Impact of IVF Protocol Selection

California
September 16 & 17th, 2009

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Disclosures

- Consultant, Ferring Pharmaceuticals
- Consultant, Gyrus ACMI/Olympus
- Consultant, Ethicon, Inc./Johnson & Johnson
- Shareholder, Gene Security Network
Outline

• Hyper-Responder
  – Selective Cut-Back Protocol
  – Ganirelix Salvage Protocol
  – Dostinex

• Importance of hCG activity

• Importance of FSH isoforms

• hMG and recFSH in the eu- and poor responder
OBJECTIVE:

Important goals for the PCOS patient undergoing ovarian stimulation for IVF include avoidance of hyperstimulation and the achievement of an ongoing pregnancy.

We sought to examine the efficacy of a once a day, single syringe, mixed preparation of gonadotropins and the potential impact of a selective cut-back regimen.

Bush et. al., *Fertility and Sterility*, Sept 07, S293.
MATERIALS AND METHODS:

PCOS patients 37 years of age or less in whom there was no prior history of endometriosis or infectious tubal disease were enrolled.

All subjects took 1500 mg of extended release metformin daily, 81 mg of aspirin daily, and underwent acupuncture with transfer.

Cycles were initiated with birth control pill, luteal GnRH agonist, and a starting dose of 75 – 150 IU HP-FSH mixed in the same syringe with 150 IU HP-hMG (which contains 10 IU hCG activity per 75 IU).

Depending on physician judgment, either a static dose of gonadotropin was given or it was cut-back.

Bush et. al., *Fertility and Sterility*, Sept 07, S293.
For the cut-back, the HP-FSH was first removed.

The HP-hMG either remained at the same dose or was also cut-back but never discontinued.

In contrast to a protocol utilizing GnRH agonist LH suppression where FSH alone is being cut-back, this approach provided a decreasing dose of FSH while maintaining LH drive to the more mature follicular cohort expressing LH receptors.

Bush et. al., *Fertility and Sterility*, Sept 07, S293.
One patient received three day 3 embryos.

All other patients received two embryos, 75% of them on day 5.

Five patients with hyperstimulation had their oocytes retrieved but did not receive fresh transfer.

Two of these patients had the evening GnRH agonist dose replaced with 250 ug of GnRH antagonist during the final 1-2 days of stimulation in an effort to foster more favorable conditions in which to proceed with oocyte retrieval.

Bush et. al., *Fertility and Sterility*, Sept 07, S293.
CONCLUSIONS:

A once a day, single syringe, mixed protocol is efficacious for PCOS patients undergoing IVF.

As the primary follicular cohort matures, by selectively cutting back FSH dose while maintaining adequate hCG activity, low hyperstimulation rates and excellent ongoing pregnancy rates can be achieved.

Bush et. al., *Fertility and Sterility*, Sept 07, S293.
21 oocytes/ 21 PB-1/ ICSI 18 fert, 2 blast ET (9 cryo), viable twins.
PCOS Cut Back Protocol

Pearls

- Starting dose 1 vial BRAVELLE® w/ 2 vials MENOPUR®
- Rarely 2 BRAVELLE® + 2 MENOPUR® with 1mg dexamethasone PO daily during stim, stay at 0.5mg Lupron®
- Patient seen in AM on d6, reflecting 3 evening doses of med
  - E2 500-600 consider taking away med
  - E2 600-1000 take away ½ or 1 vial BRAVELLE®
  - E2 >1000 take away all BRAVELLE® and a portion of MENOPUR®, but never to 0 vials
- If cut-back too aggressive, can always add-back 24 hrs later
- Extrapolate how many days she will need and cut back in anticipation of her predicted rate of rise in E2/follicular response
- Integration of antagonist salvage tool
- 5K hCG trigger in most circumstances
Treatment with gonadotropin-releasing hormone (GnRH) antagonists in women suppressed with GnRH agonist may avoid cycle cancellation in patients at risk for ovarian hyperstimulation syndrome

Forty-seven patients at high risk for ovarian hyperstimulation syndrome because of markedly elevated serum E₂ levels on either long-luteal or microdose flare leuprolide acetate regimens were treated with ganirelix acetate. Despite being pretreated with GnRH agonist and without withholding gonadotropins, serum E₂ decreased by 49.5% and 41.0% of pretreatment values (long luteal and microdose flare, respectively) after initiation of ganirelix, and 68.1% of the patients became pregnant. (Fertil Steril® 2006;85:251–4. ©2006 by American Society for Reproductive Medicine.)
Ganirelix® cycle salvage
Gustofson, Larsen, Bush, Segars; F&S, Jan 06, pgs 251-4.

- OCP lead-in
- Luteal Lupron® (0.5 mg/d to 0.25) n = 8
- MDF (40 ug/BID) n = 39

- If serum E2 level >2,000 d6 and/or projected E2 >5000 and >25 follicles day of hCG, then Ganirelix® 250/d replaced Lupron® until day of hCG

- Gonadotropins maintained at 37.5 – 225 IU/d
# Ganirelix® cycle salvage
Gustofson, Larsen, Bush, Segars; F&S, Jan 06, pgs 251-4.

## Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>LL (n = 8)</th>
<th>MDF (n = 39)</th>
<th>Total (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age in y (Mean ± SD)</td>
<td>29.6 ± 3.2</td>
<td>34.9 ± 3.1</td>
<td>34.0 ± 3.7</td>
</tr>
<tr>
<td>recFSH 75 IU (ampules)</td>
<td>17.1 ± 4.9</td>
<td>28.1 ± 10.9</td>
<td>26.2 ± 10.9</td>
</tr>
<tr>
<td>HMG 75 IU (ampules)</td>
<td>7.9 ± 7.2</td>
<td>10.4 ± 6.5</td>
<td>9.9 ± 6.6</td>
</tr>
<tr>
<td>Oocytes retrieved(^a)</td>
<td>23.6 ± 8.7</td>
<td>24.6 ± 9.1</td>
<td>24.4 ± 8.9</td>
</tr>
<tr>
<td>Ganirelix initiation day(^a)</td>
<td>10.5 ± 2.2</td>
<td>9.0 ± 1.6</td>
<td>NA</td>
</tr>
<tr>
<td>E(_2) on ganirelix initiation (pg/mL)(^b)</td>
<td>5,252.6 ± 1,360.2</td>
<td>4,203.9 ± 1,034.1</td>
<td>NA</td>
</tr>
<tr>
<td>E(_2) on ganirelix + 1 d (pg/mL)(^c)</td>
<td>2,560.0 ± 1,120.9</td>
<td>2,444.2 ± 1,229.2</td>
<td>NA</td>
</tr>
<tr>
<td>Percentage change</td>
<td>-49.5</td>
<td>-41.0</td>
<td>NA</td>
</tr>
<tr>
<td>E(_2) on post-hCG day (pg/mL)</td>
<td>5,686.9 ± 2,081.4</td>
<td>4,995.8 ± 2,302.9</td>
<td>NA</td>
</tr>
<tr>
<td>Ongoing pregnancy rate, %(n)(^a)</td>
<td>75.0 (6)</td>
<td>59.0 (23)</td>
<td>61.7 (29)</td>
</tr>
<tr>
<td>OHSS, n (%)</td>
<td>1 (12.5)</td>
<td>2 (5.1)</td>
<td>3 (6.3)</td>
</tr>
</tbody>
</table>

*Note:* Values are average ± SD. LL = long-luteal regimen; MDF = microdose flare regimen; NA = not applicable.

