TIME INTERVAL BETWEEN OVULATION TRIGGERING AND OOCYTE INJECTION: DOES IT AFFECT THE EMBRYOLOGICAL AND CLINICAL OUTCOME?

I declare that no commercial or financial interest has influenced the content of this presentation
Timings in ART

1. Ovulation triggering
2. Oocyte retrieval
3. Denudation/Injection
4. Clinical outcome
INTRODUCTION

- In vivo oocyte maturation

Ovulation triggering

Oocyte retrieval

36-38h

Optimal maturation rates
(Bosdou et al., 2015; Garor et al., 2015)

- Nuclear maturation (MII)
- Cytoplasmic maturation

vs. Risk of spontaneous ovulation
(Nargund et al., 2001; Raziel et al., 2006)
INTRODUCTION

*Is in vitro oocyte incubation necessary to accomplish full competence?*

= *In vitro* oocyte maturation

---

**Oocyte Retrieval**

2

**Denudation/Injection**

3

---

**ESHRE Guidelines**

Timing of denudation should be kept close to timing of injection

*(ESHRE Guideline Group on Good Practice in IVF labs, 2015)*
INTRODUCTION

Is in vitro oocyte incubation necessary to accomplish full competence?

= In vitro oocyte maturation

Oocyte Retrieval

Denudation/Injection

1-3h

Yes! Fertilization
(Falcone et al., 2008; Pujol et al., 2018)

No! Pregnancy and Live Birth
(Garor et al., 2015; Barcena et al., 2016)
INTRODUCTION

How much time do we actually have?

1. Ovulation triggering: 36-38h
2. Oocyte Retrieval
3. Denudation/Injection: 1-3h

Total: 37-41h (Dozortsev et al., 2004; Barcena et al., 2016)
INTRODUCTION

Timings in UZ BRUSSELS

Ovulation triggering
1

36h

Oocyte Retrieval
2

2-3h

Denudation/Injection
3

38-39h

X 15 OPUs/day (max. 22)

In large ART centers with heavy workloads respecting these exact time intervals is frequently challenging
What is the *safe* time range between ovulation triggering and oocyte injection regarding embryological and clinical outcome?
STUDY DESIGN

Single-centre retrospective analysis
STUDY DESIGN

Single-centre retrospective analysis

Period: 2010-2015
STUDY DESIGN

Single-centre retrospective analysis

Period: 2010-2015

8811 ICSI cycles
STUDY DESIGN

Single-centre retrospective analysis

8811 ICSI cycles

Period: 2010-2015

Inclusion/exclusion criteria

- Fresh oocytes and ejaculated sperm
- Testicular/epididymal sperm
- Natural cycles
- PGT, IVM, IVF, IVF/ICSI
- Donation (sperm/oocyte)
STUDY DESIGN

Time intervals trigger - ICSI
STUDY DESIGN

Time intervals trigger - ICSI: <36h, 36h, 37h, 38h, 39h, 40h and ≥41h

Outcome measures:
- Oocyte maturation
- Fertilization
- Embryo Utilization rate
- Clinical pregnancy rate (CPR)
- Live birth rate (LBR)
STUDY DESIGN

Time intervals trigger - ICSI: 
<36h, 36h, 37h, 38h, 39h, 40h and ≥41h

Statistics: multivariate multilevel mixed modeling regression analysis

Outcome measures:
- Oocyte maturation
- Fertilization
- Embryo Utilization rate
- Clinical pregnancy rate (CPR)
- Live birth rate (LBR)
STUDY DESIGN

Time intervals trigger - ICSI: <36h, 36h, 37h, 38h, 39h, 40h and ≥41h

Statistics: multivariate multilevel mixed modeling regression analysis

Outcome measures:
- Oocyte maturation
- Fertilization
- Embryo Utilization rate
- Clinical pregnancy rate (CPR)
- Live birth rate (LBR)

Adjusting for confounders
- female age,
- number of oocytes,
- day of transfer,
- number of embryos transferred
- embryo quality
## RESULTS

### Demographic and Cycle Characteristics

<table>
<thead>
<tr>
<th></th>
<th>&lt;36h</th>
<th>36h</th>
<th>37h</th>
<th>38h</th>
<th>39h</th>
<th>40h</th>
<th>≥41h</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>72</td>
<td>826</td>
<td>1341</td>
<td>1344</td>
<td>1944</td>
<td>1797</td>
<td>1487</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>35.6</td>
<td>35.2</td>
<td>34.5</td>
<td>34.6</td>
<td>34.8</td>
<td>34.4</td>
<td>34.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>#COC</td>
<td>8.3</td>
<td>7.6</td>
<td>8.8</td>
<td>8.6</td>
<td>8.3</td>
<td>8.3</td>
<td>8.9</td>
<td>0.02*</td>
</tr>
<tr>
<td>Day of ET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>79.4%</td>
<td>76.4%</td>
<td>69.7%</td>
<td>73.5%</td>
<td>69.8%</td>
<td>66.5%</td>
<td>64.4%</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>5</td>
<td>20.6%</td>
<td>23.6%</td>
<td>30.3%</td>
<td>26.5%</td>
<td>30.2%</td>
<td>33.5%</td>
<td>35.6%</td>
<td></td>
</tr>
</tbody>
</table>

