Pushing the limits of detection: investigation of cell-free DNA for aneuploidy screening in embryos

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Pushing the limits of detection: investigation of cell-free DNA for aneuploidy screening in embryos

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- cfDNA: cell-free DNA
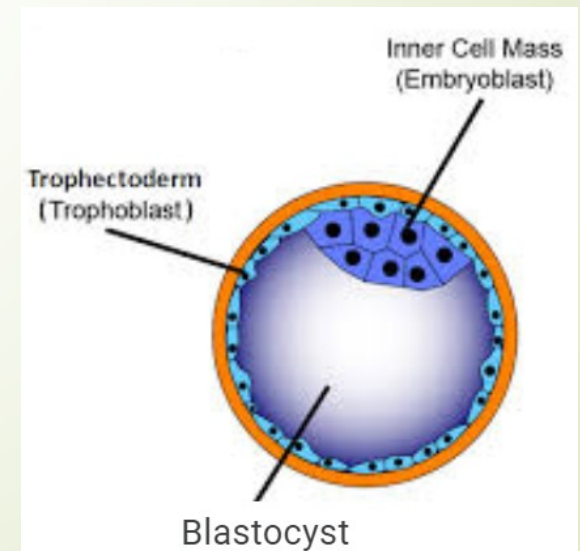
- SEM: Spent Embryo Medium

- PGT-A: preimplantation genetic testing for aneuploidy (aCGH, SNP, NGS)
    - aCGH: array Comparative Genomic Hybridization
    - SNP: Single Nucleotide Polymorphism
    - NGS: Next- Generation Sequencing

- TE: Trophoectoderm

Inner Cell Mass (Embryoblast)

Trophectoderm (Trophoblast)

Blastocyst

Introduction

- Selecting the best embryo is crucial for achieving a live birth with IVF
  - Blastocyst Transfer
  - PGT-A
  - Time –Lapse morphokinetics
  - Metabolomics of Spent Embryo Medium (SEM)

- Studies have investigated nucleic acid in SEM

- Next application for SEM is the use of cf DNA for aneuploidy screening

Aims of this study

1- To determine the accuracy of 'cfDNA in SEM' for ploidy and sex

detection at cleavage and blastocyst stages

2- To determine assisted hatching (AH) and morphologic
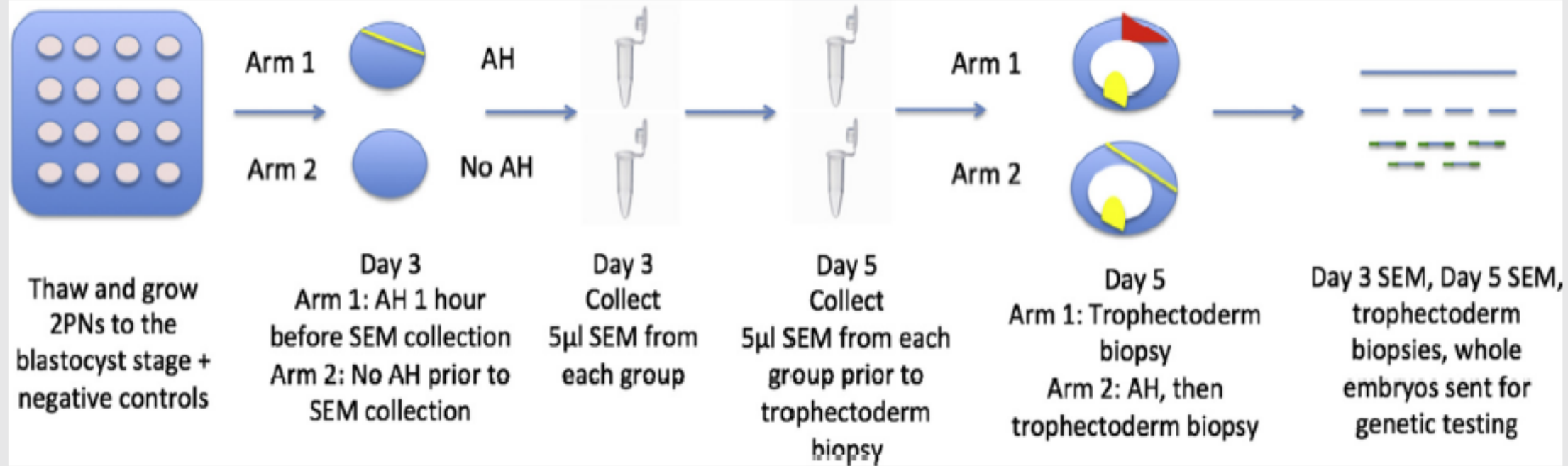
grading influence cfDNA concentration and accuracy