\(^a\) No significant difference between subgroups.

\(^b\) The E\(_2\) percentage changes represent the average percentage change for each patient and are not the percentage change of the average E\(_2\).

\(^c\) Change in E\(_2\) from day of initiation to day +1, \(P<.001\).
Ganirelix® cycle salvage
Gustofson, Larsen, Bush, Segars; F&S, Jan 06, pgs 251-4.

- E2 levels continue to rise on d1 of coasting
- Prolonged coasting may impair IVF cycle outcome
- The incidence of severe OHSS (2.1%) was lower than the published incidence in this high risk group (9-38%)
- GnRH-I and GnRH-II receptors have been found in granulosa cells, and antagonists may be acting directly on the ovary - particularly considering the reduction in pituitary cell membrane GnRH receptors with the application of GnRH agonist
- Granulosa cell GnRH receptors may follow later expression pattern as seen with LH receptors

15 oocytes/ 15 PB-1/ ICSI 14 fert, 2 blast ET (5 cryo), viable twins.

2 yrs prior, LL/rec-FSH only, hyperstim, ET w/o preg, no cryo.
Ganirelix® cycle salvage in hyper-responders and as a tool to avoid severe OHSS

- Replace evening Lupron® with Ganirelix®
- Keep gonadotropin dose the same or cut-back
- Replace Ganirelix® with hCG “trigger” shot

- Seven days of Ganirelix® to avoid severe OHSS
  - Patients cancelled prior to retrieval
  - In those patients who go to retrieval but in whom embryos are cryopreserved
  - Donors
Cabergoline (Cb2) therapy in face of OHSS

- VEGF induces VP (vascular permeability)\(^1,2\)
- Effects of Cb2 attributable to VEGF receptor dephosphorylation\(^3\)
- Cb2 prevents VP in a dose dependent manner without affecting angiogenesis and implantation in humans (n = 35 treated in face of OHSS)\(^4\)
- Cb2 reduced the amount of ascites, hemoconcentration and incidence of moderate-severe OHSS\(^5\)
- Cb2 0.5 mg x 8 days (total of 4 mgs) starting day of trigger

At 31, peak E2 3707, 10K, 12 2PN, 2 8-cell ET, singleton (2 blast cryo)

Now at 36, 24 2PN, 20 biopsy 13/18/21/X/Y, 4 blast XX, 1 blast ET, +7wk
OBJECTIVE:
We compared the efficacy of alternative medications for oocyte maturation in IVF cycles in high responders with significant risk factors for ovarian hyperstimulation syndrome (OHSS). These medications were leuprolide acetate in conjunction with 1000-2500 IU of hCG (dual trigger) or leuprolide acetate alone.

DESIGN:
Retrospective study of 164 fresh autologous IVF cycles. 119 of which used leuprolide acetate alone and 45 of which used dual triggers.

MATERIALS AND METHODS:
Patients were stimulated with gonadotropins and pituitary suppression was achieved with ganirelix acetate. For final oocyte maturation, patients were given either 4 mg leuprolide acetate (prior to 2006) or else 4 mg leuprolide acetate plus 1000 to 2500 IU hCG (from 2006).

The hCG dose was modulated based on body mass, and was approximately 12 IU per pound of body mass. All patients received luteal phase progesterone and estradiol supplements from retrieval until 8-10 weeks gestation, so that serum levels were maintained above 15ng/ml and 200 pg/ml, respectively.

Pregnancy was determined by rising serum titers of hCG 10 days after blastocyst transfer. Ongoing pregnancy was determined by the presence of viable fetal heart motion at 10 weeks gestation.

Early pregnancy losses included pregnancies that did not achieve ongoing pregnancy. Wilcoxon’s test was used to compare numeric variables and chi-square tests were used to compare categorical variables.

Shapiro et. al., *Fertility and Sterility*, Sept 07, S121.
RESULTS:
Patients receiving leuprolide alone and those receiving dual triggers did not differ significantly in serum E2 concentration on the day of trigger (mean 5,285 vs. 4,870 pg/ml, respectively), in the number of blastocysts developed (7.2 vs. 5.3) or in the number of embryos transferred (1.8 in both groups), but did have a greater number of mature follicles (30.9 vs. 27.3, \(P=0.004\)) and oocytes retrieved (26.0 vs. 20.4, \(P=0.004\)).

The two groups had similar rates of pregnancy (64.5% per transfer in both groups) but those receiving dual triggers had a lower rate of early pregnancy loss (17.2% vs. 56.3% of pregnancies, respectively, \(P=0.0004\)) and a higher ongoing pregnancy rate (53.3% vs. 26.1%, \(P=0.001\)). No significant OHSS occurred.

CONCLUSIONS:
Including 1000 to 2500 IU hCG with leuprolide acetate for oocyte maturation appears to reduce pregnancy loss rates and increase ongoing pregnancy rates when compared to leuprolide acetate alone.

This represents lower risk of pregnancy loss than leuprolide acetate alone and may represent lower risk of OHSS than a standard dose of hCG alone.

Shapiro et. al., *Fertility and Sterility*, Sept 07, S121.
Extragonadal Functions of the LH/hCG Receptor

- LH/hCG receptors in multiple uterine cell types, highest levels in endometrial epithelial cells

- LH/hCG receptor knockout models have abnormal uterine function, including decreased ability to implant donor blastocysts, which cannot be reversed by normalizing E+P levels

- Uterine Lh/hCG receptor activation upregulates COX-2, increases stromal cell differentiation, modulates pro- and anti-implantation cytokine production, increases blood flow through vasodilation and angiogenesis, inhibits myometrial contractions, and influences resident macrophage populations

Filicori et al., F&S, Vol 84, No 2, August 2005, 275-284
Extragonadal Functions of the LH/hCG Receptor

- The expression of *estrogen and progesterone receptors* in the human endometrium has been reported to be *altered* in ovarian stimulation cycles using GnRH agonists/antagonists, in which *serum LH concentrations in the follicular phase are low (concept of hCG prep)*

