

REDUCING THE IMPACT OF OVARIAN STIMULATION

PH.D. PROJECT 2016-2019
UNIT OF REPRODUCTIVE MEDICINE
HERLEV/GENTOFTE HOSPITAL
COPENHAGEN UNIVERSITY



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AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY

AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY

STUDY BACKGROUND

TAKE HOME BABY RATES STILL SUBOPTIMAL



Laboratory techniques



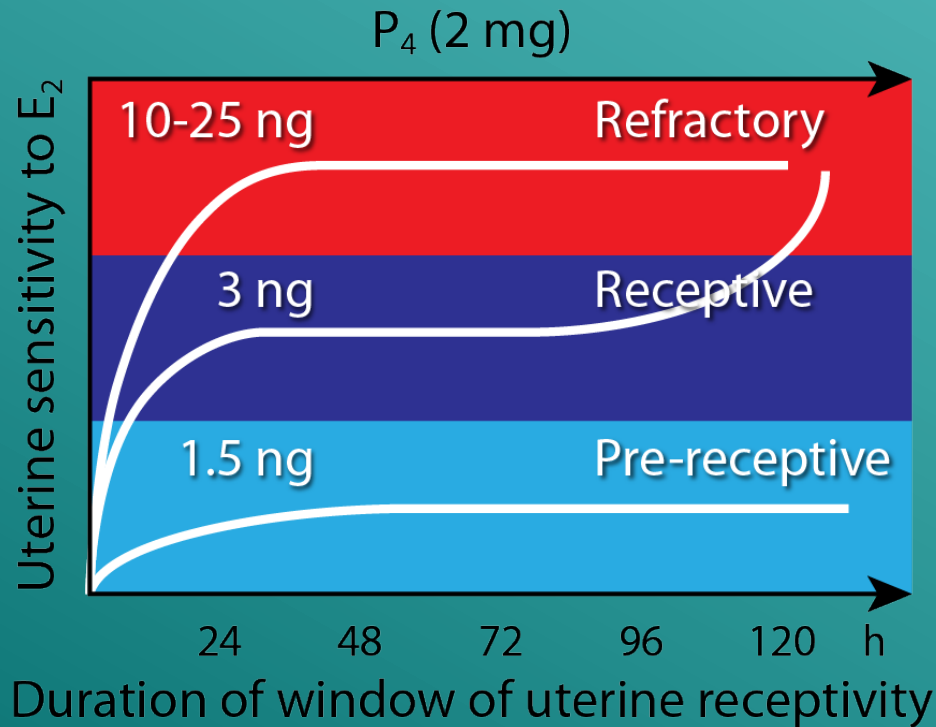
Ovarian stimulation regimens



More
physiological
conditions?



STUDY BACKGROUND ENDOMETRIUM



Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation

Wen-ge Ma*, Haengseok Song[†], Sanjoy K. Das, Bibhash C. Paria, and Sudhansu K. Dey[‡]

Proc Natl Acad Sci U S A. 2003 Mar 4;100(5): 2963-8

AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

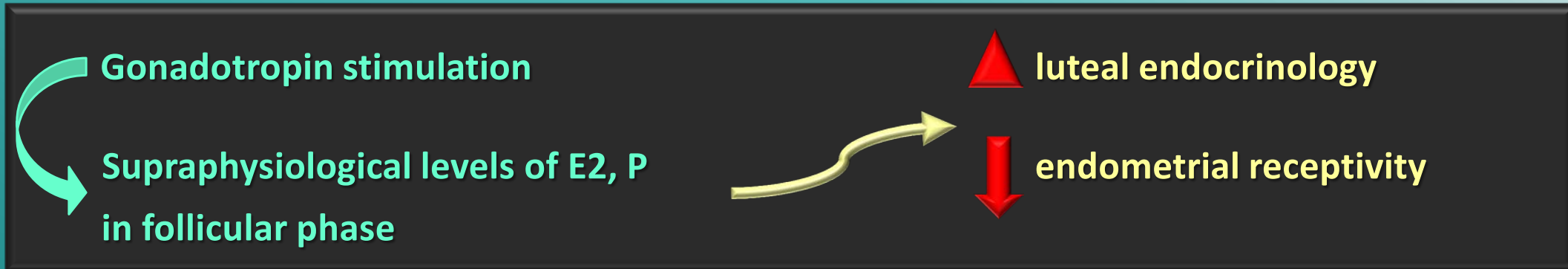
BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY

OBJECTIVE RATIONALE



Frozen Embryo Transfer Cycle

Modulation of endocrinology in Fresh Embryo Cycle

Suppression of
supraphysiological E2 and P
with
Aromatase inhibitor

OBJECTIVE

RESEARCH QUESTIONS

Can the detrimental effects of ovarian stimulation on outcomes in fresh embryo transfer cycles be ameliorated by co-treatment with aromatase inhibitor?

