

MINI-IVF AND EPIGENETIC PERTUBATIONS

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OVARIAN CLUB VIII

BUILDING A BRIDGE BETWEEN SCIENCE
AND CLINICAL PRACTICE

4-6 NOVEMBER, 2016 • PARIS, FRANCE

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MINI-IVF AND EPIGENETIC PERTUBATIONS

Disclosure information: Nothing to declare.

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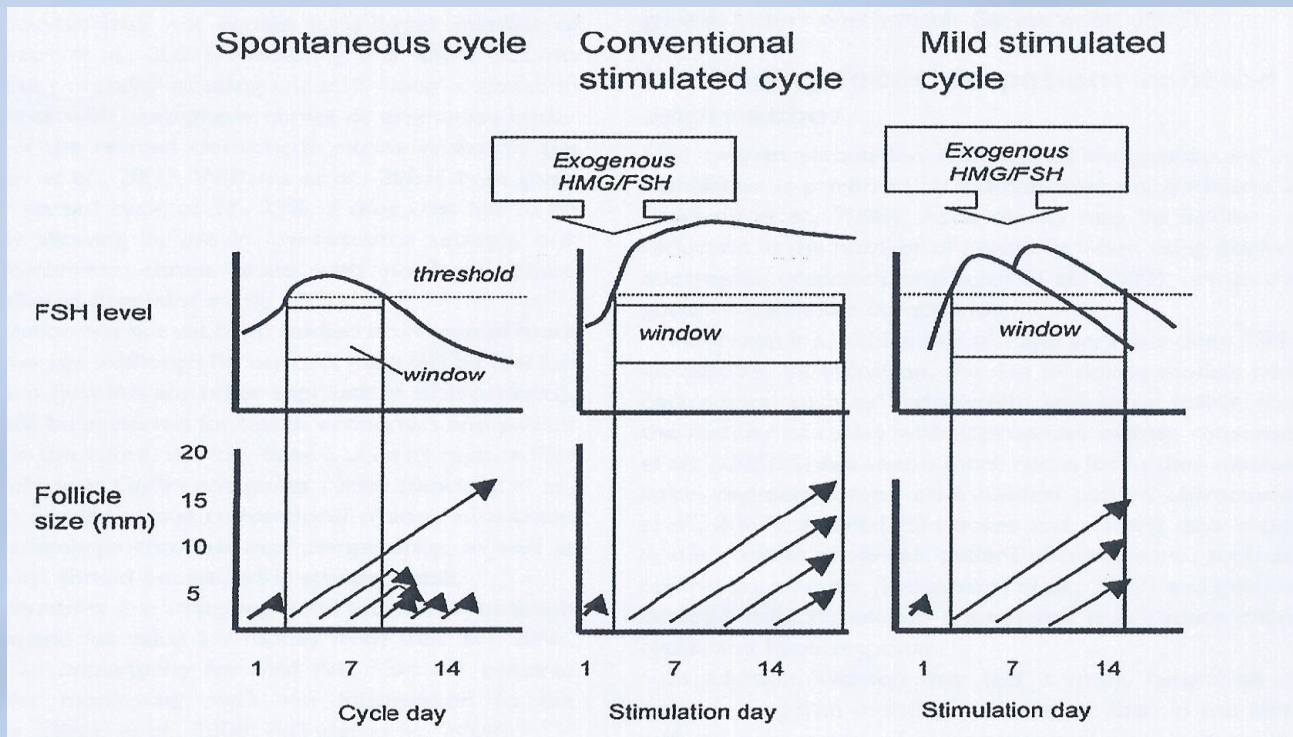
GONADOTROPINS

- Late 1990's widespread increase in daily dosages of gonadotropins
 - Increase the number of oocytes
 - Retrieve an excess number of embryos for cryopreservation
- An increase in the recruitment of multiple fertilizable oocytes
 - Better success rates
 - Increased cost
 - Increased OHSS and multiple pregnancies

In 1996, Edwards et al. were the first to express concern with regard to the contemporary ovarian stimulation approaches for IVF and called for the use of milder stimulation protocols

(Edwards et al., 1996)

Fauser, et al in 1993, introduced the “Window Concept” which emphasizes the importance of a transient increase of FSH above the threshold level in order to gain single dominant follicle selection. He was the first to develop the strategy of using mild ovarian stimulation in IVF.



Fauser et al., Human Reproduction, Vol 25, No 11, pp 2678-2684

THE IDEAL COS REGIMEN FOR IVF

- Minimize cost
- Reduction in amount of COS medications
- Reduction in duration of stimulation
- Limit monitoring for patient convenience
- Lower side effects and risk
- Maximize embryo quality
- Maximize Live Pregnancy Rates

LATE FOLLICULAR PHASE hCG/LH

Filicori et al. 1999

- LH promotes folliculogenesis in synergy with FSH
- Shortened COH timing
- Decreased FSH requirements
- Decreased COH cost

First to show that LH activity enhances the efficacy and improves ovulation induction outcomes.

