ESHRE CAMPUS 2010
MARIBOR

INFLUENCE of stimulation on oocyte quality ?

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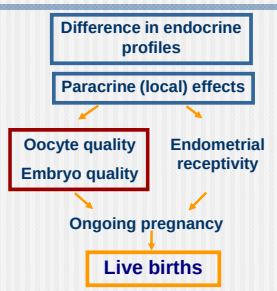
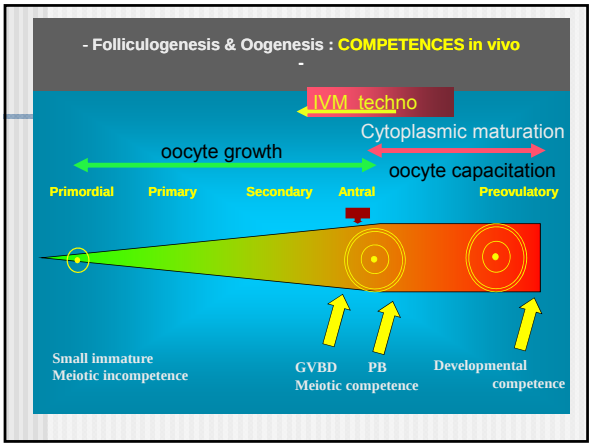
Concept : effects by treatment



OUTLINE

- Oocyte- and follicle growth relationship
- follicle growth: regulation
- stimulate the ovary: why ? how ? when?
- oocyte quality
- Effects downstream the use of gonadotrophins :
  - Quantity and quality



Folliculogenesis : regulation

Is driven by a stage-specific interaction between endocrine hormones and local factors

Consider : the "3 layers of regulation"

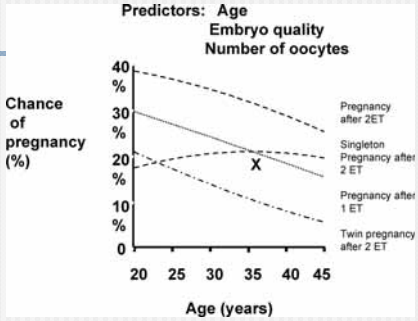
- LAYER 1: FSH and LH
- LAYER 2: IGF-1, E2, androgens, inhibin, activin, …
- LAYER 3 Oocyte Secretion Factors
  - ex. GDF-9, BMP-15, …

(interactions : are partially known)

Treatment-independent factors affecting oocyte developmental competence

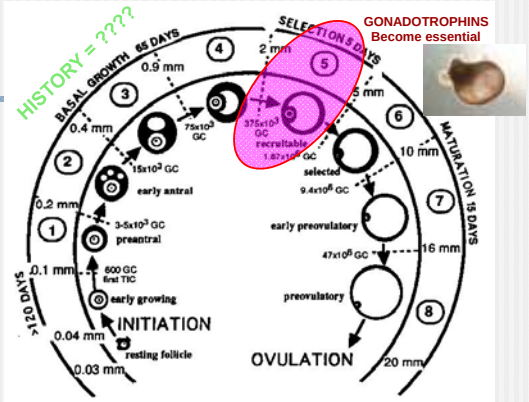
Season	Rensis, 2003
Follicle size	Pavlok, 1992
Stress	Rensis, 2003
Nutrition	Boland, 2001
Health	Saito, 2001
Age	Gandolfi, 2000

Multivariate prediction (based on woman's age, quality score of embryos to be transferred, and number of oocytes obtained at retrieval) of the chance for a singleton or twin pregnancy should one or two embryos be transferred



Macklon, N. S. et al. Endocr Rev 2006;27:170-207

ENDOCRINE REVIEWS



FSH support is critical, but gets modulated

survival of the small antral follicle:

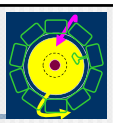
means: - proliferation GC and TC , increased vascularisation,
- minor oocyte growth, but major follicle growth

