of chromosomal abnormalities occurring in first trimester miscarriage and ART, but their results are somewhat controversial [10,11]. This study aims at building up an association between the ART used and the incidence of cytogenetic abnormality in the products of conception of both first and second trimester in pregnant women of West Bengal.

MATERIALS AND METHODS

The present study was conducted in Anatomy department of a Government Hospital in Kolkata, and the aborted fetal samples were obtained from the Obstetrics & Gynaecology Department of the same Hospital. The study included 50 aborted fetal samples, obtained over a period of one year. The chromosomal pattern of the aborted fetal samples of spontaneous abortion were analysed to determine the strength of association of chromosomal abnormality as a cause of spontaneous abortion,especially in cases of ART.

Samples from 50 cases of spontaneous abortion with less than 20 weeks of gestational age were collected either by sponta0neous evacuation or by uterine curettage. Gestational age was calculated from the first day of the last menstrual period.First trimester terminations of pregnancy and early abortus material are referred to as product of conception (POC)[13].It is usually possible to select fetal tissue or membranes from a first trimester or fresh abortus sample.In case of second trimester spontaneous abortion, the the whole fetus and placenta are often received. If so,the following biopsies are taken with sterile forceps and scissors:-[14,16]

i. Skin biopsy taken from thigh buttock or back of shoulder about 5 mm sq.
ii. Cord biopsy 2 cm long.
iii. Amnion biopsy taken from as near to the umbilical origin as possible to ensure that it is of fetal origin and at least 1 cu cm.

Using multiple tissues increases the chance of success, especially in case of IUD when the fetus is often macerated [17]. Enzymatic dissociation of the solid tissue biopsies, tissue sample is first washed with normal saline and then 90% alcohol to wash off the blood clots and to sterilize the specimen once[18].Then the tissue sample is minced well with a small sterile scissor. The tissue biopsy is then placed into a sterile centrifuge tube with 1 ml of prewarmed and reconstituted collagenase enzyme(125 U/ml). The tissue is mixed well and kept for 24 to 48 hours at 4 degree Celsius depending on tissue density. When the tissues are broken up by the stipulated time depending on the tissue type,the collagenase becomes cloudy and particulate. At that point 5 ml of Hams F10 media is added to stop the action of collagenase.Then it is centrifuged for 200g for 5 minutes. Thereafter the supernatant is poured of and the pellet is flicked up. The entire process involves the following steps [19,20,21].

WORKING DAY 1: For setting up the culture the following reagents are added to the pellet which is transferred to centrifuge tube.

1. RPMI-5ml
2. Fetal Bovine Serum -1 ml
3. Penicillin and Streptomycin combination (PENSTREP)-50 microlitres.
4. Phytoagglutinogen-75 microlitres.

All the reagents are mixed well and kept in the incubator at 37 degree Celsius and 5% CO2 for 72 to 96 hours for culture.

WORKING DAY 2: As the cells have divided adequately depicted by yellowish discoloration of the suspension,0.05ml of 4*10^ -5M colchicine solution is added and incubated for 1 hour.Then the tube is centrifuged at 3000rpm for 15 minutes. Then the cells are put to hypotonic treatment with 0.56% potassium chloride solution and

again incubated for 45 minutes to 1 hour.After thatacetoethanol is added in a ratio of 1:3 and centrifuged for 15 minutes at 1200 rpm. Such wash is given for another time till the suspension is crystal clear.The suspension thus obtained is kept at 4 degree centigrade overnight.

WORKING DAY 3: The suspension is now ready for dropping on alcohol treated chilled slides.Once the dropping is done,the slides are air dried and immersed in freshly prepared Giemsa stain for 10 minutes.After this,the slides are washed in running water and dried and seen under microscope for karyotype analysis.

RESULTS

Out of the 50 samples collected for study,Artificial Reproductive Techniques were applied to 11 (22%) samples. Out of the 26 aborted fetal samples of 1st trimester, ART was done in 4(15.4%); while among the 26 samples of 2nd trimester, 7(29.2%) had undergone ART. Thisgestational age variation of such samples are depicted in Table 1.

The variable that is considered in this study is the association of chromosomal abnormality found among the aborted fetal samples which had undergone assisted reproduction. It is seen that out of the 39 cases where ART was not done, karyotype abnormality was present in 17 (43.6%) of them, while the remaining 22 (56.4%) did not show any such abnormality. Among the 11 cases with ART, chromosomal abnormality occurred in 4 (36.4%)samples, and it was absent in the other 7 (63.6%) samples. This relation is depicted in Table 2.

