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The importance of hormonal stimulation

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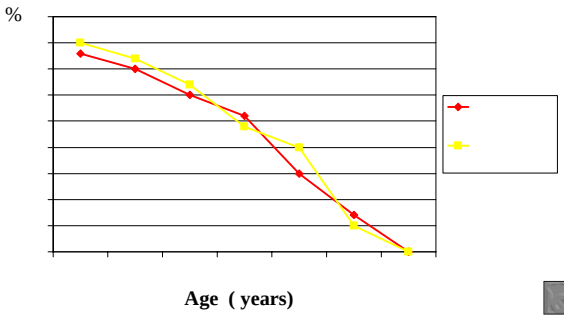
In vivo and in vitro fecundity (pregnancy rate in one cycle)



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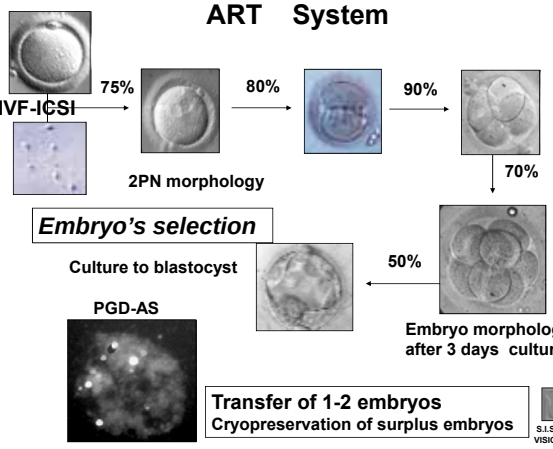
ART System



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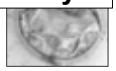
ART system

To have a baby we need :

One embryo ⇨ One baby

2-3 blastocysts →



3-4 eight cells embryos →

8-10 eggs

One egg ⇨ One baby

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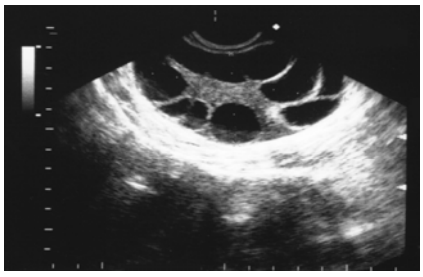
COH is still crucial in ART



COH is used in 99% of the cycles performed in the world

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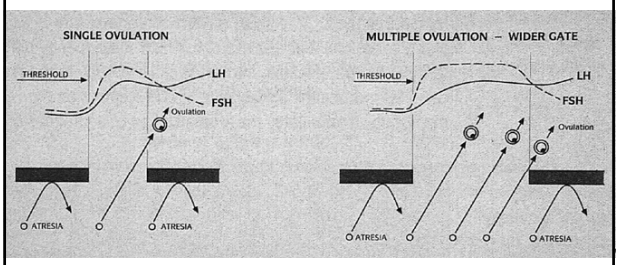

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COH
Drugs to increase FSH levels

- Clomifene citrate
- Urinary FSH
- Recombinant FSH
- Urinary HMG
  - FSH:LH=1:1
  - FSH:LH=2:1
- Recombinant FSH/LH

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Charge distribution of FSH isoform

Preparation	pI<4.0 (%)	pI>4.0 (%)
Rec - Follitropin α	9	91
Rec- Follitropin β	24	76
u-hFSH	40	60
u-hFSH HP	74	26

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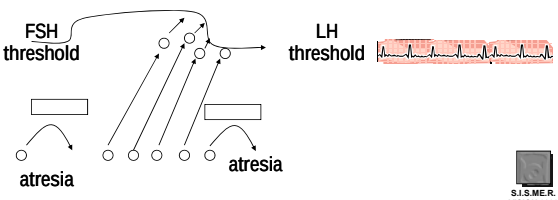
COH concepts

FSH threshold concept

LH ceiling concept



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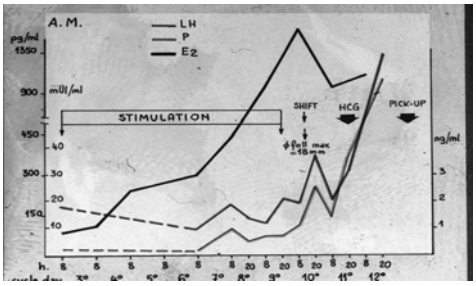
Pre- GnRH analogue period : endogeneous feed-back mechanisms not suppressed



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Early Luteinization (E.L.)

