How to improve ART outcomes?

X.TURKISH-GERMAN GYNECOLOGY CONGRESS
ANTALYA,TURKEY/2014

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For better outcome?

- **Age (<37)**
- **First cycle**
- **High quality embryo at previous cycle**
- **Normal uterine cavity and absence of hydrosalphinx**
- **Absence of endometriosis**
- **Absence of endometrial factor**
- **Absence of abnormal gamet morphology**
- **High quality embryo for freezing**

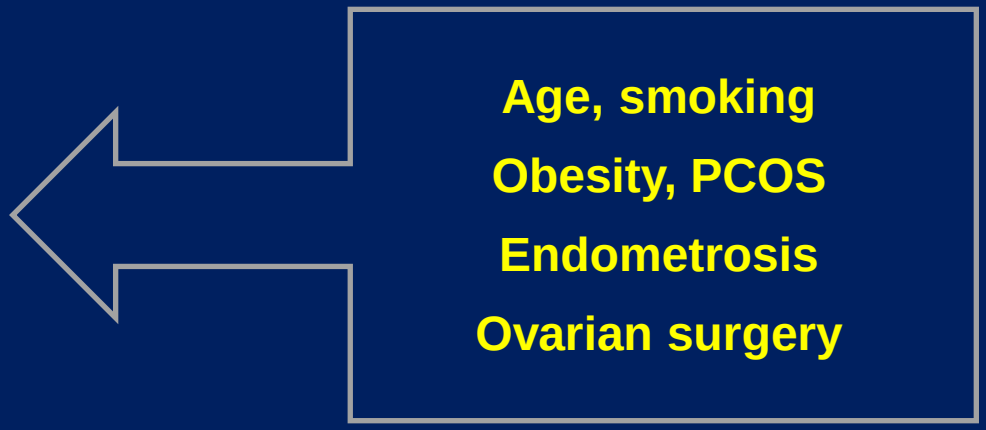
How to improve ART outcomes?

- Ovarian reserve evaluation
- Immunological screening
- A ‘must’ before ART?

AMH
AFC
FSH,E2

H/S
L/S
Myomectomy?
Polypectomy?
HSG?

- Oocyte factor

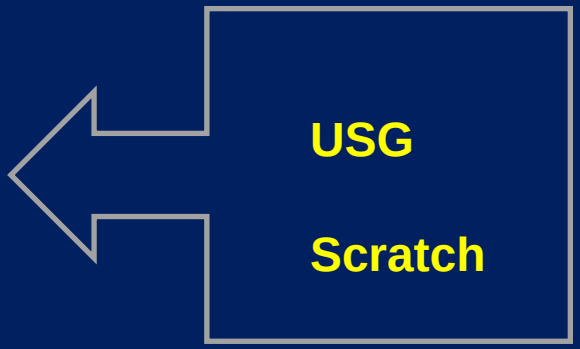


- Adjuvant therapies

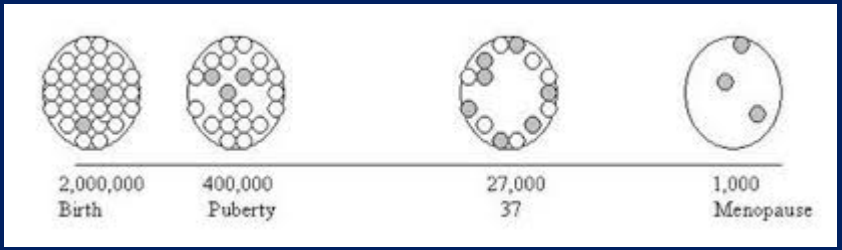
- Outcome measurements

- Embryo transfer

- Endometrial factor



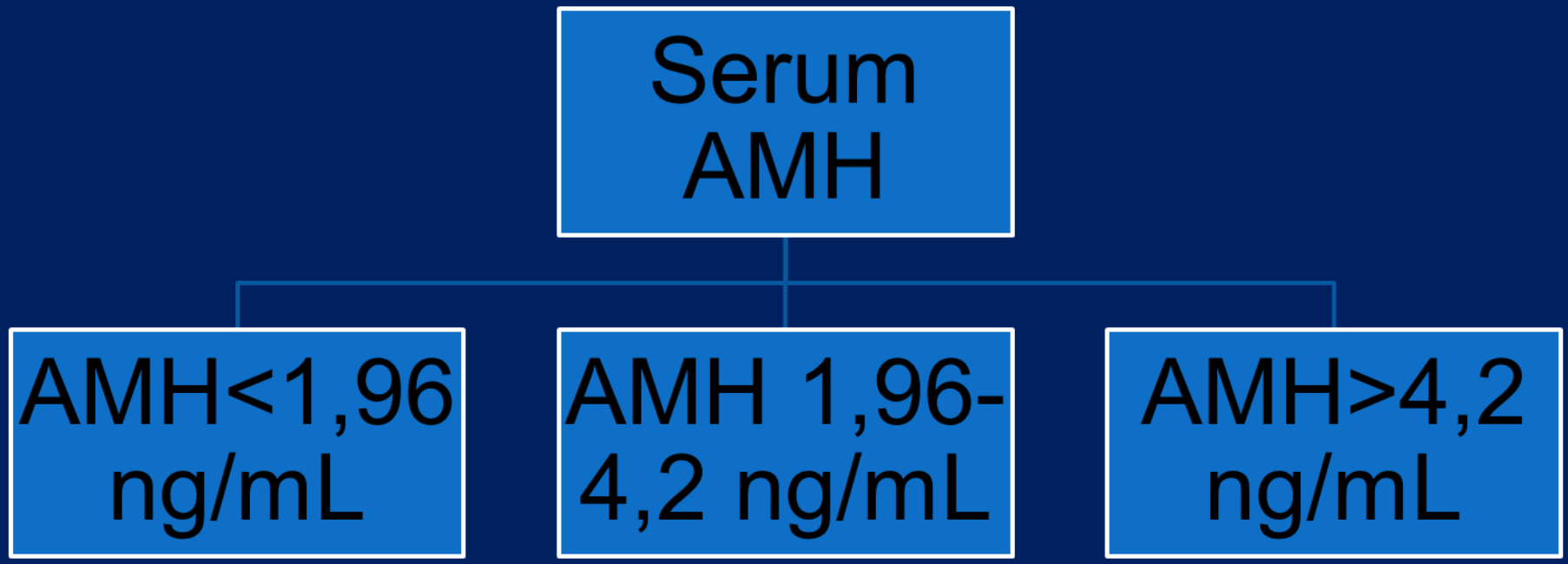
OVARIAN RESERVE EVALUATION



Ovarian reserve: AMH, D3 FSH, E2 inhibin B

AMH< 1,96ng/mL → (<4 oocytes) →300IU/day

AMH> 4,2 ng/mL →150 IU→100-125 IU/day



- AMH<0,01 ng/mL

Poor reserve

- FSH <15 IU/L

High dose gonadotropin

Gonadotropin dosage should be decided according to age

- High OHSS risk
- low FSH dose (125 IU/gün)
- GnRH ant. protocol
- Agonist trigger

Antral Follicle Count

- AFC <4 → cycle cancellation 37x
- 3D and 2D USG same but AFC is less effective for predicting ART outcome.
- AMH and AFC have similar efficacy for predicting pregnancy outcome.

Gibreel A, Hum Fertil 2009

Ovarian Reserve Tests (ORT)

- AMH, is better than D3 FSH, estradiol and inhibin B for predicting ART outcome.

Seifer DB, Fertil Steril, 2002
Hazout A, Fertil Steril, 2004
Muttukrishna S, BJOG, 2004
Penarrubia J, Hum Reprod, 2005
Tremellen KP, Obstet Gynecol, 2005

- For predicting high response AMH >FSH, AFC
- For predicting poor response AMH=AFC>FSH

Nardo LG, Fertil Steril,2009

IMMUNOLOGICAL SCREENING

Immunologic screening Before ART

Autoantibodies	Frequency in infertile women	Correlation with Infertility	Other
Antiphospholipid		-	Habituel abortion
Antithyroid	Minimally 	-	Tiroiditis, abortion
Antigliadin	Minimally 	-	Celiac disease
Antisperm	No effect	-	Fertilisation failure
Antinuclear	Minimally 	-	Otoimmune disease
Antiovarian	Minimally 	-	Otoimmune disease

Immunologic screening Before ART

- *APA screening is not necessary before ART!*

ASRM

→ APA(+) is not associated with poor outcome.

Buckingham KL, J Reprod Immunol 2009

→ Anticoagulant therapy does not change outcome of APA(+) patients.

ASRM, 2008

- *Routine tyroid autoantibody* screening is not suggested.
- If habituel abortion history is positive; autoantibody evaluation may be valuable

Immunological screening Before ART

Which patients should be screened?

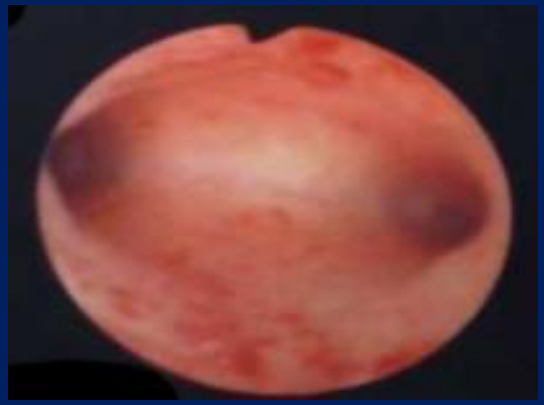
- History of venous thromboembolism
- Presence of high risk trombophilia(+) at 1. degree relatives
- Venous thromboembolism (age of <50 (+))

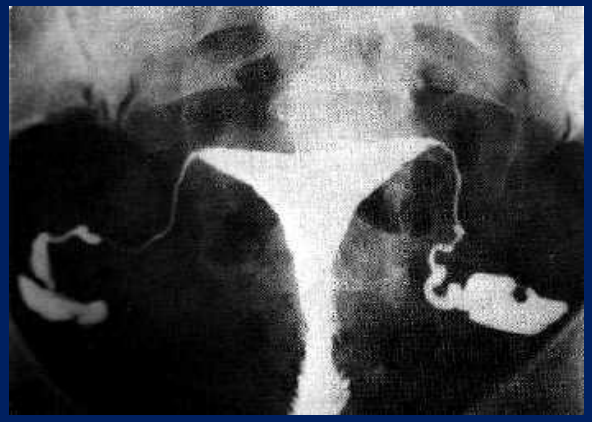
Tyroid Functions? Antibodies ? Before ART

Thyroid Function Tests:

- Anovulatuar and idiopatic infertile patients 5-6%
- Tubal and male factor (+) 2%

TSH, sT4 must be evaluated before ART





BEFORE ART;

H/S?

L/S?

Myomectomy?

Polypectomy?

HSG?

Is hysteroscopy routine before ART?

Not suggested !

- No signs
- For uterine cavity evaluation US/HSG/H/S
- Age <35, male factor (+), family history (-)

Hysteroscopy suggested !

1.Age >35 , abnormal uterine bleeding,
abnormal clinical symptoms
2. 2 IVF failure
3. Sign + (Intrauterine polyp, submucosal leiomyoma, uterine septum, Ashermans syndrome…)

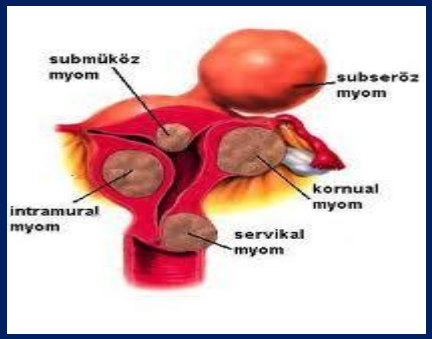
Is laparoscopy routine before ART?

If the etiology of the infertility is clear it is unnecessary

Laparoscopy is suggested;

1. **Presence of pelvic inflamatory disease**
2. **Presence of hydrosalpinx, endometriosis or endometrioma**

Myomectomy before ART



- Intramural & >4 cm distorting cavity/degenerated:::::::::Myomectomy
- Intramural & >5cm not distorting cavity::::::::::::::::::?
- Subserosal & >5cm ::?
- Submucosal & >1cm Myoma / Polyp ::::::::::::::::::::::::::Myomectomy

IM Leiomyoma: does not change pregnancy rates

SM leiomyoma: increases pregnancy rates but same abortion rates

Pritts et al, Fertil Steril, 2009

Fibroids and infertility: an updated systematic review of the evidence

Elizabeth A. Pritts, M.D.,[a] *William H. Parker, M.D.,*[b] *and David L. Olive, M.D.*[a]

[a] Wisconsin Fertility Institute, Middleton, Wisconsin; and [b] Department of Obstetrics and Gynecology, University of California, Los Angeles, California

TABLE 6

Effect of myomectomy on fertility: submucosal fibroids.

Outcome	Number of studies/ substudies	Relative risk	95% confidence interval	Significance
A. Controls: fibroids in situ (no myomectomy)				
Clinical pregnancy rate	2	2.034	1.081–3.826	*P*=.028
Implantation rate	0	—	—	—
Ongoing pregnancy/live birth rate	1	2.654	0.920–7.658	Not significant
Spontaneous abortion rate	1	0.771	0.359–1.658	Not significant
Preterm delivery rate	0	—	—	—
B. Controls: infertile women with no fibroids				
Clinical pregnancy rate	2	1.545	0.998–2.391	Not significant
Implantation rate	2	1.116	0.906–1.373	Not significant
Ongoing pregnancy/live birth rate	3	1.128	0.959–1.326	Not significant
Spontaneous abortion rate	2	1.241	0.475–3.242	Not significant
Preterm delivery rate	0	—	—	—

Pritts. Fibroids and infertility. Fertil Steril 2009.

Myomectomy before ART

- Postoperative synechia with monopolar cautery % 35–45 , bipolar cautery %7.5

Touboul C.Fertil Steril 2009

- Second look H/S is suggested after 6-8 weeks after prosedure (%10 synechia +)

Capmas M, Curr Opinion,2013

Polypectomy before ART

- Polyp:
  - Abnormal implantation
  - Irreguler bleeding
  - Inhibition of sperm transport
  - Inflammatory process
  - Glycodelin secretion
- → Excision (H/S)
- RCT: H/S polypectomy improves pregnacy outcomes when compered with biopsy.(%63 vs %28)

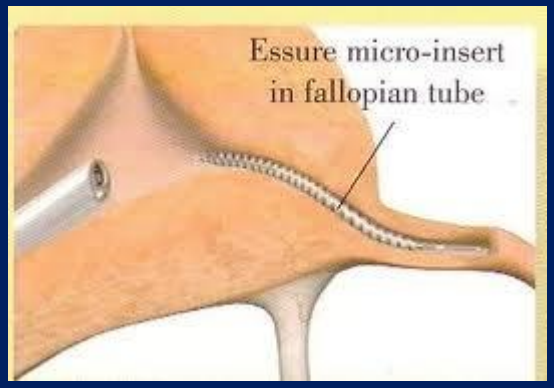
Perez-Medina T et al. Hum Reprod 2005

Hydrosalpinx before ART?

- *Hydrosalpinx :* Decreased implantation rates.
- Decreases IVF success rates up to 50% :
  - Mecanical or toxic effect
  - ‘Wash-out’ effect to the embryo

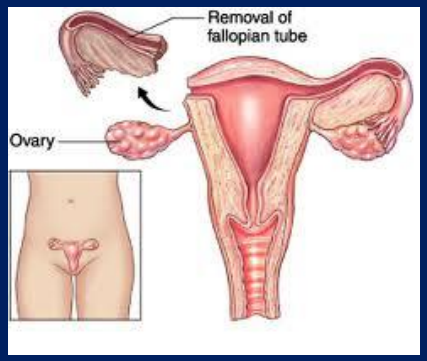
Hydrosalpinx therapy

1. Reconstructive surgery
2. Medical therapy (doxicycline)
3. Salphingectomy
4. Proximal Tubal Occlusion
   - Clips
   - Electrocautery
   - Hysteroscopy : *Essure*, Adiana, Ovabloc
5. Salphingostomy
6. Aspiration



Johnson N et al. Cochrane Database Syst Rev 2010

Hydrosalpinx Therapy



- Laparoscopic salphingectomy is suggested.
- Laparoscopy eases OPU and lessens possible complications

like abscess, torsion.

