Intensified ovarian stimulation in a GnRH antagonist protocol with agonist triggering: A prospective, clinical feasibility study

G Griesinger et al.
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Introduction

- Aim of *ovarian stimulation* for IVF: induce *multifollicular growth* → retrieval of multiple oocytes for extracorporal fertilization
- ↑ *discomfort* & risk of adverse events
- Threat of *severe OHSS*, in young patients → limited the feasibility of maximizing the *oocyte yield / single treatment cycle*
GnRH antagonist ovarian stimulation protocol + **agonist triggering**

- Intensified ovarian stimulation
- Without ↑ the likelihood of severe OHSS

- GnRH agonist (instead of hCG) bolus → Triggering of final oocyte maturation
  - Pituitary remains responsive
  - ↔ ↓ risk of moderate-to-severe OHSS
Cryopreserved all fertilized oocytes by **vitrification** for a later transfer

- GnRH agonist triggering in OHSS risk patients as a safe & efficacious option
  
  *(Griesinger et al., 2007, 2010)*

- Circumvents the impaired luteal phase after agonist triggering
  
  *(Babayof et al., 2006; Humaidan et al., 2009; Nevo et al., 2003)*

- Eliminates the risk of late-onset OHSS
Highly efficacious cryopreservation technique

- Had potential to allow *temporally splitting* ovarian stimulation and embryo transfer

- Without a significant loss of treatment efficacy
This prospective clinical study ...

- Exploring the feasibility of such **ovarian stimulation approach**
  - Creating a maximally large number of **fertilized** oocytes from a **single** ovarian stimulation cycle
  - For later transfer in **repetitive** vitrified-warmed cycles
- No previous experience → non-randomized study → derive a first estimate of the **tolerability, safety and efficacy**
Questions:

(i) Is an intensified ovarian stimulation protocol with agonist triggering safe in terms of OHSS occurrence?

(ii) Is this approach acceptable to the patient in terms of discomfort?

(iii) What is the cumulative live birth rate from multiple vitrified–warmed embryo replacement cycles per single oocyte retrieval?
Materials and methods
Patient population – **Inclusion criteria**

(i) ≤ 36 y/o, indicated for IVF or ICSI

*intracytoplasmic sperm injection*

(ii) No expected or previous poor response

(≥ 3 oocytes at retrieval)

(iii) Both ovaries present

(iv) No endometriosis American Fertility Society grade III–IV

(v) neither uterine nor ovarian abnormality on transvaginal sonography

(vi) informed consent
Study protocol

- D2 or 3 of MC (spontaneous or induced)
- ↓ Progesterone, oestradiol, LH (to confirm reference range values)
- 225–375 IU r-FSH or human menopausal gonadotrophin (HMG) or combination → once daily s.c.
- (Chosen dose) Aim: inducing ≥20 follicles
- Expected normo-responders ≤36 y/o → 150 IU daily (non-intensified stimulation)
After 5–6 days FSH/HMG

- Start **GnRH antagonist** 0.25 mg daily s.c. → until the day of **triggering final oocyte maturation**
- D7 or 8 of stimulation → TVS, serum oestradiol, progesterone and LH → leading follicle 17–18 mm
oocyte retrieval

- 0.2 mg GnRH agonist (triptorelin) bolus (single s.c. injection)

- Approximately 36 h later ➔ IVF or ICSI
Endometrial transformation

- After agonist triggering
  - Medroxyprogesterone acetate (MPA)
    - 10mg 10–14 days oral daily
- Oestradiol < 4000 pg/ml during stimulation
  - Low-molecular weight heparin (dalteparin 5000 IU/day) self-administered daily sc
  - Continued until menstruation
Vitrification

- 20 h after IVF or ICSI
- Oocytes at the 2 pronuclear (2PN) stage → vitrified

(Kuwayama et al., 2005)
Cryopreserved embryo transfer

- After spontaneous or induced menses
  \textit{(Bals-Pratsch et al., 1999)}

- Preparation of the endometrium:
  - 14 days x Transdermal oestradiol patches \textit{(Estraderm TTS 100, Novartis Pharma)} or Oral oestradiol \textit{(Progynova, Bayer Vital)}
  - Since day 15 Add vaginal progesterone 
    \textit{(Crinone 8\%, Merck Serono or Utrogest, Kade/Besins)}
Embryo transfer

