Cumulus Cells Gene Expression in Patients Attending IVF

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Background

- Infertility is an expanding problem in modern society
- Despite great improvement
- The success rate of IVF is still sparse

Background

- However, our understanding of oocyte maturation process and quality acquirement is still limited

- So far the evaluation of the oocyte and embryo quality is still based on subjective analysis of embryiologist

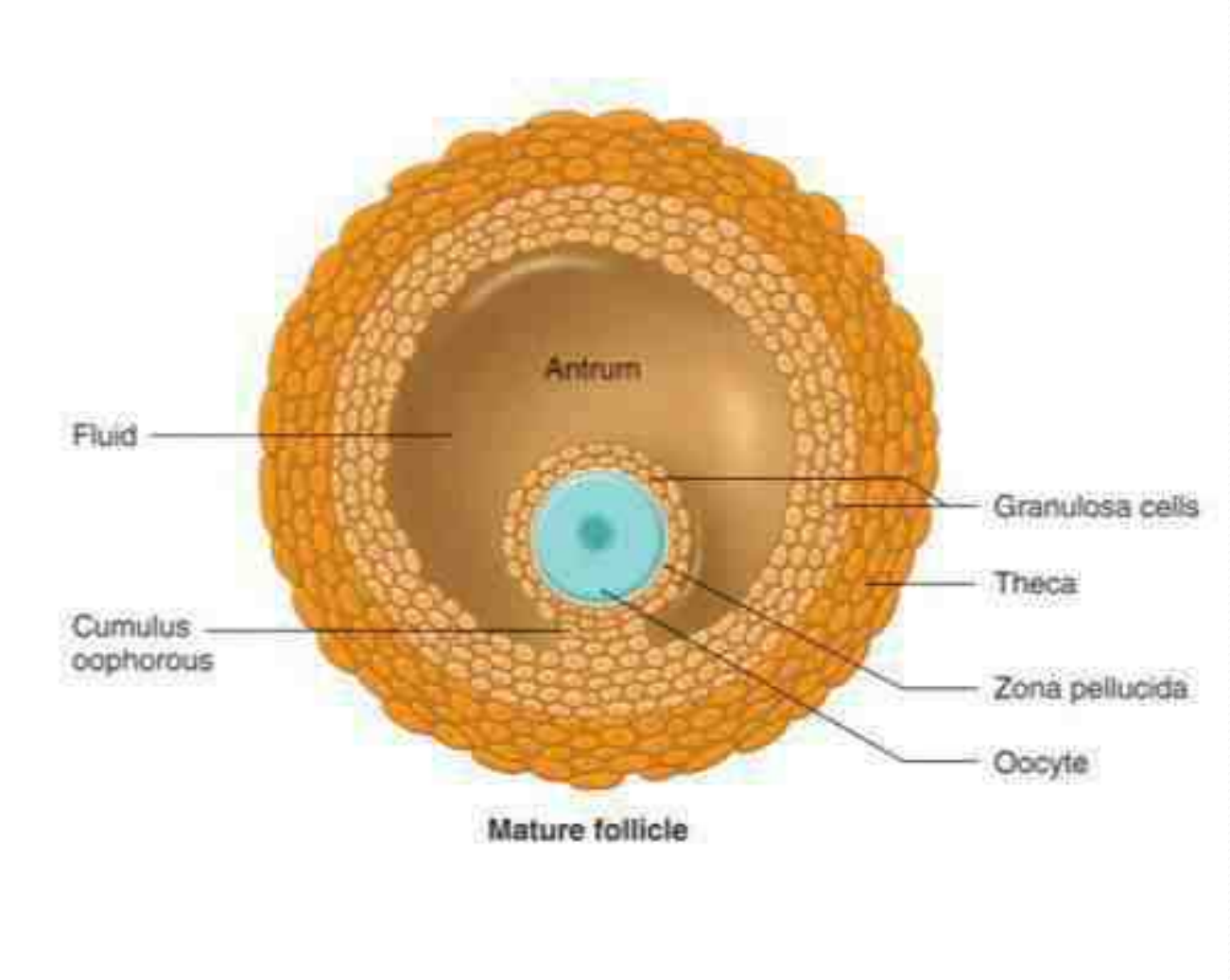
Background

- Development of “omics” technology (transcriptomics, metabolomics, proteomics) has enabled analysis of physiological processes on molecular level
- In reproductive medicine this technology is being used for understanding of oocyte maturation process and for discovery of biomarkers of oocyte and embryo quality

