Short Communication

Epimutations and genetic aberration adversely affect ART outcome

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Summary

In the last decade the advent of ART has proven to be a boon for the infertile couple. In a large number of infertile couples there may be a genetic basis. Such couples who harbor genetic abnormalities need to be provided comprehensive counseling prior to opting for ART. Despite state of art technology and professional expertise, the carry home live-birth rate in ART is low. One of the factors responsible for low success rate of ART in couples opting for ART could be genetic. We analyzed 350 couples with recurrent spontaneous abortions (RSA) among whom 86 were couples with recurrent ART/ICSI failure. All couples with 3 or more ART failures were referred for cytogenetic, *Yq* microdeletion and mitochondrial mutation analysis. Among these 86 couples cytogenetic abnormalities were detected in 32 (20 cases in the male and 12 cases in the female). *Yq* microdeletions were detected from genomic DNA isolated from blood in 7 men whereas 9 men showed *Yq* microdeletions in DNA isolated from spermatozoa. High frequency of mitochondrial mutations and deletion of *4977* were also detected in sperm DNA (n=3) and blood of the female partner (n=4). These genetic abnormalities may be iatrogenically transmitted to the offspring. In cases with sex chromosomal and autosomal aberrations there is probability of poor embryo development and, consequently, implantation failure and early fetal loss. ART is a very expensive technique and recurrent ART/IVF failure would result in severe financial burden and physical stress, coupled with emotional stress. It is suggested that all couples opting for ART must undergo genetic analysis.

Key words*:* ART, chromosome, epigenetics, genetic counseling, ICSI, implantation failure, recurrent miscarriage

ART (Assisted reproductive technologies) has revolutionized the management of severe male and female factor infertility. The major advancement in ART is in vitro fertilization (IVF) and Intra-Cytoplasmic Sperm Injection (ICSI). ICSI is a boon for men with obstructive azoospermia, asthenozoospermia or teratozoospermia. It is also very useful in women in whom even after ovarian hyper-stimulation very few ova are retrieved or there is failure of 3 attempts of intrauterine insemination (IUI).

During the last 5 years, despite major technological advancements in the field of assisted reproduction, procreation techniques and professional expertise, the carry home live birth rate after several ART cycles is only 27 to 35%. The vast majority of embryos produced in vitro and transferred to the uterus fail to develop into an infant, supporting the concept that only a small fraction of embryos is destined to become a live-birth. One of the main reasons for such a low embryo to infant ratio is that a remarkably high number of embryos are found to harbor genetic abnormalities. In a recent study, Patrizio et al., (2007) reported that only 18% embryos conceived by ART were cytogenetically normal. Thus there is enormous biological wastage during assisted reproduction. This results in severe physical, emotional and financial stress to the couple. Recent studies by Dada et al. (2006) and Fluka and Fluka (2007) reported that the embryos, egg and sperm used for ART may be intrinsically abnormal.

In a 4 year study to understand the genetic etiology of reproductive failure, we analyzed 350 couples with recurrent spontaneous abortions (RSA) of which 86 were couples with recurrent ART/ICSI failure. All couples with 3 or more ART failures were referred for cytogenetic, Yq microdeletion and mitochondrial mutation analysis. These cases were referred from the department of Urology,

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Endocrinology, Obstretics and Gynaecology and ART Centre of Army Research and Referral hospital. In these 86 couples with recurrent ART/ICSI failure, cytogenetic abnormalities were detected in 32, among whom 20 were in the male (Figure 1) and 12 were in the female (Figure 2). Yq microdeletions were detected from genomic DNA isolated from blood in 7 men whereas 9 men showed Yq microdeletions in DNA isolated from sperms. High frequency of mitochondrial mutations and deletion of 4977 were also detected in sperm DNA (n=3) and blood of the female partner (n=4). Thus, these genetic abnormalities may be iatrogenically transmitted to the offspring. Recent studies have shown an increased prevalence of neural tube defects, hypospadias, imprinting defects, retinoblastoma and sex chromosomal abnormalities in babies conceived by ART. Therefore, since we are bypassing several natural steps of selection we are iatrogenetically transmitting genetic abnormalities from parents to offspring. Nature by making such couples infertile prevents the transmission of such genetic abnormalities.