Johnson N et al. Cochrane Database Syst Rev 2010

Human Reproduction vol.14 no.5 pp.1243–1249, 1999

Pregnancy rates after in-vitro fertilization in cases of tubal infertility with and without hydrosalpinx: a meta-analysis of published comparative studies

Table II. Pregnancy rates

First author and year of publication	Hydrosalpinx group No. (%)	Group without hydrosalpinx No. (%)	Odds ratio (95% CI)
Andersen[a] (1994)	9/91 (9.8)	224/744 (30.1)	0.25 (0.13–0.52)[b]
Strandell (1994)	14/121 (11.57)	89/367 (24.25)	0.41 (0.22–0.75)[b]
Sims (1993)	43/234 (18.37)	341/1287 (26.49)	0.62 (0.44–0.89)[b]
Blazar[a] (1995)	39/161 (24.22)	116/385 (30.13)	0.74 (0.44–1.13)
Van Dromme (1995)	7/69 (10.14)	14/61 (22.95)	0.38 (0.14–1.01)
Sharara (1996)	27/103 (26.21)	30/89 (33.70)	0.70 (0.38–1.30)
Akman (1996)	1/14(7.1)	24/98 (24.5)	0.24 (0.03–1.91)
Murray (1996)	8/45 (17.77)	57/141 (40.42)	0.32 (0.14–0.73)[b]
Katz (1996)	16/95 (16.84)	467/1268 (36.82)	0.35 (0.20–0.60)[b]
Fleming (1996)	18/77 (23.37)	63/212 (29.71)	0.72 (0.39–1.32)
Wainer[a] (1997)	49/267 (18.35)	199/867 (22.95)	0.75 (0.53–1.07)
Barmat[a] (1997)	42/106 (39.62)	502/1150 (43.65)	0.85 (0.56–1.27)
Ng[a] (1997)	9/41 (21.95)	11/92 (11.96)	2.07 (0.78–5.47)
De Witt[a] (1997)	41/224 (18.3)	66/326 (20.25)	0.88 (0.57–1.36)
Total	323/1642 (19.67%)	2203/7061 (31.2%)	0.64 (0.56–0.74)[b]

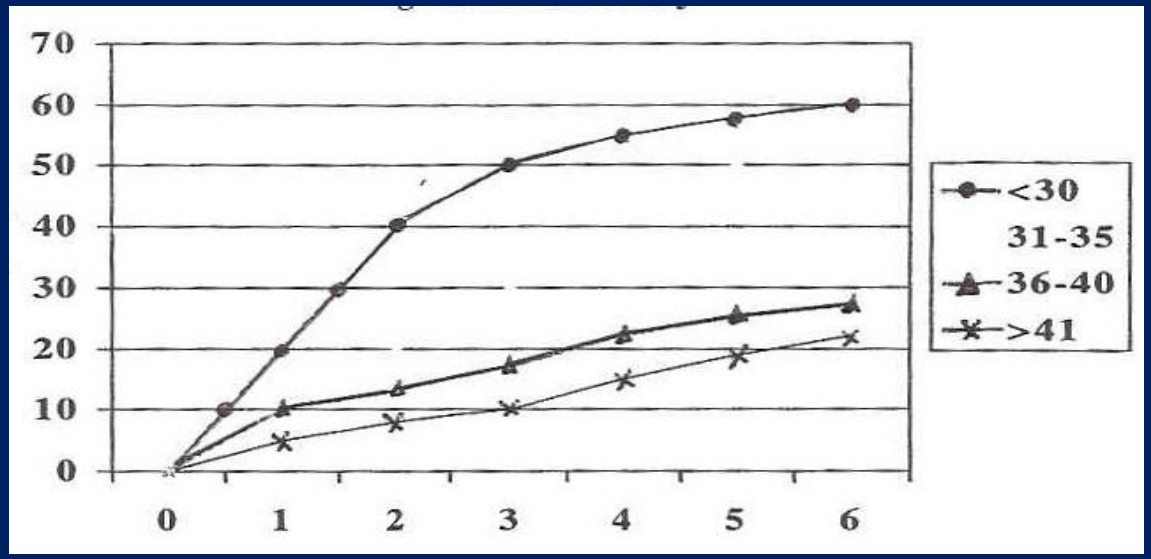
[a]Excluding biochemical pregnancies.
[b]Odds ratio significantly different from 1 ($P < 0.05$).
χ^2-test for heterogeneity (with 13 df) = 29.2 ($P < 0.05$).
CI = confidence interval.

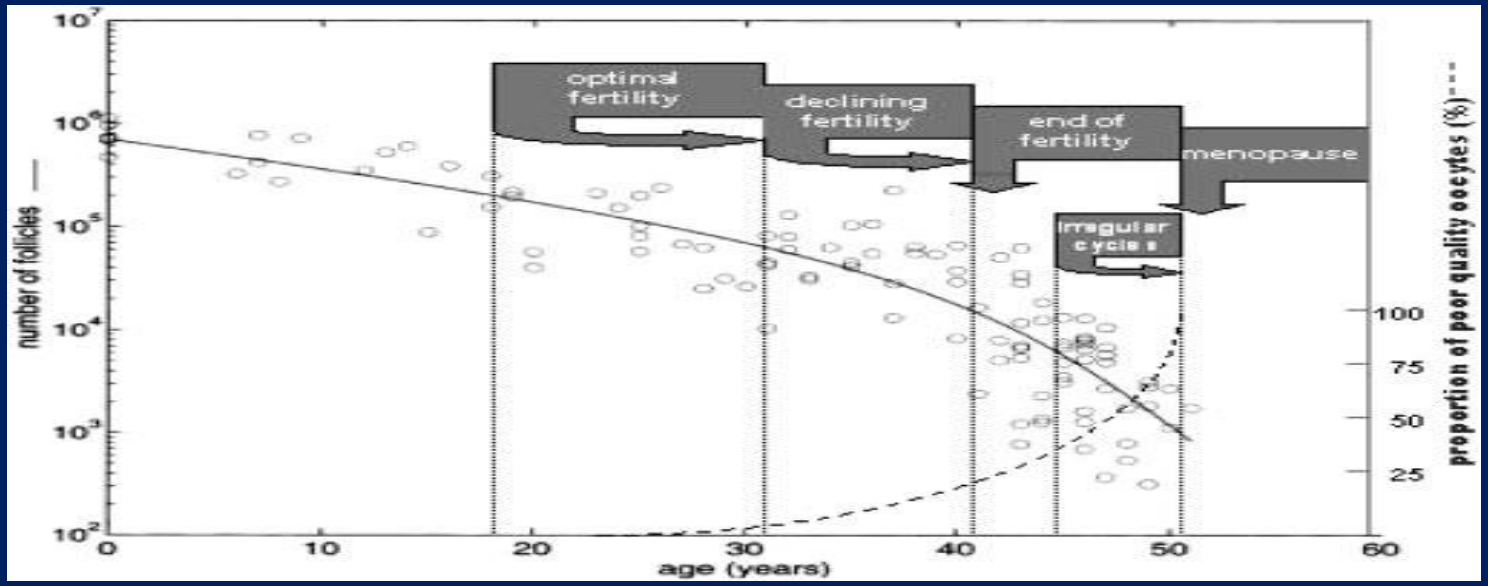
E.Camus[1,4], C.Poncelet[1], F.Goffinet[3], B.Wainer[1], F.Merlet[2], I.Nisand[1] and H.J.Philippe[1]

OOCYTE FACTOR

Age
Smoking
Obesity
PCOS
Endometriosis
Ovarian surgery

Age


Pregnacy %
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
<30
31-35
36-40
>41
Cycle number


optimal fertility
declining fertility
end of fertility
menopause
irregular cycles
number of follicles
proportion of poor quality oocytes (%)
age (years)
0
10
20
30
40
50
60
100
75
50
25

Analysis of 2,386 consecutive cycles of in vitro fertilization or intracytoplasmic sperm injection using autologous oocytes in women aged 40 years and above

Gamal Serour, M.D.,[a,b] *Ragaa Mansour, Ph.D.,*[b] *Ahmed Serour, M.D.,*[a,b] *Mona Aboulghar, M.D.,*[b] *Yahia Amin, M.D.,*[b] *Omnia Kamal, B.S.,*[b] *Hesham Al-Inany, M.D.,*[b] *and Mohamed Aboulghar, M.D.*[b]

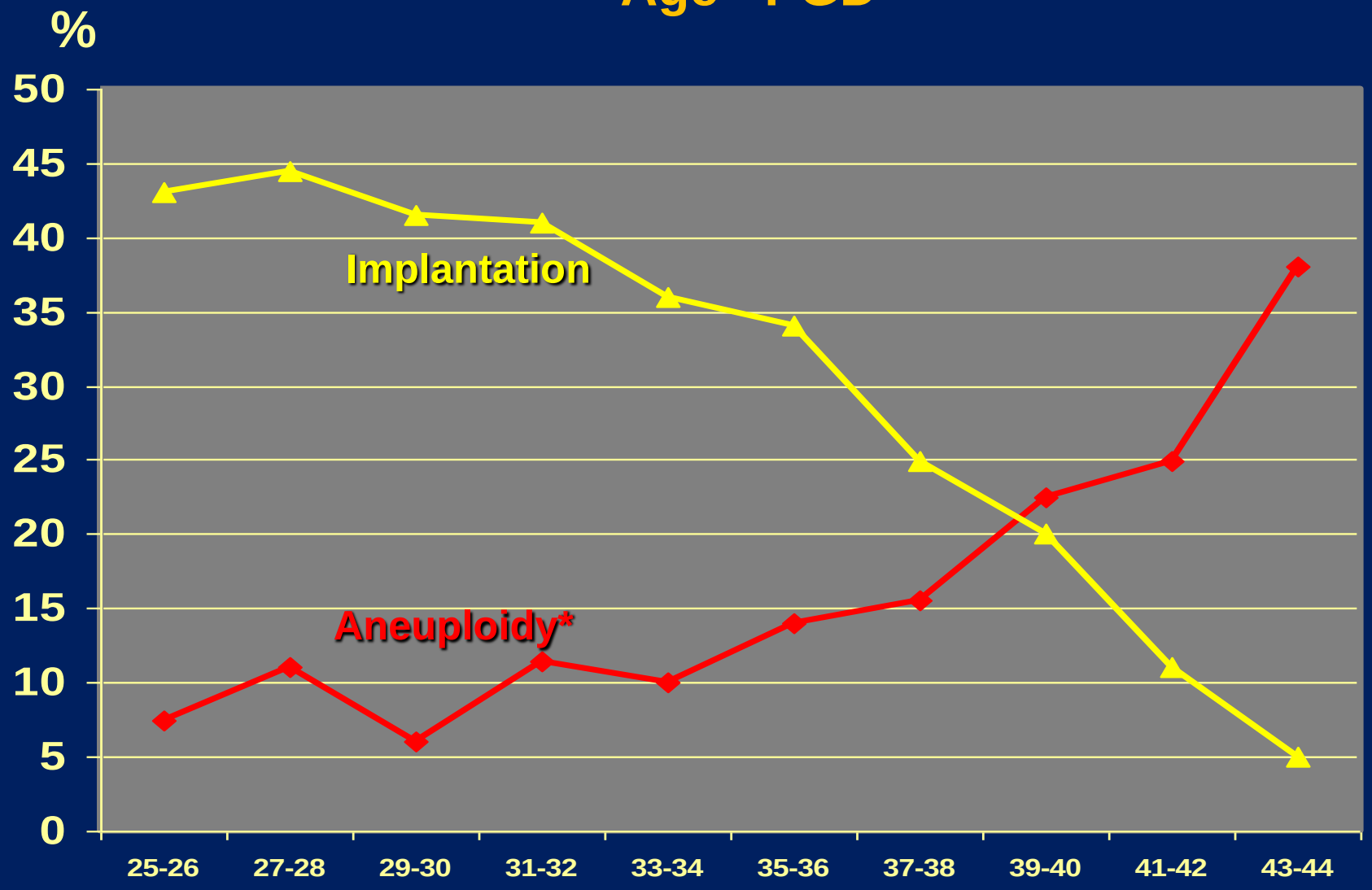
[a] Al Azhar University, and [b] Egyptian IVF and ET Center, Cairo, Egypt

Cycle outcomes based on 1-year age increments for women aged ≥40 in 2386 IVF/ICSI cycles.

	Age (y)						
Outcome	**40**	**41**	**42**	**43**	**44**	**≥45**	**Total**
No. of initiated cycles	742	595	429	251	150	219	2386
Number of pickup cycles	673	536	379	206	111	99	2004
Cancellation rate	9.3%	10%	12%	18%	26%	55%	16%
No. of embryo transfer cycles	601	480	337	178	101	86	1783
Positive β-hCG	190	125	61	32	12	5	425
No. of clinical pregnancies	148	92	55	16	4	3	318
Clinical pregnancy rate per pickup	22.4%	17.2%	14%	7.8%	3.6%	3	17.9
Miscarriage rate	39%	44.4%	51.3%	64.3%	75%	67%	44.8%
No. of deliveries	72	40	20	5	1	1	139
Live birth per initiated cycle	10%	7%	5%	2%	0.7%	0.5%	6.7%
Live birth rate per oocyte pickup	11%	7.5%	5.3%	2.4%	0.9%	0.5%	8%
Live birth per embryo transfer	12%	8.5%	5.9%	2.8%	1%	1.1%	8.8%

Serour. IVF/ICSI cycles in women ≥40 years. Fertil Steril 2010.

Age - PGD



*XY, 13, 15, 16, 18, 21, 22 *(Munne et al, 2003) aneuploidi*

Smoking

Human Reproduction Update, Vol.15, No.1 pp. 31–44, 2009
Advanced Access publication on October 15, 2008 doi:10.1093/humupd/dmn046

human reproduction update

Effects of cigarette smoking upon clinical outcomes of assisted reproduction: a meta-analysis

A.L. Waylen[1,6], M. Metwally[2], G.L. Jones[3], A.J. Wilkinson[4], and W.L. Ledger[5]

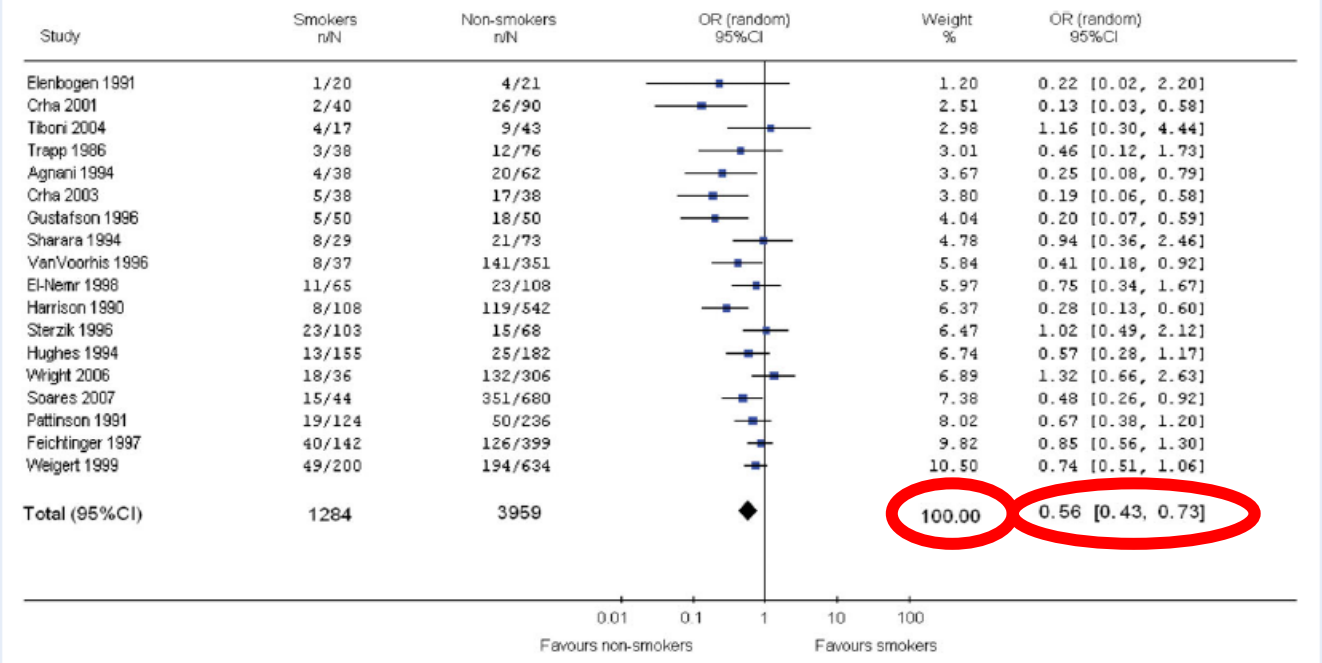


Figure 2 Odds ratio of clinical pregnancy rate per cycle.

