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Oral Presentations

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Non-invasive preimplantation genetic diagnosis (PGD) for X-linked disease by sex determination through cell-free DNA

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Background: Preimplantation genetic diagnosis (PGD) is a useful clinical tool to identify embryos with or at risk of specific genetic malady before embryo implantation. Current procedures for embryo chromosomal screening require an invasive biopsy of the embryo. Blastomere biopsy has a potential lesion to the embryos may result in developmental defects or abortion. Thus, a non-invasive PGD is needed.This study hypothesized that embryonic DNA is present in the spent culture medium. We focused on X-linked disorders, these single-gene diseases due to the presence of defective genes on the X chromosome are dominant in males.

Objective: Therefore, the objective of this study was to discriminate between female (XX) and male (XY) embryos by detecting Y chromosome-specific genes in cell-free DNA and comparing to PGD results. It opens a new window for the development of a non-invasive PGD method.

Materials and Methods: Embryo´s spent media from day 3 and day 5 embryos development were collected. The modified phenol-chloroform solution was used for DNA extraction from spent media. DNA from spent media was evaluated using SRY, TSPY, and AMELOGENIN as targets using the qPCR method. IBM SPSS and Medcals were used for statistical analyses, to compare sex determination of embryos using spent medium with PGD results.

Results: Yield and purity of the extracted DNA as well as repeatability of the method were performed well using the modified phenol-chloroform solution. The amount of DNA at day 5 embryo culture medium was significantly higher than day 3. Results of sex determination using spent medium by Q-PCR were consistent with the results of PGD and 12th wk sonography. This invasive PGD method using a spent culture medium gave a sensitivity of 66.7%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 67.6 (N = 56, Nxx = 23, Nxy = 33).

Conclusion: This investigation provides a potentially effective procedure that can help to avoid the invasive preimplantation genetic diagnosis, especially about X-linked diseases. Results of sex determination using spent medium by Q-PCR were consistent with the results of PGD. Improvements in DNA collection, amplification, and testing may allow for PGD without biopsy in the Future.

Key words: *PGD, X-Link diseases, cfDNA.*