Serum and follicular hormonal profile in natural IVF cycles

Vrtačnik Bokal E, Jančar N, Virant Klun I

Reproductive Unit, Department of Obstetrics and Gynaecolgy, University Medical Centre Ljubljana

Aim

- serum and follicular hormone status
- women attending IVF program
- focus on AMH and LH

AMH

- a member of the transforming growth factor beta family of growth factors produced in ovarian granulosa cells
- one of the most important markers of ovarian reserve - marker of ovarian aging
- stronlgy related to the number of retrieved oocytes (IVF) – marker of ovarian response in IVF treatment

Van Rooij, HR 2002
De Vet, Fertil Steril 2002

AMH

- related to ovarian follicular development

Knight, Reproduction 2006

Follicular AMH

- positively associated with fertilization rate

Takahashi, Fertil Steril 2008

- predicts the implantation potential

Fanchin, J Clin Endocrinol & Metabol 2007

FSH,LH

- are required for oocyte and embryo developement
- granulosa cells in small antral follicles start to express FSH receptors which are dependent on FSH
- FSH stimulation causes GC to express aromatase and LH receptors
- converts androgens to estrogens

Aim

- the pregnancy rate is lower in natural cycles (NC) than in stimulated cycles (COH)
- we aimed at finding an explanation for this difference by comparing follicular and serum AMH, LH, FSH, E2, P, AND concentrations between the NC and COH groups

Inclusion criteria

- tubal factor infertility
- FSH, LH, PRL (day 3)- normal
- semen analysis had to be normal

Design

- we included 30 women undergoing NC IVF
- and 30 women undergoing COH IVF

Monitoring - NC

- on day 9: US,E2, the urine sample was tested for the presence of LH surge
- dominant follicle ≥ 16 mm, serum E2 exceeded 0.40 nmol/l, and no LH surge was detected, 5000 IU of HCG
- OR was done 31-32 hours after HCG administration

Ovarian stimulation - COH

- GnRH ant. and rFSH
- 225 IU of gonadotropin folitrophin alpha - on day 2
- GnRH antagonist cetrorelix acetate (0.25 mg)- dominant follicle 13 mm
- When at least three follicles measured ≥ 17 mm - HCG 10 000 IU
- OR was carried out 34-36 hours after HCG

Folicular fluid analysis

- Each follicle was aspirated separately
- Immediately after removal of the oocyte, volume of FF was measured, and 1 ml from each aspirate containing an oocyte was separated.
- We pooled FFs from empty follicles - separated 1 ml of FF.
- FFs were centrifuged and supernatants were stored at -20 °C for subsequent collective analysis of AMH, LH,FSH, E2, P, AND

Serum analysis

- on the day of OR blood samples were obtained
- AMH, LH,FSH, E2, P, AND

Oocyte quality

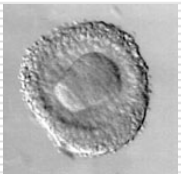
- mature
- immature
- degenerated

Maturity of oocytes (IVF)

according to the cumulus mass appearance and consisted of three components:

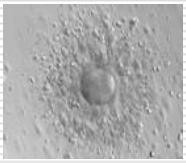
number of cumulus mass layers
cumulus expansion
contact between cumulus cells and oocyte

Immature oocyte



tight, dense cumulus cells
poor expansion around oocyte
3 or less layers of cumulus cells
cumulus appearance is dark

Mature oocyte



fully expanded cumulus cells
10 or more layers of cumulus cells
cumulus appearance is bright and transparent

Degenerated oocyte

characterized by multiple morphological aspects, ranging from darkened, vacuolated and irregular ooplasm.

Embryo quality

was assesed on day 5 according to developmental stage of embryos and were divided to:

- arrested embryos
- < 10 cells
- morulae
- blastocysts

General data

Parameter	NC group (n = 30)	COH group (n = 30)	*P* value
Age (years)	34.9 ± 3.4	33.6 ± 4.1	NS
BMI (kg/m2)	21.9 ± 2.5	23.6 ± 4.3	NS
FSH (IU/l)	6.7 ± 4.1	6.7 ± 2.4	NS
LH (IU/l)	5.1 ± 3.4	6.0 ± 3.9	NS
Prolactin (ng/ml)	12.2 ± 5.7	11.0 ± 5.9	NS
E2 (nmol/l)	0.6 ± 0.2	4.3 ± 3.1	< 0.001

