

IVF Protocols: Hyper & Hypo-Responders, Implantation

**Midwest Reproductive Symposium
June 4-5, 2010**



CONCEPTIONS

REPRODUCTIVE ASSOCIATES
OF COLORADO



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P-560

SELECTIVE CUT-BACK PROTOCOL FOR IN-VITRO FERTILIZATION (IVF) OVARIAN STIMULATION IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME (PCOS). M. R. Bush, M. S. Swanson, S. Stephan, B. H. Albrecht. Conceptions Reproductive Associates, Denver, CO.

MATERIALS AND METHODS:

PCOS patients 37 years of age or less taking 1500 mg of extended release metformin daily.

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

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

Technique	Hyper-stimulation	Pregnancy Rate (PR)/Cycle	PR/embryo transfer (ET)	Miscarriage Rate	Ongoing PR/ET	Patients with cryo	Avg# cryo
Cut-Back (n=8)	0/8 (0%)	8/8 (100%)*	8/8 (100%)	0/8 (0%)	8/8 (100%)	7/8 (88%)	6
Static Dose (n=14)	5/14 (36%)	8/14 (57%)*	8/9 (89%)	2/8 (25%)	6/9 (67%)	11/14 (79%)	8

*Statistically significant (χ^2). All other comparisons NS.

CONCLUSIONS:

A once a day, single syringe, mixed protocol is efficacious for PCOS patients undergoing IVF allowing for low hyperstimulation rates and excellent ongoing pregnancy rates.

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

CONCEPTIONS

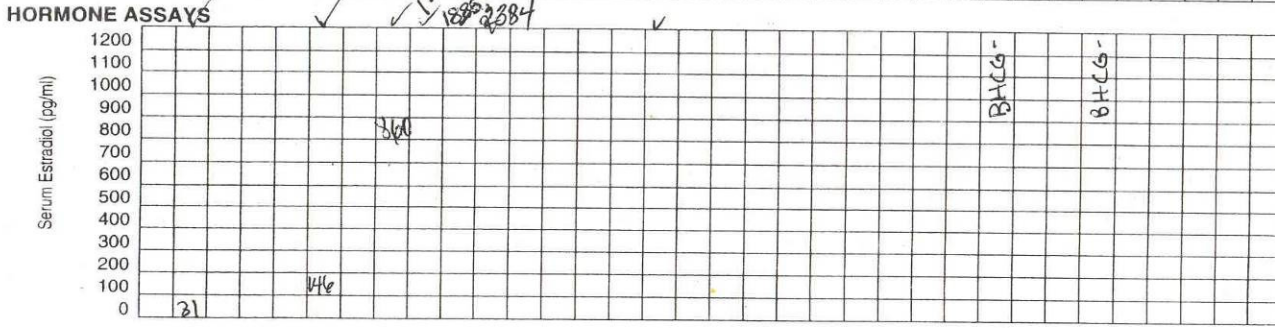
Women's Health and Fertility Specialists, P.C.

A.R.T. CYCLE MONITORING CHART 2-7-77

Patient: [Redacted] Age 30 Weight [Redacted] Cycle A LMP 5-12-07
 M.D. MLO Office Lat Comments IVF-ET #1

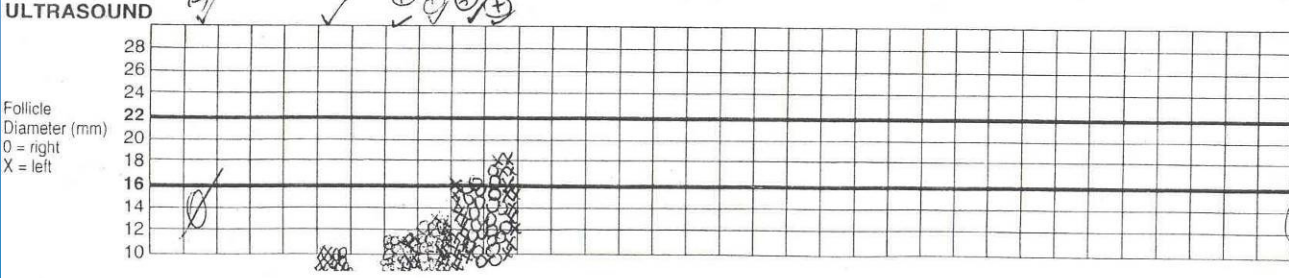
Date	12	14	16	18	20	22	24	26	28	30	1/1	3	5	7	9	11	13																	
Cycle Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34

TREATMENTS	OCP 4/19 to 5/9										Lupron starts 5/5										Acupuncture													
Lupron	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5																								
Bravelle	75 75 75 75 75 75 75 75 75 75																																	
Menopur	150 150 150 150 150 150 150 150 150 150																																	
HCG	5,000																																	
Hematocrit																																		
Insemination																																		



ULTRASOUND

FSH																																		
OO																																		
LH																																		
Progesterone																																		
P ₄ /E ₂																																		
Endo thickness	4.2	4.4	6.4	7.6	9.6	10.6																												



SPECIAL COMMENTS
 Reduce ?
 Transfer ASAM
 Cryo Yes
 Uterus WNL
 Male ICSI
 Sp. anti. _____

DIAGNOSIS:
Pco

PLAN:
 Screening US d 2
 LH serum d _____
 E₂ d 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34
 FSH d _____
 US for develop - d 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34
 hCG 10K units
 - trigger foll. Cigna
 Medrol 64 mg x 4 days
 (start eve of retrieval)
 Luteal progesterone No
 Luteal progesterone Pyinol
 Dose _____
 Route _____
 P₄/E₂ _____
 βhCG luteal d _____
 Baby ASA one daily to begin Day _____

CYCLE COMMENTS
 # follicles ≥14 mm _____
 # ova retrieved 21
 # fertilized _____
 # embryos _____
 # transferred _____
 # frozen _____

3 extra tubes
 Consents signed OK
 Antibiotics _____
 Metformin
 1500 XR daily
 3-255-7170
 Rite Aid
 408010865-4

21 oocytes/ 21 PB-1/ ICSI 18 fert, 2 blast ET (9 cryo), viable twins.

Antagonist cycle salvage

Gustofson, Larsen, Bush, Segars; F&S, Jan 06, pgs 251-4.

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

- If projected E2 >5000 and >25 follicles day of hCG, then antagonist 250/d replaced agonist until day of hCG

- Gonadotropins maintained at 37.5 – 225 IU/d

- 47 patients, PR of 68.1%

Antagonist cycle salvage

Gustofson, Larsen, Bush, Segars; F&S, Jan 06, pgs 251-4.