Modulators of FSH

- Pos: auto- and paracrine: ACT, INH, GDF9, BMP15
  - Differential exposure of follicles to these factors makes them different (… "selection" process)
- Neg: - FSH responsiveness gets reduced by AMH **
  - BMP 6 : suppresses FSH action

***AMH is a survival factor for small follicles, either directly or indirectly (AMH-null mice have an increased amount of oocyte 'remnants')*

The significance of OSF



"Oocytes benefit their own development by enhancing metabolic cooperativity between granulosa-cumulus and oocytes"
Sigiura and Eppig, 2005

- OSF regulate the 'outsourced functions" in CC:
  - Activity of glycolysis
  - Uptake of L-alanine and L-histidine
  - Rate of cholesterol biosynthesis

transfer of essential metabolites to oocyte

Oocyte's capacity to regulate cc is lowest during oocyte growth and highest throughout antral phase

Getting a 'competent' oocyte

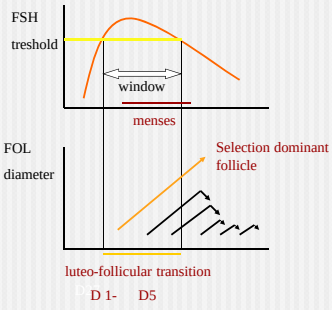
Need to take into account …..the 3rd layer of regulation

- Oocyte Secretion Factors : ex. GDF-9, BMP-15,…
  -maintain a CC micro-environment required for appropriate 'oocyte capacitation'
  -are an important component of 'cytoplasmic' maturation

- These communication factors are least understood
- when OSF are added in IVM media : improved embryo quality

Rob Gilchrist, Jeremy Thompson (Adelaide, AUS)

Selection in normal cycle FSH major player



Stimulation : WHY ?

- Stimulation in 2010 in human IVF
  - single ET
  - E's surviving cryopreservation

- qualitative dimension
  - obtain a number of competent oocytes to develop good quality embryos

Armamentarium of the gynaecologist :

GnRH analogs, FSH, LH, hCG

Variables : when, how much, how long ?

Stimulation : what & how ?

- Change the hormonal condition to optimize the normal selection process
- Interact at different levels
  - CNS : e.g. clomiphene, GnRH,…
  - OVARY : e.g. gonadotrophins, oestrogens, …
    luteal phase !
- Prohibit spontaneous LH rises
  - GnRH analogs

Start of Stimulation : When ?

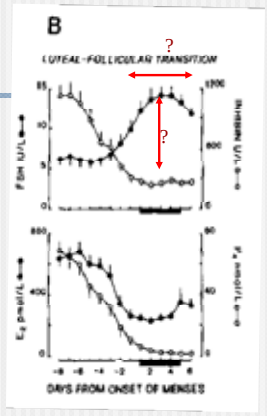
'background'

- Natural background (D1 or D2): is a dynamic situation
- Pretreatment : induces a basal situation
  - GnRH analogs
  - Gives a different type of 'cohort'

NATURAL BACKGROUND =

Inter-cycle FSH rise

CL function dept: Prog

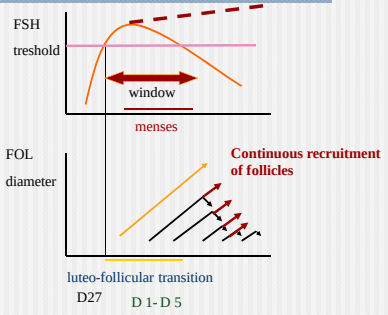


Prevailing AMH tonus:

Is : **-high in PCO ovary**
-increased if high basal LH
-augmented by androgens

(Pellatt, 2007;Andersen,2007)