LATE FOLLICULAR PHASE hCG/LH

Filicori, et al., 2005

Showed the efficacy of a stimulation protocol with complete replacement of FSH with LH/hCG from a follicle size of 12mm in combination with a long GnRH agonist down regulation protocol.

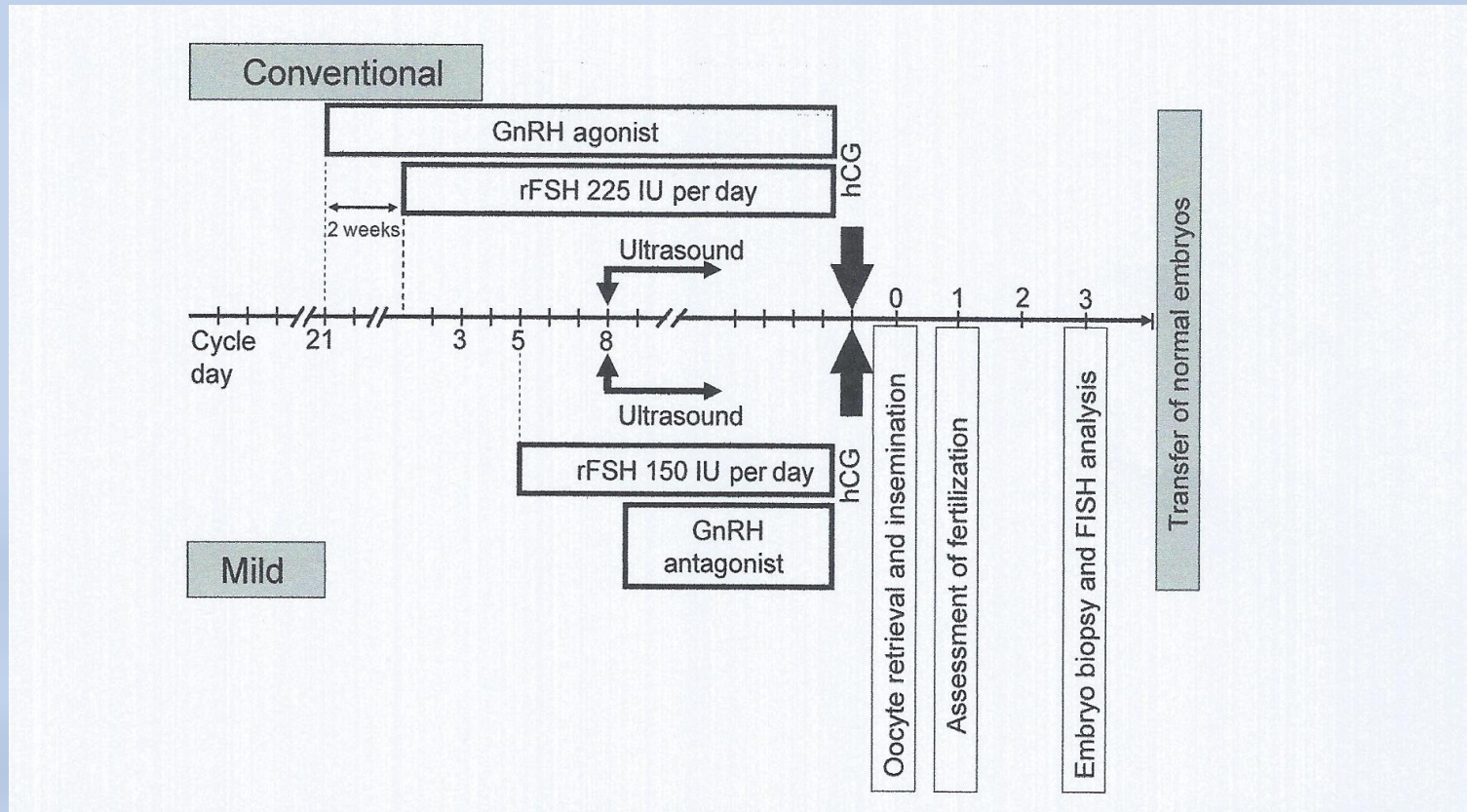
- Significant reduction in FSH needed
- Less small follicles at final maturation
- No difference in pregnancy rates
- A reduction in the incidence of OHSS could not be established

MILD OVARIAN STIMULATION

- The availability of GnRH antagonists for the acute suppression of a premature LH rise enabled this concept to be introduced into IVF.
- Low dose gonadotropin administration is delayed until the mid-follicular phase is based on the “FSH window effect”.
- Low dose of gonadotropins to produce a maximum of 10 oocytes.

