Forty-second Annual
Postgraduate Program

October 17, 2009
Atlanta, GA

Improving the Outcome of IVF from Ovarian Stimulation to Luteal Phase Support

Course 9

Developed in Cooperation with the Middle East Fertility Society

Sponsored by the American Society for Reproductive Medicine

New Procedure to Obtain CME Credits

Dear Postgraduate Course Participant:

The Accreditation Council for Continuing Medical Education now requires that ASRM document learning for participants in CME programs. Thus, the procedure for claiming CME credits has changed. We ask your cooperation in following the steps below to ensure that your credits are provided correctly to you.

1. Within 3 days after the Annual Meeting you will be sent an email asking you to complete an online evaluation of this postgraduate course. A personalized Web link to the evaluation will be provided in your email. Please do not share this unique link.
2. In late November you will be sent a second email with a personalized Web link asking you to complete the post-test on the content of the course. This test is identical to the pre-test and will enable ASRM to assess the effectiveness of this postgraduate course as a learning activity. For your convenience, the test questions are printed in the course syllabus.

After both steps have been completed, you will be able to claim your CME credits and/or ACOG Cognates and receive a printable CME certificate. Please note that you must provide your 10-digit ACOG Membership Number to have your ACOG Cognates reported to ACOG. Results of both the course evaluation and the post-test are anonymous.

Both steps must be followed completely by **December 31, 2009** in order to receive CME credits. A maximum of 6.5 CME credits can be claimed for the postgraduate course. Please be aware that some email systems flag emails with Web links as junk mail, and you may need to check your junk-email folder for your notifications.

Please DO NOT forward the links. In case of difficulty please email *pfenton@asrm.org*

********Deadline for receiving CME credits = December 31, 2009********

Continuing Medical Education

Continuing medical education is a lifelong learning modality to enable physicians to remain current with medical advances. The goal of ASRM is to sponsor educational activities that provide learners with the tools needed to practice the best medicine and provide the best, most current care to patients.

As an accredited CME provider, ASRM adheres to the Essentials and policies of the Accreditation Council for Continuing Medical Education (ACCME). CME activities now must first, address specific, documented, clinically important gaps in physician competence or performance; second, be documented to be effective at increasing physician skill or performance; and third, conform to the ACCME Standards for Commercial Support.

AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE

Developed in Cooperation with the

MIDDLE EAST FERTILITY SOCIETY

ANNUAL MEETING POSTGRADUATE COURSE

ATLANTA, GA

OCTOBER 17, 2009

"IMPROVING THE OUTCOME OF IVF FROM OVARIAN STIMULATION TO LUTEAL PHASE SUPPORT"

Chair: **Botros Rizk, M.D.**
University of South Alabama
Department of Obstetrics and Gynecology
Phone: 251-415-1491
Fax: 251-415-1552
botros3@aol.com

Co-Chair: **Mohamed Aboulghar, M.D.**
Cairo University and the Egyptian IVF Center
10 Geziret El Arab Street
Mohandessin 12411 Cairo
Egypt
Phone: 20 2 37498488
Fax: 20 2 33383049
Email: ghar@link.net

Faculty: **David R. Meldrum, M.D.**
Scientific Director
Reproductive Partners Medical Group, Inc.
510 North Prospect Avenue, Suite 202
Redondo Beach, California 90277
Phone: 310-318-3010
Fax: 310-798-7304
Email: davidmeldrum@earthlink.net

William Schoolcraft, M.D., H.C.L.D.
Colorado Center for Reproductive Medicine
10290 RidgeGate Circle
Lone Tree, Colorado 80124
Phone: 303-788-8300
Fax: 303-788-8310
Email: bschoolcraft@colocrm.com

All speakers at the 2009 ASRM Annual Meeting and Postgraduate Courses were required to complete a disclosure form. These disclosures were reviewed and potential conflicts of interest resolved by the Subcommittee on Standards of Commercial Support of the Continuing Medical Education Committee. The faculty has revealed the following information as potential conflicts of interest:

Botros Rizk, M.D.: Boehringer-Ingelheim, Solvay, Proctor and Gamble and Eli-Lilly: Research/Principal Investigator, Wyeth, Proctor and Gamble/Sanofi-Aventis, Duramed, Myriad, Warner-Chilcott: Speakers' Bureau

Mohamed Aboulghar, M.D.: Nothing to disclose

Botros Rizk, M.D.: Boehringer Ingleheim, Solvay: Research support /principal investigator

David R. Meldrum, M.D.: Nothing to disclose

William Schoolcraft, M.D., H.C.L.D.: Nothing to disclose

This activity may include discussion of off-label or otherwise non-FDA approved uses of drugs or devices.

Accreditation statement:
The American Society for Reproductive Medicine is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

Designation statement:
The American Society for Reproductive Medicine designates this educational activity for a maximum of 6.5 *AMA PRA Category 1 Credits™*. Physicians should only claim credit commensurate with the extent of their participation in the activity.

American College of Obstetricians and Gynecologists (ACOG)
The American College of Obstetricians and Gynecologists has assigned 6.5 cognate credits to this activity.

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Please turn off/mute cell phones and pagers during the postgraduate course and all Annual Meeting sessions.

Thank you.

IMPROVING THE OUTCOME OF IVF FROM OVARIAN STIMULATION TO LUTEAL PHASE SUPPORT

NEEDS ASSESSMENT AND COURSE DESCRIPTION

From the start of ovarian stimulation to the luteal phase, there are many procedures to be executed during each IVF cycle. Stimulation protocols differ considerably in type of gonadotropins, type of GnRH analogue and use of a variety of adjuncts to ovarian stimulation. The performance of clinicians also varies. Why do some use agonists, others use antagonists, and why use urinary FSH or recombinant FSH, or a combination of both? Which adjuncts should be used, if any? How do they deal with poor responders? How and what dose is used to trigger ovulation for final maturation of oocytes? Is LH supplementation needed during stimulation? Is in vitro maturation of oocytes an accepted routine procedure or still an experimental treatment? Physicians vary widely in their answers to these critical therapeutic questions.

This course is aimed at IVF specialists, clinical endocrinologists, fellows in reproductive medicine, infertility specialists, scientists and biologists in IVF laboratories and residents in obstetrics and gynecology. Participants will learn to differentiate between what is practiced and what is evidence-based, helping to improve their practice of medicine and benefit their patients. The faculty will clarify the role of preimplantation genetic testing in IVF and will address the huge knowledge gap regarding the duration of luteal phase support.

ACGME COMPETENCY

Patient Care
Medical Knowledge

LEARNING OBJECTIVES:

At the conclusion of this course, participants should be able to:

1. Describe the role of GnRH antagonists in ovarian stimulation for IVF.
2. Compare different gonadotropins used for ovarian stimulation.
3. Implement treatment protocols for patients who respond poorly to gonadotropins.

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OCTOBER 17, 2009

"IMPROVING THE OUTCOME OF IVF FROM OVARIAN STIMULATION TO LUTEAL PHASE SUPPORT"

Botros Rizk, M.D., Chair

Mohamed Aboulghar, M.D., Co-Chair

Saturday, October 17, 2009

08:15 – 08:30	Course Introduction and Orientation **Mohamed Aboulghar, M.D.**
08:30 – 09:00	Different Gonadotropins for Ovarian Stimulation: How Can We Choose? **Botros Rizk, M.D.**
09:00 – 09:30	Current Role of GnRH Antagonist for ART **Mohamed Aboulghar, M.D.**
09:30 – 10:00	Panel Discussion, Questions and Answers **Moderator: David R. Meldrum, M.D.**
10:00 – 10:30	Break
10:30 – 11:00	Uses of Various Adjuncts for Optimizing Ovarian Stimulation and Embryo Quality **David R. Meldrum, M.D.**
11:00 – 11:30	Ovarian Reserve Testing and the Treatment of Poor Responders **William Schoolcraft, M.D., H.C.L.D.**
11:30 – 12:00	Panel Discussion, Questions and Answers **Moderator: Botros Rizk, M.D.**
12:00 – 13:00	Lunch
13:00 – 13:30	Triggering Ovulation for Final Maturation of Oocytes **Botros Rizk, M.D.**
13:30 – 14:00	Lifestyle, Acupuncture, Stress Management, Erectile Function, Nutrition and Supplements In the Management of Infertility **David R. Meldrum, M.D.**

Saturday, October 17, 2009 (continued)

14:00 – 14:30	Critical Evaluation of the Use of LH **Mohamed Aboulghar, M.D.**
14:30 – 15:00	Panel Discussion, Questions and Answers **Moderator: William Schoolcraft, M.D., H.C.L.D.**
15:00 – 15:30	Break
15:30 – 16:00	Limits of Day 3 Biopsy for PGS **William Schoolcraft**
16:00 – 16:30	Luteal Phase Support in Reproduction: Why, When, What and How? **Mohamed Aboulghar, M.D.**
16:30 – 17:00	Panel Discussion, Questions and Answers **Moderator: Mohamed Aboulghar, M.D.**

DIFFERENT GONADOTROPINS FOR OVARIAN STIMULATION: HOW CAN WE CHOOSE?

Botros Rizk, M.D., M.A.
Professor And Director,
Division of Reproductive Endocrinology and Infertility
University of South Alabama College of Medicine
Mobile, Alabama

LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

1. Review the function and pharmacodynamics of follicle-stimulating hormone (FSH) and luteinizing hormone (LH).
2. Explain ovarian response in relation to FSH-receptor polymorphisms.
3. Describe the historical development of gonadotropins.
4. Perform a 10-year review of meta-analyses on gonadotropins.
5. Discuss future developments in gonadotropins.

Different Gonadotropins for Ovarian Stimulaton: How Can We Choose?

Botros Rizk, M.D., M.A., F.R.C.O.G., F.R.C.S.(C), H.C.L.D., F.A.C.O.G., F.A.C.S.

Professor and Director
Division of Reproductive Endocrinology and Infertility
University of South Alabama College of Medicine
Mobile, Alabama

Learning Objectives

At the conclusion of this presentation, participants should be able to:

- Review the function and pharmacodynamics of follicle-stimulating hormone (FSH) and luteinizing hormone (LH).
- Explain ovarian response in relation to FSH-receptor polymorphisms.
- Describe the historical development of gonadotropins.
- Perform a 10-year review of meta-analyses on gonadotropins.
- Discuss future developments in gonadotropins.

Objectives

- Compare the efficacy and safety of human menopausal gonadotropin (hMG) vs. recombinant FSH (rFSH) in in vitro fertilization-intracytoplasmic sperm injection (IVF-ICSI) cycles.
- Compare the efficacy and safety of hMG vs. rFSH in IVF cycles and ICSI separately.
- Compare the efficacy and safety of urinary FSH vs. rFSH.
- Assess the efficacy and safety of recombinant LH (rLH) supplementation
- Compare the efficacy and safety of highly purified rFSH (HP-rFSH) in gonadotropin-releasing hormone (GnRH) antagonist cycles.

DISCLOSURE

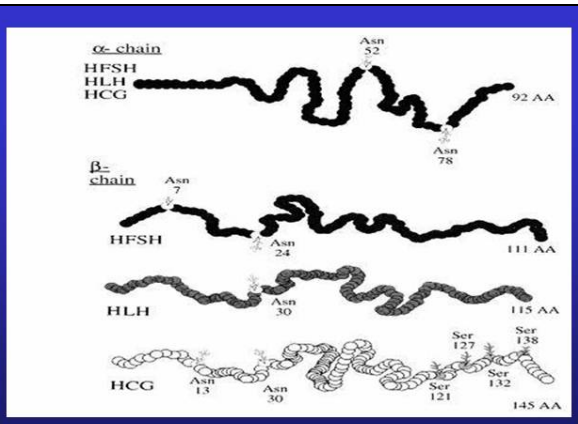
- Research/Principal Investigator: Boehringer-Engelheim, Solvay, Proctor and Gamble and Eli-Lilly
- Speaker honoraria: Wyeth, Proctor and Gamble/Sanofi-Aventis, Duramed, Myriad, Warner-Chilcott.

Follicle-Stimulating Hormone

FSH plays a central role in oogenesis. It triggers the maturation of follicles, proliferation of granulosa cells and aromatase enzyme induction. Its role is pivotal in the recruitment of the dominant follicle.

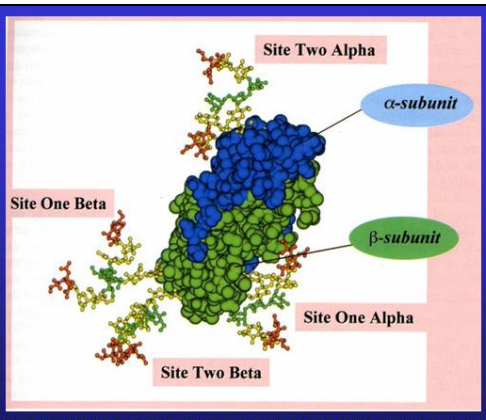
FSH, LH and Human Chorionic Gonadotropin (hCG)

FSH and LH are produced and secreted by the pituitary gland as a highly heterogenous glycoprotein. FSH, LH and hCG consist of a common alpha subunit and a receptor-specific beta subunit.



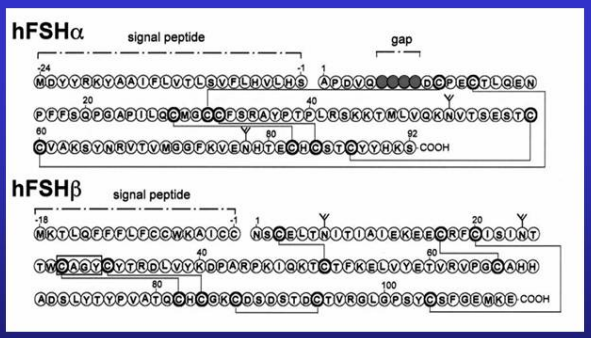
Schematic Representation

Olijve et al. 1996;Mol Hum Reprod 2:371-382



Model of a Fully Glycosylated and Sialylated Human FSH Molecule

Edwards and Risquez (Eds) 2003 Reproductive Biomed Online

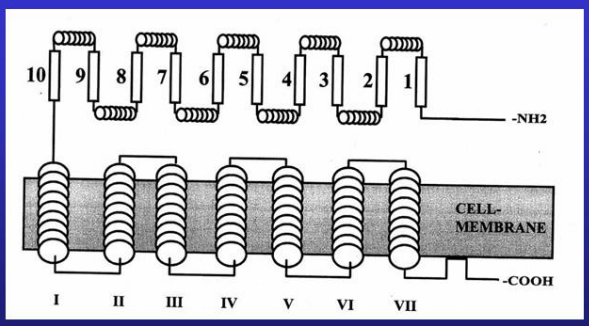


The Sequence of the Common Human α-subunit

Edwards and Risquez (Eds) 2003 Reproductive Biomed Online

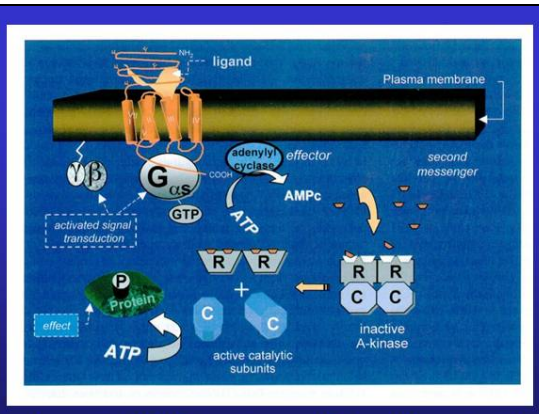
FSH Receptor

- The FSH receptor is a glycoprotein belonging to the family of G-protein coupled receptors. Complex transmembrane proteins are characterized by seven hydrophobic helices inserted in the plasmalemma, and by intracellular and extracellular domains



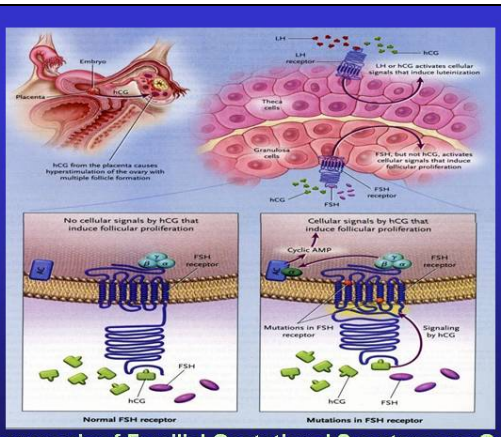
FSH Receptor

Simoni et al. 1997: Endocrine Rev 18:739-773



Attachment to FSH Receptor and Signal Transduction

Edwards and Risquez (Eds) 2003 Reproductive Biomed Online



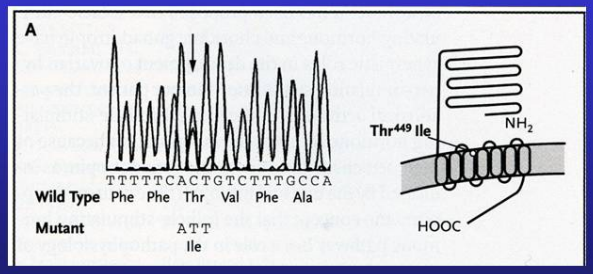
Pathogenesis of Familial Gestational Spontaneous Ovarian Hyperstimulation Syndrome (OHSS)

Kaiser B (2003) N Engl J Med 349:729-732

Activating Mutations of FSH Receptor (FSHR) and OHSS

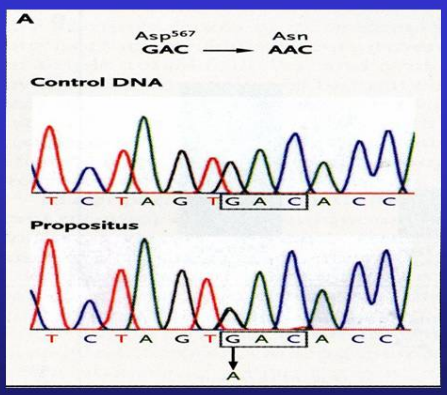
Asp567 → Asn	*FSHR increased sensibility to FSH or HCG* *Spontaneous or iatrogenic OHSS*
Thr 449 → Ile	*FSHR increased sensibility to FSH, HCG or thyroid stimulating hormone (TSH)*
Thr 449 → Ala	*Spontaneous or iatrogenic OHSS, even due to hypothyroidism*
Ile 545 → Thr	*FSHR increased sensitivity to FSH, HCG, or TSH* *Spontaneous or iatrogenic OHSS, even due to hypothyroidism*

Rizk B (2009) Reproductive Biomed Online



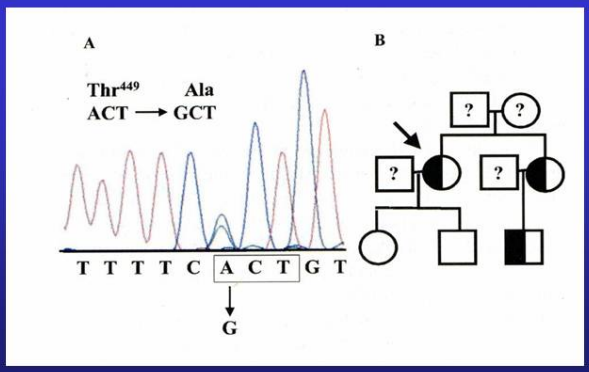
FSH-Receptor Mutation and Spontaneous OHSS

Vasseur et al. (2003) N Engl J Med 349:753-759



FSH-Receptor Mutation and Spontaneous OHSS

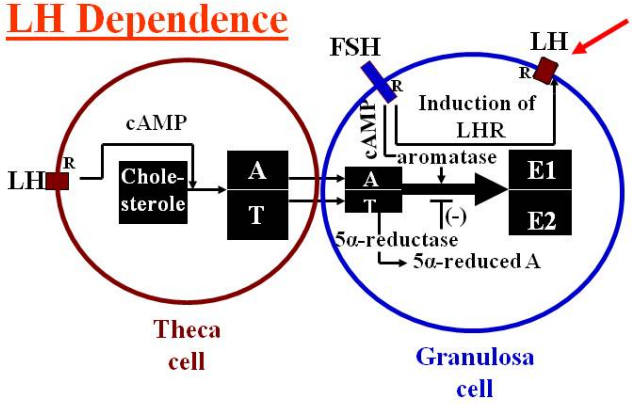
Smits et al. (2003) N Engl J Med 349:760-766



FSH-Receptor Mutation and Spontaneous OHSS.
Detection of the T449 A Mutation.

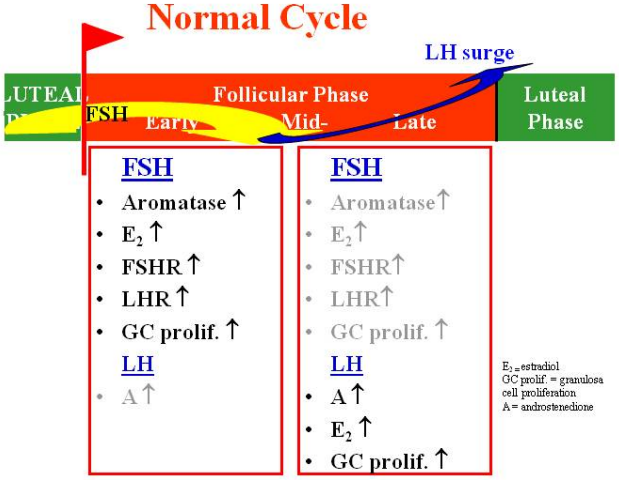
Montanelli et al. (2004) J Clin Endocrinol Metab 89:1255-1288

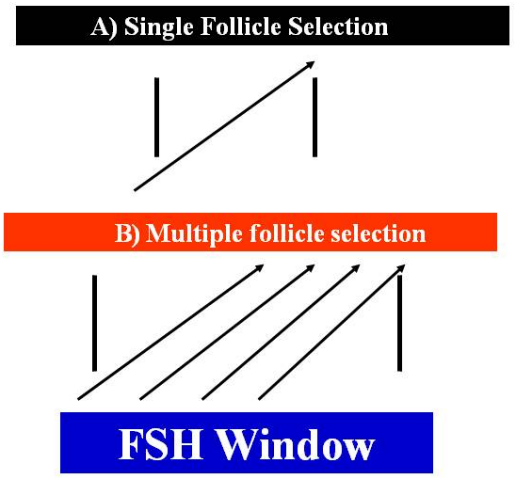
LH Dependence



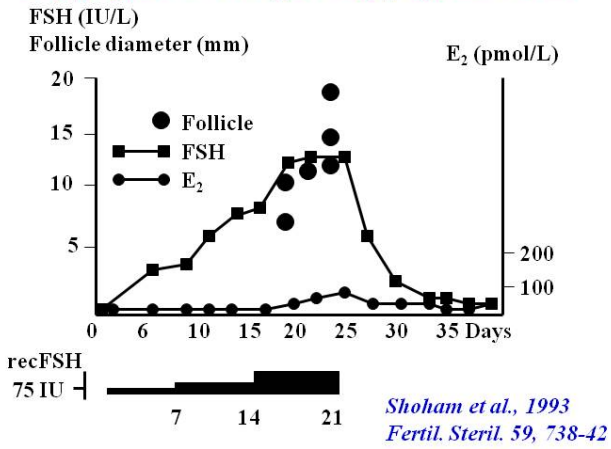
Mid- to Late Follicular Phase
(Dominant Follicle)

Normal Cycle





Hypogonadotropic Hypogonadism



Shoham et al., 1993
Fertil. Steril. 59, 738-42

Hypogonadotropic Hypogonadism (Use of rFSH with rLH)

	FSH IU	LH IU	E2 (pmol/L)
Couzinet et al., 1988	225 (hMG)	225 (hMG)	2753
Kousta et al.,1996	150	225	780
El-Shawarby et al., 2004	50-75	75	3155

Messinis, 2005;Hum. Reprod. 20, 2688-97

Suppression of LH during ovarian stimulation: effects differ in cycles stimulated with purified urinary FSH and recombinant FSH

Fleming et al. Hum Reprod 2000;15(7):1440-1445

LH Thresholds Studied

- 3.0 mIU/mL *(Esposito et al., 2001)*
- 1.2 mIU/mL *(O'Dea et al., 2008)*
- 1.0 mIU/mL *(Cabrera et al/. 2005)*
- 0.7 mIU/mL *(Balasch et al., 2001)*
- 0.5 mIU/mL *(Westergaard et al., 2000)*

Urinary HMG and FSH

	Brand	Molecule	Company
hMG	Repronex	FSH and LH and 95% urine proteins	Ferring
hMG	Humegon	FSH and LH and 95% urine proteins	Organon
hMG	Pergonal	FSH and LH and 95% urine proteins	Serono
Highly purified hMG	Menopur	FSH and LH and <5% urine proteins	Ferring
Purified FSH	Metrodin	Urofollitropin and <5% urine proteins	Serono
Highly purified FSH	Bravelle	Urofollitropin and <5% urine proteins	Ferring
Highly purified FSH	Metrodin HP	Urofollitropin and <5% urine proteins	Serono

Urinary HCG

	Brand	Molecule	Company
hCG	Novarel	Choriogonadotropin and <5% urine proteins	Ferring
hCG	Profasi	Choriogonadotropin and <5% urine proteins	Serono
hCG	Pregnyl	Choriogonadotropin and <5% urine proteins	Organon

Recombinant FSH, LH and HCG

	Brand	Molecule	Company
FSH	Gonal-F	Follotropin α	Serono
FSH	Puregon	Follotropin β	Organon
LH	Luveris	Lutropin α	Serono
HCG	Ovidrel	Choriogonadotropin α	Serono

rFSH versus Urinary FSH Meta-analysis

Daya and Gunby published the first meta-analysis comparing urinary FSH with rFSH. Daya updated the meta-analysis: rFSH produced higher pregnancy rates per cycle than urinary FSH (OR 1.21; 95% CI 1.04-1.42), with a 3.7% absolute increase in pregnancy rate with rFSH. He included both randomized and quasirandomized trials (n=18) including 3,421 cycles.

OR= odds ratio
CI= confidence interval

Daya and Gunby Cochrane Database Syst Rev. 2000;(4):CD002810
Daya. Fertil Steril 2002;77(4)711-714

Meta-analysis of hMG vs. rFSH

Van Wely et al. (2003) compared the effectiveness of hMG versus rFSH in a meta-analysis including six studies with 2,030 IVF/ICSI cycles. They concluded that hMG resulted in a higher clinical pregnancy rate than rFSH in IVF/ICSI cycles downregulated by the long protocol. However, one of the studies included was quasirandomized, and if the data of this study were excluded, there would be no significant difference in clinical pregnancy rate between hMG and rFSH.

Van Wely et al. Fertil Steril 2003;80(5):1121-1122

Human Menopausal Gonadotrophins vs. rFSH: a Meta-analysis

- *Primary outcomes:*
  - *The probability of a live birth following hMG administration was significantly higher than with rFSH [OR=1.20, 95% CI=1.01-1.42]*
  - *The reates of OHSS (OR=1.21, 95%CI=0.78-1.86) were similar between the treatment options and were not significantly different.*

Human Menopausal Gonadotrophins versus rFSH: a Meta-analysis

- *Secondary Outcomes:*
  - *There was statistical significance with regard to the clinical pregnancy rate in favor of the hMG grouop [OR=1.22, 95% CI= 1.03-1.43].*
  - *There were significantly fewer treatment days [WMD= -1.21, 95% CI= -1.35—1.06], and a lower total dose (IU) [WMD= -282.50, 95% CI= -311.36 to -253.65], but embryos produced (WMD=0.80, 95%CI=0.56-1.05) was not significantly different between the two groups.*

WMD = weighted mean difference

rFSH vs. Urinary FSH and hMG Meta-analysis

Al-Inany et al. published a meta-analysis comparing urinary FSH with rFSH. They included 20 randomized studies (4,610 IVF/ICSI cycles using long GnRH downregulation protocol) and found no statistically significant difference in the pregnancy rate per started cycle between rFSH and urinary-derived FSH gonadotrophins (OR 1.07; 95% CI 0.94-1.22). Subgroup analysis showed no significant difference in the pregnancy rate per started cycle between recombinant FSH and hMG (OR 0.81; 95% CI 0.63-1.05). The conclusion was that there is no evidence for superiority in terms of clincial pregnancy rate between rFSH and urinary FSH.

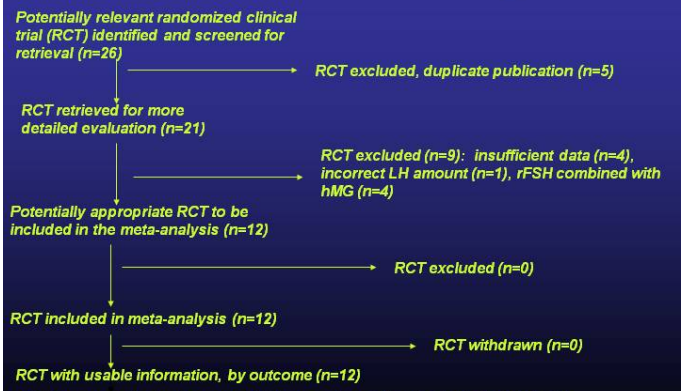
Al-Inany, et al. Hum Reprod 2003;18(2):305-313

Recombinant FSH vs. Urinary hMG

Different concepts of the role of LH in controlled ovarian stimulation (COS) resulted in performing several clinical randomized studies comparing rFSH vs. hMG. A meta-analysis including 2031 patients showed no significant difference in ongoing or live-birth rate between rFSH and hMG (OR 1.18; 95% CI 0.93-1.50).