	E.L.		Control
Cleavage rate	37%	p< 0.01	66%
Pregnancy rate	8%	p< 0.05	22%

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GnRH agonist (Long –protocol)

Pre-treatment
- to synchronize follicles
- to increase oocyte quality
- to synchronize patients

During treatment
- to avoid LH surge
- to avoid early luteinization

HCG ⇩

Agonist

Ovarian stimulation

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GnRH agonist (Long –protocol)

The degree of pituitary suppression is variable but concentration > 0.3 mIU/ml are usually observed

HCG

Agonist

Ovarian stimulation

FSH monotherapy is able to produce competent oocytes in almost all patients

Is addition of LH needed to increase oocyte quality ?

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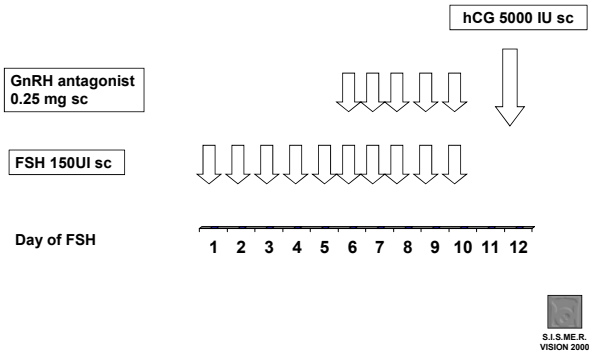
GnRH Antagonist protocols



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Drugs for COH

Clomiphene HMG FSH	HP FSH Agonist Rec FSH	Rec LH Antagonist Rec FSH/LH HP HMG
Early days	Mid-1980s	Twenty first century

Next : Long acting FSH

Future : Oral Gonadotropins ?? (FSH agonist ?)

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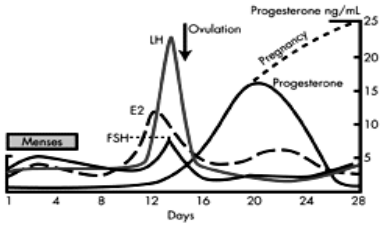
COH
interferes with the balance of forces within the ovary and overridens their endogenous pattern of control



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Hormonal Control of Folliculogenesis 5

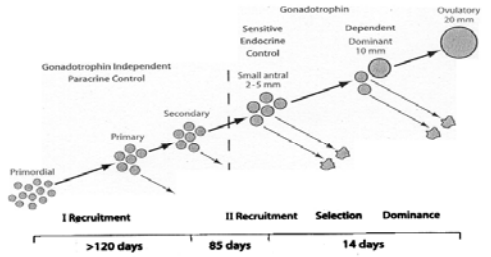

Fig. 1. Folliculogenesis in women. The *dashed line* indicates the time of antrum formation (0.1–0.2 mm diameter)

Baird and Mitchell, 2002

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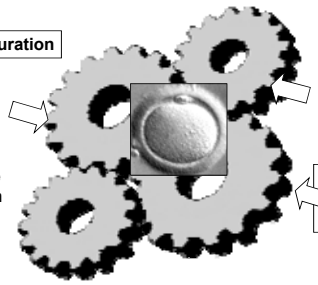
Oocyte competence



Meiotic maturation

Cytoplasmatic maturation

Membrane maturation

Epigenetic maturation

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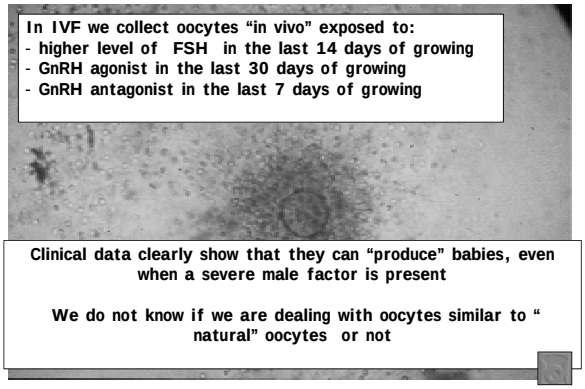

Clinical data clearly show that they can "produce" babies, even when a severe male factor is present

We do not know if we are dealing with oocytes similar to " natural" oocytes or not

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Several questions are still open :

- Are oocytes matured by COH similar to "natural" oocytes ?