MILD OVARIAN STIMULATION

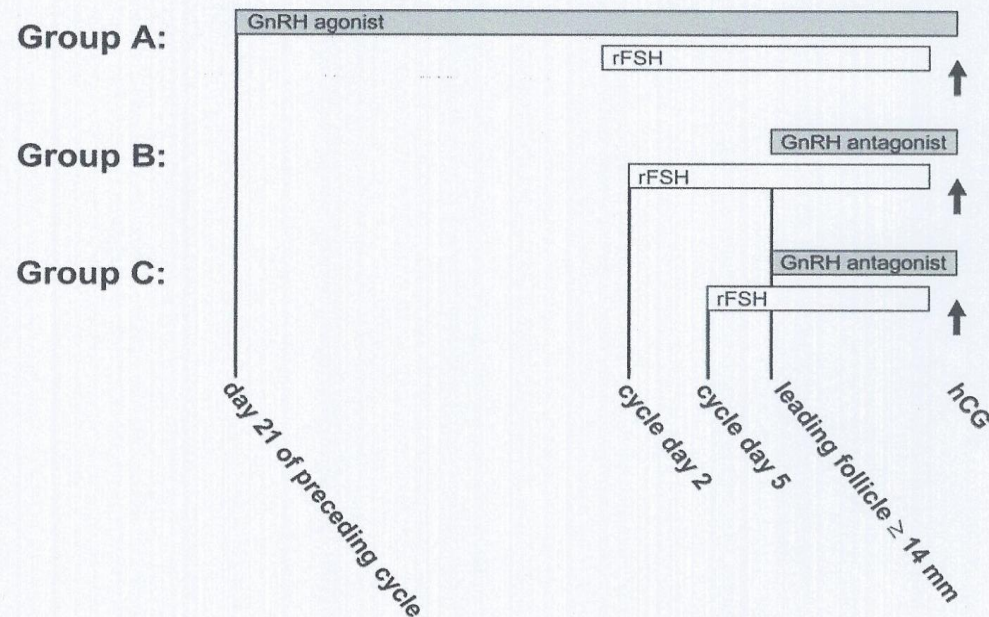
GnRH ANTAGONIST PROTOCOL VS STANDARD AGONIST PROTOCOL



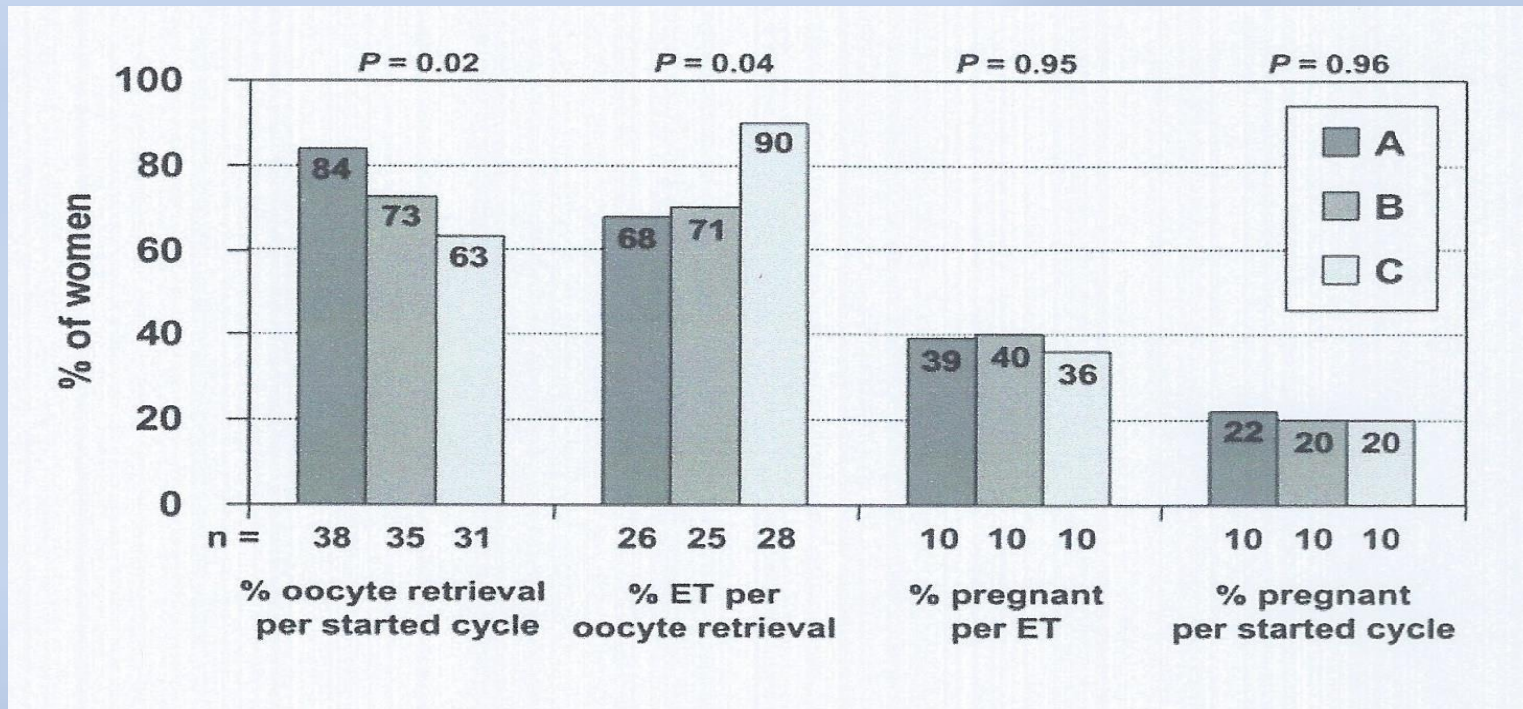
MILD OVARIAN STIMULATION

Hohmann et al, 2003: a prospective randomized study involving 142 patients divided into three groups.

FIG. 1. Schematic representation of the three studied stimulation regimens: a GnRH agonist long protocol and two late follicular phase GnRH antagonist protocols with start of a fixed dose of rFSH on cycle d 2 or cycle d 5.



MILD OVARIAN STIMULATION



The Mild protocol vs. The Conventional protocol resulted in:

- Comparable pregnancy rates
- Reduction in duration of stimulation
- Marked reduction in amount of exogenous FSH needed

Hohmann, et al

MILD OVARIAN STIMULATION

Heijnen et al., 2007