- Day 3 of progesterone administration
- Day 2 of preimplantation development
- 2PN oocytes → viable after thawing (maximally 3) → further culture → transferred to the uterus at the embryo stage (no selection of embryos according to morphology)
Supplementation of early pregnancy: IM progesterone + transdermal oestradiol → GA 8–10 weeks
Safety & tolerability assessment

- D3 or 4 after oocyte retrieval
- Signs & symptoms of OHSS (Golan et al., 1989) & treatment tolerability
- TVS → Ovarian volume & the presence of free abdominal fluid (ascites)
- WBC, CRP, Hct, oestradiol, LH, progesterone

→ In case of Abdominal distension/pain, nausea, vomiting, diarrhoea or headache during later luteal phase → advise to visit
Tolerability assessment questionnaire

Do you experience today or did you experience within the previous 5 days:

(i) Abdominal distension
(ii) Lower abdominal pain
(iii) Nausea/vomiting
(iv) Headache

Scale: 1 (perfect wellbeing) ~ 5 (maximum discomfort)
Outcome parameters

- Primary efficacy outcome: Cumulative live birth rate per patient undergoing oocyte retrieval
- Live birth rate per embryo transfer
- Time-to-conception (duration in weeks agonist administration → + PPT → live birth)
- Number of Biochemical pregnancies
- Number of Clinical pregnancies (+ fetal sac)
- Number of Clinical abortions (+fetal sac → no progression to live birth)
- **Fertilization rate**
  - Number of 2PN oocytes / number of MII oocytes injected or cumulus–oocyte–complexes inseminated per patient

- Proportion of 2PN oocytes cryopreserved per cumulus–oocyte–complex retrieved

- Proportion of 2PN oocytes cryopreserved per MII oocyte retrieved (ICSI cases)

- Survival rate after cryopreservation
  - Number of vital embryos/number of thawed 2PN oocytes
Sample size and statistics

- 30 patients
- Mean ± SD, median ± interquartile range or proportions with 95% CI
- Linear relationship between two variables → Pearson’s correlation coefficient (Pearson’s r)
- Number of oocytes retrieved ⇔ number of oocytes available for freezing ( & later transfer) → regression modelling
Results
Demographic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study population (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statistic</td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.0 ± 3.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.6 ± 12.0</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.9 ± 4.3</td>
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<tr>
<td>Duration of infertility (months)</td>
<td>39.5 ± 22.6</td>
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<tr>
<td>Cycle length (days)</td>
<td>33.7 ± 12.8</td>
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<tr>
<td>Cycle rank</td>
<td>2.0 ± 1.3</td>
</tr>
<tr>
<td>No. of previous pregnancies</td>
<td>3/30 (10)</td>
</tr>
<tr>
<td>No. of previous live births</td>
<td>2/30 (6.7)</td>
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</tbody>
</table>

- 19 x regular cycle, 11 x irregular cycle
- 13 x 1st IVF Tx attempt, 11 x 2nd, 3 x 3rd, 3 x more
<table>
<thead>
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<tbody>
<tr>
<td></td>
<td>Statistic</td>
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<tr>
<td></td>
<td>Range</td>
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<tr>
<td>Stimulation (days)</td>
<td>9.9 ± 2.0</td>
</tr>
<tr>
<td>Total FSH (IU)</td>
<td>2564.9 ± 722.4</td>
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<tr>
<td>FSH/day (IU)</td>
<td>273.3 ± 65.5</td>
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<tr>
<td></td>
<td>6–17</td>
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<tr>
<td></td>
<td>1350–3750</td>
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<tr>
<td></td>
<td>150–375</td>
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<tr>
<td>On day of final oocyte maturation</td>
<td></td>
</tr>
<tr>
<td>Oestradiol (pg/ml)</td>
<td>3619.9 ± 2123.0</td>
</tr>
<tr>
<td></td>
<td>478–9799</td>
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<tr>
<td>Progesterone (ng/ml)</td>
<td>1.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>0.6–4.6</td>
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<tr>
<td>No. of follicles &gt;10 mm</td>
<td>18.3 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>9–40</td>
</tr>
<tr>
<td>No. of COC</td>
<td>17.0 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>4–42</td>
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<tr>
<td>No. of MII oocytes (n = 29)</td>
<td>13.4 ± 6.6</td>
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<tr>
<td></td>
<td>3–26</td>
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<tr>
<td>Fertilization rate (%)</td>
<td>65.4 ± 20.5</td>
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<tr>
<td></td>
<td>19–100</td>
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<tr>
<td>No. of 2PN vitrified&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.4 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>3–22</td>
</tr>
<tr>
<td>2PN oocyte vitrified per COC (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.9 ± 15.2</td>
</tr>
<tr>
<td></td>
<td>15.8–100</td>
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<tr>
<td>2PN oocyte vitrified per MII oocyte (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.0 ± 20.0</td>
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<tr>
<td></td>
<td>18.8–100.0</td>
</tr>
<tr>
<td>Survival rate (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.3 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>50–100</td>
</tr>
<tr>
<td>No. of embryos transferred&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>2–3</td>
</tr>
<tr>
<td>Modified cumulative embryo score&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.3 ± 8.3</td>
</tr>
<tr>
<td></td>
<td>6–42</td>
</tr>
<tr>
<td>Luteal-phase haematocrit (%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>37.4 ± 3.8</td>
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<td></td>
<td>28–43</td>
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</tbody>
</table>
Stimulation characteristics