Material and Methods

- Prospective study comparing accuracy of aneuploidy screening with '**cfDNA** in SEM' compared with '**TE biopsy** and **whole embryo**' by NGS

- This study had two portions

  ➢ Pilot study

  ➢ Clinical study

Pilot Study: All on cryopreserved embryos donated for research

FIGURE 1

Experimental diagram for pilot study. 2PN = two pronuclei; AH = assisted hatching; SEM = spent embryo medium.

Ho. Cell-free DNA for aneuploidy screening.. Fertil Steril 2018.

Clinical Study: All on patients planning to undergo PGT-A as a part of their IVF cycle

- All blastocysts underwent AH on day 5 and TE biopsy

- SEM was collected after embryos were removed for AH and biopsy

Grading & analysis

➡ Morphologic grading

    Day 3: I or II or III for: cell number, fragmentation, symmetry

     shape

    Day 5: expansion stage, quality of inner cell mass, quality of

     TE

➡ Genetic analysis: whole genome amplification

Outcome measurements

Concordance rate were calculated for **aneuploidy and sex** between following groups:

➡ For pilot samples:

> Day 3 SEM # whole embryo

> Day 5 SEM # whole embryo

➡ For clinical samples:

> Day 5 SEM # TE biopsy

> TE biopsy # whole embryo

Concordance rates

- **TE biopsy** and **whole embryo:**

  ploidy **93%** and **sex 96.3%**

- **day 3 cfDNA** and **whole embryo:**

  ploidy **56%** and **sex 81.3 %**

- **day 5 cfDNA** and **whole embryo:**

  ploidy **45.5%** and **sex 78.8%**

Results
Sensitivity , specificity, PPV, NPV

Reference for day 3 cf DNA: whole embryo

Referenc for day 5 cfDNA: TE biopsy

Day 5 cfDNA for aneuploidy detection:

- **sensitivity 0.8**
- **specificity 0.6**
- **PPV of 0.47**
- **NPV 0.88**

Day 5 cfDNA had an overall better performance than day 3 cfDNA

Aneuploidy embryos had a higher number of reads on day 3

Conclusions

- AH was not associated with a difference in cfDNA concentration either on day 3 or on day 5

- Concordance rate for ploidy and sex were not significantly different between AH and no AH groups for day3 and day 5

- Morphology and fragmentation were not associated cfDNA concentration or with concordance rate

- Concordance rate for ploidy were not significantly different between good versus poor morphology embryos with use of day 5 cfDNA

DISCUSSION

- Age related aneuploidy cause decrease pregnancy rate and higher miscarriage rate

- To obviate this problem , PGT-A recommended to improve the selection euploid embryo

- TE biopsies from blastocysts were better , safer and more accurate than cleavage stage biopsies

- There is still debate regarding the efficacy of PGT-A and whether it improves the LBR

- cfDNA has emerged as a noninvasive strategy for aneuploidy screening

- SEM recently showing a high accuracy for ploidy screening with the use of NGS (86%)

- Specificity and NPV of cfDNA on day 5 , are still not high enough to reassure patient that euoploid embryo is being selected

- In a similar study (XU etal.) specificity and NPV are higher than this study due to methodological differences (day 0 or day 3 frozen embryos >> contamination with maternal cumulus cells)

IN CONCLUSION

cfDNA in SEM is not currently optimized for aneuploidy screening in embryo , but with further improvement it remains a promising tool for non invasive PGT-A