How to interpret hormonal analyses in the IVF cycle

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What does the evidence say in 2015?

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Monitoring of IVF/ICSI cycles

- Centre specific

- Transvaginal ultrasonography plus serum estradiol levels most commonly used

- It has been suggested that combined monitoring is time consuming, expensive and inconvenient for women and that simplification of IVF and ICSI therapy by using TVUS only should be considered. (Cochrane review , Kwan et al., 2014)

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Monitoring of IVF/ICSI cycles:

- 6 RCTs, 781 women

- TVUS vs TVUS and estradiol monitoring

- No difference in clinical pregnancy rates between TVUS vs. TVUS +E2 (OR) 1.10; 95% CI 0.79 to 1.54; four studies; N = 617; I² = 5%; low quality evidence).

- CPR in TVUS+E2 34%, TVUS only from 29-44%

- No difference in OHSS between the two arms (OR 1.03; 95% CI 0.48 to 2.20; six studies; N = 781; I² = 0%; low quality evidence),

- 4% chance of OHSS TVUS +E2, 2-8% with TVUS only

Cochrane review , Kwan et al., 2014

Hormonal analyses in the IVF cycle- GnRH antagonist cycles/ clinicac aspects

- Basal measurements, estradiol and progesterone

- Drop of estradiol before day of HCG administration

- Risk of OHSS, estradiol and coasting

- LH during ovarian stimulation

- Progesterone levels before hCG administration

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Prognostic value of day 3 estradiol on in vitro fertilization outcome

- 225 patients, 292 cycles

- Long agonist protocol, basal E2 in the cycle preceeding ET

- E2 > 80pg/ml achieved a lower PR per initiated cycle (14.8% versus 37.0%) and had a higher cancellation rate (18.5% versus 0.4%) compared with those with E2 < 80 pg/mL

- Same effect if excluded cycles with elevated FSH

Smotrich et al., 1995

Pre-stimulation parameters predicting live birth after IVF in the long GnRH agonist protocol

Göran Pettersson [a,*], Anders Nyboe Andersen [b], Per Broberg [a], Joan-Carles Arce [a]

Endocrine profile					
FSH (IU/l)	3.9 ± 1.4	4.1 ± 1.5	NS[b]	1.07	0.95—1.20
LH (IU/l)	2.3 ± 1.3	2.4 ± 1.6	NS[b]	1.07	0.95—1.21
Progesterone (nmol/l)	1.3 ± 0.6	1.2 ± 0.6	0.012[b]	0.68	0.50—0.93
Androstenedione (nmol/l)	4.6 ± 1.9	4.2 ± 1.8	0.021[b]	0.90	0.81—0.99

Table 3 Multivariable logistic regression analysis with odds ratios of pre-stimulation parameters for the prediction of live birth in IVF.

Predictors	Estimate	P-value	OR	95% CI
Intercept[a]	1.782	—	—	—
Age	−0.062	0.023	0.94	0.89—0.99
Duration of infertility	−0.068	NS	0.93	0.86—1.01
Tubal infertility	−0.462	0.024	0.63	0.42—0.94
Mild male factor infertility	−0.883	0.008	0.41	0.21—0.77
Other infertility diagnosis	−0.010	NS	0.99	0.55—1.74
Pre-stimulation endometrial thickness	−0.157	NS[b]	0.85	0.73—1.00
Pre-stimulation total testosterone	−0.575	0.039	0.56	0.32—0.96
Pre-stimulation sex hormone-binding globulin	0.005	NS	1.01	0.99—1.01

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Basal hormonal values

- ## Basal estradiol
  - → When can we start with stimulation
  - → UZ Brussel E2 <80pg/ml

- ## Basal progesterone
  - → UZ Brussel Pg <1.5pg/ml

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Elevated progesterone at initiation of stimulation is associated with a lower ongoing pregnancy rate after IVF using GnRH antagonists

