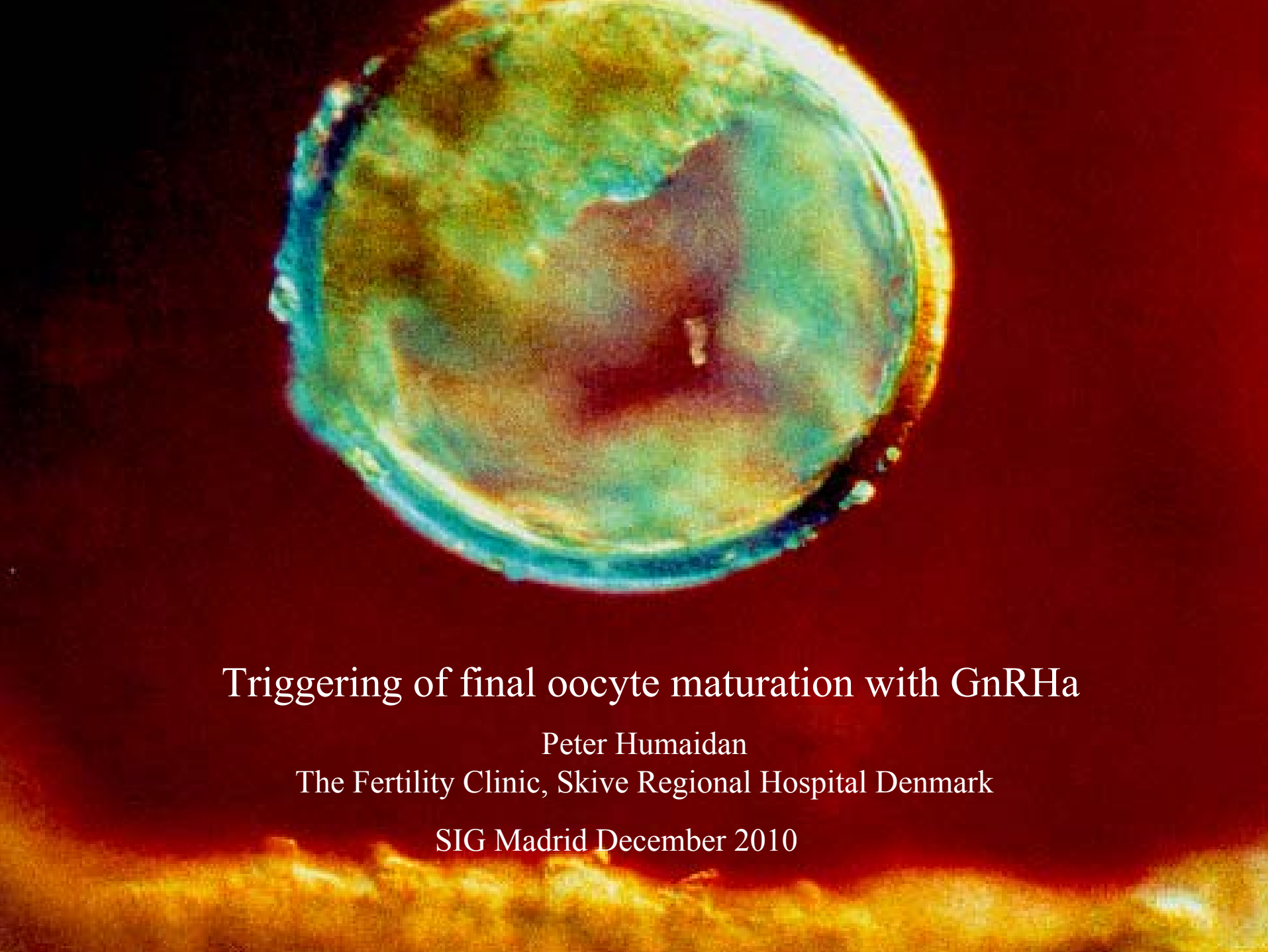


Triggering of final oocyte maturation with GnRHa

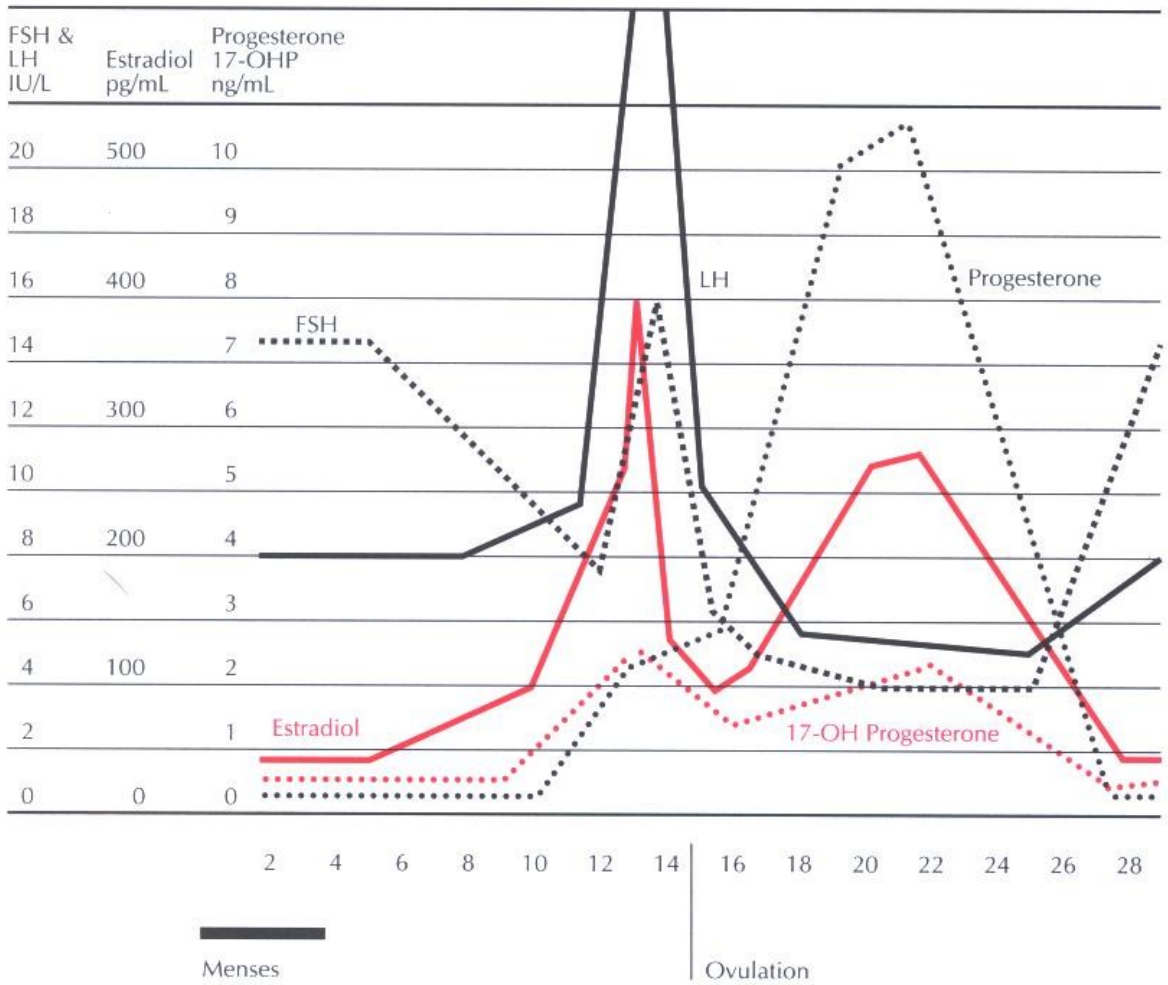
Peter Humaidan
The Fertility Clinic, Skive Regional Hospital Denmark