- A large randomized efficacy study
- Analyzed whether a mild IVF strategy with a single embryo transfer would lead to a similar overall outcome while reducing a patient's discomfort, multiple pregnancies and costs when compared with standard conventional stimulation and the transfer of two embryos.

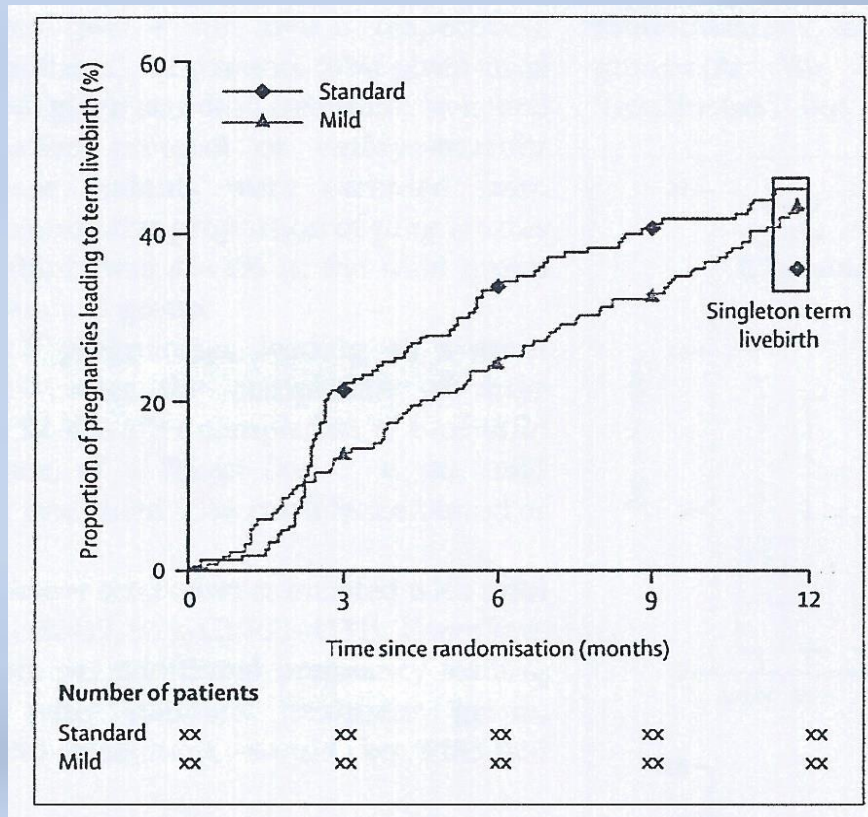
MILD OVARIAN STIMULATION

	MILD	CONVENTIONAL	
Cumulative Pregnancy Rate	43.4%	44.7%	NS
Multiple Pregnancy Rate	0.5%	13.1%	
# Days of Ovarian Stimulation	8.3	11.5	P< 0.001
# Of Injections	8.5	25.3	P< 0.001
Proportion of pregnancies leading to a term live birth	52.4% after 4 cycles	50.3% after 3 cycles	

CONCLUSION: Mild stimulation with single embryo transfer and a standard protocol with double embryo transfer had equivalent pregnancy rates.

