Follicular fluid hormonal and non-hormonal composition after GnRHa

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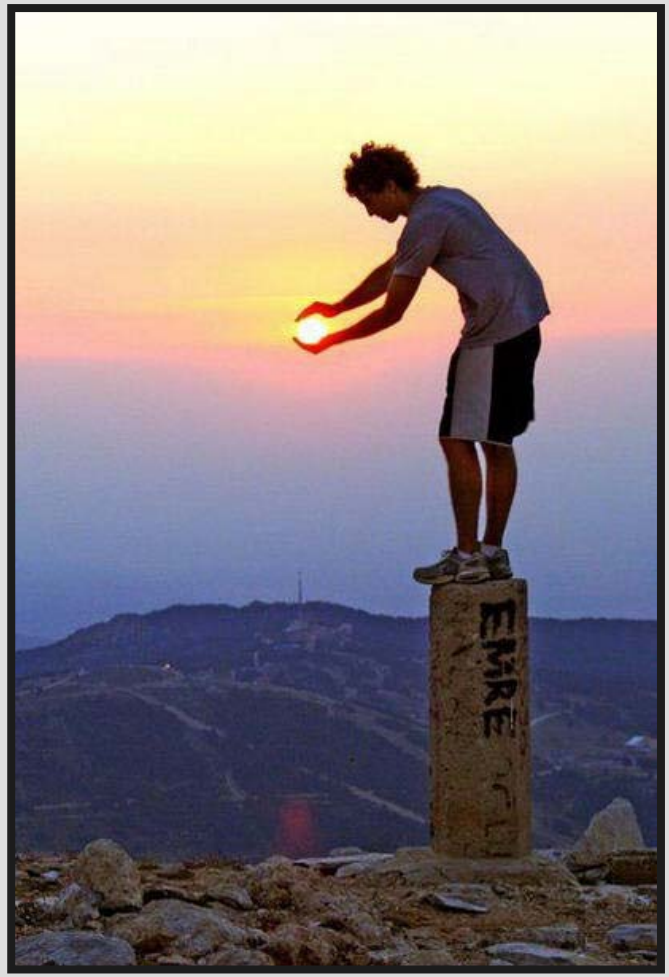
ESHRE Campus "GnRH Agonist for Triggering of Final Oocyte Maturation Time for a Paradigm Shift" Madrid, Spain – 3 December 2010

Outline

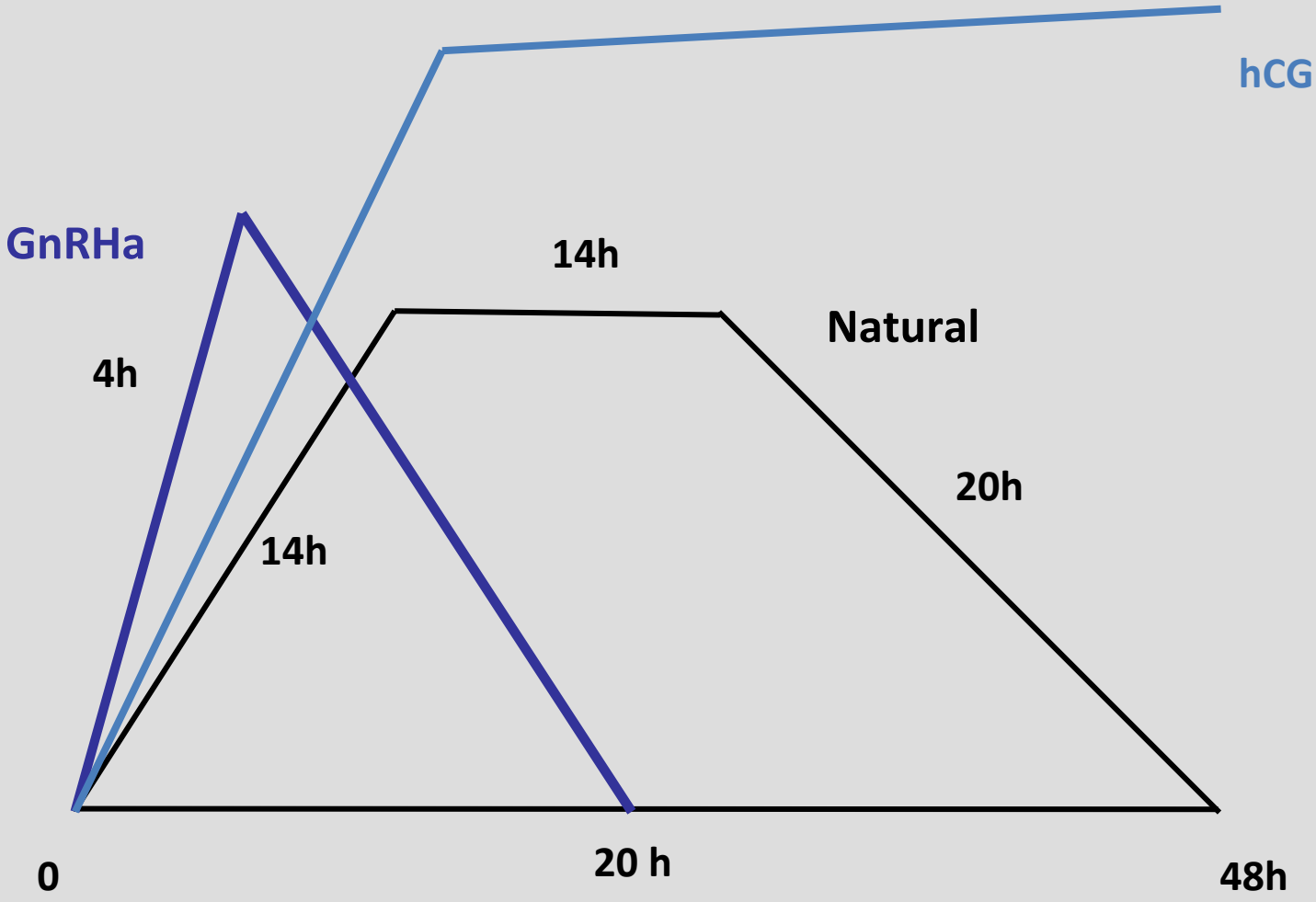
- Effects of the mid-cycle surge of gonado-tropins on the preovulatory follicle
- Follicular fluid hormone profile from GnRHa and hCG induced patients
- Profile of factors in follicular fluid from GnRHa and hCG induced patients of importance for oocyte maturation