	Normal-P group	High-P group	*P*
Ongoing pregnancy rate			
Per started cycle % (*n*)	31.8 (124/390)	5.0 (1/20)	0.011
Per oocyte retrieval % (*n*)	33.8 (124/367)	6.3 (1/16)	0.026
Per embryo transfer % (*n*)	36.9 (124/336)	6.3 (1/16)	0.014
Ongoing implantation rate % (*n*)	21.1 (151/714)	3.6 (1/28)	0.028

Kolibianakis et al, 2004

Administration of GnRH antagonists in case of elevated progesterone at the initiation of the

	Normal P Group (n=454)	High P Group (n= 30)			
	On Day 2 of the Cycle	On Day 2 of the Cycle**		After 3 Days of Antagonist**	
P (ng/ml)	0.8 ± 1.0	3.3 ± 1.5	$p < 0.05$	0.8 ± 0.4	$p = 1.0$
E_2 (pg/ml)	34.3 ± 17.4	48.4 ± 21.1	$p < 0.05$	19.5 ± 13.8	$p < 0.05$
FSH (IU/L)	7.19 ± 2.7	5.14 ± 2.3	$p < 0.05$	5.92 ± 1.2	$p < 0.05$
LH (IU/L)	5.13 ± 2.5	4.8 ± 2.5	$p = 0.46$	3.5 ± 1.6	$p < 0.05$

*Mean Values ± SD per patient.

**Mean values ± SD per patient and P-value compared with the normal P group.

Blockeel et al, 2011

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Table 4.   Serum Hormone Levels on the Day of hCG*

	Normal P Group	High P Group	p - Value
P (ng/ml) *	1.47 (1.9)	1.5 (1.8)	0.94
E_2 (pg/ml) *	1628 (1049)	1629 (956)	1.0
FSH (IU/L) *	13.1 (7.4)	14.2 (5.5)	0.46
LH (IU/L) *	1.8 (2.3)	2.5 (3.3)	0.16

* Mean Values (SD) per patient.

Blockeel et al, 2011

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Table 5.   Clinical Outcome Measures

	Normal P Group	High P Group	p Value
Positive hCG			
Per started cycle % (n)	31.9 (145/454)	23.3 (7/30)	0.33
Per pickup % (n)	32.1 (145/452)	26.9 (7/26)	0.58
Per embryo transfer % (n)	36.1 (145/402)	29.2 (7/24)	0.49
Outcome for patients with positive hCG test			
Biochemical pregnancy % (n)	10.3 (15/145)	42.9 (3/7)	0.01
Miscarriage % (n)	9.7 (14/145)	0.0 (0/7)	0.38
Ectopic pregnancy % (n)	2.8 (4/145)	0.0 (0/7)	0.66
Ongoing pregnancy % (n)	77.2 (112/145)	57.1 (4/7)	0.22

Blockeel et al, 2011

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Estradiol drop prior to hCG administration

- Falling estradiol levels as a result of intentional reduction of gonadotrophin dose
- Spontaneously falling estradiol levels

Falling estradiol levels during stimulation

- Retrospective cohort study 1993-2002, 112 patients

Table I. Patient demographics and cycle parameters

	Control (n = 112)	Protocol modification (n = 34)	Spontaneously falling estradiol (E_2) (n = 78)	P
Age (years), mean ± SD	34.4 ± 3.9	33.3 ± 3.5	34.9 ± 4.3	0.157[a]
Diagnosis, n (%)				
Tubal	78 (70)	24 (70)	59 (75)	0.005[b]
Unexplained	26 (23)	3 (9)	13 (17)	
Anovulatory	3 (3)	6 (18)	1 (1)	
Male factor	5 (4)	1 (3)	5 (7)	
Protocol, n (%)				
Long	84 (76)	28 (82)	57 (72)	0.500[b]
Flare	26 (24)	6 (18)	22 (28)	
Peak estradiol (pmol/l), median (range)	8134 (1022–37 446)	14 626 (3315–53 970)	5470 (298–24 144)	< 0.0001[c,d]
Estradiol at hCG (pmol/l), median (range)	8134 (1022–37 446)	8046 (782–46 304)	4928 (290–19 300)	< 0.0001[c,e]