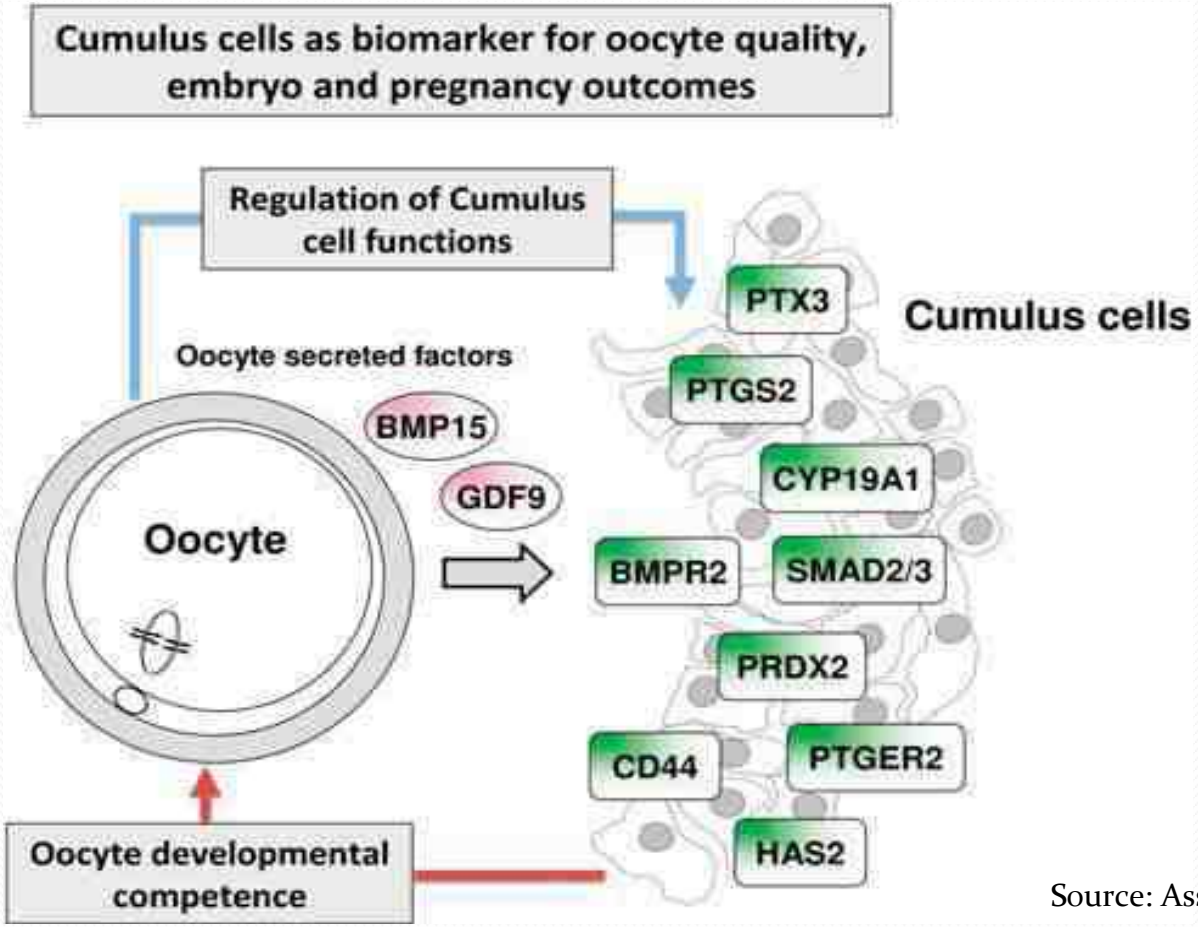
Background

- GC and CC are in direct contact with oocytes during the oocyte maturation process and play an esential role in this process
- Oocyte regulates GC and CC functions and processes through gene expression
- Gene expression in GC and CC therefore indirectly reflects oocyte's quality
- These cells are easily accessible and discarded during IVF procedure – appropriate material for analysis

