IVF Protocols: Hyper & Hypo-Responders, Implantation

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Subset: Hypo-Responders

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Hypo-responder
Identification

1. Age
2. Clinical History
   Prior failed stim, prior decreased MII egg quantity/quality, longstanding unexplained with poor prognosis testing, miscarriage
3. AMH
   < 1.0, > 1.5
   Correlates with age, oocytes, peak E2, number of HQ embryos; IR & PR sig better if > 2.7 (1)
4. RFC
   < 4, > 8
5. d3 FSH
   > 10
   Absence of functional cyst where E2 > 80 drives down FSH and is also indicative of DOR

Hypo-responder
Overview of options

- DHEA
- Luteal E2/antagonist prep
- Dexamethasone 1 mg d1 to trigger
- Letrozole
- Growth hormone
- 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, double rec-hCG
DHEA

- DHEAS levels decrease by 50% from age 25 – 45.
- 50% of follicular fluid T is derived from circulating DHEA; it is also a substrate for E (1).
- FSH receptors are reduced in poor responders and androgen increases FSH receptors on granulosa cells.
- Granulosa cell apoptosis increases as women age.
- Androgen receptor mRNA correlates positively with proliferation and negatively with apoptosis in primate granulosa cells (2).
- Advancement from primordial oocyte pool to recruitable oocyte pool.

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

**Table 1. Comparison of results of IVF before and after treatment with dehydroepiandrosterone (DHEA)**

<table>
<thead>
<tr>
<th></th>
<th>Pre-DHEA</th>
<th>Post-DHEA</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.9 ± 0.8</td>
<td>40.4 ± 0.8</td>
<td>–</td>
</tr>
<tr>
<td>Weeks of DHEA</td>
<td>–</td>
<td>17.6 ± 2.13</td>
<td>–</td>
</tr>
<tr>
<td>Cancellation</td>
<td>8/25 (32%)</td>
<td>1/25 (4.3%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Peak estradiol (pmol/l)</td>
<td>3493 ± 512</td>
<td>4065 ± 589</td>
<td>Not significant</td>
</tr>
<tr>
<td>Oocytes</td>
<td>3.4 ± 0.5</td>
<td>4.4 ± 0.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fertilized oocytes</td>
<td>1.4 ± 0.3</td>
<td>3.0 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percentage of fertilized oocytes</td>
<td>39</td>
<td>67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Day 3 embryo blastomeres</td>
<td>3.4 ± 0.4</td>
<td>4.7 ± 0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Day 3 embryo grade</td>
<td>2.9 ± 0.1</td>
<td>3.4 ± 0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>Cumulative embryo score per oocyte retrieved</td>
<td>8.4 ± 1.5</td>
<td>16.1 ± 1.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Transferred embryos</td>
<td>1.4 ± 0.2</td>
<td>2.4 ± 0.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Normal day 3 embryos</td>
<td>1.2 ± 0.2</td>
<td>2.7 ± 0.4</td>
<td>0.001</td>
</tr>
</tbody>
</table>

- 25 women with DOR. 450 rec-FSH + 150 hMG/d.
- Second cycle had an average of 4 ½ months DHEA lead-in with 75/d.

DHEA

- 89 women with DOR took 75 mg/d DHEA for up to 4 months prior to IVF, controls proceeded straight to IVF without DHEA.

- Day 3 FSH 16 (+/- 1.2) in treatment v. 13.6 (+/- 1.0) controls.

- Cumulative PR (+CA after 6 weeks) 28.4% v. 11.9% (relative hazard of pregnancy, HR, 3.8; 95% CI 1.2 – 11.8, p < 0.05).

- Half of the pregnancies in the DHEA group occurred before IVF; yet strong trend toward higher PR remained with DHEA at IVF (20.6% v. 11.9%).

Barad et al, JARG 2007; 24: 629-34.
DHEA
Treatment effect on AMH

- AMH was evaluated in 55 women with DOR at IVF, given DHEA lead-in of 75/d for average of 2 ½ months.
- AMH significantly improved after DHEA (p=0.002). Age (p=0.007) and length of DHEA treatment (p=0.019) were independently associated with increasing AMH.
- Under age 38 all ages demonstrated higher AMH levels, and improved AMH proportionally more than females > 38.
- Longitudinal, AMH improved by ca. 60% from 0.22 ± 0.22 ng/mL to 0.35 ± 0.03 ng/ml (p<0.0002). Those reaching IVF had pregnancies in 23.64%. Pregnant women showed more improvement in AMH than those who did not conceive (p=0.001).

DHEA
Treatment effect on AMH

• Presents objective evidence for improvements in ovarian reserve (OR) after DHEA at all ages.
• Concurrent with reported clinical outcomes, it is more pronounced in younger (premature aging) than older (physiologic aging) women.
• DHEA-associated improvements in OR statistically reflect subsequent improvements in cumulative clinical pregnancy rate.
• AMH, therefore, is reflective of improving OR and pregnancy chances in women who receive DHEA supplementation.

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** (progestin 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.

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

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

Letrozole

Poor Responders

Letrozole
GH (thru hepatic IGF-1 production) reduces apoptosis and improves the health and proliferation of granulosa cells.
Growth Hormone

- Meta-analysis of RCTs in poor responders GH increased pregnancy and live-birth rates 3x and 4x compared with placebo.

- RCT, > 40, poor responders GH increased pregnancy and live-birth rates 4X and 5x over patients not given GH with otherwise identical stim regimen (450 recFSH + 150 hMG/d). Significantly increased intrafollicular E2 levels and a trend towards better embryo quality was also observed.
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

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 HP-FSH/HP-hMG has 16 isoforms (3,4)
- Hamster derived recFSH has 11 isoforms (5)
- An additional difference is that HP-hMG contains 10IU of LH activity (as hCG) per 75 IU

FSH Isoform Profile

- Isoforms characterized by isoelectric focusing according to pH
- HP-FSH 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>Bravell&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 (HP-hMG 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).