No data available on control (" natural" ovulatory oocytes)

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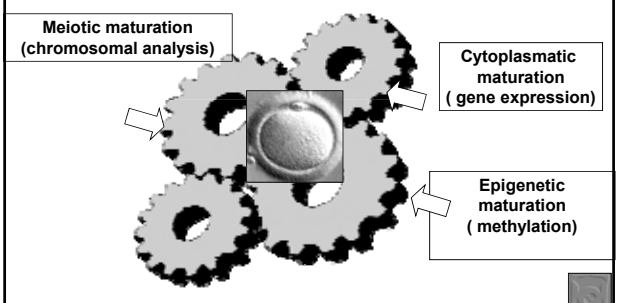
Oocyte competence



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Oocyte Meiotic maturation Aneuploidy incidence by analysis of 6 chromosomes in the First Polar Body

(SISMER – 30.000 observations)

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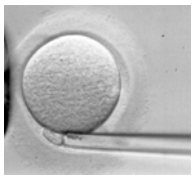
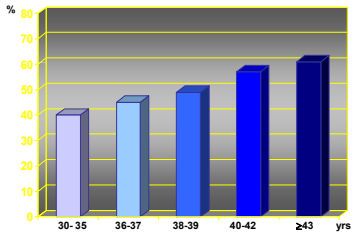
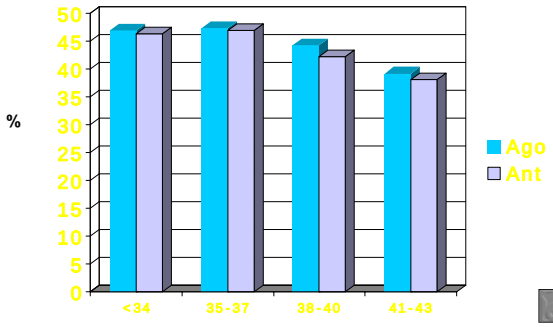




FIRST POLAR BODY ANALYSIS

CHROMOSOMALLY NORMAL OOCYTES ACCORDING TO AGE AND TO PROTOCOL



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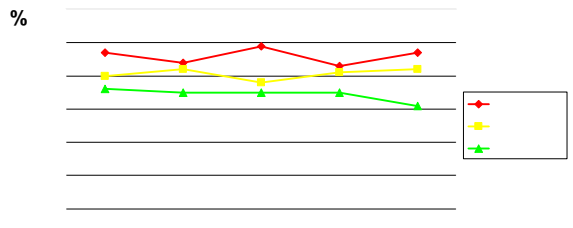
Percentage of euploid oocytes depending on different E2 levels exposure and different amount of FSH administered



E2 at HCG (pg/ml)	< 500	500-1000	1000-1500	1500-2000	> 2000
Tot FSH IU	3550	3300	3100	2500	2300
Eggs/PU	4.2	6.2	7.7	9.2	10.8

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Aneuploidies in human oocytes (Full karyotyping)

Not only chromosomes 13, 18, 21, X and Y are involved but also chromosomes 1, 4, 22, 6, 16 ***(Fragouli et al., 2006)***

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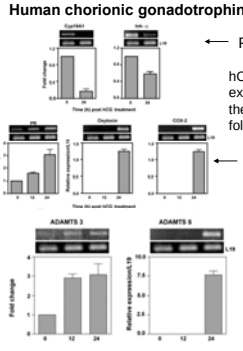
By the use of gene expression analysis it could be possible to demonstrate the molecular basis of hormones action

Human chorionic gonadotrophin (hCG) treatment effect on follicles (bovine)



← Preovulatory follicle marker

hCG treatment induce ovulation, demonstrated also by the expression of ovulatory markers (cox-2, PR, oxytocin) and the decrease of gene specific to pre-ovulatory bovine follicles (Cyp19A1, Inhα)

← Ovulatory Marker

Treatment with hCG induce the expression of genes involved in extracellular matrix remodelling, a process that occurs during cumulus expansion and follicular rupture leading to ovulation

(Jyotsna and Medhamurthy 2008)

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DRUGS USED IN IVF AND GENE EXPRESSION

An effect on gene expression has been found in animals models and in humans after the treatment with different drugs commonly used in IVF practice, and this can be observed at different levels:

Endometrium

Granulosa Cells (GCs)

Oocyte (?)