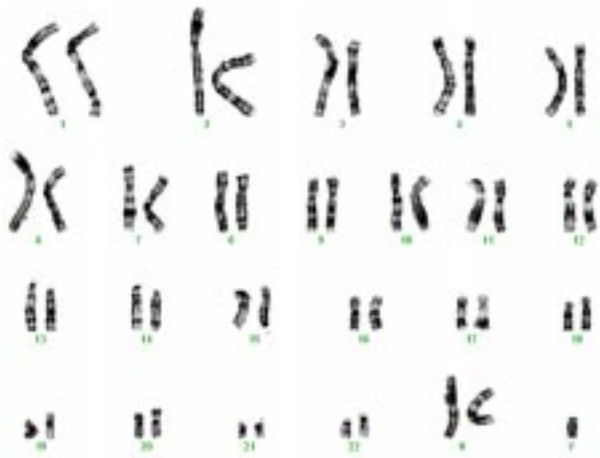

Figure 1. G-banded karyotype from an infertile male with 47, XXY chromosomal complement.

Genetic integrity is required for ovarian follicle growth, normal sperm and ovum development, differentiation, ovulation, fertilization and pre-implantation and post- implantation growth. Therefore, the very aim of ART to give birth to a healthy baby is defeated in such cases (Dada et al., 2006).

Morphologically normal spermatozoa may even harbor DNA damage. This damage of DNA does not affect fertilization or early embryonic development till 4 cell stages since paternal genome expression begins only after 4 cell stage but this has detrimental effect on implantation, embryogenesis and take home live birth rate. Kuo and Guo (2004) reported that chromosomal abnormalities predispose to embryonic lethality or result in birth of babies with chromosomal imbalances with growth and mental retardation.

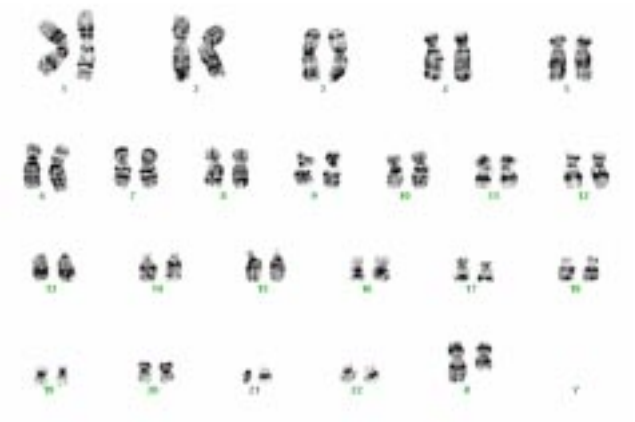

Figure 2. G-banded karyotype in an infertile female with 46, XX (Xqdel) karyotype

Epigenetics is the way the chromatin is packaged. It is the natural and heritable change in gene function that does not involve change in gene sequence. Such epigenetic programming is very active in pre-implantation embryos and the concerned genes may play a key role in embryonic growth and development (Edwards et al., 2006). During fertilization and early stages of embryogenesis certain portions of genome are transcriptionally active and certain areas are silenced by methylation or acetylation. However, an increased incidence of imprinting defect in children conceived by ART shows that there are disturbances in epigenetic control in babies conceived via ART. This loss in epigenetic control is known as epimutation. It is well known that infertile couples have a genetic predisposition to epigenetic instability. Also, ovarian hyperstimulation results in retrieval of epigenetically aberrant oocytes. Thus, though genetic abnormalities may lead to post-implantation failure and early fetal loss, altered epigenetic states are transmitted to the offspring and can be passed to successive generations. This is the trans-generational effect of epigenetically aberrant germ cells. The environment (ART culture media), ultracentrifugation and hormonal priming of the uterus in ART/IVF can result in altered gene expression in progeny by affecting the epigenetic status of the genome. The altered hormonal profile can adversely affect the cellular programmes that attach and detach methyl groups (CH_3) and, thus, alter the imprinting status of several genes. Till date over 500 such endocrine sensitive genes have been discovered. Thus, alterations in the

epigenetic process controlling fertilization, implantation, placentation, organogenesis, tissue growth and development have life-long implications on health. These altered epigenetic states can switch off master developmental genes and, thus, result in severe intrauterine growth retardation.

Thus, genetic alterations and epimutations adversely affect outcome of assisted reproduction procreating techniques. Hence, the results of our study strongly indicate that all infertile couples opting for ART must undergo genetic screening and should undergo pre-implantation genetic diagnosis.

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