SIG Madrid December 2010

Overview
GnRHa for triggering of ovulation

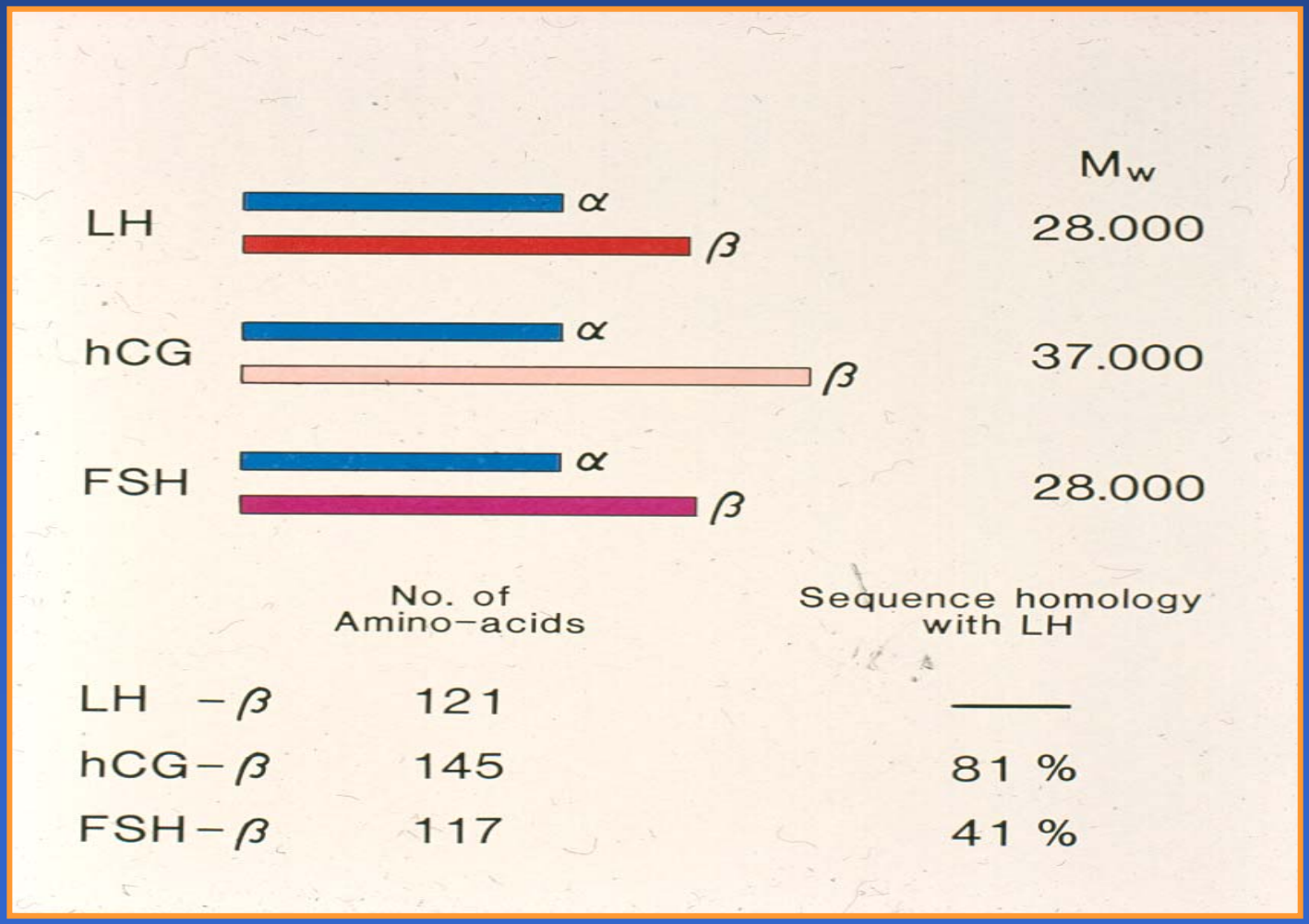
- Interpretation of previous findings
- ”Luteal phase rescue” - development of present protocol for normo-responder patients
- Luteal phase rescue in OHSS-risk patients
- Concept of ”personalized” luteal phase support

Natural menstrual cycle



From Speroff et al.

Peptide composition of gonadotrophins



Characteristics of gonadotrophins

	FSH	LH	hCG
No. of sugar residues	4	3	7
Initial half life	3–4 hours	20 min?	12 hours
Chromosome localization of the gene for the α-chain	6q21.1-23.	6q21.1-23.	6q21.1-23.
Chromosome localization of the gene for the β-chain	11	19q13.3	19q13.3
No. of copies of the gene	1	1	6

LH/hCG receptor

Sharing the same α subunit and 81% of the aminoacid residues of the β subunit, LH and hCG bind to the same receptor: LH/hCG receptor

(Kessler et al., 1979)

Effects of the mid-cycle LH surge

- Oocyte maturation (cytoplasmic and nuclear)
- Induction of ovulatory cascade
- Luteinization of theca/granulosa cells
- Support of the corpus luteum

Effects of the mid-cycle FSH surge

- Nuclear maturation (resumption of meiosis)

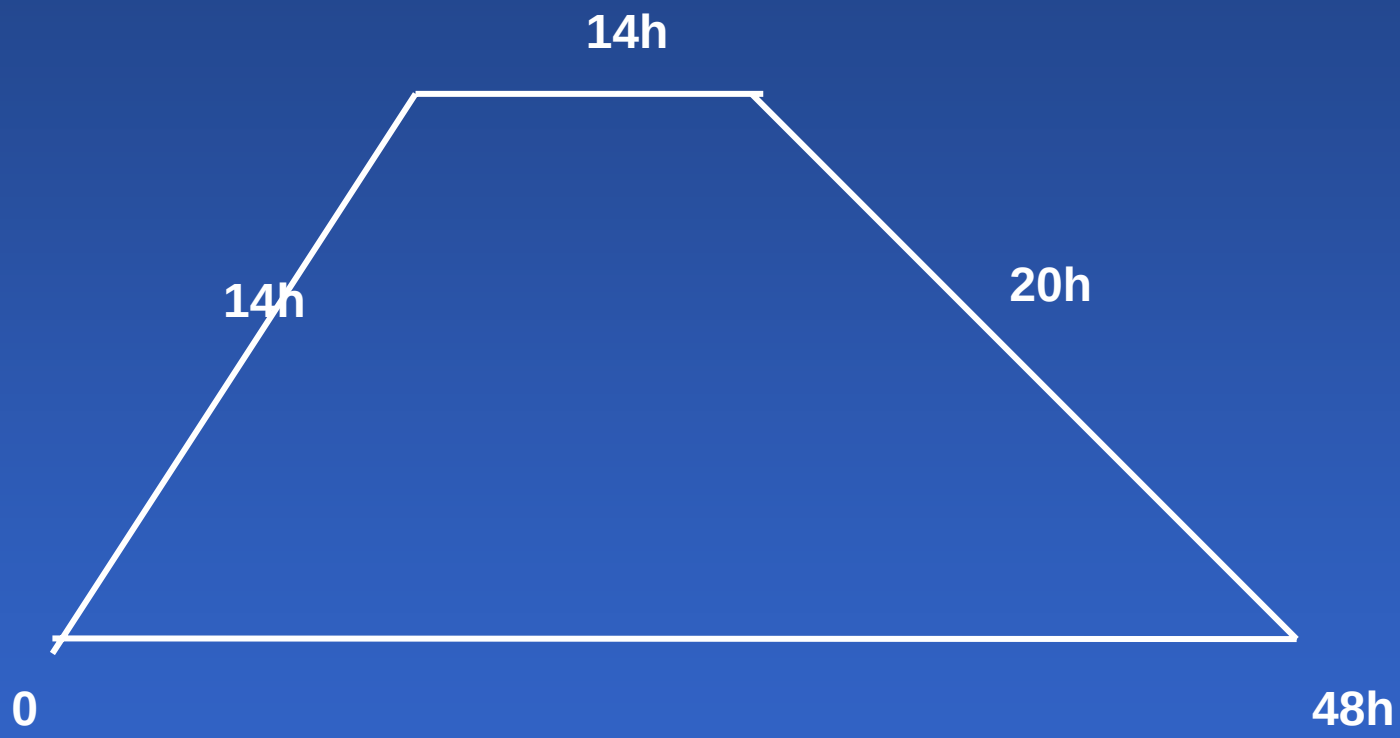
  (Zelinski-Wooten et al., 1995, Yding Andersen et al., 1999)

- LH R induction in granulosa cells

- Cumulus expansion

  (Stickland et al., 1976; Eppig, 1979)

Natural mid-cycle surge of gonadotrophins



Hoff et al., 1983

HCG for triggering of ovulation

hCG effectively induces:

- Final oocyte maturation
- Ovulation
- Luteinization of theca/granulosa cells
- Corpus luteum formation

due to structural and biological similarities with LH

HCG for triggering of ovulation - drawbacks

- Due to the longer half-life of hCG it is detectable up to 6 days following a single injection of 5000 IU
- Supraphysiological steroid levels (estradiol and progesterone), leading to disrupted luteal phase
- No FSH surge
- The sustained luteotropic effect may facilitate OHSS