Even if discordant data are present in the literature, a major effect reported by different papers regards the **apoptotic process**.

It has been shown that the expression of proapoptotic factors like Bax and FasL increase after treatment with GnRH agonist and antagonist in endometrium, whereas antiapoptotic genes like Bcl-2 decrease

A proapoptotic effect of GnRH has also been documented in animals and human cultured GCs, and no differences were found between agonist and antagonist.

The same effect was seen in rats treated with the GnRH analogue leuprolide acetate (LA): the expression of the antiapoptotic factor Bcl-xL was decreased and apoptotic indices were increased in preovulatory ovarian follicles.

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IMPRINTING DEFECTS AND ART

In the past 25 years, the frequency of assisted reproductive technology (ART) births has increased rapidly to account for 1–3% of all births in many developed countries.

ART procedures such as *in vitro fertilization* (IVF) and *intracytoplasmic sperm injection* (ICSI) are generally considered to be safe, but recent studies suggest a small excess of birth defects and low-birth weight in ART children
(Schieve et al., 2004)

In addition, several clinical studies have reported an increased frequency of ART conceptions among children with Beckwith–Wiedemann syndrome or Angelman syndrome caused by an imprinting defects.

(Halliday et al., 2004)

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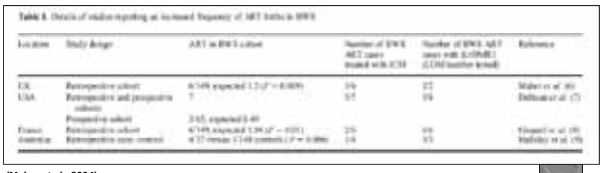
IMPRINTING DEFECTS AND ART

However, of 23 ART-related BWS cases reported in the four recent studies, only 10 have involved ICSI. Thus, *ICSI per se is not the major determinant of the observed association between ART and imprinting disorders.*



(Maher et al., 2004)

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IMPRINTING DEFECTS AND ART

Evidence that male subfertility might increase risk of imprinting defects

↓

Abnormal methylation (*H19* and *MEST*) of imprinted genes in human sperm is associated with moderate or severe oligozoospermia.

(Kobayashi et al., 2007) (Marques et al., 2008)

↓

Global methylation level of sperm DNA can influences only the pregnancy rate in IVF

(Benchaib et., 2005)

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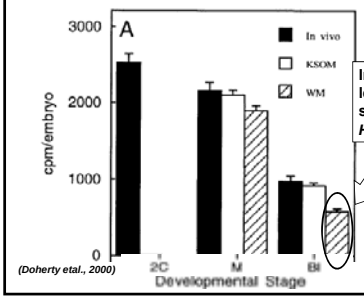
Loss of imprinting of H19 was enhanced by different culture media.



Inability of Whitten's to maintain levels of DNA methylation sufficient to support monoallelic *H19* expression in embryos.

(Doherty etal., 2000)

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IMPRINTING DEFECTS AND ART

The increased risk of an imprinting disorder following ART might be because of an association with superovulation and in vitro embryo culture.

⇩

Use of gonadothropins might be implicated in epigenetic defects increasing risk of imprinting diseases.