Table 1: Gestational age and art status.

		ASSISTED REPRODUCTION		TOTAL
		NO	YES	
GESTATIONAL AGE OF ABORTUS	1ST TRIMESTER	22	4	26
	2ND TRIMESTER	17	7	24
TOTAL		39	11	50

Table 2: Art status and chromosomal abnormality.

		CHROMOSOMAL ABNORMALITY		TOTAL
		PRESENT	ABSENT	
ASSISTED REPRODUCTION	NO	17	22	39
	YES	4	7	11
TOTAL		21	29	50

Fig. 1: Showing the Trisomy 15.

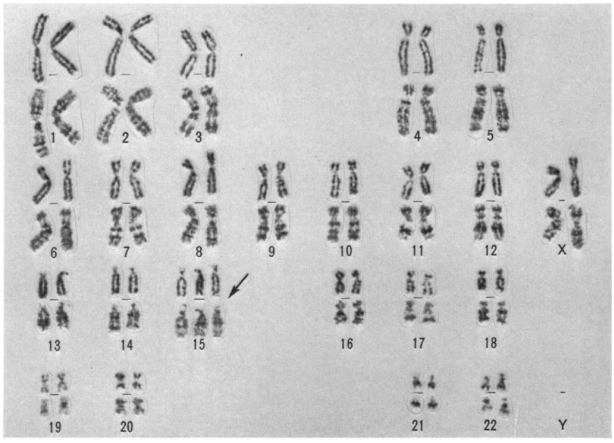
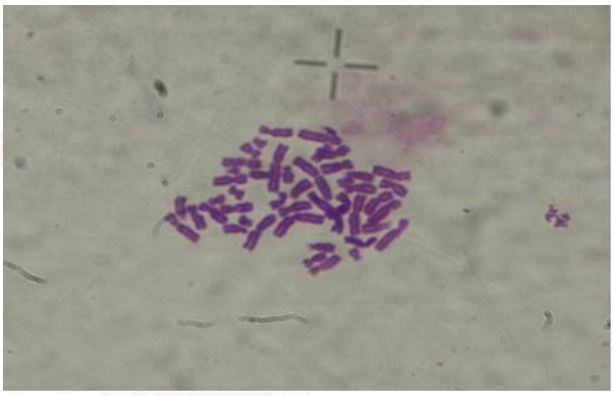


Fig. 2: Showing Mosaicism.



DISCUSSION AND CONCLUSION

The rate of successful tissue culture in the present study is 68% which is higher than the 53% rate reported by Creasy et al(1976) but lower than the 91.8% rate described by Hassold et al(1978)[22,23]. It is well known that the incidence of first-trimester abortion is much higher following IVF treatment than in natural pregnancy. There are many reasons for this spontaneous abortion, including chromosome abnormalities, maternal age, medical illness, intrauterine adhesion, infections, and immunological, endocrine, and psychological factors. Among these, chromosome abnormality is believed to be the most common etiological factors behind spontaneous abortion and may account for up to 50% of miscarriages. Compared with the natural pregnancy, we know little about the cytogenetic information in the aborted embryos achieved by IVF. It is important to know the mechanism for the spontaneous abortion after IVF. Based on the cytogenetic findings, the physician could select the further treatment scheme, and the patients might have their anxiety decreased and continue to accept the therapy with

confidence. Moreover, cytogenetic analysis of the spontaneously aborted tissue can effectively prohibit the mother from giving birth to a trisomic infant, which increases risk about 1% in subsequent trisomic conceptus [23].

Conventional cytogenetic analysis on aborted materials includes tissue sampling, cell culturing, metaphase spread preparation, and karyotype analysis.In the present study, there were 11 samples were spontaneous abortion occurred after ART.Out of these 11 sampleschromosomal abnormality occurred in 4 (36.4%), and it was absent in the other 7 (63.6%) samples. The incidence of chromosome abnormalities is similar to that in the natural aborted fetuses reported by Ohno et al. The most frequent abnormality was mosaicism. These results show that, just like in spontaneously aborted fetuses in natural pregnancy, chromosome abnormality is one of the causes of early spontaneous abortion following IVF and that mosaicism and Trisomy 21 are the most frequently detected abnormalities.

Conflicts of Interests: None

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