HEIJNEN, ET AL., LANCET 2007; VOL 369

MILD OVARIAN STIMULATION



Heijnen et al: Lancet 2007; vol 369

MILD OVARIAN STIMULATION

Verberg et al, 2009

- A meta-analysis of 3 studies comprising of 592 patients
- Purpose: to investigate whether retrieving low number of oocytes following mild IVF is associated with impaired implantation rates
- Optimal embryo implantation rates were observed with 5 oocytes retrieved with mild (31%) versus 10 oocytes following conventional stimulation (29%)

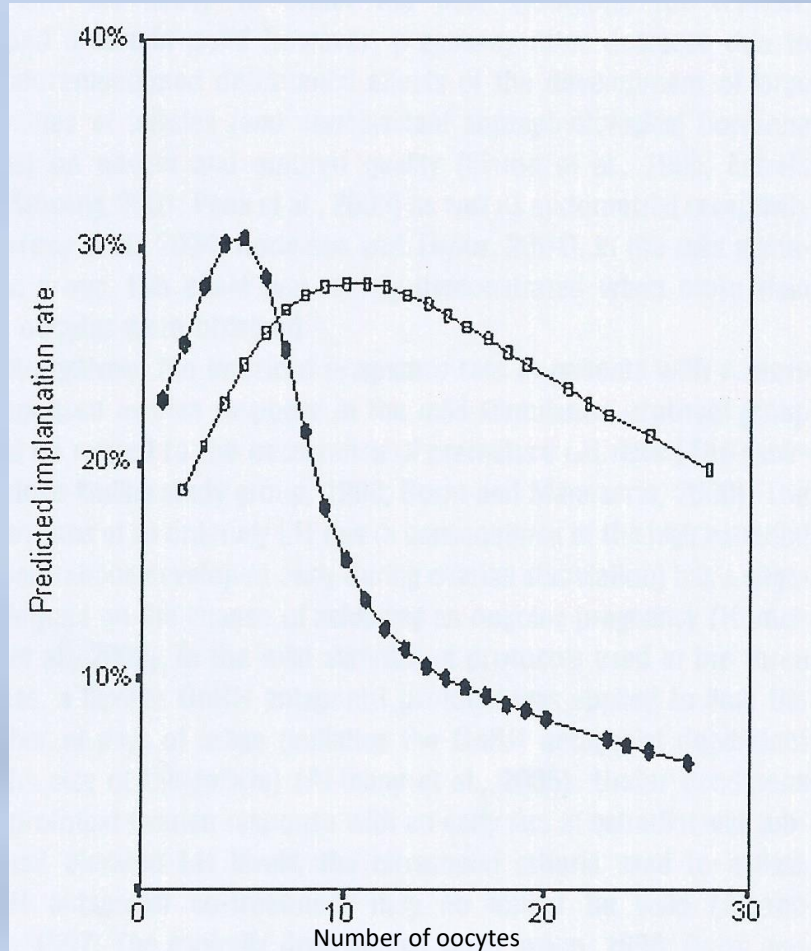
MILD OVARIAN STIMULATION

Table III Ongoing pregnancy rate per embryo transferred as a function of the number of retrieved oocytes following mild or conventional ovarian stimulation for IVF

Number of retrieved oocytes	Conventional stimulation			Mild stimulation		
	Implantation failure	Ongoing pregnancy/ embryo transferred		Implantation failure	Ongoing pregnancy/ embryo transferred	
	<i>n</i>	<i>n</i>	%	<i>n</i>	<i>n</i>	%
1-3	21	4	16	38	15	28
4-6	64	21	25	46	19	29
7-9	69	26	27	43	10	19
10-12	58	27	32	22	3	12
13-15	41	12	23	14	3	18
16-18	20	10	33	9	2	18
19-21	11	0	0	4	0	0
22-24	1	3	75	4	0	0
25-27	2	0	0	5	0	0
28-30	1	0	0	0	0	0
Total	288	103	26	185	52	22

Verberg et al, Human Reproduction Update, Vol 15, No.1 pp5-12,2009

MILD OVARIAN STIMULATION



Verberg et al., Human Reproduction Update, Vol15, No.1 pp 1-5, 2009

MILD OVARIAN STIMULATION

CONCLUSION

Mild Stimulation Protocols

- Reduce the number of days of ovarian stimulation
- Reduces the number of injections
- Decreases cost (less medication/monitoring)
- Less negative psychological impact
- Preserves pregnancy rates
- Decreasing OHSS

MINI-IVF

Clomiphene Citrate

- Discovered in late 1950's
- First method of ovarian stimulation used in IVF
- Two isomers: Enclomiphene and Zuclomiphene
- Increase pituitary FSH by reducing negative feedback of Estrogen
- Advantages:
 - Oral administration
 - Low cost
 - Widely available

MINI-IVF

In 1993, Corfman et al., introduced the term “Minimal Stimulation”.

