Letrozole and gonadotropins versus luteal estradiol and gonadotropin-releasing hormone antagonist protocol in women with a prior low response to ovarian stimulation

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Introduction

• Low **ovarian response**:  
  ➔ Lack of uniform definition  
  ➔ Difficulty in comparing treatment outcomes

• Tests for **ovarian reserve**:  
  ➔ Actual ovarian response to stimulation  
  ➔ Reasonable capacity to predict poor response  
  ➔ Low ability to predict the occurrence of pregnancy
• Poor responders
   ➥ Generally resistant to a multitude of intervention strategies
   ➥ Sometimes difficult to identify before controlled ovarian hyperstimulation
• Poor response to COH
   ⇔ \( \downarrow \) E2 & follicle response to gonadotropins
   ⇔ \( \downarrow \) number of retrieved oocytes & available embryos for transfer
Ovarian Stimulation Protocols

• Several are proposed to improve IVF outcomes in patients with low ovarian response
• Some may enhance the ovarian response
• None have demonstrated a significant improvement in pregnancy rates
Antagonist protocols

• Luteal-phase estradiol (E2) and gonadotropin releasing hormone (GnRH) antagonists before gonadotropin stimulation

⇒ Synchronization of early antral follicle growth in the luteal phase before COH

⇒ Subsequent increase in oocyte

⇒ Improvement in pregnancy rates
• Estrogen
  ➔ Prevent luteal FSH ↑
  ➔ May also ↑ sensitivity to gonadotropins
• Luteal GnRH antagonist
  ➔ Induces luteolysis & prevents the FSH ↑
  ⇔ Lower basal FSH and inhibin levels
  ⇔ ↓ size and variability in the diameter of antral follicles
Aromatase inhibitors (AIs)

- Recognized by Mitwally et al.
- Few studies so far have used in low responders

- **Aromatase (in Fibroblasts, Osteoblasts, liver, breast):**
  - \( \text{Androstendione} \rightarrow \text{estrone} \)
  - \( \text{Testosterone} \rightarrow \text{Estrodiol} \)
Aromatase inhibitors (AIs)

- **Central mechanism:** E2 hypothalamus → Augment follicular growth → Release endogenous gonadotropins (↑FSH) ↔ r-FSH chemically different (carbohydrate moiety)

- **Peripheral mechanism:** Accumulation of intra-follicular androgen substrate → ↑FSH receptors expression ↔ intraovarian factors (Gn surge attenuating factor ↔ premature LH surge)

↑ Response to gonadotropins
Aromatase inhibitors

- Alternative ovulation-induction agents
- Alone or adjunct to gonadotropins
- Without apparent adverse effect on endometrium (as antiestrogen therapies / Clomifene citrate )

- AIs + r-FSH in IVF cycles $\rightarrow$ ↓ the total dose of gonadotropins $\rightarrow$ ↓ cost of IVF (also ↓ OHSS )
• Letrozole/antagonist protocol (LA) ↔
luteal E2/GnRH antagonist protocol (LPG)

✓ in women who had exhibited low ovarian response in prior IVF attempts
MATERIALS AND METHODS
Patients

- Retrospective cohort study
- University of Connecticut institutional review board
- January 2009 ~ October 2010
- 99 low-responder patients, < 42 year old
  - $\geq 2$ prior ovarian stimulation cycles at a starting dose of gonadotropins $\geq 300$ IU $\rightarrow < 5$ oocytes
  - One prior cycle cancellation due to low follicular recruitment (after 10 days of stimulation, $\leq 3$ follicles, $\geq 15$ mm in diameter)
• **Prior failed cycles:** GnRH agonist down-regulation, GnRH antagonist, and microdose leuprolide

• **No prior ovarian OP or exposure to C/T or R/T**

• **Flexible antagonist protocol**
  - Luteal E2 patch and GnRH antagonist (LP G)
  - Early follicular letrozole with no luteal pretreatment (LA)

• **Each patient → single cycle → no crossover**

• **Assignment of protocol as physician’s discretion**
(Average values. Durations and values may differ between different females or different cycles.)
Stimulation Protocols

LPG group (n = 52)

Previous cycle

• Day 10 after the LH surge → Initiated Transdermal E2 (Vivelle Dot 0.1 mg; Novartis, Miami, FL) every other day

• 11th day → began daily administration of ganirelix acetate (Ganirelix; Organon Pharmaceuticals, Roseland, NJ), 0.25 mg SC for 3 consecutive days

