DNA in culture media

Arne Sunde, PhD
Professor, Head of Department of Obstetrics and Gynaecology
St. Olav's University Hospital in Trondheim
Norway

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Conflict of interest?

- Worked for:
  - MediCult (Origio)
    - 1999 full time
    - 2000-2003 Consultant
  - CellCura
    - 2005-2010 Consultant
- Fee for lectures from:
  - Astellas, Ferring, IBSA, Merck Serono, MSD

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DNA in culture media(?)

- I have taken the title quite literally
  - "DNA in culture media?"
- I do understand that your interest is primarily from a NIPT-perspective,
  - but I won't let you go that easily ☺

DNA i culture media?

- In the culture media itself?
- From gametes and gamete collection?
- From the embryo?

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The issue..

- Can DNA found in the embryo culture media be used to:
  - Rank embryos according to their implantation potential?
    - ”PGS”
  - Identify embryos with genetic defects?
    - ”PGD”

DNA in culture media itself?

- In the culture media itself?
- During production
  - Culture media are not in general produced under conditions that necessarily renders them ”DNA-free”

Production of embryo culture media

- Generally in GMP-facilities
  - Rigorous quality control
  - Focus on:
    - Sterility
    - Human pathogens (virus/prions)
    - Biochemical and biophysical parameters

- Presence of cell-free DNA is in general not an issue concerning ingoing material QC or production QC (?)
  - I will be surprised if commercial culture media may contain some cell-free DNA - including human DNA.

DNA in culture media itself ?

- In the culture media itself?
  - Ingredients:
    - Small and well defined molecules
    - Macromolecules
      - Some of them not well defined

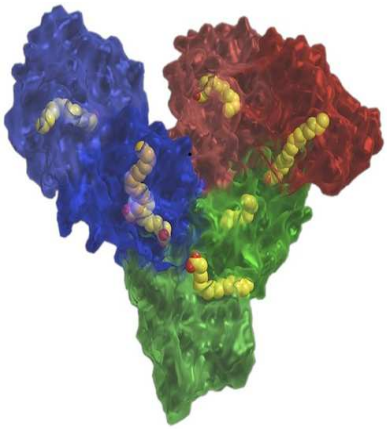
DNA in culture media?

- In the culture media itself?
  - Ingredients
  - Small molecules
    - Salts, sugars, amino acids etc.
    - In general sourced from non-animal origin
    - "free" of human pathogens (prions)
    - not necessarily guaranteed DNA-free
      - plant or microbiological origin
      - unlikely to be a major contributor to "DNA-contamination"

DNA in culture media?

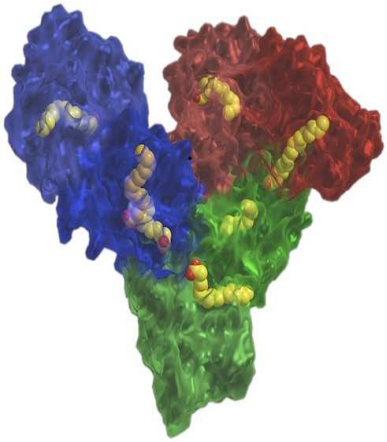
- Macromolecules
  - Growth factors, hormones etc..
  - Usually recombinant
    - In some cases traces of DNA can be found originating from the organism it is expressed in…
  - Serum albumin, serum globulins
    - Extracts from pooled human blood
      - May contain human DNA/RNA

Human serum albumin



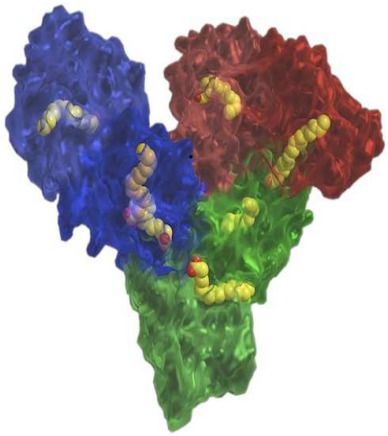
- Pleiotrophic molecule
  - May regulate
    - pH and osmolality
  - May donate
    - Lipids
    - Carboxylic acids
    - Steroids and Thyroxin
  - ***May adsorb/bind***
    - ***DNA!***
  - **Major contributor to batch-to-batch variation**

Rec-Human serum albumin



- Expressed in different cell systems
  - Native hSA is toxic to embryos
    - Needs to add molecules to render it OK
  - Contamination of DNA a known issue
    - from the cell-system it is expressed in

Human serum albumin



- Extracted from pooled serum
- Purified, but no pure
  - That's why it is beneficial
  - Tested for pathogens and biochemical, biophysical parameters
  - *I would be surprise if it is DNA/RNA-free*

Background noise?

