Resistant Ovaries: the endocrinology of poor responders

by

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Resistant Ovaries

**definition**

Despite containing primordial follicles these ovaries have functioned normally after the menarchie but, soon after, entered into endocrine (?) and exocrine hypofunction,

or that

after GnRH “down-regulation” did not respond to FSH stimulation.
Resistant Ovaries
clinical picture

Secondary amenorrhea ?
Hypoestrogenism ?
Vasomotor symptoms ?
Resistant Ovaries

• In naturally occurring anovulation
  [Savage Syndrome, Premature Ovarian Failure (POF), Premature Menopause (PM)]

• In “down-regulation” induced anovulation
Resistant Ovaries

is
Premature Ovarian Failure (POF)
the same as
Premature Menopause (PM)?
No !
Spontaneous premature ovarian failure is not an early menopause

The disorder may be associated with other conditions that require evaluation and management, including hypothyroidism, dry eye syndrome, abnormal karyotype, or a premutation of the FMR1 gene. Finally, clinicians need to be sensitive to the emotional aspects of this disorder when delivering the diagnosis and during subsequent management.

Hypergonadotrophic hypogonadism with preserved fertility--a new syndrome?

The aetiology of the resistant ovary syndrome is not known. Several theories have been proposed, such as abnormal endogenous gonadotrophins, lack of gonadotrophin receptors of the target cells, inhibitors preventing the normal action of the gonadotrophins on the target cell as well as the possibility of an autoimmune disease. But as yet no conclusive evidence of the validity of any of these theories has been produced.

An unusual case of ovarian resistance syndrome

An animal model has been developed to show what happens when the FSH receptor is missing or nonfunctional. **In FSH receptor knockout in mutant female the ovary is atrophic, there are ovulatory and skeletal defects.**

Mechanisms of premature ovarian failure

Many transgenic "knock-out" animals have been created with deficient ovarian function. Most interesting along these lines is the heterozygous FSH receptor knock-out, which exhibits a reduced follicle reserve and early ovarian depletion.

Santoro N. Ann Endocrinol (Paris)2003;64(2):87-92
Resistant Ovaries
Premature ovarian failure

- Idiopathic
- Genetic
- Iatrogenic
- Immunologic
- Enzyme defects (17αOH-ase, aromatase)
- Viral (mumps)
Autoimmune endocrinopathies associated with R.O.

- Hipocorticalism, Hipoparathyroidism
- Schmidt Syndrome (*Addison’s disease* + chronic lymphocytic thyroiditis +/- *Diabetes mellitus*)
- Idiopathic Addison’s disease + hyperthyroidism and DM
- Autoimmune thyroid diseases
- Pernicious anemia (*thyrogastric antibodies* +/- D.M.)
- D.M., vitiligo
- Miastenia gravis
- Antibodies to FSH/LH receptors or postreceptor dysfunctions
Regulators of follicular recruitment, selection, growth, dominance leading to ovulation of a fertilizable oocyte

- FSH and LH (pulsatility, isoform distribution, B/I)
- FSH and LH gradients in the ovary (circulation)
- Receptors for FSH, LH, estrogens (theca and granulosa)
- Biosynthesis of estrogens (two cell theory)
- Intrafollicular milieu (oocyte maturation inhibitor, luteinization inhibitor, ???)
Diagnostic Strategies

- RIA vs. Bioassays of FSH and LH (rythms)
- DHEA, 17αOH-Prog, estradiol, androgens
- Insulin
- TSH, fT3, fT4
- Antibody assays (e.g. ovary, thyroid, etc.)
- Ultrasound imaging
- Doppler of the ovaries
- Biopsy of the ovaries
Ovarian biopsy for differential diagnosis

Are there false positives for PM?
(absence of primordial follicles)
Yes !
Factors that may interfere with ovulation

Clinical evidence
Therapeutic approaches

A. The quality of the stimulation
B. The quality of the ovarian response
C. Conditioners of the ovarian response
FOLLICULAR MATURATION

FSH

ESTROGENS

LH

OVULATION
A. The quality of stimulation

- LHRH pulsatile
- HMG vs p-FSH vs r-FSH; r-hCG vs r-LH
- Isoform distribution (B/I)
- Dose and regimen \((\text{step up vs step down})\)
- Clomiphene → HMG or FSH
- Clomiphene or tamoxifen +/- dexamethasone and/or metformin \((\text{doses, duration})\)
- Aromatase inhibitors
- Oral contraceptives
Time of initiation of clomiphene citrate and pregnancy rate in PCOS

100 mg/day on days 1 through 5 of the menstrual cycle (group 1);
41 collectively underwent 73 cycles of CC at the same concentration on days 5 through 9 (group 2).