Obesity

- Weight ⬆ Fertility ⬇
- High gonadotropin and cost
- Diet, exercise, stress management, habituel education
- 5-7 kg weight loss, decreases insulin resistance, induces spotaneous ovulation and pregnancy.

Clark AM, Hum Peprod,1998

PCOS-COH

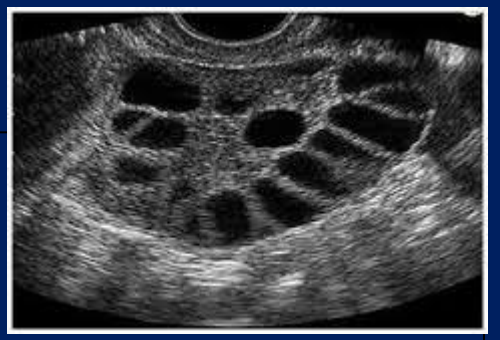
COH PROTOCOLS

- Minimal-mild stimulation
- GnRH agonist
- GnRH antagonist

MODIFIED PROTOCOLS

- GnRH agonist trigger
- Metformin
- IVM
- Embryo freezing

‘PCOS’: Metformin



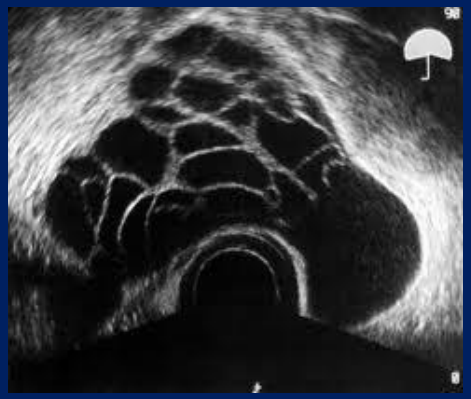
- PCOS; ***Insulin resistance***
- RCT
- Metformin started 16 weeks before cycle
- Dose and duration heterogenous (2x500mg, 3x850mg)

- ***Metformin > placebo***
- ***Increased live birth rates***

Tso LO, Cochrane Database Syst Rev 2009

Metformin and OHSS

- Metformin ↓ hCG day testosteron, free androgen
- hCG day E2 and VEGF OHSS ↓
- Long GnRH agonist protocol OHSS ↓



TSO LO, Cochrane Database Syst Rev 2009

OHSS Prediction

- AMH (sens. %90.5,spes. %81.3)
- AMH cut-off 3,36 ng/mL

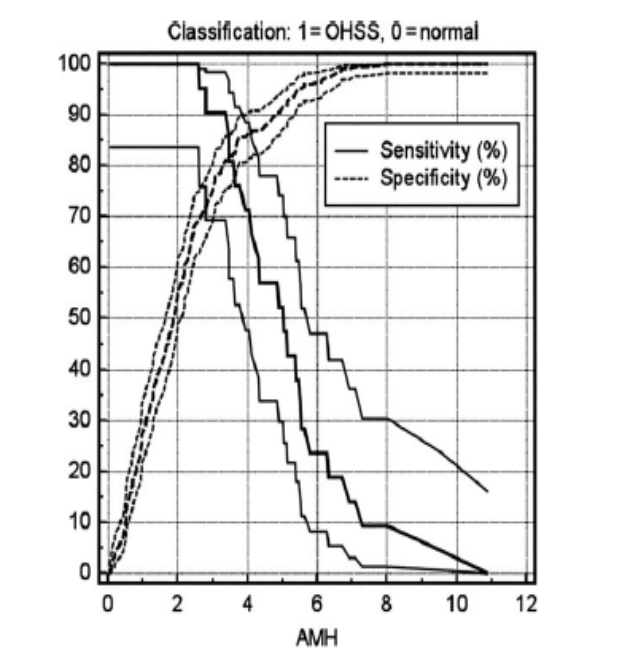


Figure 2: Selection of cut-off value for basal serum AMH to predict OHSS by ROC curve analysis
The selected value was 3.36 ng/ml, with a sensitivity of 90.5% (95% CI 69.6–98.5) and a specificity of 81.3% (95% CI 75.8–86.0).

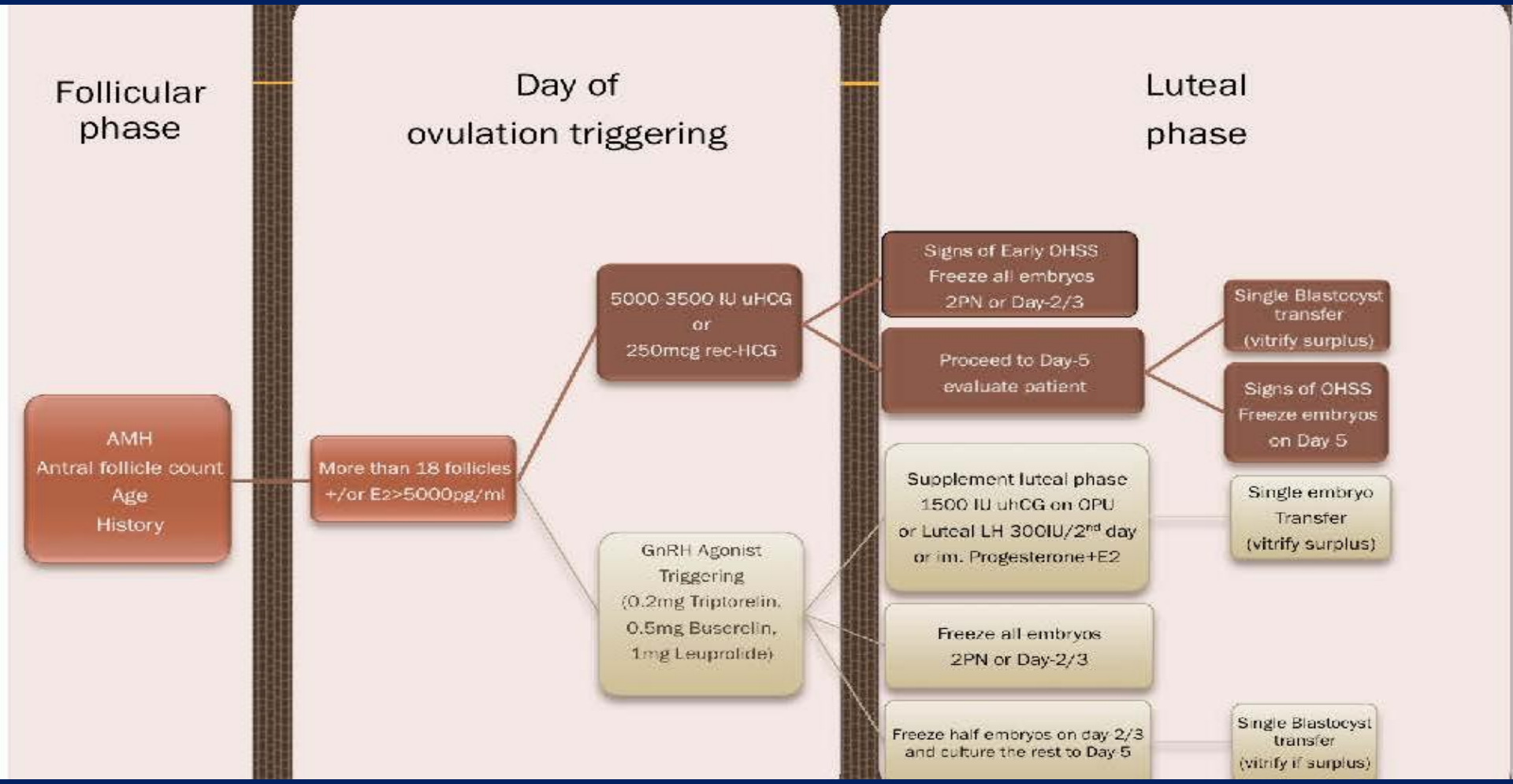
Lee et al,2008

OHSS Prevention

- PCOS and OHSS history
- Mild stimulation
- Antagonist protocols
- Coasting
- Analog trigger
- IV albumin-OPU
- Cryopreservation
- In vitro maturation
- Low dose hCG
- Metformin and dopamine

Aboulghar, Rep Biomed Online, 2009

OHSS Prevention



Papanikolaou EG, Reproductive Biology & Endocrinology, 2011

Endometriosis and ART

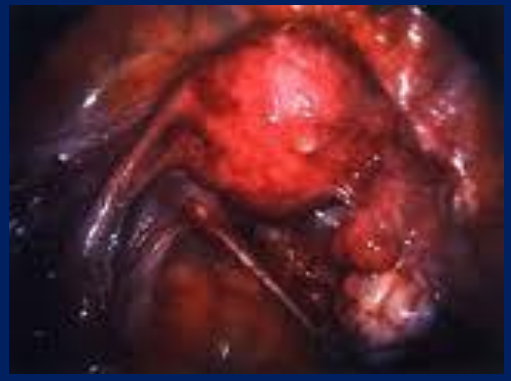
Ultra-long protocol

- GnRH agonist 3-6 months or OCs 6-8 weeks before ART

Surrey ES et al. Fertil Steril 2002
Sallam HN et al. Cochrane Database Syst Rev 2006

De Ziegler et al. Fertil Steril 2010

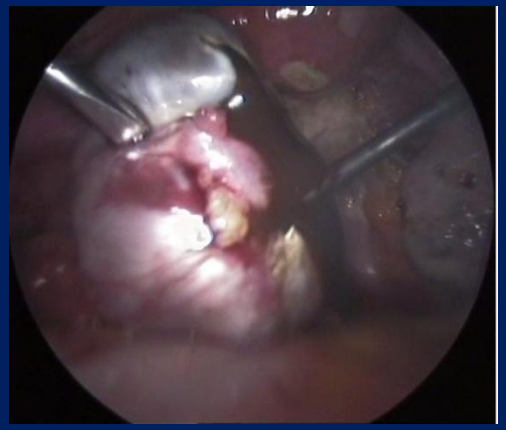
Ongoing pregnancy rates and
MII oocyte and number of embryos



Endometriosis: surgery before ART?

Similar success rates after surgical management

Garcia-Velasco et al. Fertil Steril 2004



- ***Recurrent IVF failure*** → Pregnancy after surgery %72

Littman E et al. Fertil Steril 2005

- **There is no consensus!**
  - **Unilateral vs bilateral**
  - **Size, age, previous surgery**
  - **Surgical technique**
- **>4 cm endometrioma → SURGERY**

ESHRE Consensus

ADJUVANT THERAPIES

DHEA ?

Luteal Phase Support ?

GH ?

DHEA

REVIEW Open Access

The role of androgens in follicle maturation and ovulation induction: friend or foe of infertility treatment?

Norbert Gleicher[1,2,3*], Andrea Weghofer[1,4] and David H Barad[1,2,5]

DHEA increases FSH activity at granulosa cells during preantral and antral period by binding androgen receptors.

Gleicher, Reproductive Biology and Endocrinology, 2011

DHEA

- Meta-analysis/ 3 RCT
- 4-10 weeks 75mg/day DHEA

DHEA and control group;

Spontanous

Microdose flare up FSH 300–450 + hMG 150

Long GnRH protocol rFSH450+rLH150

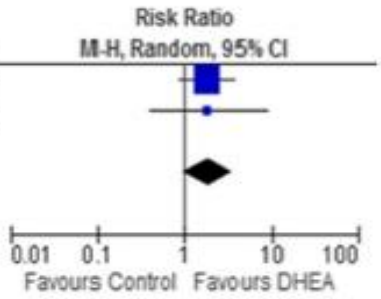
Microdose agonist flare FSH 300-450 + hMG 150

Narkwichean A, Rep Bio & Endocrinol, 2013

A: Clinical Pregnancy Rate

Study or Subgroup	DHEA Events	DHEA Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Barad et al. 2007	13	64	11	101	81.5%	1.87 [0.89, 3.91]	2007
Wiser et al. 2010	4	17	2	16	18.5%	1.88 [0.40, 8.90]	2010
Total (95% CI)		81		117	100.0%	1.87 [0.96, 3.64]	
Total events	17		13				

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.00$, $df = 1$ ($P = 0.99$); $I^2 = 0\%$
Test for overall effect: $Z = 1.83$ ($P = 0.07$)



B: Miscarriage Rate

Study or Subgroup	DHEA Events	DHEA Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Barad et al. 2007	5	25	4	11	85.5%	0.55 [0.18, 1.66]	2007
Wiser et al. 2010	1	17	1	16	14.5%	0.94 [0.06, 13.82]	2010
Total (95% CI)		42		27	100.0%	0.59 [0.21, 1.65]	
Total events	6		5				

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.13$, $df = 1$ ($P = 0.71$); $I^2 = 0\%$
Test for overall effect: $Z = 1.00$ ($P = 0.32$)

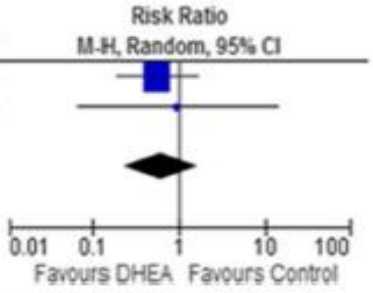


Figure 1 Meta-analysis of clinical pregnancy and miscarriage rates. Meta-analysis of studies of DHEA supplementation versus controls for outcome of **A**) clinical pregnancy rates and **B**) Miscarriage rates in DOR or poor responders undergoing IVF cycle.

200 cycle;
DHEA no significant advantage!

Narkwichean A, Rep Bio & Endocrinol, 2013

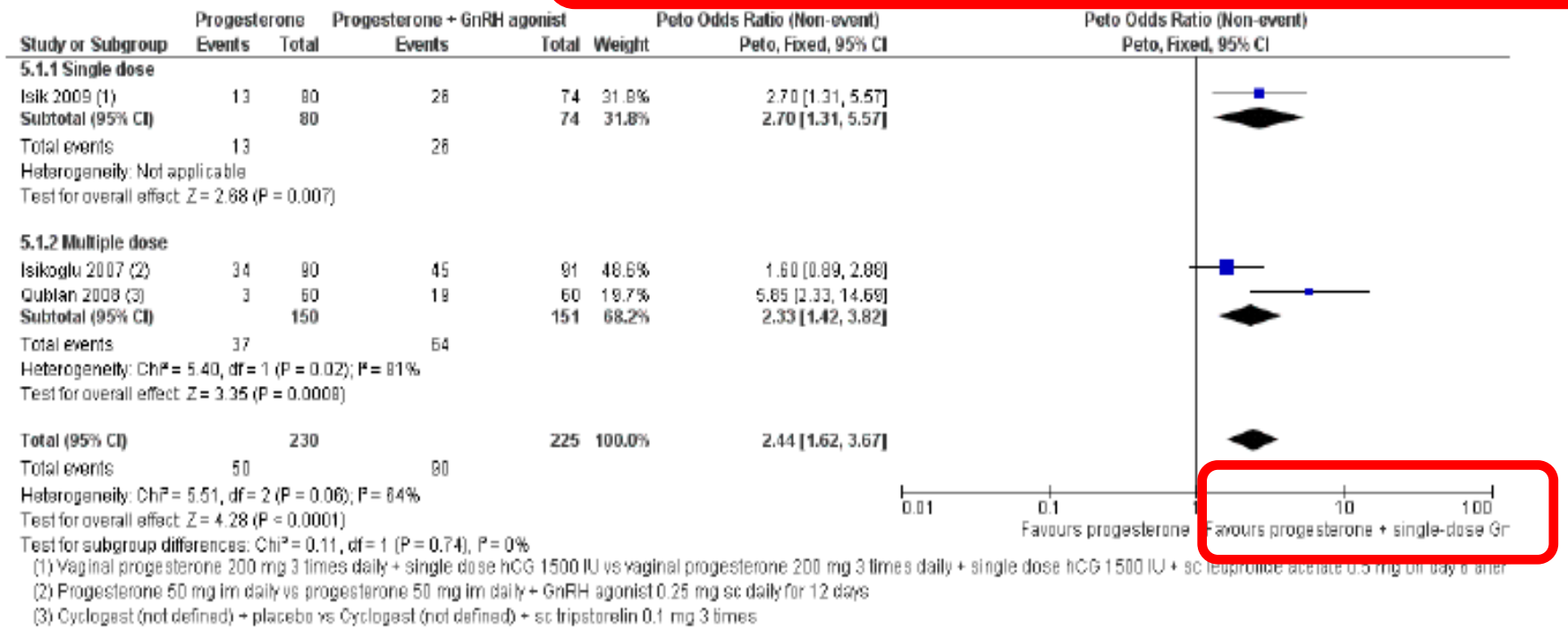
Luteal Phase Support

- Synthetic progesterone is (oral dydrogesterone) more benefical than micronised progesterone.
- Estrogene and hCG do not effect outcomes.
- Route of progesterone administration does not change the results.

