RATE OF FOLLICULAR GROWTH FOLLOWING OVULATION TRIGGER IN IN VITRO FERTILISATION CYCLES

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Size of the Graafian follicle directly correlated with oocyte maturity in NATURAL CYCLES\(^1\)

Extrapolation to stimulated cycles\(^2\)

However

- Different intrafollicular milieu\(^3\)
- Asynchronous nuclear and cytoplasmic maturation\(^4\)
- Non-spherical follicles; need for measurement of follicles in longitudinal and transverse planes
Introduction

Spherical and non-spherical follicles

- **Natural Cycle**

- **Stimulated Cycle**
Introduction

Effect of Controlled Ovarian Hyperstimulation

- Correlation between follicular size and oocyte maturity controversial\textsuperscript{5,6}
- Multi-follicular growth:
  - Follicular asynchrony
  - Heterogeneous cohort of follicles
  - Variable growth rate
  - Secondary/ tertiary cohorts
- Leading follicle does not always yield the oocyte with the greatest potential\textsuperscript{6,7}
Nuclear and cytoplasmic maturation

Effect of gonadotrophin stimulation: increased follicular growth rate
Follicular growth rate

- Rate of follicular growth in natural cycles
  - “Conceptual pattern\textsuperscript{10}”
    - Rapid early growth
    - Stabilisation in 24h preceding ovulation
- Rate of follicular growth following stimulation
  - Replication of natural cycle findings\textsuperscript{11}
  - Rate of follicular growth immediately prior to hCG influences oocyte competence\textsuperscript{12}
Introduction

Role of hCG

- Resumption of meiosis in the oocyte
- Synthesis of Progesterone
- Luteinization of granulosa cells
Introduction

Follicular growth rate: Hypothesis

“The growth rate of the ovarian follicle following exogenous ovulation trigger correlates with oocyte competence”
Method

- **Participants:**
  - 20 women undergoing IVF / IVF-ICSI
  - Women with PCOS excluded

- **Protocol for IVF / IVF-ICSI:**
  - **Long protocol**
    - Pituitary down-regulation: GnRH agonist (Leuprolelin acetate SR 3.75 mg)
    - Controlled ovarian hyperstimulation: HMG (Menopur®) at individualised dose
    - Ovulation trigger: hCG (Pregnyl® 5000 IU)
    - UDOR 35-37h after hCG (09:00-11:00)
    - Standard IVF or ICSI techniques
    - ET on D2, 3 or 5
Method

Ultrasound follicular assessment

- ALOKA SSD 1700
- Individual operator
- Follicular monitoring
  - TV-USS from D8 of COH (daily when lead follicle >14mm)
  - Measurement of follicle:
    - 4x measurements in sagittal and transverse planes
    - Mean Maximum Diameter (MMD) calculated
Method
Ultrasound follicular assessment

Tranverse

Longitudinal

R Follicles
RT-1-ave 21.6 mm

1a 23.3 mm
1b 16.7 mm
1c 31.8 mm
1d 14.5 mm
Method

Ultrasound follicular assessment

- ALOKA SSD 1700
- Individual operator
- Follicular monitoring
  - TV-USS from D8 of COH (daily when lead follicle >14mm)
  - Measurement of follicle:
    - 4x measurements in sagittal and transverse planes
    - Mean Maximum Diameter (MMD) calculated
    - Recorded for all visible follicles
- hCG administration:
  - 1 or more follicles MMD ≥17 mm
MMD recorded for all visible follicles

Spatial arrangement was catalogued for largest four follicles on both ovaries
  - Detailed images in longitudinal and transverse planes recorded to be used as a reference and comparison on day of UDOR
Method

Follicular assessment at UDOR

- Particular attention paid to spatial arrangement of follicles, and positions correlated with records from USS on hCG day to identify specific follicles

- Immediately prior to aspiration of an individual follicle MMD calculated and recorded
Method

Spatial arrangement of follicles

hCG

UDOR

Leading

2nd

3rd

4th

New Leading

2nd (Previous leading)

3rd

4th
Method

Longitudinal tracking

- The oocyte / embryo retrieved from individual follicles cultured in single droplets enabling longitudinally tracking to its fate.
Method

Statistical Analysis

- Analysis of rate of individual follicular growth and Patient and cycle demographics
  - Linear mixed model (Stata/SE 11.1, Texas, USA)