Ovulation rates were 72.8% in group 1 and 70.8% in group 2 (P=.78), and pregnancy rates were 40.5% in group 1 and 19.5% in group 2 (P=.04). CONCLUSION: Treatment with CC is associated with higher rates of pregnancy if started early (days 1-5) in the menstrual cycle.

An extended regimen of clomiphene consisting of 250 mg of clomiphene for 8 days followed by the administration of 10,000 IU of human chorionic gonadotropin (hCG) 6 days later was administered to 13 oligomenorrheic women who had previously failed to ovulate when treated with 250 mg of clomiphene for 5 days and hCG. Eight of these 13 women ovulated.

An extended 10-day course of clomiphene citrate (CC) in women with CC-resistant ovulatory disorders

At least one cycle of 100 mg CC from days 3 to 12. Fourteen patients (47%) ovulated during 31 of their 48 cycles (65%). Five women (17%) conceived a total of seven singleton pregnancies, including five term deliveries and two spontaneous abortions.

Use of micro-dose human chorionic gonadotropin (hCG) after clomiphene citrate (CC) to complete folliculogenesis in previous CC-resistant anovulation

Patients who had failed to ovulate after 150 mg CC.

The study patients were treated with 100 mg CC but started a 200 IU hCG I.M. injection daily when the largest follicle was ≥ 12mm mean diameter

Branigan EF and Estes A. Am Journal Obst Gynecol 2005;192(6):1890-4
Use of micro-dose human chorionic gonadotropin (hCG) after clomiphene citrate (CC) to complete folliculogenesis in previous CC-resistant anovulation

The low-dose hCG group had significantly higher percentage of ovulatory cycles (57% vs 7%, P < .001), peak E2 levels (378 pg/mL vs 125 pg/mL, P < .01), and pregnancy rates (18% vs 0%, P < .001).

Branigan EF and Estes A. Am Journal Obst Gynecol 2005;192(6):1890-4
Use of micro-dose human chorionic gonadotropin (hCG) after clomiphene citrate (CC) to complete folliculogenesis in previous CC-resistant anovulation

The use of micro-dose hCG in the late follicular phase results in continued follicle growth, increased E2 levels, ovulation and pregnancies.

This treatment offers an efficient and cost-effective alternative before gonadotropin therapy for this type of patient.

Branigan EF and Estes A. Am Journal Obst Gynecol 2005;192(6):1890-4
Extended clomiphene citrate (CC) and *prednisone* for the treatment of chronic anovulation resistant to CC alone

Treatment consisted of CC given on cycle days 3 through 9 (extended) at a starting dose of 100 to 150 mg/d. **Additionally, patients were given prednisone 5 mg orally each night throughout the cycle.**

Extended clomiphene citrate (CC) and prednisone for the treatment of chronic anovulation resistant to CC alone

A total of 60 cycles were available for review. Forty-four of these cycles were ovulatory (73%) and 11 patients (46%) conceived on this therapy.

Use of dexamethasone and clomiphene citrate in the treatment of clomiphene citrate-resistant patients with PCO and normal dehydroepiandrosterone sulfate levels

Two hundred thirty women with PCOS and normal DHEAS who failed to ovulate after routine protocol of CC. **80% of the treatment group** and **20% of the control group** had evidence of ovulation.

Clomiphene citrate and dexamethasone in treatment of clomiphene resistant PCO

100 mg CC from day 3 to 7 of the cycle and dexamethasone 2 mg/day from day 3 to day 12 of the cycle.

Elnashar A et al. Hum Reprod 2006;21(7):1805-8
Clomiphene citrate and dexamethasone in treatment of clomiphene resistant PCO

The high-dose, short-course regimen is more convenient to the patient with higher ovulation (75–88%) and pregnancy rates (40–40.5%) according to the results of the present study.