- 1. Levels of sex steroids and endometrial receptivity in late follicular phase**
- 2. Luteal phase endocrinology**

AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY

STUDY DESIGN

RANDOMIZED PLACEBO CONTROLLED CLINICAL TRIAL
The role of aromatase inhibitor in ovarian stimulation

128 patients
5 ReptoUnion Centres

Skåne

Rigshospitalet

Hvidovre

Holbæk

Herlev/Gentofte Hospital

FSH/LH receptor
polymorfism



AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY

INTERVENTION

AROMATASE INHIBITOR

Mechanism



aromatization of androgens to estrogens

Expected Effects



E2 late follicular phase

+



premature **P4** rise



endometrial receptivity

INTERVENTION

AROMATASE INHIBITOR



Letrozol[®] 5 mg/day

GnRH Antagonist 0,25mg

recFSH 150 IU

hCG inj

Progesterone



CD 2-3

Stim. Day
5

Day before
hCG

OPU

ET

7 days
post hCG

10 days
post hCG

INTERVENTION

AROMATASE INHIBITOR

SAFETY

Publication of an abstract on outcome of **150 babies** following the treatment with letrozole or letrozole and gonadotropins.

Biljan MM, Hemmings R, Brassard N. Fertil Steril 84 (Suppl 1.1): O-231. 2005



Concerns of teratogenicity of letrozole

“Major methodological flaws in this study as the intervention group was not well controlled”

(Franik et al 2014. Cochrane Database Syst Rev)

2 studies including 911 and 470 infants compared use of letrozole to CC and spontaneously conceiving women: **no higher levels of minor or major congenital malformations or cardiac abnormalities in newborns after usage of letrozole for ovulation induction.**

Tulandi et al. Fertil Steril. 2006, Formann et al. J Obstet Gynaecol Can. 2007

623 children born to infertile women. Overall, congenital malformations, chromosomal abnormalities were found in 5 out of 171 (**2.9%**) **babies in natural conception** group and 5 out of 201 babies in the **letrozole group (2.5%)** and in 10 of 251 babies in the **CC group (3.9%)**. Sharma S et al. PLoS One. 2014

Double-blind, multicenter trial randomized **750 women** to receive letrozole or clomiphene. “letrozole was associated with higher live-birth and ovulation rates among infertile women with the polycystic ovary syndrome.....and **no significant differences in overall congenital anomalies**”. Legro RS et al. N Engl J Med. 2014

INTERVENTION

AROMATASE INHIBITOR

SIDE EFFECTS in < 10 %

Nausea

Vomiting

Diarrhea

Abdominal pain

Bloating

Mild headache

Joint- and musclepain

All reported transient

AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY

STUDY POPULATION

POWER



Reduction in incidence of serum P rise > 1.5 ng/ml on day of hCG

P rise > 1.5 ng/ml in 24%

Papanikolaou EG et al, 2012. GnRH-agonist versus GnRH-antagonist IVF cycles: is the reproductive outcome affected by the incidence of progesterone elevation on the day of hCG triggering? A randomized study. Hum Reprod 2012; 0:1-7

Variations in measurement \longrightarrow estimate of incidence 15 %

Cut off for estradiol on day of hCG of 1750 pg/ml for premature rise in progesterone > 1.5 ng/ml

Kyrou D et al. The relationship of premature progesterone rise with serum estradiol levels and number of follicles in GnRH antagonist/recombinant FSH stimulated cycles. Eur J Obstet Gynecol Reprod Biol 2012; 162:165-168

Potential to achieve reduction \longrightarrow estimate of incidence 2 %

128 patients

Reduction in the incidence of serum progesterone > 1.5 ng/ml on the day of hCG from 15% to 2%, with 80% power at a p - value of < 0.005 .

Drop out rate ≈ 10 %

STUDY POPULATION

INCLUSION

- Normal ovulatory cycles
- Male factor indication for IVF
- Tubal factor indication for IVF
- Medical indication for IVF
- Age <40
- AFC 10-25

EXCLUSION

- Poor responders
 - < 4 oocytes obtained
 - AMH < 4pmol/l OR
CD 2-3 FSH > 12 IU
- ≥ 3 IVF with negative serum hCG
- PCOS
- Smoking
- BMI ≥ 31

AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY

OUTCOME MEASURES

PRIMARY

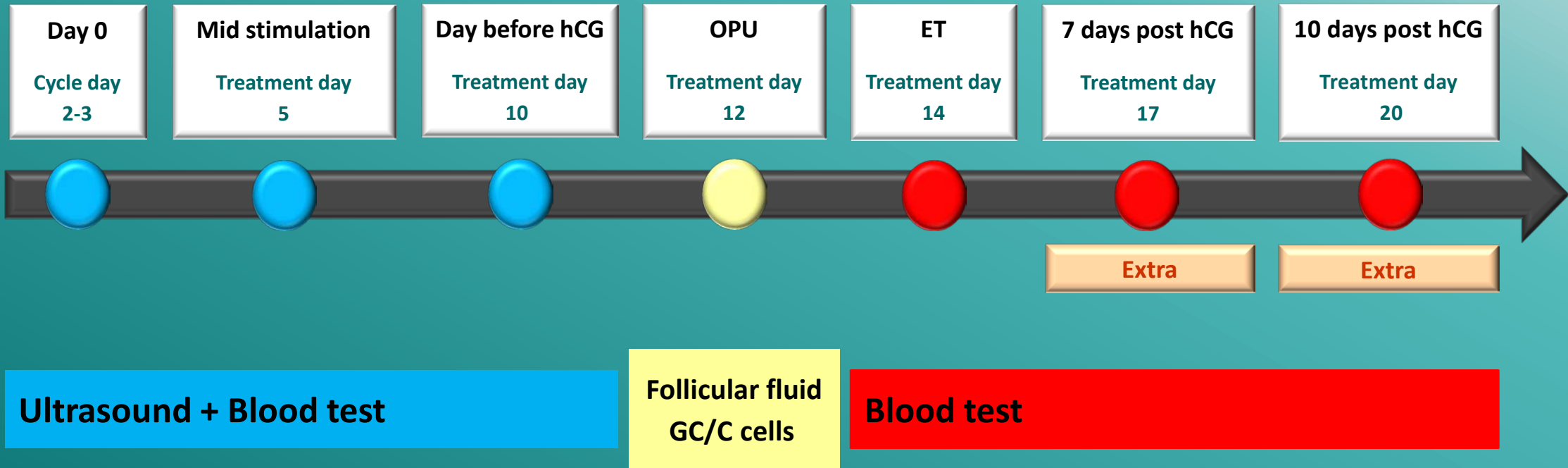
Serum P on day of hCG

SECONDARY

- **P, E2, Testosterone** levels day 4 – 14 (AUC)
- **Follicles > 12 mm** on day of hCG
- **Endometrial thickness** on day of hCG
- **Follicular fluid**
 - P, E2, Testosterone
 - AMH
 - PAPP-A, Inhibin
- **Oocytes** retrieved
- **Embryo** quality
- **Implantation** rate
- **Clinical pregnancy** rate

OUTCOME MEASURES

HOW TO GET THERE...



AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY

TIMELINES

THE RIOT PROJECT

Danish Medicines Agency

Ethics Committee

Danish Data Protection Agency

EudraCT: 2015-005682-24
2015-005683-41

GCP monitor: Unit of GCP
Bispebjerg Hospital

Data collection

August 2016 – March 2018

**Data analysis &
publication**

Dec. 2018

Ph.d. Thesis

Dec. 2018 – March 2019

AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

BACKGROUND

OBJECTIVE

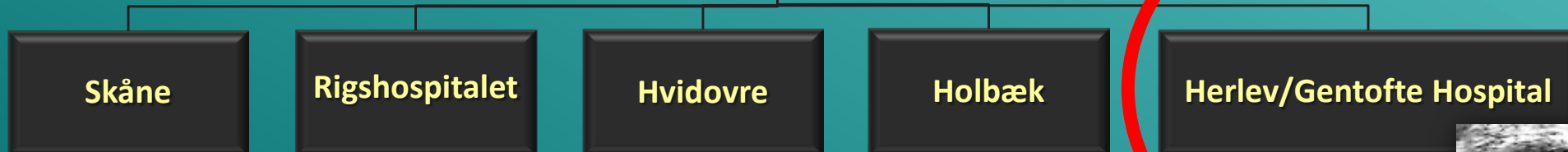
OUTCOME MEASURES

SUMMARY

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EXPLORATORY STUDY



AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

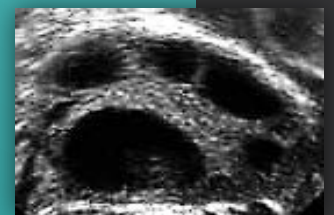
NESTED STUDY

BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY



BACKGROUND

TO GET IT JUST RIGHT...