Al-Inany et al. Gynecol Endocrinol 2005;20(3):161-169

Recombinant FSH vs. Urinary hMG



Al-Inany et al. Reprod Biomed online 2008;16:81-88

Recombinant FSH vs. Urinary hMG

Efficacy and safety of human menopausal gonadotropins vs. rFSH were recently evaluated in a meta-analysis. The live-birth rate was significantly higher with hMG [OR=1.2; 95% CI=1.01-1.42] vs. rFSH, but ovarian hyperstimulation syndrome (OHSS) rates were not significantly different [OR=1.21, 95% CI = 0.78-1.86]. There were significantly fewer treatment days, total dose and embryos produced in the rFSH group compared with the hMG group

Al-Inany, et al. Reprod Biomed online 2008;16:81-88

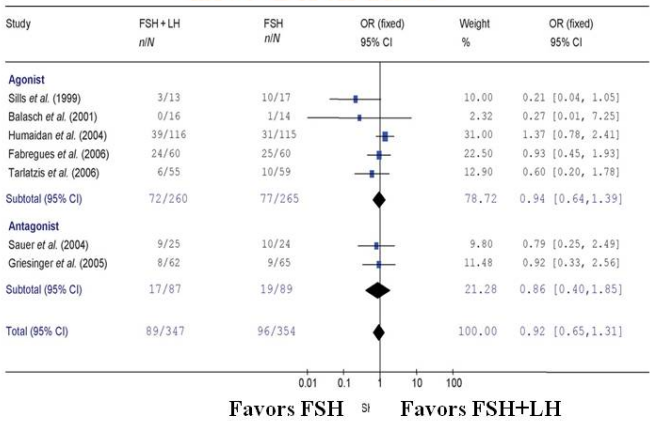
Recombinant FSH VS. Urinary hMG

Another meta-analysis of 2159 randomized patients showed that hMG increases the live-birth rate by 4% compared to rFSH. [RR=1.18; 95% CI=1.02-1.38; P=0.003].

RR = relative risk

Coomarasamy, et al. Hum Reprod 2008;23:310-315

Live-Birth Rate



Kolibianakis et al., Hum Reprod Update. 2007, 13, 445-52

Highly Purified (HP) hMG vs. rFSH in IVF

	HP-hMG (n=363)	rFSH (n=368)	P
Clinical pregnancy/cycle started	100/363	87/368 (24%)	0.263
Ongoing pregnancy/cycle started	97/363	82/368 (24%)	0.204
Moderate/severe early OHSS	5/363	6/368	1.000
Moderate/severe late OHSS	3/363	2/368	0.773

Andersen et al. Hum Reprod 2006;21:3217-3227

The Influence of HP-hMG or rFSH ON Embyro Quality

- Randomized, assessor-blind, multinational trial (n=731) in women undergoing IVF after stimulation with HP-hMG (n=363) or rFSH (n=368)
- Ongoing pregnancy was the primary endpoint [HP-hMG, 27%; rFSH 22%; odds ratio (OR 1.25; 95% CI 0.89-1.75).
- 7535 oocytes retrieved and evaluated daily until day 3 (embryo transfer) in a blinded manner by local-site embryologists and a central panel of 3 embryologists.

MERIT Trial Zieve et al. Hum Reprod 2007;1-10

The Influence of HP-hMG or rFSH on Embryo Quality

- The proportion of top-quality embryos/oocytes retrieved was higher with HP-hMG (11.3%) compared with rFSH (9.0%) (p=0.444) in the local assessment, but comparable in the central assessment (9.5% and 8%, respectively).
- The number of blastomeres and degree of fragmentation were significantly different in favor of the HP-hMG group.
- The uniformity of blastomere size, localization of fragments, frequency of multinucleation and homogeneous cytoplasm were comparable between HP-hMG and rFSH.

The Influence of HP-hMG or rFSH on Embyro Quality

- The live-birth, ongoing pregnancy and ongoing implantation rates for top-quality embryos were higher with HP-hMG than rFSH [4% vs. 32% (p=0.038), 48% vs. 32% (p=0.038), 41% vs. 27% (p=0.032)].
- Both the proportion of embryos with at least 50% surviving blastomeres after cryopreservation and embryos resuming mitosis were more frequent with HP-hMG compared with rFSH.

HP-hMG vs. rFSH in Ovarian Hyperstimulation with GnRH Antagonists:

I. *Serum LH determination throughout the follicular phase*

II. LH administration

III. Randomized study of hMG vs. rFSH

HP-hMG vs. rFSH in Ovarian Hyperstimulation with GnRH Antagonists:

Serum LH determination throughout the follicular phase

Kolibianakis et al. (2004)
Merviel et al. (2004)
Bosch et al. (2005)

HP-hMG vs. rFSH in Ovarian Hyperstimulation with GnRH Antagonists:

LH Administration

Cedrin-Durenerin et al. (2004)
Griesinger et al. (2005)

HP-hMG vs. rFSH in Ovarian Hyperstimulation with GnRH Antagonists:

A randomized study

Bosch et al. (2008)

HP-MG vs. Recombinant FSH in Ovarian Hyperstimulation with GnRH Antagonists: :

RCT comparing the ongoing pregnancy rate, the primary endpoint in 280 patients undergoing IVF-ICSI after stimulation with HP-hMG or rFSH in GnRH antagonist cycles

- No significant differences were observed, 35.0% vs. 32.1% respectively.
- No differences in implantation, clinical pregnancy and pregnancy loss rates.
- More oocytes were obtained in rFSH than in hMG, 14.4% VS. 11.3%.
- Estradiol (E_2) was higher at the end of stimulation in the hMG group, whereas progesterone (P_4) was higher in the FSH group.

HP-hMG vs. rFSH

- Prospective RCT
- ICSI only
- Single center only
- Fixed protocol: 150 IU/day
- Long downregulation protocol: triptorelin, 3.75 mg

Kilani, et al. Hum Reprod (2003),18:1194-1199

HP-hMG vs. rFSH

	Group A (rFSH-alpha)	Group B (HP-hMG)	P
Patients recruited	50	50	NS
Poor responders (treatments suspended before oocyte retrieval) (%)	14	12	NS
Pre-ovulatory follicles >14mm	8.4+/-0.6	8.5+/-0.6	NS
Oocytes retrieved	6.8+/-0.6	7.9+/-0.7	NS
Metaphase II oocytes	5.2+/-0.5	6.3+/-0.5	NS
Patients not reaching transfer (%)	7.0	2.3	NS
Fertilization rate (%)	87+/-5	76+/-3	NS
Embryos transferred	1.83+/-0.06	1.93+/-0.04	NS
Moderate OHSS	1	3	NS
Twin gestations	2	1	NS
Pregnancy rate per started cycle (%)	28	30	NS
Pregnancy rate per transfer (%)	35	35	NS
Miscarriage rate per started cycle (%)	6	6	NS
Delivery rate per started cycle (%)	22	24	NS

Kilani, et al. Hum Reprod (2003),18:1194-1199

HP-hMG vs. rFSH

- hMG was associated with shorter duration, lower gonadotropin requirement and more efficient response.
- Increased serum levels of hCG, E_2 and immunoreactive FSH
- ICSI outcome indistinguishable from rFSH

Kilani, et al. Hum Reprod (2003),18:1194-1199

HP-hMG vs. rFSH: European and Israeli Study Group

- Prospective RCT
- Multicenter
- Flexible gonadotropin protocol
- Different downregulation protocols

The European and Israeli study group. Fertil Steril, 2002;78:520-528

HP-hMG vs. rFSH: European and Israeli Study Group

	HP-hMG n (%)	rFSH n (%)
Positive hCG test	114 (35.5)	98(32.8)
Miscarriage rate	29 (25.4)	27 (27.6)
Clinical pregnancies	95 (29.6)	76 (25.4)
Multiple gestation rate	30 (31.6)	27 (35.5)
Completed cycles	344 (96.4)	317 (94.3)
OHSS rate	7 (1.9)	4 (1.2)

The European and Israeli study group. Fertil Steril, 2002;78:520-528

HP-hMG vs. rFSH: European and Israeli Study Group

- The inclusion of LH in gonadotropin preparation is not detrimental to IVF outcome.
- High HP-hMG is at least as effective as rFSH and has comparable safety and tolerability.

The European and Israeli study group. Fertil Steril, 2002;78:520-528

A Reanalysis of the European and Israeli Study Group: Exogenous LH may Influence Outcome in IVF but Not ICSI

	IVF			ICSI		
	HP-hMG	*rFSH*	*P*	*HP-hMG*	*rFSH*	*P*
Positive beta hCG	48(40%)	30(27%)	.035	71(30%)	70(32%)	NS
Clinical pregnancy	42(35%)	22(20%)	.009	56(24%)	55(25%)	NS
Ongoing pregnancy	38(31%)	22(20%)	.037	49(21%)	50(23%)	NS
Implantation rate	21%	15%	.054	12%	13%	NS

NS = not statistically significant

Plateau et al. Fertil Steril. 2004;81:1401-1404

Reanalysis of the RCT in Wely's Meta-analysis

Study	ICSI %	hMG	rFSH
Gordon	0	38%	28%
Westergaard	25%	40%	34%
Euro-Israeli	64%	26%	22%
Ng	100%	25%	20%

Is There a Subset of Normal Gonadotropic Women Who Would Benefit from LH Supplementation?

- In about 10% of normal gonadotropic patients, an initial response during the first days of stimulation is followed by a plateau in which there is no significant increase in follicular size or estradiol production in the next 3 or 4 days of stimulation.

de Placido et al. Hum Reprod 2001;16:1875-1879
de Placido et al. Hum Reprod 2005;20:390-396

Is There a Subset of Normal Gonadotropic Women Who Would Benefit from LH Supplementation?

- In a prospective, randomized trial, women who had no follicle with a mean diameter >10 mm and E_2 of 180 pg/mL on day 8 were randomized to receive LH supplementation in the form of hMG or an increase in the rFSH activity.

de Placido et al. Hum Reprod 2001;16:1875-1879
de Placido et al. Hum Reprod 2005;20:390-396

Is There a Subset of Normal Gonadotropic Women Who Would Benefit from LH Supplementation?

- The mean number of oocytes retrieved was significantly higher in women treated with hMG supplementation than in women who received rFSH step-up.
- The outcome of the hMG group was comparable with the normo-responders.

de Placido et al. Hum Reprod 2001;16:1875-1879
de Placido et al. Hum Reprod 2005;20:390-396

Is There a Subset of Normal Gonadotropic Women Who Would Benefit from LH Supplementation?

- In a dose-finding study, the efficacy of recombinant LH (rLH) supplementation in women with an initial steady response to rFSH was examined.
- The randomization occurred on day 8 to rLH, 75 IU or 150 IU.
- The number of oocytes in the 150 IU group was similar to normo-responders and significantly higher than the group receiving 75 IU.

de Placido et al. Hum Reprod 2001;16:1875-1879
de Placido et al. Hum Reprod 2005;20:390-396

Exogenous LH in ART

- Women showing hyporesponsiveness to FSH in a GnRH downregulation protocol were randomized into 3 groups:
- Group A: received an increased dose of FSH.
- Group B: rLH was added to the increased dose of FSH
- Group C: hMG was given as an additional FSH/LH.

Ferraretti et al. Fertil Steril 2004;82;1152-1156

Exogenous LH in ART

- The pregnancy rates and implantation rates were statistically higher in Group B, women receiving rLH in addition to rFSH when compared with Group A and Group C.
- There was no difference from the control group (Group D).
- The live-birth rate was similar in Groups B and D, and significantly lower in groups A and C.

Ferraretti et al. Fertil Steril 2004;82;1152-1156

Exogenous LH in ART

	Group A (n=50)	Group B (n=54)	Group C (n=22)	Group D (n=54)
Number of fresh ETs	45	41	18	41
Pregnancy rate/ET	11(24.4%)	22(54%)	2 (11%)	17 (41%)
Implantation rate (%)	14.1 (12/85)	36.8 (24/65)	7.4 (2/27)	35.4 (29/79)
Live-birth rate/started cycle	22% (11)	40.7% (22)	18% (4)	37% (20)

ET = embryo transfer

Ferraretti et al. Fertil Steril 2004;82;1152-1156

Exogenous LH in ART

- The data suggest that the use of rLH seems to be more effective than urinary LH (hMG). There was a significantly lower implantation rate in group C compared with group B and the controls. The hCG content of hMG usually contributes to the LH activity, and in other studies, low-dose hCG does not adversely affect folliculogenesis.

Ferraretti et al. Fertil Steril 2004;82;1152-1156

Future of Gonadotropins

Two new long-acting gonadotropins developed by fusing the carboxyterminal peptides (CTP) of human chorionic gonadotropin (hCG) to native recombinant hFSH have been reported.

Princivalle M. In: Rizk, Garcia-Velasco, Sallam, Makriagiannakis (Eds). Infertility and Assisted Reproduction. Cambridge University Press, 2008 Chapter 26;233-240.

Future of Gonadotropins

Small molecule gonadotropin mimetics, FSH-receptor and LH-receptor modulators are currently reported to be in development.

Princivalle M. In: Rizk, Garcia-Velasco, Sallam, Makriagiannakis (Eds). Infertility and Assisted Reproduction. Cambridge University Press, 2008 Chapter 26;233-240.

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NOTES

CURRENT ROLE OF GNRH ANTAGONISTS FOR ART

Mohamed Aboulghar, M.D.
Professor of Obstetrics and Gynecology, Cairo University
Clinical Director, The Egyptian IVF center
Cairo, Egypt

LEARNING OBJECTIVES
At the conclusion of this presentation, participants should be able to:

1. Compare basic differences between gonadotropin-releasing hormone (GnRH) antagonists and GnRH agonists.
2. Describe different protocols for the use of GnRH antagonists.
3. Suggest other options for the use of GnRH antagonists.

Current Role of Gonadotropin-Releasing Hormone (GnRH) Antagonists for ART

Mohamed Aboulghar, M.D.
Cairo, Egypt

Learning Objectives

At the conclusion of this presentation, participants should be able to:

- Compare basic differences between GnRH antagonists and GnRH agonists.
- Describe different protocols for the use of GnRH antagonists.
- Suggest other options for the use of GnRH antagonists.

Disclosure:
Nothing to disclose

No available data in SART /ASRM IVF registry or ESHRE European IVF registry on the percentage of IVF/ICSI cycles stimulated with GnRH antagonist protocols.

SART = Society for Assisted Reproductive Technology
ESHRE = European Society of Human Reproduction and Embryology
IVF/ICSI = in vitro fertilization/intracytoplasmic sperm injection

Mechanism of Action

- **Antagonist**
- Receptor blockage
- Competitive inhibition
- Immediate suppression
- Rapid reversibility

- **Agonist**
- Initial flare-up
- Receptor down-regulation
- Pituitary desensitization
- Slow reversibility

Advantages of Antagonist Protocols

- Shorter treatment (several weeks)
- Smaller doses of gonadotropins
- No ovarian cyst formation
- Lower incidence of ovarian hyperstimulation syndrome (OHSS)
- Immediate recovery by pituitary

Why Were Agonists Not Replaced by Antagonists for Controlled Ovarian Stimulation (COS) in ART cycles?

Cochrane Review: Pregnancy Outcome. Al-Inany and Aboulghar 2002

Study	GnRH antagonist N/H	GnRH agonist N/H	OR (95% CI Fixed)	OR (95% CI Fixed)
Albano 2000	42/198	22/95		0.89 (0.49,1.61)
European Orgalutran	101/463	67/237		0.70 (0.49,1.01)
European-Middle East	73/226	40/111		0.85 (0.52,1.37)
North American	66/196	36/99		0.87(0.53,1.45)
Olivennes 2000	26/126	11/43		0.75(0.33,1.73)
Total (95% CI)	308/1211	176/585		0.79(0.63,0.99)

Cochrane Review: Pregnancy Outcome. Al-Inany and Aboulghar 2002

- The clinical pregnancy rate was significantly lower in the antagonist group. The absolute treatment effect (ATE) was calculated to be 5%. The number needed to treat (NNT) was 20. This means that for every 20 subfertile couples undergoing IVF/ICSI program, one additional successful pregnancy was added to the 5-8 expected pregnancies in the GnRH agonist treated group.

Lower Pregnancy Rate in Antagonist Cycles??

Effect of Antagonist on Endometrium and Implantation

- Dose-finding study: subjects taking 2 mg of ganirelix had very low pregnancy rate (Ganirelix Dose-Finding Study Group, 1998).
- Negative effect on endometrial receptivity (Hernandez et al. 2000; however, this was criticized by Mannaerts and Gordon 2000).
- Pregnancies from frozen-thawed embryos from antagonist cycles are similar to those from agonist cycles, suggesting an effect of the antagonist on endometrium and not on oocytes (Kol 1999).

Learning Curve and Fine-Tuning

- Some major European clinics use antagonist-only with good results.
- Recent meta-analysis comparing agonists and antagonist showed the difference in pregnancy rate to be very small (Fauser and Devroey 2005).

Trials to Improve Pregnancy Rate in Antagonist Protocol

- Several studies investigated different options to improve the pregnancy rate.
- Flexible protocol
- Early start of GnRH antagonist.
- Use of oral contraception.
- Increase dose of folilcle-stimulating hormone (FSH) at start of antagonist.

Flexible Protocols

- In a prospective cohort study, GnRH antagonist was administered on day 4, 5 and 6 of start of stimulation. Ongoing pregnancy rates were 37.3%, 34.7% and 18.6%, respectively.
- Conclusion: In a flexible GnRH antagonist protocol, initiating GnRH antagonist before stimulation day 6 was associated with a higher pregnancy rate *(Lainas et al. 2005)*.

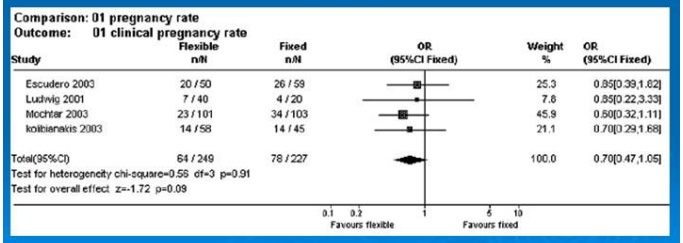
Day 1 versus Day 6 GnRH Antagonist Prospective Randomized Study *(Kolibianakis et al. 2003)*

- Fixed dose 200 IU recombinant FSH (rFSH) on day 2 of cycle
- GnRH antagonist day 1 or day 6 of cycle
- Lower estradiol (E_2) and luteinizing hormone (LH) in day 1 antagonist
- Similar number of oocytes, and fertilization rate (FR) and ongoing pregnancy rate (PR)
- No advantage of GnRH antagonist day 1

Recent studies have raised concern regarding an unfavorable effect of too-late administration of antagonist. Kolibianakis et al. (2003) reported lower implantation rate with flexible GnRH antagonist protocol.

Meta-analysis of Clinical Pregnancy Rate in Fixed and Flexible GnRH Antagonist Protocols

Al-Inany et al. 2005



GnRH Antagonist Fixed versus Flexible Protocols: Meta-analysis

(Al-Inany et al 2005)

- Only 4 randomized studies met the criteria.
- There was no statistically significant difference in pregnancy rate between fixed and flexible protocol (0.7, 95% CI 0.42-1.1).
- There was a trend towards higher PR with fixed protocols, particularly if antagonist was started after day 8.

Increasing FSH Dose with Start of GnRH antagonist

- In a randomized study, increasing the dose of human menopausal gonadotrophins (hMG) on day of GnRH antagonist administration had no effect on improving the pregnancy rate *(Aboulghar et al. 2004)*
- In a randomized study, increasing the dose of rFSH after starting GnRH antagonist did not alter E_2 response or improve implantation and pregnancy rates *(Propst et al. 2006)*.

Recombinant LH (rLH) Supplementation During GnRH Antagonist Protocol: Meta-analysis of 5 Randomized Studies (*Baruffi et al 2007*).

- 5 trials included
- Significantly higher E_2 level in rLH arm ($p < 0.001$) and significantly higher mature oocytes ($P<0.0098$)
- No significant difference in implantation or pregnancy rate.
- Conclusion: rLH supplementation does not affect IVF endpoints.

rLH supplementation in GnRH antagonist cycles: a Cochrane review (*Mochtar et al 2007*)

- Three randomized trials are included (216 patients).
- There is no evidence of a difference in clinical pregnancy rate (OR 0.79, 95% CI 0.95 -1.56) or ongoing pregnancy rate (0.83, 95% CI 0.39-1.80).

OR= odds ratio
CI= confidence interval

The pooled pregnancy estimates of trials including only poor responders showed significant increase in pregnancy rate in favor of co-administrating rLH (3 trials; OR 1.85; 95% CI 1.10-3.11) (Mochtar et al. 2007)

Poor Responders: Agonist versus Antagonist Protocol: a Randomized Study (*Schoolcraft et al. 2007*)

- Microdose flare-up GnRH agonist (GnRH-a) protocol, versus GnRH antagonist/letrozole protocol
- 534 patients randomized
- No significant difference in the number of oocytes, FR, number of embryos transferred or embryo score
- E_2 level and ongoing pregnancy rate were significantly higher in flare-up protocol.

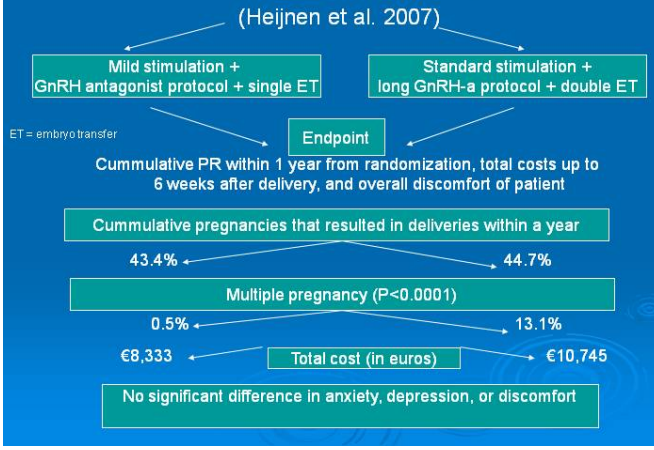
Meta-analysis of Agonist versus Antagonist in Poor Responders

- 6 trials included; there was no significant difference between GnRH antagonist and agonist long or flare-up protocol with respect to cycle cancellation rate, number of oocytes and clinical pregnancy rate per cycle initiated (*Franco et al 2006*).

Addition of estradiol, 4 mg orally daily, to progesterone for luteal phase support in GnRH antagonist cycles did not enhance the probability of pregnancy (*Fatemi et al 2006*).

Soft Protocol Randomized Trial for IVF (404 Patients)

(Heijnen et al. 2007)

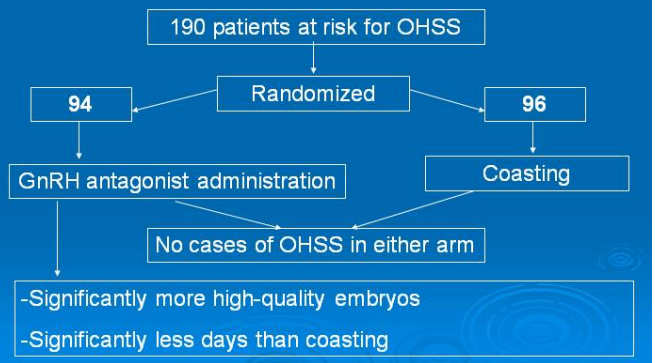


Effect of GnRH Antagonists in FSH Mildly Stimulated Intrauterine Insemination Cycles: a Multicenter Randomized Trial (Crosignani et al. 2007)

299 couples with unexplained or mild male-factor infertility enrolled in a randomized trial. The GnRH antagonist group (n=148) received 50 IU rFSH starting on day 3 of the cycle and ganirelix 0.25 mg daily when a follicle reached 13-14 mm. The control group (n=151) received only 50 IU rFSH starting on day 3 of the cycle. Clinical pregnancy rates were 12.2% and 12.6%, respectively. There is no benefit of antagonist on mild COS/IUI.

Antagonist for Prevention of OHSS

Aboulghar et al. 2007



GnRH Antagonist for Treatment of Early OHSS (Lainas et al. 2009)

- In 3 patients with severe early OHSS, GnRH antagonist was given daily for a week. Symptoms subsided and embryos were cryopreserved at blastocyst stage for future ET.

In a systematice review of 23 studies (*Griesinger 2006*)

- GnRH agonist versus human chorionic gonadotropin (hCG) were compared for triggering final oocyte maturation in GnRH antagonist protocol. Triggering ovulation by GnRH agonist reduced significantly the pregnancy rate (OR 0.21; 95% CI 0.05-0.84; p = 0.03).
- The odds of first trimester pregnancy loss is also increased (p < 0.05).

Outcome of Cryopreserved Embryos Following Triggering Ovulation by GnRH-a in GnRH Antagonist Cycles (*Eldar-Geva et al. 2007*)

- Elective cryopreservation of all pronuclear (PN) oocytes after GnRH agonist triggering of ovulation in patients at risk of OHSS in GnRH antagonist cycles reults in high pregnancy rate of 31.6% per ET of cryopreserved embryos (Griesinger et al 2004).
- The lower PR after triggering ovulation by GnRH agonist appears not to be related to an adverse effect on oocyte quality.

Live Birth after IVF: Agonist/Antagonist: a Meta-analysis (Kolibianakis et al 2006)

- 22 RCTs
- 3176 subjects
- Live birth (from manuscript in 10 studies and by conversion of pregnancy rate to live-birth rate using special formula in 12 studies (Arce et al. 2005).
- Both long and flare-up agonist protocols were included.
- No significant difference between PR in agonist and antagonist protocols (OR, 0.86; 95% CI, 0.72-1.02).

Al-Inany et al. 2006 – Cochrane Review

- 27 RCTs included.
- Only long GnRH protocol was included.
- Published studies and abstracts at major meetings were included.
- Clinical pregnancy rate was significantly lower in the antagonist group (OR = 0.84, 95% CI = 0.72-0.97)
- Ongoing pregnancy rate and live birth rate showed the same significant lower pregnancy rate in the antagonist group (P = 0.03; OR 0.82, 95% CI 0.69-0.98).
- OHSS was significantly lower in the antagonist arm (P=0.01; RR 0.61; 95% CI 0.42-0.89).

RR = relative risk

Authors' Conclusions (Al-Inany et al 2006)

- GnRH antagonist protocol is a short and simple protocol with good clinical outcome, with significant reduction in OHSS and amount of gonadotropins used, but with significantly lower pregnancy rate.

Conclusion 1

- GnRH antagonist protocol provides significant advantages:
  - Shorter stimulation periods
  - Option for the use of soft, friendly protocol
  - No cyst formation
  - Lower incidence of OHSS

Conclusion 2

- GnRH antagonist disadvantages
  - Lower pregnancy rates
  - More difficult to control the start of the cycle and the timing of hCG.

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NOTES

USE OF VARIOUS ADJUNCTS FOR OPTIMIZING OVARIAN STIMULATION AND EMBRYO QUALITY

David R. Meldrum, M.D.
Clinical Professor, UCLA and UCSD
Scientific Director, Reproductive Partners Medical Group
California, U.S.A.

LEARNING OBJECTIVES:
At the conclusion of this presentation, participants should be able to:

1. List proven adjuncts to gonadotropin stimulation for IVF.
2. Apply proven adjuncts to specific patient groups.
3. Assess the literature regarding other evolving adjuncts not yet proven.

Use of Various Adjuncts for Optimizing Ovarian Stimulation and Embryo Quality

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Disclosure

David Meldrum, M.D.

Grant support: Serono, Organon

None of the adjuncts I will be discussing (except antagonists and LH) are approved by the U.S. Food and Drug Administration (FDA) for the uses described.

Advantages of Gonadotropin-Releasing Hormone (GnRH) Agonists

- Marked reduction in cancellation for poor response and luteinizing hormone (LH) surges
- Flexibility for program and patients by varying the duration of GnRH agonist (GnRH-a) suppression.
- Two-fold increase in pregnancy rate*

*Hughes EG, et al: Fertil Steril 1992; 58:988

Advantages of GnRH Agonists

- More follicles and oocytes
- More embryos from which to select the best for transfer
- More extra embryos for cryopreservation, resulting in a higher pregnancy rate per frozen embryo transfer

Oral Contraceptive (OC) Pretreatment

	Cysts %	Days of GnRH-a	Days of stim.	Amps
No OC	27	21	12	44
OC	0 *	7 *	10 *	33 *

*P<0.0001

Biljan et al: Fertil Steril 1998; 70: 1063

GnRHa vs. OC/GnRHa Stimulation Parameters

	GnRH-a	OC/GnRH-a
Peak E_2	1688 ± 188	2431 ± 501*
Days of stim	9.1 + 0.4	8.9 ± 0.2
Amp hMG	41.9 ± 1.4	38.9 ± 1.2
Oocytes	9.9 ± 1.0	11.8 ± 1.2*
Fertilization(%)	55.7	69.1*

E_2 = estradiol
hMG = human menopausal gonadotropin
stim = stimulation

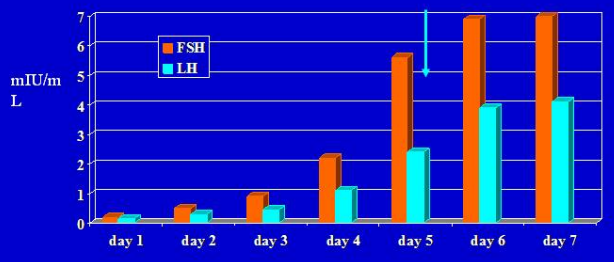
P<0.05 Gelety TJ et al: ASRM abstract 010 1997

OC Pretreatment

	Normal responder	Normal responder	Poor responder	Poor responder	No OC
	day 0	day 3	day 0	day 3	day 3
E2	28±4	39±3	**11±1**	**11±1**	41±7
FSH	8.4±1	13.6±1	**2.8±1**	**7.2±2**	16±2
LH	6.5±1	7.9±1	2.5±.4	6.1±1	12±2

Benadiva CA, et al: Fertil Steril 1988; 50:516

Follicle-Stimulating Hormone (FSH) and LH : Desogen®-free Interval



Van Heusden AM, Fauser BCJM: Contraception 1999; 59: 237

OC/GnRHa for High Responders

- OC for 25 days, 1 mg leuprolide last 5 days and until human chorionic gonadotropin (hCG)
- 150 IU FSH or hMG starting day 3 of menses
- 99 cycles, 13 cancellations (4 for poor response)
- Ongoing pregnancy rate (PR) 40.4%, higher fertilization
- 8 mild-moderate ovarian hyperstimulation syndrome (OHSS)

Damario MA, et al: Hum Reprod 1997;12:2359

OC Pretreatment

- Occasionally menses do not occur
- May be due to either the endometrium being too thin or unrecognized ovulation during the OC
- When used prior to mini-flare or antagonist, we scan the ovaries before stopping OC, and if menses do not occur, we check serum estradiol and progesterone to assure the absence of a corpus luteum.

Need for LH with Agonist?

- Studies vary in choice of agonist, dose and regimen, all of which affect gonadotropin suppression.
- OC pretreatment further suppresses LH.
- Assays for LH vary, and some may cross-react with LH fragments that are stimulated by agonist.
- Negative studies usually have limited statistical power.
- Therefore I will concentrate on studies showing a significant impact of added LH, accepting that this question remains largely unresolved due to the difficulty extrapolating from one regimen to another.