- Influence of follicular growth on follicular outcome
  - Mixed-model multivariate logistic regression analysis

- Follicular growth rate:
  - Percentage increment of MMD calculated by absolute difference between MMD on hCG day and UDOR
Results

Patient and cycle demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.65</td>
<td>3.07</td>
<td>28</td>
<td>40</td>
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<tr>
<td>Baseline FSH (IU/L)</td>
<td>7.00</td>
<td>2.96</td>
<td>3.5</td>
<td>15.8</td>
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<tr>
<td>Starting dose of gonadotrophin (IU)</td>
<td>326.25</td>
<td>133.88</td>
<td>150</td>
<td>600</td>
</tr>
<tr>
<td>Total dose of gonadotrophin (IU)</td>
<td>3623.75</td>
<td>1468.03</td>
<td>1300</td>
<td>7275</td>
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<tr>
<td>Day of hCG</td>
<td>11.05</td>
<td>1.47</td>
<td>7</td>
<td>13</td>
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<tr>
<td>Total oocytes retrieved</td>
<td>8.5</td>
<td>4.23</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>
## Results

### Patient and cycle demographics

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Category</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF/ICSI</td>
<td>IVF</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>ICSI</td>
<td>8</td>
<td>40</td>
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<tr>
<td>Subfertility aetiology</td>
<td>MFI</td>
<td>7</td>
<td>35</td>
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<tr>
<td></td>
<td>Unexplained</td>
<td>7</td>
<td>13</td>
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<tr>
<td></td>
<td>Reduced ovarian reserve</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>PID</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Endometriosis</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Cycle outcome</td>
<td>No embryo transfer*</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Miscarriage</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Singleton implantation</td>
<td>3</td>
<td>25</td>
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<tr>
<td></td>
<td>Twin implantation</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Negative PT</td>
<td>12</td>
<td>60</td>
</tr>
</tbody>
</table>

*Failed fertilization (Aetiology >100% due to multiple aetiologies in some women)
## Results

### Follicle demographics

<table>
<thead>
<tr>
<th>Follicle Outcome</th>
<th>Category</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicles monitored</td>
<td></td>
<td></td>
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<tr>
<td>n=141</td>
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<tr>
<td>Oocyte retrieved</td>
<td></td>
<td>117</td>
<td>83</td>
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<tr>
<td>No oocyte retrieved</td>
<td></td>
<td>24</td>
<td>17</td>
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<tr>
<td>Oocyte maturity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=117</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal oocyte</td>
<td></td>
<td>5</td>
<td>4.27</td>
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<tr>
<td>Germinal vesicle</td>
<td></td>
<td>4</td>
<td>3.42</td>
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<tr>
<td>Metaphase I</td>
<td></td>
<td>13</td>
<td>11.1</td>
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<tr>
<td>Metaphase II</td>
<td></td>
<td>85</td>
<td>72.65</td>
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<tr>
<td>Unknown*</td>
<td></td>
<td>10</td>
<td>8.55</td>
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<tr>
<td>Fertilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=95)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normally fertilized</td>
<td></td>
<td>64</td>
<td>67.37</td>
</tr>
<tr>
<td>Abnormally fertilized**</td>
<td></td>
<td>12</td>
<td>12.63</td>
</tr>
<tr>
<td>Unfertilized</td>
<td></td>
<td>12</td>
<td>12.63</td>
</tr>
<tr>
<td>Damaged in ICSI</td>
<td></td>
<td>6</td>
<td>6.32</td>
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<tr>
<td>Cleavage</td>
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<td></td>
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<tr>
<td>(n=64)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Normally cleaved</td>
<td></td>
<td>54</td>
<td>84.37</td>
</tr>
<tr>
<td>Uncleaved</td>
<td></td>
<td>10</td>
<td>15.63</td>
</tr>
<tr>
<td>Fate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=53)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Embryo transfer</td>
<td></td>
<td>24</td>
<td>45.3</td>
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<tr>
<td>Discarded</td>
<td></td>
<td>26</td>
<td>49.1</td>
</tr>
<tr>
<td>Frozen</td>
<td></td>
<td>3</td>
<td>5.6</td>
</tr>
</tbody>
</table>

*Following IVF, failed fertilization and uncertain oocyte maturity. Included in oocytes inseminated and assessed for fertilization. **1PN or ≥3PN embryos.
## Results