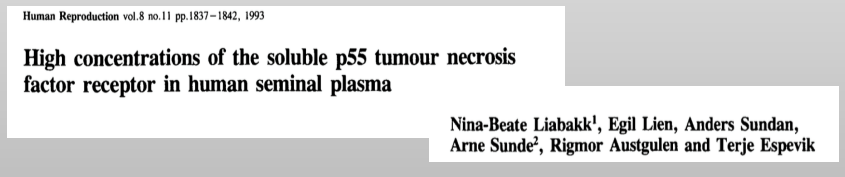
- Embryo culture media will likely contain DNA (and RNA) in small quantities
  - Even some human DNA/RNA
- Background noise (?):
- " we have problems with the controls…"

DNA in culture media

- DNA contamination during gamete retrieval
  - Personnel in the lab
  - Semen
    - Somatic cells, microorganisms
    - Seminal plasma contains cell-free DNA and miRNA
    - Sperm cells may adsorb “foreign” DNA
  - Follicular aspirates
    - Microorganisms in the follicle and in the vagina fluid
    - Somatic cells from the follicle and vagina
    - Cell-free DNA (miRNA) in follicular fluid

Seminal fluid

- Normal procedures for isolating sperm cells from the seminal fluid are not designed to remove *all* components from the seminal fluid (including DNA/RNA)
  - A large dilution, but not totally “clean”
  - An example, we were looking for embryonic interleukins..

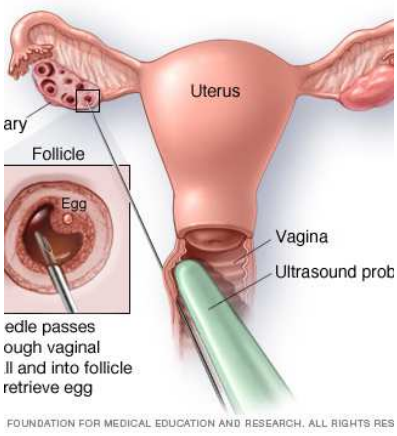


Human Reproduction vol.8 no.11 pp.1837–1842, 1993

High concentrations of the soluble p55 tumour necrosis factor receptor in human seminal plasma

Nina-Beate Liabakk[1], Egil Lien, Anders Sundan, Arne Sunde[2], Rigmor Austgulen and Terje Espevik

Follicar puncture



- Follicular fluid
- Vaginal fluid
  - Maternal DNA
    - Follicular cells
    - Vaginal cells
  - Microbiological DNA
    - Candida Sp
    - Bacteria
  - miRNA

Follicular aspirates

- Frequently contaminated with vaginal fluid
  - Microorganisms (bacteria and yeast)
  - And of cause somatic cells
- Normal ”washing” procedures of oocyte-cumulus complexes dos not remove everything

So the start is rather messy

- After fertilization:
  - DNA/RNA from
    - Microorganisms
    - Culture media
    - Gametes
      - Paternal and maternal DNA/RNA

So the start is rather messy

- Potential confounders?
- Cell-free DNA and miRNA in follicular fluid and seminal plasma seems to be correlated to gamete and embryo quality.
  - Some of the DNA/RNA will be carried over til the embryo culture

DNA from the embryo itself

- From genomic DNA
- From mitochondrial DNA

Cell-free DNA from the embryo

- Necrosis
  - DNA fragments of various lengths
- Apoptosis
  - DNA 180-200 bp fragments
- Fragmentation
  - Possibly whole chromosomes (?)
- Active secretion of specific genes/DNA regions (?)
  - Not documented

DNA/RNA in culture media

- For embryo selection- ”PGS”

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Human Reproduction, Vol.28, No.10 pp. 2652–2660, 2013
Advanced Access publication on July 25, 2013 doi:10.1093/humrep/det314

human reproduction

ORIGINAL ARTICLE *Embryology*

Mitochondrial DNA content in embryo culture medium is significantly associated with human embryo fragmentation

S. Stigliani[1], P. Anserini[1], P.L. Venturini[1,2], and P. Scaruffi[1,*]

This will be the topic of session 7 later today
-I will not spoil the show

BUT an example of how an analysis of DNA in the culture medium can be used to rank embryos (“PGS”)

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miRNA for embryo selection?

- miRNA plays a central role in control of gene expression
  - Conceivable that miRNA profile in embryos may reflect developmental potential
  - miRNA can be detected in embryo culture media
  - Source?
    - Extra-embryonic or embryonic?

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Human embryos secrete microRNAs into culture media—a potential biomarker for implantation

Evan M. Rosenbluth, M.D., Dawne N. Shelton, Ph.D., Lindsay M. Wells, M.D., Amy E. T. Sparks, Ph.D., and Bradley J. Van Voorhis, M.D.
Department of Obstetrics and Gynecology, University of Iowa Carver College of Medicine, Iowa City, Iowa

F&S 2014

"of the 10 miRNAs identified, only two (miR-372 and miR-191) was confirmed ….. to be solely in spent media
The rest was detected in unexposed media samples

..we assayed both protein-free media and media with added protein substitute and only detected RNA in the latter"*

*Serum Protein Substitute
(hSA and α- and β-globulins from Sage)

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DNA in culture media

- For PGD

OPEN

Medium-Based Noninvasive Preimplantation Genetic Diagnosis for Human α-Thalassemias^{-SEA}