GnRH agonist used for ovulation induction in women following a GnRH antagonist protocol

- GnRH antagonists protocols allows ovulation to be induced with either hCG or GnRH agonists
- GnRHa induces an endogenous flare-up of gonadotropins
- but also a pituitary down-regulation resulting in an attenuated surge of gonadotropins

The midcycle surge of gonadotropings: GnRHa versus the natural cycle



Hoff *et al*., 1983 Itskovits *et al*., 1991

Differences between GnRH agonist and hCG induced trigger of ovulation

- Duration and amplitude of the GnRHa induced ovulation is a lot shorter
- The total LH-like activity in hCG stimulated ovulation induction is a lot higher
- The GnRHa induced ovulation produces a combined FSH and LH surge

Main physiological functions of the midcycle surge of gonadotropins

- Induce resumption of meiosis of the enclosed oocyte
- Induce the ovulatory process itself and expulsion of oocyte
- Prepare the function of the corpus luteum in order to sustain the luteal phase

These functions have different sensitivity towards the amplitude and composition of the midcycle surge of gonadotropins – at least in rodent models!

Sensitivity of preovulatory follicles to gonadotropins

- At around 30 % of the preovulatory surge meiotic resumption is induced
- At around 85 % of the preovulatory surge ovulation itself is induced
- FSH induces LHR formation in the developing corpus luteum

Dose of LH (μg)	N	Number of follicles	Percentage luteinized	Percentage ovulated
0.25	5	12.6 ± 0.8	41.3 ± 19.6	–
0.50	5	12.0 ± 0.8	90.1 ± 5.0	–
1	4	11.5 ± 0.6	100	–
2	4	13.0 ± 0.4	94.4 ± 3.6	5.6 ± 3.6
4	4	13.5 ± 0.6	71.5 ± 19.7	28.5 ± 19.7
8	4	12.8 ± 1.0	54.0 ± 10.0	46.0 ± 10.0

Mattheij JA *et al.,* Eur J Endocrinol, 1995;**132:**91
Vermeiden JPW and Zeilmaker GH, Endocrinol, 1974; **95**:341

Does GnRH agonist for triggering of ovulation induce changes in the preovulatory follicle different from those of hCG?

And to what extent does a possible change affect oocyte maturation and implantation potential?