Van der Linden M, Cochrane, 2012

Luteal Phase Support

Figure 10. Forest plot of comparison 5 Progesterone versus progesterone + GnRH agonist, outcome: 5.1 Live Birth Rate.



GnRHa for luteal phase support, positively effects the outcomes.

Van der Linden M, Cochrane, 2012

GH

Growth hormone

- Insulin like growth factor-1 ↑
- FSH effect ↑
- Oocyte maturation ↑

Kucuk T, J Assist Reprod Genet ,2008
Bachelot A, Endocrinology, 2002

- Embryo quality and implantation rates ↑

Mendoza C,Human Reprod,2002
Mendoza C,Human Reprod,1999

GH

- Meta-analysis;

For Poor responders;

GH addition increases clinical pregnancy and live birth rates!

Kolibianakis, Hum Rep, 2009

Growth Hormone (GH) ?

- *Growth hormone ;*
- GH- Poor responder ; positive effects

Kyrou D, Fertil Steril 2009

- GH- Normo responder ; not effective!

Duffy JMN et al. Cochrane Rev

GH

Variables	GH/HMG/GnRHant group (group I) $n = 40$	HMG/GnRHant group (group II) $n = 42$	p value
Fertilization rate (%)	65 %	69 %	0.638
Implantation rate (%)	7.2 %	6.6 %	0.240
Chemical pregnancy rate/cycle, *n/n* (%)	6/40 (15 %)	6/42 (14.3 %)	1.000
Clinical pregnancy rate/cycle, *n/n* (%)	5/40 (12.5 %)	5/42 (11.2 %)	1.000
Chemical pregnancy rate/transfer, *n/n* (%)	6/33 (18.2 %)	6/33 (18.2 %)	1.000
Clinical pregnancy rate/transfer, *n/n* (%)	5/33 (16.2 %)	5/33 (15.2 %)	1.000
Abortion rate, *n/n* (%)	1/6 (16.7 %)	1/6 (16.7 %)	1.000

Not effective within antagonist protocol!

Eftekhar M, Arch Gynecol Obstet, 2013

CYCLE SUCCESS

Progesterone
Estradiol

Progesterone levels

- Meta-analysis/ 6 RCT
- Late follicular phase P levels are low?

- Age of <39 , IVF cycle (n=1866)
- **rFSH +GnRH ant** protocol
- P : **1.5ng/ml**

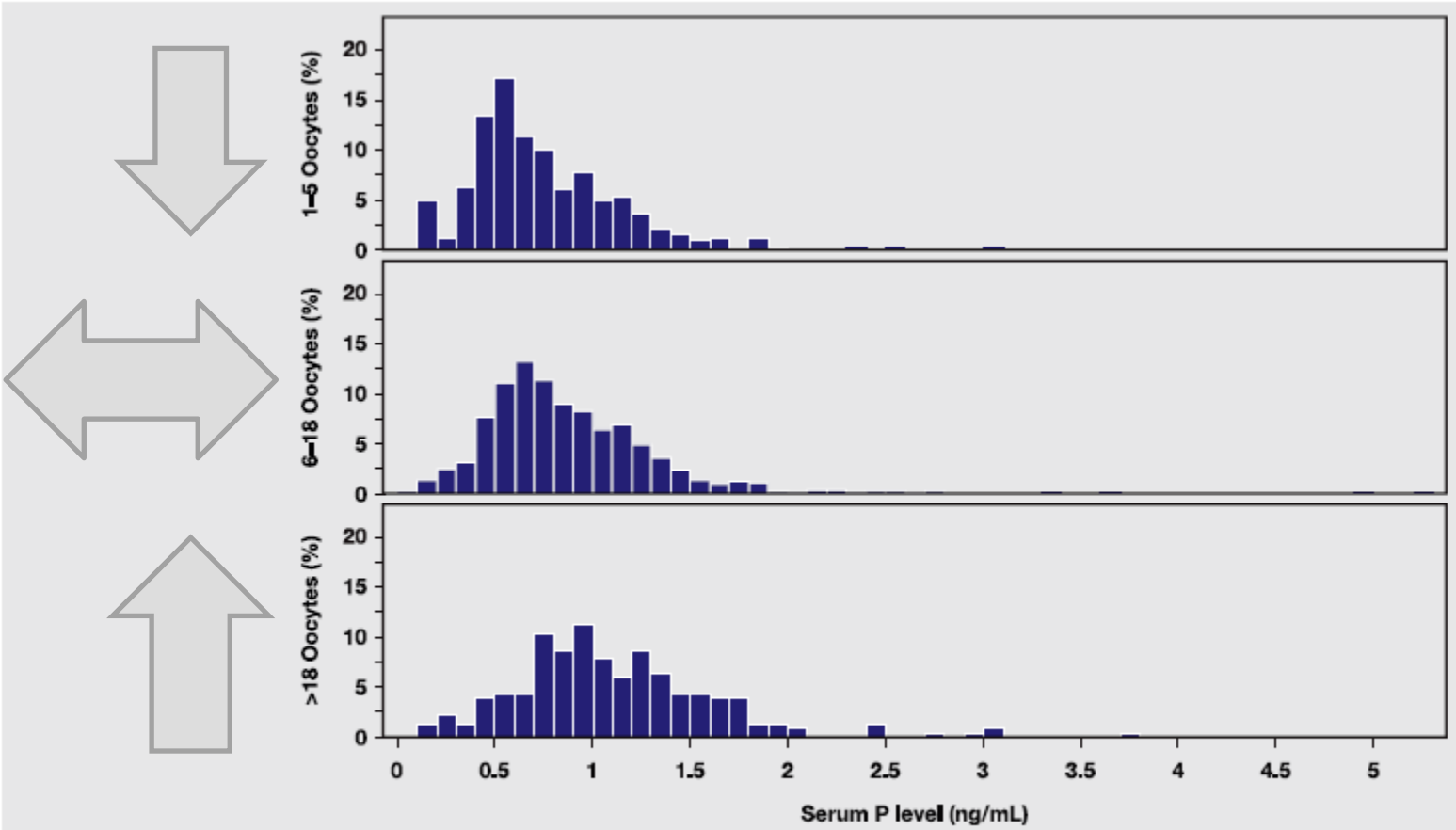
Griesinger G, Fertil Steril, 2013

Progesterone levels

	On day hCG P >1.5ng/ml 157/1866 (%8.4)
POOR RESPONSE (1-5 oosit)	%4.5
HİGH RESPONSE(>18 oosit)	%19

Griesinger G, Fertil Steril, 2013

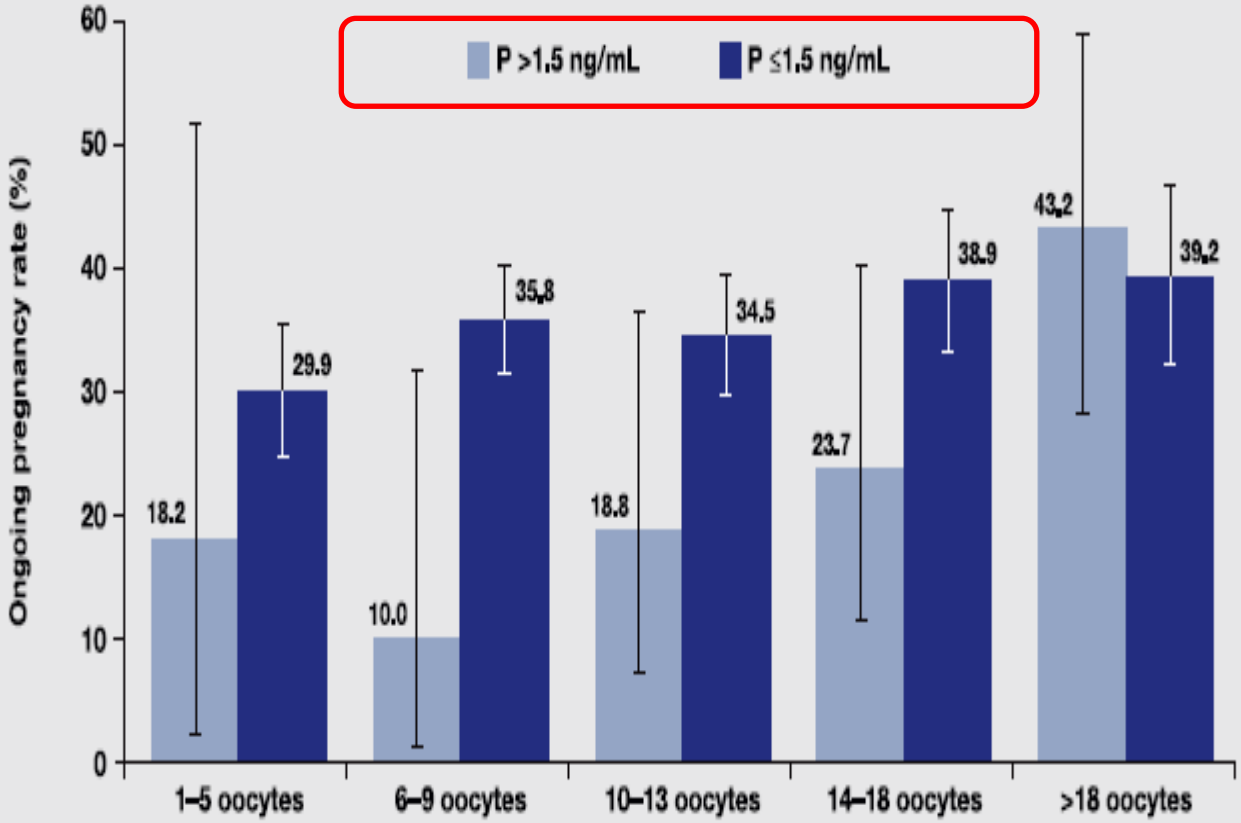
Progesterone levels



Frequency distribution of serum P levels on the day of hCG administration for women with low ovarian response (<6 oocytes), normal ovarian response (6–18 oocytes), and high ovarian response (>18 oocytes).

Griesinger. Elevated P and ongoing pregnancy. Fertil Steril 2013.

Progesterone levels



Ongoing pregnancy rate per embryo transfer and associated 95% confidence interval by number of oocytes retrieved and serum P level on the day of hCG.

Griesinger. Elevated P and ongoing pregnancy. Fertil Steril 2013.

Progesterone levels

- POOR and NORMO responder patients

High P levels are assosiated with low pregnancy rates

- HIGH responder patients High P levels are not associated with pregnancy rates.

Griesinger G, Fertil Steril, 2013

Estradiol after hCG

n= 1712 IVF cycle

- Retrospective /age ;21-45 /
- GnRH agonist protocol
- GnRH antagonist protocol or 'microdose flare'

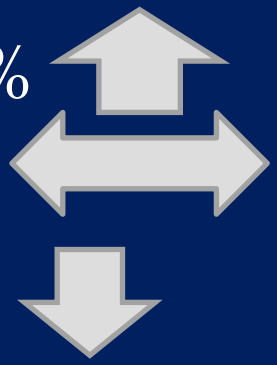
- **Estradiol levels ;**
  - On hCG day (early in the morning)
  - Post-hCG (after 10-12 h)

Kondapalli , Hum Rep, 2012

Estradiol after hCG

E2 levels;

- Group A: 1065 >10%
- Group B: 525 plato
- Group C: 122 >10%



Kondapalli , Hum Rep, 2012

Estradiol after hCG

Table I Patient characteristics in three groups stratified by serum estradiol response to hCG administration.

	Group A (>10% rise), *n* = 1065	Group B (± 10% plateau), *n* = 525	Group C (>10% fall), *n* = 122	*P*-value
Diminished ovarian reserve	16.9	23.4	25.4	0.002[a,†,b]
Polycystic ovarian syndrome	13.9	5.3	7.4	<0.001[a,†,b]
Ovarian stimulation protocol (%)				
GnRH agonist	81.0	71.2	75.4	<0.001[a,†]
GnRH antagonist	3.8	5.1	5.7	0.37
Microdose GnRHa Flare	15.2	23.6	18.8	<0.001[a,†]

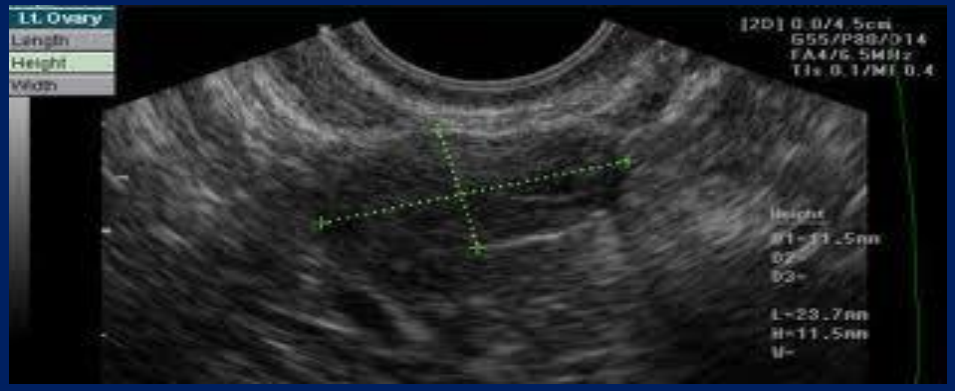
Kondapalli , Hum Rep, 2012

Estradiol after hCG

Estradiol;

- >%10 decrease → Clinical pregnancy& live birth%40-50
- Plato (± %10) → Clinical pregnancy& live birth >25%.

Kondapalli , Hum Rep, 2012



POOR RESPONSE

Protocol shift

Mild stimulation

Antagonist protocol

Microdose protocol

Oocyte pooling?

IVF? ICSI?

Poor Responder

- Suggested gonadotropine dose 300 IU/day
- >300 IU doses are not effective

Centre for Clinical Effectiveness
Cohrane Database 2000

- Why?

FSH rec. gen polymorphism ?

Ser/Ser allel variants are gonadotropin insensitive

Cai J, Fertil Steril 2007

Poor Responder

- rFSH or hMG have same outcomes for IVF/ICSI cycles.

Al-Inany H, Gynecol Endocrinol 2005

- There is insufficient data for addition of LH to ART outcomes.