* One-way ANOVA **Pearson’s χ² test
## RESULTS

### Demographic and Cycle Characteristics

<table>
<thead>
<tr>
<th></th>
<th>&lt;36h</th>
<th>36h</th>
<th>37h</th>
<th>38h</th>
<th>39h</th>
<th>40h</th>
<th>≥41h</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>72</td>
<td>826</td>
<td>1341</td>
<td>1344</td>
<td>1944</td>
<td>1797</td>
<td>1487</td>
<td></td>
</tr>
<tr>
<td><strong>#Embryos transferred</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>41.7%</td>
<td>37.5%</td>
<td>38.6%</td>
<td>37.4%</td>
<td>39.8%</td>
<td>39.2%</td>
<td>40.7%</td>
<td>0.127**</td>
</tr>
<tr>
<td>2</td>
<td>34.7%</td>
<td>43.6%</td>
<td>41.9%</td>
<td>43.0%</td>
<td>43.5%</td>
<td>44.5%</td>
<td>44.5%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11.1%</td>
<td>11.7%</td>
<td>10.4%</td>
<td>11.7%</td>
<td>11.2%</td>
<td>9.3%</td>
<td>9.6%</td>
<td></td>
</tr>
<tr>
<td><strong>EQ for ET (best)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>76.2%</td>
<td>71.2%</td>
<td>70.8%</td>
<td>71.3%</td>
<td>69.1%</td>
<td>69.2%</td>
<td>69.6%</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>22.2%</td>
<td>23.5%</td>
<td>24.4%</td>
<td>24.7%</td>
<td>26.8%</td>
<td>25.1%</td>
<td>25.3%</td>
<td>0.469**</td>
</tr>
<tr>
<td>C</td>
<td>1.6%</td>
<td>5.2%</td>
<td>4.2%</td>
<td>3.7%</td>
<td>3.4%</td>
<td>5.0%</td>
<td>4.0%</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.0%</td>
<td>0.1%</td>
<td>0.6%</td>
<td>0.2%</td>
<td>0.6%</td>
<td>0.7%</td>
<td>1.1%</td>
<td></td>
</tr>
</tbody>
</table>

* One-way ANOVA  **Pearson’s χ² test
RESULTS

The interval of 36h and 36h occurred only if OPU was carried out before the planned 36h trigger interval and followed by immediate injection.
## RESULTS

### Outcome parameters (after adjusting for confounders)

<table>
<thead>
<tr>
<th></th>
<th>&lt;36h</th>
<th>36h</th>
<th>37h</th>
<th>38h</th>
<th>39h</th>
<th>40h</th>
<th>≥41h</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>72</td>
<td>826</td>
<td>1341</td>
<td>1344</td>
<td>1944</td>
<td>1797</td>
<td>1487</td>
<td></td>
</tr>
<tr>
<td><strong>Maturation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>76.4%</td>
<td>80.7%</td>
<td>79.9%</td>
<td>80.9%</td>
<td>81.5%</td>
<td>82.7%</td>
<td>83.2%</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>Fertilization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>69.2%</td>
<td>75.7%</td>
<td>74.7%</td>
<td>75.5%</td>
<td>77.1%</td>
<td>77.1%</td>
<td>79.3%</td>
<td>&lt;0.020*</td>
</tr>
<tr>
<td><strong>Utilization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>72.9%</td>
<td>77.0%</td>
<td>76.9%</td>
<td>79.0%</td>
<td>77.8%</td>
<td>75.0%</td>
<td>76.0%</td>
<td>&lt;0.020*</td>
</tr>
<tr>
<td>Day 5</td>
<td>62.1%</td>
<td>49.9%</td>
<td>51.1%</td>
<td>50.3%</td>
<td>52.6%</td>
<td>50.4%</td>
<td>48.8%</td>
<td>0.072*</td>
</tr>
</tbody>
</table>

*Multivariate multilevel logistic regression; predicted probabilities

Utilization rate: number of embryos utilized (transferred or cryopreserved) per number of 2PN zygotes
## RESULTS