- Uterine receptivity is induced by blastocyst hCG signaling superimposed on *E/P primed endometrium*

- LH/hCG receptors are *upregulated* by progesterone in the luteal phase, underscoring the role for this ligand-receptor complex during implantation and placentation

Bourgain et al., *F&S*, Vol 78, No 2, August 2002, 237-244
Filicori et al., *F&S*, Vol 84, No 2, August 2005, 275-84
hCG affects Uterine Receptivity Independent of Ovarian Function

- Donor oocytes were split between two sets of recipients, those that ovulated and those that did not (FSH > 30)

- The ovulating recipients received triptorelin 3.75 mg, and both recipient groups received oral estradiol valerate and vaginally administered micronized progesterone

- Within each of these groups, half of the recipients received 5000 hCG at the same time as the donor

hCG affects Uterine Receptivity Independent of Ovarian Function

Donor Oocytes

Recipient: Ovulating
3.75 mg Triptorelin

Recipient: Non-Ovulating (FSH >30)
Oral Estradiol and Micronized Vaginal Progesterone

Received 5000 IU hCG same time as Donor
No hCG
Received 5000 IU hCG same time As Donor
No hCG

Tesarik et al., Repro BioMed Online, Vol 7, No 1, April 2003, 59-64
hCG affects Uterine Receptivity Independent of Ovarian Function

- Implantation rate, ovulatory recipients
  - Received hCG: 30.6%
  - Did not receive hCG: 20.7% ($P<0.01$)

- Endometrial thickness on transfer day (d19), ovulatory recipients,
  - Received hCG: 9.7mm +/- 0.9
  - Did not receive hCG: 8.2mm +/- 0.8 ($P<0.01$),
- day 14 “stagnation” was ameliorated

- There was no difference in the non-ovulatory recipients with tonically elevated LH levels, signifying the effect was mediated through the LH/hCG receptor

Adjunctive hCG in Donor Oocyte Recipients, FET and IVF cycles

- Donor oocyte recipients and FET cycles receive lupron down-reg, estradiol patches for endometrial proliferation (+ vaginal estradiol as needed), luteal progesterone and estradiol
- Patients receive 5,000 IU hCG SQ one day before they begin progesterone (for donor recipients this is the same day the donor receives her hCG)
- Patients then receive 1000 IU days 4, 7 and 10 after the initial dose
- Fresh IVF cycles utilize 1000 IU days 4, 7 and 10 after the initial hCG dose, with luteal progesterone and estradiol
- Second serum β-hCG pregnancy test is 8 days after the last 1000 IU injection, allowing for complete clearance (first test greater than 25 c/w pregnancy)
Impact of FSH Isoforms and hCG activity

• Can choice of gonadotropin preparation affect rates?
• What are the biochemical differences between the preparations?
FSH Isoforms

- 20-30 isoforms of both FSH and LH are present in the bloodstream throughout the menstrual cycle (1)
- The human isoforms arise from varying 5’ promoter influence (hormonal - transcriptional) and post-translational carbohydrate changes: endoplasmic reticulum (glycosylation), Golgi apparatus (further processing of the carbohydrate component)
- The carbohydrate component affects the biological activity (potency) of the hormone-receptor complex after binding, with a critical role in activation (coupling) of the adenylate cyclase system (3)
- Sialic acid is the critical determinant of half-life (duration of action). LH has less than half the sialic acid of FSH leading to a half life of 20 minutes for LH compared to 3-4 hours for FSH
- The highest bioactive levels of FSH are midcycle and in post-menopausal women (including women with POF) (2)
- Recombinant FSH: a/b chain expression vectors integrated into CHO DNA with cultured hamster cell glycosylation and modification

Differences in the Preparations: FSH Isoforms and LH activity

- Human FSH has ~ 20 isoforms (1,2)
- Human derived FSH in BRAVELLE®/MENOPUR® has 16 isoforms (3,4)
- Hamster derived recFSH has 11 isoforms (5)
- An additional difference is that MENOPUR® contains 10IU of LH activity (as hCG) per 75 IU

FSH Isoform Profile

- Isoforms characterized by isoelectric focusing according to pH
- BRAVELLE® has a much greater % (100% vs. 56%) of less acidic isoforms (PI > 4.25)
- Less acidic forms are more biologically active at the level of the receptor

**Table 8. Percentage of FSH with isoelectric points (pI) > 4.25 and pI range for different gonadotrophin preparations.**

<table>
<thead>
<tr>
<th></th>
<th>Gonal-f&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Puregon&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Metrodin HP&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Metrodin&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Bravelle&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>pI &gt; 4.25 (%)</td>
<td>56</td>
<td>64</td>
<td>~15</td>
<td>~40</td>
<td>~100</td>
</tr>
<tr>
<td>pI range</td>
<td>3.5–6.1</td>
<td>3.2–5.7</td>
<td>3–5.5</td>
<td>–</td>
<td>3.5–7.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Horsman et al. (2000).
<sup>b</sup>Lambert et al. (1995).
<sup>c</sup>Internal Data Instituto Masdone (isoelectric focusing).

Wolfenson et al, 2005
Glycosylation variants are age and cycle sensitive (Ulloa-Aguirre et al, Endocr Rev 1995;16:765-87)
Resumption of meiosis is isoform dependent (Yding et al, Reprod Med Online 2002;5:232-9)

All patients d3 FSH/E2 of <8/<80, mean ages 34 and 27
Group 1:  DOR, ET of 2-3 poor quality, fragmented embryos, NP
Group 2:  Donor, ET of 2 high quality, clinical pregnancy

After second spontaneous menses, d3 FSH isoforms compared
Sig difference in % of FSH eluted in pH range 4.51-5 (12.6 vs. 18.1)
Differential secretion of FSH isoforms may exist in DOR
Potential benefit in covering isoform gaps throughout cycle

HP hMG vs. recFSH ICSI cycles
Differences in FSH clearance and potency

- RCT (MENOPUR® vs. G-F)
- 50 patients each arm, long luteal, 150 IU/d fixed until trigger at >3 follicles >17 mm

FSH levels \((P < 0.001)\) and E2 levels \((P < 0.05)\) higher despite equal and static amounts of FSH administered. Displays decreased clearance of FSH isoforms \((t^{1/2})\) Displays increased potency of FSH isoforms on aromatase. Not entirely explained by increased substrate (notice flat T).