Loutradis, Curr Op Obstet Gynecol, 2008

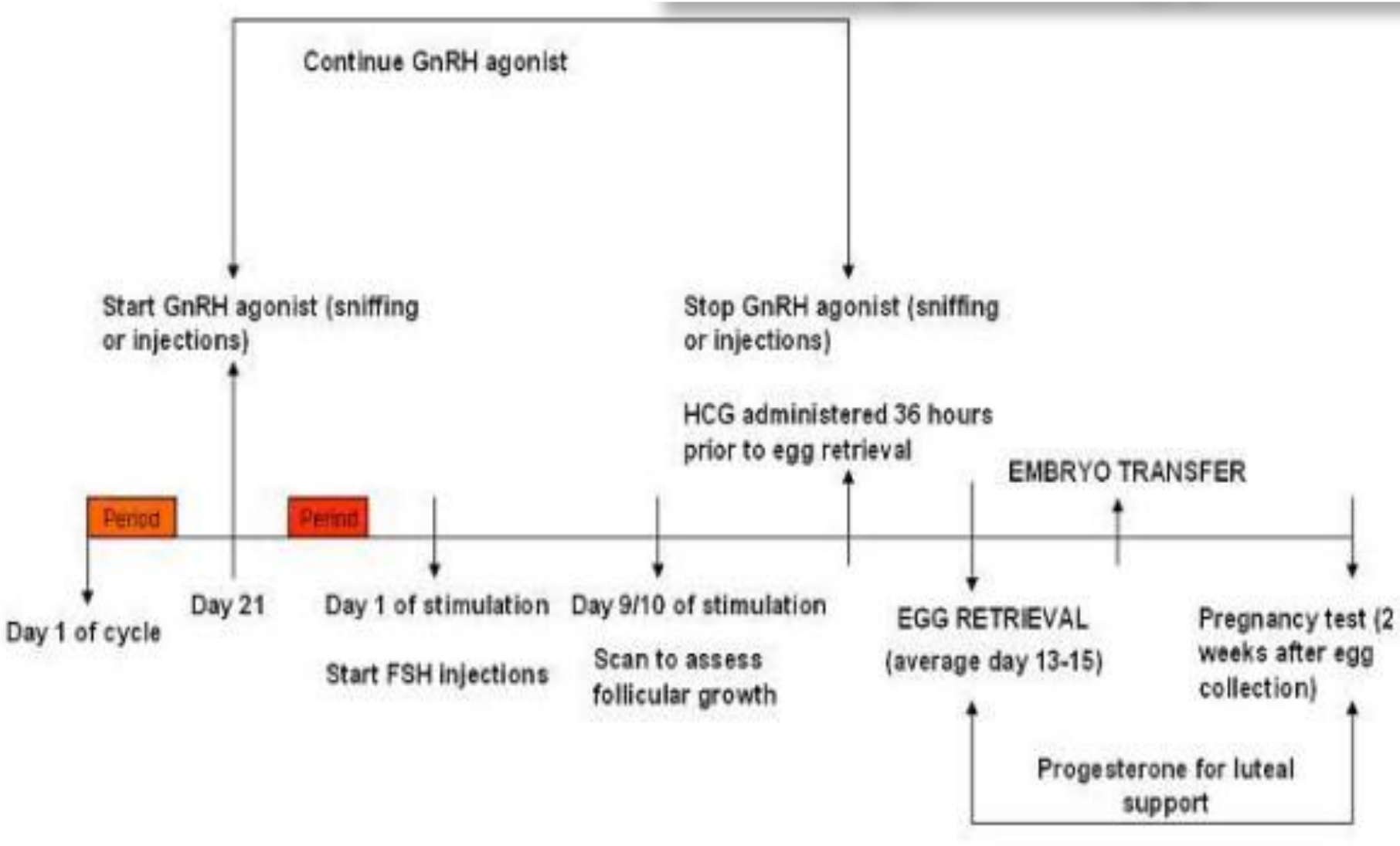
Poor Responder

- PRINT trial (n=111) poor responder (RCT)

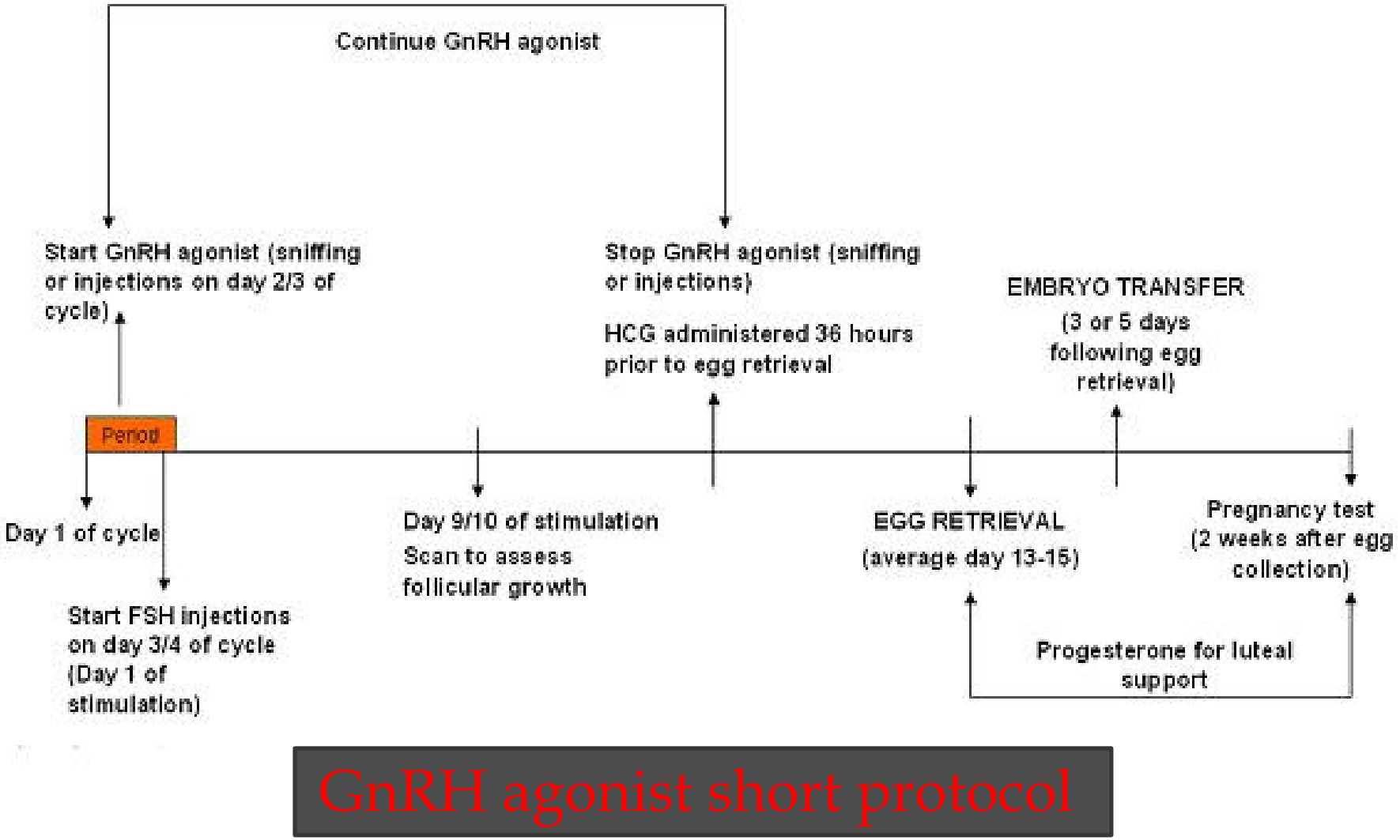
- (1) the GnRH agonist long protocol
- (2) the GnRH agonist short protocol
- (3) the GnRH antagonist protocol

Sunkara SK Fertil Steril 2014

GnRH agonist long protocol



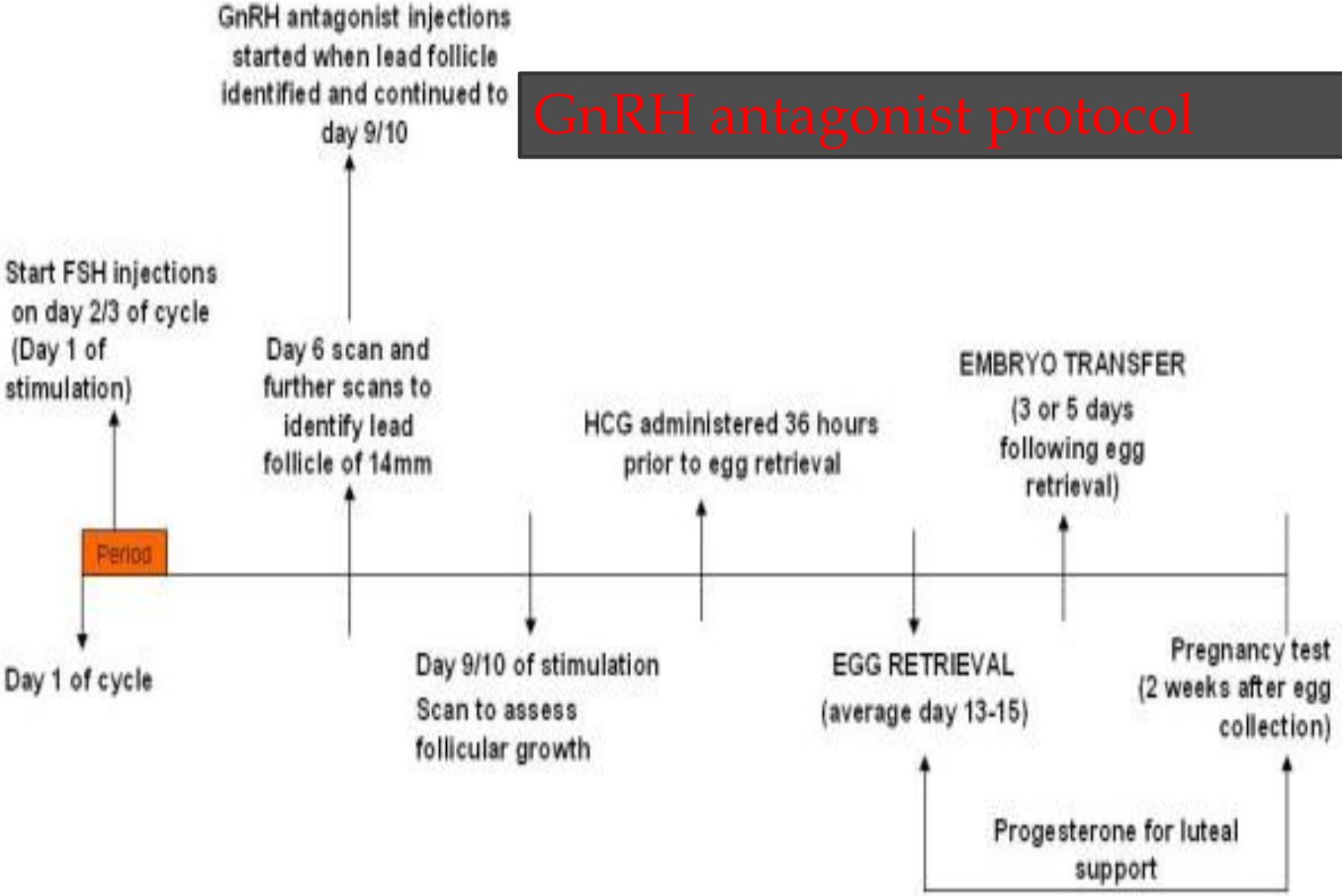
Sunkara SK Fertil Steril 2014



GnRH agonist short protocol

Sunkara SK Fertil Steril 2014

GnRH antagonist protocol



Sunkara SK Fertil Steril 2014

Poor Responder

- Oocyte number

Long agonist = Antagonist >Short agonist

- Gonadotropine dose

Long agonist > Short agonist & Antagonist

- Ongoing pregnancy

Short and Long agonist - %8.1 Antagonist - %16.2

Long agonist and antagonist protocols are more efficient

Sunkara SK Fertil Steril 2014

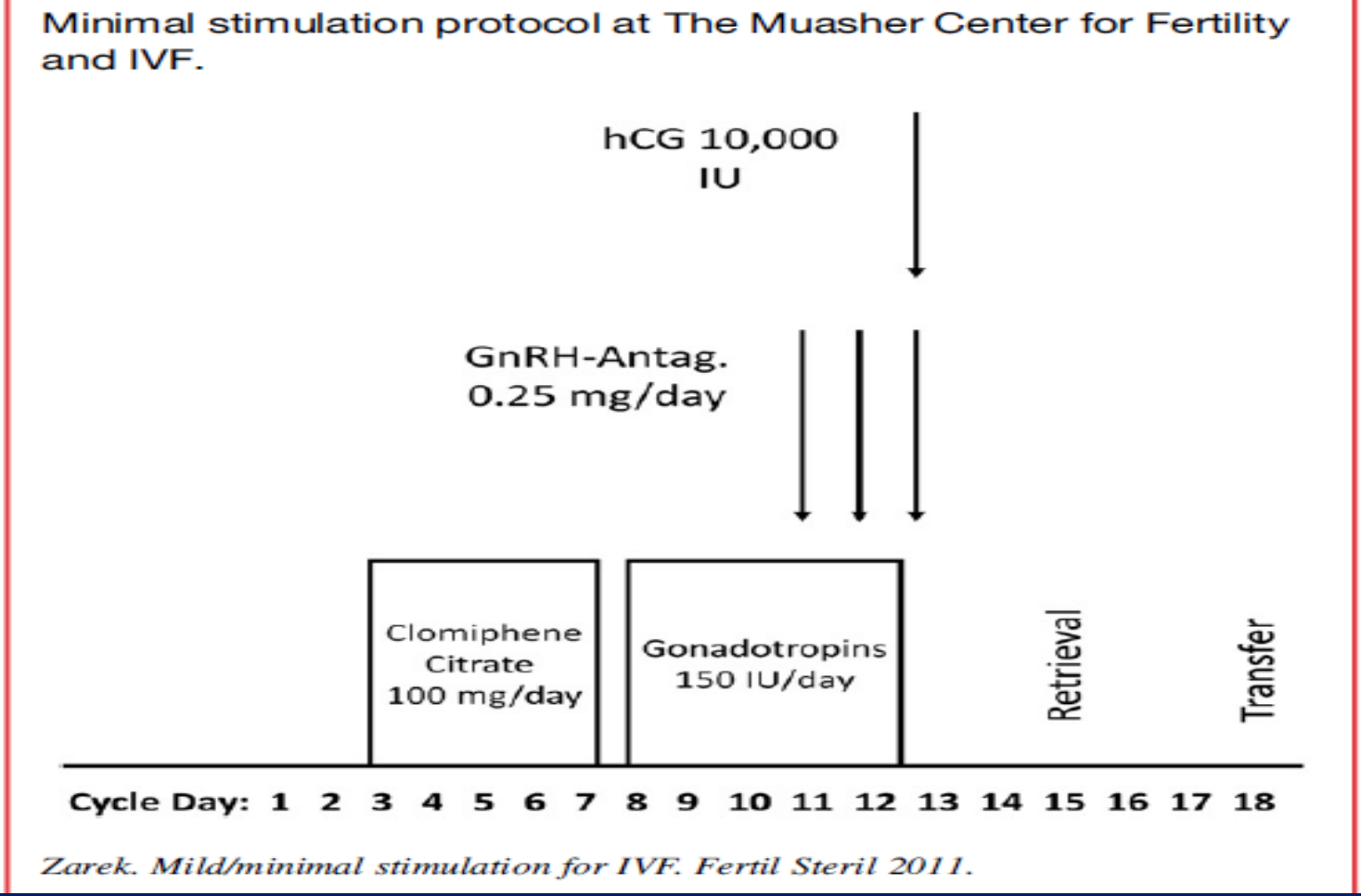
Table 2: ART outcome in two groups (Mean ± SD)

	P value	GnRH agonist/ antagonist	Microdose flare-up
No. of used gonadotropin ampoules	0.591	44.12 ± 8.20	45.20 ± 6.93
Duration of stimulation (Days)	0.610	11.60 ± 1.32	11.42 ± 1.61
No. of retrieved oocytes	0.802	4.61± 3.53	4.42 ± 3.63
No. of transferred embryos	0.954	2.44 ± 2.10	2.31 ± 2.41
Fertilization rate (%) (Per cycle)	0.458	62 ± 27	58 ± 30
Clinical pregnancy rate (%) (Per cycle)	0.389	13.3%	10%

Results: There were no significant differences between the groups in the number of used gonadotropin ampoules (p=0.591), duration of stimulation (p=0.610), number of retrieved oocytes (p=0.802), fertilization rate (p=0.456), and the number of transferred embryos (p=0.954). The clinical pregnancy rates were statistically similar in group I (10%) compared with group II (13.3%, p=0.389).

Eftekhar M, Int J Fertil Steril 2013

Minimal Stimulation/poor responder



Zarek Fertil Steril 2011

Minimal Stimulation/poor responder

Minimal stimulation versus full stimulation in low responders at the Muasher Center for Fertility and IVF, 2009–2010.