GnRH agonist for triggering of ovulation

- Of interest in the late eighties/early nineties
- Not applicable in cycles down-regulated with an agonist
- Renewed interest with the introduction of GnRH antagonist protocols

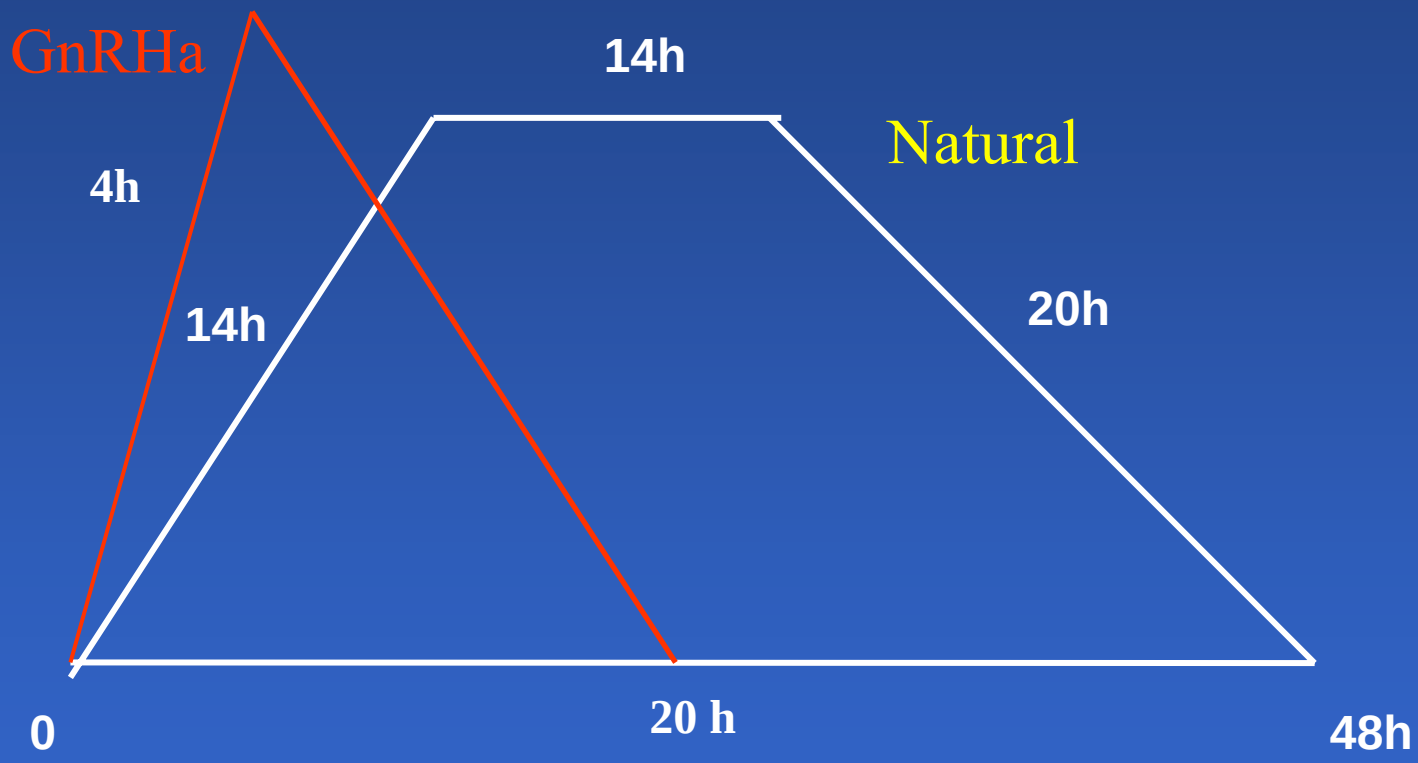
GnRH agonist for triggering of ovulation

GnRHa displaces the GnRH antagonist from the GnRH receptors in the pituitary triggering a surge (flare-up) of both LH and FSH:

- Resembling the surge of gonadotrophins of the natural cycle
- Effectively stimulation of ovulation and final oocyte maturation

(Gonen et al., 1990; Itskovitz et al., 1991)

LH-surge differences in GnRHa and natural cycle



Hoff et al., 1983; Gonen et al., 1990; Itskovitz et al., 1991

GnRH agonist for triggering of ovulation

Why??

- Negative impact of hCG on endometrial receptivity
  (Forman et al., 1988; Fanchin et al., 2001; Fatemi et al., 2010)

  Negative impact of hCG on oocyte quality
  (Valbuena et al., 2001)

- Expected decrease in the incidence of OHSS
  - T1/2 endogenous LH shorter than T1/2 hCG,
  - 20 min versus 33 hours

- More MII oocytes harvested in IVF
  (Imoedemhe et al., 1999; Humaidan et al., 2005; Humaidan et al., 2010; Oktay et al., 2010)

- More physiological
  - Endogenous FSH surge
  - Steroid level in luteal phase closer to physiological condition

Use of a GnRH agonist (Buserelin) versus hCG for ovulation induction : A prospective randomised study

Humaidan P.[1], Ejdrup Bredkjær H.[2], Bungum L.[1], Bungum M.[1],

Grøndahl M.L.[2], Westergaard L.[3] and Yding Andersen C.[4]

[1]The Fertility Clinic, Viborg Hospital (Skive), Denmark ,[2] The Fertility Clinic, Hvidovre Hospital, Denmark, [3]The Fertility Clinic Trianglen, Copenhagen, Denmark and [4] Laboratory of Reproductive Biology, University Hospital of Copenhagen, Denmark

Humaidan et al.,2005

Stimulation:

- **Fixed rFSH (150-200 IU/day) – from cd 2 for 6 days**
  Leading follicle, max 14 mm – GnRH antagonist s.c., 0.25 mg/day
- **Diam. ≥ 17 mm, ≥ 2 follicles randomized to either:**
- **hCG (10.000 IU) or Buserelin (0.5 mg) s.c.**
  Oocyte retrieval 34 h after ovulation induction
- **A maximum of two embryos transferred**

Luteal phase support:

- **Micronized vaginal progesterone, 90 mg/day**
- **Oestradiol 4 mg/day per os from day of OPU+1 until OPU+14**

Use of a GnRH agonist (Buserelin) versus hCG for ovulation induction : A prospective randomised study

	GnRHa	hCG	P-value
Patients, n	**55**	**67**	
Rate of transfer, n (%)	**48 (87)**	**57 (85)**	**NS**
Pos. hCG per ET, n (%)	**14 (29)**	**25 (44)**	**> 0.10**
Clinical pregnancy, n (% per cycle)	**3 (6)**	**24 (36)**	**P=0.002**
Implantation rate, n (%)	**3/89 (3)**	**33/97 (34)**	**P=0.0001**
Early pregnancy loss, n (%)	**11 (79)**	**1 (4)**	**P=0.005**

*) Fishers exact test

Humaidan et al., 2005

Conclusions

- Significantly more MII oocytes (16%) in the GnRHa group, indicating a positive effect of the mid cycle FSH surge on oocyte maturation
- Significantly lower implantation - and clinical pregnancy rate in the GnRHa group (fresh cycle)
- Significantly higher rate of early pregnancy loss in the GnRHa group
- Low reproductive outcome attributed to a luteal phase insufficiency despite supplementation with progesterone and oestradiol

Humaidan et al., 2005

GnRH agonist for triggering of ovulation

Additional studies in:

- Follicular fluid
  (Yding Andersen et al., Hum Reprod 2006)
- Live birth after GnRHa versus hCG triggering
  (Griesinger et al., Fertil Steril 2007)

Luteal phase insufficiency

Physiology

- Supraphysiological steroid level (oestradiol and progesterone) in early-mid luteal phase exert a negative feed-back on the hypothalamic-pituitary axis reducing LH secretion in early luteal phase.

  (Tavaniotou and Devroey, 2006; Tavaniotou et al., 2001)

- GnRHa triggering leads to significantly reduced total amounts of gonadotrophins (LH and FSH) released by the pituitary due to profile and duration of surge

  (Gonen et al., 1990; Itskovitz et al., 1991)

The role of LH in the luteal phase

LH plays a crucial role in the luteal phase

- Totally responsible for steroidogenic activity of the corpus luteum
  (Casper and Yen, 1979)
- Upregulation of growth factors, VEGFA, FGF2
  (Sugino et al., 2004; Wang et al., 2002)
- Upregulation of cytokines involved in implantation
  (Licht et al., 2001)
- Stimulation of LH receptors in endometrium
  (Rao, 2001; Tesarik et al., 2003)

LH levels

LH mean mid-luteal phase

- 6.0 IU/l in natural cycle (Tavaniotou and Devroey 2003)
- 1.5 IU/l in GnRHa group (Humaidan et al, 2005)
- 0.2 IU/l in hCG group (Humaidan et al, 2005)

How can we rescue the luteal phase?