Fischer et al., 2005

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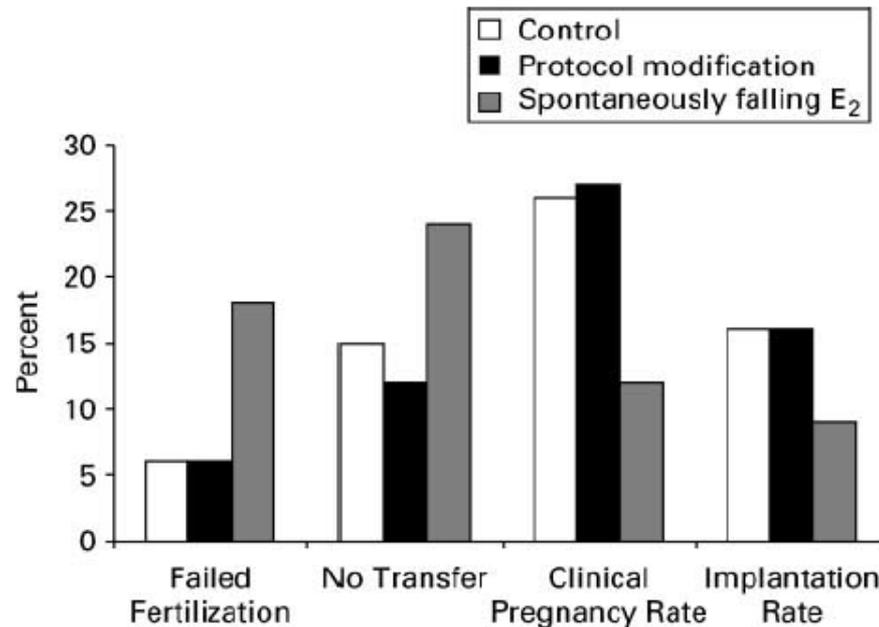
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Figure 1. IVF outcomes including the rate of failed fertilization, percentage of patients with no embryos for transfer, clinical pregnancy rate and implantation rate. Significantly higher rates of failed fertilization and no embryo transfer, and lower clinical pregnancy and implantation rates were observed in the spontaneously falling estradiol group compared to the protocol modification and control groups.

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	GROUP A (CONTROL)	GROUP B (E_2-DROP)	GROUP C (MATCHED-CONTROL)	p-VALUE (A vs. B/B vs. C)
General cycle characteristics (mean ± standard deviation)				
n	1066	83	83	-
Patient age on oocyte retrieval	30.7 ±3.5	30.2 ±3.6	30.5 ±3.7	NS/NS
Total FSH dose (IU)	1587.8 ±610.1	1698.0 ±726.5	1514.1 ±362.5	NS/NS
Oocytes retrieved	11.0 ±6.8	11.0 ±7.8	10.4 ±6.3	NS/NS
Mature oocytes	8.6 ±5.6	10.0 ±6.9	8.1 ±4.5	NS/NS
Late-follicular endocrine profile (mean ± standard deviation)				
Trigger E_2 (pg/mL)	1935.3 ±1143.6	1194.9 ±826.6	1276.9 ±563.8	<0.01/NS
Trigger progesterone (ng/mL)	1.02 ±0.52	0.97 ±0.52	0.95 ±0.60	NS/NS
IVF/ICSI details (%)				
ICSI	82.2%	79.5%	78.3%	NS/NS
Maturation rate (only-ICSIs)	79.2%	82.7%	82.7%	NS/NS
Fertilisation rate	74.3%	75.3%	71.2%	NS/NS
Details of fresh embryo transfer (%)				
Cancelation rate (no embryo transfer)	9.6%	9.6%	9.6%	NS/NS
Single embryo transfer	72.1%	84.0%	73.3%	NS/NS
Day 3 embryo transfer	49.5%	37.3%	37.3%	0.04/NS
Miscarriage rate per embryo transfer	17.7%	21.3%	17.3%	NS/NS
Cycle outcome (% per cycle)				
Clinical pregnancy rate	34.1%	28.9%	31.3%	NS/NS
Live birth rate	22.1%	16.9%	20.5%	NS/NS

