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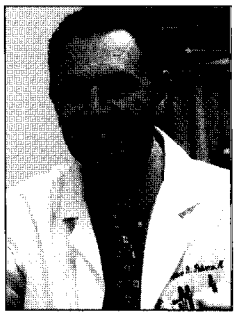

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Genetic and Epigenetic of ART

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INTRODUCTION

The etiology of compromised spermatogenesis is often genetic. Since male subfertility has been associated with a higher incidence of genomic defects, ranging from aneuploidy to Yq microdeletions, concerns have been raised as to the risk of transmitting genetic defects to the offspring. Thus, screening for such defects can be important for appropriate counseling prior to ART treatment. However, only a few reports of father/son cohorts have evaluated the heritability of mutations associated with male factor infertility as well as the well-being of the children. Due to the unnatural conception and requirement for *in vitro* steps, the ARTs and ICSI in particular have generated concerns in relation to the offspring's health. Although the early studies on neonatal outcomes failed to reveal any differences between ICSI and IVF babies, some recent reports claim a greater incidence of abnormalities in ART children compared to those conceived normally. Anxieties have been voiced also in regard to rare imprinting disorders, as well as cancer following ART.

Here we have assessed the genetic profiles of infertile men treated by ART, and the profiles of the pregnancies they generated, including obstetrical and perinatal outcomes and the developmental milestones in children born from ART. These children were also subjected to genetic and epigenetic analysis as well as to pediatric and psychological examinations.

MATERIALS AND METHODS

Oocyte retrieval was performed after down-regulation with a GnRH-agonist and superovulation with gonadotropins. For ICSI, a single immobilized spermatozoon was injected into the cytoplasm of a metaphase II oocyte. Morphologically good quality embryos were transferred 3-5 days after ICSI. Clinical pregnancy was indicated by a fetal heartbeat at ultrasonographic screening. Finally, pregnancy and obstetrical outcomes as well as congenital malformations were analyzed.

The development of ICSI offspring at 3 years of age was monitored through parent-administered questionnaires. In addition, a selected group of 5 year old children was monitored in-house by a developmental psychologist and a pediatrician.

Cytogenetic screening was carried out on peripheral lymphocytes obtained from ART couples and

their children. Yq deletion was assessed on lymphocytes, cheek cells, and spermatozoa by multiplex PCR while chromosomal analysis of spermatozoa was performed by FISH. CAG repeat length of the androgen receptor gene was measured by PCR and gene sequencing. Epigenetic analysis was carried out by methylation-specific PCR (chromosome 15q11-13) on peripheral blood while on fetal placenta, by RNA isolation and qRT-PCR for key developmental genes (IGF2, H19, KCNQ1OT1, and CDKN1C).

RESULTS

A total of 8,850 ICSI cycles were performed with ejaculated spermatozoa, 579 cycles with epididymal spermatozoa, and 527 cycles with testicular spermatozoa. A viable fetal heart was observed by ultrasound in 4,318 patients, of which 377 miscarried and 46 had an ectopic pregnancy. The ongoing pregnancy rate was 39.1% per retrieval and 42.0% per replacement procedure. Among the 5,248 neonates, the overall incidence of congenital neonatal abnormalities was 2.8%.

A follow-up of the ICSI and IVF children revealed no abnormalities above the threshold in cognitive, social, and physical development at 3 years of age. When a random sample of children were assessed in-house at 5 years of age, the average performance IQ for the ICSI group was 110 and for those conceived naturally was 108, with both groups scoring appropriately for their age in fine and gross motor skills. Cytogenetic analyses were performed on a total of 251 blood samples from mothers (n=100), fathers (n=68), daughters (n=47) and sons (n=36). All the adults and 96.6% of the children proved to have normal karyotypes.

In 87 men and 47 boys, 22 STS were analyzed by multiplex PCR and deletion breakpoints were tested with additional loci. Microdeletions in Yq AZF were detected in 3.4% of 87 adults and in 2.1% of their sons (n=47). FISH performed on spermatozoa revealed up to 2.8% incidence of germ-line aneuploidy.

Screening for Angelman/Prader-Willi syndromes carried out on 53 children (27 boys and 26 girls) did not reveal any methylation abnormalities.

Among the patients recruited for Beckwith-Wiedemann syndrome screening, 61 were conceived normally and 49 were treated by ART (13 IUI, 22 IVF, and 14 ICSI). The average maternal age was higher in the ART population (35.9 $\pm$ 5 vs 32.3 $\pm$ 4, respectively; $P < 0.01$), but the placental weight, gestational age, and neonatal weight were comparable. When the ART offspring were compared to the naturally conceived, overall there were only minor differences in expression of the key developmental genes.

CONCLUSIONS

Screening for any genetic component contributing to the etiology of male infertility is paramount during counseling of infertile couples and when evaluating possible transmission to their offspring. Although the incidence of sex chromosomal abnormalities was slightly increased in male children of these infertile men, Yq deletions were within the expected range for the azoo-/oligo-spermic population.

Evaluation of ART children at two key developmental checkpoints, IQ and motor development did not reveal any unusual abnormalities among them or those conceived naturally. Further, there was no indication among ART children of a higher occurrence of congenital defects, or a compromised psychomotor development. Although, no imprinting disorders were identified in the ART children analyzed, these epigenetic screening techniques may prove useful in monitoring ART procedures and performance. Nonetheless, the relatively early stage of the investigation, genetic screening and counseling of couples undergoing ART treatment is prudent. appropriate.