Administration of 1500 IU hCG 12hrs after triggering of ovulation with 0.1mg Triptorelin normalized luteal phase in 34 cycles (IUI)

(Emperaire et al., 2004)

2500 IU hCG - 6 and 10 days after triggering of ovulation with 0.5mg Leuprolide normalized luteal phases in 22 cycles (IUI)

(Penarrubia et al., 1998)

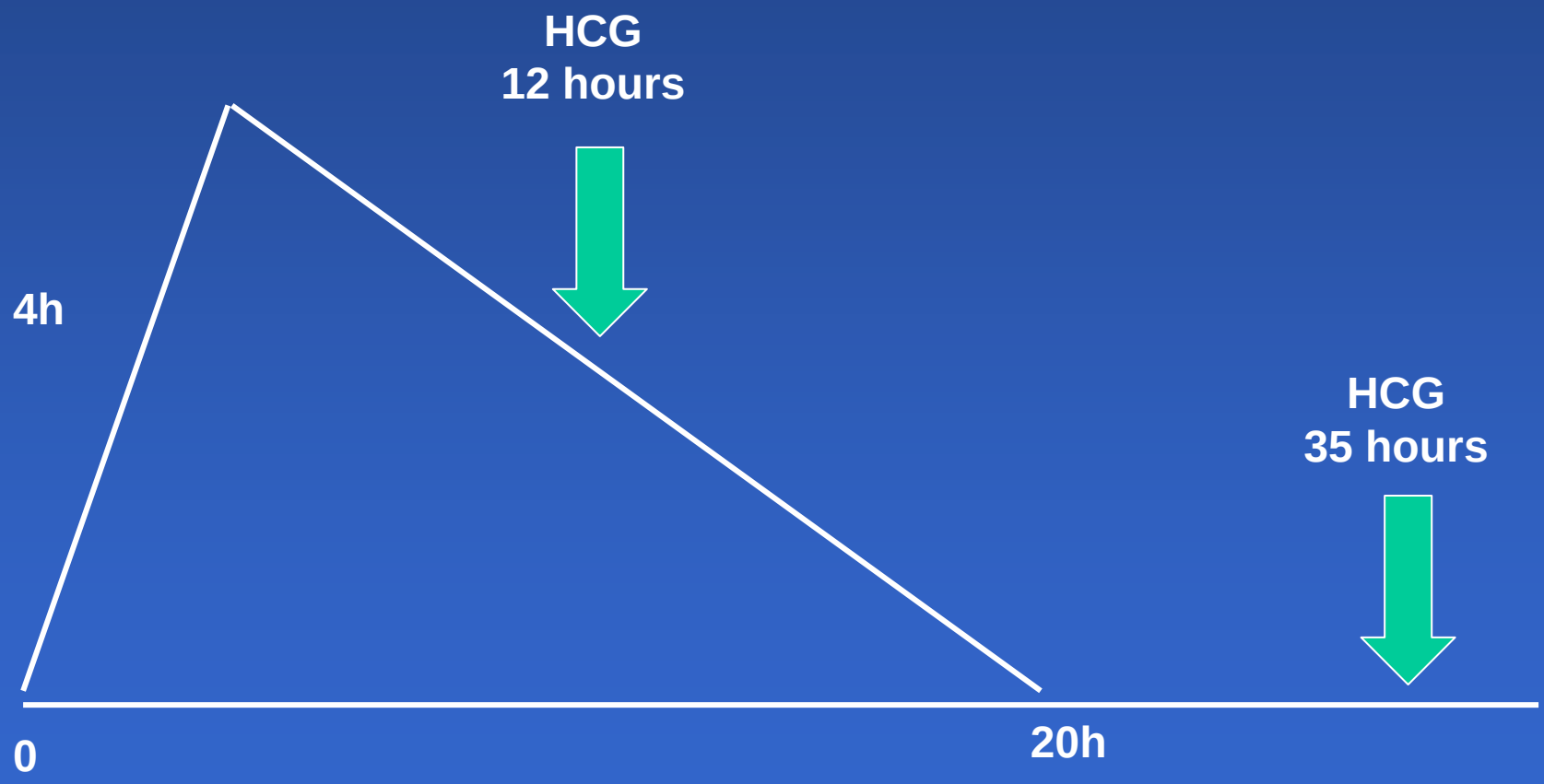
Rescue of corpus luteum function with periovulatory hCG supplementation in IVF/ICSI GnRH antagonist cycles in which ovulation was triggered with a GnRH agonist – A pilot study

Humaidan P.[1], Bungum L.[1], Bungum M.[1], and Yding Andersen C.[2]

[1]The Fertility Clinic, Viborg Hospital (Skive), Denmark , [2] Laboratory of Reproductive Biology,, University Hospital of Copenhagen, Denmark

Humaidan et al.,2006

Rescue of luteal phase



Humaidan et al., 2006

GnRHa triggered ovulation and the effect of hCG 1500 IU on mid-luteal progesterone and pregnancy outcome

	No cycles	OPU + 7 progesterone, mean,nmol/l	ET, No	Pos hCG, n (% per ET)	CPR/Cycle (%)
hCG	**15**	**248 ±125[c]**	**12**	**9/12 (75)**	**8/15 (53)[a]**
GnRH+ hCG 12 hrs	**17**	**60 ±33[d]**	**9**	**3/9 (33)**	**2/17 (12)[b]**
GnRH+ hCG 35 hrs	**13**	**103 ±70[e]**	**12**	**6/12 (50)**	**6/13 (46)[a]**

[c,d,e] $p<0.05$ [a,b] $p<0.02$

Humaidan et al., 2006

1500 IU hCG secures a normal pregnancy outcome in IVF/ICSI GnRH antagonist cycles in which ovulation was triggered with GnRH agonist

Humaidan P.[1], Ejdrup Bredkjær H.[2], Westergaard L.G.[3] and Yding Andersen C.[4]

[1]The Fertility Clinic, Viborg Regional Hospital (Skive), Denmark ,
[2]The Fertility Clinic, Holbæk Hospital, Denmark,
[3]The Fertility Clinic, Odense University Hospital, Denmark,
[4]Laboratory of Reproductive Biology, University Hospital of Copenhagen.

Humaidan et al., Fertil Steril, 2010

Aims of study

- To compare reproductive outcome and luteal phase endocrine profiles in patients undergoing IVF/ICSI following a flexible GnRH antagonist protocol

- Randomisation to final oocyte maturation with either 10.000 IU hCG or a single bolus of GnRHa (Buserelin) followed by a bolus of 1500 IU hCG given i.m. 35 hours after triggering of ovulation.

- Open label prospective randomised three-centre study including a total of 305 cycles.

Results
Oocyte maturation, fertilization and cleavage in GnRHa/hCG vs. hCG-group

	GnRHa/hCG	hCG	P –value *
Patients	**152**	**150**	
Oocytes (Mean)	**1361 (8.9)**	**1420 (9.3)**	NS
M II , only ICSI (%)	**465/546 (85)**	**468/574 (81)**	P= 0.06
2 PN oocytes total (%)	**790/1361 (58)**	**780/1420 (55)**	P= 0.05
Good available embryos, %	**30**	**30**	NS

*) Fishers exact test

Reproductive Outcome

	GnRHa/hCG	hCG	P-value
Patients, n	152	150	
Rate of transfer, n (%)	130/152 (86)	138/150 (92)	0.054
Pos. hCG per ET, n (%)	63/130 (48)	66/138 (48)	0.36
Ongoing PR per patient (%)	40/152 (26)	49/150 (33)	0.69
Delivery rate/patient	36/152 (24)	47/150 (31)	0.16
Early pregnancy loss, n (% of pos)	13/63 (21)	11/66 (17)	0.36

*) Fishers exact test

Humaidan et al., Fertil Steril, 2010

Reproductive Outcome

	GnRHa (2005)	GnRHa + hCG 1500	hCG
Patients, n	55	152	150
Rate of ET, n (%)	48/55 (87)	130/152 (86)	138/150 (92)
Pos. hCG/ET, n (%)	14/48 (29)	63/130 (48)	66/138 (48)
Ongoing PR per pat (%)	3/55 (6)	40/152 (26)	49/150 (33)
Delivery rate per pat (%)	3/55 (6)	36/152 (24)	47/150 (31)
Early PL, n (%)	11/14 (79)	13/63 (21)	11/66 (17)

Reduction of OHSS ?