(Maher et al., 2003)

⇩

Sato et al., found an abnormal demethylation of *PEG1* and an abnormal gain in the methylation of *H19* in oocytes in some ART-treated infertile women

(Sato et al., 2007)

⇩

Premature release of oocytes that did not complete the imprinting process *(while meiotic maturation is completed)*

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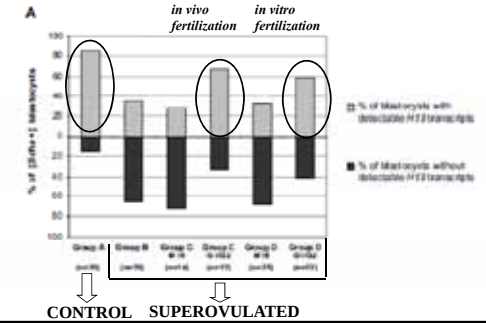
IMPRINTING DEFECTS AND ART

Superovulation and in vitro mice embryo culture on imprinted gene expression: it's possible to minimize the effects of superovulation



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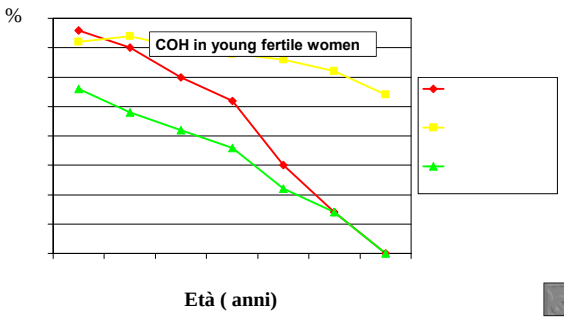
In vivo fecundity vs in vitro implantation rate



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Questions still open :

- Are the oocytes matured by COH similar to "natural" oocytes ?
- **Did the new drugs available during the years improved the oocyte quality ?**
- **Does it exist the " optimal stimulation protocol "**

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Several studies comparing :

- **Agonist vs antagonist protocols**
- **FSH alone vs FSH+LH**
- **Urinary FSH vs recombinant FSH**

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Agreement points

- For safety reasons, there is a general trend to move away from drugs extracted from biological material such urine
- No infection have been associated with urinary gonadotropins used for more than 40 years
- The purification process is able to remove prion proteins
- Rec products are more expensive
- Rec products made production independent by urine collection, guarantees pure preparation and no batch-to-batch variation
- Preparation containing LH activity reduce the amount of FSH needed in down regulation
- Antagonist reduces the amount of gonadotropins needed and avoids estrogens deprivation symptoms

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Several studies comparing :

- Agonist vs antagonist protocols
- FSH alone vs FSH+LH
- Urinary FSH vs recombinant FSH

Controversial results on clinical outcomes

Systematic review and meta-analysis

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Most up-to-date meta-analysis and systematic reviews

- No differences in the ongoing PR per started cycle between rFSH and uFSH (Al-Inany, 2005)
- HMG vs rFSH in long agonist protocol (Coomarasamy, 2008) : higher ongoing PR with HMG
- Antagonist vs agonist
  - Kolibianakis,2006 (all type of protocols) : similar live birth rate
  - Al-Inany,2006 (antagonist vs long agonist) : lower PR with antagonist

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GnRH antagonist may affect endometrial receptivity (Rackow and al. Fertil Steril May 2008)

- **HOXA10 (marker of endometrial receptivity) expression was significantly decreased in endometrial stromal cells in GnRH antagonist cycles compared to GnRH agonist cycles or natural cycles control subjects**
- **Molecular explanation for the lower IR seen clinically with antagonist**

Schachter et al (Ferti Steril October 2008) suggest to administer GnRH agonist before oocyte retrieval in GnRH antagonist cycles to displace antagonist from the endometrial receptors

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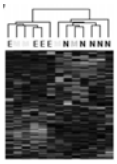
The comparison between natural and gonadotropin stimulated **human peri-implantation endometria** showed a significant difference in the expression patterns of 411 genes among the samples, with the classification of the differentially expressed probes into different functional groups:

Enzyme
Signal transducer
Ion-binding protein
Transporter
Receptor
Transcription factor
Cell adhesion molecules
Regulatory proteins
Other and unknown functions

65%



(Liu et al., 2008)

A high number of genes involved in endometrial receptivity were **aberrantly expressed** in endometria following ovarian stimulation with **GnRH agonist** (342 genes), showing the expression levels to be more similar to those in a **non-receptive endometrium** (Horcajadas et l., 2005)