Background


Antrum
Fluid
Granulosa cells
Theca
Cumulus oophorous
Zona pellucida
Oocyte
Mature follicle

Background



Source: Assou *et al.*, MHR,16,531–538,2010

Our studies

- Our group conducted 3 studies of genome wide gene expression analysis of human GC and CC using microarrays
- Patient inclusion criteria: less than 35 years old, BMI 17-26 kg/ m2, tubal/unexplained infertility, first/second IVF cycle, normal partner‘s semen analysis

Comparison of cumulus cell gene expression in controlled ovarian stimulation with GnRH agonists and antagonists

GnRH analogues

- Controlled ovarian stimulation: gonadotropins in combination with GnRH antagonists or GnRH agonists
- Comparison of GnRH agonists and antagonists:
  - Pregnancy rate and delivery rate: Kolibianakis et al. (2006), Al Inany HG et al. (2009)
  - OHSS: Al Inany HG et al. (2011)
- A comparison at CC gene expression level has not been done yet.

Cumulus cells biomarkers

- CC gene expression can predict oocyte maturation, developmental potential and pregnancy outcome
- The results are very controversial
- Different women age, protocols, male factor of infertility

  - McKenzie et al. (2004), Zhang et al. (2005), Hamel et al. (2008, 2010), van Montfort et al. (2008), Assou et al. (2008)

Aims

- To determine differences in CC gene expression depending on GnRH analogue used for ovarian stimulation protocol

- To determine whether a type of GnRH analogue used in COS affects expression of CC biomarkers for oocyte maturation and developmental potential

Sample collection

- CC from individual cumulus – oocyte complex
  - CC from MI oocytes
  - CC from MII nonfertilized oocytes
  - CC from mature MII oocyte developed to blastocyst

Sample analysis

- **Microarray analysis**: GeneChip® Human Gene 1.0 ST (Affymetrix) for whole – transcript expression analysis