Characteristics of follicular fluid from women receiving either Buserelin or hCG to induce ovulation

Claus Yding Andersen[1],

P Humaidan[2], H Ejdrup Bredkjær[3], L Bungum[2], M Bungum[2], ML Grøndahl[3], J Ingemanssen[3] & LG Westergaard[4]

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Yding Andersen et al., Hum Reprod, **21**, 2006

Aim

- **To compare hormonal profiles of follicular fluid from women undergoing IVF/ICSI following a flexible GnRH antagonist protocol – randomized to final oocyte maturation with either hCG or a single bolus of GnRHa (Buserelin)**

Open label prospective randomised two-centre study

FF from our original study in which the reproductive outcome was poor

Serum concentrations of oestradiol and progesterone (nmol/l) in GnRHa versus hCG-group (mean ± SD)

Day	Oestradiol		Progesterone	
	Buserelin	hCG	Buserelin	hCG
Ovulation induction	7.1 ± 4	6.4 ± 3	5.5 ± 5.8	5.0 ± 3.0
OPU	4.2 ± 2	4.4 ± 2	28 ± 18[c]	49 ± 33[c]
OPU + 7 days	2.9 ± 2[a]	7.1 ± 4[a]	39 ± 30[d]	283 ± 205[d]
OPU + 14 days	2.7 ± 2[b]	5.6 ± 5[b]	ND	ND

[abcd]: P<0.001 Student t-test

Hormonal profile of follicular fluid from women receiving either Buserelin or hCG to induce ovulation

Number of samples

Agonist		hCG	
64 (32)		74 (37)	
Neg. hCG	Pos. hCG	Neg. hCG	Pos. hCG
44 (22)	20 (10)	44 (22)	30 (15)

Hormonal profile of follicular fluid from women receiving either Buserelin or hCG to induce ovulation

Agonist			hCG		
FSH	LH	hCG	FSH	LH	hCG
6,3 ± 0,6	11,1 ± 0,6	ND	3,3 ± 0,2	3,6 ± 0,3	139 ± 8
M II oocytes : 84 %			M II oocytes : 63 %		

No significant differences between pregnant and non-pregnant women

Hormonal profile of follicular fluid from women receiving either Buserelin or hCG to induce ovulation

Agonist		hCG	
Oestradiol (µmol/l)			
1,9 ± 0,2		1,8 ± 0,2	
Neg. hCG	Pos. hCG	Neg. hCG	Pos. hCG
1,9 ± 0,2	1,9 ± 0,9	1,9 ± 0,2	1,7 ± 0,2
Progesterone (µmol/l)			
70 ± 4*		90 ± 6*	
Neg. hCG	Pos. hCG	Neg. hCG	Pos. hCG
73 ± 5**	48 ± 5**	86 ± 4	90 ± 6

Data: Mean ± SEM

Hormonal profile of follicular fluid from women receiving either Buserelin or hCG to induce ovulation

Agonist		hCG	
Inhibin-A (ng/ml)			
37,4 ± 4,8		40,1 ± 3,1	
Neg. hCG	Pos. hCG	Neg. hCG	Pos. hCG
44,7 ± 7,1*	24,8 ± 2,3*	37,6 ± 4,3	43,6 ± 5,1
Inhibin-B (ng/ml)			
35,6 ± 2,8		40,1 ± 3,1	
Neg. hCG	Pos. hCG	Neg. hCG	Pos. hCG
35,3 ± 3,3	36,7 ± 5,5	39,2 ± 4,2	41,3 ± 4,7

Data: Mean ± SEM

Conclusions

- The poor reproductive outcome of inducing ovulation by a GnRH agonist does not seem to be caused by inappropriate intrafollicular oocyte maturation
- Despite significant differences in FF levels of progesterone they were too small to explain a difference
- On the contrary more oocytes may actually reach M II
- What about factors affecting oocyte maturation directly and substances that affect the corpus luteum function.

Yding Andersen et al., Hum Reprod, **21**, 2006

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

P.Humaidan [1], L.G.Westergaard [2], AL.Mikkelsen [3], M. Fukuda [4] and
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[5] Laboratory of Reproductive Biology, University Hospital of Copenhagen.

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 the GnRHa and hCG group:

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

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 12-15 mm preovulatory follicles prior to ovulation induction

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

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

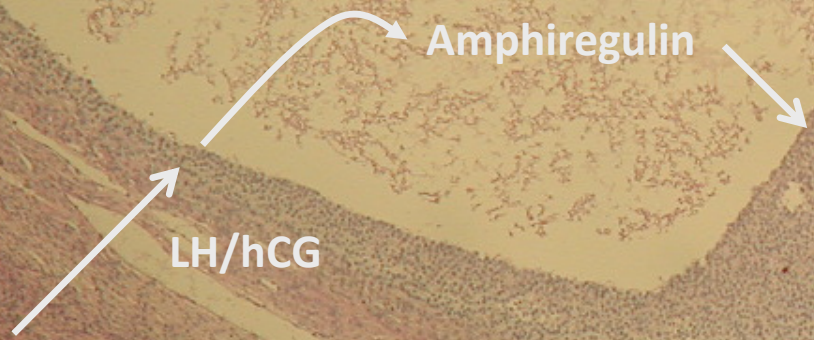
Regulation of oocyte maturation

Amphiregulin:

- EGF-like peptide produced by the mural granulosa gells but not the cumulus cells
- Released by the LH-activity of the mid-cycle surge
- Induce resumption of meiosis

FSH:

- Induce yet unidentified substances that promote oocyte maturation (e.g. meiosis activating substance)



Prolactin:

- Produced by the pituitary, endometrium and granulosa cells
- PRL inhibits estradiol production and stimulates progesterone production in granulosa cells
- PRL also interferes with FSH ± action by suppressing LH receptor expression at sites downstream of cAMP synthesis in GC
- Analysis of PRL knockout mice models has led to a conclusion that PRL is essential for corpus luteum functions

Follicular Fluid concentration of E2, P4, Inhibin B, VEGF, Amphiregulin and Prolactin

	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
Prolactin, ng/ml	17,2 ± 1,9	17,2 ± 2,3	17,3 ± 3,1	NS

Data are mean ± SEM

Follicular fluid concentration of VEGF and Amphiregulin in controls versus GnRHa and hCG

	Small antral follicles	Preovulatory follicular fluid			
		Before the midcycle surge	Natural cycle	GnRHa	hGG
No. FF aspirates	15	15	12	73	73
VEGF, pg/ml	527 ± 517[a]	ND	2248 ± 924[b]	1199 ± 83[c]	1192 ± 91[c]
Amphiregulin, ng/ml	1.5 ± 1.5[d]	< 0.5	68 ± 25[e]	51 ± 3.5[f]	71 ± 6.0[g]

Groups with a different letter differ significantly ($p < 0.003$)

Data are mean ± SD

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

Triggering ovulation with GnRHa does not compromise embyro implantation rates

TABLE 2

Triggering ovulation with GnRH agonist does not compromise embryo implantation rates.

	GnRH agonist	hCG	*P*
Oocytes (n)	327	288	ns
Oocytes (x)	9.1 ± 4.01	10.3 ± 6.3	ns
Fertilization rate (%)	80	65	ns
Oocytes MI (%)	6	8	ns
Oocytes MII (%)	70	76	ns
Embryo quality (%)			
Grade 1	35	50	ns
Grade 2	52	41	ns
Grade 3	7	8	ns
Multinucleated	4	1	ns
Embryos with >8 blastomeres (x)	1.5 ± 1.2	1.9 ± 1.3	ns

TABLE 3

Triggering ovulation with GnRH agonists does not compromise embryo implantation rates.

	GnRH agonist	hCG	*P*
No. recipients	30	30	
Oocytes donated	5.9 ± 2.4	5.4 ± 3.1	ns
Pregnancies/transfer (%)	55	59	ns
Biochemical pregnancy (%)	5	9	ns
Clinical pregnancy (%)	84	90	ns
Implantation rate (%)	29	32	ns

Acevedo *et al.*, *Fertil. Steril*, **86**,2006,1682

Conclusions

- Despite lower levels of progesterone in the GnRHa group, the differences in FF hormone profiles determined up until does not raise concern on the use of GnRHa for ovulation induction
- hCG and GnRHa induce oocyte maturation with similar efficacy and resulting embryos are equally good
- The GnRHa trigger may create slightly more embryos perhaps through a combined effect of LH and FSH
- Results are encouraging for a continued development of GnRHa protocols to triggering ovulation

Laboratory of Reproductive Biology

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Course dates:
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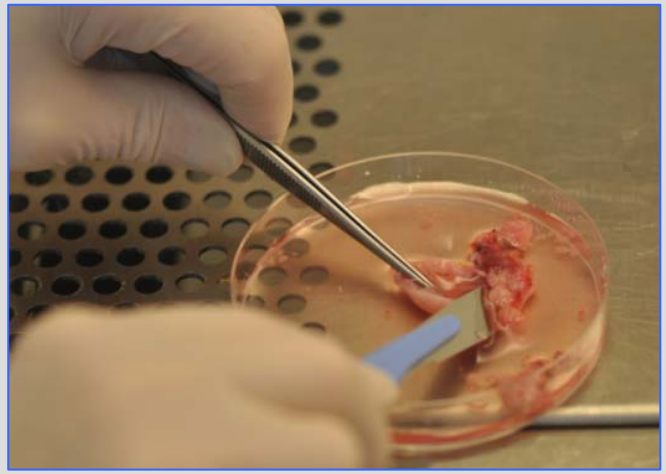

CryoOvary.dk

For more information go to

www.cryoovary.dk

or contact the course coordinator:
stine.gry.kristensen@rh.regionh.dk


Although it is freezing cold in Copenhagen at the moment it is not as bad as this yet