HCG triggering :

3/150: 2% (1 severe/2 moderate)

GnRHa triggering:

0/152

Humaidan et al., fertil Steril 2010

Conclusions

Supplementation with 1500 IU hCG at 35 hours

- Provides a clinical alternative to hCG induced ovulation
- 4% more MII oocytes
- Reduction of OHSS?

Humaidan et al., Fertil Steril 2010

Luteal phase rescue in high-risk OHSS patients by GnRHa triggering in combination with low-dose HCG - a pilot study.

- 12 patients with > 25 follicles > 11 mm prospectively enrolled to have final oocyte maturation with 0.5 mg Buserelin followed by a bolus of 1500 IU hCG 35 hours later
- All patients transferred

Humaidan RBM Online 2009

Luteal phase rescue in high-risk OHSS patients by GnRHa triggering in combination with low-dose HCG - a pilot study.

Stimulation, oocytes and fertilization

Stimulation (days)	10.8 ± 4.9
Total FSH (IU)	1141.9 ± 460.3
Total dose of antagonist (mg)	1.0 ± 0.0
Serum oestradiol day of ovulation induction (nmol/l)	18.6 ± 10.5
Oocytes, n	21.5 ± 6.0
Fertilization rate, n (%)	138/258 (53.5)
Cleavage rate, n (%)	120/258 (46.5)
Embryos transferred, n	1.7 ± 0.5

Values are mean ± SD

Pregnancy outcome

Pos HCG/cycle, n (%)	10/12 (83.3)
Early pregnancy loss, n (%)	4/10 (40.0)
Clinical ongoing/cycle, n (%)	6/12 (50.0)
Live birth /cycle, n (%)	6/12 (50.0)

Humaidan RBM Online 2009

Luteal phase rescue in high-risk OHSS patients by GnRHa triggering in combination with low-dose HCG - a pilot study.

OHSS?

- No patient developed early onset OHSS
- One patient developed moderate, late onset OHSS

Humaidan RBM Online 2009

GnRH agonist for triggering of ovulation

Personalized luteal phase support:

- Normo-responder patient (< 14 follicles)
- ✓ Repeat bolus of hCG (1500 IU, OPU + OPU+5) + E2/P4 until 7 weeks

- OHSS risk patient
- ✓ One bolus of hCG (1500 IU, OPU) + E2/P4 until week 7
- ✓ Total freeze

GnRH agonist for triggering of ovulation

Which dose and regimen of GnRHa is the optimal?

- 123 women treated with 14 different regimens:
  Buserelin, Triptorelin, Leuprolide, Nafarelin, Buserelin i.n.
  No difference regarding duration of surge of gonadotrophins
  (Parneix et al., 1996)

Most commonly used GnRHa triggering doses:

- Buserelin 0.5mg s.c. – 0.2mg i.n.
- Triptorelin 0.2mg s.c.
- Leuprolide 1mg s.c.

Conclusion
GnRH agonist versus hCG for triggering of ovulation

GnRHa triggering:

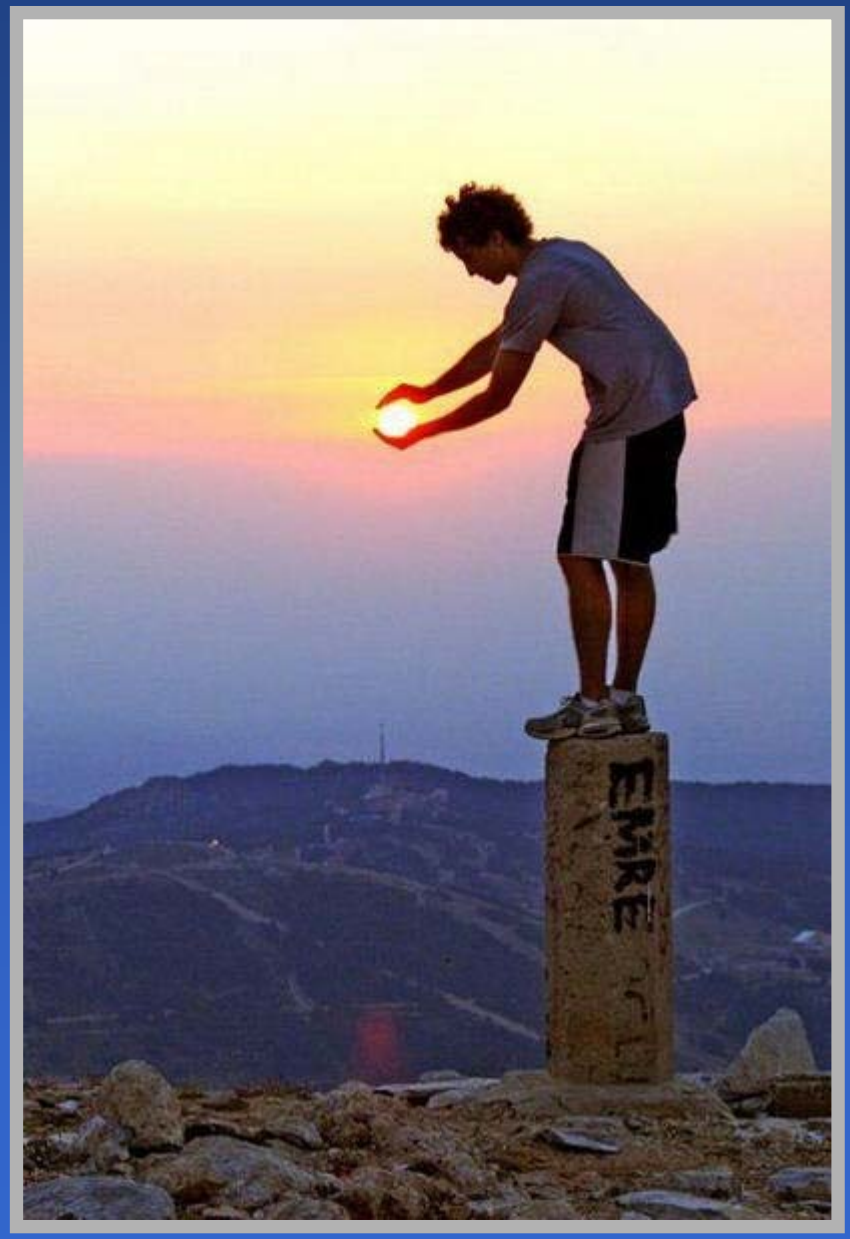
- More physiological
- More MII oocytes
- Expected decrease in moderate/severe OHSS
- Less abandonned cycles
- Higher patient convenience?
- The option to perform a total freeze in cases with an excessive response to stimulation with no risk of OHSS in the patient
- The protocol of choice in oocyte donors

GnRHa triggering of final oocyte maturation

Golden opportunity for:

Paradigm shift of ovulation triggering concept in ART

Hopefully for the benefit of our patients



Thank You for Your attention

Peter.Humaidan@viborg.rm.dk

The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study

Lawrence Engmann, M.D., Andrea DiLuigi, M.D., David Schmidt, M.D., John Nulsen, M.D., Donald Maier, M.D., and Claudio Benadiva, M.D.