FSH : rescues Follicles from atresia

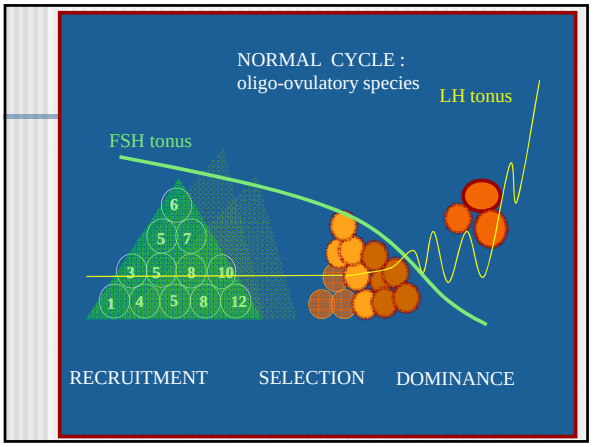


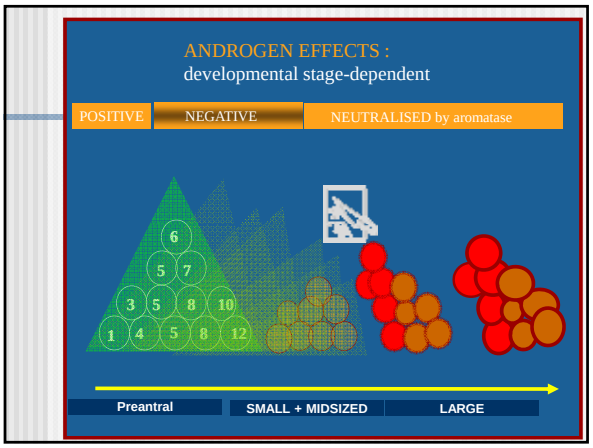
LH-action : will determine E/A

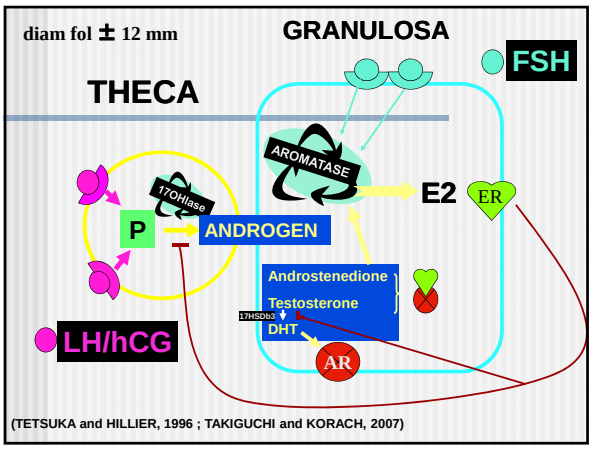
Still poorly understood & controversial
LH-R triggered by hLH or hCG

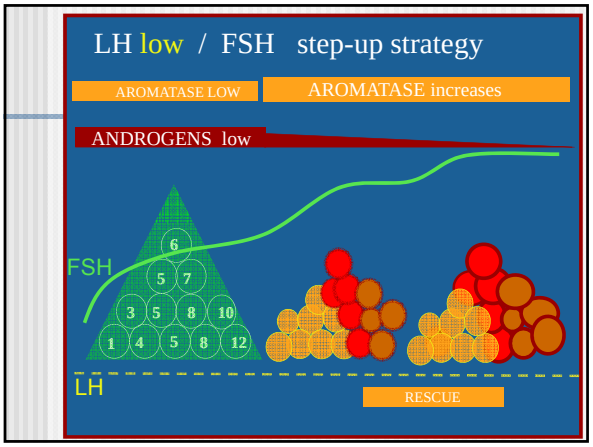
- De-selects the smaller follicles that were rescued by FSH : less oocytes
- If hCG : adds a qualitative dimension to cycle outcome*