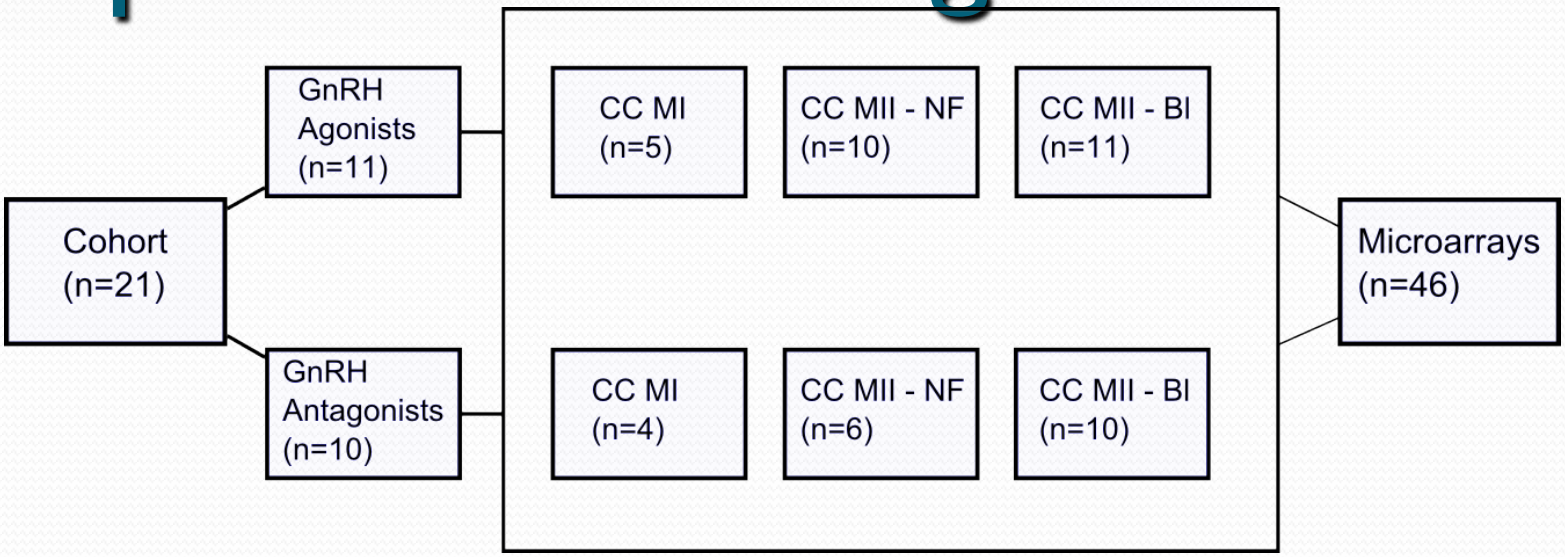
- **qPCR** of selected genes

Results I

- Tabela I: Patients characteristics

	GnRH agonists	GnRH antagonists	p value
Age (years)	30.7 ± 3.88	30.5 ± 3.03	0.88
BMI (kg/m2)	23.1 ± 2.68	22.7 ± 2.80	0.72
Retrieved oocytes (n)	11.7 ± 5.24	8.0 ± 3.89	0.08
Fertilized (ratio)	0.65 ± 0.14	0.50 ± 0.19	0.06
Degenerated (ratio)	0.02 ± 0.05	0.04 ± 0.11	0.46
MII: fertilized and nonfertilzed (ratio)	0.92 ± 0.08	0.82 ± 0.21	0.18
MI (ratio)	0.07 ± 0.08	0.14 ± 0.15	0.19
Blastocyst (ratio)	0.23 ± 0.08	0.20 ± 0.07	0.31
Pregnancy rate (ratio)	0.55	0.60	0.81
Delivery rate (ratio)	0.55	0.40	0.53

Experimental design



Legend:
Cumulus cells from immature MI oocyte – **CC MI**
Cumulus cells from mature MII oocyte nonfertilized – **CC MII – NF**
Cumulus cells from mature MII oocyte developed to blastocyst – **CC MII – Bl**