- 3x150 IU (hyperresponse) → 7, 18, 23 oocytes retrieved
- 1x fertilization failure → 29x at least one 2PN oocyte vitrified (8.4 2PN)
- Mean number of oocytes at retrieval: 17
- Number of oocytes at retrieval ↔ ovarian volume on days 3–4 after OR: significantly correlated (Pearson’s r 0.50, P = 0.02).
oocytes at retrieval $\Rightarrow$ linearly

oocytes available for cryopreservation

Quadratic equation in the bivariate regression model ($R = 0.598$;
Tolerability and safety

- No severe OHSS (0%, 95CI 0–11.4%)
- No patient required hospitalization
- Mean luteal phase CRP 5.0 mg/l, WBC 9098/μl, progesterone 12.9 ng/ml, oestradiol 620 pg/ml, LH 13.3 IU/l, Hct 37.4%
- Total mean ovarian volume: 158 ± 122 mm³
- Free abdominal fluid: in 32% patients (mean value of the largest diameter of free fluid pocket: 23 ± 7.2 mm)
On D3 or 4 after OR

- Abdominal pain: 1.6 ± 2.5
- Abdominal distension: 1.6 ± 2.1
- Nausea/vomiting: 1.0 ± 0.5
- Headache: 1.0 ± 0.5

From 26 returned questionnaires
Significant correlation
- Abdominal pain ⇔ distension (Pearson’s r 0.75, P < 0.01)
- Abdominal pain ⇔ nausea/vomiting: (Pearson’s r 0.51, P = 0.01)
No significant correlation
- Number of oocytes retrieved ⇔ abdominal pain, distension, nausea or headache
Live birth rate

- 3x not undergo vitrified–warmed ET (1x divorce, 2x spontaneous pregnancy)
- 26x at least one vitrified–warmed ET
- Mean number of transfers: 2.4 ± 1.7
- 0.5x 2PN oocyte → cryopreserved per cumulus–oocyte–complex
- Survival rate after thawing: 96%
<table>
<thead>
<tr>
<th>Cryopreserved cycle rank</th>
<th>Live birth rate per total cryopreserved cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (n/total)</td>
</tr>
<tr>
<td>1</td>
<td>7.7 (2/26)</td>
</tr>
<tr>
<td>2</td>
<td>13.6 (3/22)</td>
</tr>
<tr>
<td>3</td>
<td>9.1 (1/11)</td>
</tr>
<tr>
<td>4</td>
<td>0.0 (0/7)</td>
</tr>
<tr>
<td>5</td>
<td>0.0 (0/3)</td>
</tr>
<tr>
<td>6</td>
<td>50.0 (1/2)</td>
</tr>
<tr>
<td>7</td>
<td>9.7 (7/72)</td>
</tr>
</tbody>
</table>
72 ET

5 biochemical pregnancies (5/30, 16.7%, 95%CI 16.3–33.6),

2 x first-trimester abortions (2/30, 6.7%, 95% CI 1.8–21.3%)

7 x live birth (7/30, 23.3%, 95% CI 11.8–40.9%)
Cumulative live birth rate

- Strict intention-to-treat approach of analysis (with spontaneous pregnancies): 9/30 (30%, 95% CI 16.7–47.9%) = cumulative clinical pregnancy rate