TABLE 1

Cycle characteristics and outcomes.

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

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

^a No significant difference between subgroups.

^b The E₂ percentage changes represent the average percentage change for each patient and are not the percentage change of the average E₂.

^c Change in E₂ from day of initiation to day +1, P<.001.



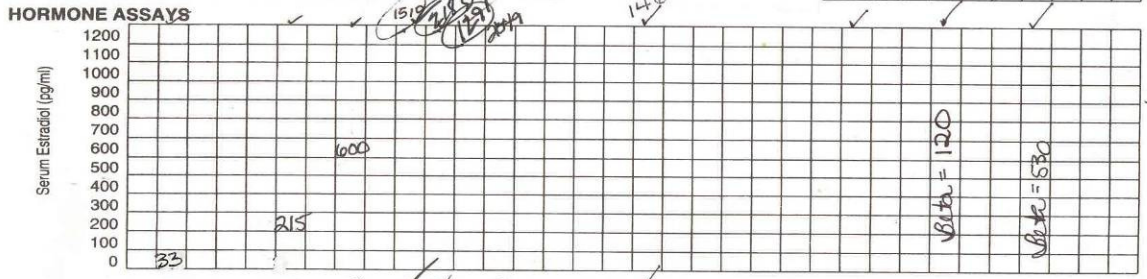
A.R.T. CYCLE MONITORING CHART

3-23-76
 Age 31 Weight _____ Cycle C LMP 11/7/07
 M.D. MRB Office LAF Comments IVF #2

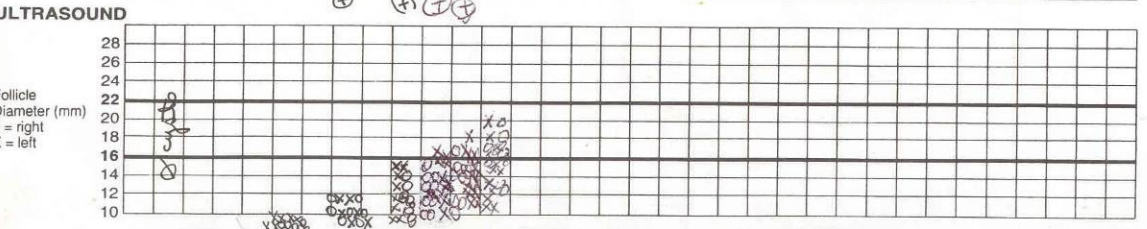
Date	11/7	9	11	13	15	17	19	21	23	25	27	29	30	1	3	5	7	9
Cycle Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

TREATMENTS OCP'S 10/15 to 11/4 (24) upon 10/31 Synthroid metformin EcoTID, Folgard 2-2 BID

Lupron	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
menopur	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Bravelle	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75
Dexameth	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Medrol then prednisone 10mg BID																		
Hematocrit HCG	10K																	
Insemination																		



FSH																		
LH																		
Progesterone																		
P/E ₂																		
Endo thickness	4.0		6.1	7.6	10.8	11.3	12.0											



SPECIAL COMMENTS

Reduce No

Transfer ?

Cryo Yes

Uterus SHG

Male ICSI

Sp. anti. _____

DIAGNOSIS: Dur, Pco, male factor

PLAN:

Screening US d 2

LH serum d _____

E₂ d 2, 6, PEN

FSH d _____

US for develop - d 6, PEN

hCG 10K units SK

- trigger foll.

Medrol 64 mg x 4 days → prednis (start eve of retrieval)

Luteal hCG _____

Luteal progesterone Both

Dose 50mg

Route ceibra) P/O

P₄/E₂ _____

βhCG luteal d 13, 16

Baby ASA one daily to begin Day of Ret

CYCLE COMMENTS

follicles ≥14 mm 15

ova retrieved _____

fertilized _____

embryos _____

transferred 2

frozen 2

3 extra tubes _____

Consents signed _____

Antibiotics _____

405011020-6

LABS ENTERED ✓

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

2 yrs prior, LL/rec-FSH only, hyperstim, ET w/o preg, no cryo.

Antagonist cycle salvage

Gustofson, Larsen, Bush, Segars; F&S, Jan 06, pgs 251-4.

- E2 levels continue to rise on d1 of coasting
- Prolonged coasting may impair IVF cycle outcome
- Implantation rate declines with duration of coast
- The incidence of severe OHSS (2.1%) was lower than the published incidence in this high risk group (9-38%)
- GnRH-I and GnRH-II receptors have been found in granulosa cells; antagonists may be acting directly on the ovary - particularly considering the reduction in pituitary cell membrane GnRH receptors with the use of GnRH agonist.

Egbase et al, *Hum Reprod* 2000; 15: 2082-6.

Tortoriello et al, *F&S* 1998; 69: 454-60.

Levinsohn-Tavor et al, *Hum Reprod* 2003; 18: 937-40.

Morris et al, *Hum Reprod* 1995; 10: 811-4.

Asch et al, *Hum Reprod* 1991; 6: 1395-9.

Khosravi et al, *JCEM* 2003; 88:663-72.

Leung et al, *Mol Cell Endocrinol* 2003; 202: 145-53.

Kang et al, *Endocr Relat Cancer* 2003; 10: 169-77.

Cabergoline (Cb2) therapy in face of OHSS

- VEGF induces VP (vascular permeability)^{1,2}
- Effects of Cb2 attributable to VEGF receptor dephosphorylation³
- Cb2 prevents VP in a dose dependent manner without affecting angiogenesis and implantation in humans (n = 35 treated in face of OHSS)⁴
- Cb2 reduced the amount of ascites, hemoconcentration and incidence of moderate-severe OHSS⁵
- Cb2 0.5 mg x 8 days (total of 4 mgs) starting day of trigger

1) McClure, et al, *Lancet*, 1994; 344: 235-236.

2) Bates, et al, *Vascul Pharmacol*, 2002; 39: 225-237.

3) Gomez, et al, *Endocrinology*, 2006; 147: 5400-5411.

4) Alvarez, et al, *Hum Reprod*, 2007; 22: 3210-3214.

5) Alvarez, et al, *J Clin Endocrinol Metab*, 2007; 92: 2931-2937.