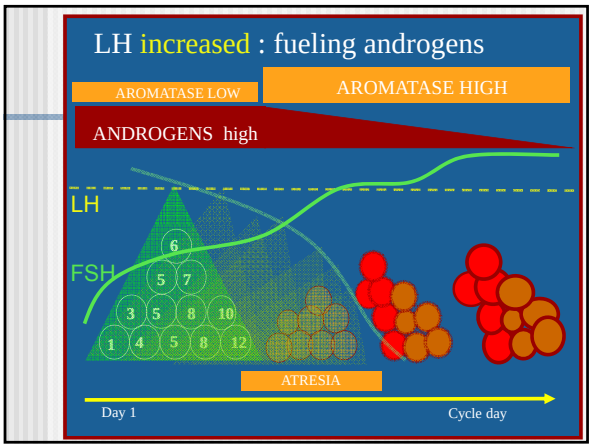
(* 3 independent studies)


NORMAL CYCLE :
oligo-ovulatory species
LH tonus
FSH tonus
6
5 7
3 5 8 10
1 4 5 8 12
RECRUITMENT
SELECTION
DOMINANCE


ANDROGEN EFFECTS :
developmental stage-dependent
POSITIVE
NEGATIVE
NEUTRALISED by aromatase
6
5 7
3 5 8 10
1 4 5 8 12
Preantral
SMALL + MIDSIZED
LARGE


diam fol ± 12 mm
GRANULOSA
FSH
THECA
AROMATASE
E2
ER
17OHlase
P
ANDROGEN
Androstenedione
Testosterone
17HSDb3
DHT
LH/hCG
AR
(TETSUKA and HILLIER, 1996 ; TAKIGUCHI and KORACH, 2007)



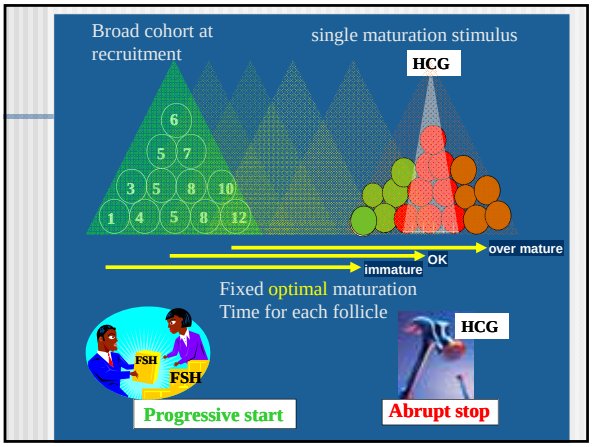


ending FSH stimulation : when ?

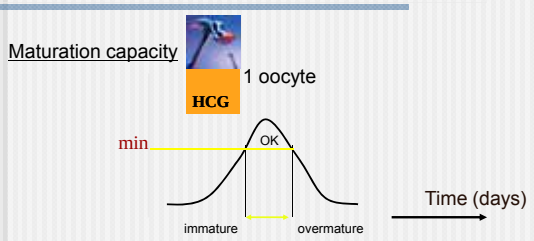
AIM = 'obtain 7-10 competent oocytes'

- When sufficient follicles are ≥15mm - <20mm
- Before sustained rises of Progesterone (>4nMol) (*)

(*) MERiT® dataset



the 'plasticity' of oocyte maturation



For how long does the oocyte in the follicle keep its full developmental potential 2-3 days ?

Known effects of being "outside"maturation window

- Immature

  - Cytoplasmic immature
  - Deficient Ca waves
  - Deficient pool of essential transcripts
  - Insufficient synthesis and storage of GSH
    - Meiotic arrest
    - Developmental arrest

- Over- mature

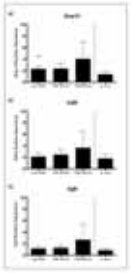
  - Prolonged transcriptional inactivity
  - Deficient activation machinery
  - degradation mRNA pools (are labile)
    - defects in E development

stimulation failures

- Low (≤ 3) and high(≥15) responses
- High immaturity (GV, M1) rates
- Repeatedly poor embryonic development
- Increased early pregnancy losses

Does stimulation by itself lead to an increase in oocyte aneuploidy rate ?