	Stimulation protocol		
	Minimal	Full	*P* value
No. of patients	13	42	
Age (y)	38.7 ± 3.7	38.9 ± 2.9	NS
Day-3 FSH (mIU/mL)	12.1 ± 2.7	10.1 ± 3.7	NS
E_2 at hCG (pg/mL)	808 ± 353	1,082 ± 561	< .05
Vials of gonadotropins	9.7 ± 3.3	49.8 ± 7.4	< .01
Days of monitoring	3	6	
Mature oocytes	2.4 ± 1.6	3.8 ± 2.3	< .05
Embryos transferred	2.0 ± 1.1	2.1 ± 1.2	NS
Clinical pregnancy/ cycle	38% (5/13)	36% (15/42)	NS
Clinical pregnancy/ transfer	42% (5/12)	47% (15/32)[a]	NS

Note: E_2 = estradiol; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin.

[a] Four patients canceled before retrieval. Six patients had retrieval without transfer.

Zarek Ferti/ Steril 2011

‘Oocyte Pooling’

- N=724 poor responder
- <5 oocytes
- D3 FSH >11 IU/ml
- AFC <6
- AMH < 0.7ng/ml

Group I: ‘low response, accumulation of oocytes and vitrification’ n=242 Group II: low response, fresh oocytes n=482

Cobo A, Rep BioMed Online, 2012

‘Oocyte Pooling’

- OCs 15-21 day
- Flexible antagonist protocol

- Fertilisation rates same
- Cycle cancellation 4x in Low Response-fresh group

Cobo A, Rep BioMed Online, 2012

Table 3 Live birth rate per patient and per embryo transfer.

	LR-Accu-Vit	*LR-fresh*
Embryo transfers (*n*)	220	318
Transfer cancellations/patient (%, 95% CI)	9.1 (6.8—11.4) [a]	34.0 (29.8—38.2) [a]
Implantation rate		
n/total	110/440	138/540
% (95% CI)	25.0 (20.7—30.0)	25.6 (21.9—29.3)
Embryos transferred (mean, 95% CI)	2.0 (1.9—2.1) [b]	1.7 (1.6—1.8) [b]
Live-birth rate/embryo transfer		
n/total	73/220	108/318
% (95% CI)	33.2 (25.7—38.0)	34.0 (28.7—39.1)
Live-birth rate/patient		
n/total	73/242	108/482
% (95% CI)	30.2 (24.3—35.9)	22.4 (18.7—26.1)
Cumulative live-birth rate/patient[c]		
n/total	88/242	114/482
% (95% CI)	36.4 (30.3—42.4) [d]	23.7 (19.9—27.4) [d]

LR-Accu-Vit = low response, accumulation of oocytes and vitrification; LR-fresh = low response, fresh oocytes.
[a,b,d]Same superscript letters in a row indicate a statistically significant difference ($P < 0.05$).
[c]Calculated considering the additional number of babies born after subsequent embryo cryotransfers.

Cumulative pregnancy rates are higher with oocyte pooling

Cobo A, Rep BioMed Online, 2012

IVF or ICSI?

- ICSI indications;
  Male factor
  Conventional IVF failure,
  Surgically achieved spermatozoa
  Before PGD

Borini A,Reprod Biomed Online, 2009; Griffiths TA, Hum reprod,2000

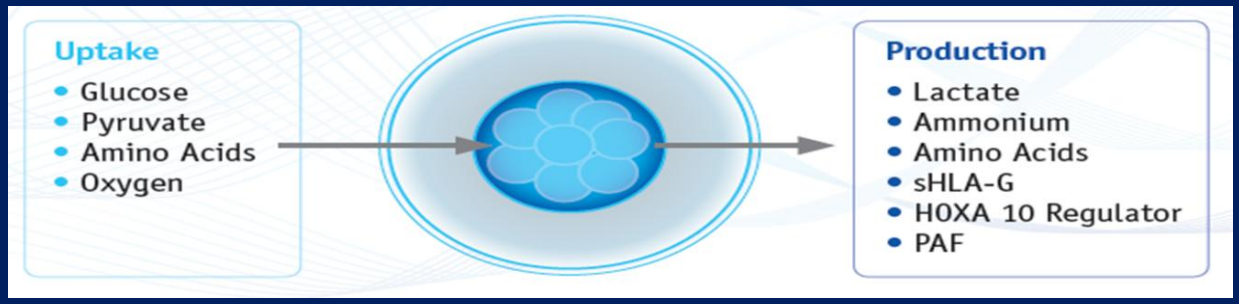
Intracytoplasmic sperm injection (ICSI) for non-male factor infertility: a committee opinion

The Practice Committees of the American Society for Reproductive Medicine and Society for Assisted Reproductive Technology

American Society for Reproductive Medicine, Birmingham, Alabama

CONCLUSIONS

- There are no data to support the routine use of ICSI for non-male factor infertility.
- ICSI may be beneficial for patients using PGT, IVM, or cryopreserved oocytes.
- The safety and cost of ICSI in the setting of non-male factor infertility must be considered.



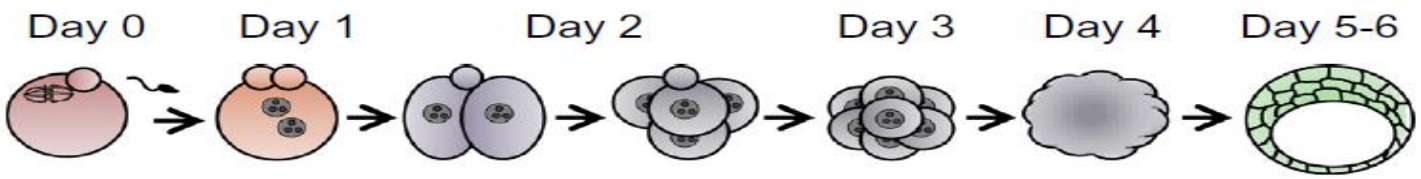
EMBRYO QUALITY

PGD

CGH

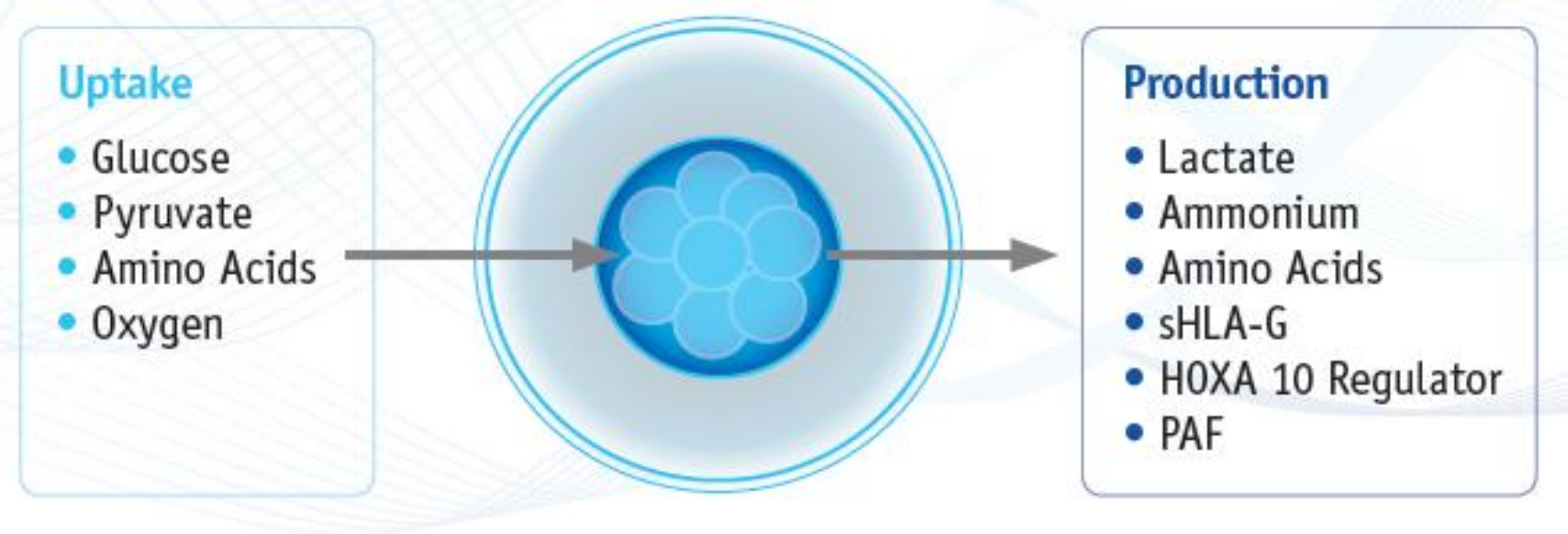
Time-lapse embryoscopy

Metabolomics



Morphology assessment:	PS, ZP, PB cytoplasm and spindle grading	PN grading	Cleavage rate, assessment of fragmentation	ICM size
PGD / PGS:	Polar body biopsy		Blastomere biopsy	TE biopsy
Transcript-omics:	mRNAs in follicular cells			
Analysis of metabolism:			Puryvate and glucose uptake	
			Oxygen consumption	
			Amino acid turnover	
			HLA-G levels	Leptin levels
			Metabo-lome	Secrotome and metabo-lome
Time-lapse imaging:		Cyto-plasmic flows	Rate and synchrony of the first cleavage divisions	

Embryo Quality



Embryo Selection

- Implantation failure → aneuploidi

- 'Comprehensive chromosome screening' (CCS) tecniques
  - single nucleotide polymorphism (SNP) array
  - quick polymerase chain reaction analysis,
  - comparative genomic hybridization (CGH) array

.

Genetic Tests- Embryo

Table I Overview of most common techniques used in genetic testing of embryos.

	Amplification-based PCR (multiplex PCR and PGH)	Fluorescent *in situ* hybridization (FISH)	Array CGH	SNP array	Quantitative SNP array analysis and karyomapping
Single-gene defects	x			x	x
HLA typing	x			x	x
Chromosome screening					
Aneuploidy	x	x (5–12 chr)	x (24 chr)	x (24 chr)	x (24 chr)
Duplication/deletions	x		x	x	x
Reciprocal/Robertsonian imbalance	x	x	x	x	x

Hens K, Hum Rerod Update,2013

Embryo Selection

- Blastocyst transfer:
  - First clivage time,
  - Time between 2. stage to 3. stage ,
  - Time between ICSI to 5 cells

Meseguer MHuman Reprod 2011;
Hashimoto S, Fertil Steril 2012;
Wong CC, Nat Biotechnol 2010;

Embryo Selection
PGD vs PGS

- Preimplantation Genetic Diagnosis(PGD)
  Preimplantation Genetic Screening (PGS)
- PGD;

  For patients who may have a genetic defect

  PGS;

  For evaluation embryos in terms of chromosomes

Harper, Segupta, 2012

Kahraman, 2011

Embryo Selection
PGD

- Implantation rate 5X,
- Pregnancy rate 2x

- Translocation (+);
  Abortion rate 6x
  Pregnancy rate 7x

Verlinsky Y, Preimplantation Genetic Diagnosis, 2006

Gianaroli L, Reprod BioMed Online,2004

Embryo Selection
CGH

- **CGH array**
  - polar body
  - Blastocyst trophoectoderm cells

- Less mozaisism
- Less harm to the embryo

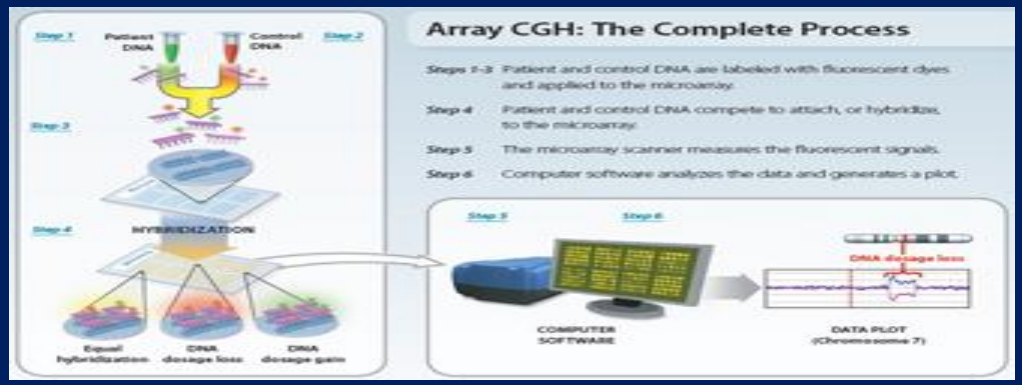
Santos MA, Hum Reprod 2010
Forman EJ, Semin Reprod Med 2012

Embryo Selection
CGH

- CGH increases pregnancy rates.

Forman EJ, Hum Reprod 2012
Yang Z, Mol Cytogenet 2012

- Prospective RCT – Ongoing pregnancy rate (%69 vs %42) (P=0.009)



Yang Z, Mol Cytogenet 2012

Genetic Tests- ‘Embryoscope’

- Morphological properties
  - Non-invasive
  - Analysis of ‘snapshot ‘ images
- Time-lapse images of embryonic development.
- Higher implantation rates with embryoes selected by embryoscope

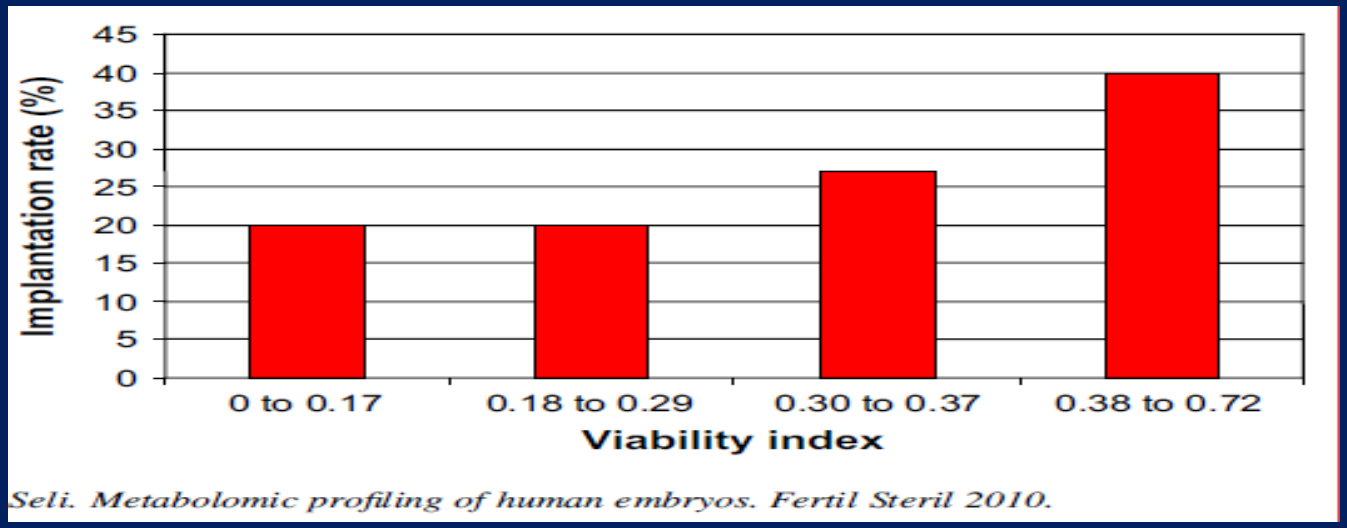
Metabolomics

- Metabolomic characteristics are different in implanted and non implanted groups

  Sakkas D,Current Opin Obstet Gyn,2005; Botros L, Mol Hum Reprod,2008

- Embryo viability indexes, Raman and NIR are high due to spectroscopic analysis.