Hypo-responder

Identification

1. Age

2. Clinical History

Prior failed stim, prior decreased MII egg quantity/quality, longstanding unexplained with poor prognosis testing, miscarriage

3. AMH

$< 1.0, \geq 1.5$

Correlates with age, oocytes, peak E2, number of HQ embryos; IR & PR sig better if > 2.7 (1)

4. RFC

$\leq 4, \geq 8$

5. d3 FSH

≥ 10

Absence of functional cyst where $E2 > 80$ drives down FSH and is also indicative of DOR

(1) Silberstein et al, Hum Reprod 2006 21: 159-63.

Hypo-responder

Overview of options

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

DHEA

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

(1) Hillier et al, Mol Cell Endocrinol 1994; 100(1-2):51-4.

(2) Weil et al, J Clin Endocrinol Metab 1998; 83: 2479-85.

DHEA

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

	Pre-DHEA	Post-DHEA	<i>P</i> -value
<i>n</i>	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

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

DHEA

- 89 women with DOR took 75 mg/d DHEA for up to 4 months prior to IVF, controls proceeded straight to IVF without DHEA.
- Day 3 FSH 16 (+/- 1.2) in treatment v. 13.6 (+/- 1.0) controls.
- Cumulative PR (+CA after 6 weeks) 28.4% v. 11.9% (relative hazard of pregnancy, HR, 3.8; 95% CI 1.2 – 11.8, $p < 0.05$).
- Half of the pregnancies in the DHEA group occurred before IVF; yet strong trend toward higher PR remained with DHEA at IVF (20.6% v. 11.9%).

DHEA

Treatment effect on AMH

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

Poor Responders

Luteal E2/antagonist prep

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

Poor Responders

Luteal E2/antagonist prep

- Compared to prior cycle, cancellation rate was decreased from 33.3% to 13.6% ($P < .05$).
- Higher mean number of oocytes retrieved (8.3 vs. 6.4 $P < .05$) and embryos produced (4.5 vs. 2.4 $P < .05$)
- Acceptable clinical PR in this group of 30.3%
- Luteal E2 results in a reduction in antral follicle heterogeneity and more synchronized maturity via decrease in luteal FSH curve
- Antagonist may increase FSH receptor sensitivity (decreases circulating levels of ligand)
- E2 may foster expression of FSH receptors (progestin in OCP would antagonize this effect)

Poor Responders

Luteal E2 prep

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

Hill MJ, et. al., *Fertility and Sterility*, March 09, 739-43.

Poor Responders

Dexamethasone

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

Poor Responders

Letrozole

- 147 prior poor response patients, 71 treated with FSH/hMG/antagonist alone and 76 with same regimen + letrozole with significant difference in oocytes retrieved (6.1 vs. 4.3) and IR (25% vs. 9.4%) observed with letrozole
- Unex infert, prior poor response, n = 12 at IUI, addition of letrozole saw more mature follicles produced with less gonadotropin used
- In 27 prior poor response patients, a 25.9% PR at IVF was observed with the addition of letrozole in the cycle following a failed cycle
- Adding 2.5 mg letrozole to FSH/hMG antagonist protocol offers an attractive alternative after a prior cancelled cycle

Garcia-Velasco JA, et al, *F&S*, July 2005, p. 82-7.

Farouk M, et al, *F&S*, April 2002, p. 776-780.

Quintero R, et al, *F&S*, P-33, S28.

Guillen A, et al, *F&S*, P-485, S326.

Growth Hormone

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

Dimitra Kyrou, M.D., Efstratios M. Kolibianakis, M.D., M.Sc., Ph.D., Christos A. Venetis, M.D., M.Sc., Evangelos G. Papanikolaou, M.D., M.Sc., Ph.D., John Bontis, M.D., Ph.D., and Basil C. Tarlatzis, M.D., Ph.D.

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

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

Design: Systematic review and meta-analysis.

Setting: University-based hospital.

Intervention(s): None.

Main Outcome Measure(s): Pregnancy rate.

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

Conclusion(s): Insufficient evidence exists to recommend most of the treatments proposed to improve pregnancy rates in poor responders. Currently, there is some evidence to suggest that addition of GH, as well as performing embryo transfer on day 2 versus day 3, appear to improve the probability of pregnancy. (Fertil Steril® 2009;91: 749–66. ©2009 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, live birth, ovarian stimulation, poor response

GH (thru hepatic IGF-1 production) reduces granulosa cell apoptosis, amplifies FSH action and improves the health and proliferation of granulosa cells.

Growth Hormone

- Meta-analysis of RCTs in poor responders GH increased pregnancy and live-birth rates 3x and 4x compared with placebo.
 - Harper et al, Growth hormone for in-vitro fertilization. Cochrane Database Syst Rev 2003; 3: 1-33.
- RCT, > 40, poor responders GH increased pregnancy and live-birth rates 4X and 5x over patients not given GH with otherwise identical stim regimen (450 recFSH + 150 hMG/d). Significantly increased intrafollicular E2 levels and a trend towards better embryo quality was also observed.
 - Tesarik et al, Improvement of delivery and live-birth rates after ICSI in women > 40 years by ovarian co-stimulation with growth hormone. Hum Reprod 2005; 20: 2536-41.

Premature luteinization

- Prospective cohort, day of SET defined by physician at start of cycle
- 482 patients, antagonist/recFSH protocol, <36, FSH <12
- Incidence of P > 1.5 ng/mL on day of trigger 18.2%
- P assayed with hospital based Elecsys immunoanalyser (Roche Diagnostics, Mannheim, Germany)

Premature luteinization

TABLE 1

Pregnancy outcome and stimulation characteristics in patients with or without premature luteinization on the day of hCG administration.