Bio-LH and Leuprolide (LA)/hMG

		Before hMG	Day of hCG
hMG	LH	3.8 ± 0.3	5.8 ± 0.9
	Bio-LH	**76 ± 6**	160 ± 17
LA/hMG	LH	2.4 ± 0.3	1.5 ± 0.1
	Bio-LH	69 ± 10	**51 ± 7**

Cedars, MI, et al: Fertil Steril 1990; 53:627

hMG vs. FSH

	hMG	FSH	P
Days stim	9.3	9.3	
Amp	28.6	28.6	
Peak E_2 (pmolL)	3324	2160	<0.001
Fertilization (%)	56	50	<0.005

Transferable embryo, 4 vs. 3.2, p<.01; failed fertilization 6% vs. 18%, p<.05

Westergaard LG et al: Hum Reprod 1996; 11:1209

Outcome vs. LH levels

	Day 8 LH <0.5 IU/L	Day 8 LH >0.5 IU/L	p
Cycles	98	102	
Early pregnancy loss (% of + tests)	**45**	9	**<0.005**

Westergaard: Hum Reprod 2000; 15:1003

Outcome vs. LH levels

	LH < 3 (mIU/mL)	LH > 3 (mIU/mL)	P
Cycles	116	50	
Fertilization (%)	**52**	58	.03
Spontaneous abortion (SAB) (%)	22	20	
Chemical pregnancy	**7**	0	.07

Esposito MA, et al: Fertil Steril 2001; 75:519

Outcome vs. LH Dose

- Patients randomized to four LH doses with FSH dose constant; a multidose regimen of buserelin was used.
- Implantation rate increased with increasing LH dose (p=0.035).

Gordon UD, et al: Fertil Steril 2001; 75:324

IVF Clinical Pregnancy Rate: FSH versus hMG

- Five randomized controlled studies, 2030 women
- Clinical PR higher with hMG (OR 1.22, CL 1.03-1.44)
- Delivery rate NS (OR 1.20, CL 0.99-1.45)

Van Wely M, et al: Fertil Steril 2003; 80:1086

OR = odds ratio
CL = confidence level
NS = not statistically significant

IVF Clinical Pregnancy Rate: FSH versus hMG

- Meta-analysis of 7 randomized trials
- OR for birth 1.18 (CL 1.02-1.38) favoring hMG
- Risk difference 4% (1-7%)

Coomarasamy A, et al: Hum Reprod 2008:310-15

Use of LH for Agonist Regimens

- Full-dose leuprolide (1.0 mg, reducing to 0.5 mg)
- Potent agonists such as buserelin and tripterelin, particularly multidose or depot regimens
- Combinations of OC and agonists

Adjunctive LH and GnRH Antagonist (Fixed Day 6)

	OC	No OC
Patients	214	211
FSH (IU)	1943 ± 402	1818 ± 398
Days of **FSH** (n)	9.7 ± 2	9.1 ± 2
Oocytes (n)	12.8 ± 7.7	13.2 ± 8.8
Embryos transferred	1.6 ± .7	1.7 ± .9
Ongoing implantation rate (IR) (%)	**18.2**	24.4
Early SABs (%)	**36.4***	21.6

Kolibianakis, et al: Hum Reprod 2006; 21:352

LH Levels in OC/Antagonist Cycles

	LH < 1.0 mIU	P
Day 3/ant (%)	9/21 (43)	
Day 3/ant/ OC	21/29 (72)	P < 0.05
Day of hCG/ant	1/29 (3.4)	
Day of hCG/ant/OC	11/29 (38)	P < 0.01

ant = antagonist

Dickey RP, et al: Fertil Steril 2001; 76: S237

OC/FSH/Antagonist

- 86 cycles, age < or = 35 years
- OC for 14-21 days (mean 18 days)
- FSH, 225 IU, starting evening of day 4
- Ganirelix, 250 µg, day 6 or 12 mm (evening)
- hCG when 2 follicles were at least 17 mm
- 9.5 +/- 1.6 days until hCG
- 3.4 +/- 1.1 days of ganirelix

Meldrum DR, Scott RT, Levy MJ, Alper MM, noyes N; Fertil Steril, in press

OC/FSH/Antagonist

- 2 cancelled due to risk of OHSS
- Median of 14 oocytes (4-42)
- 72% fertilized
- 8 +/- 5 good quality embryos
- 2.3 +/- 0.5 embryos transferred
- 2.9 +/- 4 embryos cryopreserved

OC/FSH/Antagonist

- 40% ongoing pregnancy rate per retrieval
- 36% implantation rate
- Biochemical PR was 22%.
- LH concentration was < or = 0.4 mIU/mL in 7 of 8 (88%) biochemical (BC) pregnancies vs 13/32 (41%) clinical pregnancies (p = 0.017).
- More FSH was used (2512 vs 1931 IU, [p = 0.01]) in BC pregnancy cycles.

OC/FSH/Antagonist

- OC programming was convenient and effective with excellent outcomes.
- An interaction of reduced response and low LH was associated with biochemical pregnancy.
- This and other studies suggest that supplementing LH may be helpful when OC pretreatment is used.

OC/Antagonist

- Donors randomized to antagonist (ant) (n=20) or ant + recombinant LH (rLH) (n=22), 75 IU
- All received OC pretreatment (confirmed with author but not in article); stimulation on day 3-4 of menses
- FSH only; cetrorelix, 250 µg day 6

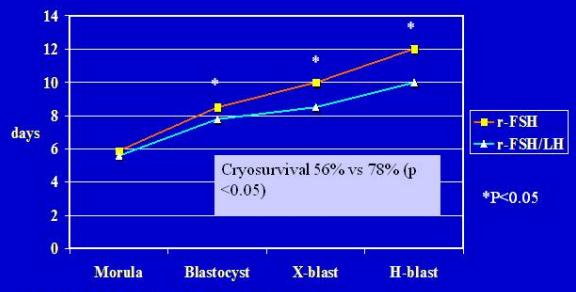
Acevedo B, et al: Fertil Steril 2004; 82:343-7

OC / Antagonist

	OC/Ant	OC/Ant/rLH	P value
Fertilization (%)	71	83	< .05
Metaphase II (%)	71	80	< .05
Grade 1 embryo	3	17	< .05
Biochemical pregnancy	28	10	< .05
IR	15	35	< .05

Acevedo B, et al: Fertil Steril 2004; 82:343-7

LH and Blastocyst Development



Weston,AM, et al: Hum Reprod 1996; 11:608

Role of LH with OC Pretreatment

- Addition of LH (e.g., 75-150 IU hMG or 10-20 IU hCG) appears to be warranted.
- It is not clear whether it is better to add LH with the onset of rFSH or only with initiation of antagonist .
- Flexible administration appears to be OK, since LH is lower following OC.
- OC with a reduced antagonist dose (e.g., 125 µg) could be examined.

Metformin and IVF

	n	Follicles	E_2 max	Oocytes	Mature
Control	30	33	5315	20.3	13
Metformin	30	23*	3981*	22	18.4*

Stadtmauer LA, et al: Fertil Steril 2001; 75: 505-9

Metformin and IVF

- Case- control study of 59 cycles in polycystic ovary (PCO) women who were coasted
- Maximum estradiol levels and days of coasting were significantly lower in the women on metformin.

Stadtmauer LA et al: Reprod Biomed Online 2002;5:112

Metformin (MET) and OHSS

- Meta-analysis of 5 trials has shown a very highly significant ($p < 0.00001$) decrease of ovarian hyperstimulation syndrome (OHSS) with MET (OR 0.21, CL 0.11-0.41) in women with polycystic ovary syndrome (PCOS) having IVF.
- Insulin, which is reduced with MET, is one of the principal factors that stimulates the production of vascular endothelial growth factor (VEGF) by luteinized granulosa cells

Costello MF, et al: Hum Reprod 2006;21:1387-99 Agrawal R, et al: Fertil Steril 2002;78:1164-9

Metformin and IVF

	n	Embryos > or = 4C	Fert (%)	Clin Preg(%)
Control	30	5.9	43	9/30 (30)
Metfor-min	30	12.5*	64*	21/30** (70)

* p< 0.001 ** p< 0.05

Stadtmauer LA, et al: Fertil Steril 2001; 75:505-9

Metformin and IVF

- 48 PCO women studied before and after 500 mg metformin or placebo
- Insulin area under the curve (AUC) decreased by two thirds, luteal serum glycodelin increased 3-fold
- Uterine resistance index decreased 20% ($p < 0.001$)

Jakubowicz DA, et al: J Clin Endocrinol Metab 2001; 86: 1126

Metformin and IVF

- 73 patients randomized to 1000 mg twice a day or placebo
- Implantation rate (IR) (38% vs. 33%), pregnancy rate (PR) (48% vs. 44%), and delivery rate (DR) (39% vs. 34%) were not different.
- In the 4-month period prior to IVF, 4 conceived with metformin and 2 with placebo.

Kjotrod S, et al: Fertil Steril 2004;81:S7

Metformin and IVF

- 101 women with PCO, long protocol
- Randomized to metformin, 850 mg twice a day or placebo
- Ongoing pregnancy higher per cycle (38.5% vs. 16.3%, p = .023) and per ET (p = .022)
- OHSS was significantly reduced (3.8% vs. 20.4%, p = .023)

Tang T, et al: Hum Reprod 21: 1416-1425, 2006

Metformin and Clomiphene Citrate (CC)

- Meta-analysis of 17 studies and 1,639 women with PCO
- Metformin vs. placebo: OR for ovulation 2.94 (CL 1.43-6.02)
- Metformin and CC vs. CC and placebo
  - OR for ovulation 4.39 (CL 1.94-9.96)
  - OR for **pregnancy** 2.67 (CL 1.45-4.94)

Creanga AA, et al: Obstet Gynecol 2008; 111:959-68

Low-dose FSH/Metformin

	FSH	FSH/Metf.
Cycles	19	18
FSH(amp)	26 ± 12	22 ± 4.8
Days	16 ± 3	14 ± 3
Foll>15mm	4.5 ± 1	2.5 ± 0.7*
Preg(%)	2(10.5)	2(16.6)
No hCG(%)	6(31.5)	0

* P< 0.001, De Leo,V, et al: Fertil Steril 1999; 72: 282

Metformin and Miscarriage

	Metformin	Control	P
Number	65	31	
SAB all (%)	8.8	41.9	<0.001
SAB EPL+	11.1	58.3	0.002
SAB EPL-	6.3	31.6	0.04

EPL = early pregnancy loss

Jakubowicz DJ, et al: J Clin Endocrinol Metab 2002; 87:524

Dexamethasone and Cycle Cancellation

- 290 subjects, randomized to dexamethasone or placebo
- 1 mg each night from day 1 of stimulation until oocyte retrieval
- Cancelled cycles decreased from 12.4% to 2.8% ($p < 0.002$)
- Median oocyte number increased by 1 (NS)
- Clinical pregnancy rate higher (29% vs. 17%, $p < 0.05$) in first cycles

Keay SD, et al: Hum Reprod 2001; 16: 1861-4

Low-dose Aspirin and IVF

- Buenos Aires, 298 cycles randomized, PR 45% vs. 28%, $p < 0.05$*
- Reanalysis of randomized clinical trials (RCTs) by the Division of Epidemiology of the National Institutes of Health (NIH)** RR 1.15 (1.03-1.27) concluded "there is no reason to change clinical management and discontinue the use of aspirin."

*Rubinstein M, et al: F&S 1999;71:825-9
**Ruopp MD, et al: F&S 2008;90:71-6

RR = risk ratio

Growth Hormone (GH) and IVF

- Multiple randomized studies have not shown improved stimulation in poor responders, with the exception of a sub-set of poor-responding PCO women.
- Until recently, use of GH as an adjunct to improve embryo quality and pregnancy outcome has not been examined.

Growth Hormone and IVF

- Cochrane Review of 6 randomized studies
- No effect on stimulation parameters
- Three trials reported live-birth rates
- OR for live birth was 4.37 (CL 1.06 to 18.01)

Harper K, et al: Cochrane Database System Rev, 2003, issue 3

GH in Women over 40

- 100 women having intracytoplasmic sperm injection (ICSI), age over 40
- Randomized to GH, 8 units subcutaneous, daily from day 7 of stimulation until oocyte retrieval, or placebo
- In addition to the usual clinical criteria, estradiol and GH were measured in follicular fluid
- Long protocol, FSH 450 IU/hMG 150 IU

Tesarik J, et al: Hum Reprod 2005; 20:2536

GH in Women over 40

- Mean ages were similar (42.2, 42.3)
- Previous attempts were 2.8 and 2.9
- Mean FSH 10.1 and 10.2
- Duration of stimulation, doses of gonadotropins were not different
- Number of oocytes and MII oocytes were the same

Tesarik J, et al: Hum Reprod 2005; 20:2536

GH in Women over 40

	Placebo	GH
Peak E_2	912 +/- 129	1523 +/- 208*
Follicular fluid (FF) E_2	578 +/- 85	921 +/- 98*
FF GH	1.7 +/- 0.3	3.7 +/- 0.4*

* P<0.01 Tesarik J, et al: Hum Reprod 2005; 20:2536

GH in Women over 40

	Placebo	GH
Clinical PR (%)	6	26*
IR (%)	1.7	6.2*
Delivery rate (%)	4	22*

* P<0.05 Tesarik J, et al: Hum Reprod 2005; 20:2536

Androgens and Ovarian Stimulation and Cycle Outcome

FSH-Receptor Activity and Ovarian Response

- 100 women, ages 20-35
- FSH < 10 and antral follicle count (AFC) > 5
- Cycles every 27-35 days
- Response defined as poor < or = 3 follicles > or = 14 mm; high response as > 12
- Luteal decapeptyl, FSH 300 IU for 3 days, tapered as necessary

Cai J, et al: Fertil Steril 2007;87:1350-6

FSH-Receptor (FSHr) Activity and Ovarian Response

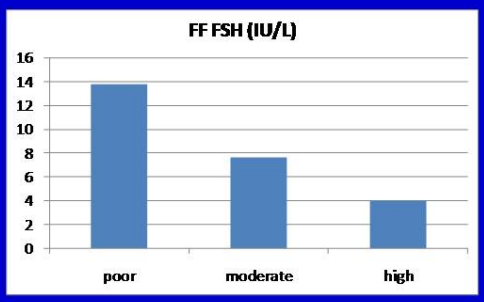
- **Pooled** FF from each patient
- Centrifuged and supernatant stored at -70, pellet resuspended and granulosa cells separated on Ficoll column
- E_2 and FSH levels measured on supernatant
- FSHr messenger RNA (mRNA) and FSHr protein expression measured on extractions of granulosa cells

FSH-Receptor Activity and Ovarian Response

Groups AFC	Poor response 9.2	Moderate response 10.7	High Response 12.7	P
Total FSH dose	3288	2573	2310	< 0.001
Peak E_2 (pmol/L)	4,043	8,415	24,842	< 0.001
Number of Mature Oocytes	2.3	8.3	22.2	<0.001

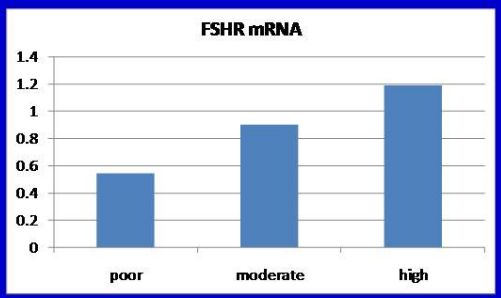
Cai J, et al: Fertil Steril 2007;87:1350-6

FSH-Receptor Activity and Ovarian Response



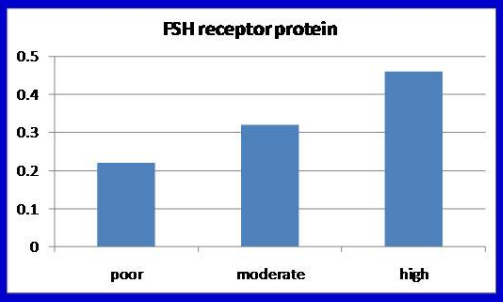
Cai J, et al: Fertil Steril 2007;87:1350-6

FSH-Receptor Activity and Ovarian Response



Cai J, et al: Fertil Steril 2007;87:1350-6

FSH-Receptor Activity and Ovarian Response



Cai J, et al: Fertil Steril 2007;87:1350-6

FSH-Receptor Activity and Ovarian Response

- FF from **mature follicle > 17 mm**
- No differences in FSH or LH receptors between poor, moderate and high responders
- Poor responders had significantly higher LH receptor (LHr) expression ,consistent with more advanced luteinization due to the higher FSH dose used.

Thiruppathi P, et al: Mol Hum Reprod 2001;7:697-704

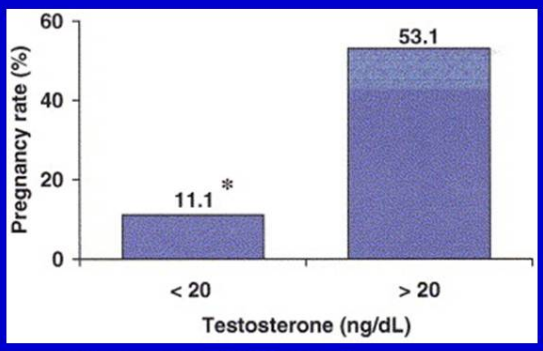
Androgens and IVF Outcome

- Serum levels of testosterone (T) and number of oocytes decline with age.
- Using multivariate analysis, baseline levels of T independently correlated positively with the number of oocytes retrieved.

Barbieri RL, et al: Fertil Steril 2005;83:302-8

Frattarelli JL, Gerber MD: Fertil Steril 2006;86:51-7

Androgens and IVF Outcome



Frattarelli JL, Peterson EH: Fertil Steril 2004;811713

Androgens and Embryo Quality

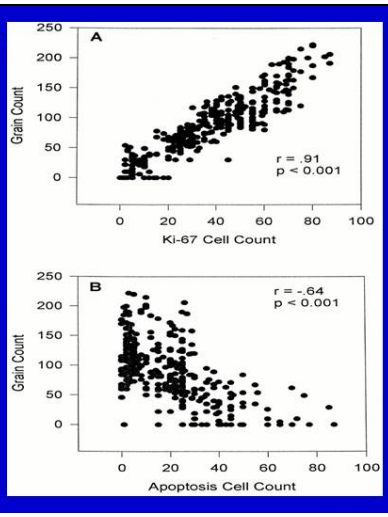
- Dehydroepiandrosterone-sulfate (DHEAS) levels decrease by approximately 50% from age 25 to 45.
- 50% of follicular-fluid testosterone is derived from circulating dehydroepiandrosterone (DHEA).
- Androgen increases FSH receptors on granulosa cells (GC).
- FSH receptors are reduced in poor responders.
- Apoptosis is increased in granulosa cells from older women.
- Androgen-receptor (AR) mRNA correlates negatively with apoptosis in primate granulosa cells. [a]

[a] Weil SJ, et al: J Clin Endocrinol Metab 1998;83:2479-85

Androgen Receptor mRNA and GC Function

Proliferation vs. AR mRNA

Apoptosis vs. AR mRNA



Weil, S. J. et al. J Clin Endocrinol Metab 1998;83:2479-2485

Testosterone for Poor Responders

- 25 women ages 31-39 (mean 35.6)
- FSH < 10 IU/L
- Body mass index (BMI) 21.2-27.4
- First cycle mid-luteal phase leuprolide 1 mg to 0.5 mg/FSH 450, 300, 150/150
- Second cycle mid-luteal phase leuprolide 0.5 mg to 0.25 mg/300 FSH plus 300 hMG for 2 days then 300 hMG
- Third cycle same as first cycle plus testosterone patch, 2.5 mg/day for 12 hours for 5 days (approximately 20 ug per kg per day)

Balasch J, et al: Hum Reprod 2006;21:1884-1893

Testosterone for Poor Responders

Cycle	1	2	3
Days of stimulation	12.2	11.9	10.2
Total rFSH (IU)	3653	4005	3570
Peak E_2 (pg/mL)	342	393	1397*
Follicles	1.6	1.6	8.5*

Balasch J, et al: Hum Reprod 2006;21:1884-1893

Testosterone for Poor Responders

Cancelled (%)	5(20)
Oocytes	5.8 +/- 0.4
High quality embryos	1.75 +/- 0.2
Clinical pregnancy per retrieval (%)	30
Twins (%)	3(50)

Balasch J, et al: Hum Reprod 2006;21:1884-1893

Testosterone for Poor Responders

Hormone (area under curve)	Cancelled	Not cancelled	P
Testosterone (ng/dL)	1392	1647	
IGF-1 (ng/mL)	910	1620	< 0.05

IGF-1 = insulin-like growth factor type 1

Balasch J, et al: Hum Reprod 2006;21:1884-1893

Testosterone for Poor Responders

- 62 women cancelled for poor response, randomized to the same protocol plus T pre-treatment or a reduced dose of suppression with an increased stimulation
- Days of stimulation, FSH dose, and % of poor responders were significantly reduced
- Number of mature oocytes increased from 3.6 to 4.1 (NS)

Fabregeus F, et al. Hum Reprod 2009;24:349-59

DHEA and Embryo Quality

(n)	Pre-DHEA (25)	Post-DHEA (25)	P-value
Age	40	40	
Oocytes	3.4	**4.4**	< 0.05
Fert (%)	39	**67**	< 0.001

Barad D, Gleicher N: Hum Reprod 2006;21:2845-9

Letrozole for Poor Responders

- 147 poor responders
- OC/hMG 150/FSH 225; antagonist at 14 mm
- 71 also received letrozole
- FSH dose slightly higher without letrozole

Garcia-Velasco JA, et al: Fertil Steril 2005;84:82-7

Letrozole for Poor Responders

Groups	Letrozole (n=71)	Control (n=76)	P
Oocytes	**6.1**	4.3	0.03
PR per cycle (%)	22	15	NS
Implantation rate	**25**	9	0.009
Multiple pregnancy	**47**	8	0.04

Garcia-Velasco JA, et al: Fertil Steril 2005;84:82-7

Letrozole for Poor Responders

Group	letrozole	control	P
Serum E_2	384	485	NS
Serum T (pg/mL)	1.7	1.1	0.07
FF testosterone	**80**	44	0.004
FF androstene-dione	**58**	37	0.004

Garcia-Velasco JA, et al: Fertil Steril 2005;84:82-7

Adjuncts Proven to be Effective

- Agonist, antagonist, OC, LH, metformin, dexamethasone, growth hormone, aspirin

Adjuncts Worthy of Further Investigation

- Testosterone
- DHEA
- Aromatase inhibitors

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44. Weston,AM, Zelinski-Wooten MB, Hutchison JS, Stouffer RL, Wolf DP. Developmental potential of embryos produced by in-vitro fertilization from gonadotrophin-releasing hormone antagonist-treated macaques stimulated with recombinant human follicle stimulating hormone alone or in combination with luteinizing hormone. Hum Reprod 1996; 11:608-13.

NOTES

NOTES

OVARIAN RESERVE TESTING AND THE TREATMENT OF POOR RESPONDERS

William Schoolcraft, M.D., H.C.L.D.
Colorado Center for Reproductive Medicine

LEARNING OBJECTIVES
At the conclusion of this presentation, participants should be able to:

1. List the common risk factors for decreased ovarian reserve.
2. Discuss the controversy for the utilization of follicle-stimulating hormone (FSH) as a screening test.
3. Describe the common elements of optimal protocols for the treatment of poor responders.

Ovarian Reserve Testing and The Treatment of Poor Responders

William Schoolcraft, M.D., H.C.L.D.
Director, Colorado Center for Reproductive Medicine

Learning Objectives

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- List the common risk factors for decreased ovarian reserve.
- Discuss the controversy for the utilization of FSH as a screening test.
- Describe the common elements of optimal protocols for the treatment of poor responders.

Disclosure

- Nothing to disclose

Ovarian Aging

- It has been suggested that critical number of follicles rather than age determines menopause (Faddy, 1992).
- The time between the onset of sub-fertility (25,000 follicles, average 37.5 years) and menopause (1000 follicles, average 51 years) is approximately 13 years.
- Best follicles recruited first; higher proportion of poorer quality oocytes as women age
- Unilateral oophorectomy in the mouse (Brook, 1984), and human (Freeman, 2000) result in early onset of cycle irregularity, increase in aneuploidy, reduced fertility and increased follicle-stimulating hormone (FSH) levels.

- 10% of population become menopausal by age 45.
- Therefore, an estimated 10% of the population at risk by age 32 for declining fertility.
- From onset of diagnosis, fertility will not be completely lost for another 4 years.
- This group at risk for:
  - Increased aneuploidy
  - Increased miscarriage
  - Poor response to controlled ovarian hyperstimulation (COH).

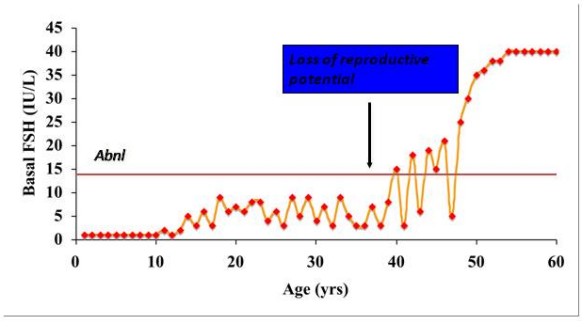
Risk Factors That Suggest Early Screening

- Family history of early menopause
- Chemotherapy, radiation
- Pelvic surgery
- Pelvic infection
- Severe endometriosis
- Smoking

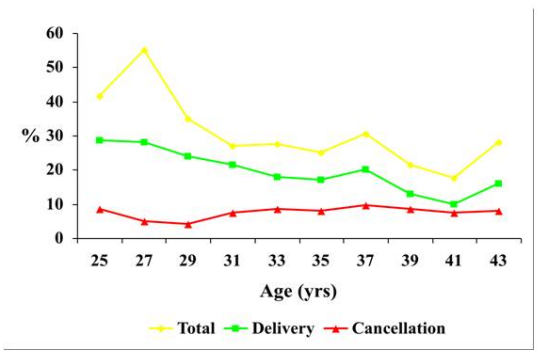
Methods of Ovarian Reserve Assessment

- Biochemical:
  - FSH, estradiol, inhibin B, anti-müllerian hormone (AMH)
- Dynamic tests:
  - Gonadotropin-releasing hormone (GnRH) agonist stimulation test (GAST), exogenous follicle stimulating hormone ovarian reserve test (EFORT)
- Sonographic:
  - Antral follicle count, ovarian volume
- Histologic
  - Ovarian biopsy

Basis of Ovarian Reserve Testing

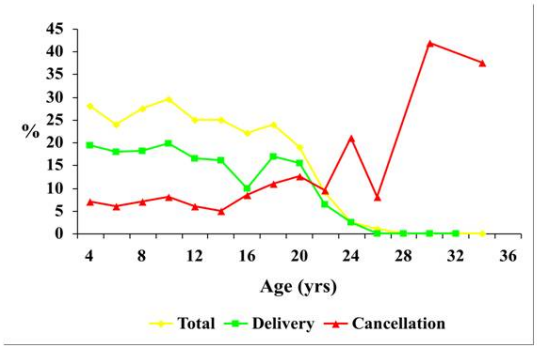


Pregnancy Rates Relative to Age in IVF



Toner JP et al. *Fertil Steril* 1991; 55:784-91

Pregnancy Rates Relative to Basal FSH Levels in IVF

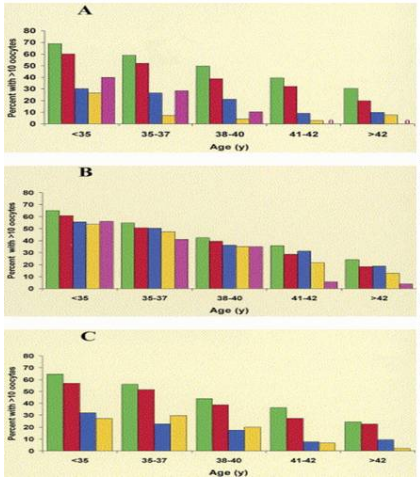


Toner JP et al. *Fertil Steril* 1991; 55:784-91

Follicle-stimulating hormone and estradiol levels independently predict the success of assisted reproductive technology treatment
Fraizer, Fertil Steril, 2004

- Women receiving 19,682 ART procedures performed in 135 clinics
- **Main outcome measure(s):** Rates of clinical pregnancy, live-birth delivery, and high ovarian response (≥ 10 oocytes retrieved after stimulation).
- **Result(s):** The ratio of each FSH or estradiol (E_2) value to the respective upper limit of normal value for the clinic was computed.

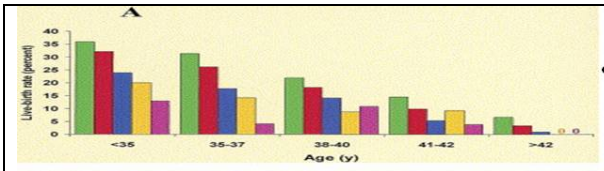
Ovarian Response Rates by Age.



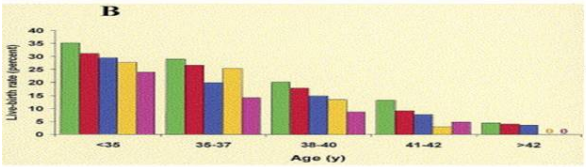
Rates by FSH ratio are shown in (A)

Rates by E_2 ratio are shown in (B), where ratios of 0–0.5, >0.5–1.0, >1.0–1.5, >1.5–2.0, and >2.0, and are represented by the green, red, blue, yellow, and violet bars, respectively.

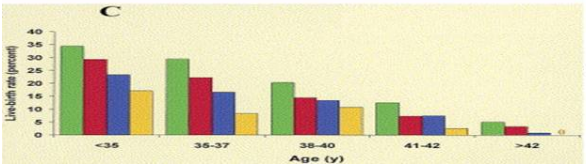
Rates by a combination of these ratios is shown in (C), where the green, red, blue, and yellow bars represent FSH and E_2 ratios both <1, only the estrogen ratio >1, only the FSH ratio >1, and both ratios >1, respectively.