TABLE 1 – Sample demographics and pregnancy outcomes
NS, not significant

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Does estradiol level on the day of hCG predict the risk of OHSS?

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TABLE 2

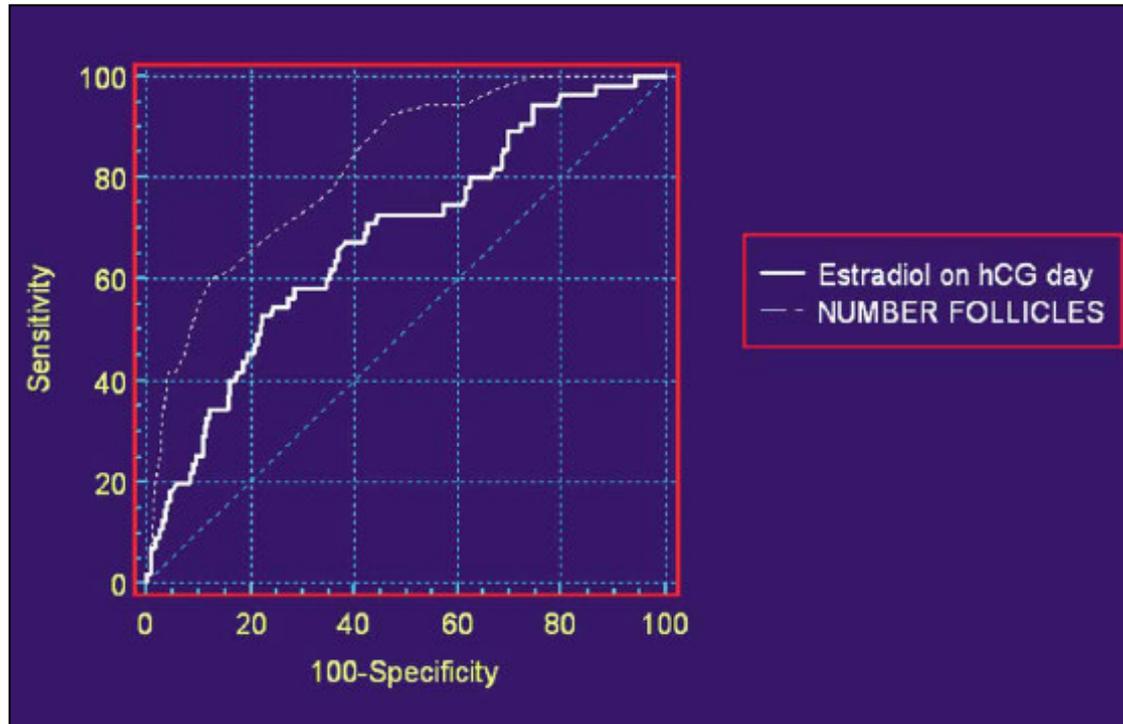Cycle characteristics and stimulation parameters.