Figure 1: Experimental design of performed study

Results

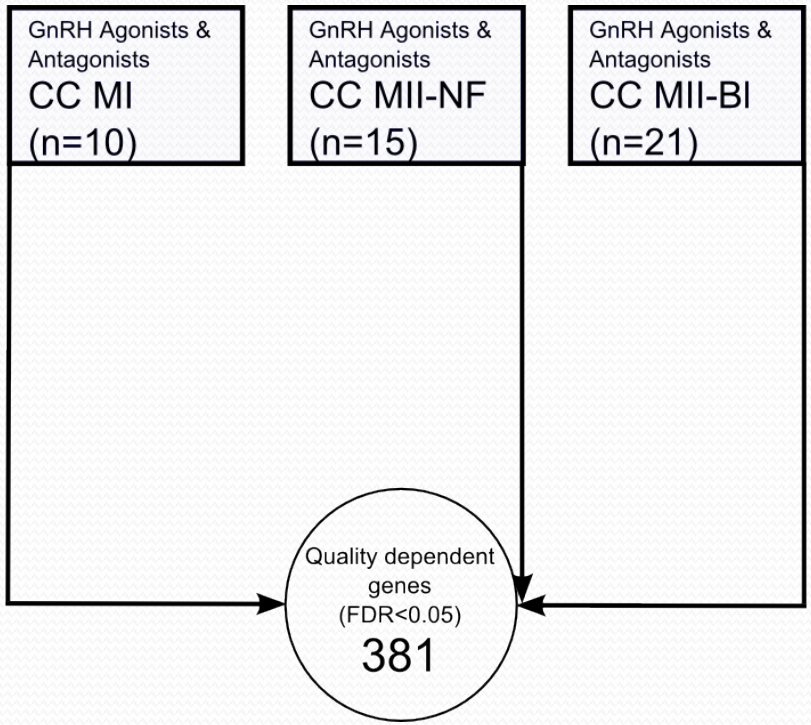


Figure 2: Differential expression among CC MI, CC MII – NF and CC MII – BI in GnRH agonists and GnRH antagonists groups together.