- FSH



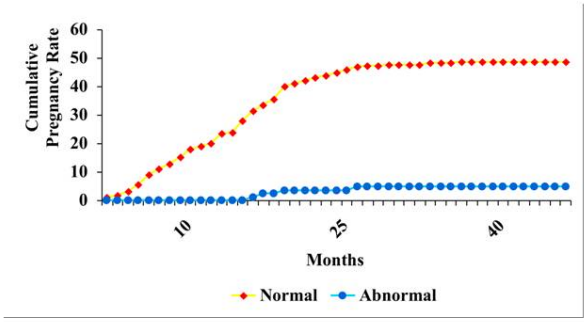
- E_2



- FSH/ E_2

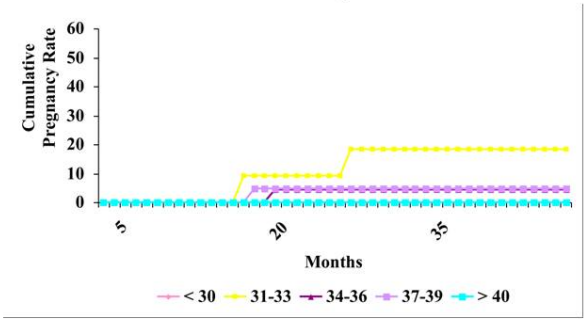
Prospective Evaluation of the Clomiphene Citrate Challenge Test (CCCT) in a General Infertility Population

589 patients evaluated by life-table analysis over 45 months

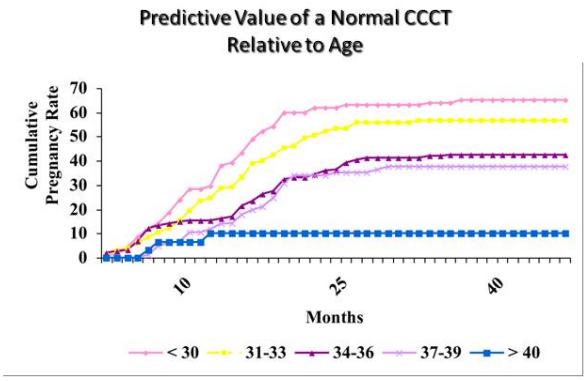


Scott et al. *Hum Reprod* 1995; 10:1706-10

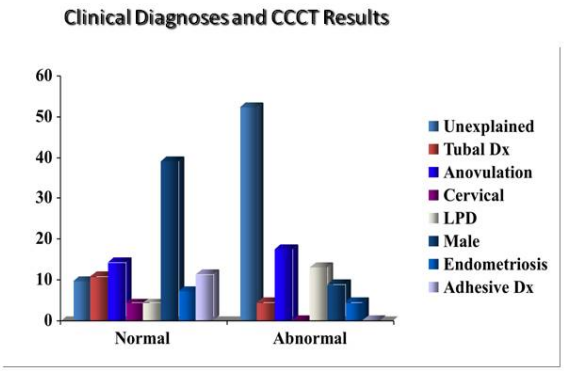
Predictive Value of an Abnormal CCCT Relative to Age



Scott et al. *Hum Reprod* 1995; 10:1706-10



Scott et al. *Hum Reprod* 1995; 10:1706-10



Scott et al. *Obstet Gynecol* 1993; 82:539-45

Case Against FSH as a Screening Tool for IVF

FSH vs. Age
Chuang, Fertil Steril, 2003

	FSH > 10		FSH < 10			
	<35	35-39	≥40	<35	35-39	≥40
Oocytes (n)	13.3	10.2	6.9	5.5	5.2	3.8
Implantation rate	17.6	13.4	7.0	23.2	14.5	7.1
Ongoing pregnancy rate (PR)	38.6	27.7	10.1	27.8	19.0	4.5

Conclusions

- Young women with high FSH have favorable IVF outcome.
- Postulate: decreased follicle pool, but normal quality
- FSH better predictor of quantity rather than quality

Young Age Does Not Protect Against the Adverse Effects of Reduced Ovarian Reserve (El-Toukhy, HR, 2002)

- 762 pts with elevated FSH (>10 IU/L) or history of poor response (≤ 3 oocytes)
- Young = <30 years
- Intermediate = 31-38 years
- Older = >38 years

	Young	Intermediate	Old
Oocytes	7.8	6.5	6.0
Canceled cycles	25	18	16
Implantation rate	13	9.6	9.8
Live birth	7.4	7.3	6.8

- High FSH dose used in all groups did not compensate for the decline in ovarian reserve of the younger age group.
- Ovarian age is an independent marker of IVF outcome and more important than chronologic age.

Elevated FSH Reflects Quantity, Not Quality

Abdalla, HR, 2004

All ages

FSH	<10	10-15	15-20	>20
Cancellation (%)	6	14	33	42
Oocytes	9.9	5.6	3.8	2.5
LBR/Cycle	25	13	14	3

Stratified by age pregnancy rates

FSH	<10	10-15	15-20	>20
Age<38	32	22	20	17
Age≥38	12	8	10	0 (0/27)

Better To Be Young with Elevated FSH Than Old with Normal FSH

	Young(<41) Elevated FSH>15	Old(≥41) Normal FSH<15	P value
N	36	50	
Cancellation rate	31%	8%	.06
Implantation rate	34%	11%	.003
Ongoing PR/cycle	25%	10%	.08
Ongoing PR/embryo transfer (ET)	40%	13%	.01

Van Rooji, F&S, March 2003

FSH as a predictor of poor response and failure to conceive after IVF - A meta-analysis
Bancsi et al, F&S, 2003;70:1091

- The summary receiver operating characteristic (ROC) curve indicated a moderate predictive performance for poor response, and a low predictive performance for non-pregnancy.
- Predictions with a substantial shift from pre-FSH-test probability to post-FSH-test probability are only achieved at extreme cut-off levels for basal FSH. Sensitivity of such cut-off levels, for both the prediction of poor response and non-pregnancy, is limited.
- Conclusion: Clinical value of testing for basal FSH is restricted to a small minority of patients. Basal FSH should not be regarded as a useful routine test for the prediction of IVF outcome. The development of better tests to assess ovarian reserve remains of importance.

Discrepancy in Predictive Value of FSH

- Different threshold values/assays used by various studies
- Different stimulation protocols depending on age
- Heterogeneity of young high-FSH patients
  - Variations in FSH-receptor genotype
  - Spurious elevation due to heterophilic antibodies

FSH "FUNDAMENTALISTS"

- Ovarian reserve testing developed to define women with poor prognosis for pregnancy
- Later, FSH used to predict ovarian response
- Problems:
  - Use test to predict response, and then use the poor correlation to invalidate any use of FSH
  - Equate normal FSH with good reproductive potential
  - Utilize arbitrarily defined threshold values

- Those claiming high success rates with elevated FSH simply reflect the choice of an inappropriate "cut-off" value.
- Artificially low threshold discourages patients from pursuing IVF who have a reasonable chance of success.
- Pregnancies in the "abnormal" FSH group give false hope to patients who truly have diminished ovarian reserve.

FSH "Critics"

- FSH better predictor of response.
- Age better predictor of implantation and miscarrage.
- Both quality and quantity important; therefore, FSH and age influence delivery rates.
- Young women with elevated FSH should be allowed to cycle.

- These women will have fewer eggs and higher cancellation rates, but if successful retrieval occurs, reasonable pregnancy rates.
- Agree that extremes of FSH (>20 IU/L), or age (>43 years) equate with poor prognosis

Both FSH and AGE are Important in Predicting Fertility Potential

Better markers of ovarian reserve are needed, particularly in the younger patient.

New Approach to Ovarian Reserve Testing

Sun, Fertil Steril, Dec 2008

Changes in the Predictive Ability of Screening Tests Based on Changes in Prevalence of Disease.

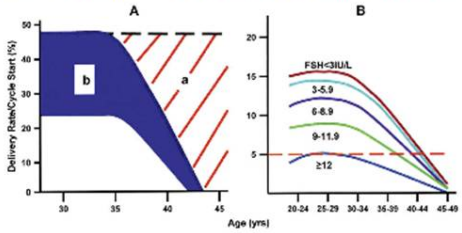
Probability of a test with 95% sensitivity and specificity

Age (years)	Pretest probability	Post-test odds	Positive Odds	Negative predictive value	Positive predictive value
30	0.02 (2/100)	0.02	0.39	0.27	0.99
38	0.10 (10/100)	0.11	2.09	0.68	0.99
40	0.30 (30/100)	0.43	8.14	0.89	0.98

Sun, Fertil Steril, Dec 2008

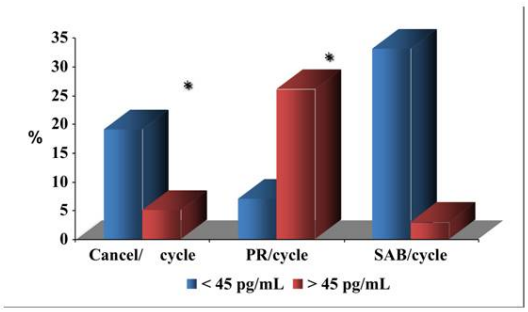
FIGURE 1

Combined effect of age and FSH on expected delivery rate with assisted reproduction. (**A**) Delivery rate per cycle-start versus age. Optimal success at assisted reproductive technology (ART) is present at age 30 years, denoted by the *dotted line*. Physiologic reduction delivery rate due to aging and the associated ovarian insufficiency is indicated by the *hatched area (a) in red*; and can be conceptualized as a reduction from that *dotted line*. In contrast, nonphysiologic ovarian insufficiency associated with increased basal FSH (or other markers of ovarian insufficiency) indicated marked by *(b) in the blue area* is also associated with a reduction in live birth rate. (**B**) Nomogram of delivery rate per cycle-start versus age including basal FSH after Akande et al. (32). The *dotted line* represents a 5% expected delivery rate per cycle-start. Note that the 5% expected delivery rate crosses FSH values at different ages. This nomogram (or a similar nomogram specific for an ART program) can be used to plot patient values of FSH and age to assess the likelihood of live birth with assisted reproduction. Use of a nomogram may provide more accurate assessment of ART outcome than either age or basal FSH as singular values.



Sun. New approach to ovarian reserve testing. Fertil Steril 2008.

Day 3 Inhibin Levels and Ovarian Reserve



*Significantly different

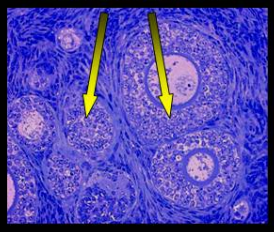
Seifer DB *et al. Fertil Steril* 1997; 67:110-4

Introduction

Müllerian inhibiting substance (MIS, AMH):

- Glycoprotein that belongs to transforming growth factor-beta (TGF-β) superfamily

Granulosa cells



In women: expressed in the granulosa cells of pre-antral and early antral follicles

*On **cycle** day 3, peripheral MIS levels:*

- Decline with advancing age

De Vet et al. Fertil Steril 2002

- Correlate with number of oocytes retrieved for IVF

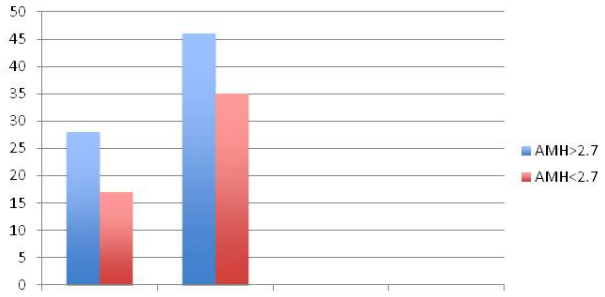
Seifer et al. Fertil Steril 2002

AMH and Clinical Outcome

- AMH levels correlated with
  - Age
  - Oocytes
  - Peak E_2
  - Number of high-quality embryos

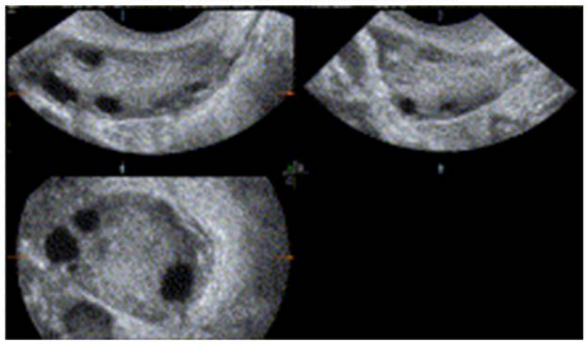
Silberstein, T. et al. Hum. Reprod. 2006 21:159-163

Implantation Rate (IR) and Ongoing Pregnancy Rate (OPR) for Patients whose Müllerian Inhibiting Substance (MIS) Values Were >2.7 or <2.7 ng/mL



Silberstein, T. et al. Hum. Reprod. 2006 21:159-163; doi:10.1093/humrep/dei270

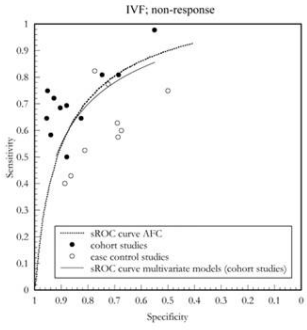
Three-dimensional multiplanar display of the ovary demonstrating three mutually related orthogonal image planes at 90° to one another: **(A) longitudinal view, or A-plane; (B) transverse view, or B-plane; and (C) coronal view, or C-plane. Antral follicle measurements are made in the A-plane while scrolling through the B-plane with simultaneous reference to all three planes.**



Jayaprakasan, F&S, December, 2008

The accuracy of multivariate models predicting ovarian reserve and pregnancy after *in vitro fertilization: a meta-analysis*

Verhagen, Human Reproduction Update 2008 14(2):95-100



- 11 studies analyzed
- Evaluated
  - Age
  - Inhibin B
  - Basal FSH
  - CCCT
  - GAST
  - AMH
  - Ovarian volume
  - Antral follicle count (AFC)
- Antral follicles best predictor for ovarian reserve

Mean Value and Intercycle Variability of Markers of Ovarian Reserve, as Assessed by Limits of Agreement

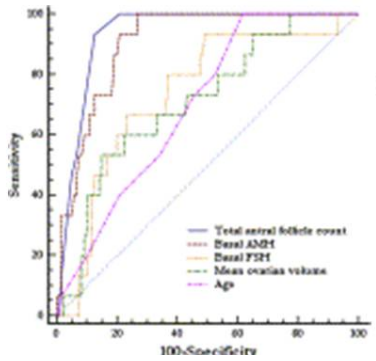
Variable	Mean	Mean difference	Upper limit of agreement (ULA)	Lower limit of agreement (LLA)	Range between ULA and LLA	MoMs
Total AFC (n)	15.98 ± 8.7	0.16 ± 1.98	+4.03	−3.71	7.75	0.48*
Mean ovarian volume (cm^3)	7.81 ± 3.12	−0.18 ± 1.46	+2.67	−3.03	5.70	0.73
Basal FSH levels	6.87 ± 1.68	−0.08 ± 2.27	+4.36	−4.52	8.89	1.29

Note: MoM = multiples of the mean.

*The AFC showed the least degree of variation, with a range of 0.48 times its own mean, in contrast to corresponding values of 0.73 and 1.29 for ovarian volume and basal FSH levels

Jayaprakasan, F&S, December, 2008

A prospective, comparative analysis of anti-Mullerian hormone, inhibin-B, and three-dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation
Jayaprakasan, Fertil Steril, 2008



AFC and AMH were equally predictive of poor ovarian response

Treatment of Poor Responders

Gonadotropin Response to Ultra-low Dose Leuprolide
Scott, Fertil Steril, 1993

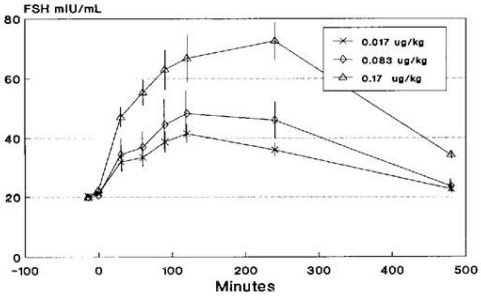
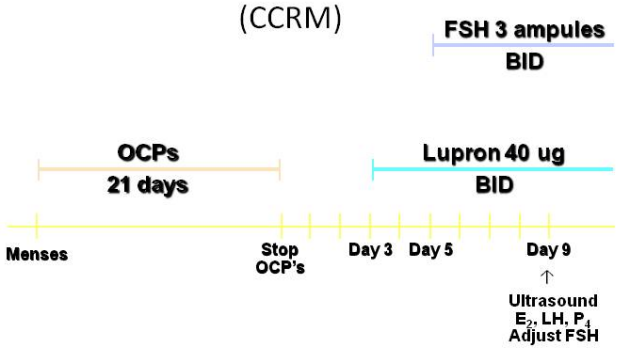


Figure 1 Serum LH (**A**) and FSH (**B**) concentrations after injection of three low doses of LA. There was an increase in both LH ($P < 0.05$) and FSH ($P < 0.02$) concentrations after administration of each of three doses studied (0.17 μg/kg > 0.083 μg/kg > 0.017 μg/kg).

Microdose GnRH agonist (GnRH-a)/FSH Protocol Colorado Center for Reproductive Medicine (CCRM)



OCPs = oral contraceptive pills
P_4 = progesterone
BID = twice a day

Microdose Flair Protocol CCRM

Patients	512
Age (years)	38.4
Prior canceled cycle	29%
FSH	9.1
Prior IVF failure	53%

Microdose Flair Protocol

Amps of FSH	51
Oocytes	11.4
Fertilization	6.9
Embryos transferred	3.1
Ongoing pregnancy	261/512 (51%)
Implantation rate	(24%)

Summary of Micro-Flare Literature

- Microdose flair protocols result in improved ovarian response in poor responders compared to mid-luteal GnRH-a and standard flair protocols.
- OCP pre-treatment avoids follicular increase in LH, P_4, androstenedione (A), and T and their effects on oocyte and embryo quality as well as the endometrium
- Microdoses of GnRH-a result in sustained pituitary release of FSH > LH, yet prevent premature LH surges.

Use of a luteal estradiol patch and a gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation for in vitro fertilization in poor responders

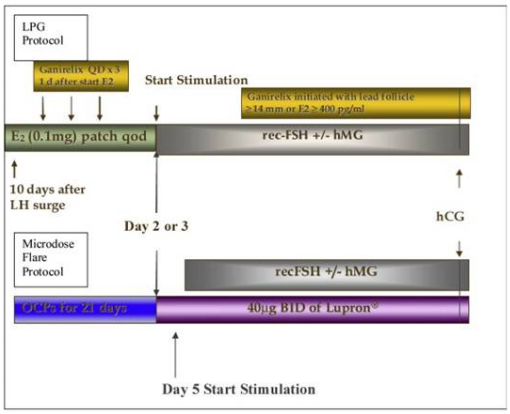
TABLE 1

Stimulation parameters for completed cycles.

Cycle parameter	Prior cycle (n = 66)	E_2 patch cycle (n = 66)	*P* value
Cancellation rate (%)[a]	33.3%	13.6%	<.05
Ampules of gonadotropins	53.0 ± 21.3	70.5 ± 16.8	<.05
Days of stimulation	10.8 ± 2.4	11.0 ± 1.5	NS
Ampules of gonadotropins/day	5.5 ± 1.4	6.9 ± 1.2	<.05
E_2 day of hCG (pg/mL)	873.0 ± 603.2	931.3 ± 562.3	NS
Oocytes retrieved	6.4 ± 4.3	8.3 ± 5.3	<.05
Mature oocytes	5.2 ± 3.4	6.8 ± 4.4	<.05
Two pronuclei[b]	2.4 ± 2.5	4.5 ± 3.2	<.05
Mean fertilization rate	64.7%	68.5%	NS
Embryo grade	2.3 ± 0.5	2.2 ± 1.1	NS
Embryos transferred	2.5 ± 1.7	3.1 ± 1.7	<.05

Note: Values are means ± SD. NS = not significant.
[a] $\chi^2 = 5.4$, $P<.05$.
[b] Embryo grade on a scale of 1–5, with 1 being the highest.

Dragisic. Luteal E_2 patch in poor responders. Fertil Steril 2005.



Weitzman, F&S, 2008

LPG Protocol = luteal E_2 patch/GnRH-a protocol

Comparison of luteal estradiol patch and gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation versus microdose gonadotropin-releasing hormone agonist protocol for patients with a history of poor in vitro fertilization outcomes

Weitzman, F&S, 2008

	LPG group	Microdose group	*P*
Number of cycles	45	76	
Peak E_2	1533	2141	<.05
Oocytes	9.1	8.9	NS
Embryos transferred	2.5	2.7	NS
Cancellation rate	29%	30%	NS
Implantation rate	15%	12.5%	NS
Ongoing pregnancy	30%	26%	NS

NS = not statistically significant

Agonist "flare-up" versus antagonist in the management of poor responders undergoing in vitro fertilization treatment
Mohamed, F&S, February, 2005

TABLE 1

Outcome of short flare-up vs. antagonist protocol.

	Group 1 (flare-up) 77 cycles	Group 2 (antagonist) 57 cycles	*P* value
Age	38 ± 4	38 ± 4	NS
Early follicular phase FSH	9.3 ± 3.3 IU/L	8.6 ± 3.2	NS
Early follicular phase LH	5.3 ± 2.8 IU/L	4.5 ± 1.9	NS
Gonadotropin/day	303 ± 63 IU	318 ± 68 IU	NS
Days of stimulation	11 ± 2 days	9.8 ± 1.6 days	.01[a]
Endometrial thickness	10.3 ± 2 mm	10.2 ± 2 mm	NS
Peak E_2 level	5,910 ± 3,162 pmol/L	3,923 ± 2,040 pmol/L	.001[a]
No. of follicles	6 ± 3	6 ± 3	NS
No. of oocytes	5.2 ± 2.6	5.4 ± 3.6	NS
Fertilization rate (FR)	65.4%	57.4%	NS
No. of embryos transferred	2.1 ± 0.8	1.9 ± 1.1	NS
No. of patients reaching ET	73/77 (94.8%)	47/57 (82.5%)	.02[a]
Implantation rate	21/165 (12.8%)	14/109 (12.8%)	NS
Failed oocyte retrieval	0	2/53 (3.8%)	NS
Failed fertilization	3/77 (3.9%)	4/51 (7.8%)	NS
Failed cleavage	1/74 (1.4%)	0	NS
Cancellation (poor response)	None	4 (7%)	.03[a]
Clinic pregnancy rate/cycle	19/77 (24.7%)	10/57 (17.5%)	NS
Clinic pregnancy rate/ET	19/73 (26%)	10/47(21.3%)	NS

[a]Significant.

A controlled trial of natural cycle versus microdose gonadotropin-releasing hormone analog flare cycles in poor responders undergoing in vitro fertilization
Morgia, Fertil Steril, 2004

Parameters	Natural cycle	COH	P
Number of patients	59	70	—
Number of cycles	114	101	—
Cycles with oocytes (%)	77.2	82.2	—
Cycles with transfer (%)	41.2	68.3	NS
No. of embryos/transfer	1.0	1.8	NS
Pregnancy/cycle (%)	6.1	6.9	NS
Pregnancy/transfer (%)	14.9	10.1	NS
Implantation rate (%)	14.9	5.5	.05

Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders
Mohamed Farouk, M. Mitwally, M.D., and Robert F. Casper, M.D.

Variable	FSH	FSH+Let	P
Total FSH	1590	616	.001
Days of stim	9	6.6	.03
Mature follicles	1.9	3.3	.03
E_2 (pmol/L)	2471	1786	NS

Let = letrozole

Antagonist/Letrozole vs. Microdose Leuprolide Flare (CCRM)

TABLE 2
Stimulation results.

Protocol	Gonadotropin dose, 75 IU ampules	Duration of stimulation, days	Peak E_2, pg/mL	Oocytes retrieved	% Metaphase II oocytes
AL	56.3 ± 9.9	9.9 ± 1.3	1,403 ± 965	12 ± 6	70 ± 20
ML	52.5 ± 13	10.1 ± 1.6	3,147 ± 1,189	13 ± 5.3	79 ± 15
P	NS	NS	<.05	NS	NS

Schoolcraft. Letrozole/antagonist for poor responders. Fertil Steril 2008.

TABLE 3
Treatment outcome.

	Fertilization, %	Day 3 embryo score	Embryos transferred	Implantation rate, %	Ongoing pregnancy rate, %
AL	71	3.48 ± 0.27	3.5 ± 1.3	15	37
ML	73	3.47 ± 0.28	3.7 ± 1.3	21	52
P	NS	NS	NS	NS	<.05

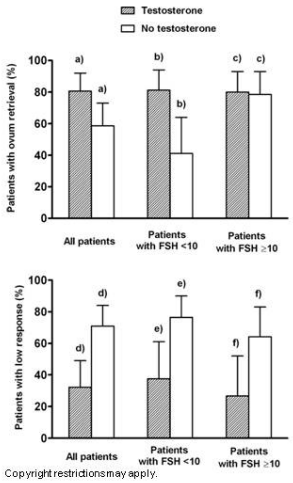
Schoolcraft. Letrozole/antagonist for poor responders. Fertil Steril 2008.

Transdermal testosterone may improve ovarian response to gonadotrophins in low-responder IVF patients: a randomized, clinical trial

Fábregues, Human Reproduction 2009 24(2):349-359

- **Randomized clinical trial including 62 infertile women who had a background of the first IVF treatment cycle canceled because of poor follicular response.**
- **In patients in Group 1 (*n = 31), transdermal application of testosterone preceding standard gonadotrophin ovarian stimulation under pituitary suppression was used.***
- ***In Group 2 (n = 31 patients), ovarian stimulation was carried out with high-dose gonadotrophin in association with a minidose GnRH agonist protocol.***

- Transdermal testosterone treatment was carried out using a daily single patch with a 2.5 mg/day nominal delivery rate of testosterone , which was applied on the thigh at night and always removed at 9:00 in the morning (20 µg/kg per day for 5 days).



Effect of Transdermal Testosterone Administration on the Percentage of IVF Patients Reaching Ovum Retrieval or Being Low Responders in the Whole Study Population and According to Basal FSH Levels

Fabregues, F. et al.

Human Reproduction

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

Barad, Human Reproduction Vol.21, No.11 pp. 2845–2849, 2006

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

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

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

Barad, Human Reproduction Vol.21, No.11 pp. 2845–2849, 2006

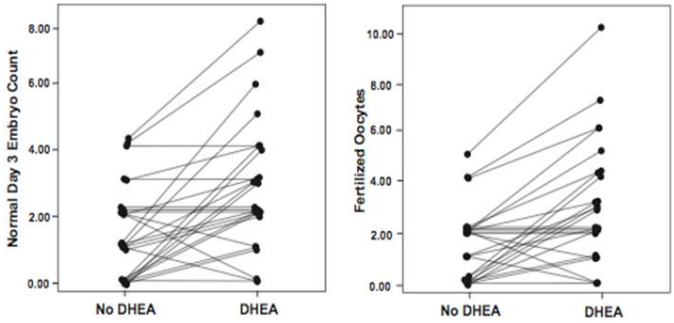


Figure 1. Paired comparison of fertilized oocytes (average increase 1.6 ± 0.37; $P < 0.001$) and normal day 3 embryo count (average increase 1.2 ± 0.4; $P < 0.001$) among 25 patients with pre- and post-dehydroepiandrosterone (DHEA) treatment IVF cycles.

Natural-cycle in vitro fertilization in poor responder patients: a survey of 500 consecutive cycles

- Inclusion criteria in the study were patient age < 44 years and a previous IVF cycle performed in our IVF center that was canceled due to no follicle activation or only one follicle recruited.
- Mean age was 39.3 years (range: 30 to 43 years); their duration of infertility was 4.6 years.

Schimberni, IVF with natural cycle in poor responder women. Fertil Steril 2008.

Natural-cycle in vitro fertilization in poor responder patients: a survey of 500 consecutive cycles

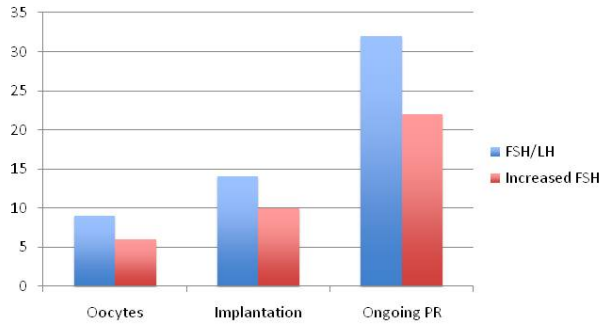
TABLE 1

Data on poor responder women treated with natural-cycle IVF in all cases, stratified by women's age.

Parameters	All cases	≤35 years	36–39 years	≥40 years
No. of patients	294	60	69	165
No. of cycles	500	105	120	275
Cycles without oocytes	21.9%	19.1%	19.6%	24.0%
Cycles without embryos	21.0%	19.1%	23.5%	20.6%
Cycles with transfer	57.0%	61.8%	56.9%	55.4%
No. of embryos	285	65	68	152
Embryo A type	37.0%	43.1%	49.0%	30.7%
Embryo B type	51.9%	41.1%	41.5%	58.7%
Embryo C type	11.1%	15.7%	9.4%	10.6%
No. of embryos/transfer	1.0	1.0	1.0	1.0
Pregnancy/cycle	9.8%	18.1%	11.7%	5.8%
Pregnancy/transfer	17.1%	29.2%	20.6%	10.5%
Pregnancy/patient	16.7%	31.7%	20.3%	9.7%
Implantation rate	17.1%	29.2%	20.6%	10.5%
Abortion rate	16.3%	10.5%	14.3%	25.0%

Schimberni. IVF with natural cycle in poor responder women. Fertil Steril 2008.

De Placido, Human Reproduction 2005 20(2):390-396



Comparison of rLH and rFSH versus rFSH Alone for COH in GnRH Agonist Dowregulated IVF/ICSI Cycles in Poor Responders, Outcome 1: Ongoing Pregnancy per Woman Randomized.

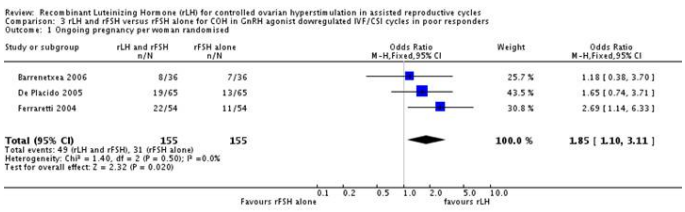


TABLE 3

Interventions proposed for the treatment of poor responders and their effect on primary and secondary outcome of this systematic review and meta-analysis.