Elnashar A et al. Hum Reprod 2006;21(7):1805-8
Ovulation induction in clomiphene-resistant anovulatory women with normal dehydroepiandrosterone sulfate levels: beneficial effects of the addition of dexamethasone during the follicular phase

To evaluate the effect on ovulation of a 10-day course of dexamethasone (DEX) initiated concurrently with a 5-day course of clomiphene citrate (CC) in CC-resistant patients with normal DHEAS levels.

Ovulation induction in clomiphene-resistant anovulatory women with normal dehydroepiandrosterone sulfate levels: beneficial effects of the addition of dexamethasone during the follicular phase

Treatment consisted of 50 mg CC on menstrual cycle days 3 through 7 with 0.5 mg DEX on days 3 through 12. The dose of CC was increased to 100 mg and then 150 mg if anovulation persisted.

Ovulation induction in clomiphene-resistant anovulatory women with normal dehydroepiandrosterone sulfate levels: beneficial effects of the addition of dexamethasone during the follicular phase

Eleven of 13 women had evidence of ovulation. Five clinical pregnancies were achieved.

B. The quality of the ovarian response

- Number of FSH receptors (*give estrogens*)
- Gradient of FSH in the ovary = circulation (*give estrogens, NO suppliers ? short wave diathermy??*)
- HRT, or ET
- Raloxifene ?
- Aromatase inhibitors ?
- Testosterone ?
- Ovarian drilling ?
FSH receptor gene polymorphisms have a role for different ovarian response to stimulation in patients entering IVF/ICSI-ET programs.

GR patients carry more often the Asn/Ser genotype. The latter is correlated with more follicles and oocytes in both OD and GR patients. The Ser/Ser variant might be related to higher serum FSH levels, while the Asn/Ser with lower.

Ovarian response to gonadotropin stimulation depends on the level of follicle stimulating hormone receptor on granulosa cells

The expression of FSHR was significantly different among the 3 groups, being 0.19 +/- 0.07, 0.34 +/- 0.16, and 0.45 +/- 0.18 for poor, moderate and high responders, respectively. Poor responders had the lowest peak levels of serum E(2) and the least number of follicles. (2) The FSHR was positively correlated with peak level of E(2) in serum and the number of follicles.

C. Conditioners of the ovarian response

- Insulin resistance (metformin)
- ↑ plasma androgens (dexamethasone)
- ↓ androgens in the ovary (aromatase inhibitors)
- ↓ gradient of FSH in the ovary = ↓ circulation (estrogens, raloxifene, NO suppliers?)
Efficacy of combined metformin–letrozole in comparison with metformin–clomiphene citrate in clomiphene-resistant infertile women with PCO

Nestler et al. (1998) showed that metformin increases ovarian response to clomiphene citrate in obese women with PCOS.

Efficacy of combined metformin–letrozole in comparison with metformin–clomiphene citrate in clomiphene-resistant infertile women with PCO

In clomiphene-resistant PCOS patients the combination of letrozole and metformin leads to higher full-term pregnancies (34.5% vs. 10%).

Comparisons of follicular levels of sex steroids in poor responder and normoresponder patients undergoing ovarian stimulation with GnRH antagonist

Serum and follicular levels of E(2) and progesterone were significantly lower in the poor responder group compared to the normoresponder group. **Follicular level of testosterone was also lower in poor responders**, but not to a level of statistical significance. **The serum FSH level was higher in the poor responder group**, but **follicular levels of gonadotropins did not differ between the two groups.**

Androgen priming using aromatase inhibitor and hCG during early-follicular-phase GnRH antagonist down-regulation in modified antagonist protocols

Temporary exposure of follicles to increased levels of androgens may enhance their sensitivity to FSH. The aim of this study was to increase the intraovarian androgen level using aromatase inhibitors and hCG before controlled ovarian stimulation (COH) and to test this concept clinically.

Lossl K et al. Hum Reprod 2006 Jul 10; [Epub ahead of print]
Pretreatment with transdermal testosterone may improve ovarian response to gonadotrophins in poor-responder IVF patients with normal basal concentrations of FSH

All patients received transdermal testosterone treatment (20 µg/kg per day) during the 5 days preceding gonadotrophin treatment.

Balaisch J et al. Hum Reprod 2006;21(7):1884-93
Pretreatment with transdermal testosterone may improve ovarian response to gonadotrophins in poor-responder IVF patients with normal basal concentrations of FSH.