Variable	OHSS (n = 53)	Non-OHSS (n = 2,471)	Early OHSS (n = 31)	Late OHSS (n = 22)	P value (OHSS vs non-OHSS)	P value (early vs. late OHSS)	P value (early vs. non-OHSS)	P value (late vs. non-OHSS)
Age (y ± SEM)	30.2 ± 0.5	33.1 ± 0.2	29.7 ± 0.6	30.6 ± 0.7	<.01	NS	<.05	<.05
BMI (± SEM)	23.13 ± 0.8	23.05 ± 0.1	23.09 ± 0.9	23.23 ± 0.9	NS	NS	NS	NS
Follicles on day of hCG (mean ± SEM)	21.1 ± 1.4	11.9 ± 0.3	22.6 ± 1.3	18.2 ± 1.6	<.001	.05	<.001	<.05
Oocytes retrieved (mean ± SEM)	21.3 ± 1.4	11.0 ± 0.1	24.2 ± 1.9	16.0 ± 1.5	<.001	<.05	<.001	<.05
E_2 level on day of hCG (ng/L ± SEM)	2,650 ± 174	1,739 ± 49	2,970 ± 233	2,127 ± 219	<.001	<.05	<.001	.04
Gonadotropin dose (IU ± SEM)	1,860 ± 89	1,875 ± 86	1,947 ± 122	1,732 ± 125	NS	NS	NS	.05
Days of stimulation (d ± SEM)	11.0 ± 0.2	11.1 ± 0.3	11.4 ± 0.3	10.5 ± 0.5	NS	NS	NS	NS

Note: NS = not significant; BMI = body mass index.

Papanikolauo. OHSS in GnRH-antagonist IVF cycles. Fertil Steril 2006.

Papanikolau et al, 2006

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OHSS prediction:E2 or follicles on day of hCG?

Analysis of ROC curve for E2 of 2,560 ng/L (area under the curve, 0.680) and for number of follicles 13 (area under the curve, 0.823). Difference between the two areas, 0.144 (95% CI 0.055 to 0.232; *P*.001). Solid line, E2 on hCG day; broken line, number of follicles.

Papanicolaou et al., Fertil Steril 2006

OHSS prediction:E2 or follicles on day of hCG?
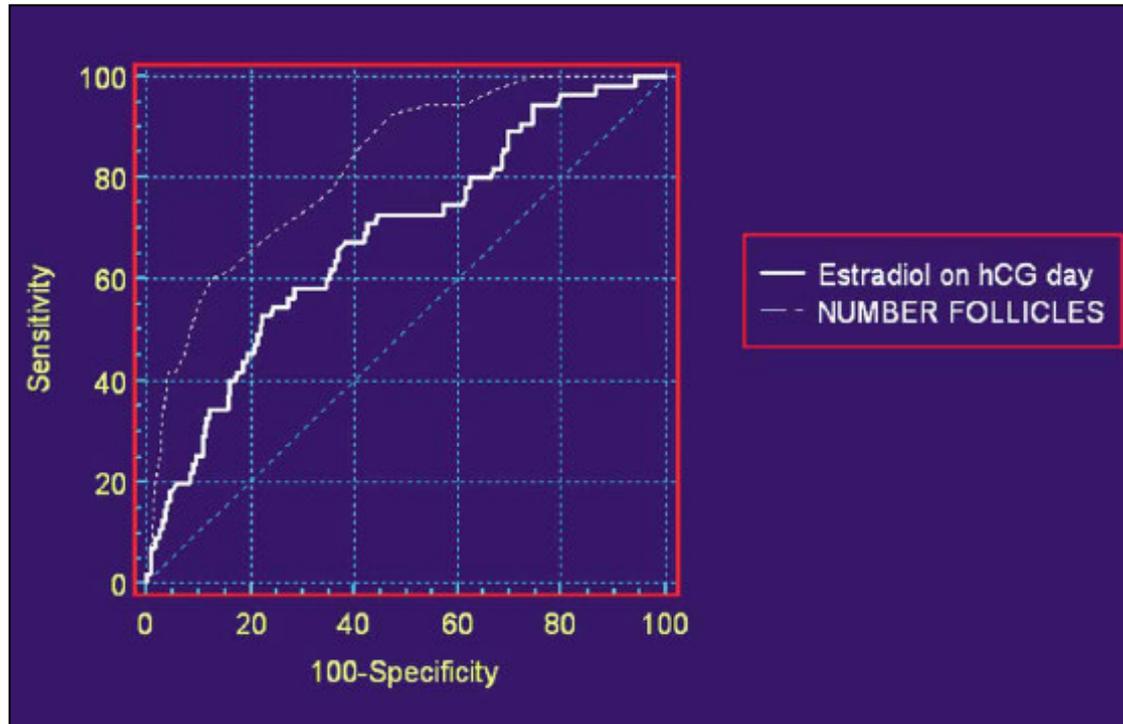
18 follicles >10 mm or E2 > 5,000 pg/l:

sensitivity = 83%, specificity = 84%

5/53 cases of severe OHSS missed with these criteria

Papanicolaou et al., Fertil Steril 2006

Coasting

- Initiated when follicles are 15 to 16 mm in diameter and serum E2 levels > 3000pg/ml

- Large follicles low dependancy on FSH , can tolerate few days without gonadotrophins

- Immature follicles enter atresia

- E2 <3000pg/ml limit below which coasting can be terminated and hCG administered

Coasting in GnRH antagonist protocols

- With the develpoment of good cryo/ vitrification programmes coasting is becoming redundant

- Patients at risk of OHSS, GnRH agonist trigger, freeze all and transfer in natural or HRT cycle

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LH rise during COS

- During induced multiple follicular development, when FSH concentrations are maintained because of exogenous gonadotropin administration, natural selection of a single dominant follicle does not occur and multiple follicles continue to grow.

- This increased number of follicles produces higher serum estradiol concentrations and consequently, the serum estradiol concentration that triggers the preovulatory LH surge is reached prematurely, i.e., before the follicles have fully developed

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Table 2 Incidence of LH rises (≥10 IU/L) and of LH rises with P rises (≥3.18 nmol/L) measured at stimulation day 5 or 6 prior to the start of ganirelix treatment and during ganirelix treatment

		Ganirelix start day	% (95% CI), n/N	P value*
LH rises	Early	Day 5	2.3 (1.4-3.4) 22/955	< 0.01
		Day 6	6.6 (5.2-8.2) 74/1113	
	Late	Day 5	1.2 (0.6-2.0) 11/949	0.06
		Day 6	2.3 (1.4-3.3) 25/1096	
LH+P rises	Early	Day 5	1.0 (0.5-1.9) 10/955	0.11
		Day 6	2.0 (1.2-2.9) 22/1113	
	Late	Day 5	0.5 (0.2-1.2) 5/949	0.44
		Day 6	0.9 (0.4-1.6) 10/1096	

Early indicates on start day of ganirelix treatment; Late indicates during ganirelix treatment. *CI* confidence intervals, *LH* luteinizing hormone, *P* progesterone. *Day 5 versus day 6.

Fratarelli et al., 2013

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Table 3 Ovarian response and clinical outcome according to early or late LH rise versus no LH rises

	Early LH rises (n = 96)	Late LH rises (n = 36)	No LH rises (n = 1962)
Follicles ≥11 mm on day of hCG, mean ± SD	13.2 ± 7.8	8.9 ± 6.6	11.6 ± 6.1
Serum estradiol on day of hCG, pmol/L, median (P5, P95)	7303 (3171, 16478)	5391 (573, 15047)	4367 (1369, 10955)
Oocytes retrieved, mean ± SD	12.9 ± 8.5	7.5 ± 6.7	10.2 ± 6.4
Ongoing pregnancy rate, %	26.0	16.7	29.9

hCG human chorionic gonadotropin, P5, P95 5th and 95th percentiles, SD standard deviation.