Intervention proposed	Number of eligible RCTs	Current evidence: Pregnancy	Current evidence: Secondary outcomes: Significant effect detected	Current evidence: Secondary outcomes: No significant effect detected
1 Addition of GH	5	Significantly increases live birth rate		
2 Addition of GHRF	1	No statistically significant difference in live birth rates		No significant differences in the total number of ampoules administered for ovarian stimulation
3 Addition pyridostigmine	1	No statistically significant difference in ongoing pregnancy/delivery rates	Significantly shorter duration and less ampoules of gonadotropins required for ovarian stimulation and significantly higher number of COCs retrieved when pyridostigmine was added	
4 Addition of oral L-arginine	1	No statistically significant difference in pregnancy rates	Significantly higher number of COCs retrieved and number of embryos transferred when oral L-arginine was added	No significant differences in the duration and the number of gonadotropin ampoules required
5 Addition of transdermal testosterone	1	No statistically significant difference in delivery rates		No significant differences in the duration, the number of gonadotropin ampoules required and COCs retrieved
6 Addition of letrozole	1	No statistically significant difference in pregnancy rates		No significant differences in COCs retrieved
7 Short GnRH protocol (after oral contraceptives pretreatment) versus long GnRH agonist protocol (after medroxyprogesterone acetate pretreatment)	1	No statistically significant difference in clinical pregnancy rates	Significantly less COCs retrieved with the short GnRH-a protocol	No significant differences in the duration and the number of gonadotropin ampoules required

Kyrou. Poor responders and pregnancy. Fertil Steril 2008.

TABLE 3

Continued.

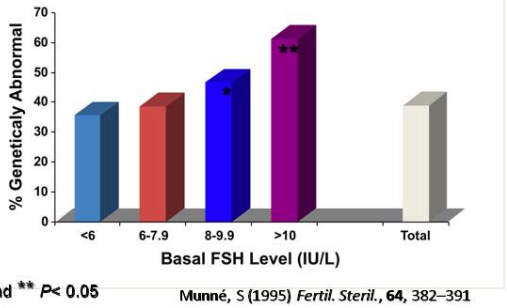
Intervention proposed	Number of eligible RCTs	Current evidence: Pregnancy	Current evidence: Secondary outcomes: Significant effect detected	Current evidence: Secondary outcomes: No significant effect detected
8 GnRH antagonist versus long GnRH agonist protocol	1	No statistically significant difference in ongoing pregnancy rates	Significantly shorter duration and less ampoules of gonadotropins required and significantly more COCs retrieved in the GnRH antagonist group	
9 Combination of clomiphene citrate with rFSH in a flexible GnRH antagonist protocol versus long GnRH agonist protocol	1	No statistically significant difference in pregnancy rates	Significantly less ampoules of gonadotropins required for ovarian stimulation and more COCs retrieved when clomiphene citrate with rFSH in a flexible GnRH antagonist protocol was used	
10 GnRH antagonist versus short GnRH agonist	3	No statistically significant difference in clinical pregnancy rates	Significantly less COCs retrieved in the GnRH antagonist group	No significant differences in the duration and the number of gonadotropin ampoules required.
11 Natural cycle IVF versus long GnRH-a	1	No statistically significant difference in clinical pregnancy rates		
12 Stop versus non-stop long GnRH-a protocol	2	No statistically significant difference in clinical pregnancy rates		No significant differences in the duration, the number of gonadotropin ampoules required and COCs retrieved
13 rFSH versus uFSH	1	No statistically significant difference in pregnancy rates	Significantly shorter duration and less ampoules of gonadotropins required, as well as significantly more COCs retrieved in the rFSH group	
14 ICSI versus conventional insemination	1	No statistically significant difference in pregnancy rates		

Kyrou. Poor responders and pregnancy. Fertil Steril 2008.

Summary

- Based on limited evidence, the only interventions that appeared to increase the probability of pregnancy were the addition of growth hormone (GH) to ovarian stimulation (OR for live birth: 5.22; 95% CI, 1.09–24.99) and the performance of embryo transfer on day 2 compared with day 3 (ongoing pregnancy rate: 27.7% and 16.3%, respectively.
- 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, appears to improve the probability of pregnancy.

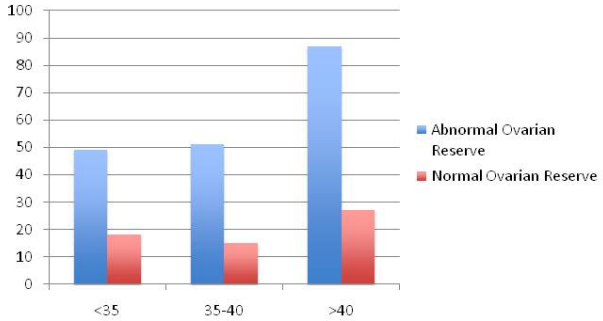
Overall Genetic Abnormality Rate Among 604 Discarded Oocytes and Embryos from Women Under the Age of 40
(Analysis of 13,18,21,X and Y by FISH)



* and ** $P < 0.05$

Munné, S (1995) *Fertil. Steril.*, **64**, 382–391

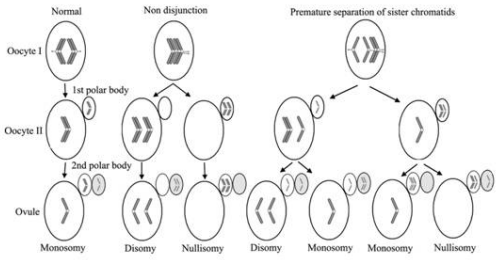
Women with Abnormal Ovarian Reserve Had a Significantly Higher Rate of Reproductive Loss Compared to Patients with Normal Ovarian Reserve (*P < .01 for all groups*).



Levi, F&S, 2001

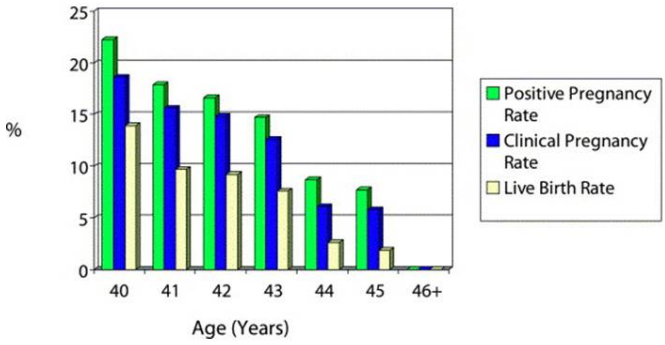
Schematic Representation of the Origin of Meiosis Oocyte Aneuploidies

Vialard, F. et al. Hum. Reprod. 2006



Pellestor *et al.* (2002) recently analyzed a series of 1397 karyotypes of unfertilized oocytes after IVF attempts (women aged 19–46 years) and showed that precocious separation of sister chromatids (PSSC) was more frequent than non-disjunction. Moreover, PSSC seems to be more influenced by maternal aging than mal-segregation (Pellestor *et al.*, 2003).

One last chance for pregnancy: a review of 2,705 in vitro fertilization cycles initiated in women age 40 years and above
Klipstein, F&S, 2005



What About Preimplantation Genetic Screening (PGS) for Patients with Diminished Ovarian Reserve (DOR)

Currently 6 randomized clinical trials (RCTs) regarding PGS for aneupliody; none show benefit

Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial

	PGS group (n = 56)	Control group (n = 53)	P-value
Normal embryos	1.75		
No. of transfers	45 (80.3%)	53 (100%)	0.001
Embryos transferred/ET	1.5 (0.5)	1.8 (0.4)	0.003
No. of live births	3 (5.4%)	10 (18.9%)	0.039
(% per randomized)			
Implantation rate (%)	8/70 (11.4%)	18/95 (18.9%)	0.19
Spontaneous abortions (%)	7/10 (70.0%)	6/16 (37.5%)	0.11

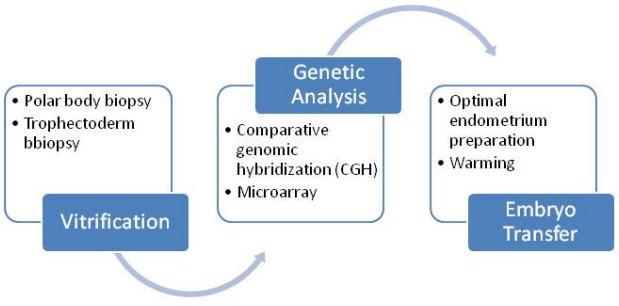
Hardarson, Hum. Reprod. Advance Access published online on June 25, 2008

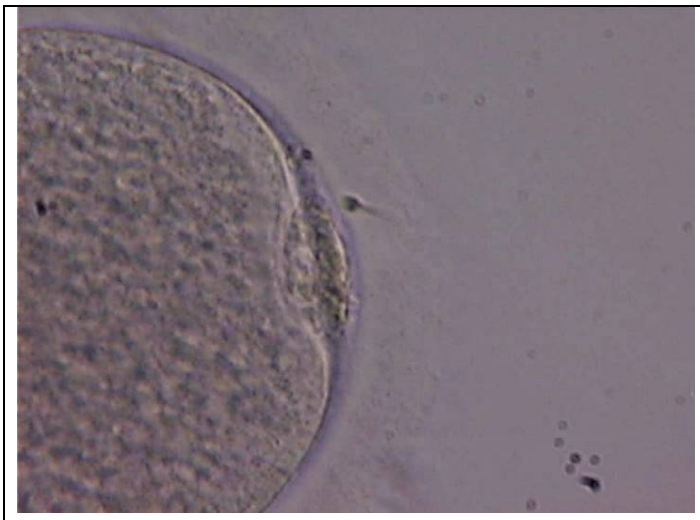
Significant Increase in the Possibility of Performing an Embryo Transfer with Increasing Numbers of Biopsied Embryos

- **1 Embryo = 33% Chance of Transfer**
- **2 Embryos = 43% Chance of Transfer**
- **3-4 = 46% Chance of Transfer**
- **5-7 = 79% Chance of Transfer**
- **8-9 = 78% Chance of Transfer**
- **≥10 = 92% Chance of Transfer**

Kearns, Shady Grove

Change in Paradigm for Embryo Transfer





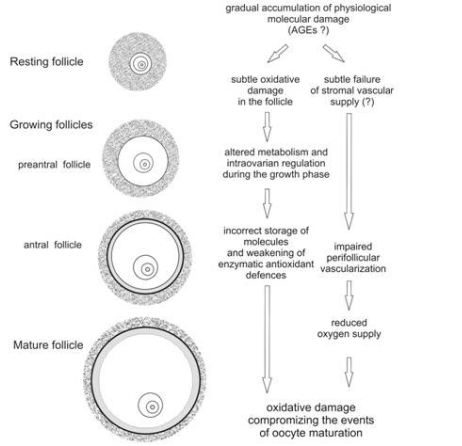
Comprehensive Aneuploidy Screening of Pronuclear Embryos

- We clinically applied a comprehensive aneuploidy screening method to first and second polar bodies (PBs) in 50 poor-prognosis patients
- Average age: 41 years
- Multiple failed IVF attempts or multiple abortions
- Mean FSH: 13.
- PB DNA was subjected to whole-genome amplification and microarray or CGH analysis.
- Zygotes were cryopreserved while PBs underwent testing.
- Normal embryos were thawed and transferred in subsequent cycles.

- CGH provided a full chromosome screening for 94% of zygotes.
- The aneuploidy rate for metaphase I (MI) and metaphase II (MII) was similar (43.3% and 39.8%).
- The total oocyte abnormality rate was 65%, with most errors involving the smaller chromosomes (i.e., 13-22).
- Unbalanced chromatid predivision was rare among larger chromosomes (i.e., 1-12).
- 9-chromosome fluorescent in situ hybridization (FISH) would have failed to detect 48% of abnormalities.
- Implantation rate: 11%

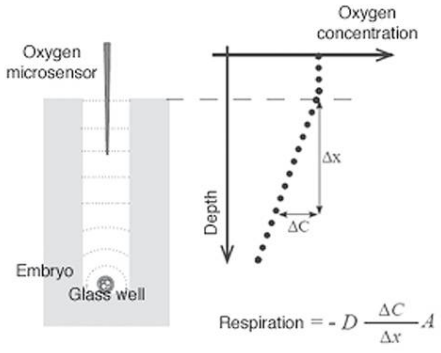
But
Is the Poor Outcome of Patients with DOR Entirely Due to Aneuploidy?

Possible Mechanisms Underlying the Process of Follicle Aging



Tatone, C. et al. Hum Reprod Update 2008 14:131-142; doi:10.1093/humupd/dmm048

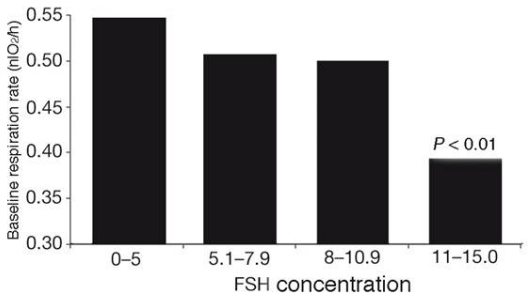
Human Oocyte Respiration-Rate Measurement



$$\text{Respiration} = -D\frac{\Delta C}{\Delta x}A$$

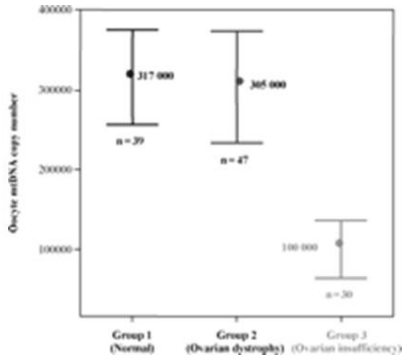
Scott, RBMonline, Oct. 2008

Baseline Respiration Rates of Oocytes According to FSH Concentration.



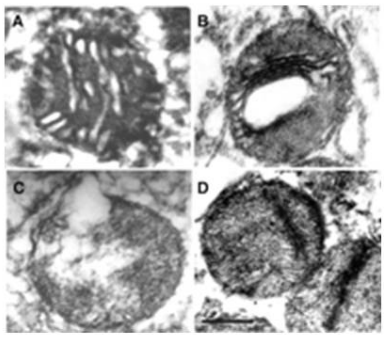
Low Oocyte Mitochondrial DNA Content in Ovarian Insufficiency

May-Panloup Human Reproduction 2005 20(3):593-597



Cellular and Molecular Aspects of Ovarian Follicle Aging

Tatone, Human Reproduction Update 2008 14(2):131-142



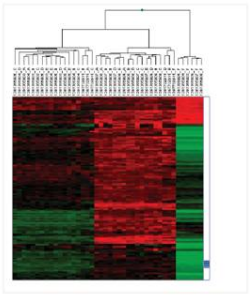
Representative electron micrographs showing granulosa cell mitochondria in younger and older women: in the younger group, intact tubular cristae are evident (**A**) Several defects of mitochondrial structure are observed in the older group including vacuolization (**B**), ruptured membranes (**C**) and degeneration of both cristae and matrix (**D**).

Differential Cumulus Gene Expression

Gene expression with advanced maternal age (greater than 40 years)

UPREGULATED	
Carbonic anhydrase 9	9-fold
Zinc finger protein 582	6-fold
Hyaluronan synthase-2	2-fold
DOWNREGULATED	
Bone morphogenic protein 1	4-fold
Syndecan 3	2-fold

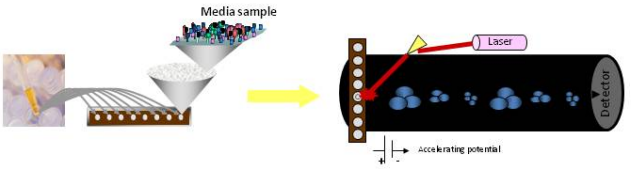
Illumina Human V-2 Genechip



Submitted ASRM 2008
McKenzie

Proteomics – a More Global Approach

Surface-Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry (TOF-MS)



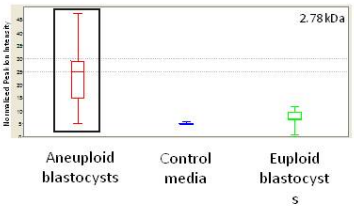
Specific protein capture
Anionic (negatively charged)
Cationic (positively charged)
Normal phase
Metal ion

TOF-MS analysis

Correlation of Variance = 6 to 10%

Non-Invasive Preimplantation Genetic Screening Assay

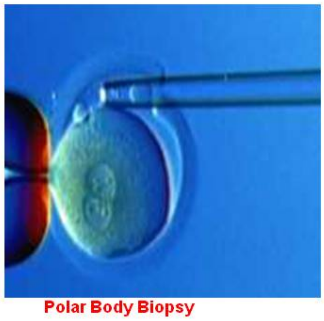
Candidate for Upregulated Protein in Aneuploid Blastocysts ($P < 0.05$)



- A potential identifying candidate for the 2.78kDa protein: early placenta insulin-like peptide (EPIL)
- EPIL is a secreted protein and is encoded by the INSL4 gene.
- Mock et al. 2000 JCEM:

"...Pro-EPIL peptide levels were significantly higher in amniotic fluids from chromosomally abnormal pregnancies from chromosomally normal pregnancies..."

The Future of IVF?

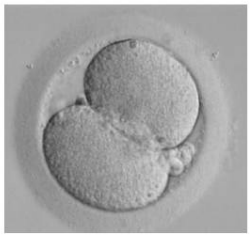


Polar Body Biopsy

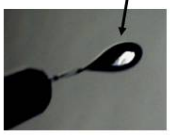
The Future of IVF?

Media sampled for:
Metabolic analysis
Metabolome
Secretome
Other factors

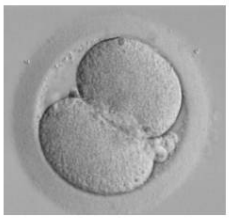
The Future of IVF?



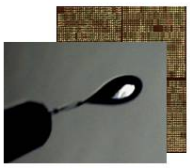
Cleaving embryo then vitrified



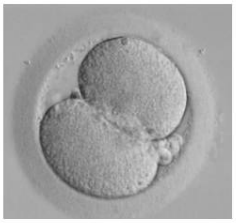
The Future of IVF?



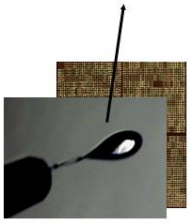
Complete karyotype and gene expression analysis



The Future of IVF?



Assessed embryo then warmed and transferred in a subsequent natural cycle



Colorado Center for Reproductive Medicine

Medical Director, William Schoolcraft, M.D., H.C.L.D.

Clinical

- Dr. Eric Surrey
- Dr. Deb Minjarez
- Dr. Rob Gustofson

Research

- Director of Research, Mandy Katz-Jaffe
- Scientific Director, Dr. David K. Gardner
- Laboratory Director, John Stevens

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NOTES

NOTES

TRIGGERING OVULATION FOR FINAL MATURATION OF OOCYTES

Botros Rizk, M.D., M.A.
Professor And Director,
Division of Reproductive Endocrinology and Infertility
University of South Alabama College of Medicine
Mobile, Alabama

LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

1. Discuss the pharmacodynamics of human chorionic gonadotropin (hCG).
2. Critically review the use of gonadotropin-releashing hormone (GnRH) agonist to trigger ovulation in ART.
3. Assess the role of recombinant luteinizing hormone (rLH) in triggering ovulation.

Triggering Ovulation for Final Maturation of Oocytes

Botros Rizk, M.D., M.A., F.R.C.O.G., F.R.C.S.(C), H.C.L.D., F.A.C.O..G, F.A.C.S.

Professor and Director,
Division of Reproductive Endocrinology and Infertility
University of South Alabama College of Medicine
Mobile, Alabama

4.

Different Gonadotropins for Ovarian Stimulation: How Can We Choose?

LEARNING OBJECTIVES

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- Discuss the pharmacodynamics of human chorionic gonadotropin (hCG).
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Disclosure

- Research/Principal Investigator: Boehringer-Ingelheim, Solvay, Proctor and Gamble and Eli-Lilly
- Speaker honoraria: Wyeth, Proctor and Gamble/Sanofi-Aventis, Duramed, Myriad, Warner-Chilcott.

Structure and Pharmacokinetics of Chorionic Gonadotropin (CG)

hCG is administered during controlled ovarian stimulation. It can be administered prior to oocyte retrieval to mimic an endogenous luteinizing hormone (LH) surge if the oocytes will be used for IVF or prior to intrauterine insemination if fertilization is attempted in the patient's uterus. It is also administered after fertilization to support the function of the corpus luteum.

Shuler and Scammel. 2008; In: Rizk, Garcia-Velasco, Sallam, Makrigiannakis (Eds) Infertility and Assisted Reproduction Cambridge University Press;Chapter 25;228-234

Structure and Pharmacokinetics of Chorionic Gonadotropin

The β-subunit of hCG is 145 amino acids. Homology between the β-subunits of human CG and human LH is approximately 80%, with CG possessing a C-terminal extension of 24 amino acids. The β-subunit of CG is glycosylated at six sites. The four O-linked glycosylation sites in the C-terminus protect CG from degradation. The oligosaccharide chains form a physical barrier, protecting the protein core.

Trinchard-Lugan, etal. Reprod Biomed Online 2002;4:106-115

Structure and Pharmacokinetics of Chorionic Gonadotropin

Like recombinant human follicle-stimulating hormone (hFSH), recombinant hCG (r-hCG) is also made in a Chinese hamster ovary (CHO) cell culture system. This system yields highly purified product without many of the contaminants observed in urinary-origin hCG. The pharmacodynamics of r-hCG are similar to those of urinary hCG, but r-hCG is more potent. The half-life is biphasic; the longer phase is reported to be approximately 24 to 33 hours. Clearance is reported to be 0.3 L per hour. The liver metabolizes approximately 80% of cleared hCG.

Trinchard-Lugan, etal. Reprod Biomed Online 2002;4:106-115

r-hCG and Controlled Ovarian Stimulation (COS)

CG binds to the CG/LH receptor (LHR). Like the FSH receptor (FSHR), the LHR is also a G-protein-coupled receptor. r-hCG can be used in controlled ovarian stimulation protocols to induce maturation of the oocyte and can improve pregnancy outcome. In addition, r-hCG has significant advantages over urinary CG, including a higher serum hCG concentration, higher serum progesterone concentration and a reduction in local reactions. Exogenously administered CG in controlled ovarian stimulation has also been shown to increase endometrial receptivity to the early embryo.

Griesinger et al. Hum Reprod Update 2006;12:159-168

Human Chorionic Gonadotropin for Triggering Ovulation

hCG is commonly used for triggering ovulation and the final stages of follicular maturation because of its LH-like activity. There are some drawbacks to its use because it has biologic differences from human LH. After the administration of hCG, the LH-like activity spans several days, compared to the 48 hour duration of the endogenous LH surge. The prolonged activity of hCG is the result of its greater carbohydrate content and longer half life.

Ravel and Casper 2001; Infertility and Reproductive Medicine Clinics of North America.

hCG Dosage in the First Series of Ovarian Hyperstimulation Syndrome (OHSS)

MILD

Diagnosis	Case	Amps of Pergonal	IU of hCG	Remarks
Primary amenorrhea	Ge. E.20/31	25	25000	Pregnancy
	Le. R. 63/108	28	26000	------
Secondary amenorrhea	Ge. P. 21/32	55	29000	------
Secondary amenorrhea and galactorrhea	Sh. M. 72/121	28	29000	------
Postpartum amenorrhea and galactorrhea	Lo. S. 40/67	19	25000	Pregnancy
Anovulation	Iv. B. 1/1 Hi. E. 89/163	2927	20000	------

Rabau et al. Am J Obstet Gynecol 1967;98:92-98

hCG Dosage and the First World Series of OHSS

SEVERE

Diagnosis	Case	Amps of Pergonal	IU of hCG	Remarks
Primary amenorrhea	Do. M. 108/199	22	25000	Pregnancy
Secondary amenorrhea	Ge. P. 21/34	73	15000	------
Secondary amenorrhea MAP+	Ki. A, 59/149	14	25000	Pregnancy
Postpartum amenorrhea and galactorrhea	Bi. F. 11/17 Ba. A. 13/21	3460	21500 25000	Quadruplet abortion
Anovulation	Be. Z. 19/29	20	10000	------
Proliferative follicular phase	Po. A. 53/90	20	25000	Twin pregnancy

Rabau et al. Am J Obstet Gynecol 1967;98:92-98

Effectiveness of GnRH Agonist in Uncontrolled Studies

Study	*Criteria*	*#*	*LH Surge n (%)*	*Pregnancy rate per ET n (%)***
Lanzone, et al. (1989)		8	8 (100)	--
Emperaire and Ruffie (1991)	E_2 > 1200 pg/mL	126	--	27 (22)
Imoedemhe et al. (1991)	E_2 > 4000 pg/mL	27	--	11 (29)
Itskovitz et al. (1991)		12	12 (100)	4 (29)
Tulchinsky et al. (1991)	Pilot study	13	11 (85)	4 (36)
Van der Meer et al. (1993)	Pilot study	48	44 (92)	10 (23)
Balasch et al. (1994)	Cycles that would otherwise have been cancelled	23	17 (74)	4 (17)
Shalev et al. (1994)	E_2 > 3200 pg/mL	12	--	6 (50)
All		261	88%	29%

Rabau et al. Am J Obstet Gynecol 1967;98:92-98

ET = embryo transfer
E_2 = estradiol

Effectiveness of GnRH Agonist versus hCG in Controlled Studies

Gonen et al. (1990)	CC-hMG	9	9 (100)	0	9	9	3 (33)	NS
Segal and Casper (1992)	CC-hMG	95	--	19 (19)	84	--	18 (20)	NS
Scott et al. (1994)	CC	21	21 (100)	--	21	21 (100)	--	NS
Kulikowski et al (1995	CC-hMG	34	--	3 (9)	32	--	4 (13)	NS
Gerris et al. (1995)	hMG	10	10	--	19	--	--	<0.01
Schmit-Sarosi et al (1995)	CC	15	8 (53)	2 (13)	11	11 (100)	3 (27)	NS
Shalev et al. (1995)	CC	106	--	14 (13)	104	--	3 (12)	NS
Shalev et al.(1995)	hMG	68	--	18 (27)	72	--	11 (15)	0.0007
Romen et al. (1997)	FSH	416	413 (99)	71 (17)	345	342 (99)	93 (27)	<0.05
All		753	461/471 (98)	127/734 (16.7)	676	402/405 (99)	144/657 (22)	

Revel and Casper (2001) I

Uncontrolled Studies To Determine Whether GnRH Agonists for Triggering Ovulation Prevent OHSS

Emperaire and Ruffie (1991)	E_2 >1200 pg/mL or >3 follicles of 17 mm	37	10	
Imoedemhe et al. (1991)	E_2 >4000 pg/mL	36	0	
Iskovitch et al. (1991)	E_2 5000-13000 pg/mL	8	0	
Vander Meer et al. (1993)	--	48	3	Mild to moderate
Balasch et al (1994)	Cycles to be cancelled due to high risk	23	0	
Balasch et al (1995)		30	0	
Lanzone et al. (1989)	Polycystic ovary syndrome (PCOS)	30	0	Some GnRH agonist, some hCG
Lewit et al. (1995)	High risk?	80	0	
Shalev et al. (1994)	E_2 > 3500 pg/mL, number of follicles >20	12	0	Not IVF
Total		334	3(0.9%)	

Revel and Casper (2001)

Controlled Studies to Determine Whether GnRH Agonist for Triggering Ovulation Prevents OHSS

Gonen et al (1990)		9	0	9	0	
Segal and Casper (1992)	Randomized	84	0	95	0	
Gerris et al.	Controlled	28	1	10	0	On native GnRH
Kulikowski et al. (1995)	Non-randomized	48	0	34	4	Moderate OHSS
Shalev et al. (1995)	Randomized	72	4	84	8	Not significant
Shalev et al. (1995)	Randomized	104	0	106	0	Clomiphene cycles
Romeu et al. (1997)	Prospective, non-randomized	345	0	416	0	FSH, intrauterine insemination (IUI)
Penarrubia et al. (1998)	Prospective, non-randomized	26	0	26	0	2 doses of hCG and of LH
All		716	5 (0.7%)	780	12 (1.5%)	Significant, p=0.047 (z test)

Revel and Casper (2001)

Recombinant LH

A recent double-blind large multicenter randomized study that compared the implantation and pregnancy rates following triggering ovulation by r-hLH versus hCG was discussed in a letter to the editor in response to an article advocating the use of LH to trigger ovulation. The study is as yet unpublished.

Emperaore and Edwards Reprod Biomed Online 2004;9:480-483

Recombinant LH

A total of 437 patients were randomly allocated in a 2-to-1 ratio to either the r-hLH treatment group (291 patients). The two groups were matched for age, height, weight, body mass index (BMI), race and smoking habits at baseline. The mean ages were 31.1 +/- 4.5 years, mean height 164 +/- 7 cm, mean body weight 65.3 +/- 11.4 kg and 66.0 +/- 11.8 kg, the mean BMI 24.4 +/- 4.2 kg/m^2 and 24.4 +/- 4.1 kg/m^2 in the r-hLH and u-hCG groups respectively.

Emperaore and Edwards Reprod Biomed Online 2004;9:480-483

Recombinant LH

The majority of patients in the study population were Caucasian (95.9% and 97.9% in the r-hLH and the urinary hCG (u-hcCG) groups, respectively) and the majority did not smoke (78% and 82.2% respectively). The results showed that a clinically significant OHSS (severe and all cases) was significantly lower in the r-hLH and the u-hCG group (p=0.001); however the pregnancy rates and the clinical pregnancy rates were significantly lower in the r-hLH group compared to the u-hCG group (p= 0.018 and p=0.023, respectively). In order for the r-hLH to be as effective as hCG, the dose should be increased.

Emperaore and Edwards Reprod Biomed Online 2004;9:480-483

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NOTES

NOTES

LIFESTYLE, ACUPUNCTURE, STRESS MANAGEMENT, ERECTILE FUNCTION, NUTRITION AND SUPPLEMENTS IN THE MANAGEMENT OF INFERTILITY

David R. Meldrum, M.D.
Clinical Professor, UCLA and UCSD
Scientific Director, Reproductive Partners Medical Group
California, U.S.A.

LEARNING OBJECTIVES:
At the conclusion of this presentation, participants should be able to:

1. List lifestyle factors that impact IVF success.
2. Apply interventions to improve IVF outcomes.
3. Discuss the role of nutrition and supplements in infertility and erectile function.