Center for Advanced Reproductive Services, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Dowling South Building, University of Connecticut Health Center, Farmington, Connecticut

Engmann et al, Fertil Steril 2008, 89:84

Study Design

- **< 40years, FSH < 10 with**
- **PCOS or PCO morphology**
- **Or Previous High Response**

Randomization

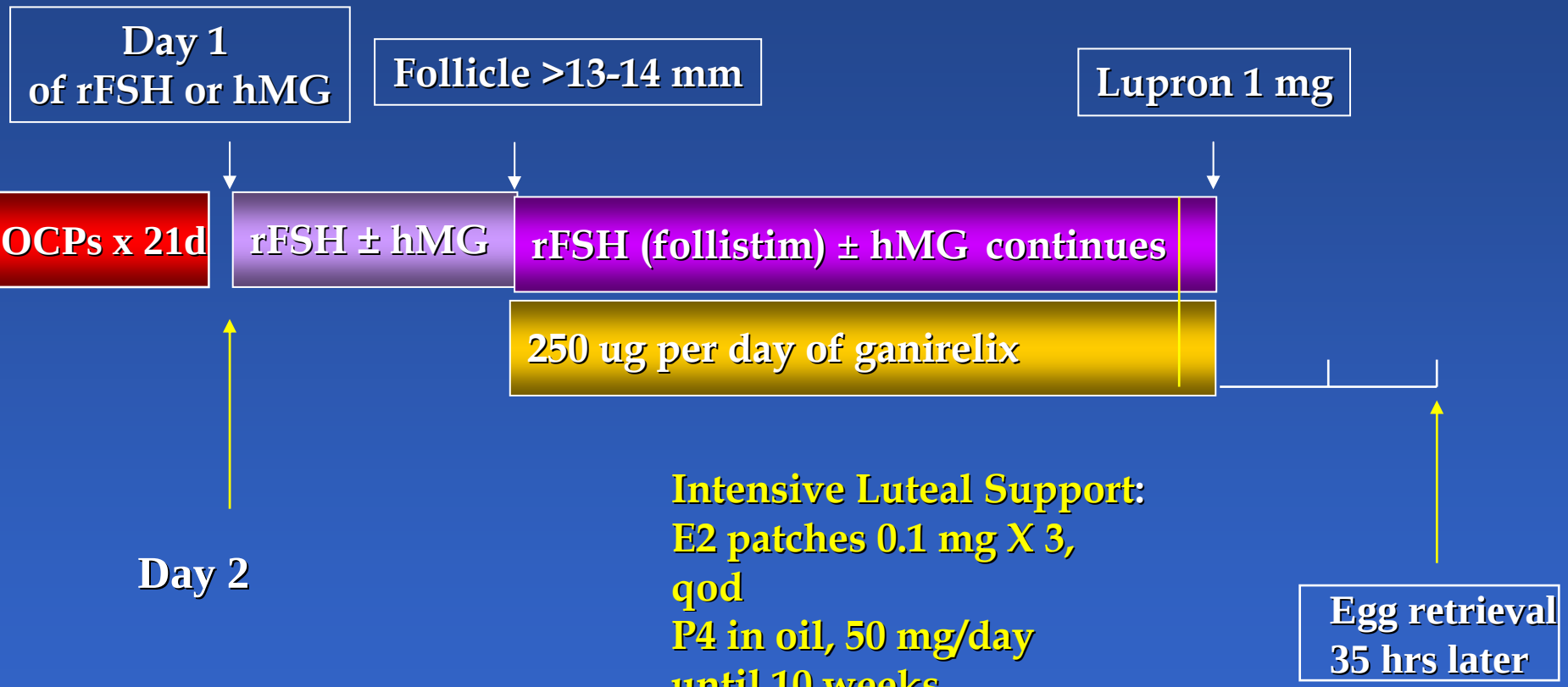
Dual suppression OCP's & Lupron

HCG trigger

OCP's + Ganirelix

Lupron trigger

GnRHa triggering protocol



Intensive Luteal Support:
E2 patches 0.1 mg X 3, qod
P4 in oil, 50 mg/day until 10 weeks.
E2+P4 levels monitored (P4 > 20ng/ml; E2 > 200pg/ml)

Results

TABLE 2

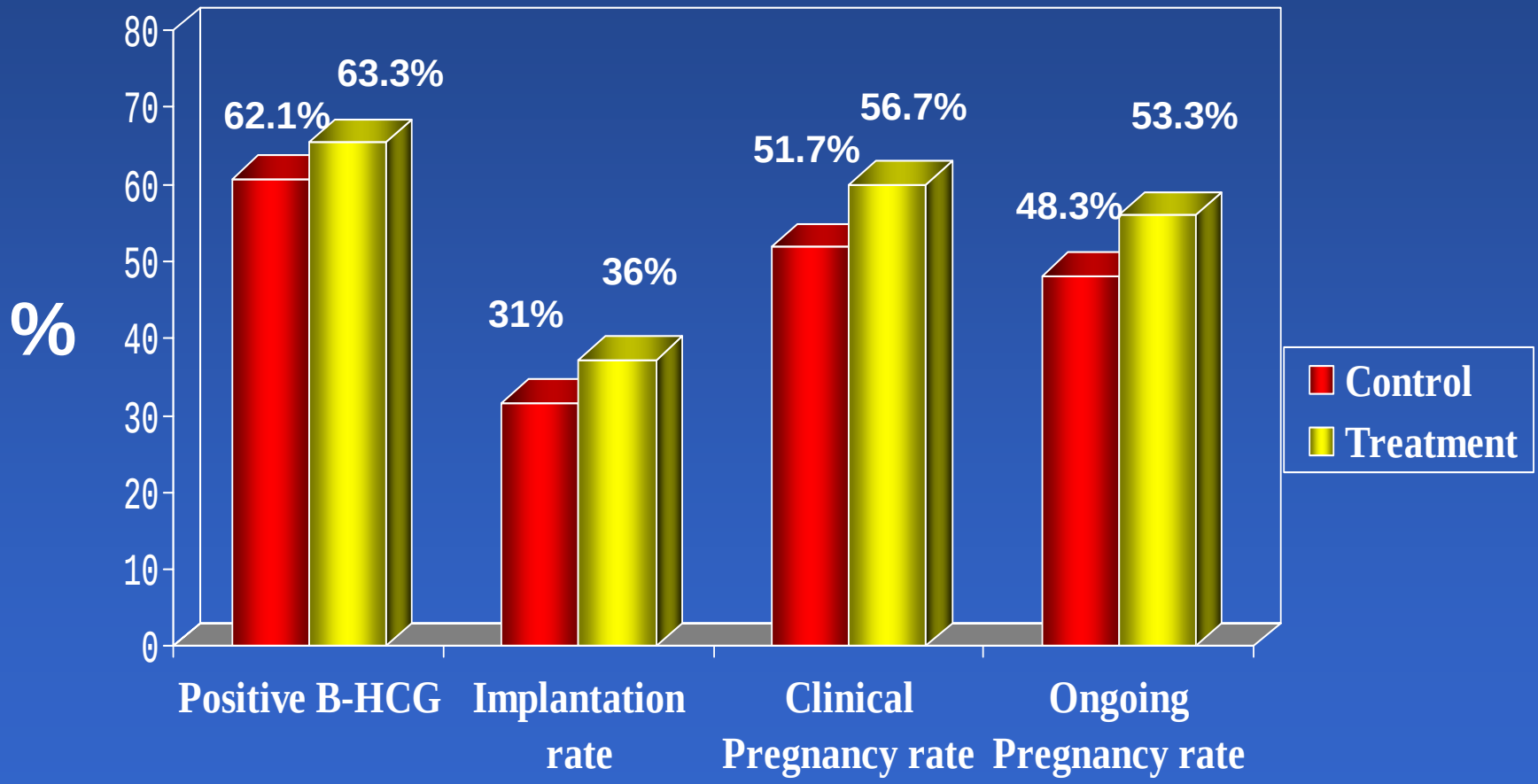
Outcome of ovarian stimulation.

	Study group (n = 30)	Control group (n = 29)	*P* value
Duration of ovarian stimulation (days)	9.9 ± 1.7	9.6 ± 1.7	NS
Total dose of gonadotropins (IU)	1589 ± 511	1527 ± 534	NS
Serum E_2 on day of trigger (pg/mL)	2645 ± 1101	2658 ± 1122	NS
Oocytes (n)	20.2 ± 9.9	18.8 ± 10.4	NS
Proportion of M11 oocytes (%)	81.0 ± 16.3	83.8 ± 13.2	NS
Fertilization rate (%)	71.6 ± 14.1	74.9 ± 17.3	NS
Embryos transfered (n)	2.0 ± 0.2	2.2 ± 0.6	NS
Embryos frozen (n)	3.9 ± 4.4	4.3 ± 4.7	NS
Midluteal ovarian volume (cm^3)	36.6 ± 22.2	129.0 ± 77.4	< .01
Serum E_2 on day of embryo transfer (pg/mL)	485 ± 219	1320 ± 695	< .01
Midluteal serum E_2 (pg/mL)	283 ± 216	663 ± 556	< .01
Serum P on day of embryo transfer (ng/mL)	25 ± 14	117 ± 61	< .01
Midluteal serum P (ng/mL)	28 ± 8	46 ± 50	NS