EISG and MERiT

- **RCTs** (Both trials MENOPUR® vs. G-F)
- Comparison of **3 vials/d for a fixed period** of 5 days
- **Same trigger criteria** within each study
- Age 18-38 (EISG), 21-37 (MERiT)
- COS with long luteal GnRH agonist, ovulatory women, up to three prior unsuccessful cycles
- Day 3 transfer of 1-3 (EISG) or 1-2 (MERiT) normally developed embryos
- Number of embryos transferred equal in both groups

Combined EISG and MERiT IVF Analysis: Pregnancy Rates

<table>
<thead>
<tr>
<th>Pregnancy rate (%)</th>
<th>HP-hMG n=491</th>
<th>rFSH n=495</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>29</td>
<td>22</td>
<td>1.42</td>
<td>1.06-1.89</td>
</tr>
<tr>
<td>Ongoing</td>
<td>27</td>
<td>21</td>
<td>1.38</td>
<td>1.03-1.86</td>
</tr>
</tbody>
</table>

Superiority

Favors HP-hMG

Favors rFSH

IVF (n=986)

Abstracted at ASRM 2005
MERiT: Greater percentage of oocytes develop into TQE

- Top Quality Embryos (TQE): 4-5 cells (d2), ≥7 cells (d3), equally sized blastomeres, ≤20% fragmentation, no sign of multinucleation

- ~25% of embryo transfers were with TQE only

<table>
<thead>
<tr>
<th></th>
<th>HP-hMG</th>
<th>rFSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>TQE (% of oocytes)</td>
<td>10.2</td>
<td>8.6</td>
</tr>
<tr>
<td>Patients w/ TQE (%)</td>
<td>48</td>
<td>44</td>
</tr>
</tbody>
</table>

Abstracted at ESHRE 2005
MERiT: TQE derived from hMG have higher implantation and pregnancy rates

**Implantation Rate**

- HP-hMG: 41%
- rFSH: 27%

**Ongoing PR**

- HP-hMG: 48%
- rFSH: 32%

*p<0.05

Abstracted at ESHRE 2005
Broader isoform coverage and/or hCG may have an impact on embryo development

- A *higher proportion of the oocytes retrieved in the HP-hMG group developed into top quality embryos*

- Differences in gonadotropin stimulation preparations influence the specific endocrine environment for the maturing oocyte that may be important for embryo development

- Top quality embryos derived from HP-hMG stimulation *are associated with a significantly improved capacity to implant and establish a pregnancy*

Abstracted at ESHRE 2005
Follicular Progesterone

- Follicular phase serum P levels are not related to the amount of LH activity administered

- Neither LH or hCG administration in the follicular phase is associated with premature endometrial luteinization

- Increased P is associated with the use of recombinant FSH and the amount of FSH activity administered

- Influence of granulosa cell steroidogenesis by FSH can result in follicular P secretion, more so for rFSH than urinary derived

Bosch et al, F&S, 2003, 80, 1444-9
# MERiT: Endocrine profile on the last day of stimulation

<table>
<thead>
<tr>
<th></th>
<th>HP-hMG (n=363)</th>
<th>rFSH (n=368)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (IU/L)</td>
<td>18.3</td>
<td>16.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>1.8</td>
<td>1.7</td>
<td>0.125</td>
</tr>
<tr>
<td>hCG (IU/L)</td>
<td>2.7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Estradiol (nmol/L)</td>
<td>7.2</td>
<td>6.6</td>
<td>0.031</td>
</tr>
<tr>
<td>Progesterone (nmol/L)</td>
<td>2.7</td>
<td>3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Androstenedione (nmol/L)</td>
<td>11.9</td>
<td>9.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total testosterone (nmol/L)</td>
<td>1.7</td>
<td>1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>89</td>
<td>89</td>
<td>0.974</td>
</tr>
</tbody>
</table>

Abstracted at ESHRE 2005
Serum Progesterone by Endometrial Echogenicity (Day of hCG)

<table>
<thead>
<tr>
<th>Echogenic pattern (Day of hCG)</th>
<th>Serum $P_4$ (nmol/L)</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoechochogenic</td>
<td>2.9 ± 2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoechochogenic</td>
<td>3.0 ± 1.6</td>
<td></td>
<td>0.0002</td>
</tr>
<tr>
<td>Hyperechochogenic</td>
<td>4.3 ± 6.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abstracted at ESHRE 2005
# Endometrial Status (Day of hCG)

<table>
<thead>
<tr>
<th></th>
<th>HP-hMG</th>
<th>rFSH</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endometrial thickness (mm)</strong></td>
<td>10.7 ± 1.9</td>
<td>10.8 ± 2.0</td>
<td>0.780</td>
</tr>
<tr>
<td><strong>Triple-layer structure</strong></td>
<td>96%</td>
<td>97%</td>
<td>0.532</td>
</tr>
<tr>
<td><strong>Echogenic pattern</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoechogenic</td>
<td>42%</td>
<td>36%</td>
<td></td>
</tr>
<tr>
<td>Isoechogenic</td>
<td>48%</td>
<td>49%</td>
<td></td>
</tr>
<tr>
<td>Hyperechogenic</td>
<td>10%</td>
<td>16%</td>
<td></td>
</tr>
</tbody>
</table>

Abstracted at ESHRE 2005
Human choriogonadotropin prior to controlled ovarian stimulation and *invitro* fertilization improves implantation and pregnancy rates

- 36 women given 250ug of Ovidrel on day 1 of menses
- 64 controls not given hCG on day 1
- All patients began Gonal-f on day 3; antagonist protocol
- Age 20-38, FSH $\leq 10$

hCG on day 1

- Average total r-FSH significantly less at 1,731 v. 2,096 IU (p < 0.5).
- More top-quality embryos (8 cell; <20% fragmentation) produced: 1.0 v. 0.5 (p < 0.001).
- Significantly higher implantation (33% v. 21%) and ongoing pregnancy rate (64% v. 41).

hCG on day 1
Impact on oocyte/embryo and endometrium

- Mimics LH wave present at beginning of normal menstrual cycles
- May synchronize early follicular cohort development
- May synchronize intra-follicular hormonal/growth factor milieu serving to enhance oocyte developmental competence
- hCG upregulates COX-2, increases stromal cell differentiation, modulates pro- and anti-implantation cytokine production, increases blood flow through vasodilation and angiogenesis, inhibits myometrial contractions, and influences resident macrophage populations

Motta et al, J Assist Reprod Genet, published online 16 June 2009
Filicori et al., F&S, Vol 84, No 2, August 2005, 275-284
Ongoing Pregnancy Rates by hCG Level on Day 6 (IVF only)

Platteau et al., F&S, Vol 81, No 5, May 2004, 1401-1404
### Clinical Pregnancy Rate by Age