Results

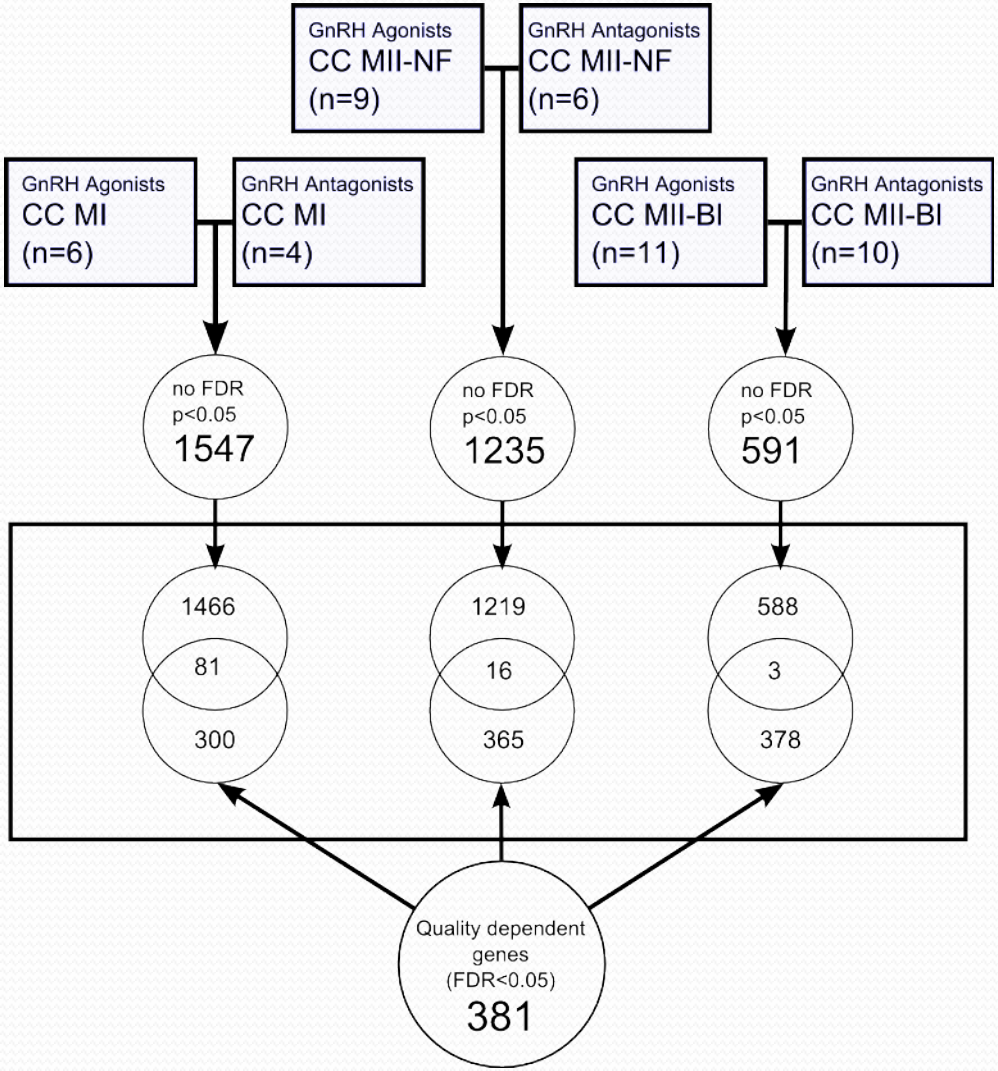


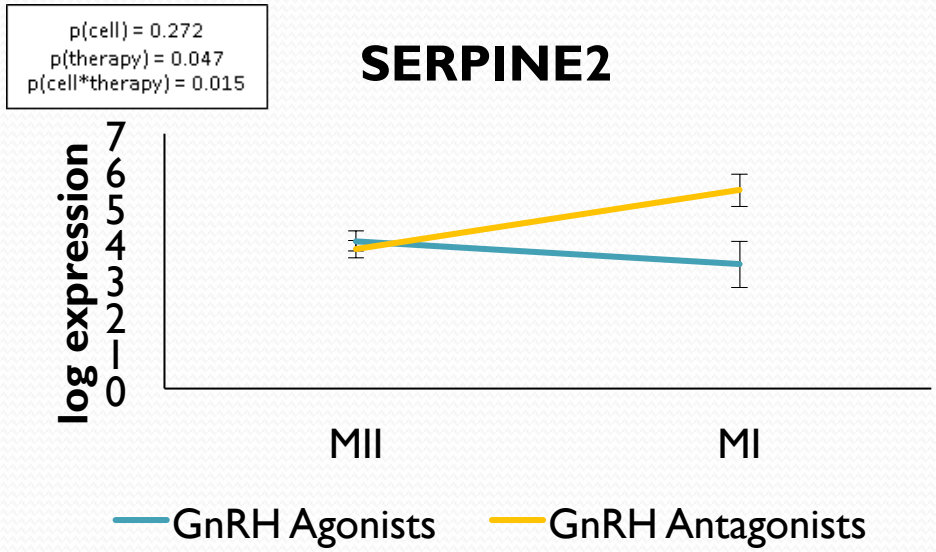
Figure 4: Venn diagrams of differentially expressed genes between GnRH agonists and GnRH antagonists group according to the oocyte stage and 381 quality dependant genes.

Q PCR validation

- EBAG9, FSHR, SERPINE2, AMHR2
- According to functional-biological value not on account of fold change of the highest expressed genes