Lifestyle, Acupuncture, Stress Management, Erectile Function, Nutrition and Supplements in the Management of Infertility

David R. Meldrum, M.D.
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Learning Objectives

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1. List lifestyle factors that impact IVF success.
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Disclosure

David Meldrum, M.D., is president of Sexuality EDucation Network (SEN)

SEN operates the web site www.Erectile-Function.com and publishes the book, "Survival of the Firmest"

Psychosocial Stress and ART Outcome

- 90 women followed prospectively.
- Univariate analysis found a negative impact of full-time employment, hostile mood, and higher anxiety on successful outcome.
- Multiple regression analysis also found depression to be a negative factor.

Sanders KA, Bruce NW: Hum Reprod 1999; 14: 1656-1662

Anxiety, Depression and IVF

- 291 women having IVF/intracytoplasmic sperm injection (ICSI)
- State and Trait Anxiety Inventory and Beck Depression Inventory
- Multiple logistic regression
- State anxiety ($p = 0.01$) and depression ($p = 0.03$) were correlated with failure.

Smeenk JMJ, et al.: Hum Reprod 2001;16:1420-3

Positive/Negative Affect and IVF

- 151 women having IVF or gamete intra-fallopian transfer (GIFT)
- Positive affect was associated with number of oocytes retrieved, embryos tranferred and live births

Klonoff-Cohen H: Fertil Steril 2001;76:675-87

Vulnerability to Stress

- Stroop Color and Word Test
- Blood pressure (BP), heart rate (HR)
- Success associated with lesser physiologic changes in response to stress

Facchinetti F, et al.: Fertil Steril 1997;67:309-14

Psychosocial Interventions and Pregnancy Rates

- Prospective, randomized study
- Interventions were associated with higher pregnancy success
- In the group having intensive cognitive and behavioral intervention, 55% conceived, and almost half those pregnancies were unassisted.

Domar AD, et al.: Fertil Steril 2000; 73:805-12

Negative Affect and IVF

- 391 women, Hospital Anxiety and Depression Scale
- Multiple stepwise regression analysis
- Less negative affect associated with poorer outcome

De Klerk C, et al.: Hum Reprod 2008;23:112-6

Acupuncture and IVF

- 10 infertile women with uterine artery pulsatility index (PI) ≥ 3.0
- Electroacupuncture twice weekly for 4 weeks in gonadotropin-releasing hormone (GnRH) agonist suppressed women
- PI decreased significantly from 3.34 to 2.68 and stayed down for 8-10 days

Stener-Victorin E, et al.: Hum Reprod 1996;1314-7

Acupuncture and IVF

- 7 trials, 1366 women, acupuncture within 1 day of embryo transfer (ET)
- Trials with mock acupuncture controls and no adjuvant therapy controls were combined
- Odds ratio (OR) 1.65 (confidence level [CL] 1.27-2.14) for clinical pregnancy
- Sham acupuncture also less effective
- Treatment effect not significant where control success rates were higher

Manheimer E, et al.: BMJ 2008;336:7346

Acupuncture and IVF

- Meta-analysis of 13 randomized trials using sham controls, 5 at retrieval and 5 at ET
- Relative risk (RR) was 1.06 for time of retrieval and 1.23 for time of ET, neither being significant
- 5 ET trials had birth data and the RR (1.34) was not significant

El-Toukhy T, et al.: BJOG 2008;115:1203-13

Acupuncture and IVF

- 150 subjects randomized to acupuncture or control; same protocol as Paulus study
- No difference in pregnancy rate (PR) (30.8% vs. 33.8% for controls)
- With at least one good quality embryo, 42% vs. 47%

Domar AD, et al.: Fertil Steril 2009;91:723-6

Acupuncture and IVF

- 370 subjects randomized to acupuncture or placebo acupuncture
- PR ***higher*** with placebo acupuncture (OR 1.58; CL 1.05-2.48; p = 0.038)
- No differences in endometrial or sub-endometrial vascularity, serum cortisol or anxiety level

So EWS, et al.: Hum Reprod 2009;24:341-8

Acupuncture and IVF

- Prospective, randomized trial
- Acupuncture group had a **reduced** pregnancy rate.
- Emphasizes the need to be sure the acupuncture technique matches that used in the original trials (see Manheimer E, et al.: BMJ 2008;336: 1746).

ASRM abstract O-106, Annual Meeting, Oct 2007

Acupuncture - Conclusions

- There is currently no consistent evidence that acupuncture increases the chance of success with IVF.
- In individual studies, acupuncture ***reduced*** the chance of IVF success.
- If acupuncture is to be used, be sure that the technique is the same as was used in studies where improved success was suggested.*

*Manheimer E, et al.: BMJ 2008;336:7346

Sexual Dysfunction and IVF

- Advise couples to freeze a specimen if there is concern.
- Allow couples to collect together, off-site, or using non-toxic condom.
- Have sildenafil available
- If all else fails, emergency sperm retrieval is an option.

Tur-Kaspa I, et al.: Hum Reprod 1999;14:1783-4

Sexual Dysfunction and IVF

- 121 infertile couples
- 22% of male partners had mild to moderate erectile dysfunction.
- Erectile dysfunction (ED) correlated with their partner's sexual satisfaction and with their own self-esteem.
- 12 % also had depression.

Shindel AW, et al.: J Urol 2008;179:1056-9

Sexual Dysfunction and Treatment of ED

- Female sexual satisfaction resulting from treatment of ED
- 38 women whose partners had ED had highly significant decreases of various indices of sexual satisfaction.
- Treatment of ED increased arousal and lubrication ($p = 0.001$, $p = 0.002$), and orgasm and satisfaction ($p < 0.001$).

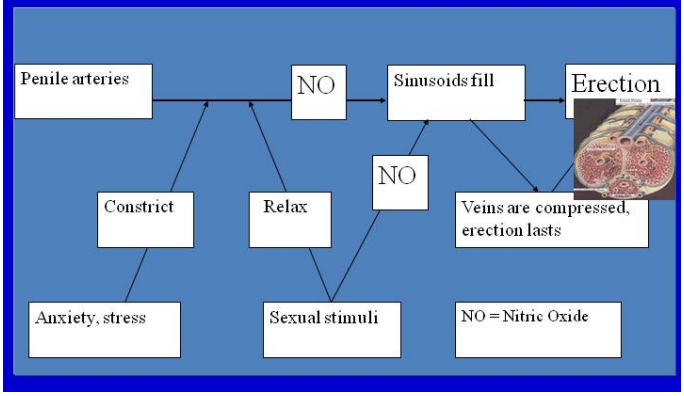
Cayan S et al.: J Sex Marital Ther 2004;30:333-41

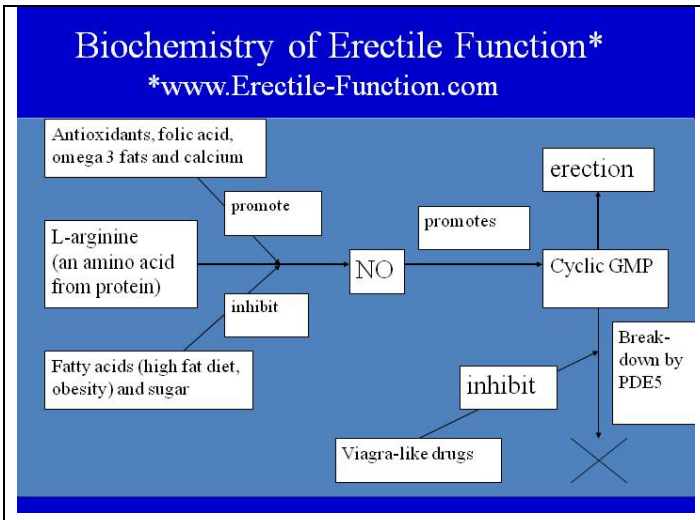
Sildenafil-like Drugs Are a "Cover-up" We Can *ILL* Afford

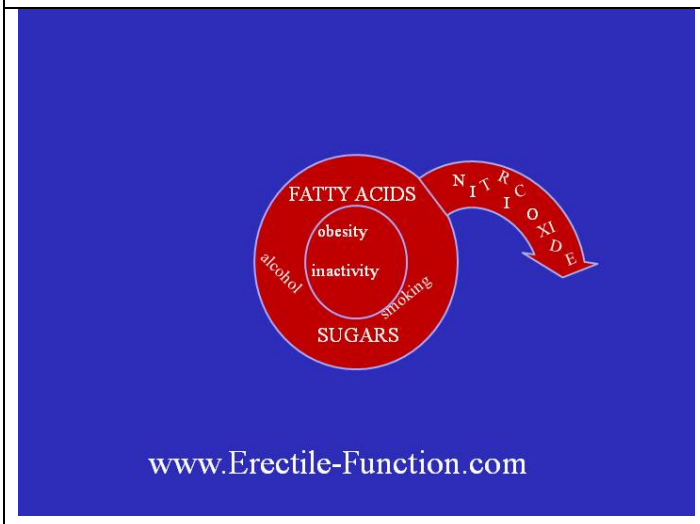
- Inhibitors of phosphodiesterase type 5 (PDE5) afford only symptomatic treatment that increases cyclic guanosine monophosphate (GMP) but does nothing to solve the underlying problem of deficient nitric oxide (NO) production by unhealthy blood vessels.
- NO both stimulates cyclic GMP ***and*** also reduces/prevents atherosclerosis, intra-arterial clotting and smooth muscle proliferation in artery/arteriolar walls.

Physiology of Erectile Function*

*www.Erectile-Function.com




Biochemistry of Erectile Function*
*www.Erectile-Function.com
Antioxidants, folic acid, omega 3 fats and calcium
promote
L-arginine (an amino acid from protein)
inhibit
Fatty acids (high fat diet, obesity) and sugar
NO
promotes
erection
Cyclic GMP
Break-down by PDE5
inhibit
Viagra-like drugs


FATTY ACIDS
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Alcohol/Caffeine and Normal Fertility

	Pregnancy (%)	Odds ratio
None (per week)	24.5	1
Alcohol 1 drink	17.3	**.43(.25-.76)**
1-7	11.9	
> 7	8.3	
Any alcohol + > 1 cup/day coffee	10.5	**.26(.13-.52)**

Hakim RB: Fertil Steril 1998; 70:632

Type of Alcohol and Fecundity

- 29,844 pregnant women answered questionaire.
- Proportion of women taking over 12 months to conceive was higher with highest alcohol intake.
- Wine associated with significantly less delay (e.g., 2.5-7 glasses per week, OR = 0.7; CL 0.64-0.69),

Juhl M, et al.: Hum Reprod 2003;18:1967-71

Alcohol and IVF

- 221 couples, multicenter trial
- Multivariate logistic regression
- Female: 13% reduction in number of eggs; risk of not becoming pregnant increased by 2.9 (0.99-8.24); increased miscarriage by 2.2 (1.1-4.5).
- Male: over 2-fold decrease of PR; over 2-fold increase of miscarriage.

Klonoff-Cohen H, et al.: Fertil Steril 2003;79:330-9

Caffeine and IVF

- 221 women studied prospectively before IVF or GIFT
- 0-2 mg of caffeine (= 1 cup of decaffeinated coffee) was associated with a higher chance of pregnancy

Klonoff-Cohen H, et al.: Hum Reprod 2002;17:1746-54

Smoking and IVF Outcome

- Meta-analysis showed an odds ratio for successful pregnancy of .54 (CL .385-.757)
- Various studies show reduced ovarian reserve, ovarian response, semen quality and fertilization rate, and increased miscarriage rate.

Hughes EG: Fertil Steril 1994; 62:807

Secondhand Smoke and IVF

- 225 women having IVF or ICSI
- Pregnancy rate similarly and significantly reduced with side-stream smoke (20%) and in smokers (19.4%), compared to non-smokers (43.8%) ($p < 0.001$).

Neal M, et al.: Hum Reprod 2005;20:2531-5

Smoking and Uterine Receptivity

- 785 egg donation cycles with non-smoking male; no donors were heavy smokers
- Heavy smoking (> 10 cigarettes per day) was associated with a lower pregnancy rate (34.1% vs 52.2%, p = 0.02)

Soares SR, et al.: Hum Reprod 2007;22:543-7

Male Smoking and IVF

- 301 couples having IVF or ICSI
- Pregnancy rates for male smokers were reduced with ICSI (22%) and IVF (18%) compared to non-smokers (38% and 32%).
- Multinomial logistic regression confirmed lower rate of viable pregnancy with male smoking (p 0.003).

Zitzmann M, et al.: Fertil Steril 2003;79:1550-4

Exercise and IVF

- Cardiovascular exercise overall resulted in an OR of 0.7 (CL 0.6-0.9) for live birth.
- 4 or more hours of exercise per week was associated with increased cancellation, increased pregnancy loss and reduced live birth, but division of the data into 8 categories may have influenced the chance of a significant result.
- Exercise during the cycle was not evaluated.
- Until more information is available, it may be prudent to advise only walking, or limitation of cardiovascular exercise to 3 hours or less per week.

Morris SN, et al.: Obstet Gynecol 2006;108:938

Nutrition and IVF

- Antioxidant capacity in follicular fluid (FF) correlates with outcome and decreases with age.
- Antioxidant capacity in semen correlates with sperm quality and decreases with age.
- A nutritional supplement, fertilityblend, was compared with placebo in women attempting to conceive, and more women using the supplement conceived during the first 5 months ($p < 0.01$).

Westphal LM, et al.: J Reprod Med 2004;49:289-93

Advanced Glycation Endproducts (AGEs) and IVF

- Advanced glycation end products (AGEs) are toxic oxidative chemicals produced when foods are subjected to high heat and increase with age.
- AGEs are higher in serum and FF of women who fail to conceive with IVF.*

ASRM abstract O-17*, 2008 Annual Meeting, Fertil Steril 2008;90, suppl 1:S6

Oxidative Stress and IVF

- Oxidative stress of granulosa cells correlated positively with apoptosis and negatively with embryo quality and pregnancy rate.*
- In another study, granulosa cell apoptosis was dramatically lower (0.86%) in pregnant women compared to non-pregnant (14.4%, $p < 0.001$) women undergoing IVF.**

ASRM abstracts P-681* and P-737** 2008 Annual Meeting, Fertil Steril 2008;90, suppl 1:S336* and S356**

Nutrition and Semen Quality

- 30 men with poor semen quality, 31 normospermic controls
- In the logistic regression model, cases had lower intake of lettuce, tomatoes and fruits, and higher intake of dairy and meat processed products

Mendiola J, et al.: Fertil Steril 2009;91:812-8

Supplements and Semen Quality

- 64 men with elevated sperm DNA fragmentation (mean 22%)
- Randomized to 1 gm of vitamin E and 1 gm vitamin C versus placebo for 2 months
- % fragmentation was reduced from 22% to 9% ($p < 0.001$); sperm density increased from 19 to 28% (NS)

Greco E, et al.: J Andrology 2005;26:349-53

Obesity (BMI > 25) and IVF Outcome

- Clinical and ongoing pregnancy rates were significantly lower (31% and 25%, respectively) for obese women than for women with body mass index (BMI) $\leq$ 25 (55% and 51%, respectively).*
- Two abstracts at the 2001 ASRM annual meeting also showed a negative impact of BMI on IVF outcome.

*Loveland JB: J Assist Reprod Genetics 2001; 18: 382

Find a Young Husband

- 59 French IVF centers, 1,938 men
- Partners with absent or obstructed tubes
- OR for failing to conceive was 2.0 when the woman was age 35-40, and 5.7 when over 41 (CL 2.16-15.23)
- Older men have increased DNA damage

La Rochebrochard E et al.: Fertil Steril 2006;85:1420-4; Schmid TE, et al.: Hum Reprod 2007;22:180-7

Conclusion

- Poor nutrition, stress, smoking, caffeine and alcohol all have a negative impact on IVF outcome.
- Poor nutrition and the increase of oxidative and advanced glycation end-products with age in both partners appear to play a much larger role in IVF outcome than previously appreciated.

Conclusion

- Nutritional supplements may play an important role in the preparation of infertile couples for IVF, particularly those who are older.
- Exercise, weight loss, a more nutritious diet and supplements all enhance erectile function without the need for drugs.
- www.Erectile-Function.com will provide your patients with evidence-based information on good nutrition and the prudent use of supplements –David@Erectile-Function.com

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NOTES

CRITICAL ASSESSMENT OF THE USE OF LUTEINIZING HORMONE (LH)

Mohamed Aboulghar, M.D.
Professor of Obstetrics and Gynecology, Cairo University
Clinical Director, The Egyptian IVF center
Cairo, Egypt

LEARNING OBJECTIVES
At the conclusion of this presentation, participants should be able to:

1. Elucidate on the role of luteinizing hormone (LH) in controlled ovarian hyperstimulation for IVF, particularly with the use of the gonatropin-releasing hormone (GnRH) analogues.
2. Provide further insight into the role of LH during ovulation induction for IVF.
3. Suggest appropriate clinical criteria for LH supplementation.

Critical Assessment of the Use of Luteinizing Hormone (LH)

Mohamed Aboulghar, M.D.
Cairo, Egypt

Learning Objectives

At the conclusion of this presentation, participants should be able to:

- Elucidate the role of LH in controlled ovarian hyperstimulation for IVF, particularly with the use of the gonadotropin-releasing hormone (GnRH) analogues.
- Provide further insight into the role of LH during ovulation induction for IVF.
- Suggest appropriate clinical criteria for LH supplementation.

Disclosure

Nothing to disclose

LH exerts its activity on theca cells. It was also found that LH receptors are detectable on the granulosa cells at the intermediate follicular phase *(Hillier et al., 1994)*. Therefore, it appears that LH regulates both granulosa and theca cells, and is important in promoting follicular maturation.

The Role of High LH

- It was believed that excessive stimulation of the ovaries by LH adversely affects normal pre-ovulatory development; follicles exposed to inappropriately high concentrations of LH enter atresia or become prematurely luteinized, and oocyte development may be compromised. *(Balasch and Fabregues 2002)*.

The available recent data throw doubt on the earlier belief that too much LH is harmful.

Filicori et al. 2002

200 IU of hCG = 1200 IU rLH

↓

From day 8 of stimulation

↓

- No detrimental effect on large follicles (≥ 10 mm)
- Hastened demise of small follicles (<10 mm)

hCG = human chorionic gonadotropin
rLH = recombinant luteinizing hormone

Controlled Ovarian Stimulation (COS) and LH *(Balasch, 2004)*

Profound hypogonadotropic amenorrhea

↓

rFSH stimulation

↓

No rise of estradiol (E_2)

Some exogenous LH is necessary to optimize ovulation induction

rFSH = recombinant follicle-stimulating hormone

A Small Amount of LH Is Required for Proper Folliculogenesis

- Q1: How much LH do we need?
- A1: Probably a very small amount, but not exactly decided.
- Q2: How much LH is available after down-regulation?
- A2: Variable amounts.

How Much LH Is Available After Down-Regulation (Westergaard et al. 2001)

- It depends upon:
  - GnRH agonist (GnRHa) formulation.
  - Dose
  - Mode of administration.
- LH level reaches between 0.5 and 2.5 IU/L
- May fall to < 0.5 IU/L during intermediate-late stage of stimulation.

Large dose of GnRHa given by injection causes more suppression than the use of nasal spray for down-regulation of the pituitary gland (Westergaard 2001).

During the days when human menopausal gonadotropn (hMG) was routinely used, the problem of LH was not raised during stimulation after down-regulation, because LH actually was abundant in the administered hCG. However, the question was raised when recombinant FSH, which is free of LH activity, began to be used in many cases of COH after down-regulation with long protocol.

Thus, multiple follicular growth is induced without exogenous LH and in a low endogenous LH environment. Nevertheless, an adequate ovarian response is achieved in almost all patients *(Chappel and Howles, 1991).*

Large Multicenter Randomized Trial with Long GnRHa Protocol

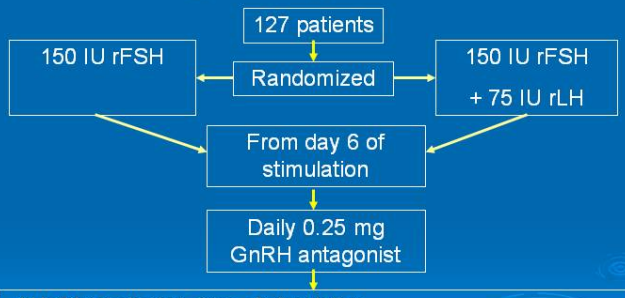
rFSH, 225 IU/day	rFSH, 225 IU/day + rLH, 150 IU/day from day 6 of stimulation

No significant difference in number metaphase II (MII) oocytes or cumulative pregnancy rate *(Marrs et al. 2004)*.

Early Follicular-Phase Recombinant Luteinizing Hormone Supplementation During Stimulation for IVF (Kovacs et al. 2009)

- Randomized controlled trial
- Long GnRHa down-regulation protocol
- Patients in the experimental group received 75 IU of rLH daily for 4 days and rFSH at a fixed starting dose of 150 IU.
- In control group, patients started rFSH at a fixed done of 150 IU for the first 5 days at suppression.
- Stimulation, embryology parameters and treatment outcome were comparable.

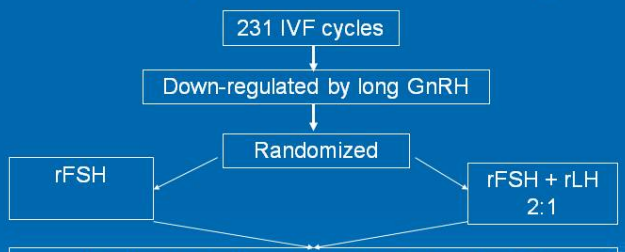
Recombinant LH Supplementation in GnRH Antagonist Cycles (Griesinger 2005)



- rLH did not shorten days of stimulation.
- Significantly higher E_2 (1924±1256 vs. 1488.3±824 pg/mL) in rLH arm

Humaidan et al. (2004) in a randomized trial studied the effects of LH activity supplementation in normogonadotrophic women, aged <40 years. Stimulated by rFSH using long GnRha protocol, they failed to show any significant difference in terms of endocrinology, ovarian response and pregnancy outcome between groups.

Recombinant LH Supplementation in IVF (Humaidan et al 2004)



- No significant difference in pregnancy rate (PR)
- Women older than 35 years had a higher implantation rate and less consumption of rFSH in the rLH arm.

In a double-blind study using long GnRHa protocol, patients were randomized when the lead follicle reached a diameter of 14 mm to receive recombinant human FSH (r-hFSH) in addition to recombinant human LH (r-hLH), 75 IU, or placebo daily for a maximum of 10 days prior to oocyte retrieval and IVF *(Tarlatzis et al 2006)*. Serum estradiol concentrations on the day of hCG administration were significantly higher in the group receiving r-hLH plus r-hFSH ($P = 0.0001$).

There were no significant differences between the groups in dose and duration of r-hFSH treatment required, oocyte maturation, fertilization rate, pregnancy rate and live birth rate (BR) *(Tarlatzis et al 2006)*. Although the study was underpowered, it was well designed and executed.

Kolibianakis et al. (2006) performed a systematic review, including 4 retrospective and 2 prospective studies, to assess whether endogenous LH levels predict ongoing pregnancy beyond 12 weeks among women with normal ovulation or World Health Organization (WHO) Group II patients undergoing ovarian stimulation in GnRH analogue IVF cycles. Their conclusion was that there was no adverse effect of low LH level on probability of ongoing pregnancy beyond 12 weeks.

No LH Cut-off PointWas Found To Identify Women Requiring LH Supplementation

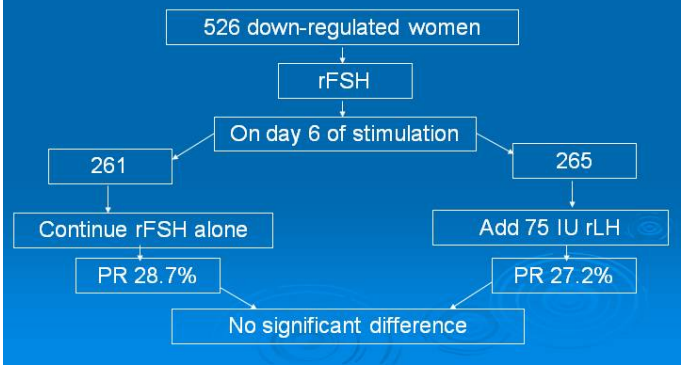
Reasons:

- Selection criteria
- Clinical endpoints
- Serum LH assay *(Alviggi et al. 2006)*

A Randomized Controlled Trial on the Effect of LH on IVF Cycles in Poor Responders (Barrenetxea et al. 2008)

- Prospective randomized trial.
- The addition of rLH at a given time of follicular development produces no further benefit in the patient population of our study. A reduced ovarian response cannot be overcome by changes in the stimulation protocol.

rLH Supplementation to rFSH during IVF Stimulation: a Multicenter Randomized Trial (Nyboe Andersen et al 2008)



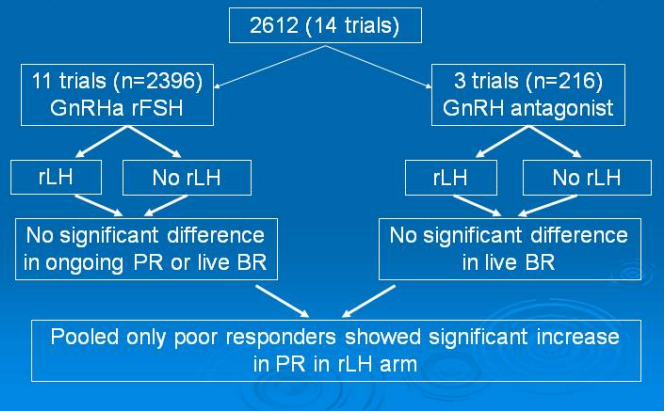
Recombinant Human LH Supplementation During GnRH Antagonist Administration in IVF/ICSI Cycles: a Prospective Randomized Study (Cedrin-Durnerin 2004)

- A total of 218 patients from 3 IVF centers were randomized to receive (n=114) or not (n = 101) a daily injection of rLH 75 IU from GnRH antagonsit initiation to hCG injection.
- The numbers of oocytes and embryos, as well as the delivery rate (25.2% vs. 24%) and implantation rate per embryo (19.1 vs 17.4%), were similar in both groups.
- There was no evident benefit to supplementing GnRH antagonist-treated cycles with rLH.

rLH Supplementation to rFSH During Induced Ovarian Stimulation in the GnRH Antagonist Protocol: a Meta-analysis (Baruffi et al 2007)

- Five trials fulfilled the inclusion criteria.
- Advantages were observed for the rLH supplementation protocol with respect to:
  - Higher serum estradiol concentration on the day of hCG ($P < 0.0001$)
  - Higher number of mature oocytes ($P = 0.0098$).
- It failed to show any statistically significant difference in implantation and pregnancy rates.

rLH in IVF Randomized Trials (Mochtar et al. 2007; a Cochrane Review)

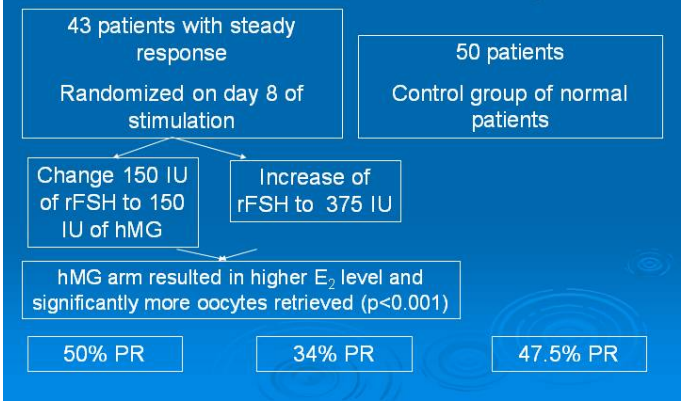


Some polymorphic variants of the FSH receptor are associated with a poor ovarian response to exogenous FSH *(de Castro et al., 2004)*. In such cases, an initial suboptimal response to r-hFSH would be rescued by LH, which is able to substitute FSH activity during the intermediate-late stages of folliculogenesis *(Filicori et al., 2003)*.

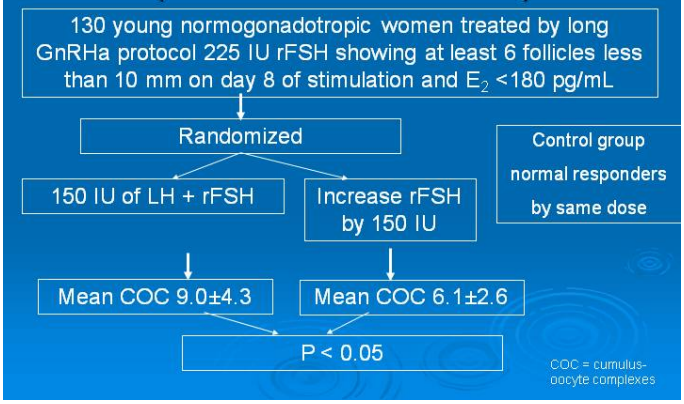
Steady Response During COS and LH

- *De Placido et al. (2001)* found that in about 10–12% of normogonadotrophic patients, an initial response (i.e., at least five 2–9 mm follicles in each ovary) during the first days of stimulation is followed by a plateau in which there is no significant increase in follicular size or estradiol production in the next 3–4 days of stimulation.

Rescue of IVF Cycles by hMG in Patients with Poor Initial Response



rLH for Steady Responders (DePlacido et al. 2005)



The effectiveness of r-hLH in 'steady responders' was evaluated in a large randomized controlled trial (RCT) *(De Placido et al., 2005)*. The number of cumulus–oocyte complexes (primary end-point) and mature oocytes retrieved was significantly higher in women receiving r-hLH than in those treated with the r-hFSH step-up protocol.

Lahoud et al 2006

In Long GnRH a Protocol + rFSH Stimulation

Patients with low LH day 7/8 resulted in:

- Lower E_2 (P<0.001)
- Lower number of oocytes (P<0.01)
- Lower number of embryos (p<0.01)
- More rFSH (430 IUI [P<0.01])
- No significant change in live-birth rate

Lahoud et al 2006 (Continued)

- Reduction of 50% of mid-follicular LH as compared to early-follicular LH resulted in a significant reduction in live-birth ratio:
- Per embryo transfer (ET), 27.3% versus 19% (P<0.05).
- Per started cycle, 22.2% versus 15.8% (P<0.05).