**SART Comparison**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Endometrin® Trial (n = 1,211)</th>
<th>SART 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRAVELLE® + minimum 1 vial MENOPUR® Luteal Lupron®</td>
<td></td>
</tr>
<tr>
<td></td>
<td># ET</td>
<td>PR</td>
</tr>
<tr>
<td>&lt; 35 years</td>
<td>2.29</td>
<td>47.1</td>
</tr>
<tr>
<td>35-37 years</td>
<td>2.43</td>
<td>39.6</td>
</tr>
<tr>
<td>38-40 years</td>
<td>2.53</td>
<td>31.8</td>
</tr>
<tr>
<td>41-42 years</td>
<td>2.83</td>
<td>32.5</td>
</tr>
</tbody>
</table>
Clinical efficacy of highly purified urinary FSH versus recombinant FSH in volunteers undergoing controlled ovarian stimulation for in vitro fertilization: a randomized, multicenter, investigator-blind trial

Valerie L. Baker, M.D., a,b Victor Y. Fujimoto, M.D., c L. Michael Kettel, M.D., d G. David Adamson, M.D., b Fred Hoehler, Ph.D., e Clarence E. Jones, Ph.D., f and Michael R. Soules, M.D. g

a Obstetrics and Gynecology, Stanford University Medical Center, Stanford, California; b Fertility Physicians of Northern California, San Jose, California; c Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, California; d San Diego Fertility Center, San Diego, California; e Biostatistician Consultant, Orange, California; f Consultant, Huntington Beach, California; and g Seattle Reproductive Medicine, Seattle, Washington
HP-hFSH v. recFSH

- RCT, BCP/lupron down-reg, 300 IU starting dose, inclusion <10 FSH, ≥ 10 AFC.
- Sample size calculated with power to detect 20% difference in mean oocytes retrieved (mean for centers 16.3; 20% difference 3.26 oocytes). No difference in mature oocytes retrieved. RecFSH not shown to offer “superior stim”.

- 152 good prognosis patients randomized, 34.3 – 34.8, AFC 17.4 – 16.4, basal FSH 6.5 – 6.3, 90% first cycle, none with prior poor response.
- With 2 HQ embryo transfer, makes sense that CPR 48.7 – 44.7%; LB same at 38.2% (LB not primary endpoint; 95% confident true diff is +/- 15.4%).
- Isoform gap not as relevant in good prognosis patients.
hMG compared to recFSH
2008 meta-analysis of 7 RCTs IVF-ICSI with luteal down reg

- $n = 2159$

- Significant increase in clinical pregnancy rates with hMG, relative risk (RR) = 1.17 (95% CI 1.03 – 1.34)

- Significant increase in live birth rates with hMG, relative risk (RR) = 1.18 (95% CI 1.02 – 1.38)

- hMG associated with a pooled 4% increase in live birth rate when compared with recFSH

hMG compared to recFSH
2008 meta-analysis 10 RCTs long lupron (xJansen) IVF/ICSI

- n = 2937

- Significant increase in live birth rates with hMG, odds ratio (OR) = 1.20 (95% CI 1.01 – 1.42)

- No difference in patient safety rates as measured by OHSS rates (xRashidi) in meta-analysis of 9 RCTs with OR 1.21 (95% CI 0.78 – 1.86)

hMG compared to recFSH
2008 meta-analysis of 10 RCTs IVF-ICSI with luteal down reg

Figure 2. Forest plot for live birth rate (see Table 2 for studies included). CI = confidence interval; HMG = human menopausal gonadotrophin; OR = odds ratio; r-FSH = recombinant FSH.

Al-Inany et al, RBM Online 2008, 16(1), pgs 81-88.
[recFSH+ HP hMG] vs. [recFSH] on euploidy rates, antagonist protocol

- 104 women, retro controlled cohort, mean age 40
- 300 – 450 total dose, one group had 150 hMG base
- Antagonist when lead 13mm
- IVF with two pass PGD for 13, 16, 18, 21, 22 and then 15, 17, X & Y
- Euploidy rates (25.7% v. 29.4%) and number of euploid embryos (1.9 v. 2.1) similar

Weghofer et. al., Fertil Steril, in press.
[recFSH+ HP hMG] vs. [recFSH] on euploidy rates, antagonist protocol

**Table 3**

Pregnancy rates of matched cohorts according to gonadotropins used (recFSH vs. recFSH/hMG) during antagonist stimulation.

<table>
<thead>
<tr>
<th></th>
<th>recFSH (n = 52)</th>
<th>recFSH/hMG (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancies per cycle start</td>
<td>34.6% (18/52)</td>
<td>11.5% (6/52)a</td>
</tr>
<tr>
<td>Pregnancies per transfer</td>
<td>40% (18/45)</td>
<td>15% (6/40)a</td>
</tr>
<tr>
<td>Implantation rate per transfer</td>
<td>23.0%</td>
<td>11.7%</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>27.8% (5/18)</td>
<td>0%</td>
</tr>
<tr>
<td>Ongoing per transfer</td>
<td>28.9% (13/45)</td>
<td>15% (6/40)</td>
</tr>
</tbody>
</table>

*a P<.05.

Weghofer et. al., Fertil Steril, in press.
[recFSH+ HP hMG] vs. [recFSH] on euploidy rates, antagonist protocol

- Need to see if these numbers hold up: reported groups have 13 and 6 ongoing pregnancies respectively
- Med dosage demonstrated a significantly negative impact on recFSH only cycles but not for recFSH + hMG (P < 0.05)
- Significant assoc between euploidy rates and implantation as well as clinical and ongoing pregnancy rates per cycle start and embryo transfer for FSH/hMG but not with recFSH only (P < 0.5)
- Antagonist cycles have initial endogenous LH activity present until leads reach 13mm
- Miscarriage rates underscore importance of hCG primed and maintained endometrium

HP hMG vs. recFSH on euploidy rates, long protocol

- 104 women, retro controlled cohort, mean age 35.5
- 100 – 300 IU/d starting dose of either recFSH (G-F or Follistim) or HP hMG (REPRONEX® and MENOPUR®)
- IVF with two pass PGD for 13, 16, 18, 21, 22 and then 15, 17, X & Y
- hMG use resulted in 69.8% of embryos euploid vs. 45.3% for recFSH ($P < 0.01$)
- Miscarriage 0% hMG, 9% recFSH
- Ongoing pregnancy rates (clinical – miscarriage):
  - Per cycle started 63% (33/52) for hMG vs. 40% (21/52) ($P < 0.02$)
  - Per embryo transferred 63% for hMG vs. 44% ($P < 0.049$)

HP hMG vs. recFSH on euploidy rates

• One of the mechanisms (with TQE, recFSH P4 influence, hCG endometrial prep) behind rate differences observed by MERiT, EISG, Coomarasamy et al, Al-Inany et al (preponderance of data demonstrating difference v. data demonstrating no diff – design difficulties/not powered to show difference)

• Oocyte quality begets embryo quality

• hCG activity and broader FSH isoform spread influences oocyte quality

• Resumption of meiosis is isoform dependent (Yding et al, Reprod Med Online 2002;5:232-9)