Day 3 SET (n = 275)				
	P rise > 1.5 ng/mL (n = 51)	Normal P level (n = 224)	OR [95%CI]	P value
Age ^a (years)	31.3 ± 0.5	31.9 ± 0.2		0.18
Stimulation ^a (days)	10.4 ± 0.4	9.7 ± 0.2		0.08
Prog on hCG day ^a (ng/mL)	2.0 ± 0.19	0.9 ± 0.02		<0.001
E ₂ on hCG day ^a (pg/mL)	2236 ± 190	1762 ± 78		<0.01
LH on hCG day ^a (IU/L)	1.7 ± 0.2	2.1 ± 0.2		0.46
Oocytes retrieved ^a (COCs)	13.8 ± 0.9	10.9 ± 0.4		<0.005
Fertilized oocytes ^a (2PN)	8.1 ± 0.7	6.5 ± 0.3		0.02
Top quality embryos	74.5%	73.7%		0.73
Positive hCG	23.5% (12)	39.7% (89)	0.46 [0.23–0.94]	0.03
Clinical pregnancy	15.7% (8)	32.1% (72)	0.39 [0.18–0.88]	0.02
Day 5 SET (n = 207)				
	P rise > 1.5 ng/mL (n = 37)	Normal P level (n = 170)	OR [95%CI]	P value
Age ^a (years)	30.3 ± 0.5	31.1 ± 0.2		0.18
Stimulation ^a (days)	9.7 ± 0.3	9.6 ± 0.2		0.73
Prog on hCG day ^a (ng/mL)	2.0 ± 0.08	0.9 ± 0.02		<0.001
E ₂ on hCG day ^a (pg/mL)	2453 ± 219	1849 ± 93		<0.001
LH on hCG day ^a (IU/L)	1.4 ± 0.2	1.6 ± 0.1		0.76
Oocytes retrieved ^a (COCs)	16.6 ± 0.4	12.5 ± 1.3		<0.001
Fertilized oocytes ^a (2PN)	10.0 ± 0.8	7.8 ± 0.4		0.04
Top quality embryos	83.8%	78.8%		0.43
Positive hCG	48.6% (18)	42.9% (73)	1.25 [0.61–2.56]	0.53
Clinical pregnancy	40.5% (15)	38.2% (65)	1.34 [0.64–2.72]	0.42

Note: CI = confidence interval; OR, odds ratio.

^a Values are expressed as mean ± SEM.

Papanikolaou. Long-term sperm storage. *Fertil Steril* 2009.

Premature luteinization

- P rise above 1.5 ng/mL had deleterious effect on day 3 transfer but not day 5
- No significant difference in PR between elevated P rise above 1.5 ng/mL and normal levels if transferred d5
- 75% of embryos in the day 3 group, with or without P elevation, were top quality – making P impact on oocyte or embryo quality unlikely
- Suggests that by the fifth luteal day the endometrium has time to recover from the effect of stimulation

“Milder” stimulation better?

- RCT, under 38
- Conventional: GnRH agonist, recFSH 225 IU/d
- Mild: GnRH antagonist, rec FSH 150 IU/d
- 11% cancelled due to poor response in mild group v. 2% cancelled due to OHSS in conventional group
- For all reasons, 15% fewer patients got to biopsy in the mild group
- FISH analysis of copy number from 10 chromosomes

"Milder" stimulation better?

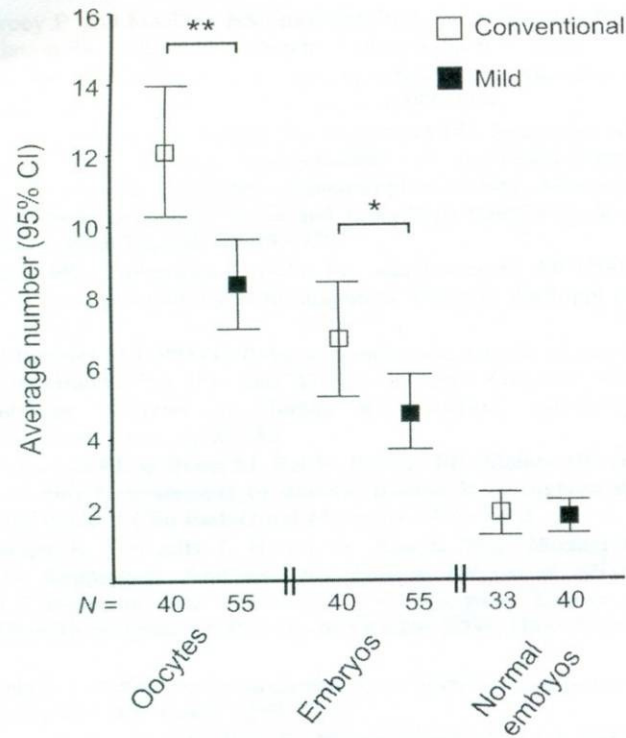


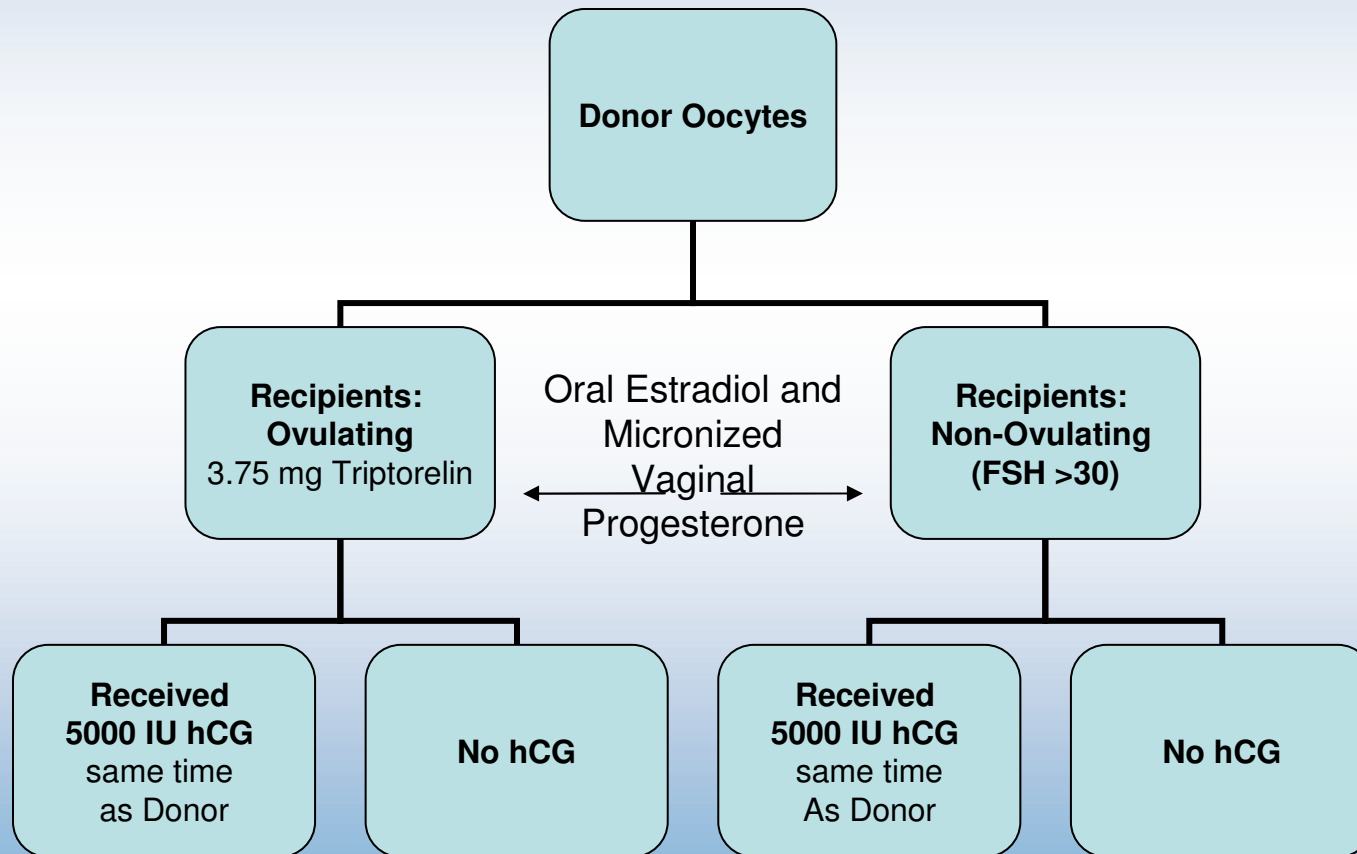
Figure 4. Oocyte and embryo yield and embryos successfully biopsied and diagnosed by fluorescent *in-situ* hybridization (FISH) as chromosomally normal on the basis of FISH results from one cell following conventional and mild stimulation. Values are expressed on a per patient basis and are represented as mean. Bars indicate the 95% confidence interval. * $P < 0.05$, ** $P < 0.01$.