Haitao Wu, MD, Chenhui Ding, MD, Xiaoting Shen, MD, PhD, Jing Wang, MD, PhD, Rong Li, MD, PhD, Bing Cai, MD, Yanwen Xu, MD, PhD, Yiping Zhong, MD, PhD, and Canquan Zhou, MD, PhD

Day 3 biopsy
biopsy lysed and analysed by PCR

Cultured to Day 5/6
Supernatant analysed by PCR

OPEN

Medium-Based Noninvasive Preimplantation Genetic Diagnosis for Human α-Thalassemias^{-SEA}

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Cell-free DNA quantification		
Time	Diagnosis Efficiency	Concentration (pg/μl)
Day 4	19.67%	14.24±4.76
Day 5	90.16%	48.78±20.45
Day 6	88.46%	54.35±22.78

Corresponding Diagnosis efficiency on biopsied cells: 100%

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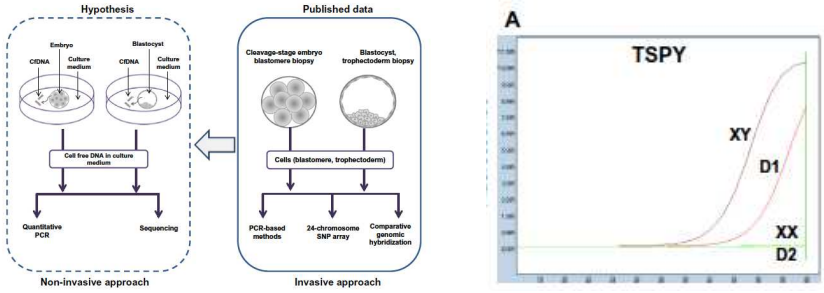
Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Non-invasive pre-implantation genetic diagnosis of X-linked disorders

Said Assou [a,b], Ounissa Aït-Ahmed [a,b], Safia El Messaoudi [c], Alain R. Thierry [c], Samir Hamamah [a,b,d,*]



Spent embryo culture media analysed by PCR

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DNA in the blastocele fluid

Reproductive BioMedicine Online (2013) 26, 603–610

ARTICLE

Genomic DNA in human blastocoele fluid

S Palini [a,*,1], L Galluzzi [b,1], S De Stefani [a], M Bianchi [b], D Wells [c], M Magnani [b], C Bulletti [a]

Table 3 DNA quantification, PCR results and microarray CGH results of blastocoele fluid samples subjected to WGA.

Sample ID	*DNA quantification (ng/μl)*	*PCR results*			*Microarray CGH results*
		MAP1LC3B	*TBC1D3*	*TSPY1*	
FOS2	117	+	+	+	47,XY,+22
NAT1	118	+	+	–	NA
NAT2	114	+	+	–	NA
VAN2	127	–	–	NA	NA
VAN5	113	+	+	+	46,XY,–1,–10,+11,+16
Negative control	127	–	–	NA	–

NA = not available.

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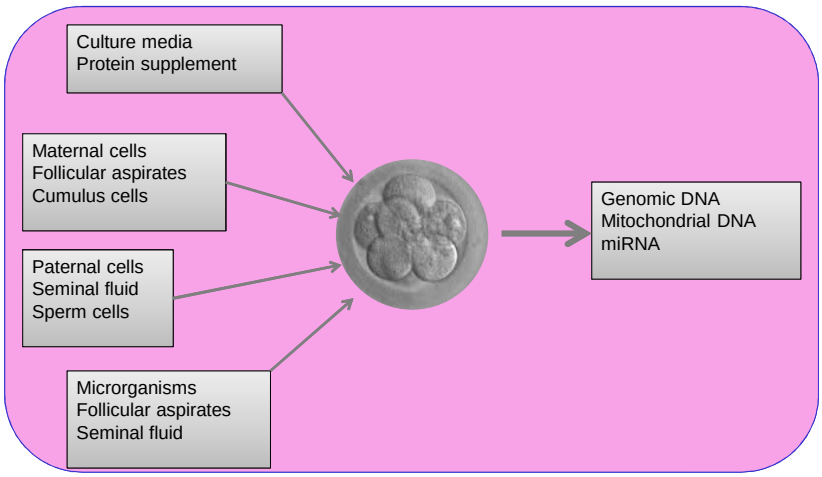
DNA in culture media



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Part of the issue II

- Highly sensitive detection methods used on:
  - DNA in the culture media
    - As a variable contaminant
      - Variable background?
  - DNA From the embryo
    - Secondary to necrosis/apoptosis
      - ”Random partial biopsy”?
  - DNA that are actively secreted(?)
    - The right reporter?
- You need to know what you are doing here…☺

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DNA in culture media - Summary

- For PGD
  - Proof of concept
  - Diagnostic specificity and sensitivity must be compared to traditional PGD using trophectoderm biopsy(or similar)
- For embryo selection ”PGS”
  - Proof of concept
  - Must be tested against alternative methods
    - Morphology
    - Morphokinetics
    - Genetic analysis
  - Independent variable?

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