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RESULTS OF PRENATAL TESTS IN CASES AFTER ASSISTED REPRODUCTIVE TECHNOLOGIES

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ABSTRACT
In vitro fertilisation (IVF) and other assisted reproductive technologies (ART) are effective treatments for infertility and are widely provided in developed countries. However recent scientific publications suggest that there is an elevated risk of major structural malformations, imprinting defects and such syndromes as Prader-Willy, Angelman, Wiedeman-Beckwith. The aim of the study was to analyse if ART are associated with increased risk of genotoxicy for chromosomal nondisjunction in meiosis. Study included analysis of 50 families that prenatally were diagnosed chromosomal anomalies (Down, Turner, Klinefelter, Edwards, Patau syndrome). And control group included 70 families with healthy children. The results of present study showed, that in chromosomal anomalies group three cases of children with chromosomal anomalies were conceived after ART. In control group all healthy children were conceived naturally ($p<0.05$). The study also showed a statistically significant relationship between: older age of the woman (> 35 years old) and the occurrence of trisomy ($p<0.05$). The number of observed pathological cases is not so big to make exact conclusions, but results of present study supports hypothesis, that ART are associated with greater risk of chromosomal anomalies in conceived children.

INTRODUCTION

The first human pregnancy after the transfer of a cryopreserved embryo was depicted in 1983 by Trounson *et al*. and the first live birth was mentioned in 1984 [1]. There are several advantages in assisted reproductive technology (ART) programs, given by embryo cryopreservation, which is followed by throwing and trasfer into the uterus. While using it the risk of multiple gestations and risk of ovarian hyperstimulation syndrome can be avoided [2].

Because multiple gestation, with its typical risk for adverse outcome, remains a major problem in ART practice, the single-embryo transfer (SET) strategy has become more and more accepted [3]. One consequence of SET is an availability of excess embryos for freezing [4] as a result more children are born after cryopreservation and there is a decrease of multiple pregnancies [5]. Cryopreservation will also increase the chance of pregnancy in a natural cycle without additional ovarian stimulation and oocyte retrieval.

The successful pregnancy rate after transfer of frozen-thawed embryos depends on which freezing program was used, the stage of the embryo at freezing, the quality of the frozen embryo and the survival rate after thawing [6] as well as the number of frozen-thawed embryos transferred. Pregnancy and birth rates of one or more frozen-thawed embryos have been conveyd as 25–30% and 15–20%, accordingly [7].

Though cryopreservation and thawing touches cellular changes, there were no reports of adverse outcome in mammalian embryos [8]. Still, a detailed study of adult mice born after cryopreservation reported about morphological and behavior disorders [8] and because some of these findings were in adult animals only, there was made a conclusion that the effects of embryo freezing and thawing may be delayed. We hypothesize, that

abortion as stressed event not only for emotional state, but for cellular signaling, may affect process of meiosis increasing the rate of chromosomal mutations.

METHODS

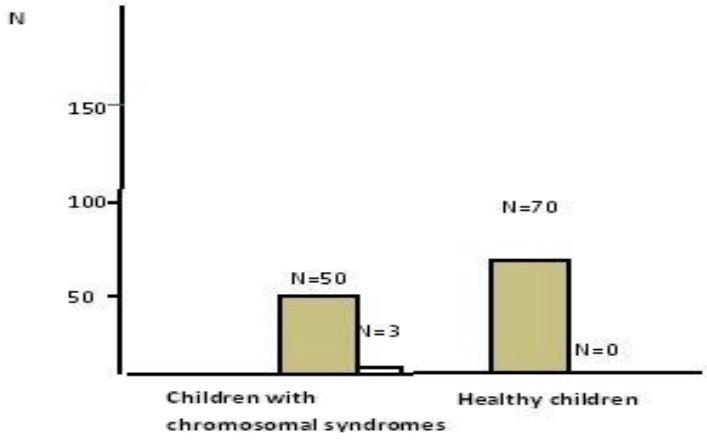
Study included analysis of 50 families that prenatally were diagnosed chromosomal anomalies (Down, Turner, Klinefelter, Edwards, Patau syndrome). And control group included 70 families with healthy children. Investigative material was amniotic fluid with fetal cells, that provide information about the fetal chromosomes. Amniotic fluids are taken from the womb during 16 – 22 weeks by using invasive prenatal diagnostics (amniocentesis). Amniocytes, that were collected in the laboratory, are divided into two parts: amniocytes for the molecular cytogenetic FISH test (5 – 8 ml), that should help to identify the concrete chromosome with its specific symptom and 5 – 10 ml of amniocytes for the cytogenetic test, that should help to identify the whole karyotype. The study design was approved by the Regional Ethics Committee and all studied subjects gave their informed consent. Statistical analysis was performed using the SPSS 14.0 program. Quantitative variables were expressed as means with standard deviation (SD). The differences for their statistical significance were analyzed with the Kruskal-Wallis test. A *P* value of less than 0.05 was considered significant.

RESULTS

The results of present study showed, that in chromosomal anomalies group three cases of children with chromosomal anomalies were conceived after ART (Fig 1.). Two cases were with Down syndrome (21 chromosome trisomy) and one with Edwards syndrome (18 chromosome trisomy).