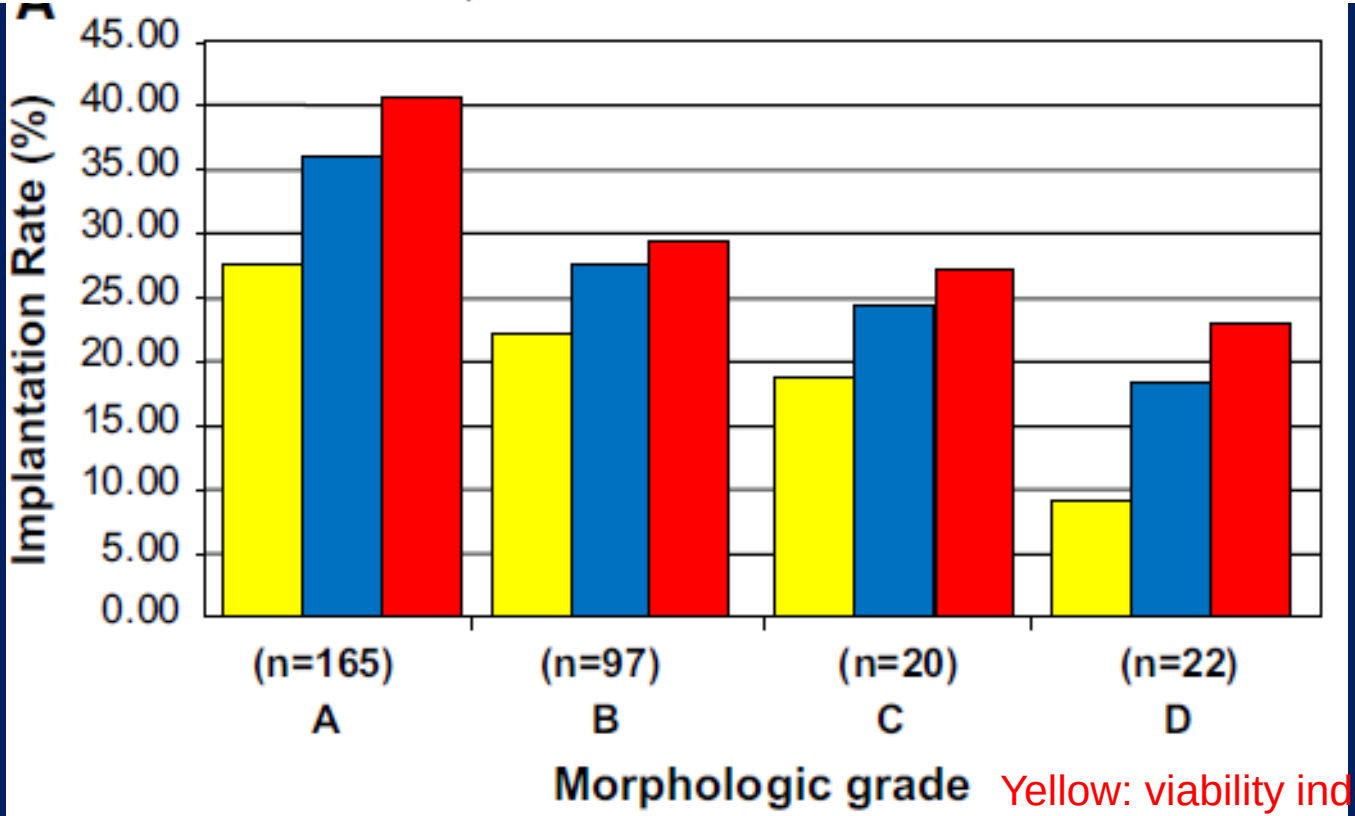
Botros L, Sakkas D, Seli E, Mol Hum Reprod, 2008



Seli. Metabolomic profiling of human embryos. Fertil Steril 2010.

Noninvasive metabolomic profiling as an adjunct to morphology for noninvasive embryo assessment in women undergoing single embryo transfer

Emre Seli, M.D.,[a] *Carlijn G. Vergouw, M.Sc.,*[b] *Hiroshi Morita, B.Agr.,*[c] *Lucy Botros, M.Sc.,*[d] *Pieter Roos, Ph.D.,*[d] *Cornelius B. Lambalk, M.D., Ph.D.,*[b] *Naoki Yamashita, M.D.,*[c] *Osamu Kato, M.D.,*[c] *and Denny Sakkas, Ph.D.*[a,d]



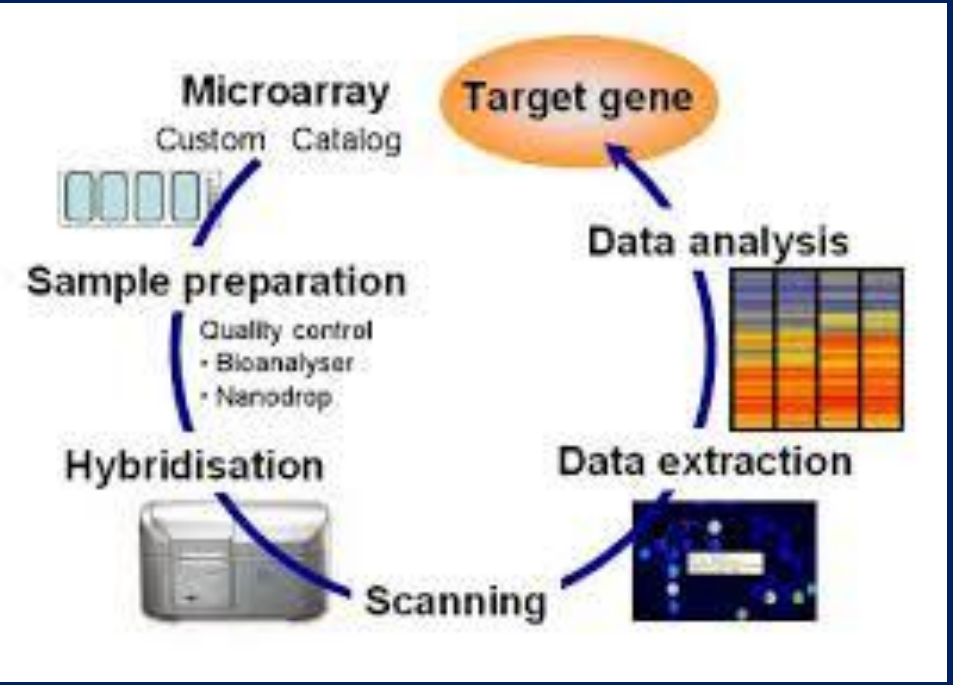
Implantation rates of day 3 embryos comparing morphological grades and a viability index of less than or greater than 0.3.

Yellow: viability index< 0.3
Red: Viability index > 0.3
Blue:implantation rates for different morphological grades

Transcriptomics

Transcriptomic analysis → Genetic expression analysis

-Cellular development
-Biological problems
-Embryonal viability
-Harmful peripheral factors
-Morphological anomalies



Genetic Tests

- For embryonal viability ⟹ Cumulus cell gen express.
- Time-lapse molecular analysis.
- Sperm count < 5 million ⟹ CGH %72 anomaly
- Sperm count > 5 million ⟹ CGH %53 anomaly

Genetic Tests

- PIF ⇒ Preimplantation genetic factor

- From endometrial stromal cells
- At first trimestr from extravillous cytotrophoblasts
- To determine placental complications
- To define embryo development

Genetic Tests

- CCS ⇒ Comprehensive Chromosomal Screening

- qpcR based CCS analysis
- %97,6 - %98,6 positive

- qpcR is more accurate than a CGH and can define genomic imprinting defects

Genetic Tests

- How to diagnose sperm dysfunction?
  - Calcium activation
  - Catsper activation
  - Proteomics (Sperm fertility array)
  - Swim-up gradient
  - IMSI, ICSI, PICSI,
  - Birefrigence
  - PLA2,ABCA, CD84, CMKLR1,
  - MAC'S (Magnetic cell sorting)

Genetic Tests

- ERA (Endometrial Receptivity Array)
- This molecular test allows to diagnose endometrium is receptive or not by analysing the expression 238 genes related to endometrial receptivity.
- Endometrial biopsy must be performed at **P+5** (hormone replacement therapy cycle) or at **LH+7** (natural cycle)

Genetic Tests

- ERA (Endometrial Receptivity Array)
- Biopsy of the uterine fundus with a Pipelle catheter or similar. About 30 mg or 3 mm tissue is enough.
- If the result of first ERA test is non receptive this means that the window of implantation may be displaced, it is necessary to validate this displacement with a second ERA test.
- This second analysis will lead to the day in which the endometrium shows a receptive status, and therefore the thawing of eggs or embryos and their transfer must be scheduled to coincide with the day in which the receptive result has been obtained.
- İn less of 1% of patients the first ERA test is non reseptive **without a recommendation for a new implantation window** which does not suggest a therapeutic solution.

EMBRYO TRANSFER

Time?

Technique?

Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis

Evangelos G. Papanikolaou[1], Efstratios M. Kolibianakis, Herman Tournaye, Christos A. Venetis, Human Fatemi, Basil Tarlatzis and Paul Devroey

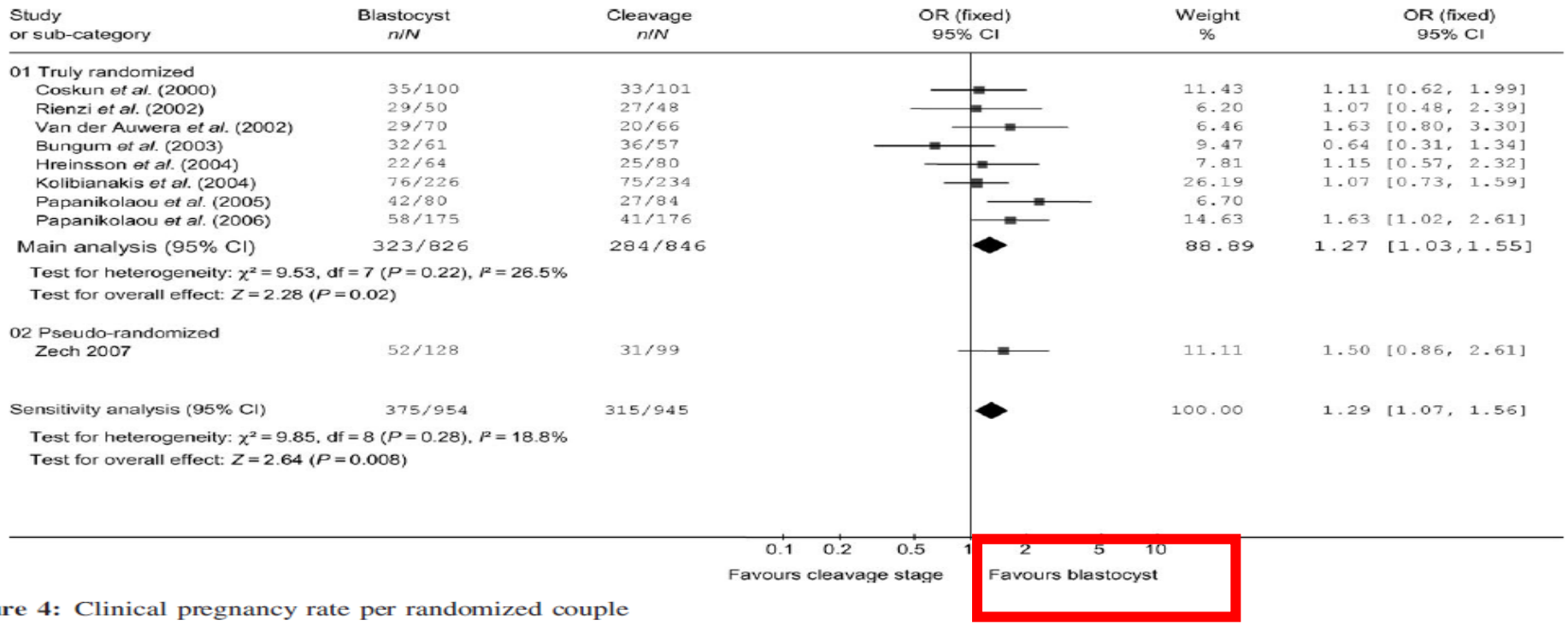


Figure 4: Clinical pregnancy rate per randomized couple

Human Reproduction Vol.23, No.1 pp. 91–99, 2008

Time for ET?

- ***Blastocyct Transfer***
  - Uterine and embryonic syncronisation
  - Uterine micro-environtment hyperstimulation
  - Uterine contraction
  - Embryo selection
  - Implantation rates

Gardner DK,Fertil Steril,1996;

Barnes FL,Theriogenology, 2000;

Fanchin R, Hum Reprod,Hum Reprod, 2001

Embryo Transfer: Technique

- ET : %85 implantation failure
- %30 transfer technique

* ***Edwards RG. Hum Reprod 1995; 10: 60-6***

** ***Li et al. J Assist Reprod Genet 2005; 22: 3-8***

Embryo Transfer

The relative importance of factors important for successful embryo transfer.[a]

Priority	Mean score[b]
Removal of hydrosalpinges	6.8
Absence of blood or mucus	6.6
Type of catheter	6.1
Not touching fundus	5.8
Avoiding tenaculum	5.7
Removal of all mucus	5.2
Ultrasonography of cavity before procedure	4.3
Leaving catheter in place for 1 minute	4.2
30 minutes of bed rest	3.8
Trial transfer	3.1
Ultrasonographic monitoring	2.6
Antiprostaglandins to prevent uterine contractions	1.9

[a] Data from reference 11.

[b] The possible score for each factor was on a scale of 1 to 10.

Schoolcraft. Embryo transfer. Fertil Steril 2001.

MODERN TRENDS

Edward E. Wallach, M.D.
Associate Editor

Optimizing the technique of embryo transfer

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SUMMARY

Evidence-based Guidelines

1. Effort should be made to avoid "difficult" transfers.
2. Ultrasound guidance will result in easier transfers with improved outcomes.
3. Soft catheters should be used when feasible.

Recommendations Based on Expert Opinion

1. Trial transfers allow better preparation for difficult transfers.
2. Cervical mucus should be removed to potentially decrease bacterial contamination and mucus plugging of the catheter.
3. Embryos should be deposited in the midportion of the uterus.
4. Negative pressure should be minimized during withdrawal of the catheter.
5. The procedure should be done in a minimum amount of time.

Transfer Method

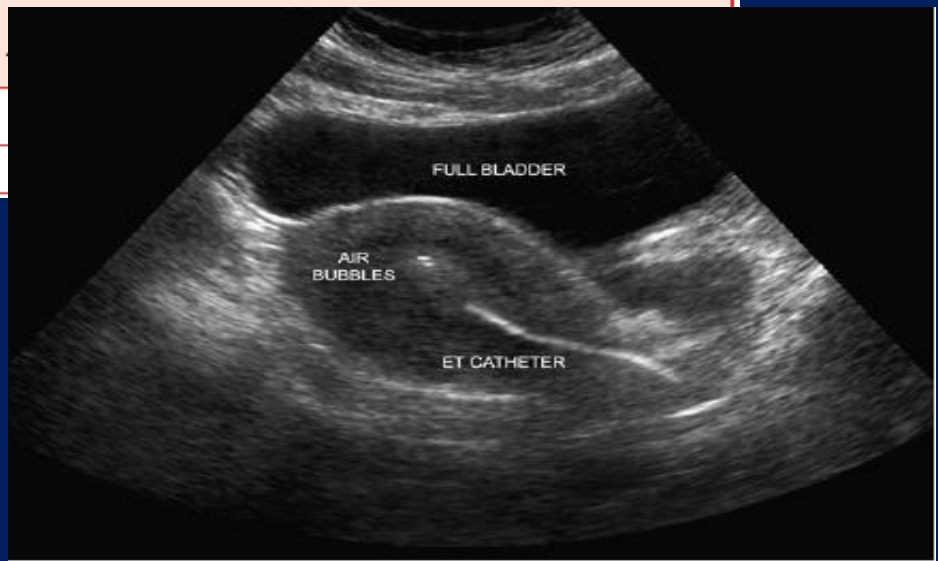
TABLE 1

Characteristics of patients, complications, and clinical outcome after ET.

	Full bladder (n = 67)	Empty bladder (n = 64)	Clinical touch (n = 40)	*P*
Mean age	34.9 ± 4.6	35.7 ± 4.9	35.9 ± 5.9	NS
BMI	24.5 ± 4.9	23.2 ± 3.8	23.7 ± 4.2	NS
Baseline FSH	6.2 ± 2.3	5.7 ± 2.2	6.1 ± 3.7	NS
No. oocytes retrieved/patient	10.7 ± 4.2	12.7 ± 5.7	10.9 ± 6.5	NS
No. embryos transferred	2.6 ± 0.8	2.4 ± 0.7	2.4 ± 1	NS
Use of obturator (%)	13.4	32.8	32.5	<.02
Use of tenaculum (%)	8.9	26.5	25	<.002
Use of hysterometer (%)	1.5	14	15	<.002
Blood in the catheter (%)	5.5	7.8	14.3	NS
Retained embryos (%)	0	3.1	5	NS
Implantation rate (%)	16.1	15.4	14.3	NS
Clinical pregnancy rate (%)	39	38.7	35.7	NS
Ectopic pregnancies (%)	0			
Abortion rate (%)	14.3			

Note: NS = not significant.

Lorusso. Ultrasound ET guidance and IVF outcome. Fertil Steril 2005.