Thus, there is a subset of normogonadotrophic women who cannot be classified as either 'poor responders' or 'normal responders.' In the case of a first ovarian stimulation cycle, early identification of women who require a high r-hFSH dose may result in timely integration with r-hLH, which, in turn, may rescue the ovarian response and improve the ovarian IVF outcome *(Alviggi et al. 2006)*.

hMG vs. rFSH in ART
(Cochrane Review) van Wely et al. 2003

- There was no evidence of a difference between hMG and rFSH in ongoing pregnancy/live-birth rate per women (OR 1.27; 95% CI 0.98 – 1.64).
- The clinical PR per woman was of borderline significance in favor of hMG (OR 1.28; 95% CI 1.00 – 1.64).
- Prescribing gonadotrophins for ovarian hyperstimulation in IVF, one should use the least expensive medication.

OR = odds ration
CI = confidence interval

Recombinant FSH versus Urinary hMG

- Different concepts on the role of LH in COS resulted in performing several clinical randomized studies comparing rFSH versus hMG. A meta-analysis including 2031 patients showed no significant difference in ongoing or live-birth rate between recombinant FSH and hMG (OR =1.18; 95% CI, 0.93-1.50) *(Al Inany et al. 2005)*.

Efficacy and Safety of hMG vs. rFSH: a Meta-analysis (Al-Inany et al. 2008)

- The live-birth rate was significantly higher with hMG (OR 1.20; 95% CI = 1.01 – 1.42) versus rFSH.

Urinary hMG vs rFSH for COS Following an Agonist Long Down-regulation Protocol in IVF or ICSI Treatment: a Systematic Review and Meta-analysis (Coomarasamy et al. 2008)

- Showed a significant increase in live-birth rate with hMG when compared with rFSH (RR = 1.18, 95% CI: 1.02-1.38, P = 0.03).
- The pooled risk difference (RD) for the outcome of live-birth rate was 4% (95% CI: 1-7%)

CONCLUSIONS I

- There is no proof that high LH level adversely affects ovarian stimulation.
- LH supplementation is essential for stimulation in profoundly hypogonadotropic women.
- There is no evidence that addition of rLH will improve outcome in COS with agonist or antagonist protocols.

CONCLUSIONS II

- There is some evidence, which is yet to be further confirmed and defined, that there is a subset of women who may require addition of rLH to improve outcome.
- There is some evidence that addition of rLH may improve IVF outcome in poor responders.
- Recent evidence showed that hMG resulted in a significantly higher pregnancy rate as compared to recombinant FSH for ovarian stimulation for IVF.

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NOTES

LIMITS OF DAY-3 BIOPSY FOR PREIMPLANTATION GENETIC SCREENING

William Schoolcraft, M.D., H.C.L.D.
Colorado Center for Reproductive Medicine

LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

1. Assess the current status of preimplantation genetic screening (PGS) using day-3 biopsy and fluorescence in situ hybridization (FISH).
2. Describe the limitations of day-3 biopsy.
3. Discuss how full karyotyping of human embryos after blastocyst biopsy may improve the outcomes of PGS.

Limits of Day-3 Biopsy for Preimplantation Genetic Screening (PGS)

William Schoolcraft, M.D., H.C.L.D.
Director, Colorado Center for Reproductive Medicine

Learning Objectives

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Disclosure

Nothing to disclose

PGS
What Are We Really Trying To Accomplish?

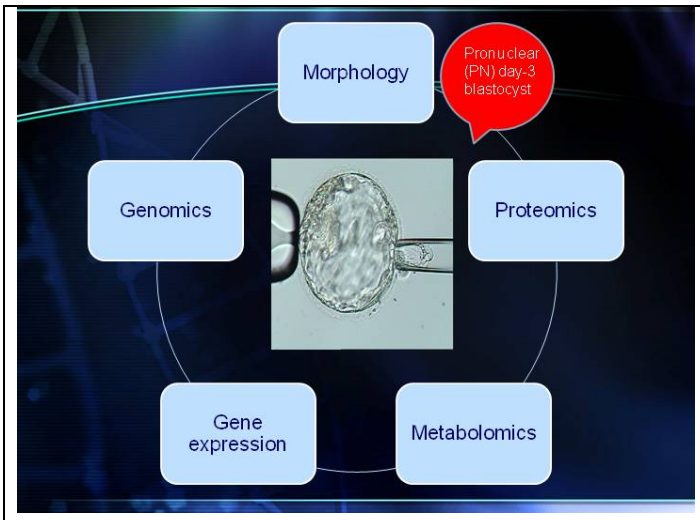
-Karyotyping

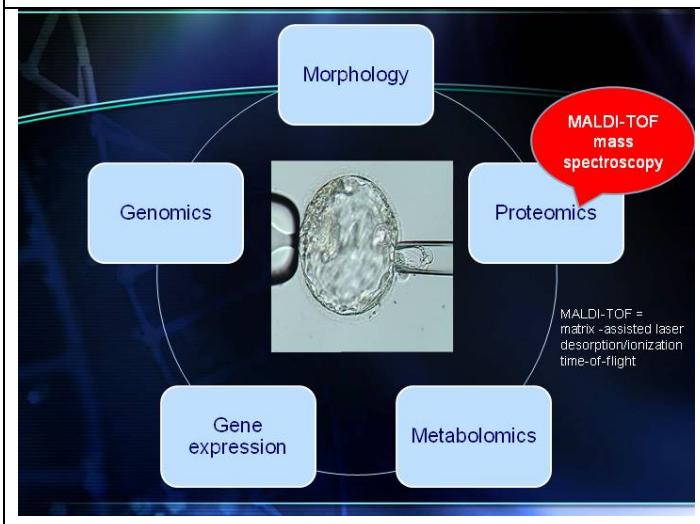
-Predict embryonic viability

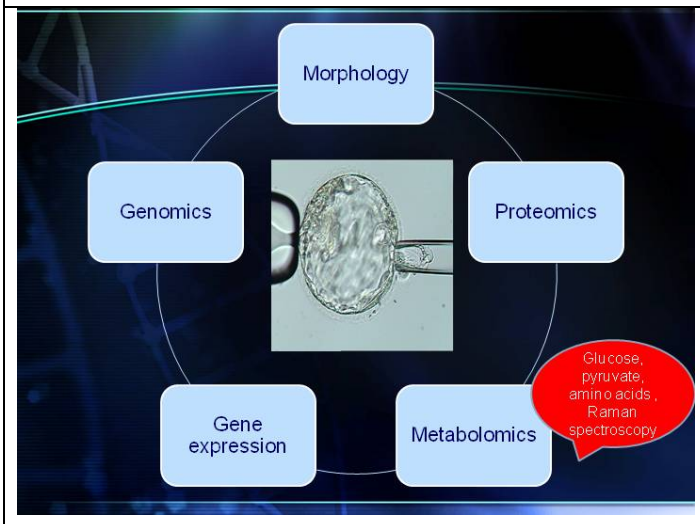
A Woman's Age and Her Risk of Having a Baby With a Chromosomal Abnormality

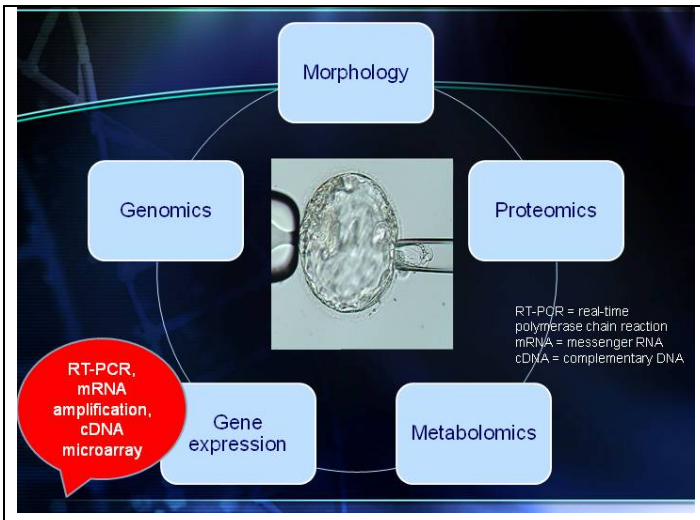
Age of woman	Risk of Down syndrome	Risk of any chromosomal abnormality
20	1 in 1,667	1 in 526
22	1 in 1,429	1 in 500
24	1 in 1,250	1 in 476
26	1 in 1,176	1 in 476
28	1 in 1,053	1 in 435
30	1 in 952	1 in 384
32	1 in 769	1 in 323
34	1 in 500	1 in 238
36	1 in 294	1 in 156
38	1 in 175	1 in 102
40	1 in 106	1 in 66
42	1 in 64	1 in 42
44	1 in 38	1 in 26
46	1 in 23	1 in 16
48	1 in 14	1 in 10

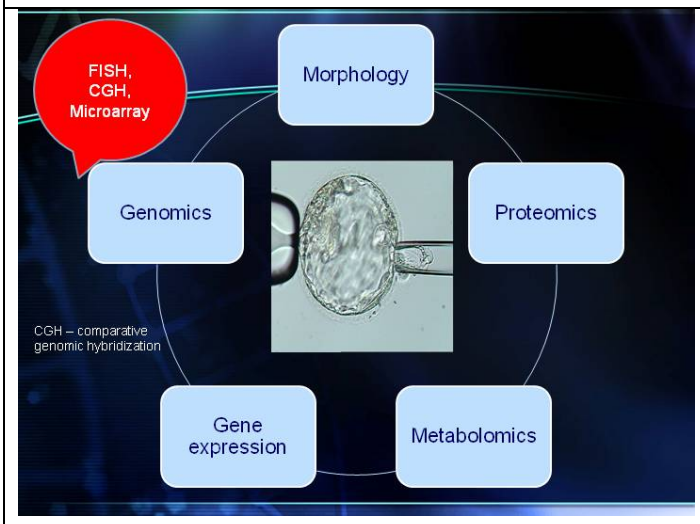
Predicting Embryonic Viability

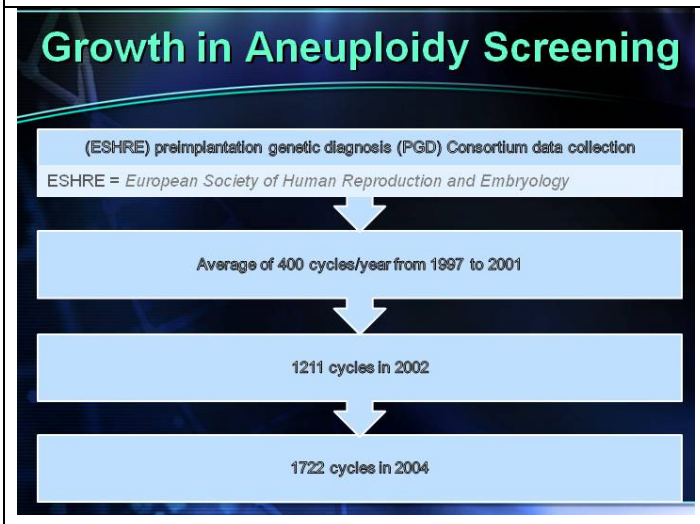

Morphology
Pronuclear (PN) day-3 blastocyst
Genomics
Proteomics
Gene expression
Metabolomics

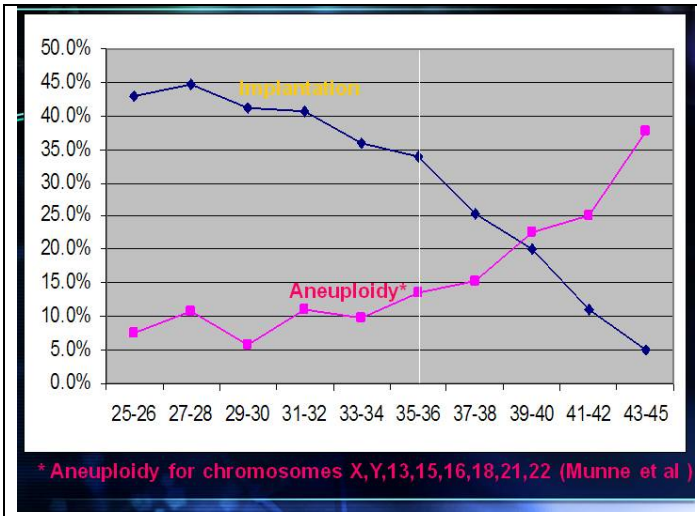

Morphology
MALDI-TOF mass spectroscopy
Genomics
Proteomics
MALDI-TOF = matrix - assisted laser desorption/ionization time-of-flight
Gene expression
Metabolomics


Morphology
Genomics
Proteomics
Glucose, pyruvate, amino acids , Raman spectroscopy
Gene expression
Metabolomics

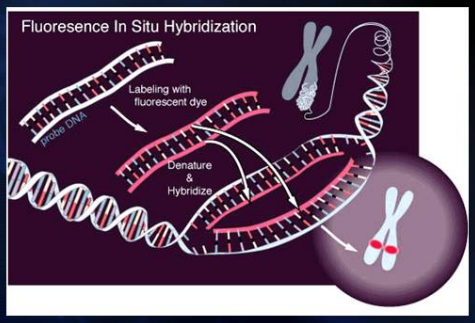

Morphology
Genomics
Proteomics
Gene expression
Metabolomics
RT-PCR, mRNA amplification, cDNA microarray
RT-PCR = real-time polymerase chain reaction
mRNA = messenger RNA
cDNA = complementary DNA


FISH, CGH, Microarray
Morphology
Genomics
Proteomics
Gene expression
Metabolomics
CGH – comparative genomic hybridization


Growth in Aneuploidy Screening
(ESHRE) preimplantation genetic diagnosis (PGD) Consortium data collection
ESHRE = European Society of Human Reproduction and Embryology
Average of 400 cycles/year from 1997 to 2001
1211 cycles in 2002
1722 cycles in 2004



FISH



Selection of FISH Probes

- The identification of chromosomal abnormalities seen in spontaneous abortion and live birth was used to select probes for FISH.
- Testing for chromosomes X, Y, 13, 16, 18, 21 and 22 enables the detection of 72% of the chromosomal abnormalities found in spontaneous abortions (Simpson, 1987).
- Re-hybridization with additional probes is possible, but leads to reduced test accuracy (Liu *et al.*, 1998)

PGD (PGS) Is a Screening Tool, Not a Diagnosis

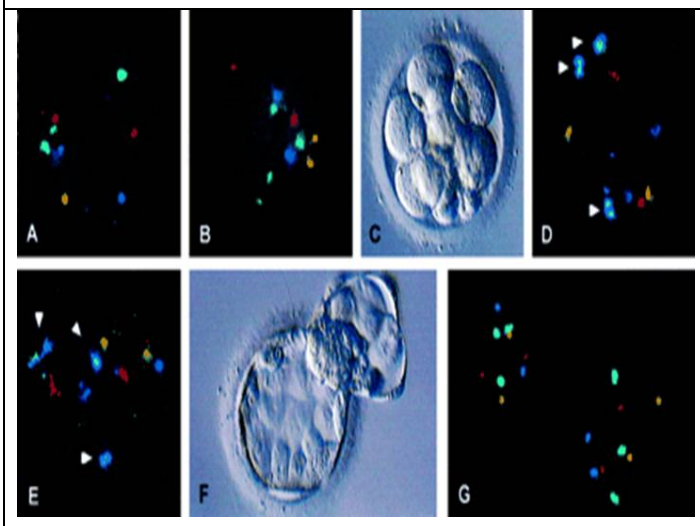
PND	PGD (?PGS)
100 cells	1-2 cells
2 weeks	10hrs
99%	90%

PND = prenatal diagnosis

Can FISH Results Be Confirmed?

E.B. Baart, Hum. Reprod. 2004 19: 685-693

Fluorescence *in situ* hybridization analysis of two blastomeres from day-3 frozen–thawed embryos followed by analysis of the remaining embryo on day 5



Overview of the Diagnosis Made on Day 3 and Rate of Cytogenetic Confirmation after Re-analysis on Day 5.

Baart, HR, 2006

Diagnosis on day 3	No. of embryos re-analyzed on day 5	No. of cases confirmed (%)
Based on two cells		
Normal	7	3 (43)
Aneuploid	11	9 (82)
Mosaic	36	18 (50)
Abnormal/normal mosaic	14	6 (43)
Abnormal/abnormal mosaic	22	12 (55)
Total	54	30 (56)
Based on one cell		
Normal	5	1 (20)
Aneuploid	24	14 (58)
Total	29	16 (55)
Overall confirmation rate	83	45 (54)

Observed a high rate of mosaic embryos after both day-3 (50%) and day-5 analysis
Overall confirmation rate 54%

Explanations for This High Rate of Discordance

- Technical problems related to the FISH procedure, especially when using more probes simultaneously in successive rounds of FISH
- The abnormal cell(s) could have been removed by the biopsy procedure, thus leaving only normal cells or cells with a different chromosome abnormality
- Apoptosis is not observed until the morula stage in human embryos (Jurisicova *et al.*, 1996; Hardy, 1997, 1999; Hardy *et al.*, 2001). This process may be responsible for the elimination of cells carrying a chromosome abnormality
- Mosaicism

High Frequency of Chromosomal Abnormalities in Embryos Obtained from Oocyte Donation Cycles

Soares,F&S, Sept. 2003

Variables	Donors	PGD
Number	15	11
Age	27	31
Abnormal embryos	56.5%	37.3%
Monosomy	36%	48%
Trisomy	26%	24%
Mosaic	21%	16%
Pregnancy rate (PR)	33%	40%
Implantation rate (IR)	25%	36%

CCRM Prospective RCT of PGD

- Sixty patients, using their own eggs, between May 2002 and April 2005 participated in the study.
- Acceptance criteria for the study were any patient age 35 and over with at least 5 good quality embryos on day 3.
- The control group was cultured to day 5 with no additional manipulation of the embryos.
- In the test group, one cell was removed from each embryo on day 3 via embryo biopsy, and embryos subsequently cultured to day 5.
- Only embryos diagnosed as "normal" using 2 rounds of FISH (Reprogenetics) for chromosomes 13, 16, 18, 21, 22 and X, Y, 15, 17 were transferred.
- Embryo biopsy was performed using either acidic tyrodes or laser.

CCRM = Colorado Center for Reproductive Medicine
RCT = randomized clinical trial

CCRM Prospective RCT of PGD

Group	# Patients	Age	% Blast	Average # transfers	Beta hCG	Ongoing PR	FHT	SAB
PGS	30	38.4	0.49	2.4	0.67	0.52	0.32	29
Control	30	38.2	0.49	2.6	0.85	0.72	0.40	42

There were no significant differences in any of the outcomes measured.

Blast = blastocyst; hCG = human chorionic gonadotropin;
FHT = fetal heart; SAB = spontaneous abortion

Comparison of Blastocyst Transfer With or Without Preimplantation Genetic Diagnosis for Aneuploidy Screening (PGD-AS) in Couples with Advanced Maternal Age: a Prospective Randomized Controlled Trial

- RCT of patients ≥ 37 years undergoing blastocyst transfer with or without PGS
- 289 couples (141 control cycles and 148 PGD-AS cycles
- From the 6-cell stage onward, two blastomeres per embryo were removed; otherwise, one blastomere was removed.
- The embryos were first incubated in calcium–magnesium-free medium before biopsy. Laser technology was used to drill a hole of 30 µm in the zona pellucida (an average of 2–3 pulses of 7 ms were applied).

Staessen, HR, 2004

PGD-Brussels Results

	Control	PGD-AS	Statistics
Cycles (*n*)	141	148	
With biopsy	–	130	
With only genetically abnormal embryos	–	38 (26%)	
Mean # embryos transferred	2.8±1.2	2.0±0.9	*P*<0.001
Implantation rate	11.5	17.1	*P*=0.09
Ongoing 12 weeks	29 (21%)	22 (15%)	NS

Multicenter RCT Comparing 3 cycles of IVF With or Without PGS in Women Ages 35-41

	Screening	Controls	
Outcome	N=206	N=202	P
Ongoing PR	52(25%)	74(37%)	.01
Live Birth	49(24%)	71(35%)	.01
SAB	18%	18%	NS
Implantation rate	11.7%	14.7%	

Mastenbroek, NEJM, July5, 2007

RCT of PGS vs. Blastocyst Culture in Patients Undergoing Elective Single Embryo Transfer (eSET)

	PGS	Blastocyst culture
Number	91	84
Mean age	30	30
% having transfer	84.6%	90.5%
Ongoing PR	37%	37%

Staessen, O-079, ESHRE, 2007

Preimplantation Genetic Screening in Women of Advanced Maternal Age Caused a Decrease in Clinical Pregnancy Rate: a Randomized Controlled Trial

	PGS group (n = 56)	Control group (n = 53)	P
Normal embryos	1.75		
No. of embryo transfers (ET)	45 (80.3%)	53 (100%)	0.001
Embryos transferred/ET	1.5 (0.5)	1.8 (0.4)	0.003
No. of live births (% per randomized)	3 (5.4%)	10 (18.9%)	0.039
Implantation rate (%)	8/70 (11.4%)	18/95 (18.9%)	0.19
Spontaneous abortions (%)	7/10 (70.0%)	6/16 (37.5%)	0.11

Hardarson, Hum. Reprod. Advance Access published online on June 25, 2008

Experience with Blastocyst Biopsy and Testing for Aneuploidy by FISH

Jansen, Human Reproduction 2008 23(7):1476-1478

- Aneuploidy screening in younger infertile women (<38 years, median 33.5 years)
- Biopsy of trophectoderm performed on day 5 or 6
- Elective single embryo transfer (eSET)
- Patients were withdrawn from the study before randomization if there were fewer than eight follicles > 1 cm diameter at 8–10 days of stimulation, fewer than four embryos with seven or more cells on day 3, or fewer than two blastocysts for biopsy on day 5 or 6.
- Biopsies consisted of 2–9 trophectoderm cells, were carried out after laser-assisted opening of the zona late on day 3 or on day 4, and were tested by 5-color fluorescent *in situ* hybridization for chromosomes 13, 18, 21, X and Y

Results

	PGS	AH only
Patients	55	46
Live birth	20 (36%)	27 (59%)
Implantation rate	22/55 (39%)	27/46 (59%)

AH = assisted hatching

ASRM Practice Committee Opinion on PGS

- Advanced maternal age: analysis based on Staessen and Mastenbroek RCTs concludes:
  - Available evidence does not support the use of PGS (aneuploidy screening) to increase live-birth rates in women of advanced maternal age.
- Recurrent pregnancy loss (RPL): no RCTs regarding RPL; based on non-randomized trials:
  - Available evidence currently does not support the use of PGS for patients with RPL because it does not improve ongoing pregnancy or live-birth rates and does not decrease miscarriage rates in such women.

ASRM Practice Committee Opinion on PGD

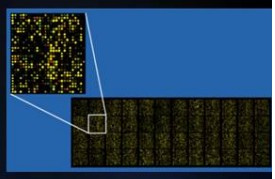
- Repeated IVF failure: No RCTs; based retrospective trials:
  - Available evidence does not support the use of PGS for patients with repeated implantation failure.
- Male factor infertility: No RCTs:
  - Available evidence does not support the use of PGS for couples receiving IVF/ICSI for male factor indications at this time.

Moving Beyond FISH

- Need to assess all 23 pairs of chromosomes
- Whole genome amplification in combination with
  - Microarray technology
  - CGH

DNA Microarray

DNA microarray = collection of microscopic DNA spots, commonly representing single genes arrayed on a solid surface



Qualitative or quantitative measurements with DNA microarrays utilize the selective nature of DNA-DNA or DNA-RNA hybridization under high-stringency conditions and fluorophore-based detection.

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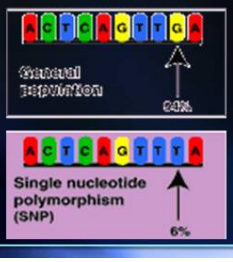
SNPs

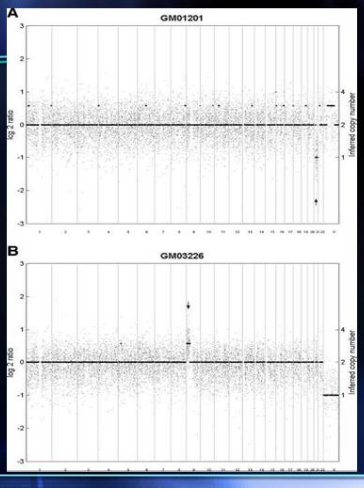
Single nucleotide polymorphisms (SNPs) are a type of genetic variation that occurs when a single base pair is mutated within a DNA sequence. To differentiate a SNP from a genetic mutation, scientists have used the expression SNP to apply to variations that occur at a frequency within the human population at more than 1%. Meanwhile, a genetic mutation is referring to a polymorphism that has a frequency of less than 1%

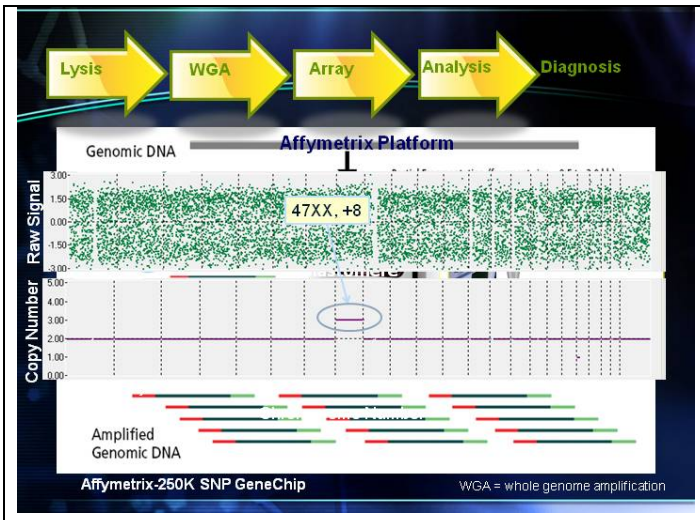
Polymorphism

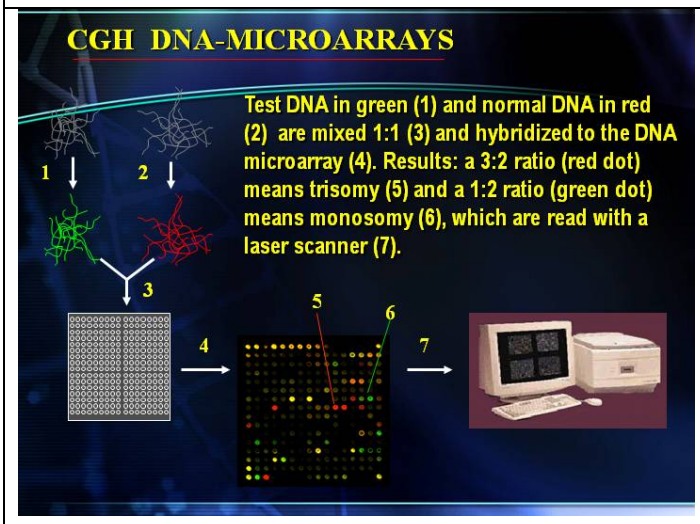
"Poly" meaning "multiple" form

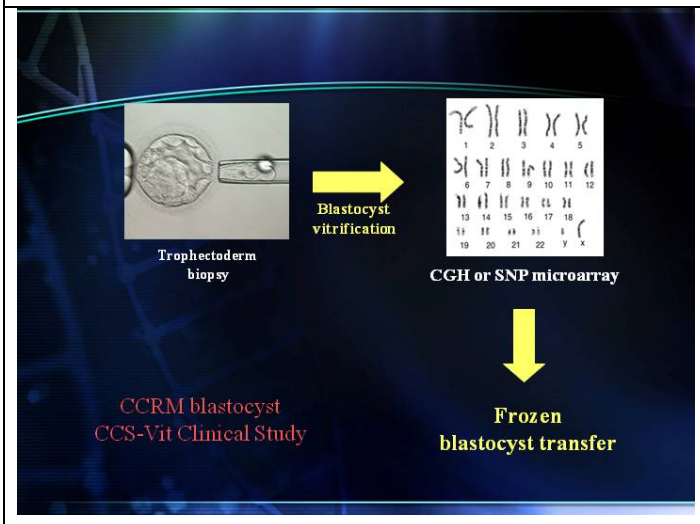


To detect copy-number alterations using a SNP array, the fluorescent signal is used to infer a copy number. This is done using the algorithm which calculates a ratio between the signal for a SNP known to have two copies to an observed signal. These ratios are converted to $\log_2$ ratios and then plotted on a scatter plot. This copy number is referred to as the "inferred copy number."




Lysis
WGA
Array
Analysis
Diagnosis
Genomic DNA
Affymetrix Platform
Raw Signal
47XX, +8
Copy Number
Amplified
Genomic DNA
Affymetrix-250K SNP GeneChip
WGA = whole genome amplification


CGH DNA-MICROARRAYS
Test DNA in green (1) and normal DNA in red (2) are mixed 1:1 (3) and hybridized to the DNA microarray (4). Results: a 3:2 ratio (red dot) means trisomy (5) and a 1:2 ratio (green dot) means monosomy (6), which are read with a laser scanner (7).
1
2
3
4
5
6
7


Trophectoderm biopsy
Blastocyst vitrification
CGH or SNP microarray
CCRM blastocyst
CCS-Vit Clinical Study
Frozen
blastocyst transfer

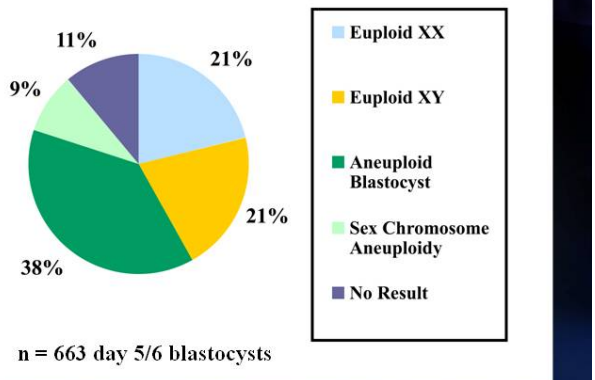
BC-CCS-Vit Study Results

unpublished data

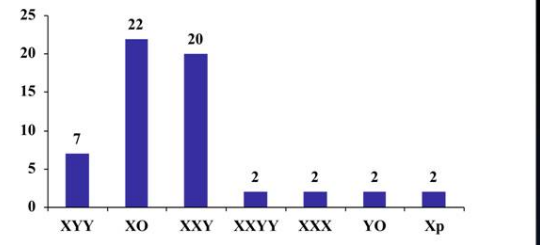
	Results
Number of cycles (2007-2008)	104
Mean maternal age (years)	37.5 years (range 30-44)
Mean # blastocysts biopsied and vitrified	6.4
% of "all aneuploid" = no transfer	6.7%
% euploid blastocyst	47% (279/589)
% blastocyst cryo survival	99% (137 out of 139)
Mean # euploid blastocysts transferred	1.99 (69 transfers)
Biochemical pregnancy	87% (60 out of 69)
Clinical pregnancy (fht)	78% (54 out of 69)
Implantation rate (fht)	63% (86 out of 137)
Missed abortion (MAB)/SAB	7% (5 out of 69)
Ongoing pregnancy rate	70% (48 out of 69)
	8 live deliveries & counting!