The endometrial development after **GnRH antagonist mimics** the **natural endometrium** more closely than after GnRH agonist at both the morphological (no relevant differences) and molecular level (only 23 genes dysregulated at high dose) (Mirkin et al., 2004)

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Effect of different preparations used for COH

Differences in gene expression of granulosa cell between rFSH and HMG (Grondahl et al, Fert Steril,2008) :
- LH/HCG receptor gene and genes involved in biosynthesis of cholesterol and steroids lower expressed in HMG
- S100-calcium-binding-protein –P (anti-apoptosis protein) higher expressed in HMG

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We are at the beginning of a new "era"

Going deeply into the (molecular) effects of COH

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Questions still open :

- Are the oocytes matured by COH similar to "natural" oocytes ?
- Did the new drugs available improved oocyte quality ?
- Does it exist the " optimal stimulation protocol "?

Do exist "optimal patients "

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Optimal patients

Normal responders

(SISMER 1999-2003)

Stimulation protocol	Agon. retard	Agon. daily	No anal.	Antag.	uFSH	rFSH
N° cycles	644	140	70	278	376	416
PR	34.5%	35%	36%	36%	33%	36%
IR	25%	24%	26%	26%	24%	26%

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"Exogeneous LH in COH for ART "
A.P.Ferraretti et al
Fertil.Steril.,82, 200

- **Patient's own response to FSH is the best biological index to identify the sub-group of women who can benefit from LH addition.**
- **Those women cannot be previously identified according to serum LH**
- **Whenever, during stimulation, increasing dosage of FSH is needed to continue and complete the growth of the recruited follicles, exogenous LH should be added as an" emergency drug " to get more competent oocytes.**

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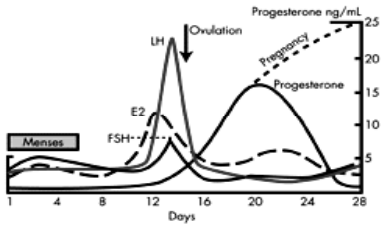
COH
interferes with the balance of forces within the ovary and overridens their endogenous pattern of control



Optimal patients : Women able to adjust the interference of COH, establishing a new equilibrium and, consequently, able to conceive

Potential "optimal" patients

- **No previous ART cycles**
- **Normovulatory patient**
- **Age ≤ 38 years**
- **BMI < 25**

New trend : Mild IVF strategy to reduce time, costs, discomfort and complications

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S.I.S.Me.R prospective study

Conventional IVF vs Lite IVF

- to plan cycle by cycle using standard GnRH analogues protocols, conventional monitoring , cryopreservation and thawing of surplus oocytes;

-to plan since the beginning three cycle with a fixed mild stimulation (clomiphene citrate 100 mg from day 3 to day 7, 150 IU of FSH on day 5,7 and 9), few monitoring from day 8 to start antagonist
- no oocyte cryopreservation. .

Primary end point :
Cumulative ongoing PR over a given period of time

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Results in 12 months	Conventional	Lite IVF
1st cycle	99	25
Fresh ongoing pregnancy	27 (27%)	7 (28%)
Thawed ongoing pregnancies	4	-
2nd or 3rd cycle	10	13
Fresh ongoing pregnancies	3 (30%)	6 (46%)
Thawed ongoing pregnancies	1	-
Cumulative OPR/ patient in one years	35% (35/99)	52% (13/25)

Positive effect on oocyte quality and endometrial receptivity ?

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Difficult patients

Old patients

Young poor responders

PCOS

Obese women

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Age group 38-43 years (342 cycles)
Agonist down regulation (half dose vs conventional dose) and Antagonist protocols

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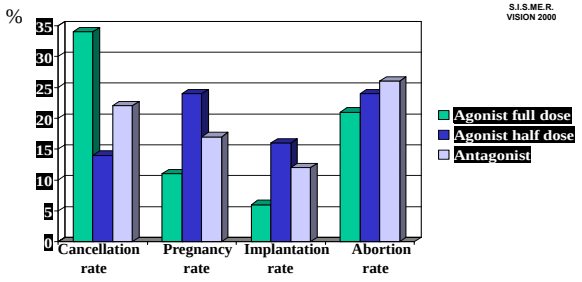