Quality of oocytes

	NC [n(%)]	COH [n(%)]	p
oocytes per puncture	0.8 ± 0.5	6.3 ± 4.3	0.001
immature	3 (12.5)	12 (9.4)	NS
mature	21 (87.5)	112(87.5)	NS
degenerated	0	4 (3.1)	NS

Quality of embryos

	NC [n(%)]	COH [n(%)]	P
fertilization	20 (83.3)	81 (63.3)	NS
≤ 10-cell embryos	4 (20%)	23 (28.4%)	NS
morulae	3 (15%)	16 (19.8%)	NS
blastocysts	11 (55%)	35 (43.2%)	NS

Implantation rate: NC-5.9%; COH-35.5% (P=0.031)

Serum hormonal levels (NS vs COH)

Parameter	NC (n=29)	COH(n=29)	*P* value
AMH (ng/ml)	2.3 ± 2.0	1.4 ± 0.9	< 0.001
LH (IU/l)	32.6 ± 19.5	0.8 ± 0.8	< 0.001
FSH (IU/l)	13.1 ± 5.4	6.5 ± 2.7	< 0.001
progesterone (nmol/l)	2.4 ± 3.5	18.7 ± 38.0	< 0.001
oestradiol (nmol/l)	0.4 ± 0.1	4.3 ± 2.1	< 0.001
androstendione (nmol/l)	6.1 ± 2.6	8.0 ± 4.0	0.01

Follicular hormonal levels (NS vs COH)

Parameter	NC (n=29)	COH(n=132)	*P* value
AMH (ng/ml)	6.1 ± 5.5	2.5 ± 1.7	< 0.001
LH (IU/l)	15.6 ± 8.6	2.0 ± 4.6	< 0.001
FSH (IU/l)	5.9 ± 3.0	7.1 ± 10.4	NS
progesterone (nmol/l)	26482.2 ± 12942.7	33276.8 ± 15827.4	0.05
oestradiol (nmol/l)	7447.5 ± 4401.4	3356.7 ± 2742.8	< 0.001
androstendione (nmol/l)	112.5 ± 16.1	102.5 ± 12.8	0.001

Follicular hormonal levels in relation to oocyte and embryo quality

	AMH (ng/ml)		*P*-value	LH (IU/l)		*P*-value	FSH (IU/l)		*P*-value
	NC	COH		NC	COH		NC	COH	
Empty follicles	4.7±4.8	**2.3±1.7[a]**	NS	15.1±4.7	1.2±1.1	0.001	6.6±4.1	8.9±12.8	NS
Mature oocytes	7.1±7.7	**2.5±1.7[b]**	0.002	15.9±9.2	2.3±5.4	0.001	6.0±2.7	6.5±10.2	NS
Immature oocytes	10.4±5.9	2.8±2.3	0.049	14.6±13.6	1.0±0.4	NS	3.7±3.1	7.6±8.2	NS
Degenerated oocytes	-	**1.2±0.5[ab]**	-	-	1.6±0.4	-	-	5.2±0.3	-
Unfertilized oocytes	11.9±10.7	1.7±1.2	0.001	15.6±2.6	2.8±5.1	0.022	4.53±1.1	7.6±5.2	NS
< 10 cells	3.3±1.6	2.7±1.6	NS	15.0±8.4	2.2±4.5	0.001	7.9±3.3	4.5±4.0	0.034
Morula	2.8±2.3	3.0±2.1	NS	14.6±15.2	4.4±10.6	NS	4.8±2.6	10.7±16.4	NS
Blastocyst	5.6±5.2	2.1±1.1	0.008	15.9±9.9	1.5±3.2	0.001	6.1±2.7	5.1±3.2	NS

Conclusions

- Hormonal status (AMH,LH, E2,P,AND) is completely different in NC vs COH.
- No effect on oocytes and embryos quality in both groups

Conclusions

- problem- endometrium as target organ
- what kind of influence very low E2 has on the endometrium?(lead to insufficient endometrium proliferation and afterwards to defective secretory differentiation and maturation)
- we don't know what is the influence of spontaneous LH surge after HCG on endometrium (LH concentrations were statistically lower in pregnant than in non pregnant women, although the quality of embryos was about the same in both groups).
- Implantation window?

Conclusion

- As to the correlation between serum and follicular AMH and LH in the NC and COH, it would be useful to follow the dynamics of periovulatory serum AMH and LH concentrations in the NC, which might provide a close to optimal time for HCG administration and oocyte retrieval.
- To modify the protocol by administration the GnRH antagonists to prevent the LH surge in NC.