Ultrasound guided embryo transfer

- Clinical pregnancy rates increases (OR:1.31, %95 CI 1.18-1.46)
- Ongoing pregnancy rates increases (OR:1.38, %95 CI 1.16-1.64)
- No difference at live birth rates (OR:1.14, %95CI 0.93-1.39)

Brown J,Cochrane Database Syst Rev, 2010

Table IV. Meta-analysis of pregnancy rates in randomized trials. Ultrasound (US)-guided transfer versus clinical touch transfer

Author	Year	US-guided transfer (%)	Clinical touch transfer (%)	*P*-value
Randomized trials				
Coroleu *et al.*	2000	50 (91/182)	33.7 (61/180)	< 0.05
Tang *et al.*	2001	26.0 (115/441)	22.5 (81/359)	NS
Matorras *et al.* (this study)	2002	26.3 (67/255)	18.1 (47/260)	< 0.05
Quasi-randomized trials				
Hurley *et al.*	1991	20.2 (19/94)	17.5 (43/246)	NS
Al-Shawaf *et al.*	1993	28.9 (44/152)	30.3 (27/89)	NS
Prapas *et al.*	1995	36.1 (22/61)	22.5 (16/71)	< 0.05
Kan *et al.*	1999	37.8 (37/98)	29.8 (28/97)	NS
Prapas *et al.*	2001	47.6 (206/433)	36.0 (229/636)	< 0.05
Global meta-analysis	2002	35.0 (601/1716)	27.5 (532/1938)	< 0.0001[a]
Meta-analysis including only truly randomized trials	2002	31.4 (273/870)	23.7 (189/799)	< 0.001[b]

[a] $\chi^2 = 20$; odds ratio (OR) = 1.4; 95% confidence interval (CI) = 1.23–1.64.
[b] $\chi^2 = 12$; OR = 1.5; 95% CI = 1.18–1.85.
NS = not significant.

Human Reproduction Vol.19, No.9 pp. 2027–2030, 2004
Advance Access publication June 10, 2004
DOI: 10.1093/humrep/deh366

Influence of the time interval between embryo catheter loading and discharging on the success of IVF

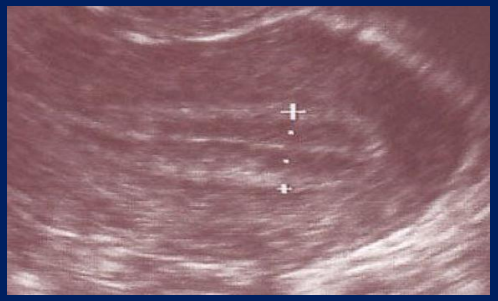
R.Matorras, R.Mendoza, A.Expósito and F.J.Rodriguez-Escudero

Table II. Duration of the interval loading discharging embryos (ILDE), cycle characteristics and cycle results

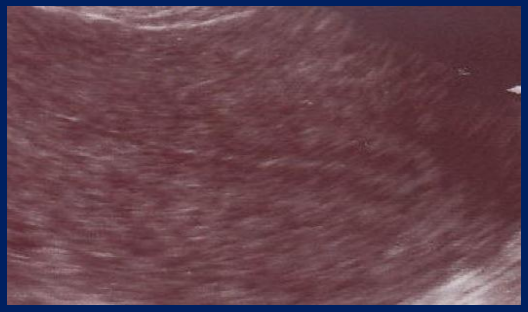
	<30 s ($n = 113$)	31–60 s ($n = 214$)	61–120 s ($n = 76$)	>120 s ($n = 47$)	*P*
Woman's age (years)	34.01 ± 3.04	34.40 ± 2.83	33.97 ± 3.57	33.93 ± 3.73	Ns
Infertility duration (years)	5.21 ± 2.54	5.07 ± 2.66	5.19 ± 2.54	5.20 ± 2.61	Ns
Primary infertility (%)	91.1	89.7	89.5	89.4	Ns
ICSI (%)	53.1	50.9	57.8	57.5	Ns
Obtained oocytes	11.00 ± 6.13	12.28 ± 6.22	12.22 ± 6.20	13.23 ± 5.83	Ns
Inseminated oocytes	9.30 ± 5.30	10.29 ± 5.30	9.92 ± 5.08	10.46 ± 4.03	Ns
Fertilized oocytes	5.30 ± 3.97	6.03 ± 4.07	5.34 ± 3.63	5.74 ± 3.16	Ns
Transferred embryos	3.05 ± 1.06	3.12 ± 0.99	3.07 ± 1.09	3.14 ± 0.85	Ns
Transferred class I embryos	1.95 ± 1.00	2.10 ± 0.98	2.37 ± 0.98	2.17 ± 0.99	Ns
% of non easy transfers	2.6	0.5	1.3	19.1	<0.001
Pregnancy rate (%)	38.9	33.2	31.6	19.1	<0.05
Implantation rate (%)	21.2	15.4	15.9	9.4	<0.01
Pregnancy rate excluding non-easy transfers (%)	40.0	33.3	32.0	19.4	<0.05
Implantation rate excluding non easy transfers (%)	21.4	15.4	16.2	8.8	<0.01

The longer the ILDE 'interval loading-discharging embryos', the lower the pregnancy and implantation rates

Endometrial pattern

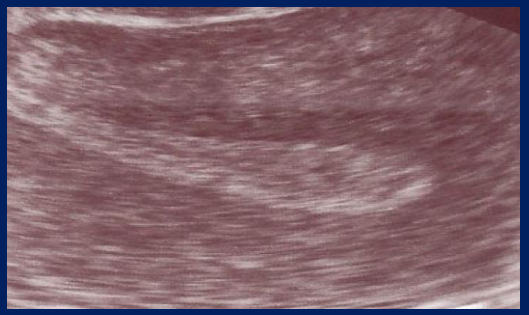


Triple-line pattern



No-triple line pattern

Iso-echoic endometrium



No-triple line pattern

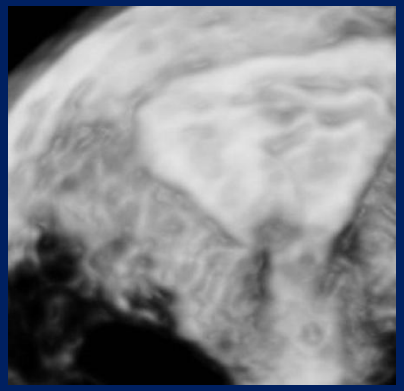
Homogeneous hyperechogenic pattern

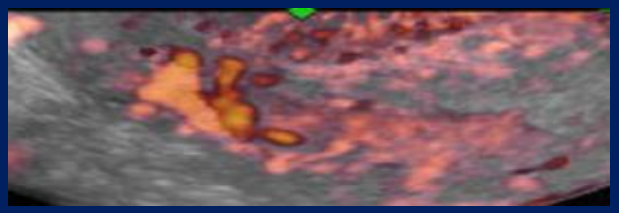
Chen et al. Reproductive Biology and Endocrinology 2010, 8:30

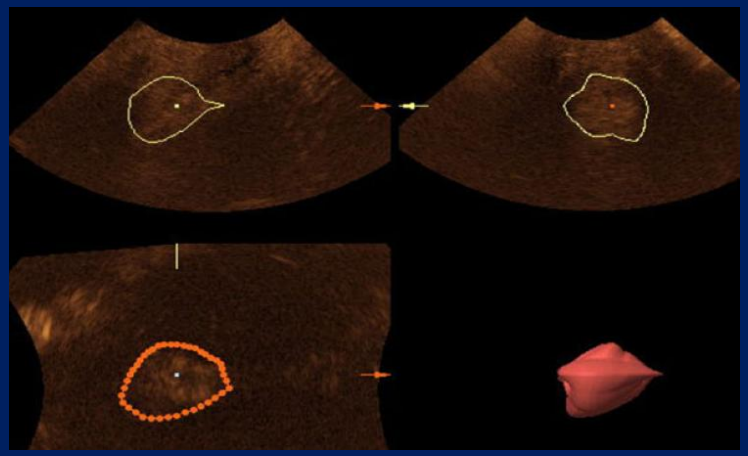
Endometrial pattern

Table 1 Three-Dimensional sonographic and Power Doppler criteria for predicting endometrial receptivity

3D Criteria	Findings	Score
Endometrial echogenicity	Hyper-echogenic	0
	Echogenic	1
	Triple line	2
Endometrial thickness	≤7 mm	0
	>7 mm	2
Endometrial volume	≤2.31 mm	0
	>2.31 mm	2
Sub-endometrial halo	Regular	0
	Disturbed	2
Endometrial flow	Yes	0
Sub-endometrial flow	No	2
RI	≥0.53	0
	<0.53	2
Vessel's architecture	Simple	0
	Complex	2







Kalmantis K .Arch Gynecol Obstet 2011

Endometrial Scratch

- 6 RCT

  Clinical pregnancy ↑

  (RR: 1.86, 95% CI 1.46–2.38]

- RIF (+)

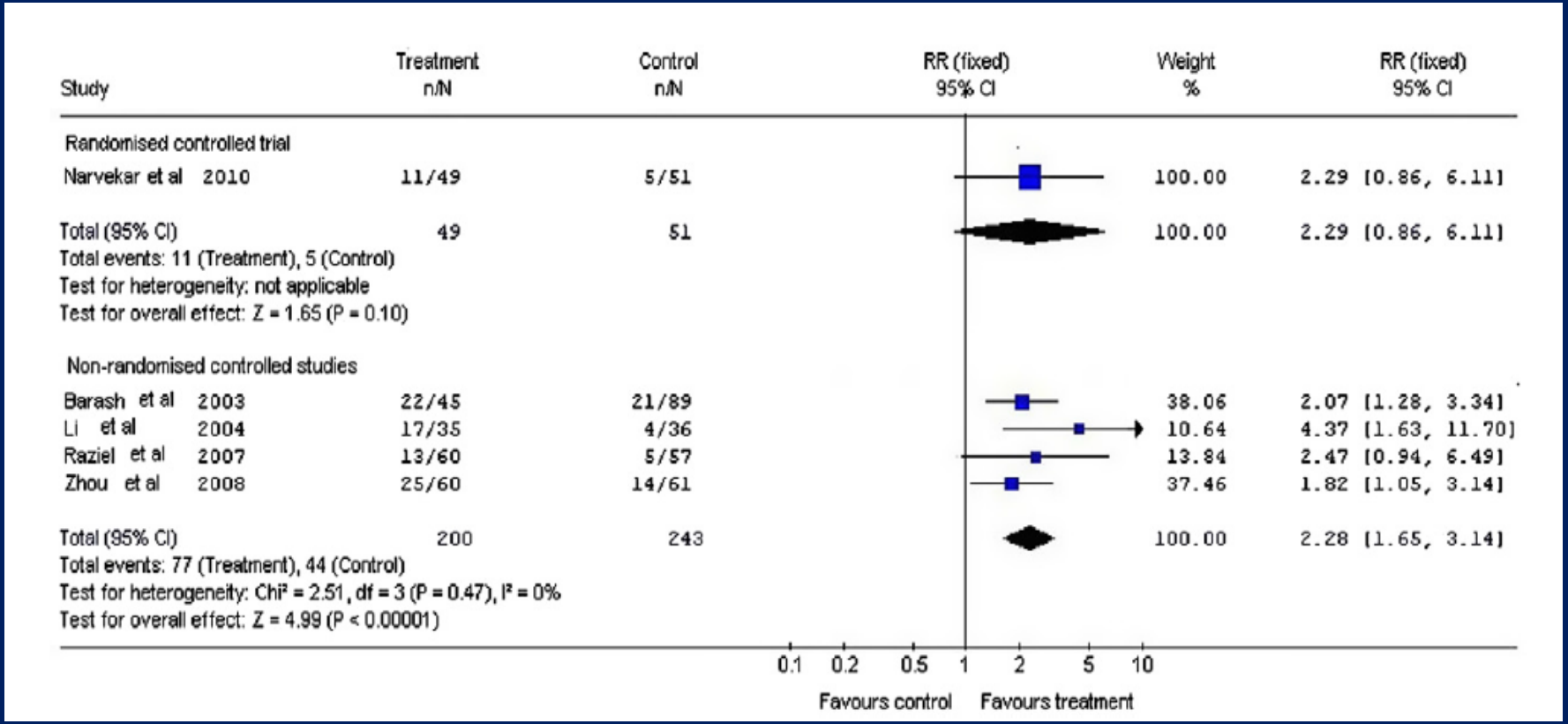
  (2 RCT and 2 nonrandomised study)

  Clinical pregnancy ↑

  (RR 2.32, 95% CI 1.72–3.13).

Nastri CO, Gynecol Endocrinol 2013

Endometrial Scratch

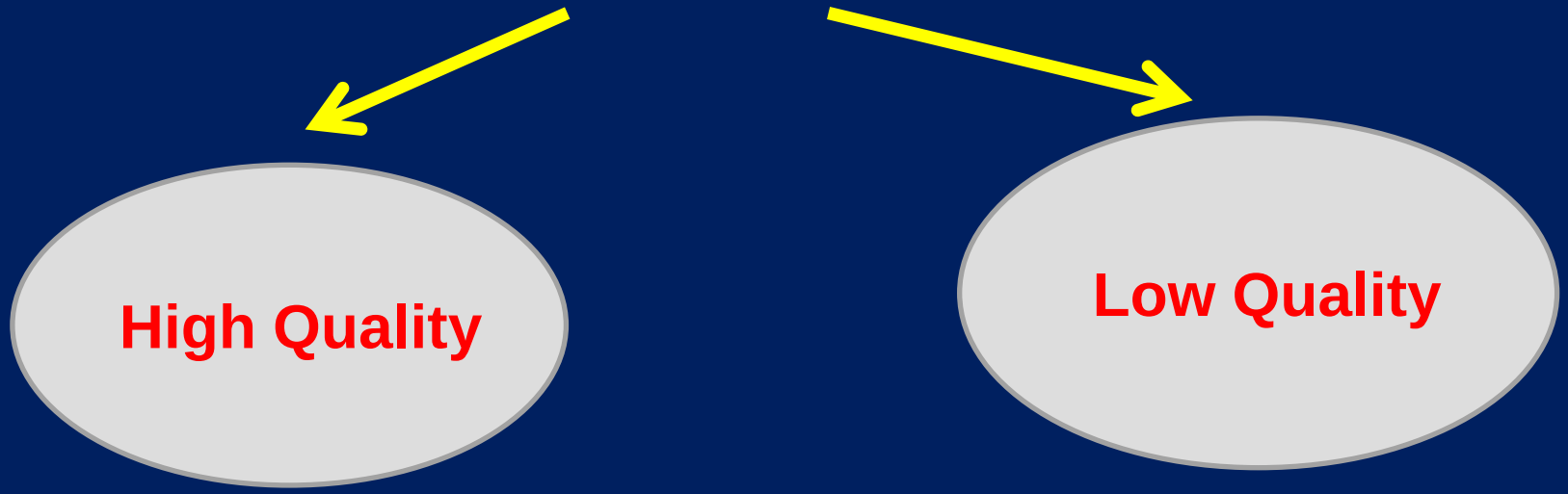


Increased live-birth and ongoing pregnancy rate

El-Toukhy T, RBM Online,2012

UNSUCCESFUL ART CYCLES;

Embyo quality should
be questioned



Embryo quality: High

- H/S
- HSG (hydrosalpinx?)
- Endometrial thickness
- IMSI? PGD? CGH? Embryoscope?
- Endometrial Receptivity Assay (ERA)
- Assisted Hatching

Embryo quality: Low

- Reassesment
- Shift to another protocol
- Look over laboratory
- Defragmantation
- Coculture
- Oocyte-Embryo pooling?

Thank you

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2008

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- **1- Prof. Dr. Recai Pabuççu**
- **2- Prof. Dr. Erol Tavmergen**
- **3- Prof. Dr. Serdar Dilbaz**
- **4- Prof. Dr. Turan Çetin**
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- **Ayrıca 07-08 Haziran 2014 tarihlerinde İstanbul Yeditepe Üniversitesi Kayışdağı Kampüsü'nde koordinatörlüğünü yine Prof. Dr. Recai Pabuçcu'nun yaptığı ve beşincisini gerçekleştireceğimiz kursumuza da Jinekolojik Mikrocerrahinin kurulucularından Sayın Prof. Dr. Victor Gomel katılacaklardır.**