- Prospective non-randomized study comparing two protocols of COS.
 - Mini-Stimulation: CC 100mg CD 3-7, followed by a single dose of 150 IU hMG on CD 9
 - hMG alone protocol starting with 150 IU hMG and individualized based on clinical data
 - 61 women/106 cycles of minimal stimulation vs 183 women/443 cycles of hMG
- Initial intent was to find different COH protocols requiring lower doses of hMG that would be attractive to patients from both a financial and patient comfort perspective without compromising outcomes

MINI-IVF

	MINI-STIM	CONVENTIONAL	
Cancellation Rates	25.8%	14.1#	NS
# Oocytes obtained/retrieval	3.4 +/- 1.6	10.1% +/- 5.4	P < 0.001
# Immature oocytes/retrieval	11.5%	18.3%	NS
# Normal ferts/# Inseminated	82%	66%	P < 0.001
Implantation rate/Embryo	16.4%	13.3%	NS
Pregnancy Rates/ Retrieval	31%	42%	NS
Delivery Rate/ Retrieval	29%	37%	NS

Corfman et al., Fertility and Sterility, Vol 60, No 5, Nov 1993, pp 864-869

MINI-IVF

Lu, 1996, later examined the effectiveness of Mini-stimulation and hMG alone using the same protocols as Corfman.

	MINI-IVF	CONVENTIONAL	
# Days of Stimulation	10.2 +/-1.4	8.6 +/- 2.5	NS
Total # of Ampules Used	2.0	16.8 +/- 8.5	P < 0.001
Cumulative PR over 5 cycles	20.8%	20.1%	NS
Singleton Pregnancy Rate	85.7%	78%	NS
OHSS	0.0 %	2.5 %	NS

LU et al., Fertility and Sterility, Vol 65, No 3, March 1996, pp 583-587

MINI-STIMULATION

Williams et al., 2002

- Retrospective Control Study
- Compared Mini-IVF protocol (Clomid 100mg CD 3-7/ 150 IU's FSH beginning CD 9 vs Standard GnRH antagonist protocol
- Compared both protocols in women < 35 y/o and > 35 y/o
- Compared the effect of GnRH antagonist on outcomes based on age

Williams et al., Fertility and Sterility, Vol 78, No 5 Nov 2002, pp 1068-1072

MINI-STIMULATION

	MINI-IVF	CONVENTIONAL	
Peak E2 levels	1523 +/- 749	2443 +/- 1231	P < 0.05
# Mature Oocytes	3.7 +/- 2.0	13.1 +/- 6.0	P < 0.05
# Embryos transferred	2.9 +/- 1.1	3.5 +/- 0.9	P < 0.05
Clinical Pregnancy Rate/transfer	37%	41%	NS
# Ampules Used	5.7 +/- 4.2	25 +/- 7.5	P < 0.05
Patients able to cryopreserve	5%	53%	P < 0.05

Williams et al., Fertility and Sterility, Vol 78, No 5 Nov 2002, pp 1068-1072

MINI-STIMULATION

Teramoto, 2007

- Large scale retrospective study
- 43,433 Patients
- Protocol
 - Clomid 100 mg CD 3-7 / 150 IU FSH on alternating days starting on CD 8

Teramoto, Reproductive Biomedicine Online. Article 2711, June 2007

MINI-STIMULATION

Conclusions

The advantages of the use of prolonged, continuous CC:

- Reduction in the dosage of hMG/FSH
 - Reduces financial and physical burden on patients
- Inhibits premature LH surge while maintaining pituitary function.
- Equivalent pregnancy rates
- Eliminates OHSS

MINI-STIMULATION

Ferraretti et al., 2015

“Lite IVF”

- 3 cycles of fresh and/or cryopreserved embryo transfers
- Reduced cost of 50% compared with 3 conventional cycles
- Stimulation:
 - CC (100mg/d CD 3-7 / 150 IU FSH CD 5,7, and 9)
 - First U/S CD 9
 - Cycle canceled if < 3 follicles
 - GnRH antagonist initiated on CD 9
 - hCG 5000 IU to trigger
 - Up to 6 oocytes inseminated (IVF or ICSI)
 - 2 embryos transferred (Day 3 or 5)

Ferraretti, et al, Fertility and Sterility, Vol 104, No 2, August 2015, pp 333-338