Twenty patients (80%) showed an increase of over fivefold in the number of recruited follicles, produced 5.8 ± 0.4 (mean ± SEM) oocytes, received two or three embryos and achieved a clinical pregnancy rate of 30% per oocyte retrieval. There were 20% cancelled cycles.

Balaisch J et al. Hum Reprod 2006;21(7):1884-93
Dehydroepiandrosterone supplementation augments ovarian stimulation in poor responders

Women were given 80 mg/day of oral micronized DHEA for 2 months. The oestradiol/ampoule ratio increased in all six cycles, by a mean of 2.94 +/- 0.50 fold (P = 0.012). One of the cycles resulted in a delivered twin pregnancy. In this small series, DHEA improved response to ovarian stimulation even after controlling for gonadotrophin dose.

Effect of **dehydroepiandrosterone** on oocyte and embryo yields, embryo grade and cell number in IVF

Twenty-five women with significantly diminished ovarian reserve had one IVF cycle before and after DHEA treatment, with otherwise identical hormonal stimulation. Women received 75 mg of DHEA daily (25 mg three times daily) for an average of 17.6 ± 2.13 weeks.

Barad D and Gleicher N  *Hum. Reprod.* Advance Access published online on September 22, 2006
Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF

After DHEA supplementation, there was a significant increases in fertilized oocytes ($P < 0.001$), normal day 3 embryos ($P = 0.001$), embryos transferred ($P = 0.005$) and average embryo scores per oocyte ($P < 0.001$) after DHEA treatment.

Barad D and Gleicher N *Hum. Reprod*. Advance Access published online on September 22, 2006
Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF

CONCLUSION: This study confirms the previously reported beneficial effects of DHEA supplementation on ovarian function in women with diminished ovarian reserve.

Barad D and Gleicher N  *Hum. Reprod.* Advance Access published online on September 22, 2006
Intraovarian blood flow during spontaneous and stimulated cycles

Preovulatory changes of blood flow in different regions of the human follicle

During the ovulatory process there are prominent changes in the regional blood flow of the follicle with a marked increase of the flow to the base of the follicle and a concomitant decrease of blood flow to the apex. These changes may be essential for the release of a mature oocyte.

Perifollicular vascularity in poor ovarian responders during IVF

Clinical pregnancy and live birth rates were 24.1% and 15.5%, respectively, in poor responders who developed ≤3 dominant follicles during ovarian stimulation for IVF treatment. There were no significant differences in the implantation, clinical pregnancy and live birth rate among poor responders with and without high-grade perifollicular vascularity.

Quantification of ovarian stromal Doppler signals in poor responders undergoing in vitro fertilization with three-dimensional power Doppler ultrasonography

The 3D power Doppler indexes of ovarian stromal blood flow in poor responders was significantly lower than normoresponders. This may help to explain the poor response during HCG administration in controlled ovarian stimulation.

A tentative explanation for the occurrence of pregnancies in Resistant Ovaries after

- Oral contraceptives (OC)
- Hormone replacement therapy (HRT or ET)
- Raloxifene?
Short-term gonadotropin suppression with oral contraceptives benefits poor responders prior to controlled ovarian hyperstimulation.

The good outcome associated with OC pretreatment may reflect production or alterations of local ovarian growth factors and/or changes in endometrial expression. Administration of exogenous estrogen may be particularly beneficial for perimenopausal women in their forties with ovarian follicular depletion.

Augmentation of low ovarian response to superovulation before in vitro fertilization following priming with contraceptive pills

Contraceptive pills were administered for 28-42 days and were immediately followed by menotropin treatment.

Thirty-three of the cycles (66%) reached the stage of ovum pick-up, compared with 22 (25%) of the previous IVF cycles in these women. The mean number of oocytes retrieved was 6.1 +/- 3.0 and 2.4 +/- 1.3 in the study and control groups, respectively (P <0.01; paired Student's t test). Embryo transfer (ET) was performed in 62% of the treatment cycles and resulted in five clinical pregnancies (16.1% per ET). No pregnancies were recorded in the control group.