Implantation efficiency and sustenance

Extragonadal function of the LH/hCG receptor

- LH/hCG receptors in multiple uterine cell types, highest levels in *endometrial epithelial cells*
- LH/hCG receptor knockout models have abnormal uterine function, including *decreased ability to implant donor blastocysts*, which cannot be reversed by normalizing E+P levels
- Uterine Lh/hCG receptor activation upregulates COX-2, increases stromal cell differentiation, modulates pro- and anti-implantation cytokine production, increases blood flow through vasodilation and angiogenesis, inhibits myometrial contractions, and influences resident macrophage populations

hCG affects Uterine Receptivity Independent of Ovarian Function



hCG affects Uterine Receptivity Independent of Ovarian Function

- Implantation rate, ovulatory recipients
 - Received hCG: 30.6%
 - Did not receive hCG: 20.7% ($P < 0.01$)
- Endometrial thickness on transfer day (d19), ovulatory recipients,
 - Received hCG: 9.7mm +/- 0.9
 - Did not receive hCG: 8.2mm +/- 0.8 ($P < 0.01$),
- day 14 “stagnation” was ameliorated
- There was no difference in the non-ovulatory recipients with tonically elevated LH levels, signifying the effect was mediated through the LH/hCG receptor

Implantation efficiency and sustenance

Myomectomy

- Distortion or disruption of the endometrium and implantation due to atrophy or venous ectasia over a myoma
- Impaired endometrial blood flow
- Endometrial inflammation and/or secretion of vasoactive substances
- Uterine artery embolization, myolysis, and MRI-guided ultrasonic treatment is not recommended for women seeking to maintain or improve their fertility because the safety and efficacy has not been established

Implantation efficiency and sustenance

Myomectomy

- Size and location
- Prospective cohort unexplained infertility, 11% conceived with myomas v. 25% without myomas v. 42% laparoscopic myomectomy (1)
- IVF rates lower with intramural myomas (2), with mean diameter 2.4 cm (3), when larger than 5 cm (4)
- 23 studies examined, fibroids with a submucosal component led to decreased clinical pregnancy and implantation rates with removal likely to improve pregnancy rates (5)

1) Donnez et al, Hum Reprod 2002; 17: 1424-30.

2) Somigliana et al, Hum Reprod Update 2007; 13: 465-76.

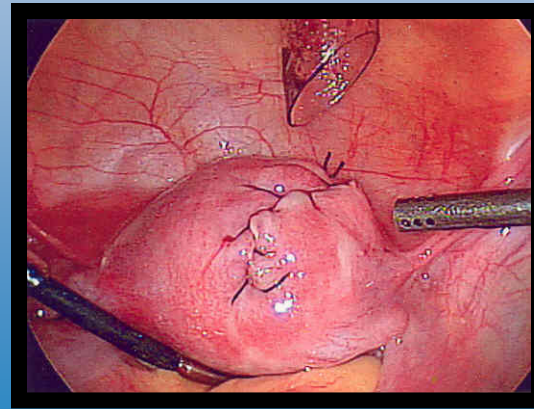
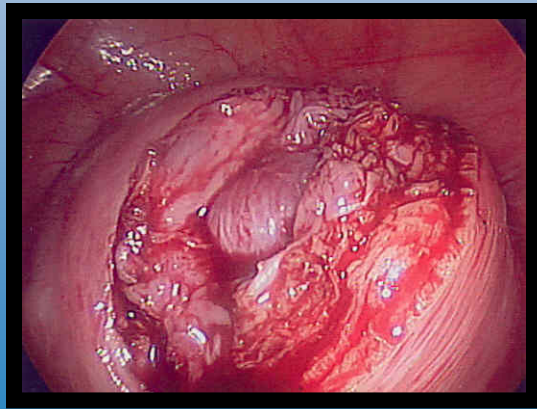
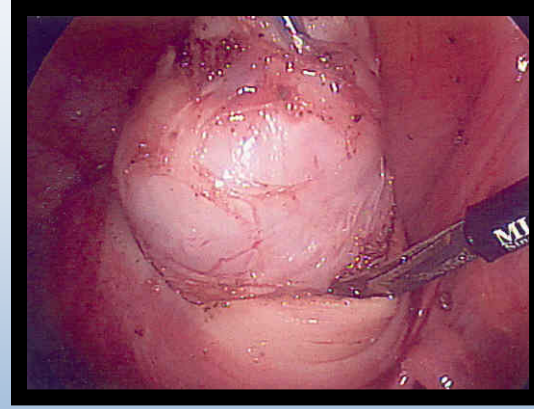
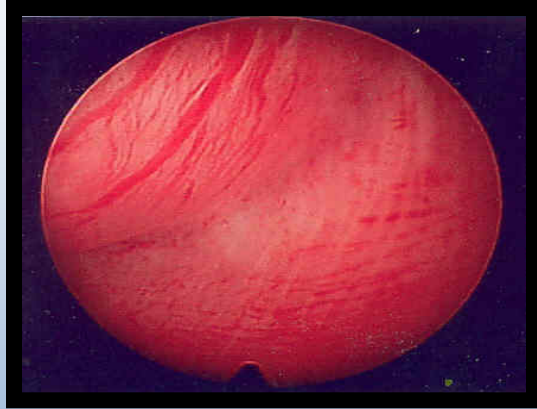
3) Eldar-Geva et al, Fertil Steril 1998; 70(4): 687-691.