**Variables impacting follicular growth rate**

<table>
<thead>
<tr>
<th>MMD (mm)</th>
<th>Follicles</th>
<th>Day of hCG</th>
<th>At UDOR</th>
<th>% Growth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (mm)</td>
<td>Standard Deviation</td>
<td>Minimum</td>
</tr>
<tr>
<td>Lead follicle*</td>
<td>18.69</td>
<td>1.50</td>
<td>16.8</td>
<td>21.6</td>
</tr>
<tr>
<td>Smallest follicle*</td>
<td>11.88</td>
<td>1.84</td>
<td>7</td>
<td>14.5</td>
</tr>
<tr>
<td>All follicles</td>
<td>15.50</td>
<td>2.58</td>
<td>7</td>
<td>21.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% Growth rate</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>P value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMD on day of hCG</td>
<td>-0.70</td>
<td>0.31</td>
<td>0.023*</td>
<td>-1.30</td>
</tr>
<tr>
<td>Follicle order**</td>
<td>0.155</td>
<td>0.35</td>
<td>0.663</td>
<td>-0.54</td>
</tr>
</tbody>
</table>

*Measured day hCG administered. Smallest follicle equates to smallest follicle in this analysis i.e. smallest follicle out of the four measured on each ovary. **Lead follicle, 2nd, 3rd etc to smallest follicle assessed.
Results

Percentage increase in MMD

Mean growth rate for lead follicles

Mean growth rate for smallest follicles

Follicle order
Results
Percentage increase in MMD for follicles on right and left ovaries
Results
MMD of largest follicles on hCG day and UDOR
Results: Summary

Growth rates for lead and smaller follicles

- Mean growth rate for smallest follicles 15.01%
- Mean growth rate for lead follicles 12.70%
  - When corrected for patient and cycle demographics, p=0.023
- Larger follicles on hCG day associated with slower growth rate compared with smaller follicles
- No differences between right and left ovarian follicular growth rate
- As a consequence of smaller follicles demonstrating higher growth rates, the lead follicles on hCG day do not necessarily equate to the largest follicle at UDOR
MMD on hCG day significantly predicted maturity of oocyte
- Higher the MMD, more likely to yield a mature oocyte (OR 1.5, p=0.002, CI: 1.16-2.00)
- The MMD on the day of the UDOR, the follicular growth rate and the ranking of the follicles were not, however, predictive
- Fertilisation, Cleavage, Transfer or cryopreservation of embryos was not influenced by follicle size or growth rate in this cohort
- No correlation between patient and cycle demographics and follicular growth rate
LH receptors predominantly in TCs of pre-antral and tertiary follicles
GCs develop increasing LH responsiveness with follicular size
Follicular growth critical step enabling successful follicular maturation
Consequently MMD at hCG frequently used to time UDOR
This study showed follicular growth rate following hCG not uniform, lead follicles grow at a slower rate. 

Lead follicle at UDOR may represent a smaller follicle at hCG that has overtaken lead follicle at hCG.

MMD at hCG positively predicts viable oocyte. NOT MMD at UDOR.

Supports findings in previous work:
- Smaller follicles 1.0-4.9 ml at UDOR yield highest percentage of viable oocytes.
- High concentrations of intrafollicular growth cytokines in follicles 1.0-4.9 ml at UDOR.
Theory:

- Disordered nuclear maturation following hCG administration in combination with rapid follicular antral expansion
- Less well established down-regulation of FSH receptors by luteinisation
- In natural cycles a complex mechanism exists to control timely ovulation involving multiple networks of cytokines
- Following COH a non-viable oocyte may disrupt this pathway resulting in asynchronous nuclear/cytoplasmic maturation
Conclusions

- Slower follicular growth beyond hCG enables optimum synchronisation between oocyte cytoplasmic and nuclear maturation

- The follicular growth rate *per se* may be a more important determinant of oocyte viability as opposed to follicular size itself
Alternatives to hCG: Is the rate of follicular growth with alternative ovulation triggers different?:

- GnRH agonists in antagonist cycles
- Kisspeptin
- Use of 3D scanning/automated scans
Thank you

- Vinay Sharma
- Nicolas Orsi
- Karen Thompson
- All the nurses and embryologists at the Assisted conception Unit, St. James’s University Hospital, Leeds
References