Ensuing menses

• Day 2 → Check FSH, LH, E2 levels, baseline echo

• Remove last E2 patch
• **Start ovarian stimulation (High-dose gonadotropins)**

  - **Average of 450 IU rFSH** *(Gonal F; Serono, Rockland, MA)*

  - **150 IU of hMG** *(human menopausal gonadotropin, Menopur; Ferring Pharmaceuticals, Tarrytown, NY)*

• **Lead follicle ≥ 13 mm or if E2 > 300 pg/mL**

  - **Restart Ganirelix** *(prevent a premature LH surge)*

  - **continued until the day of hCG administration**

• **≥ 3 lead follicles with ≥ 17-mm mean diameter**

  - **hCG 5,000–10,000 IU SC** *(human chorionic gonadotropin)*
**Stimulation Protocols**

**LA group (n = 47)**

**Spontaneous menstruation**

- **Day 2:** Initiate Letrozole *(Femara; Novartis, East Hanover, NJ)* 5 mg/day → continued for 5 days
- **Day 5:** commence 450 IU rFSH and 150 IU hMG
- **Start ganirelix as LPG protocol**
- **Criterion for hCG:** ≥ 2 follicles, ≥ 20-mm diameter
after hCG

- **35 hrs**  →  **TVOR** *(Transvaginal oocyte retrieval)*
  ⇒ **Oocyte insemination or ICSI** *(intracytoplasmic sperm injection)* as indicated

- **3rd Day**  →  **all embryos were transferred**

- **Luteal phase supplementation:**
  ⇒ **Progesterone 50 mg IM daily**
    - From the evening after oocyte retrieval
    - until negative pregnancy test or confirmed clinical pregnancy *(IUP with FHB)*
• **Primary outcome measure**
  - *Ongoing pregnancy rate (>20 weeks’ gestation) per started cycle*

• **Secondary outcome measures:**
  - *Cancellation rate*
  - *Number of oocytes retrieved and transferable embryos*
  - *Implantation and clinical pregnancy rates*
Statistical Analysis

- **Statistical Package for the Social Sciences** (release 17.0; SPSS Inc., Chicago, IL)

- **Student's t-test** — comparison of continuous variables

- **Chi-square or Fisher's exact test** — comparison of proportions

- **P < .05** — considered statistically significant

- **Data were expressed as mean standard deviation
RESULTS
• 41x in LA (47x) group (87.2%) & 43x in LPG (52x) group (82.7%) had ≥ one prior cycle cancellation due to poor follicular recruitment

• Patients with no prior cycle cancellations had ≥ two preceding IVF cycles with retrieval of < five oocytes and no pregnancies
36x (76.6%) in LA underwent a prior ovarian stimulation using the LPG protocol.

<table>
<thead>
<tr>
<th></th>
<th>LA (n = 47)</th>
<th>LPG (n = 52)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>39.1 ± 2.8</td>
<td>39.2 ± 0.2</td>
<td>.51</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 ± 7.0</td>
<td>26.7 ± 5.9</td>
<td>.90</td>
</tr>
<tr>
<td>Day-3 FSH (mIU/mL)</td>
<td>10.7 ± 5.0</td>
<td>9.5 ± 3.8</td>
<td>.27</td>
</tr>
<tr>
<td>Day-3 LH (mIU/mL)</td>
<td>4.9 ± 1.9</td>
<td>5.1 ± 2.2</td>
<td>.49</td>
</tr>
<tr>
<td>Day-3 E₂ (pg/mL)</td>
<td>46.3 ± 18.6</td>
<td>40.9 ± 25.2</td>
<td>.22</td>
</tr>
<tr>
<td>History of FSH ≥12 (%)</td>
<td>34 (16/47)</td>
<td>17.3 (9/52)</td>
<td>.06</td>
</tr>
<tr>
<td>No. of prior IVF cycles</td>
<td>4.6 ± 1.8</td>
<td>3.3 ± 1.8</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>No. of prior canceled cycles</td>
<td>1.8 ± 1.2</td>
<td>1.1 ± 0.8</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>LA (n = 47)</td>
<td>LPG (n = 52)</td>
<td>P value</td>
</tr>
<tr>
<td>--------------------------</td>
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</tr>
<tr>
<td>FSH at start of COH</td>
<td>9.2 ± 2.8</td>
<td>&gt; 4.07 ± 3.0</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Stimulation days</td>
<td>11.1 ± 2.6</td>
<td>11.3 ± 3.3</td>
<td>.88</td>
</tr>
<tr>
<td>Total gonadotropins (IU)</td>
<td>4,388 ± 1,703</td>
<td>&lt; 6,193 ± 1,018</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Peak E2 (pg/mL)</td>
<td>675 ± 458</td>
<td>&lt; 1,256 ± 799</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Cancellation rate (%)</td>
<td>55.3 (26/47)</td>
<td>36.5 (19/52)</td>
<td>.06</td>
</tr>
<tr>
<td>Canceled cycles due to poor ovarian response (%)</td>
<td>29.8 (14/47)</td>
<td>25 (13/52)</td>
<td>.6</td>
</tr>
<tr>
<td>Canceled ET after retrieval (%)</td>
<td>10.6 (5/47)</td>
<td>7.7 (4/52)</td>
<td>.6</td>
</tr>
<tr>
<td>Canceled cycles due to premature LH surge (%)</td>
<td>14.9 (7/47)</td>
<td>3.8 (2/52)</td>
<td>.06</td>
</tr>
</tbody>
</table>
### TABLE 3