MINI-STIMULATION

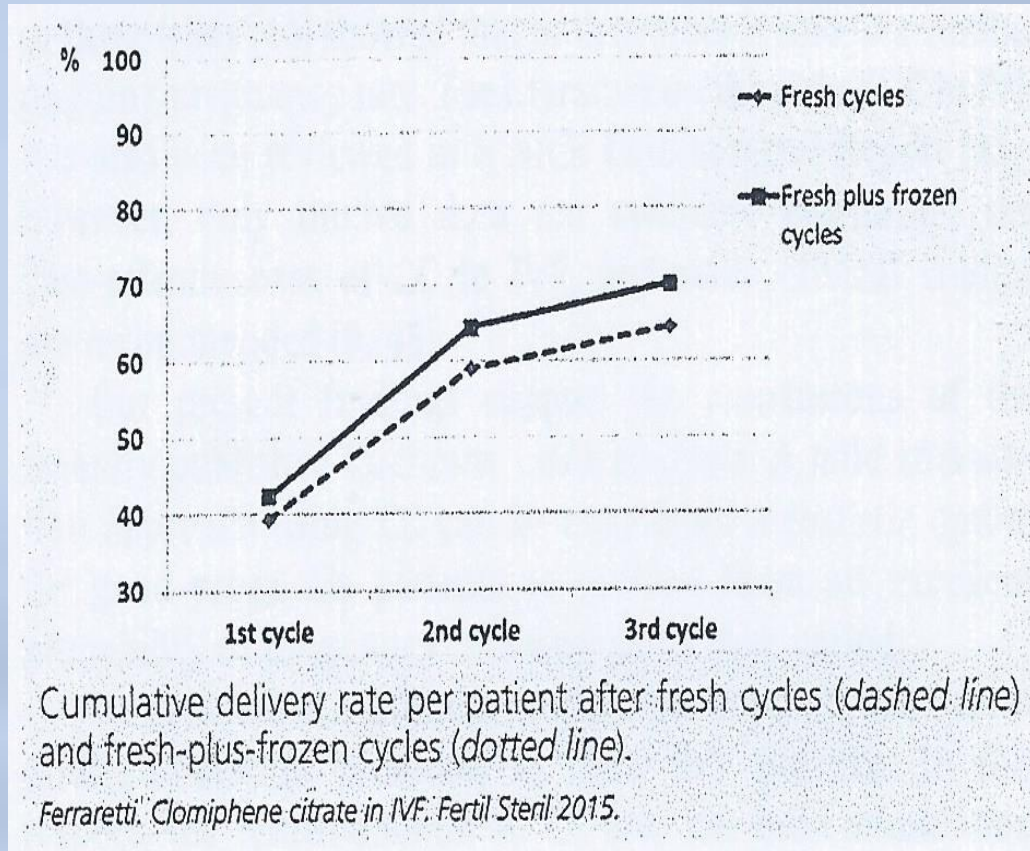
Fresh cycles.

Variable	First egg retrieval	Second egg retrieval	Third egg retrieval
No. of egg retrievals	163	94	46
E ₂ at hCG (pg/mL), mean ± SD	1,012 ± 320	996 ± 280	1,120 ± 350
Oocytes/patient (total collected), mean ± SD	5.6 ± 3.1 (894)	5.6 ± 2.8 (508)	5.5 ± 3.0 (238)
Mature oocytes, n (%)	656 (73)	398 (78)	193 (81)
Mean oocytes inseminated/patient (total inseminated)	3.7 ± 1.5 (620)	4.0 ± 1.6 (390)	3.9 ± 1.5 (180)
2PN (n), fertilization rate (%)	511 (82)	316 (81)	150 (83)
Embryos +2 (n), cleavage rate (%)	461 (90)	307 (97)	134 (89)
Grade 1, n (%)	380 (82)	235 (76)	98 (73)
No. of fresh embryo transfers (mean ± SD)	161 (1.7 ± 0.4)	93 (1.93 ± 0.1)	45 (1.98 ± 0.1)
Clinical pregnancies, n	65	32	9
PR/ET, % ^a	40.4	34.4	20
Miscarriages	1	0	0
Implantation rate, % (n) ^a	27.2 (75/276)	22.9 (44/192)	12.3 (11/89)
Twins pregnancies, n	10	12	2
Cumulative delivery rate per patient, % (n)	39.3 (64/163)	58.9 (96/163)	64.4 (105/163)

^a P < .05 for the first and second vs. third cycle.

Ferraretti. Clomiphene citrate in IVF. *Fertil Steril* 2015.

MINI-STIMULATION



Ferraretti, et al, Fertility and Sterility, Vol 104, No 2, August 2015, pp 333-338

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Per Transfer Age – Good Prognosis Patients

	Age at OPU	Clinical Pregnancy N(%)	Live Birth (among all), N(%)
Conventional (N=633)	<35 (N=499)	223(44.69)	181(36.27)
	35-39 (N=105)	37(35.24)	28(26.67)
	Total	260(43.04)	209(34.60)
Mini (N=570)	<35 (N=159)	67(42.14)	55(34.59)
	35-39 (N=163)	61(37.42)	40(24.54)
	Total	128(39.75)	95(29.50)