Fratarelli et al., 2013

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Elevated progesterone on the day of hCG

- Meta analysis of more than 60 000 cycles

- In fresh IVF cycles, a decreased probability of pregnancy achievement was present in women with PE (when PE was defined using a threshold ≥ 0.8 ng/ml) when compared with those without PE.

- The pooled effect sizes were 0.8-1.1 ng/ml: odds ratio (OR) = 0.79; 1.2-1.4 ng/ml: OR = 0.67; 1.5-1.75 ng/ml: OR = 0.64; 1.9-3.0 ng/ml: OR: 0.68 (P < 0.05 in all cases).

Venetis et al., 2013

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Elevated progesterone on the day of hCG

- PE on the day of HCG, freeze all, ET in natural or HRT cycle

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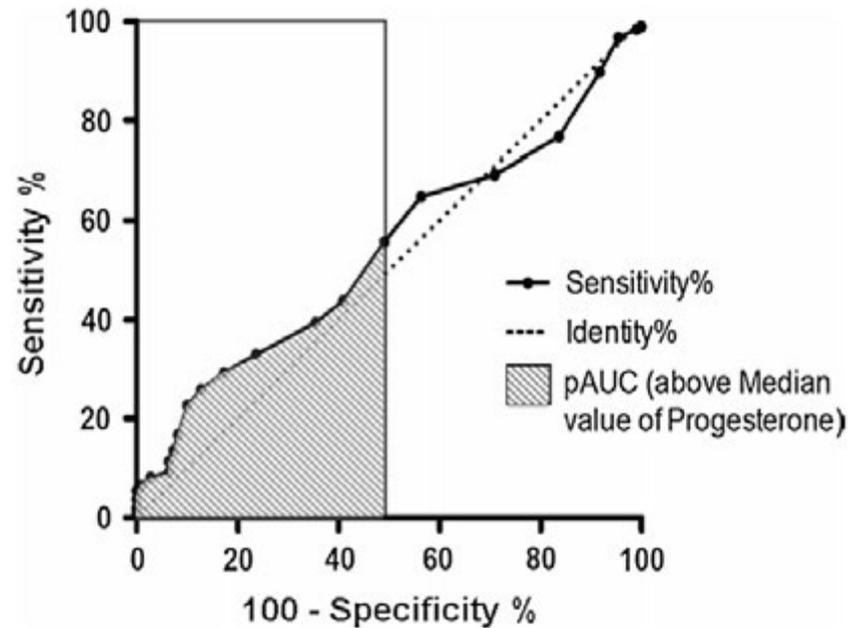Centrum voor
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Fig. 1 ROC curve for prediction of day-3 fresh embryo transfer IVF/ICSI cycle outcomes from serum progesterone levels on the day of hCG. The *highlighted rectangle* represents the area calculated from values above the median levels of progesterone in our population (median value of progesterone on day of hCG = 0.90 ng/ml, IQR = 0.60–1.30 ng/ml), which is used to define partial area under the curve (pAUC) (pAUC = 0.58, $p = 0.02$)

Papaleo et al., 2014

Table 2 IVF/ICSI cycle outcomes in women with and without elevated progesterone on the day of hCG triggering

	Positive β-hCG test		Clinical pregnancy with foetal heart beat	
	No	Yes	No	Yes
Progesterone at hCG				
≤1.35 ng/ml				
n	142	96	175	63
%	60	40**	73	27*
>1.35 ng/ml				
n	51	14	56	9
%	78	22**	86	14*

An elevated progesterone level on the day of hCG (>1.35 ng/ml) was detrimental on both positive β-hCG test (** OR = 2.46, 95 % CI 1.29–4.70, p = 0.005) and clinical pregnancy rate with foetal heart beat (* OR = 2.24, 95 % CI 1.05–4.80, p = 0.03)

Papaleo et al., 2014

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Conclusion

- Hormonal measurements remain an important factor of COS monitoring

- Fine tuning of COS on the basis of hormonal measurements has given us new treatment strategies to ensure optimal outcomes

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