4) Kolankaya et al, Obstet Gynecol Clin North Am 2006;33: 145-52.

5) Pritts et al, Fertil Steril 2009; 91(4): 1215-23.

Implantation efficiency and sustenance

Myomectomy



Implantation efficiency and sustenance

Septolysis

Table 6-4. Outcomes After Hysteroscopic Metroplasty for Septate Uterus

Author	N	Preoperative			Postoperative		
		Spontaneous Abortion (%)	Preterm Delivery (%)	Term Delivery (%)	Spontaneous Abortion (%)	Preterm Delivery (%)	Term Delivery (%)
DeCherney, 1986 ^a	103	100	—	—	2	—	80
Israel, 1984 ^b	15	80	0	—	20	—	40
Valle, 1986	12	93	—	7	16	—	83
Fayez, 1986 ^c	33	—	—	—	10	—	80
Daly, 1989	55	86	5	8	19	6	70
Choe, 1992 ^d	19	80	7	13	13	0	87
Zhioua, 1993	23	72	10	18	6	—	94
Gaucherand, 1994	78	47	17	13	—	—	90
Goldenberg, 1995 ^b	47	87	—	13	44	—	56

^a Patients with two or more first-trimester losses and otherwise normal evaluation.

^b Also includes infertility patients.

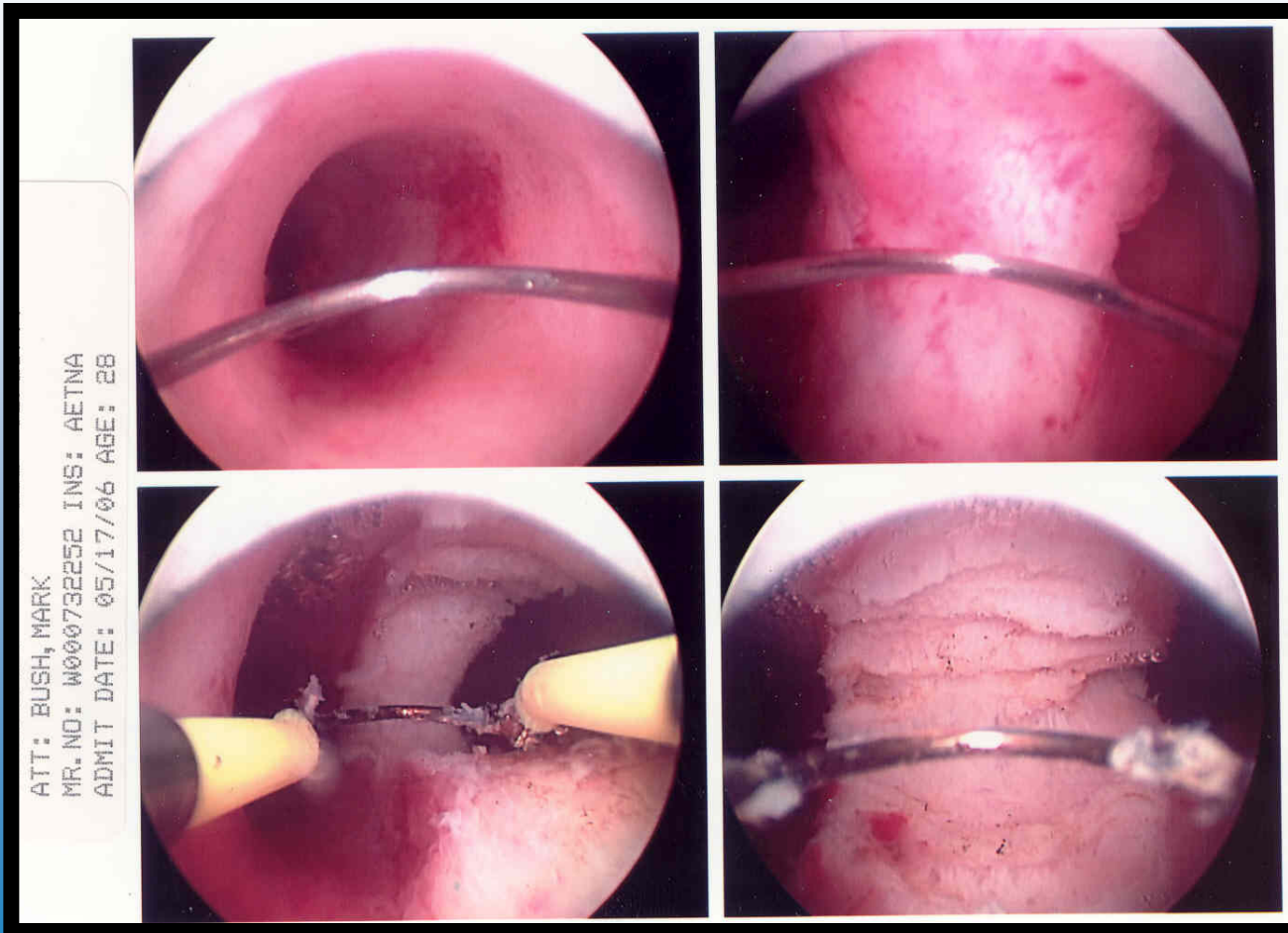
^c History of habitual abortion, preterm labor, or infertility.

^d Nd:YAG laser.