Letrozole/antagonist (LA) versus luteal-phase estradiol/gonadotropin-releasing hormone antagonist (LPG) in poor responders: in vitro fertilization outcomes.

<table>
<thead>
<tr>
<th></th>
<th>LA</th>
<th>LPG</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of oocytes retrieved</strong></td>
<td>6.1 ± 3.0</td>
<td>7.9 ± 4.8</td>
<td>.08</td>
</tr>
<tr>
<td><strong>No. of mature oocytes</strong></td>
<td>3.8 ± 2.4</td>
<td>&lt; 6.6 ± 4.3</td>
<td>&lt; .01</td>
</tr>
<tr>
<td><strong>Maturation rate (%)</strong></td>
<td>64</td>
<td>&lt; 83</td>
<td>&lt; .01</td>
</tr>
<tr>
<td><strong>No. of 2PN oocytes</strong></td>
<td>3.0 ± 2.3</td>
<td>&lt; 5.3 ± 4.1</td>
<td>&lt; .01</td>
</tr>
<tr>
<td><strong>Fertilization rate</strong></td>
<td>69</td>
<td>71</td>
<td>.9</td>
</tr>
<tr>
<td><strong>No. of embryos transferred</strong></td>
<td>2.2 ± 1.0</td>
<td>2.4 ± 1.4</td>
<td>.85</td>
</tr>
<tr>
<td><strong>Implantation rate (%)</strong></td>
<td>16.7</td>
<td>16.3</td>
<td>.96</td>
</tr>
<tr>
<td><strong>Clinical pregnancy rate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Per started cycle (%)</td>
<td>25.5 (12/47)</td>
<td>26.9 (14/52)</td>
<td>.88</td>
</tr>
<tr>
<td>- Per ET (%)</td>
<td>50 (10/20)</td>
<td>42.4 (14/33)</td>
<td>.59</td>
</tr>
<tr>
<td><strong>Ongoing pregnancy rate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Per started cycle (%)</td>
<td>19.1 (9/47)</td>
<td>13.5 (7/52)</td>
<td>.44</td>
</tr>
<tr>
<td>- Per ET (%)</td>
<td>40 (8/20)</td>
<td>21.2 (7/33)</td>
<td>.14</td>
</tr>
<tr>
<td><strong>Pregnancy loss rate (%)</strong></td>
<td>25 (3/12)</td>
<td>50 (7/14)</td>
<td>.18</td>
</tr>
</tbody>
</table>
• In the LA group
• 2x pregnancy after intrauterine insemination
• 3x multifetal gestation (2x twins, 3x triplets)
• 38x not achieve an ongoing pregnancy
  • 31x referred to donor oocyte program or opted for no further treatment
  • 7x decided to attempt an additional IVF cycle
    → 6/7 were canceled
DISCUSSION
• Poor response to COH $\Rightarrow$ ↓ follicular response in quantity $\Rightarrow$ retrieved lower number of oocytes

$\Rightarrow$ A major concern in assisted reproduction

$\Rightarrow$ The best treatment option remains controversial

• Luteal synchronization of follicular growth $\Rightarrow$ ↑ yield oocyte ... *deZiegler et al.*

• Use of luteal E2/antagonist $\Rightarrow$ ↓ cancellation rate, ↑ number of retrieved oocytes and embryos transferred ... *Dragisic et al.*
Androgen