Effects of FSH dose on COC differentiation



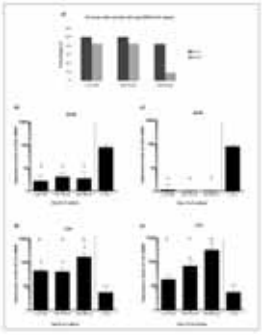
BMP-15 transcripts in oocyte

GDF-9 transcripts in oocyte

FGF-8 transcripts in oocyte

Sanchez et al., 2010

Effects of FSH dose on CUMULUS differentiation



AMH transcripts in cc

LH-r transcripts in cc

Sanchez et al., 2010

Stimulation regime in human ART : does it affect the uterus ?

- A variability can be expected due to:
  - FSH : dose, step-up / step-down
  - Presence of LH bioactivity:
    - Reduces the occurrence of HIGH Progesterone
    - use hCG = is more robust
  - Direct (?) effects of hCG (receptors on …?)
  - effects : by prolonged high E2, and premature progesterone rises

Advanced endometria: but by day 7 in Phase !

FSH-dosing : effects on human oocyte ?

Do we recruit follicles that are already compromised in quality ?
Do we induce an asynchrony in maturity of GC and oocyte ?

- Prospective studies
  Aneuploidy rate in embryo higher by 'strong' superovulation protocols
  (E. Baart et al,2006 : Verhelst et al, 2008)
- Retrospective data : hyperstimulated cycles
  Borghol et al (2006): in 5 out of 20 oocytes (collected as GV) disturbed methylation at H19
  Khoueiry et al.(2008): in vitro matured GV's : less methylation at KCNQ1OT1 than in-vivo matured

Clinical evidence: LH adds a qualitative dimension

LH activity:

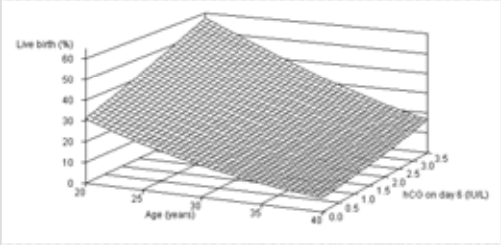
- When absent : high pregnancy losses (Westergaard, Fleming)
- When un-timely increased :
  - resumption of meiosis leads to early pregnancy loss
- Recent meta-analyses show positive effect on outcome

Use of LH-activity containing preparations
are associated with a lower frequency of aneuploidy
(A. Weghofer et al, 2008)

High ovarian response is correlated with more aneuploidy
(Haaf et al.2009)

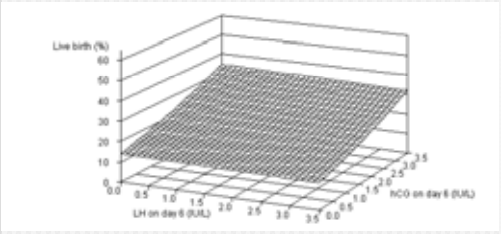
Prediction of % lifebirth: Age and conc of hCG on Day 6

HP- HMG



Prediction of % lifebirth: LH and hCG on Day 6

HP-hMG



Conclusions

- Data suggest direct and indirect effects of stimulation regimens on oocyte quality
  - Mediated by changes at level “2” and “3”
- Inter-individual differences in response
- Endocrine monitoring is needed to tailor treatments
- Better understanding of levels “2” and “3” might provide
  - Better insight in the “quality” of the oocyte
  - markers in cumulus for oocyte developmental competence
  - Innovative treatments to improve oocyte quality

How to see the future

- Aims : at a 'no-risk' treatment
  - Decrease side effects
  - Be sure our technology is 'embryo-safe'
- Pharmacogenomics : type your patient for 'response'
- Adapt treatment to the patient
- Typing the COC's retrieved by cumulus biopsy
- Differential culture dependent on maturity grade

- Dissociate stimulation cycle from transfer cycle
- Improved cryopreservation technologies offer new opportunities for improved outcomes