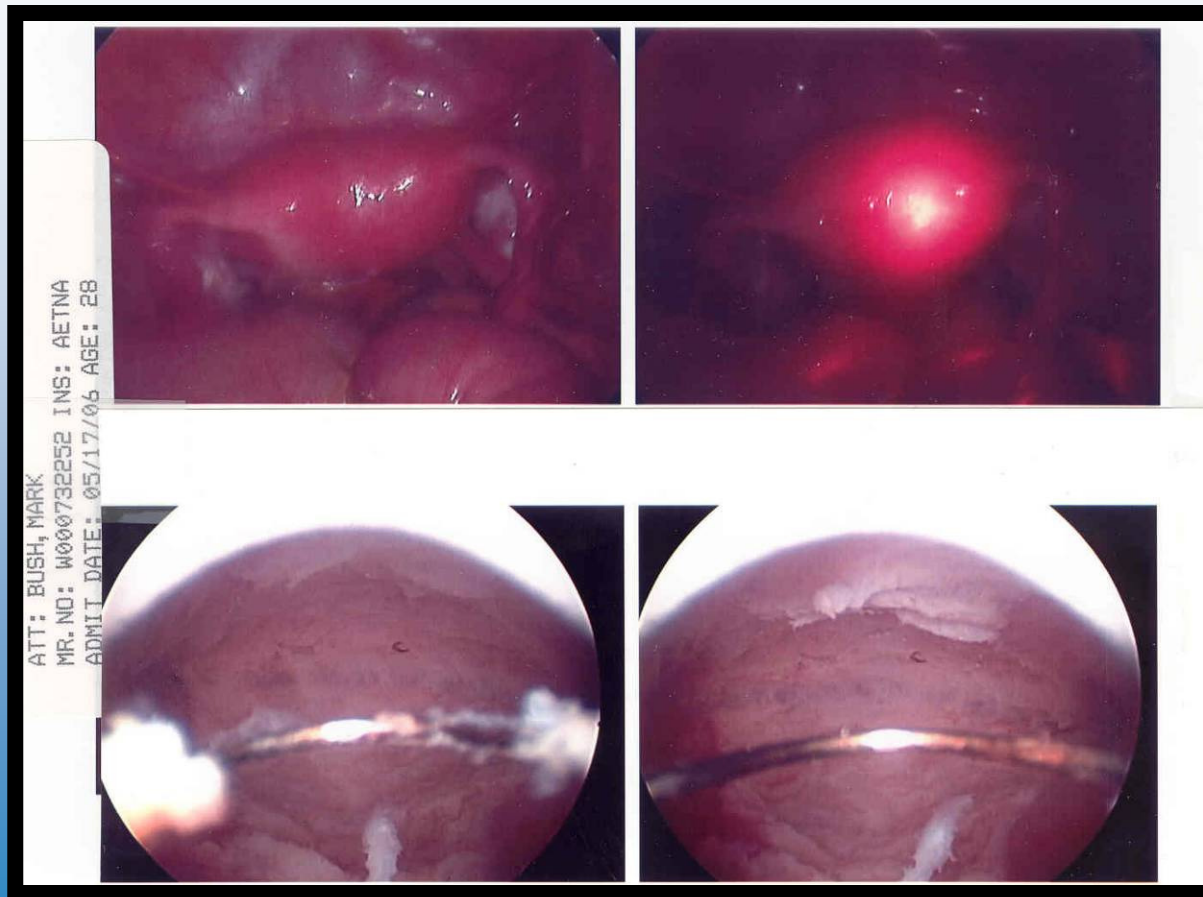
Letterie, Structural Abnormalities and Reproductive Failure, Blackwell Science, 1998.

Implantation efficiency and sustenance

Septolysis



Implantation efficiency and sustenance Septolysis



Implantation efficiency and sustenance

Septolysis

- Hysteroscopic resection with laparoscopic follow
- 5mL intrauterine stent x10 days with doxy 50mg BID
- Estrace 2mg BID for 21 days followed by Prometrium 200 qHS x 7d (2 courses) starting day after surgery
- Office hysteroscopy test of cure
- 15% fundal coaptation rate requiring H/S resection

Implantation efficiency and sustenance

Salpingectomy for hydrosalpinx

- 116 randomized to salpingectomy before IVF, 88 control
- Delivery rates 28.6% v. 16.3% ($p < .05$) (1)
- Subgroup of hydros visible on ultrasound: 40% v. 17.5% (1)
- Direct embryotoxic effect (2), decreased endometrial receptivity, mechanical disruption of implantation by fluid
- Based on the results from 3 trials, ongoing pregnancy rate after salpingectomy or proximal occlusion is two-fold higher than controls, 34% v. 17% (3)

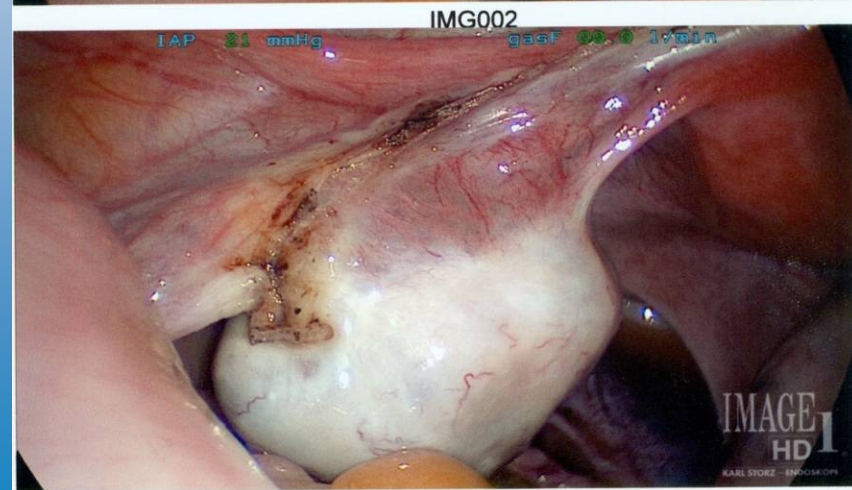
1) Strandell et al, Hum Reprod 1994; 9: 2762-9.

2) Mukherjee et al, Fertil Steril 1996; 66: 851-3.

3) ASRM Practice Committee, Salpingectomy for hydrosalpinx 2008; 90(3) S66-8.

Implantation efficiency and sustenance

Salpingectomy for hydrosalpinx



Implantation efficiency and sustenance

Coagulopathy and MTHFR defect

- Observational data suggests a relationship between elevated homocysteine levels and decreased folate levels with pregnancy loss as well as a benefit of folate, B6 and B12 supplementation (1).
- Folgard 2.2 BID (or equivalent) delivers 4.4 mg folic acid, 50mg B6 and 1mg B12. Water soluble B vitamins that can decrease intensity of N/V and be prophylactic with regard to NTD.