**Outcome parameters (after adjusting for confounders)**

<table>
<thead>
<tr>
<th></th>
<th>&lt;36h</th>
<th>36h</th>
<th>37h</th>
<th>38h</th>
<th>39h</th>
<th>40h</th>
<th>≥41h</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>72</td>
<td>826</td>
<td>1341</td>
<td>1344</td>
<td>1944</td>
<td>1797</td>
<td>1487</td>
<td></td>
</tr>
<tr>
<td><strong>CPR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>20.9%</td>
<td>27.0%</td>
<td>26.2%</td>
<td>28.7%</td>
<td>27.4%</td>
<td>26.7%</td>
<td>26.5%</td>
<td>0.860*</td>
</tr>
<tr>
<td>Day 5</td>
<td>21.2%</td>
<td>53.1%</td>
<td>49.1%</td>
<td>43.6%</td>
<td>45.8%</td>
<td>44.2%</td>
<td>49.7%</td>
<td>0.128*</td>
</tr>
<tr>
<td><strong>LBR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>12.9%</td>
<td>18.8%</td>
<td>18.5%</td>
<td>20.6%</td>
<td>19.3%</td>
<td>17.9%</td>
<td>18.2%</td>
<td>0.681*</td>
</tr>
<tr>
<td>Day 5</td>
<td>21.3%</td>
<td>41.0%</td>
<td>38.1%</td>
<td>35.3%</td>
<td>36.2%</td>
<td>34.7%</td>
<td>39.9%</td>
<td>0.449*</td>
</tr>
</tbody>
</table>

*Multivariate multilevel logistic regression; predicted probabilities*
## RESULTS

### Outcome parameters (after adjusting for confounders)

<table>
<thead>
<tr>
<th></th>
<th>&lt;36h</th>
<th>36h</th>
<th>37h</th>
<th>38h</th>
<th>39h</th>
<th>40h</th>
<th>≥41h</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>72</td>
<td>826</td>
<td>1341</td>
<td>1344</td>
<td>1944</td>
<td>1797</td>
<td>1487</td>
<td></td>
</tr>
<tr>
<td>CPR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>20.9%</td>
<td>27.0%</td>
<td>26.2%</td>
<td>28.7%</td>
<td>27.4%</td>
<td>26.7%</td>
<td>26.5%</td>
<td>0.860*</td>
</tr>
<tr>
<td>Day 5</td>
<td>21.2%</td>
<td>53.1%</td>
<td>49.1%</td>
<td>43.6%</td>
<td>45.8%</td>
<td>44.2%</td>
<td>49.7%</td>
<td>0.128*</td>
</tr>
<tr>
<td>LBR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>12.9%</td>
<td>18.8%</td>
<td>18.5%</td>
<td>20.6%</td>
<td>19.3%</td>
<td>17.9%</td>
<td>18.2%</td>
<td>0.681*</td>
</tr>
<tr>
<td>Day 5</td>
<td>21.3%</td>
<td>41.0%</td>
<td>38.1%</td>
<td>35.3%</td>
<td>36.2%</td>
<td>34.7%</td>
<td>39.9%</td>
<td>0.449*</td>
</tr>
</tbody>
</table>

*Multivariate multilevel logistic regression; predicted probabilities
## Results

### Outcome parameters (after adjusting for confounders)

<table>
<thead>
<tr>
<th></th>
<th>&lt;36h</th>
<th>36h</th>
<th>37h</th>
<th>38h</th>
<th>39h</th>
<th>40h</th>
<th>≥41h</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>20.9%</td>
<td>27.0%</td>
<td>26.2%</td>
<td>28.7%</td>
<td>27.4%</td>
<td>26.7%</td>
<td>26.5%</td>
<td>0.860*</td>
</tr>
<tr>
<td>Day 5</td>
<td>21.2%</td>
<td>53.1%</td>
<td>49.1%</td>
<td>43.6%</td>
<td>45.8%</td>
<td>44.2%</td>
<td>49.7%</td>
<td>0.128*</td>
</tr>
<tr>
<td>LBR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>12.9%</td>
<td>18.8%</td>
<td>18.5%</td>
<td>20.6%</td>
<td>19.3%</td>
<td>17.9%</td>
<td>18.2%</td>
<td>0.681*</td>
</tr>
<tr>
<td>Day 5</td>
<td>21.3%</td>
<td>41.0%</td>
<td>38.1%</td>
<td>35.3%</td>
<td>36.2%</td>
<td>34.7%</td>
<td>39.9%</td>
<td>0.449*</td>
</tr>
</tbody>
</table>

*Multivariate multilevel logistic regression; predicted probabilities*
The <36h group scores 8-10% lower in predicted LBR while all other groups differ <1%. However, the wide confidence intervals show some uncertainty in the <36h group.
STRENGTHS & WEAKNESSES

- Largest dataset
- Adjustment for potential confounders
- Only study that assesses the effect of <36h hCG-OPU timing on live birth rate
- Retrospective study
- Results cannot be extrapolated to IVM, conventional IVF or injection with testicular/epididymal sperm
CONCLUSIONS

- The interval after ovulation triggering in which insemination/injection can be performed without a negative impact on embryological and clinical outcome is 36h – 41h

- Injection <36h after trigger is not recommend

- Allows for more flexibility in IVF laboratories with an intensive workload
THANK YOU FOR YOUR ATTENTION
Questions?

KEEP CALM AND ASK QUESTIONS