In control group all healthy children were conceived naturally ($p<0.05$). The study also showed a statistically significant relationship between: older age of the woman (> 35 years old) and the occurrence of trisomy ($p<0.05$).

Figure1. Number of natural conceptions (filled bar) and after ART (not filled bar) in children with chromosomal anomalies and healthy subjects



DISCUSSION

There was a set up to analyze what was the difference in frequency of IVF conception method in two groups: prenatally detected chromosomal anomalies and healthy controls. We detected the outcome with chromosome anomalies of 3 children born after ART. Outcome measures were the neonatal outcome of children born after the use of IVF while comparing them with the outcome after natural conception. Using previously published data from other authors concentrating on neonatal outcome of 2889 ICSI and 2995 IVF children born after transfer of fresh embryos [9] and karyotype anomalies [10].

We were interested to evaluate the possible effect of IVF for congenital abnormalities. Only data concerning children's health and chromosomal malformations, although more parameters about pregnancy and the children were recorded. Spontaneous abortion rates in studied groups were similar and were also comparable with spontaneous abortion rates in the fresh groups. The cryo ICSI and the cryo IVF groups also had a compatibility in delivery rates, but were both significantly lower than in the fresh ICSI and fresh IVF group, also confirming the results published by Aytoz et al. [11].

A lower delivery rate in the frozen population means that were is a significant higher percentage of biochemical pregnancies and it tends to be higher spontaneous abortion rate in the frozen groups compared with the fresh groups. A less desirable pregnancy outcome after cryopreservation might reflect a less desirable selection of embryos for freezing when compared with fresh embryos. Also it might be a negative impact from the freezing procedure itself. It is still controversial why there is a higher risk of pregnancy loss after transfer of cryopreserved ICSI embryos compared with cryopreserved

IVF embryos [11-13] as is the difference in abortion rate between fresh and frozen embryos [14-16].

Chromosomal anomaly rate (de novo/inherited) was detected non-statistically significant increase prenatally as well as post-natally comparing cryo ICSI and cryo IVF. Overall (pre- and post-natal), cryo ICSI fetuses/infants had more chances to have a karyotype anomaly than fresh ICSI fetuses/infants. Compared with the fresh ICSI group, cryo ICSI fetuses/infants had almost double probability to have a *de novo* karyotype anomaly. The incidence of *de novo* chromosomal anomalies in cryo ICSI fetuses/children was also higher than in the general population [17]. Women after ART usually advanced maternal age at delivery, that elevates risk to have different fetal malformations, chromosomal aberrations. These women tend to stress more about their pregnancies, knowing the higher chances of malformations. So it is important to clarify patients if this condition can effect fetus development and health. Studies suggest that repeated echoscopy and second trimester biochemical markers should be performed to exclude 18 trisomy or other chromosomal abnormalities. If there are more abnormal findings in the ultrasound or CPC has not disappeared in the repeated echoscopy or there is an increased risk showed by PRISCA, then an invasive tests: fetal amniotic fluid cells FISH analysis or karyotyping, should be performed. Also, there is a non-invasive test, that are more accurate, which uses cell-free fetal DNA found in maternal circulation (Panorama by Natera) [18].

Compared with cryo IVF children and compared with fresh ICSI children were was an increase of major malformations was found in cryo ICSI children. Also, compared with the total fresh group (ICSI plus IVF), a significantly higher major malformation rate was found in the total cryo group (ICSI plus IVF). Though literature suggest that malformation rates after cryopreservation seems to be similar in those of fresh ICSI and fresh IVF [19, 20] and vary between 1% [21-25]. In the previous studies the number of infants born after cryopreservation is rather small, ranging from 105 to 270 and data on the combination of cryopreservation and ICSI are also not suficient [11, 16]. Most studies do not do a distinction between ICSI and conventional IVF. Factors that might influence the malformation rate, but did not mentioned in this study, are different cryopreservation protocols, difference in freezing day (Day 2, 3, 5 or 6) and difference in number and quality of frozen-thawed embryos transferred.

Since these factors change over time, as well as across fertility centres, it will be interesting so see what outcomes on infant health would be. In IVF group we also cannot ignore the impact of advanced ovarian stimulation and freezing-thawing protocols. From 1986 to 1991, IVF treatment was the sole ART procedure applied, mostly for patients with tubal or idiopathic infertility indications, whereas ICSI was introduced in 1991 and mainly performed for male factor infertility and less for non-male factor infertility. Since all patients were recruited from the same centre and definitions and study protocol were equally applied in all four studied cohorts While do not forgeting the weaknesses, mentioned earlier, we still consider our cohorts provide a good chance to study additional risk factors for the children from cryopreserved embryos.

CONCLUSION

We observed more fetuses conceived after IVF in chromosomal anomalies group than in healthy children group. This finding of increased frequency of chromosomal birth defects after the ART combination of cryopreservation and ICSI requires further attention and understanding.

Large follow-up studies are needed to ascertain about consequences for the children conceived with IVF embryos. Women may greatly benefit from receiving adequate information on psychological aspects of IVF, as well as for increased risk for chromosomal anomalies from the professional health care personnel.

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