HP hMG vs. recFSH on euploidy rates

- hCG throughout stimulation sets up fragile meiotic resumption in a more natural and appropriate way (1)
- Fresh cycle impact more evident in women > 35, prior failed IVF, longstanding unexplained, decreased ovarian reserve where decreased embryo production anticipated (greater benefit from increased % euploid)
- Conversely, with adequate # of embryos, effect seen in cryo program; effect can be obscured by multiple embryo transfer. Mean FET 1.9 v. 2.3 – 3.0.
- Impact also seen in young women with natural and appropriate low baseline LH levels on a long protocol (1)

Decreased Ovarian Reserve

Miami, FL
August 27, 2009

Mark R. Bush, MD, FACOG, FACS
Testing Egg Health
Summary

- FSH of less than 8 optimal, 8-10 borderline, 10–12 compromised, >12 severely compromised (d3 and/or d10).
- Basal E2 of <50 optimal, 50–80 borderline, >80 compromised (d3).
- Baseline scan to rule out E2 secreting functional cysts.
- Basal antral follicle count of 8–18 normal range, <8 compromised, ≤4 severely compromised.
- CCCT can identify up to 50% of women with DOR who will not be identified with d3 levels alone.
- AMH < 1 compromised, 1 – 1.4 borderline, ≥ 1.5 adequate. Range groups greater than 4 aid in identifying/degree hyper-response.
- Testing is interpreted within the context of age, diagnosis and fertility history.
Poor Responders
Overview of options

- Dexamethasone 1 mg d1 to trigger
- DHEA
- Growth hormone
- Luteal E2/antagonist prep
- Acupuncture, nutrition

- Adequate RFC - light application of OCP, MDF, high dose mixed, baseline 3 amps HP-hMG
- Low RFC - letrozole 2.5 d3-7 vs. GH, high dose mixed, ganirelix

- Elevated baseline FSH, E2, baseline “cysts”, failed above – no OCP, E2 patch 10d after surge, ganirelix 250/d x3d 11d after surge, high dose mixed
- Poor GV/GVB to PB1 – High dose HP-hMG, 20K hCG trigger
Poor Responders

Dexamethasone

- Prospective, double-blind, randomized, placebo controlled study. 290 patients. 1 mg dexamethasone (n = 145) or placebo tablets (n = 145).
- Significantly lower cancellation rate for poor ovarian response was observed in the dexamethasone group. 2.8% vs. 12.4% respectively, \( P < 0.002 \).
- Mechanisms of action:
  - Sensitizes the ovary to gonadotropins
  - Is a substrate for type-1 11-BHSD which is found in granulosa cells and oocytes suggesting a direct role in influencing follicular development
  - Increases serum GH and IGF-1 and follicular fluid IGF-1; IGF-1 acts synergistically with FSH

DHEA IVF Protocol Literature Synopsis as of 11/20/07
M. Bush, MD

P-377
Gleicher/Barad ASRM 2007
DHEA improves number of euploid embryos available for transfer in DOR patients.

“8 patients received at least one month of supplementation with DHEA (micronized, pharmaceutical grade; 25 mg, TID) prior to oocyte retrieval”

P-567
Gleicher/Barad ASRM 2007
Longitudinal review of 281 cycles from 2003-2006 in women above age 40 with DOR. By 2006, 45% of women above 40 were given DHEA. DHEA significantly improves CPR/ET. They have since adopted a policy of DHEA in all women above 40 undergoing IVF.

“Patients received micronized, pharmaceutical grade DHEA 25 mg TID for a minimum of two months prior to egg retrieval”

O-289
Brill/Gleicher ASRM 2006
DHEA leads to increased, and more rapid, conception rates.

“88 patients received DHEA supplementation at a dosage of 25mg TID for an average period of 3.7 +/- 0.3 months followed by IVF”

O-101
Barad/Gleicher ASRM 2005
DHEA pre-treatment for IVF in 45 DOR patients increases oocyte yield and percentage of high quality embryos.

“DHEA, at 25mg TID, for an average of 28 weeks +/- 15 wks (range 4 to 48 weeks)”

F&S, Vol. 84, No. 3, September 2005
Barad/Gleicher
Initial case report detailing a 43 year old with DOR who dramatically improved her oocyte production over the course of continuous treatment with 75 mg/d DHEA
DHEA

- Failed stim, low RFC, low AMH
- Pre-treatment RFC, AMH, DHEAS, T
- Rx DHEA 75 mg ½ tab BID (Belmar)
- Repeat DHEAS, T in two weeks with target levels of 300 – 500 and 75 – 100 respectively
- Can decrease dose for SE, i.e. acne
- Optimal ≥ 8 weeks prior to repeat stim
- Continued thru trigger (spontaneous conception stops med at +hCG)
Growth Hormone

- Nine poor responders, mean age 38.1, mean number of prior IVF attempts 5.4, fewer than 3 follicles ≥ 14mm with previous COH
- GnRH agonist stop with menses, hMG 450/d + 4 IU GH until trigger
- Significantly increased number of oocytes retrieved (4.4 v. 1.3) and number of good quality embryos produced (2.0 v. 0.9) with GH + hMG compared to previous response with hMG alone
- GH (thru hepatic IGF-1 production) amplifies FSH action on granulosa cell proliferation and differentiation
- Decreased effect if increased levels of IGFBP-3
- Conceptions regimen: Saizen vial 8.8 mg (26.4 IU), mix 5 mL diluent, 0.8 mL SQ (4.2 IU/d). Vial lasts 6 days – order two vials

How to improve the probability of pregnancy in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis

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Unit for Human Reproduction, First Department of Obstetrics & Gynecology, Papageorgiou General Hospital, Thessaloniki, Greece

Objective: To systematically review the literature to identify randomized controlled trials, which evaluate interventions aiming to improve the probability of pregnancy in poor responders undergoing in vitro fertilization (IVF).

Design: Systematic review and meta-analysis.

Setting: University-based hospital.

Intervention(s): None.

Main Outcome Measure(s): Pregnancy rate.

Result(s): Twenty-two eligible randomized controlled trials were identified that evaluated in total 15 interventions to increase pregnancy rates in poor responders. Based on limited evidence, the only interventions that appear to increase the probability of pregnancy were the addition of GH to ovarian stimulation (odds ratio for live birth: 5.22, confidence interval: 95% 1.09–24.99) and the performance of embryo transfer on day 2 compared with day 3 (ongoing pregnancy rate: 27.7% vs. 16.3%, respectively; difference: +11.4, 95% confidence interval: +1.6 to +21.0).

Conclusion(s): Insufficient evidence exists to recommend most of the treatments proposed to improve pregnancy rates in poor responders. Currently, there is some evidence to suggest that addition of GH, as well as performing embryo transfer on day 2 versus day 3, appear to improve the probability of pregnancy. (Fertil Steril® 2008;89:...)

