Lab on a Chip in Medicine

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OVERVIEW

• Spermatozoa quality analysis
Decline of the male fertility
  - 32.2% decrease in sperm concentration\(^1\)
  - 20% young adults < lower WHO reference\(^2\)

\(\text{MOTIVATION}\)

\(^1\) M. Rolland (2012)
\(^2\) Male reproductive health - ESF/EMRC (2010)
\(^3\) ART in Europe - ESHRE (1997-2010)

(Assisted reproductive technology)
SEmen Analysis

- Sperm selection
  - Concentration
  - Motility
  - Morphology

Concentration

<table>
<thead>
<tr>
<th>WHO 1999</th>
<th>≥ 20 ( \cdot 10^6 ) cells/mL</th>
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<tr>
<td>WHO 2010</td>
<td>≥ 15 ( \cdot 10^6 ) cells/mL</td>
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Motility

| a: > 20 μm/s     | a+b ≥ 50% or a ≥ 25% |
| b: 5-20 μm/s     |                    |
| c: 0-5 μm/s      |                    |
| d: 0 μm/s        |                    |

Morphology

| ≥ 15% normal     | ≥ 4% normal |

SEMEN ANALYSIS
CURRENT METHODS

- Gold standard
  - Counting chamber
- Computer assisted semen analysis system
DISADVANTAGES

- No accurate results
- Time consuming
- Patient unfriendly

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OUR SOLUTION

- The fertility chip

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CHIP DESIGN  microfluidic impedance cytometry

- Dimensions
  - Electrode: width 20 μm, interelectrode distance 30 μm
  - Channel: depth 18 μm, width 38 μm
IMPEDANCE MEASUREMENTS

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DETECTION OF CELLS

1. semen in chip

2. spermatozoon along electrode pair

3. detection of spermatozoon
CONCENTRATION DETERMINATION

- Principle of internal calibration
- Analogy of flow cytometry

\[ B = 2.2 \times 10^6 \text{mL}^{-1} \]

\[ S = \frac{N_s}{N_b} \cdot B \]

\[ N_b: 69 \]

\[ N_s: 700 \]
EXPERIMENTAL SET-UP

- Method
  - Study 1: differentiation
  - Study 2: determination of concentration
- Peak heights

Segerink et al. (2010) Lab on a Chip
RESULTS (1)

- Study 1: Differentiation between cells and beads
  - HL-60: 1730 ± 620 Ω
  - Spermatozoa (boar): 27 ± 13 Ω
  - Polystyrene beads: 240 ± 60 Ω
RESULTS (2)

- Significant differences in peak heights
RESULTS (3)

- Study 2: *(boar)* Spermatozoa concentration
  - 7 samples
  - Bead concentration: $1.1 \cdot 10^6 - 2.7 \cdot 10^6$ mL$^{-1}$
  - Spermatozoa concentration: $2.1 \cdot 10^6 - 61.4 \cdot 10^6$ mL$^{-1}$

\[ y = 0.84x + 3.70 \cdot 10^6 \quad (R^2 = 0.97) \]

*subfertile region*
SEMEN ANALYSIS – recent results and developments

- Sperm selection
  - Concentration
- Motility
- Morphology

Motility:
- a: > 20 μm/s
- b: 5-20 μm/s
- c: 0-5 μm/s
- d: 0 μm/s

Morphology:

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NEW CHIP FOR ADDITIONAL ANALYSIS

Functional diagram of the new chip

Realized chip in Glass / PDMS technology

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de Wagenaar et al., Lab on a Chip 2015, 15 (5), 1294-1301
RESULTS – focussing of spermatozoa by DEP forces

10Mhz, 5V_{pp}, 1.4 S/m
RESULTS – focusing of spermatozoa by DEP forces

Unfocused - at the detectors

DEP focused - at the detectors

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RESULTS – effect of focusing on the position at detectors

Next: motility of single sperm in trap
Electrical analysis of single sperm motility

B. de Wagenaar, D.J. Geijs, H.L. de Boer, J.G. Bomer
W. Olthuis, A. van den Berg and L.I. Segerink
ICSII: Intracytoplasmic sperm injection

- ICSI: 1 sperm cell!

- Sperm selection by technician:
  - Motility and morphology
  - Manual procedure (subjective)

**Goal:**
Design of a microfluidic platform to analyze the motility of individual sperm cells

http://dogusivfcntre.com

http://www.whatclinic.com/fertility/spain/marbella/ivf-marbella

Liquid

Air

Micro-needle
Microfluidic platform

- Individual sperm cells are captured in microfluidic traps (1 µm high)

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Electrical analysis sperm motility

- Differential impedance measurement at 1 MHz - 0.5 V (1.4 S/m)


**Electrical analysis of single sperm motility**
Optical analysis sperm motility

- Optical tracking of sperm tail at 75 fps
- Sperm beat frequency
Electrical vs optical analysis

- Identical beat frequency
Temperature stimulus

- Cooling down 0 – 300 s, warming-up 300-600 s
Temperature stimulus

- Cooling down 0 – 300 s, warming-up 300-600 s
Chemical stimulus

- Exposure to 2 mM caffeine
- Increase in sperm beat frequency
- Decrease in signal amplitude
Conclusions

- Spermatozoa were trapped individually and their motility was analyzed electrically.

- The effects of temperature and chemical stimuli were investigated on the beat frequency of individual sperm cells.

- This platform is a potential tool for non-invasive sperm analysis towards selection applications.
Acknowledgments

L.I. Segerink
W. Olthuis
A. van den Berg

Spinoza grant
Prof. A. van den Berg

Veni grant
Dr. L.I. Segerink

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Electrical analysis of single sperm motility