Unpublished data

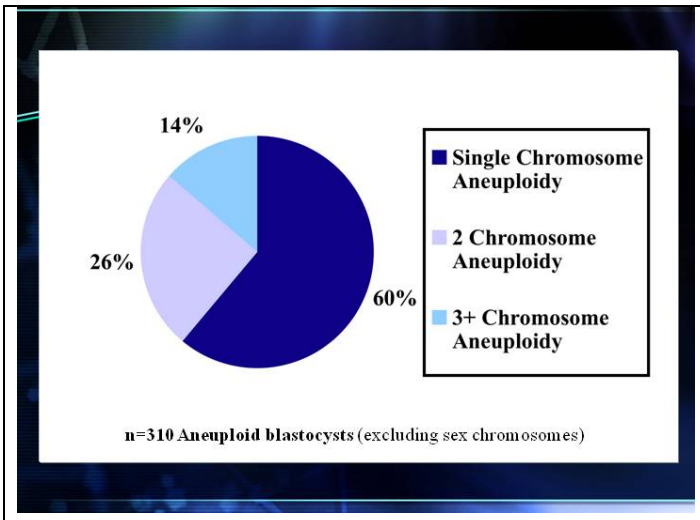
⌘A full chromosome screen was obtained for 91% of blastocysts tested

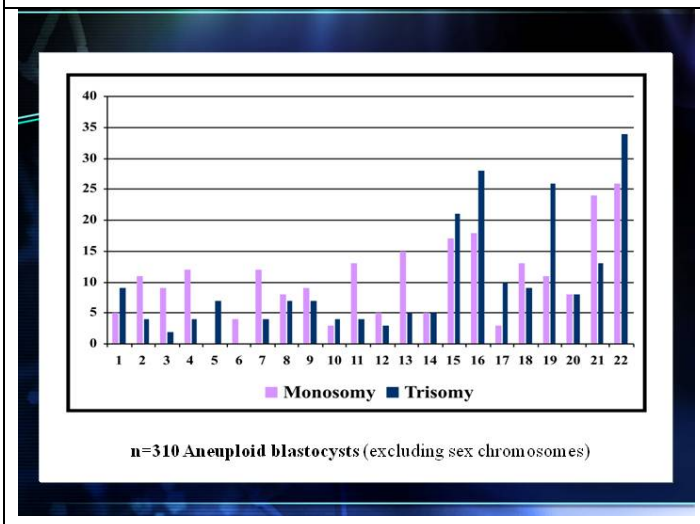


n = 663 day 5/6 blastocysts



n=57 sex chromosome aneuploid blastocysts





n=310 Aneuploid Blastocysts (excluding sex chromosomes)

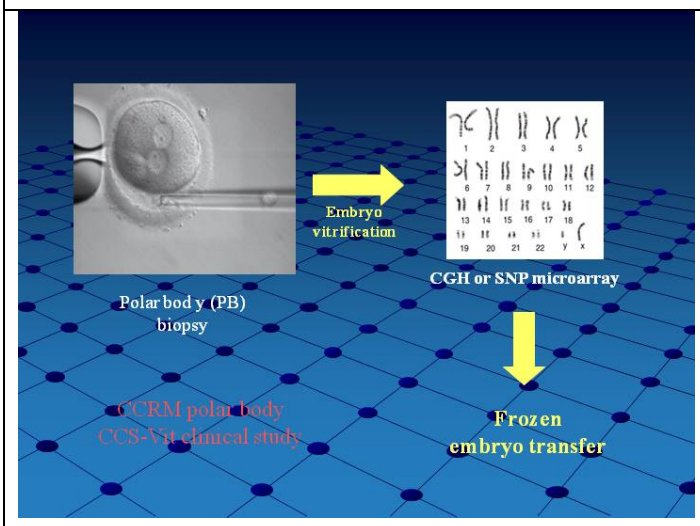
Most prevalent chromosomes involved in aneuploidy (in order) – 22, 16, 15, 19 and 21

Followed by chromosomes (in order) – 18 and 13

Then chromosomes (in order) – 11, 20, 9, 7, 4, 2, 8, 1 and 17

CGH Screening of Blastocyst-Stage Embryos: Conclusions

- **This represents** the first clinical application of a novel approach for preimplantation genetic screening, involving assessment of all chromosomes at the blastocyst stage.
- The sampling of several cells greatly reduces the risk of misdiagnosis due to chromosomal mosaicism.
- Implantation and clinical pregnancy rates were high (63% and 78%), especially given the poor reproductive history of the patients, and compare very favorably with non-PGS cycles matched for maternal age (30% and 60%, respectively).
- Despite the high implantation potential of blastocysts, it is clear that many still harbor lethal aneuploidies. It is likely that accurate, comprehensive chromosomal screening will significantly improve embryo selection and may be key to maintaining high pregnancy rates in cycles involving elective single blastocyst transfer.

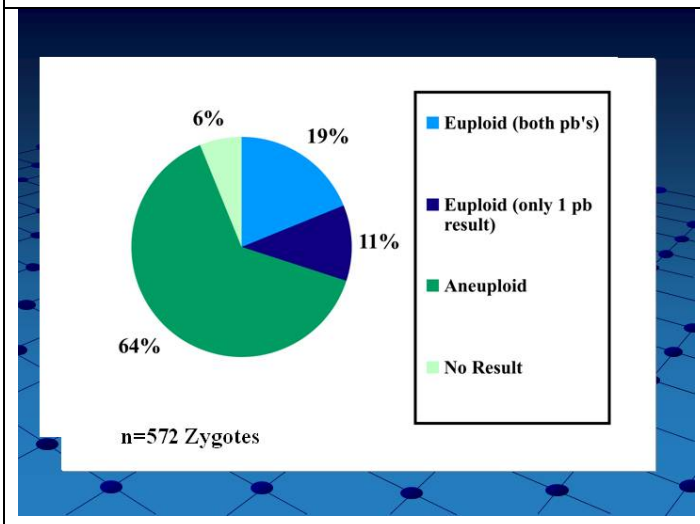


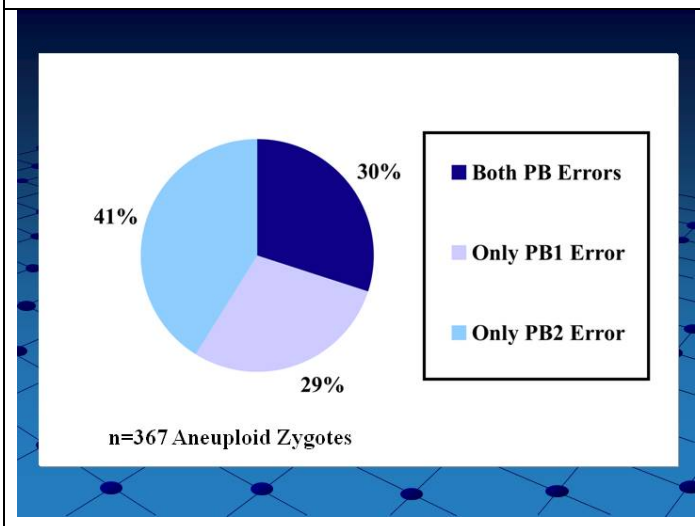
PB-CCS-Vit Study Results

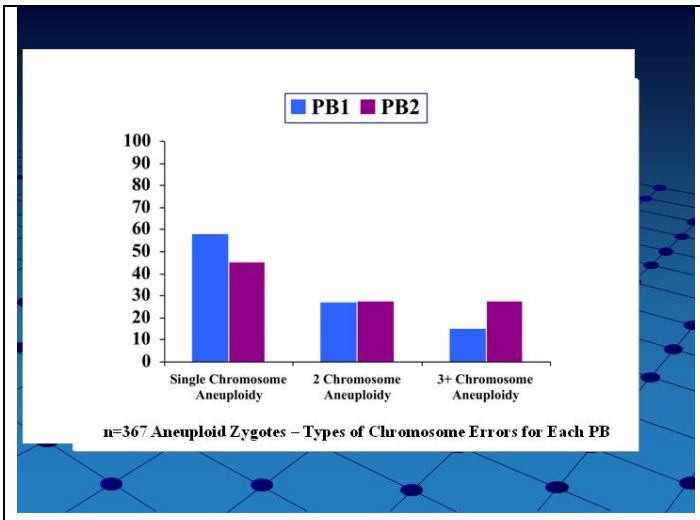
unpublished data

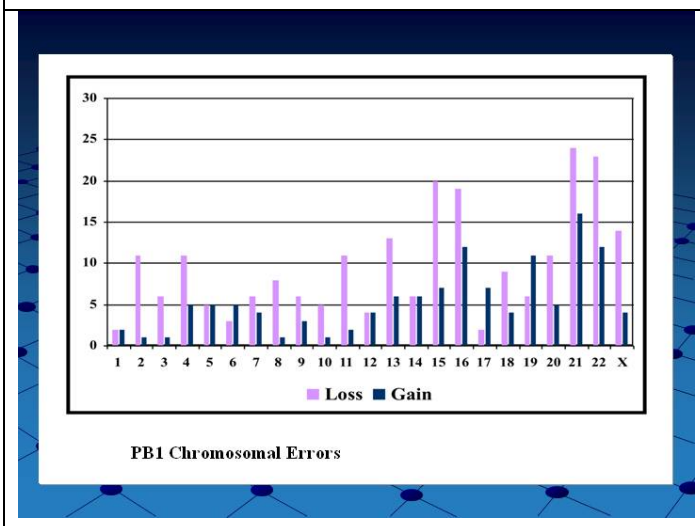
	Results
Number of cycles (2007-2008)	105
Mean maternal age (years)	40.5 years
Mean # oocytes biopsied	5.45
% of "all aneuploid" = no transfer	23% (24 cycles)
% euploid oocytes	32% (173/540)
% embryo cryo survival	96% (88 out of 92)
Mean # embryos transferred	2.26 (49 transfers)
Biochemical pregnancy	33%
Clinical pregnancy (fht)	22.5%
Implantation rate (fht)	11%
MAB/SAB	8%
Ongoing pregnancy rate	14%

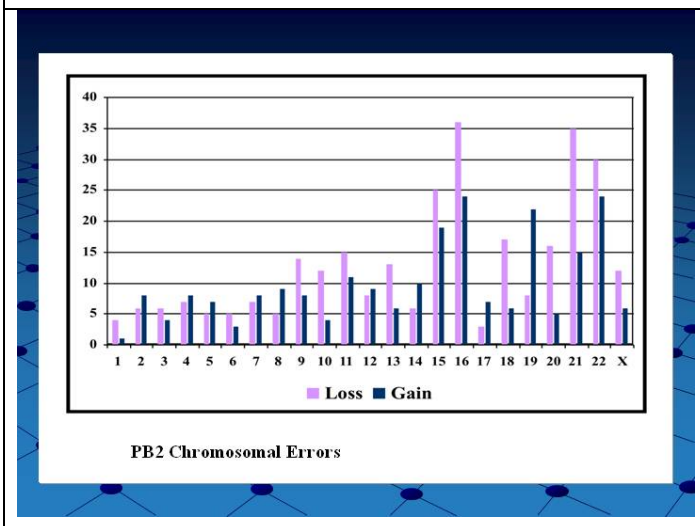
Unpublished data

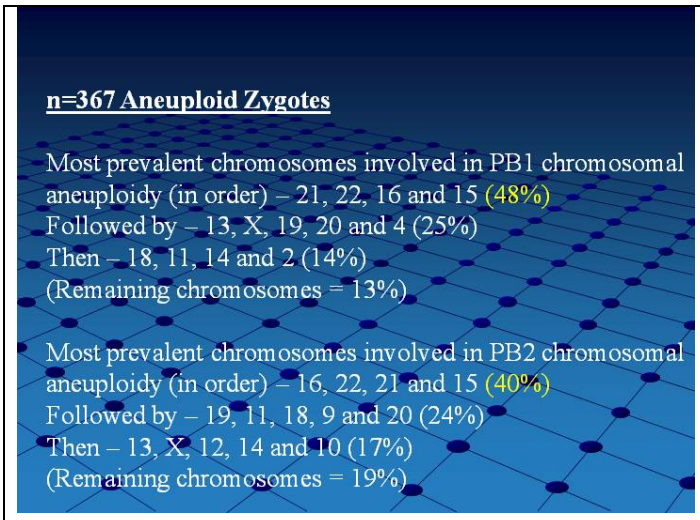





PB1 PB2
100
90
80
70
60
50
40
30
20
10
0
Single Chromosome Aneuploidy
2 Chromosome Aneuploidy
3+ Chromosome Aneuploidy
n=367 Aneuploid Zygotes – Types of Chromosome Errors for Each PB


30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
Loss Gain
PB1 Chromosomal Errors


40
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
Loss Gain
PB2 Chromosomal Errors


n=367 Aneuploid Zygotes
Most prevalent chromosomes involved in PB1 chromosomal aneuploidy (in order) – 21, 22, 16 and 15 (48%)
Followed by – 13, X, 19, 20 and 4 (25%)
Then – 18, 11, 14 and 2 (14%)
(Remaining chromosomes = 13%)
Most prevalent chromosomes involved in PB2 chromosomal aneuploidy (in order) – 16, 22, 21 and 15 (40%)
Followed by – 19, 11, 18, 9 and 20 (24%)
Then – 13, X, 12, 14 and 10 (17%)
(Remaining chromosomes = 19%)

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NOTES

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LUTEAL PHASE SUPPORT IN REPRODUCTION: WHY, WHEN, WHAT AND HOW?

Mohamed Aboulghar, M.D.
Professor of Obstetrics and Gynecology, Cairo University
Clinical Director, The Egyptian IVF Center
Cairo, Egypt

LEARNING OBJECTIVES
At the conclusion of this presentation, participants should be able to:

1. Identify the role of luteal phase support (LPS) in IVF/ICSI cycles.
2. Compare the use of progesterone and human chorionic gonadotropin (hCG) for LPS.
3. Assess the different routes and types of progesterone administration.
4. Appropriately time the starting and stopping of LPS.

Luteal Phase Support in Reproduction
Why, When, What and How?

Mohamed Aboulghar, M.D.
Cairo, Egypt

Objectives

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- Assess the different routes and types of progesterone administration.
- Appropriately time the starting and stopping of LPS.

Disclosure

- Nothing to disclose

Normal luteal function is essential for maintaining pregnancy.
Several studies have shown that removal of the corpus luteum during early pregnancy results in complete abortion (Csapo et al. 1974).

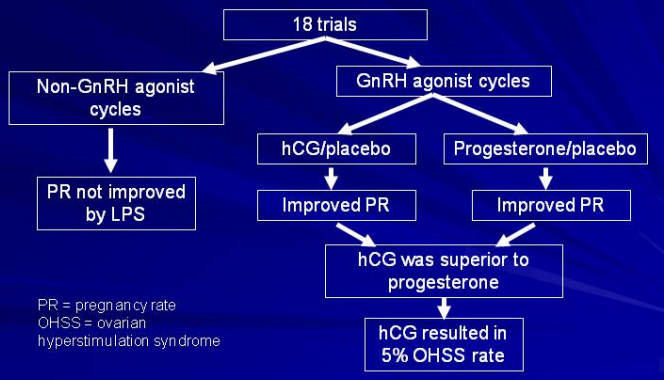
In the mid-1980s, the incorporation of gonadotropin-releasing hormone (GnRH) agonists into ovarian stimulation regimens became associated with improved outcomes after IVF (Hughes et al. 1992).

GnRH agonists, either by themselves or in connection with supraphysiological hormone profiles, may create an iatrogenic luteal phase defect (Macklon and Fauser, 2000).

Luteal-phase deficiency is a common problem in current ARTs and has been described in cycles using pituitary down-regulation with a GnRH agonist, as well as in those using GnRH antagonists

(Macklon and Fauser, 2000; Kolibianakis et al. 2003)

Luteal Phase Support: a Meta-analysis of Randomized Trials Soliman et al. 1994



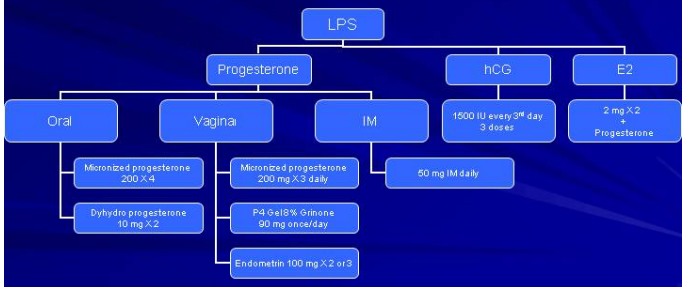
PR = pregnancy rate
OHSS = ovarian hyperstimulation syndrome

Meta-analysis of Randomized Trials on Progesterone or hCG versus Placebo (Nosarka et al. 2005)

- In 6 trials, progesterone significantly improved pregnancy rate versus placebo [OR 1.57; 95% CI 1.13-2.17 (p=0.007)].
- In 4 trials hCG significantly improved PR versus placebo [OR 2.58; 95% CI 1.41-4.73 (P = 0.002)].

OR = odds ratio
CI = confidence interval

Overview of the Hormones Used for LPS and Their Dosage



It is evident that both progesterone and hCG improve the pregnancy rate in IVF cycles down-regulated by GnRH agonists (GnRHa).

Progesterone has become the agent of choice for luteal supplementation, because hCG is associated with a higher risk of OHSS (MacDougall et al. 1992).

Oral versus Intramuscular (IM) Progesterone

A prospective randomized trial revealed a significantly higher implantation rate between women who received 50 mg IM progesterone and those who received 600 mg oral micronized progesterone preparation (Licciardi et al. 1999).

Oral versus IM Progesterone

- In a prospective randomized study (n = 430), 600 mg daily of oral micronized progesterone showed a comparable pregnancy rate and live-birth rate with 50 mg daily IM progesterone (24.1% versus 22.8%). (Chakravarty et al. 2005).

Oral vs. Vaginal Progesterone Administration for Luteal Support

(Friedler et al. 1999)

- A total of 64 high-responder patients requiring intracytoplasmic sperm injection (ICSI) were prospectively randomized for oral or vaginal administration.
- A significantly higher implantation rate in vaginal treatment (30.7% vs. 10.7%; $P<0.01$) but similar pregnancy rate(47% vs. 33.3%) and ongoing pregnancy rate (41.1% vs. 20%) were observed, compared with oral treatment.

Progesterone administered orally is subjected to first pass pre-hepatic and hepatic metabolism. This metabolic activity results in progesterone degradation to its 5α and 5β reduced metabolites (Penzias 2002).

Vaginal Progesterone Preparations

- Micronized progesterone, 600 mg daily (uterogestan)
- Crinone 8%, 90 mg daily sustained-release vaginal gel
- Endometrin, 300 mg daily: micronized natural progesterone

Vaginal Progesterone for LPS

- In four randomized studies, there were no difference s in the implantation rate and clinical pregnancy rates between daily vaginal crinone gel 8%, 90 mg and daily vaginal 600 mg micronized progesterone (Ludwig 2002, Kleinstein 2005, Simunic 2007).
- There was also no difference between endometrin 200-300 mg (micronized natural vaginal progesterone) and crinone 8%,90 mg gel (Doody 2007).

Vaginal progesterone results in high uterine progesterone concentration with low peripheral serum levels due to counter-current exchange in progesterone transport between anatomically close blood vessels (Cicenelli et al. 2000).

Advantages of Vaginal Progesterone

- Patient comfort
- High uterine progesterone concentrations.
- Uterine first-pass effect (de Ziegler et al 1995).

Vaginal versus IM Progesterone for Luteal Phase Support

- Three randomized studies have shown that 50 mg IM progesterone, when compared to vaginal micronized progesterone crinone 8%, resulted in higher implantation, ongoing and live birth rates (Propst et al. 2001, Abate et al. 1999, Perino et al. 1997).

Vaginal versus IM Progesterone for Luteal Phase Support

- In four other randomized studies, there was no significant difference in the clinical pregnancy rate between crinone 8%, 90 mg, and progesterone IM, 50 mg (Anserini et al. 2001, Artini et al. 2005, Dalprato 2008; Smitz 1992).

The main drawbacks of IM progesterone are painful injection site, local and rarely systemic allergic reactions (Hubayter 2008).

hCG for Luteal Phase Support

- Several randomized studies have shown similar ongoing pregnancy rate when LPS was done using either progesterone or hCG (Araugo et al. 1994, Ludwig et al 2001, Artini et al. 1995).
- Few randomized studies showed superior clinical pregnancy rates in favor of hCG (Claman et al. 1992).
- hCG has the drawback of increasing the incidence of OHSS.

Meta-analyses

- A meta-analysis of randomized studies showed that luteal phase support with IM progesterone or IM hCG achieved the same outcome.
- IM progesterone was superior to oral or vaginal routes of progesterone.
- hCG carries a risk of OHSS.
- Their conclusion was that IM progesterone is the best LPS.

(Pritts and Atwood 2002).

In a 2004 Cochrane review, conclusions were that luteal phase support with hCG or progesterone after ART results in an increased pregnancy rate; hCG does not provide better results than progesterone and it has higher risk of OHSS. The optimal route of progesterone administration has not yet been established.
(Daya and Gumby 2004)

Luteal Phase Support in IVF: Meta-analysis of Randomized Trials (Nosarka et al. 2005)

- 18 trials met the inclusion criteria.
- LPS is definitely indicated in IVF treatment cycles.
- This meta-analysis favored hCG over progesterone as LPS with respect to pregnancy rate.

Estradiol Luteal Phase Support

- Several randomized studies using long GnRHa protocol compared LPS with progesterone versus progestrone plus estradiol (E_2). There was no difference in the clinical pregnancy rate (Smitz et al. 1993; Levin et al. 1994; Tay et al. 2003; Farlin 2000; Lukazuk 2005; Fatemi et al. 2006).
- There is no evidence that addition of E_2 will improve the pregnancy rate.

Estradiol Luteal Phase Support

A systematic review of 10 randomized studies showed that there is no significant difference between LPS by progesterone alone as compared to estrogen plus progesterone (Gelbaya et al. 2008). Another meta-analysis of 9 studies reached the same conclusion (Jee et al. 2009).

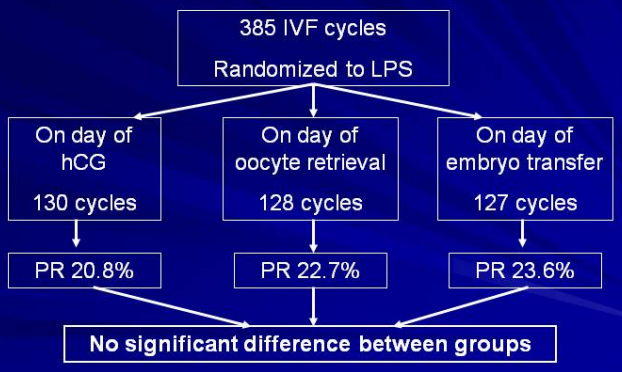
GnRHa versus Placebo

- 2 randomized studies were performed giving 0.1 mg GnRHa versus placebo in the luteal phase, in addition to the routine LPS with progesterone. One showed no effect on pregnancy rate (Ata et al. 2008) and one showed increase pregnancy rate in GnRHa arm (Tesarik et al. 2006)

When to Start LPS?

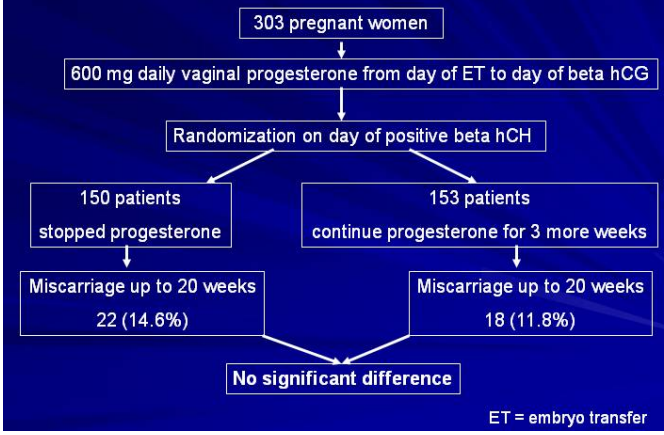
- In a prospective randomized study, it was found that delaying progesterone supplementation to day 6 after oocyte retrieval resulted in reduced pregnancy rate.
(Williams et al. 2001).

Timing LPS in GnRH Agonist Down-regulated IVF Cycles (Mochtar et al. 2006)

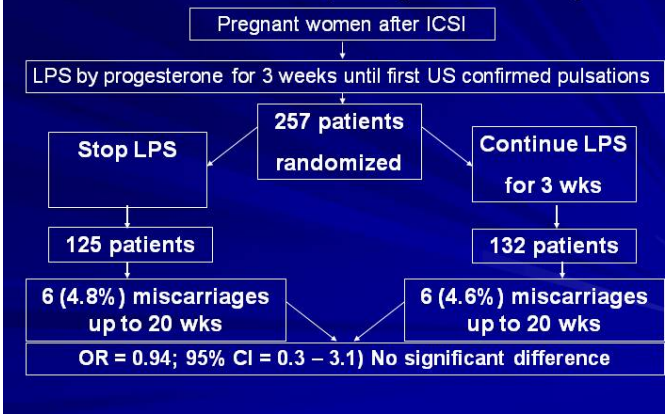


When to Stop LPS?

Stop LPS on Day of beta hCG vs. 3 Weeks Later (Andersen 2002)



Prospective Randomized Study Comparing LPS for ICSI Patients up to First Ultrasound (US) versus Three Weeks More (Aboulghar et al. 2008)



Questionnaire Sent to 21 Leading IVF Centers
(Aboulghar et al. 2008)

- 13 European centers
- 6 North American centers
- 2 Middle Eastern centers

Results of the Questionnaire

- **16** centers used vaginal progesterone
- **1** center used oral progesterone
- **3** centers used IM progesterone
- **1** center used hCG

Results of the Questionnaire

- All centers started LPS on day of oocyte retrieval or embryo transfer.
- Stopped LPS
  - 8 centers day of beta hCG
  - 4 centers 2 wks after beta hCG
  - 5 centers 2-4 wks after beta hCG
  - 3 centers 9, 10, 11 weeks
  - 1 center 12 weeks

Conclusion I

- Luteal phase support is essential for agonist and antagonist protocols.
- Progesterone and hCG are equally effective for LPS, but hCG is associated with a high rate of OHSS.
- Some studies suggest that IM progesterone is superior to vaginal routes, but vaginal progesterone is preferred to avoid IM side effects.
- No evidence that addition of E_2 increases PR.

Conclusion II

- LPS is equally effective on day of hCG, day of oocyte retrieval and day of embryo transfer.
- LPS should stop on day of beta hCG assay or day of first ultrasound.

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NOTES

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Course #9 Test Questions

1. In patients undergoing gonadotropin-releasing hormone (GnRH) agonist downregulation and recombinant follicle-stimulating hormone (rFSH) protocol for ART, the addition of luteinizing hormone (LH) in the form of recombinant LH (rLH) or human menopausal gonadotropin (hMG):

    a. Is detrimental to the outcome in terms of number of oocytes.
    b. Increases the risk of ovarian hyperstimulation syndrome (OHSS) in ART cycles.
    c. Negatively impacts oocyte maturation and pregnancy rate.
    d. Is beneficial in all patients undergoing ART.
    e. May be beneficial in a subset of patients.

2. Which one of the following accurately describes the function of FSH receptor genotype?

    a. It has only inactivating mutations.
    b. Activating mutations explain all types of iatrogenic OHSS.
    c. Polymorphisms may affect ovarian response to gonadotropins, and studies may individualize therapies in the future.
    d. FSH receptor polymorphisms do not vary according to ethnicity.
    e. It predicts with certainty which patients will develop OHSS.

3. Increasing the dose of human chorionic gonadotropin (hCG) to trigger ovulation above the standard dose (choriogonadotropin alfa, 250 µg):

    a. Does not impact OHSS as long as the dose is below 750 µg.
    b. Increases early-onset OHSS but decreases late-onset OHSS
    c. Increases OHSS even at the dose of 500 µg.
    d. Results in a lower incidence of OHSS when using recombinant HCG compared to GnRH agonist
    e. Improves the pregnancy rate because it mimics the natural cycle when used with recombinant LH.

4. The half-life of hCG is:

    a. Equal to the half-life of LH.
    b. Shorter than the half-life of LH.
    c. Longer than the half-life of LH.
    d. Independent of the carbohydrate content of the molecule.
    e. One of the reasons for having a lower OHSS rate compared to recombinant LH.

5. The reason antagonists have not replaced agonists for ovarian hyperstimulation in IVF cycles is that antagonists:

    a. Are more expensive.
    b. Are more difficult to use.
    c. Are associated with possible lower pregnancy rates.
    d. Have a higher risk of OHSS.
    e. Require higher doses of FSH.

(continued)

6. Which one of the following statements is correct regarding IVF treatment?

    a. Supplements have no effect on sperm DNA fragmentation.
    b. Acupuncture increases IVF success.
    c. Stress reduces success of IVF outcomes
    d. Alcohol intake by the female partner has no effect.
    e. Smoking by the male partner does not affect ICSI success.

7. Some randomized studies have shown a higher rate of IVF success with which one of the following treatments?

    a. Metformin in women with polycystic ovary syndrome (PCOS)
    b. Dexamethasone in women with normal androgen levels
    c. Pretreatment with oral contraceptives
    d. Growth hormone in normal responders
    e. The GnRH agonist short protocol

8. A young woman underwent her first IVF trial using the GnRH antagonist protocol and 24 oocytes were retrieved. Which one of the following is the optimal luteal phase support?

    a. No luteal phase support
    b. Vaginal progesterone
    c. Vaginal progesterone and estrogen
    d. Small doses of hCG
    e. GnRH agonist

9. During ovarian stimulation with long GnRHa protocol:

    a. All patients must take LH supplementation.
    b. LH supplementation should start from day 10 of the cycle.
    c. Patients should be tested for polymorphic variants of FSH receptor to select patients who require LH.
    d. High doses of LH adversely affect prognosis.
    e. There is no clear evidence that addition of LH is required.