Key Words: In vitro fertilization, live birth, ovarian stimulation, poor response
Objectives: To evaluate the incidence of congenital malformations among offspring of mothers who conceived with clomiphene citrate (CC) or with letrozole treatment for infertility.

Design: Retrospective study.

Setting: 5 fertility centers in Canada.

Patients: 911 newborns from women who conceived following CC or letrozole treatment.

Interventions: Examinations of medical files of both mother and newborns, and cross-checked with the reports by telephone calls.

Main Outcome Measures: Identified major and minor congenital malformations, birth weight, age of the mother, and type of treatment that led to the conception.

Results: Overall, congenital malformations and chromosomal abnormalities were found in 14 of 314 newborns in the letrozole group (4.4%) and in 37 newborns in the CC group (4.8%). The major malformation rate in the letrozole group was 1.2% (6/514) and the CC group was 3.0% (12/397). One newborn in the letrozole group was found to have a ventricular septal defect (0.2%) compared to 4 newborns in the CC group (1.0%). In addition, the rate of all congenital cardiac anomalies was significantly higher (P < 0.02) in the CC group (1.6%) compared to the letrozole group (0.9%).

Conclusion: There was no difference in the overall rates of major and minor congenital malformations among newborns from mothers who conceived after letrozole or CC treatments. However, it appears that congenital cardiac anomaly is less frequent in the letrozole group. The reason that letrozole use for ovulation induction could be teratogenic is unknown based on our data. (Fertil Steril® 2009;81:1761-5. ©2009 by American Society for Reproductive Medicine.)

Keywords: aromatase inhibitors, letrozole, clomiphene citrate, ovulation induction, infertility, congenital malformation, birth defects, congenital anomalies, congenital cardiac anomaly, congenital heart disease

A New Era in Ovulation Induction

Hannahel Hulser, M.D., 1 Robert Casper, M.D., 3 and Togas Tulandi, M.D., M.H.C.M. 3

*Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec; and 3 University of Toronto, Toronto, Ontario, Canada

Objective: To evaluate the efficacy of aromatase inhibitors in ovulation induction, superovulation, and IVF.

Design: A literature search was conducted with the key words "aromatase inhibitor," "letrozole," "anastrozole," "ovulation induction," "ovulation," and "superovulation" in MEDLINE, EMBASE, and the Cochrane Database of systematic reviews.

Result(s): Ovulation induction with letrozole is associated with an ovulation rate of 70%-84% and a pregnancy rate of 25%-30% per cycle. In one study, ovulation and pregnancy rates with letrozole seemed to be higher than those of anastrozole. In superovulation, letrozole is associated with fewer developing follicles and thinner endometrium. The use of letrozole for superovulation is associated with a pregnancy rate higher than with the use of clomiphene citrate (CC) (15% vs. 5%). The addition of letrozole to FSH treatment leads to a decreased FSH requirement. The pregnancy rate for treatment with letrozole and FSH was similar to that for FSH alone.

Conclusion(s): Aromatase inhibitors are as effective as or superior to CC in ovulation induction and in superovulation. Unlike CC, they do not carry an antiestrogenic effect on the endometrium. Given the advantages of aromatase inhibitors, they can be used to replace CC as ovulation-inducing drugs. Their role in IVF remains to be determined. (Fertil Steril® 2006;85:777-84. ©2006 by American Society for Reproductive Medicine.)

Keywords: infertility, ovulation induction, superovulation, controlled ovarian hyperstimulation, aromatase inhibitors, letrozole, anastrozole
Drugs in infertility and fetal safety

Shai E. Elizur, M.D., and Togas Tulandi, M.D., M.H.C.M.

Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada

Objective: To evaluate the safety of drugs used in infertility treatment.

Design: Literature search using the keywords birth defect, congenital malformation, clomiphene, aromatase inhibitor, letrozole, gonadotropin, metformin, gonadotropin releasing hormone agonist and antagonist, progesterone, progestin, and estrogen. We conducted the search in Medline, EMBASE, and Cochrane Database of systematic reviews.

Result(s): The available data suggest that clomiphene treatment, especially after several cycles, might be associated with a slightly higher risk of neural tube defects and severe hypospadias in the offspring. Letrozole and metformin do not appear to be teratogenic. The existing data concerning gonadotropin preparations suggest that there is no evidence of teratogenicity, yet, information after 1991 is lacking. Micronized progesterone, which is widely used in in vitro fertilization treatment, does not appear to increase the risk of nongenital birth defects; however, there might be a possible weak association between other progestational agents and hypospadias.

Conclusion(s): Infertility per se is a risk factor for congenital anomalies. Repeated clomiphene treatment might be associated with a slightly higher risk of hypospadias and neural tube defect. However, the overall increased risk related to various fertility drugs is only 1% to 2%. (Fertil Steril® 2008;89:1595–602. ©2008 by American Society for Reproductive Medicine.)

Key Words: Congenital malformation, birth defect, infertility, gonadotropin, clomiphene, aromatase inhibitors, letrozole, metformin, progesterone, progestin, estrogen
Poor Responders
Letrozole

Patient Consent for the Use of Letrozole (Femara®)

Femara (Letrozole) is approved by the FDA only for treatment and prevention of breast cancer. There are numerous medications that are prescribed for "off label" uses. (Some medications can be used for more than one problem.) Since 2001, many physicians have used Letrozole for ovulation stimulation. It has been studied in humans and had very good success with minimal side effects. Recently, a study in Canada showed that there was an increase in fetal abnormalities in women who took Letrozole compared to those who did not take the drug. There was an increase in abnormalities by 2%. (The normal risk is about 3%; the patients who took Letrozole had a 4.5% rate of abnormalities.) They did not explain how these abnormalities could be caused by the medication, nor did they explain if it could be related to other medications or environmental factors. THIS STUDY HAS NOT YET BEEN PUBLISHED.

There has been one published study that indicates there is a LOWER incidence of fetal abnormalities in pregnancies conceived with letrozole.

The most important aspect is that the study failed to explain how a medication that you take BEFORE you ovulate and conceive can cause problems to a fetus when it is out of the body by the time you are pregnant.

Regardless, the company states that "Femara (Letrozole) is contraindicated in women with premenopausal endocrine status, in pregnancy, and/or lactation due to the potential for maternal and fetal toxicity and fetal malformations."

The doctors at Conceptions Reproductive Associates are cautious in prescribing this medication. Informed consent entails discussion with your provider so that you are satisfied with the information and can make an informed decision regarding using this medication to aid in effecting ovulation or egg production.

I acknowledge that I have had an opportunity to ask questions about the use of letrozole in general and in my case specifically. I consent to the use of letrozole and understand that I may withdraw my consent at anytime. I understand the risk, consequences, and benefits as explained to me and understand that I may request information on pregnancy rates and outcomes at any time.