Treatment of clomiphene citrate-resistant anovulation with the use of oral contraceptive pill suppression and repeat clomiphene citrate treatment

Suppression of the ovary with oral contraceptives results in excellent rates of ovulation and pregnancy in patients who previously were resistant to clomiphene citrate. The decreases in ovarian androgens, luteinizing hormone, and 17 beta-estradiol may be responsible for the improved response.

OC’s

• Suppression of pituitary FSH
• Rebound with better FSH isoforms
• Release of “abnormal” FSH bound to granulosa FSH receptors
• More available unoccupied FSH receptors ready to bind better FSH molecules
OC’s

The first “phase” of triphasic preparations would seem preferable since it is more estrogenic (more EE, less progestagen)

FSH should be monitored until low levels are obtained
HRT or ET

- Synthesis of FSH receptors (granulosa)
- Stimulation of mitosis in the granulosa
- Influence on the biosynthesis of pituitarian gonadotropins?
A sequential preparation would be preferable since the lack of a withdrawal bleeding might suggest a pregnancy.
Rationale for a tentative use of Raloxifene

- Knok-out mice for the β-ER display a pattern of PCO
- The administration of a ligand (raloxifene) for the β-ER normalizes the morphology
- Raloxifene improves ovarian circulation, thus better gradients
Priming (?) effect of clomiphene prior to FSH stimulation

• Unlikely to be mediated through pituitary gonadotropin output

• **Proably due to clomiphene being a SERM, i.e. a β-ER ligand**

• Would be more appropriate to prime either with 17β-estradiol or with a better SERM like raloxifene
What about my own experience?

- A 34 year old woman who developed amenorrhea since the age of 30. She complained of vasomotor symptoms. Had very low estrogens and FSH.
- She was treated with MHG (up to 300 IU/day) for several cycles without any response.
- A sequential HRT was started and after 2 years, since there was a cycle with no withdrawal bleeding, she was found to be pregnant.
- After a full term pregnancy she delivered a normal baby.
What about my own experience?

- A 32 year old physician who had undergone several treatments elsewhere because of a long lasting amenorrhea.
- FSH was not elevated and estradiol was medium-low. No vasomotor symptoms.
- Several HMG cycles were ineffective.
- An estrogen only treatment was started. A few months later an US showed small follicles in both ovaries.
What about my own experience?

(cont.)

• Later on a good 20mm follicle was identified. hCG (5000 IU) was injected; the follicle underwent atresia.

• In subsequent cycles estrogen treatment stimulated the growth of several follicles but hCG has always induced atresia.

• Therefore I decided to keep her only on estrogens and increase their dose when a preovulatory follicle was found.

• This resulted in ovulation in several succeeding cycles (by US and high progesterone levels).

• After some more similar cycles she became pregnant and delivered a normal baby, after full term.
FOLLICULAR ATRESIA

MATURATION

LH
LHRH (?)
x ?
What about my own experience?

- Two more cases, similar to this last one, who responded favorably (ovulation) to an estrogen only treatment. One became pregnant.
and now…

to our pertinent questions …

Nature …answers …
In resistant ovaries, is the induction of ovulation the only aim?

No!

Why not?

Because the quality of the oocyte, influenced by intrafollicular regulators, is a condition of its fertilizability!
Successful pregnancies after combined pentoxifylline-tocopherol treatment in women with premature ovarian failure who are resistant to hormone replacement therapy

In women with POF and uterine resistance to HRT, combined PTX-Vit.E reduces fibroatrophic uterine lesions and improves the uterine response to HRT, thus allowing embryo implantation and ongoing pregnancy.

Is a FSH > 40 IU/L always diagnostic of a primary ovarian failure?

No!
Can high FSH levels alternate with normal levels?

Yes!
Can high FSH levels spontaneously revert to normal?  
Yes!
Can a POF spontaneously revert to a normal ovulatory ovarian function?

Yes!
If YES do these women remain normal after a full term pregnancy?

Usually … No!
In Conclusion…

and to make short
a very long and enigmatic story
about the treatment of R.O.’s …
and despite some strategies, like...

- Weight reduction in obese women
- Metformin + *stimulation protocols*
- Corticosteroids + …
- Testosterone + …
- Aromatase inhibitors + …
- DHEA + …
- T3/T4 ? + …
- Oral contraceptives
- HRT or ETR
Patients and Doctors…

must be very, very ...

LUCKY !
Thank you very much for your attention