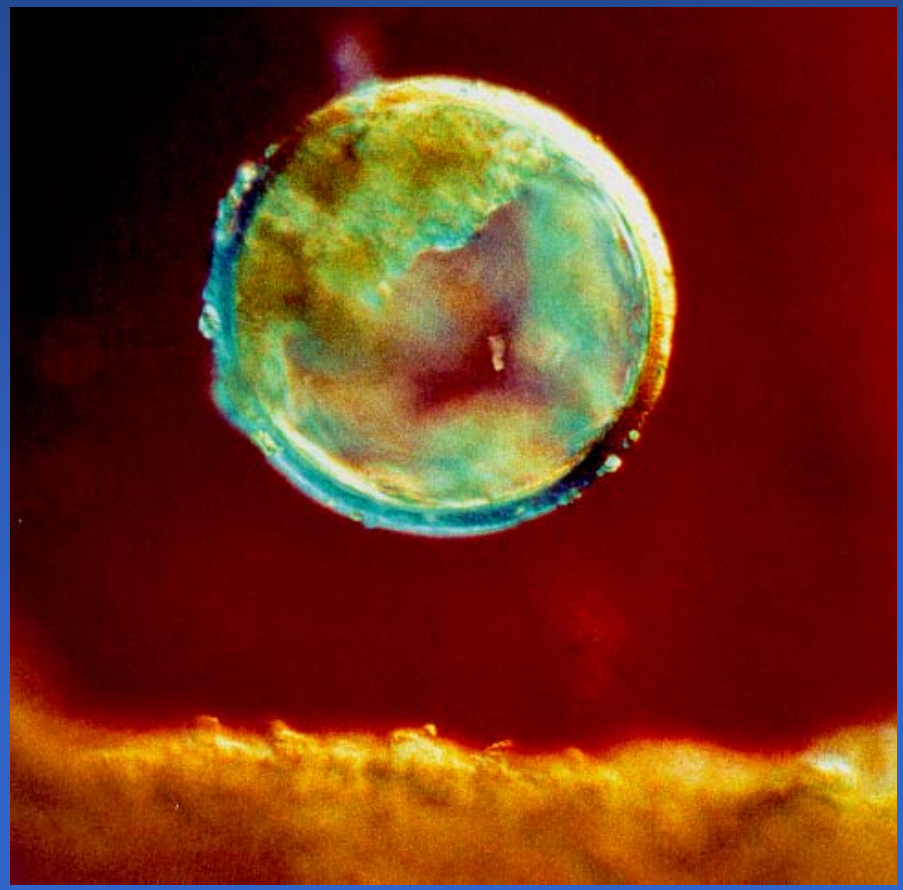
Engmann. GnRH agonist trigger and OHSS prevention. Fertil Steril 2008.

Engmann et al, Fertil Steril 2008, 89:84

Reproductive outcome



Implantation and pregnancy rates in IVF/ET



~70% of the embryos transferred in ART, do not implant.…

The luteal phase - the last black box in ART

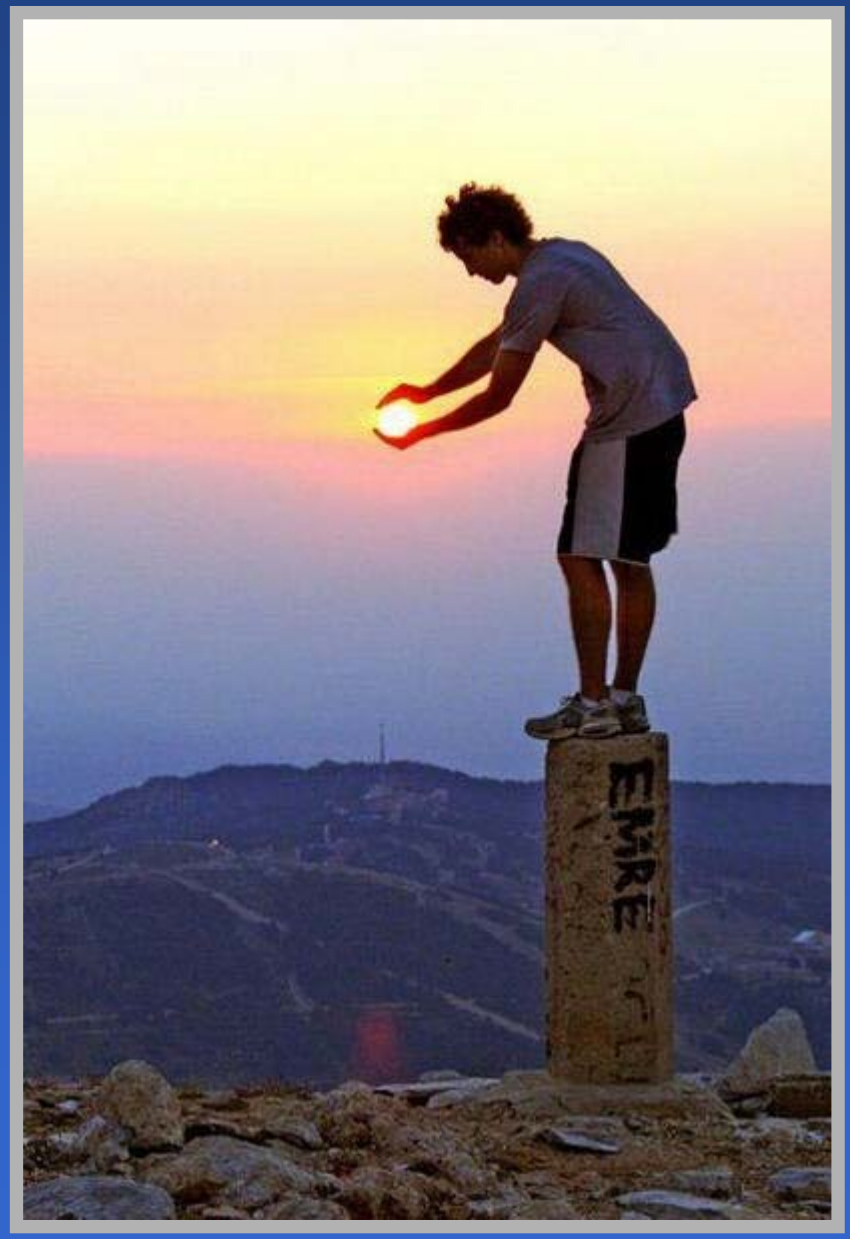
Hysteroscopic view of uterine cavity

GnRHa triggering of final oocyte maturation

Golden opportunity for:

Paradigm shift in ovulation triggering concept in ART

Hopefully for the benefit of our patients



GnRHa to trigger final oocyte maturation: a time to reconsider

Table I Main characteristics, luteal phase support and reproductive outcome of published studies on GnRHa triggering of final oocyte maturation (intention to treat)

Reference	Trial type	GnRH-antagonist protocol	Ovulation trigger	*n*	LPS	Clinical Pregnancy% (*n*)	Ongoing pregnancy% (*n*)	Delivery rate% (*n*)	*P*-value
Humaidan *et al.* (2005)	RCT	Flexible, multiple dose	GnRHa	55	P 90 mg (8%) vag + 4 mg oral E2	6 (3/55)	6 (3/55)	6 (3/55)	0.002
			hCG	67	P 90 mg (8%) vag + 4 mg oral E2	36 (24/67)	36 (24/67)	36 (24/67)	
Kolibianakis *et al.* (2005)	RCT	Fixed, multiple dose	GnRHa	50	P 600 mg vag + 4 mg oral E2	*	5.6 (1/18) (Brussels) 2.9 (1/34) (Lubeck)	*	0.005
			hCG	54	P 600 mg vag + 4 mg oral E2	*	41.7 (10/24) (Brussels) 16.7 (5/30) (Lubeck)	*	
Pirard *et al.* (2006)	RCT	Flexible, multiple dose	GnRHa	6	GnRHa nasal 100 µg IN 3×d	33 (2/6)	*	*	0.51
			hCG	6	P 600 mg	17 (1/6)	*	*	
Humaidan *et al.* (2006)	RCT	Flexible, multiple dose	GnRHa	13	1500 IU hCG OPU day + P 90 mg (8%) vag + 4 mg oral E2	46 (6/13)	38 (5/13)	38 (5/13)	0.43
			hCG	15	P 90 mg (8%) vag + 4 mg oral E2	53 (8/15)	53 (8/15)	53 (8/15)	
Babayof *et al.* (2006)	RCT	Flexible, multiple dose	GnRHa	15	P 50 i.m. → 100 mg ± 4 mg oral E2	20 (3/15)	6.6 (1/15)	6.6 (1/15)	0.46
			hCG	13	P 50 i.m. → 100 mg ± 4 mg oral E2	31 (4/13)	15 (2/13)	15 (2/13)	
Engmann *et al.* (2008)	RCT	Flexible, multiple dose	GnRHa	33	P 50 i.m. → 75 mg + E2 patches 3–4 × 0.1 mg/2d ± 4 mg oral E2	52 (17/33)	48 (16/33)	*	0.90
		OCP/GnRHa	hCG	32	50 mg P i.m.	47 (15/32)	44 (14/32)	*	
Humaidan *et al.* (2009)	RCT	Flexible, multiple dose	GnRHa	152	1500 IU hCG OPU day + P 90 mg (8%) vag + 4 mg oral E2	33 (50/152)	26 (40/152)	24 (36/152)	0.16
			hCG	150	P 90 mg (8%) vag + 4 mg oral E2	37 (55/150)	33 (49/150)	31 (47/150)	
Humaidan (2009)	Observational Uncontrolled OHSS high-risk	Flexible, multiple dose	GnRHa	12	1500 IU hCG OPU day + P 90 mg (8%) vag + 4 mg oral E2	50 (6/12)	50 (6/12)	50 (6/12)	–

LPS = luteal phase support; i.m. = intramuscular; vag = vaginal; P = progesterone; E2 = oestradiol; * = not reported; OPU = ovum pick up; OCP = oral contraceptive.