Date: ___________________ Signature of Patient: ___________________ Patient Name-Print: ___________________

As one of the staff members of Conceptions Reproductive Associates by my signature indicate that the foregoing consent was read, discussed and signed in my presence.

Date: ___________________ Signature of Witness: ___________________ Witness Name-Print: ___________________

PUBLICITY MANDATORY

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Poor Responders

Letrozole

- 147 prior poor response patients, 71 treated with FSH/hMG/anatag alone and 76 with same regimen + letrozole with significant difference in oocytes retrieved (6.1 vs. 4.3) and IR (25% vs. 9.4%) observed with letrozole

- Unex infert, prior poor response, n = 12 at IUI, addition of letrozole saw more mature follicles produced with less gonadotropin used

- In 27 prior poor response patients, a 25.9% PR at IVF was observed with the addition of letrozole in the cycle following a failed cycle
- Adding 2.5 mg letrozole to FSH/hMG antagonist protocol offers an attractive alternative after a prior cancelled cycle

Poor Responders
Luteal E2/antagonist prep

• Defined as a prior cycle with one or more of the following: 
  <4 oocytes, FSH >12, peak E2 <500
• 68 patients, 66 of which had a mean of 3 prior cycles, 
  mean of 1.1 cancelled cycles, mean age 39.7
• 10 days after surge, apply E2 patch 0.1 QOD
• 11 days after surge, antagonist 250/d for 3 days
• FSH, LH, E2 and scan on d2 of menses
• Begin high dose mixed on d2. 66 of the cycles used 8 
  amps/d.
• Antagonist when lead at 13 or E2 at 300
• Remain on last patch until falls off or until trigger
• Mean d2 FSH value 2.4, E2 110.4. The E2 will nadir by d4 
  then rise

Dragisic KG, et. al., *Fertility and Sterility*, Oct 05, 1023-6.
Poor Responders
Luteal E2/antagonist prep

• Compared to prior cycle, cancellation rate was decreased from 33.3% to 13.6% ($P < .05$).
• Higher mean number of oocytes retrieved (8.3 vs. 6.4 $P < .05$) and embryos produced (4.5 vs. 2.4 $P < .05$)
• Acceptable clinical PR in this group of 30.3%

• Luteal E2 results in a reduction in antral follicle heterogeneity and more synchronized maturity via decrease in luteal FSH curve
• Antagonist may increase FSH receptor sensitivity (decreases circulating levels of ligand)
• E2 may foster expression of FSH receptors (progesterin in OCP would antagonize this effect)

Dragisic KG, et. al., Fertility and Sterility, Oct 05, 1023-6.
Poor Responders
Luteal E2 prep

- Estrace 2mg PO BID starting on day 21 and continuing for the first 3 days of gonadotropins compared to controls without luteal E2 prep (57 subjects with 228 matched controls).
- No OCP use in luteal E2 group. Test for LH surge. If equivocal, confirm luteal with serum P4.
- Trial involved MDF and antagonist cycles. Rec FSH at 5-6 amps with or without hCG at 10-50 IU/d.
- Subjects were prior poor cycle or anticipated poor response with basal FSH > 12 or BAFC < 5.
- Percentage of embryos ≥ 7 cell stage on d3 significantly higher with luteal E2 prep (46.4% v. 40.6%; p= 0.05).
- Trend towards improved livebirth rate with luteal E2, with these patients having delivery rate increased by 25%. Power analysis to show statistical significance would require 946 subjects.
- Suppressing luteal FSH with Estrace may help prevent asynchronous follicular stim/may improve cycle outcome.

Refractory Endometrium
Including POF, longstanding hypothalamic dysfunction, Kallmann’s, empty sella syndrome, DE recipients nearing menopause with fibroatrophic endometrium

- Oral/vaginal estrace with patch to increase E2 driven proliferation
- Viagra 25mg QID PV to increase vascularity/flow
- 81 ASA QD
- Acupuncture
- 800 mgs pentoxyifylline (PTX) (Pentoxil ER 400 mg x2/d or Trental CR 400 mg x2/d) with 1000 IU tocopherol (Vit E)
  - PTX (and metabolites) improve blood flow properties by decreasing viscosity; vit E as anti-oxidant
Refractory Endometrium

- Successful pregnancies after combined pentoxifylline-tocopherol treatment in women with POF who are resistant to hormone therapy

- Case report of 3 women with echogenic endometrium mean 4.9mm despite high serum E2 levels on ERT
  - PTX 800mg with Vit E 1000mg qday for > 9 months
  - Improvement to mean 7.4mm triple layer
  - 2 embryo FET in two of the patients resulted in pregnancy
  - Fibroatrophic changes ameliorated

- Initial report of 6 women with radiation induced fibroatrophic changes responded to 12 months of therapy with increase in endometrial thickness from 3 to 6mm, 1.5 fold increase in myometrial volume, and restoration of diastolic uterine artery flow
Endometrial activation

- 134 euresponders who failed to conceive to one or more prior ETs (4.0 +/- 2.0 v. 3.9 +/- 2.1).
- 45 randomly selected to undergo biopsy in subsequent attempt; same stimulation protocol.
- 4 pipelle biopsies total on days 8, 12, 21 and 26 in the natural cycle preceding the start of lupron. After natural cycle menses, they started lupron d2 thru 17, when hypo-E2 confirmed, they began gonadotropins.
- Embryos (3.4 +/- 1.0 and 3.1 +/- 0.9), implantation rate (27.7% vs. 14.2% \( P = .00011 \)), clinical PR (66.7% vs. 30.3% \( P = .00009 \)), live birth/ET (48.9% vs. 22.5% \( P = .016 \)).
Endometrial activation
Potential modes of action

- Scratching of the progestational guinea pig uterus provokes rapid growth of endometrial cells identical to decidua cells.
- Intraperitoneal injection of the histamine releasing compound pyrathiazine in rats induced decidual response.
- Scratching induced decidualization in rats reversed by anti-histamine.
- Involves events that accompany wound healing to include secretion of cytokines and growth factors known to be involved in implantation.
Endometrial activation

  - 2 pipelle biopsies total on d21 and 26 during luteal lupron application.

  - Single biopsy in the luteal phase in the cycle preceding stimulation.

  - Biopsy induced gene modulation/factor expression validation with biopsies d11-13 and 21-24.
Endometrial activation
Variations on a theme

- Pre-IVF cold loop polypectomy, gentle curetage of the functionalis, oral estrace 2mg x 5 – 10 days while on OCP prior to cycle start
- Endometrial biopsy in the luteal phase prior to IVF cycle start
- Set up non-infectious inflammatory/repair response to potentially enhance implantation
Michael Swanson, MD; Mark Bush, MD

www.conceptionsrepro.com
Denver, Colorado