Agonist half dose vs Antagonist : less FSH IU and more oocytes

Different Protocols used in young Poor Responders (≤ 40 years) (Cumulative data 1995-2003)

Protocol	Cc + FSH	uFSH	rFSH	rFSH+ Antagonist	Agonist Low dose + rFSH	Total
N° cycles	109	151	162	267	93	782
N° of cancelled cycles	40(37%)	70(46%)	80(49%)	77 (29%)*	29 (32%)*	296(38%)
N° of oocytes / pu	3.1± 2	3.9± 2	35.1± 3	5.3 ± 3.4	5.9 ± 4.2	
N° of transfers	53	62	45	119	47	326
N° of clinic pregnancy	6 (11%)	9 (15%)	11(24%)	38 (32%)*	10 (21%)	74(23%)
N° of abortions	1 (16%)	1 (11%)	1 (9%)	12 (31%)■	1 (10%)	16(22%)
Implantation rate	7%	9%	13%	19%*	12%	
LBR / pu	7%	10%	12%	14%	15%	12%
LBR / started cycles	5%	5%	7%	10%	10%	7%

* More efficient
■ Less efficient

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Poor responders

(≤ 40 years - Antagonist or Agonist low dose protocols)

	rFSH	rFSH+rLH
N° of cycles	40	40
Cancelled cycles	12 (30%)	13 (33%)
Oocytes/pu	3.8± 1.6	3.7 ± 2.5
N° of tranfers	20	19
N° of term pregnancies	6 (26%)	1 (5%)
LBR/started cycle	13%	1.5%

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New protocol for Poor responders

- Down-regulation with daily low dose GnRH agonist ,*pre-treatment with rLH* before FSH stimulation……

- … to render the cohort of follicles more sensitive to FSH

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Results in 27 patients with repeated poor response in previous 92 cycles

	Previous cycles	New protocol (LH pre-treatment)
Started cycles	92	29
Cycles cancelled	49 (53%)	6 (21%)
Eggs retrievals (mean eggs)	43 (2.4±1)	23 (2.7±2)
Fertilization rate	67%	89%
Cleavage rate	58%	90%
Grade 1 embryos	63%	92%
Transferred cycles (mean embryos transferred)	38(1.6± 0.9)	20 (1.8 ±0.8)
Clinical pregnancies (PR/ cycle)	1 (1%)	9 (31%)
Implantation rate		27%
Live birth rate/ patient	0	30%

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New protocol for Poor responders

- Down-regulation with daily low dose GnRH agonist ,*pre-treatment with rLH* before FSH stimulation……

• … can LH pretreatment correct some ovarian defects present in these patients ?

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PCOS patients undergoing ART vs Control

	Control (tubal infertility)	PCOS with adequate response	PCOS with poor performance
No. of patients	502	32	24
No. of cycles	873	47	34
No. of cancelled cycles	137(15%)	0	21 (56%)
No of oocytes/retrieval	11.7 ± 7	13.8 ± 5	11.3 ± 4
Fertilization rate	73%	56%	48%
Cleavage rate	78%	83%	52%
No of transferred cycles	537*	44	11
No of pregnancies (%)	226(42)	18 (41)	0
Implantation rate	26%	24%	/
Abortion rate	11%	14%	/

* Note: in 169 cycles all the zygotes were cryopreserved for OHSS risk

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TVOD

Transvaginal Ovarian Drilling

Ferraretti et al Fertility and Sterility 2001;76:812

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Results (24 PCOS)

	Before TVOD	*After TVOD*
No of cycles	34	30
No cancelled*	21	5
No of eggs	11.3± 4	13.2 ± 3
Fertilization rate *	48%	70%
Cleavage rate*	52%	73%
Fresh transfers	8	21
Thawed transfers	3	10
Clin.pregnancies	0	15
Delivery rate/cycle*	0	47%
Delivery rate/patient*	0	64%

* $p < 0.05$

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Conclusions

- Hormonal stimulation is crucial in ART but several questions are still open after 30 years
- Do we need embryo selection because COH brings to maturation several non-competent oocytes ?
- At the end, it takes one egg to produce a baby …..
- … can we select it ?

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