- Undergoing at least on vitrified–warmed ET: 26.9% (7/26, 95% CI 13.7–46.1%)
Mean time-to-conception (→ live birth): 13 weeks

Dashed lines (n = 30)
solid lines = (n = 26)
B

Live birth rate [%]

Transfer cycles

0 1 2 3 4 5 6 7

0 5 10 15 20 25 30 35
End of the f/u period ➔ 14x patient still had mean 5.8 ± 4.1 further 2PN oocytes cryopreserved

Pregnancy outcome:
- 5 singleton live births, 2 twins (28.6% twin rate)
- **All** achieved live births ➔ 2 ET in the successful cycle
Discussion

- Intensified ovarian stimulation
  - Retrieved oocytes
    - Average 17 (Relatively young patients)
    - 9/30 patients \(\geq 20\) (maximum: 42)
  - Safe in terms of OHSS occurrence
  - Confirm the ability of GnRH agonist triggering \(\rightarrow\) Totally prevent severe OHSS, even in high-risk patients

(Engmann et al., 2006; Griesinger et al., 2007, 2010; Manzanares et al., 2010)
Maximizing the oocyte yield from a single oocyte retrieval ➔ Maximize the chance of the patient becoming pregnant from a single treatment cycle

↓ the need for subsequent IVF cycles with injections, oocyte retrieval procedures and the associated financial cost
Limitation – 1st

- An uncontrolled study for feasibility of intensifying ovarian stimulation
- No previous experience on intensified ovarian stimulation + agonist triggering + cryopreservation of all available oocytes
- Need control group
Limitation – 2\textsuperscript{nd}

- German embryo protection law:
  - All 2PN oocytes viable after warming
  - Transfer to the uterus \textit{(regardless of the morphological appearance)}

- Vitrified–warmed ET: $\bar{A}$ 2.5 per patient

- Conceived: majority within 2x ET, only one from 6x
Limitation – 2\textsuperscript{nd}

- **Maximizing the oocyte yield** → a worthless exercise for some patients (other patients could not be ‘pushed’ to conceive even by offering them a high number of vitrified–warmed ET)
Limitation – 2\textsuperscript{nd}

- 15/26 patients $\rightarrow$ only 2 transfers
- 11/26 patients $\rightarrow$ $\geq 3$ ET $\rightarrow$ only 2 additional live births

- A relatively large number of oocytes remained cryopreserved at the end of the follow-up period
- If \textbf{embryo selection} $\rightarrow$ same number of live births by a much smaller number of ET
Limitation – 3rd

- The number of oocytes retrieved → not predictive of pregnancy (Griesinger et al., 2010; Verberg et al., 2009)
- ↑ numbers of oocytes → 
  ↑ immature/degenerated oocytes and unfertilized oocytes after IVF or ICSI
- **Low number of oocytes at retrieval** → not a representation of advanced biological age
Limitation – 3rd

- Potential benefit of (Intensifying ovarian stimulation $\rightarrow$ ↑ the oocyte pool $\rightarrow$ available for fertilization & later transfer)
- Mean proportion of fertilized oocytes per cumulus–oocyte–complex: 0.5 ⇔ Standard GnRH antagonist protocol with daily 200 IU of FSH and hCG triggering of final oocyte maturation (Devroey et al., 2009)
Higher stimulation dose

- Lower ⇔ higher dose of FSH for ovarian stimulation, (100 IU ⇔ 200 IU) (Rombauts, 2007)
- Higher stimulation dose → higher number of oocytes → no differences in pregnancy rates (Hoomans and Mulder, 2002; Out et al., 1999, 2001)

- If higher number of surplus embryos?
- No cumulative pregnancy rates (including cryopreserved transfers)
‘Patient-friendly’ approach

- Cycle using cryopreserved embryos → less stressful for the patient (no need for injections, oocyte retrieval and the financial costs associated with a fresh treatment attempt)
- Each unsuccessful cryopreserved ET → significant psychological burden
- Cumulative live birth rate: 27%, in patient with minimum 1x ET
- Others need further treatment attempts:
'Patient-friendly’ approach

- **Intensifying** ↔ ‘conventional’ ↔ ‘mild’ ovarian stimulation
  - Tolerability, health risks, financial costs, efficacy in terms of cumulative live birth achievement, psychological distress
  - Further Well-designed RCT ideally undergoing a reduced-dose (‘mild’) ovarian stimulation protocol
Thank you for listening