Anti-phospholipid syndrome

- Sydney criteria (1)

 - Clinical

 - arterial or venous thrombosis
 - ≥ 3 unexplained miscarriages ≤ 10 wks
 - unexplained fetal death ≥ 10 wks
 - premature birth (≤ 34 wks) complicated by severe pre-eclampsia or placental insufficiency

 - Laboratory

 - ACA IgG or IgM ≥ 40 GPL or MPL
 - Anti-B2 glycoprotein-1 IgG or IgM $> 99^{\text{th}}$ percentile
 - LAC

- Treatment (2,3)

 - Livebirth rates of 69-78% with lovenox and ASA

(1) Miyakis et al, J Thromb Haemost 2006. (2) RCT Farquharson et al 2002. (3) RCT Stephenson et al 2004.

Maternal immune acceptance of placenta

Controversial

- Thyroid function and TPO/TG
- Prednisone
- Intralipids
- IVIG

Thyroid function and TPO/TG

- Miscarriage rate in TPO positive women was significantly higher than in those with no antibody, 13.8 versus 2.4 %, (RR 4.95, CI 2.59-9.48) (1)
- Levothyroxine dose requirements can increase as much as 50% during pregnancy. With the importance in implantation, maintenance of pregnancy and fetal cognitive development, one group's approach is to increase levothyroxine dosing 30% at positive pregnancy test

(1) Negro R, et al. Levothyroxine treatment in euthyroid pregnant women with autoimmune thyroid disease: effects on obstetrical complications. J Clin Endocrinol Metab 2006; 91:2587.

(2) Alexander EK, et al. Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. N Engl J Med 2004; 351:241.

Prednisone

- Safety and efficacy of prednisone 10mg BID thru 12 weeks demonstrated with a livebirth rate of 77% in 80 women with therapy as opposed to a 35% pregnancy rate in 52 matched women without therapy. Concurrent use of 5mg folate QOD, 100mg ASA QD, 20mg PO progesterone QD; autoimmunity not tested (1).
- Reznikoff-Etievant et al, Human Reproduction 1999, Vol 14(8) p. 2106 also demonstrated safety of prednisone at 20mg/d in 277 women.
- Category B. Drugs in Pregnancy and Lactation, 5th ed, Briggs, Freeman, Yaffe. Williams & Wilkins, 1998, p. 884-5.

(1) Tempfer et al, Fertil & Steril 2006, Vol 86(1) p. 145.

Prednisone

- Women with recurrent loss had significantly more uNK than controls ($p = 0.008$).
- Prednisone treatment (20 mg/d for 21 days) significantly reduced the number of CD56 cells in the endometrium, from a median of 14% before to 9% after treatment ($p = 0.0004$).
- Demonstrated that high numbers of uterine natural killer cells in preimplantation endometrium of women with recurrent miscarriage can be reduced with the administration of prednisone.

Intralipid Infusion

- 79 patients with elevated NK-cell activity ($> 10\%$) treated with 2-4 mL IV of 20% intralipid.
- 68 with implantation failure (cumulative total of 8 cleaved or 4 blasts without + hCG) – 27/68 (40% PR) following intralipid with IVF.
- 11 with RPL (≥ 2) – 10/11 (91%) had a LB with treatment.
- Intralipids suppress Nka for approx 40 days possibly through NK-cell nuclear PPARs.

IVIg

- Human IgG derived from pooled plasma with \$ 7 -14,000. per single course of therapy.
- Five RCTs of patients with RPL reported on 121 treated vs. 125 placebo controlled. Live birth rate was 62% (95% CI 53% - 71) vs 54% (95% CI 45% - 62).
- IVIG is not effective for primary recurrent pregnancy loss with likelihood of live birth 0.98 (95% CI 0.45 – 2.13).
- In secondary RPL (with at least one LB), likelihood 2.19 (however 95% CI crosses 1 at 0.65 – 7.39 denoting that patient number insufficient to rule out a chance finding).

IVIg

- History of pregnancy of at least 20 weeks followed by 3 consecutive first trimester losses.
- 38 randomized to treatment with 39 normal saline controls. Gamimune or Gamunex 500 mg/kg prior to ovulation and continued q4 weeks until 81-20 wks gestation.
- No significant difference in live birth rate at 70% for treated vs. 63% controls. 94% live birth rate in each group if pregnancy achieved 6 weeks. This phase III multi-center trial ended early on interim A-B analysis (designed to enroll 178).
- Findings may be influenced by not selecting out patients that had prior aneuploid losses (only 18% had placental karyotype) and the beneficial effect of frequent ultrasounds and supportive care.

Stephenson et al, Fertil Steril 2009; 92(3): pS244-5 (O-230).

Refractory Endometrium

Including POF, longstanding hypothalamic dysfunction, Kallmann's, empty sella syndrome, DE recipients nearing menopause with fibrotrophic endometrium

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

Refractory Endometrium

- Successful pregnancies after combined pentoxifylline-tocopherol treatment in women with POF who are resistant to hormone therapy
Letur-Kornisch et al, *Fertil Steril* 2003;79:439-411.
- Case report of 3 women with echogenic endometrium mean 4.9mm despite high serum E2 levels on ERT
- PTX 800mg with Vit E 1000mg qday for \geq 9 months
- Improvement to mean 7.4mm triple layer
- 2 embryo FET in two of the patients resulted in pregnancy
- Fibroatrophic changes ameliorated
- Initial report of 6 women with radiation induced fibroatrophic changes responded to 12 months of therapy with increase in endometrial thickness from 3 to 6mm, 1.5 fold increase in myometrial volume, and restoration of diastolic uterine artery flow
Letur-Kornisch et al, *Fertil Steril* 2002;77:1219-26.

Endometrial activation

Barash et al, *Fertil Steril* 2003; 79: 1317-22.

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

Endometrial activation

- Raziel et al, *Fertil Steril* 2007; 87: 198-201.
 - 2 pipelle biopsies total on d21 and 26 during luteal lupron application.
- Esfandiari et al, *Fertil Steril*, 2004, Vol 82, Suppl 2, S152.
 - Single biopsy in the luteal phase in the cycle preceding stimulation.
- Kalma et al, *Fertil Steril*, 2008, in press.
 - Biopsy induced gene modulation/factor expression validation with biopsies d11-13 and 21-24.

Endometrial activation

- Pre-IVF cold loop polypectomy, gentle curettage of the functionalis, oral estrace 2mg x 5 – 10 days while on OCP prior to cycle start
- Biopsy at the time of pre-IVF office hysteroscopy
- Involves events that accompany wound healing to include secretion of cytokines and growth factors known to be involved in implantation.
 - Sharkey. *Rev Reprod* 1998;3: 52-61.
 - Basak et al, *Am J Reprod Immunol* 2002; 47: 2-11.
- Set up non-infectious inflammatory/repair response to potentially enhance implantation



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