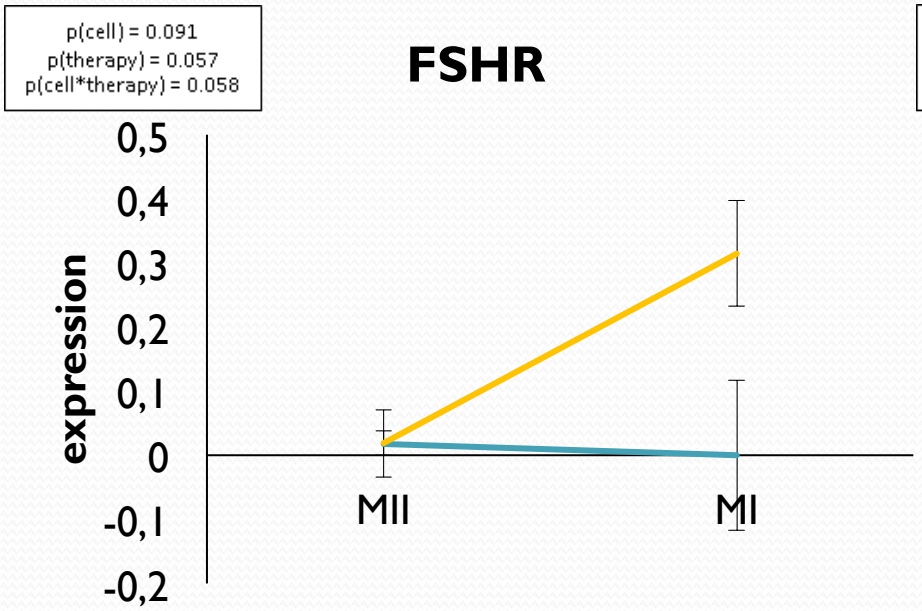
qPCR validation

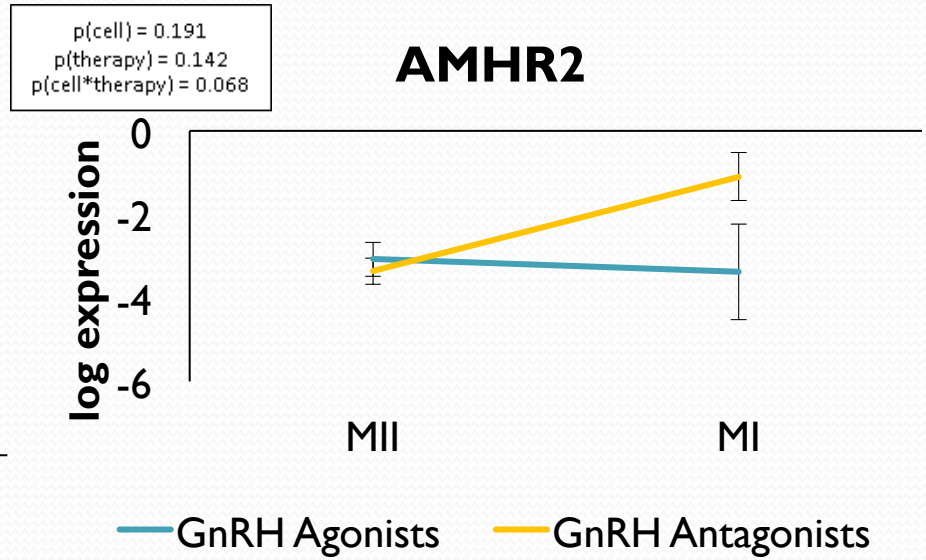
- SERPINE2: Hamel et al. (2008)



qPCR validation

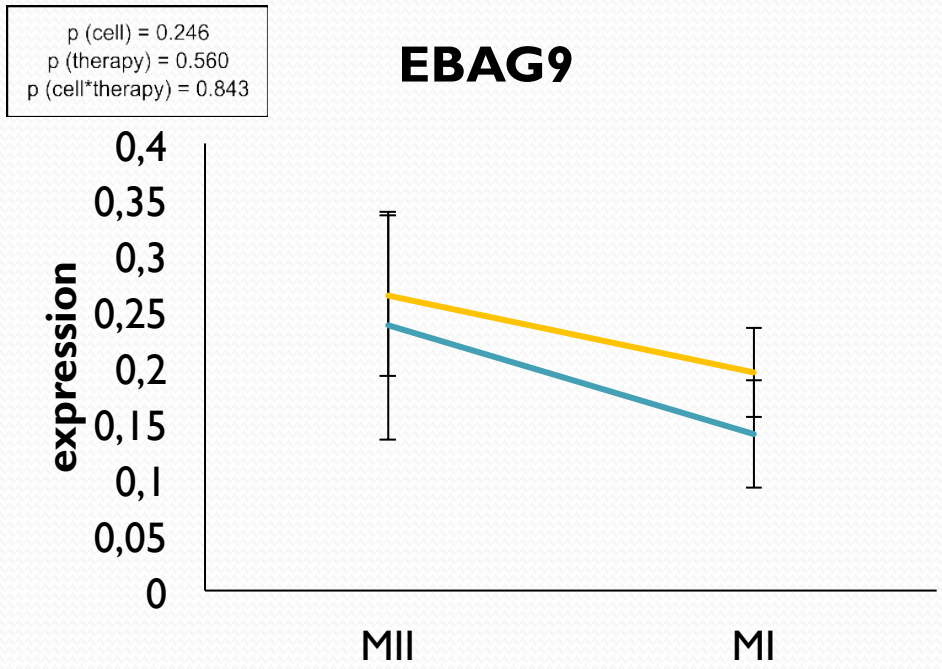
- FSHR and AMHR2: Grøndahl ML et al. (2011)





qPCR validation

- EBAG9



Conclusion

- GnRH agonist and GnRH antagonists showed
  - **a minimal impact** on CC gene expression of **MII oocyte**
  - but showed **sub maturation of MI oocytes** in GnRH antagonists group
- Clinical relevance of MI oocytes?
- Overcoming sub maturation by prolonged hCG exposure? Vrtačnik Bokal et al. (2005), Raziel A et al. (2006) and Raichman DE et al. (2011)