- Stimulates theca/granulosa cell proliferation & inhibits apoptosis $\Rightarrow$ \( \uparrow \) preantral & small antral follicles
- Accumulation of follicular androgens $\Rightarrow$ \( \uparrow \) FSH-R gene expression or stimulate IGF-I (insulin-like growth factor 1) system $\Rightarrow$ may act in synergy with FSH $\Rightarrow$ promote follicular steroidogenesis $\Rightarrow$ \( \uparrow \) follicular sensitivity
- Some reports: before FSH treatment $\Rightarrow$ Transdermal testosterone $\Rightarrow$ \( \uparrow \) ovarian response
Letrozole

• *3rd generation highly selective non-steroidal AI*
use in postmenopausal women with breast cancer

• **Competitively binding to the heme of the**
cytochrome P450 subunit of *aromatase*

⇒ *Androstenedione* ⇒ *E2*

⇒ ↑ intraovarian androgens

⇒ **Profound effect on early follicle growth**

⇒ **May up-regulate androgen-receptor gene expression** in preantral and antral follicles
serum [E2]

May limit (cumulative [E2] → negative effect

→ oocyte quality & endometrial receptivity)

Maintaining an adequate follicular development and estrogen biosynthesis
Study for Letrozole co-treatment

- Improved response to FSH stimulation
- ↑ number of oocytes retrieved and implantation rate
- ↓ mean total dose of gonadotropins
- ↓ cancellation rate
- ↓ cost of achieving a clinical pregnancy
- ↓ dose of gonadotropins and terminal E2
• Previous study: ↓ ongoing pregnancy rate ⇒ broad definition of poor response (suspected poor responder)

• Recent large retrospective study:
  ⇒ ↑ fertilization rate, implantation, cancellation rate
  ⇒ Similar PR per started cycle
In this study

• Letrozole (LA) ↔ luteal E2/GnRH antagonist (LPG) in women with known prior low response

• 5 mg/day x 5 days since MCD2 ↔ 2.5 mg/day

⇒ Intrinsic potency of letrozole: 2.5 mg/day
⇒ inhibit 97% estrogen (on nonstimulated granulosa cells)

⇒ (actively dividing granulosa cells ⇒ need higher dose of AI ⇒ aromatization attenuation)

⇒ One study for women undergoing COH and IUI: 5 mg required a lower dose of FSH
• **Started gonadotropins**: 3 days after initiation of letrozole \(\rightarrow\) allow the release of endogenous gonadotropins before initiation of exogenous stimulation \(\Leftrightarrow\) *(most of the other studies: started gonadotropins simultaneously with letrozole)*

• **Administration of hCG**: 2 follicles \(\rightarrow\) \(\geq 20\) mm \(\Leftrightarrow\) *(All prior studies: 17 or 18 mm diameter without reported \(\uparrow\) proportion of immature oocytes) \(\rightarrow\) still \(\downarrow\) Metaphase II oocytes*
Premature LH surge

- Trend toward ↑ incidence (LH ≥ 10 mIU/mL): LA vs. LPG (14.9% vs. 3.8%) (not addressed in most studies)

- Normal responder: Letrozole ➔ ↑ median [LH]

- ↑ in AI protocols (tends to occur at lower [E2])

- Ovaries with diminished ovarian reserve ➔ prone to a premature LH surge (presumably due to ↓ GnRH-attenuating factor/GnSAF production)

∴ An early start and possibly a higher dose of the antagonist should be considered when using letrozole
Safety issues

• ↑ risk of congenital cardiac malformations

  - Reassuring data among a large number of children born to women treated with letrozole: no such ↑ in the overall rates of congenital malformations or chromosomal abnormalities

  - In this study ➔ discontinued letrozole on day 6 or ≥1 week before ET (half-life 45 hours)
LA protocol

- ↓ Gonadotropins used and E2 levels
- ↓ Metaphase II oocytes (despite give hCG at lead follicle $\rightarrow$ 20mm, similar to previous study)
  A trend toward... (lack of a statistically significant)
- ↑ Cancellation rate (possibly due to inclusion of more severe poor responders)
- ↓ Miscarriage rate (possible improvement in endometrial receptivity or oocyte quality)
- ↑ Ongoing pregnancy rate per ET
• Non statistic significance between the ongoing pregnancy rates → May be type II error – (not powered enough to detect a difference of 20% in the ongoing pregnancy rate per ET between the 2 groups)
Conclusion

• Not able to identify a subgroup of low responders (who benefit from AI + antagonist-based protocol)

• Showed reasonable IVF outcomes of letrozole/gonadotropins for COH in low responders

• Using letrozole may require optimization → avoid a premature LH surge & ↑ the yield of mature oocytes

• Need prospective randomized trials with adequate power to test the efficacy of AI-based protocols ↔ other interventions in low responders
THANK YOU FOR LISTENING