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Oral Presentations

O-55
Evaluating cell free DNA in spent embryo culture media in cleavage and blastocyst stage

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Background: Chromosomal abnormalities are one of the most important causes of failure in in vitro fertilization. Preimplantation genetic testing can be a way to prevent the transfer of aneuploid embryos. It entails the use of invasive techniques to obtain embryonic DNA, with major technical limitations and ethical issues today. Therefore, the use of new non-invasive methods is a suitable solution to this problem. One of the non-invasive methods is to use the embryo spent culture medium. The origin of cell free DNA in embryo spent culture medium is trophectoderm cells and the internal cell mass.
Objective: Cell-free genomic DNA in the embryonic culture medium can be a non-invasive method for genetic assessment.
Materials and Methods: This study reviewed 25 spent embryo culture mediums. The spent culture medium used between day 3 and day 5 of embryonic development. Patients were undergoing intracytoplasmic sperm injection, and each embryo was in one drop of culture medium. We had two control samples: the culture medium contaminated with purified DNA from human blood and the culture medium without embryonic development. All samples were evaluated with nanodrop for dsDNA and ssDNA concentration. Among the collected medium, ten samples (group 1) concentrated by heating, then evaluating *SRY* and *FMR1* genes with real-timepolymerase chain reaction (RT-PCR) (group 1). Six samples were three days, and four samples were five days. The rest of the samples were classified into three groups. The cell-free DNA from the medium was purified with the blood DNA extraction kit. in group 2 with Genet bio kit, group 3 with YTzol pure DNA kit (yekta Tajhiz), and group 4 with High Pure Viral Nucleic Acid extraction kit (Roche). They evaluated by RT-PCR. Nine samples were three days, and six samples were five days.
Results: Although cell-free DNA was confirmed in the samples using nanodrop (with a range of 160 to 225 ng per microliter), the cycle of threshold did not observe in the RT-PCR product of group 1. The Purified samples were amplified in group 2,3 and 4 for *SRY* and *FMR1* genes with RT-PCR and observed only acceptable cycle of threshold in the fourth group.
Conclusion: The high protein and solutes in the culture medium and the low amount and quality of DNA are restrictive. For better results, it is necessary to purify the genomic DNA and amplify it with precise kits. Our research is underway to improve DNA collection, amplification, and testing to isolate genomic DNA.
Key words: *Cell free DNA, Spent embryo culture media, Preimplantation genetic testing.*