Comparison of gene expression in CC between modified natural (MNIVF) and stimulated IVF cycles

Study # 2

- **Comparison of gene expression in human CC between modified natural (MNC-IVF) and stimulated IVF cycles**
- There has been a tendency in recent years towards the use of milder ovarian stimulation protocols, as they are patient friendlier and cheaper than stimulated IVF cycles
- MNC-IVF cycles however, have high cancellation and low pregnancy rates

Monitoring - MNC

- 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 rFSH - 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

Quality of oocytes

	MNC [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

	MNC [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 (MNC vs COH)

Parameter	MNC (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 (MNC vs COH)

Parameter	MNC (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

Conclusions

- Hormonal status (AMH,LH, E2,P,AND) is completely different in MNC 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?

Study # 2

- We aimed to determine whether there are any gene expression differences between CC derived from MNC-IVF and COH- IVF cycles whose oocytes developed to morulae or blastocyst stage
- We aimed to determine whether we could find the reason for lower success rates of MNC-IVF cycles by analysing CC gene expression

Study # 2

- 5 individual CC samples from stimulated IVF + 3 individual CC samples from MNC-IVF cycles were used for microarray experiments
- 18 individual CC samples from stimulated IVF + 15 individual CC samples from MNC-IVF cycles were used for qPCR validation

Study # 2

- 66 differentially expressed genes between MNIVF and stimulated IVF cycles (2 decreased and 64 increased expression in MNC-IVF)
- Among overrepresented biological processes were glutathione metabolic process and oxidation reduction process
- Genes related to these processes higher expressed in MNC-IVF were: *GPX3*, *GSTA1*, *GSTA2*, *GSTA3*, *SOD2*

Study # 2

- Ovarian production of reactive oxygen species (ROS) is triggered by LH and is essential for ovulation
- Pathological levels of ROS however, diminish oocyte and embryo quality
- Our findings suggest that the developing follicle is exposed to higher levels of LH and ROS in MNC-IVF cycles than in stimulated cycles

Comparison of gene expression in GC and CC between implanted and non-implanted embryos

Study # 3

- The aim of our study was to determine potential gene expression signatures in GC/CC that could be used for prediction of embryo implantation and oocyte fertilisation
- 41 patients included in the study; short GnRH antagonist with rFSH used for ovarian stimulation
- 64 individual GC/CC samples used for microarray analysis
- 55 individual CC samples used for qPCR validation

Study # 3

- 546 genes in GC and 629 genes in CC differentially expressed between non-implanted and implanted embryos
- After the correction for multiple testing none of the genes surpassed the adjusted significance threshold (FDR≤0.05)
- No differentially expressed genes between non-fertilised and fertilised oocytes (FDR≤0.05)

Study # 3

- Possible causes for not finding differences:

1. Exclusion of factors that are known to affect gene expression (age, stimulation protocol, etiology of infertility)
2. Correction statistical analysis
3. Embryo implantation depends on various factors not related to GC/CC gene expression (chromosomal status, endometrial receptivity, embryo culture conditions, embryo transfer technique, patient‘s lifestyle)

Conclusions

1.New knowledge on molecular level of folliculogenesis and impact of gonadotrophins and Gn RH analogues on oocyte and embryo quality; it is of great and applicable importance in studing different therapeutic protocols.

2.Still we did not find any usefull clinical available biomarkers to predict high quality embryos and consequently sucssesful implantation rate.