Humaidan, et al., Hum Reprod 2009

GnRHa to trigger final oocyte maturation: a time to reconsider

Table II Main characteristics and ovarian hyperstimulation syndrome (OHSS) rate in studies on GnRHa triggering of final oocyte maturation

Reference	Trial type	GnRH-antagonist protocol	Ovulation trigger	*n*	Moderate–severe OHSS % (n)
Humaidan *et al.* (2005)	RCT	Flexible, multiple dose	GnRHa	55	0 (0/55)
			hCG	67	0 (0/67)
Kolibianakis *et al.* (2005)	RCT	Fixed, multiple dose	GnRHa	50	*
			hCG	54	*
Pirard *et al.* (2006)	RCT	Flexible, multiple dose	GnRHa	6	*
			hCG	6	*
Humaidan *et al.* (2006)	RCT	Flexible, multiple dose	GnRHa	13	0 (0/13)
			hCG	15	0 (0/15)
Babayof *et al.* (2006)	RCT	Flexible, multiple dose	GnRHa	15	0 (0/15)
			hCG	13	31 (4/13)
Engmann *et al.* (2008)	RCT	Flexible, multiple dose OCP/GnRHa	GnRHa	33	0 (0/33)
			hCG	32	31 (10/32)
Humaidan *et al.* (2009)	RCT	Flexible, multiple dose	GnRHa	152	0 (0/152)
			hCG	150	2 (3/150)
Humaidan (2009)	Observational uncontrolled OHSS high-risk	Flexible, multiple dose	GnRHa	12	8 (1/12- late)

* = not reported. OCP = oral contraceptive.

Humaidan, et al.,Hum Reprod 2009

GnRHa to trigger final oocyte maturation: a time to reconsider

Table III Main characteristics and reproductive outcome in recipients and ovarian hyperstimulation syndrome (OHSS) rates in donors in studies on GnRHa triggering of final oocyte maturation in oocyte donors and after 'total freeze'

Reference	Trial type	GnRH-antagonist protocol	Ovulation trigger	*n*	Clinical pregnancy/ transfer in recipients%	Moderate–severe OHSS % (*n*)
Acevedo *et al.* (2006)	RCT oocyte donors	Fixed, multiple dose	GnRHa	30	46	0 (0/30)
			hCG	30	53	16.6 (5/30)
Bodri *et al.* (2008)	Retrospective cohort study, oocyte donors	Flexible, multiple dose	GnRHa	1046	38.8	0 (0/1046)
			hCG	1031	42.4	1.3 (13/1031)
Griesinger *et al.* (2007a, b)	Observational un-controlled, OHSS high-risk patients with 'total freeze'	Fixed, multiple dose	GnRHa	20	–	0 (0/20)

Humaidan, et al., Hum Reprod 2009

Levels of the EGF-like peptide amphiregulin are significantly reduced in follicular fluid after GnRHa triggering of final oocyte maturation

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Background

Amphiregulin (AR)

- EGF-like growth factor
- Synthesized in granulosa cells
- Mediator (transmittor) of LH effects during periovulatory period
- Involved in oocyte maturation
- Expression rapidly and transiently increased in follicular fluid (FF) in response to LH/hCG

Background

FF Amphiregulin (AR) after hCG triggering of final oocyte maturation in IVF/ICSI:

Inversely related to

oocyte quality
fertilization rate
pregnancy rate

Inoue et al., Fertil Steril, 2009

Aims of study

To compare FF levels of amphiregulin in patients undergoing IVF/ICSI following a flexible GnRH antagonist protocol:

- Randomisation to final oocyte maturation with either 10.000 IU hCG or 0.5 mg of GnRHa (Buserelin)
- Open label prospective randomised three-centre study
- Controls:

  15 FF from small (1-8 mm) antral follicles (natural cycle)

  12 FF from preovulatory follicles - aspirated after endogenous surge (natural cycle)

Materials and methods

FF study samples:

- Two FF samples collected from each patient
- First follicle punctured bilaterally - without contamination of flushing media, centrifuged (500 x *g*), supernatant stored at - 20°C.

Results

96 patients randomised

hCG	GnRHa
48 cycles	48 cycles

No significant differences between GnRHa and hCG group:

- Age, BMI, base-line FSH and LH
- Infertility diagnosis
- Previous IVF/ICSI attempts
- Stimulation

TABLE I

FF concentration: E2, P4, Inh B,VEGF and Amphiregulin

Data are mean ± SEM

	TOTAL	GnRHa	hCG	GnRHa vs hCG T-test
N: OPU	96	48	48	
N: FF aspirates	146	73	73	
E2, ng/ml	305 ± 17	328 ± 20	282 ± 28	NS
P4, ng/ml	13297 ± 466	11758 ± 629	14835 ± 647	0.0004
Inhibin B, ng/ml	27 ± 1.6	25 ± 1.9	29 ± 2.5	NS
VEGF, pg/ml	1195 ± 61	1199 ± 83	1192 ± 91	NS
Amphiregulin, ng/ml	62 ± 3.5	51 ± 3.5	71 ± 6.0	0.003

TABLE II

FF concentration: VEGF and Amphiregulin in controls vs GnRHa and hCG

Data are mean ± SD

	Small antral foll.	Preov. foll. Natural cycle	GnRHa	hGG
N: FF aspirates	15	12	73	73
VEGF, pg/ml	527 ± 517[a]	2248 ± 924[b]	1199 ± 83[c]	1192 ± 91[c]
Amphiregulin, ng/ml	1.5 ± 1.5[d]	68 ± 25[e]	51 ± 3.5[f]	71 ± 6.0[g]

[abcde] Groups with a different letter differ significantly ($p < 0.001$)

[fg] Groups with a different letter differ significantly ($p = 0.003$)

TABLE III

OOCYTE MATURATION, FERTILIZATION AND EMBRYO DEVELOPMENT

	TOTAL	GnRHa	hCG	Fisher's Exact Two-tailed
IVF:				
N: oocyter	685	329	356	
N: Fertilized (%)	471 (69%)	237 (72%)	234 (66%)	0.08
IVF: N embryos (% of fertilized)	405 (86%)	210 (89%)	195 (83%)	NS
ICSI:				
N: oocyter	145	78	67	
N: MII (%)	124 (86%)	72 (92%)	52 (78%)	0.017
N: Fertilized of M II (%)	112 (90%)	61 (85%)	51 (98%)	0.013
ICSI: N embryos (% of fertilized)	107(96%)	57 (93%)	50 (98%)	NS
IVF + ICSI: Transferrable N (% of oocytes retrieved)	309 (45%)	167 (51%)	142 (40%)	0.005
Transferrable per cycle (mean±SEM)	3.2 ± 0.3	3.5 ± 0.4	3.0 ± 0.4	NS
Transferred per cycle (mean±SEM)	1.65 ± 0.06	1.67 ± 0.07	1.63 ± 0.1	NS

GnRH agonist for triggering of ovulation

GnRHa versus hCG triggering summary:

- FF AR significantly reduced ($p = 0.003$)
- 14 % more MII oocytes ($p = 0.017$)
- 11 % more transferable embryos ($p = 0.005$)

Conclusion

- Confirms the up-regulation of amphiregulin in final oocyte maturation
- Mode of triggering has an impact on amphiregulin synthesis
- Accumulating doses of LH activity increase concentrations of amphiregulin
- No difference in FF VEGF
- After GnRHa triggering more MII oocytes (ICSI) and transferable embryos (IVF + ICSI) – impact of AR?