INFERTILITY CENTER OF ST. LOUIS

Probabilities of pregnancy/live birth based on regression model estimates

		Per Cycle		Per Transfer	
Stimulation	Age of Egg & AFC	Clinical pregnancy (%)	Live birth (%)	Clinical pregnancy (%)	Live birth (%)
Conventional	<35, <25	40.7	34	40.7	33.2
	<35, ≥25	47.5	39.1	47.4	38.5
	35-39, <25	37	26	35.5	24.6
	35-39, ≥25	43.7	30.4	41.9	29.1
	≥40, <25	21.6	10.6	16.8	8.1
	≥40, ≥25	26.7	12.8	20.9	9.9
Mini	<35, <25	37.2	31.5	39.5	32.3
	<35, ≥25	43.9	36.4	46.1	37.5
	35-39, <25	33.7	23.9	34.4	23.8
	35-39, ≥25	40.1	28.1	40.7	28.2
	≥40, <25	19.3	9.5	16.2	7.7
	≥40, ≥25	24	11.6	20.1	9.5

MINI-STIMULATION

CONCLUSIONS

The reintroduction of CC into the mild stimulation protocols has distinct advantages when compared to conventional stimulation including:

- Oral administration
- Significantly reduce use of FSH
- Low-cost for patients and society
- Increases patient satisfaction
- Similar ongoing pregnancy rates
- Elimination of severe OHSS

EMBRYO QUALITY

Munne et al., in 1997 evaluated donated embryos for genetic aberrations and mosaicism using preimplantation genetic screening. He found that chromosomal abnormalities, particularly increased mosaicism, was suggested at higher stimulation conditions. COS might disrupt the mechanism involved in maintaining accurate chromosomal segregation.

Munne et al., Human Reproduction, Vol 12, No 4, pp 780-784, 1997

EMBRYO QUALITY

Baart, et al, 2007

- Purpose: To test if COS for IVF affects oocyte quality and thus chromosomal behavior during meiosis and early embryo development.
- Prospective, randomized controlled trial
 - Mild Stimulation using GnRH Co-treatment (67 patients)
 - Conventional high dose GnRH agonist protocol (44 patients)
 - 10 Chromosomes analyzed

Baart et al., Human Reproduction, Vol 22, No 4, pp 980-988, 2007

EMBRYO QUALITY

	CONVENTIONAL	MILD STIMULATION	
Oocytes obtained/patient	12.1	8.2	
Fertilization Rates	57 +/- 28	55 +/- 30	
Embryos with normal morphology	35 %	51 %	P=0.04
% abnormal embryos relative to the # of embryos diagnosed	63 %	45 %	P=0.02
Average Chromosomally normal embryos obtained per patient	1.8	1.8	
Overall abnormality rates (abnormal and mosaic embryos)	73 %	55 %	P=.046
Mosaic embryos/patient	65%	37%	P=.004

Baart et al., Human Reproduction, Vol 22, No 4, pp 980-988, 2007

EMBRYO QUALITY

While mild stimulation resulted in significantly less oocytes and embryos, the proportion of chromosomally normal embryos is significantly increased. Therefore, the number of chromosomally competent embryos obtained per women were similar (1.8).

In addition, after analyzing two cells per embryo, the increase in chromosomal abnormalities observed after conventional stimulation, was mainly due to and increase in chromosomal mosaicism.

In minimal stimulation, follicular recruitment and initial stages of selection remain unaffected, where as, in conventional stimulation natural follicle recruitment and selection is completely overruled. This may in fact disturb the complex interplay of folliculogenesis and oocyte maturation increasing the risk for chromosomal abnormalities.

EMBRYO QUALITY

Haaf, et al, 2009

Maternal age-related increase of chromosome errors in oocytes (1 or more ICSI cycles)

	<35 years	35 - 40 years	>40 years
First ICSI chromosomal error rates	34.4%	44.5%	50.0%
2 nd -4 th ICSI chromosomal error rates	43.8%	53.5%	54.8%

Relationship between chromosome error rates and oocyte yield with one ICSI cycle

	<35 years	35 -40 years	>40 years
Chromosomal error rate 1-5 oocytes	23% +/- 5%	42.1% +/- 2.9%	49.6% +/- 4
Chromosomal error rate 6-10 oocytes	39% +/- 4%	43/8% +/- 2.1%	50% +/- 4%
Chromosomal error rate >10 oocytes	60% +/- 6%	54% +/- 4 %	54% +/- 12%

Haaf, et al., Fertility and Sterility, Vol 91, Issue 3, pp 733-738, March 2009

THE IDEAL COH REGIMEN FOR IVF

- Minimize cost
- Reduction in amount of COS medications
- Reduction in duration of stimulation
- Limit monitoring for patient convenience
- Lower side effects and risk (OHSS/multiple pregnancies)
- Better embryo quality
- Comparable Live Pregnancy Rates

MILD/MINI- IVF

“MORE IS LESS AND LESS IS MORE.”

Blumenfeld, Zeev, J Assist Reprod Genet (2015) 32:1713-1719