Individualizing stimulation protocols for IVF treatment
on the basis of
specific clinical biomarkers of ovarian reserve

Predicting the ovarian reserve

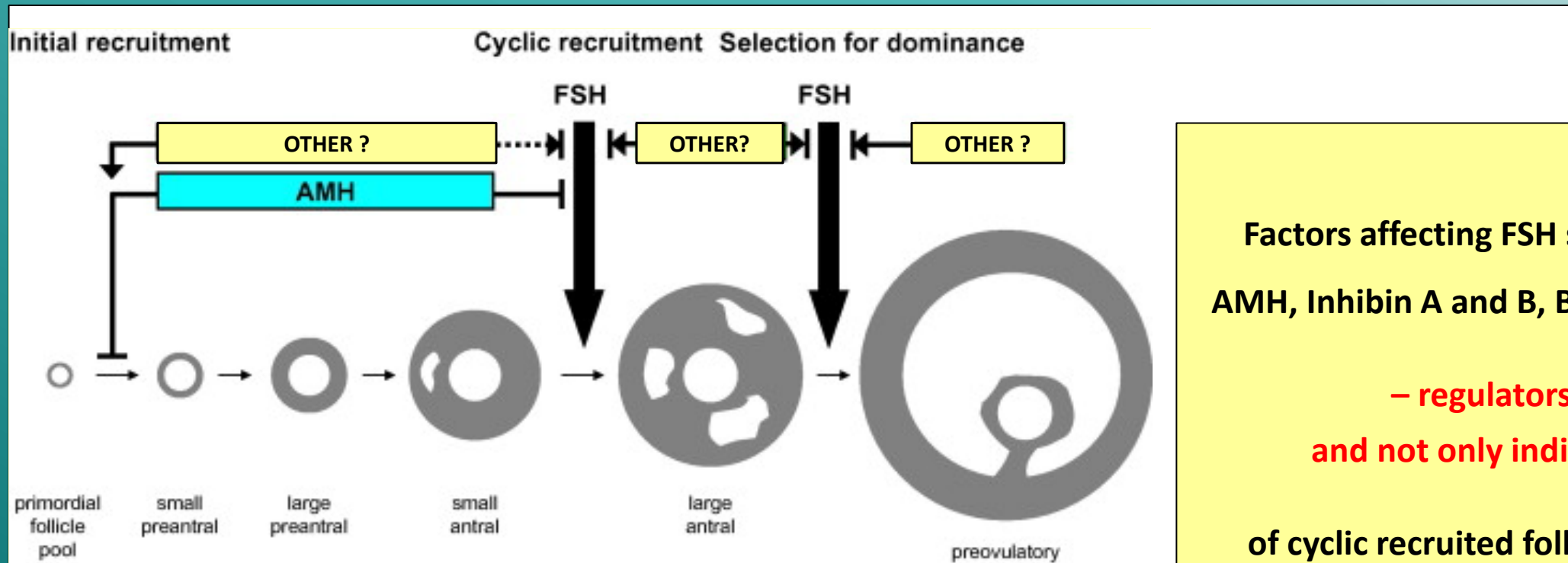
AMH

AFC

Determining the follicle pool

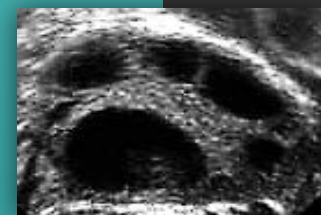


BACKGROUND MAPPING FOLLICLE GROWTH



Factors affecting FSH sensitivity
 AMH, Inhibin A and B, BMP, PAPP-A
 – regulators
 and not only indicators
 of cyclic recruited follicle pool?

Adapted from Visser J, Themmen A 2014.
 Role of anti-Müllerian hormone and bone morphogenetic proteins in the regulation of FSH sensitivity.
 Molec Cell Endocrinol. 2014;382(1):460-5.



AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

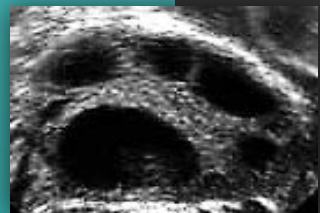
NESTED STUDY

BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY



OBJECTIVE

ENDOCRINE GATEKEEPERS TO FOLLICLE RECRUITMENT ?
ARE THEY DISRUPTED BY OVARIAN STIMULATION ?

Endocrine and follicular parameters

Gonadotropins

Sex steroids (E2, P, 17-HP, T)

AMH, BMP, PAPP-A

AFC



Uterine factors

Endometrial secretion

Uterine contractibility



Impact of supraphysiological estradiol?



OBJECTIVE

RESEARCH QUESTIONS

1. Endocrine regulators of cycle wave secondarily recruited follicles
2. Disruption by ovarian stimulation?
3. Correction with Aromatase inhibitor?
4. Impact of correction with Aromatase Inhibitor on uterine factors



AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

BACKGROUND

OBJECTIVE

OUTCOME MEASURES

SUMMARY



OUTCOME MEASURES

Study parameters for analysis

Size of follicle cohort in relation to serum endocrine and paracrine markers and its inter-cyclic variability

Expression of cytokine and growth factors in endometrial secretions, uterine contractility and follicular fluid endocrine and paracrine markers

AMH

FSH, LH

Estradiol

Progesterone

17-Hydroxyprogesterone

Testosterone

PAPP-A

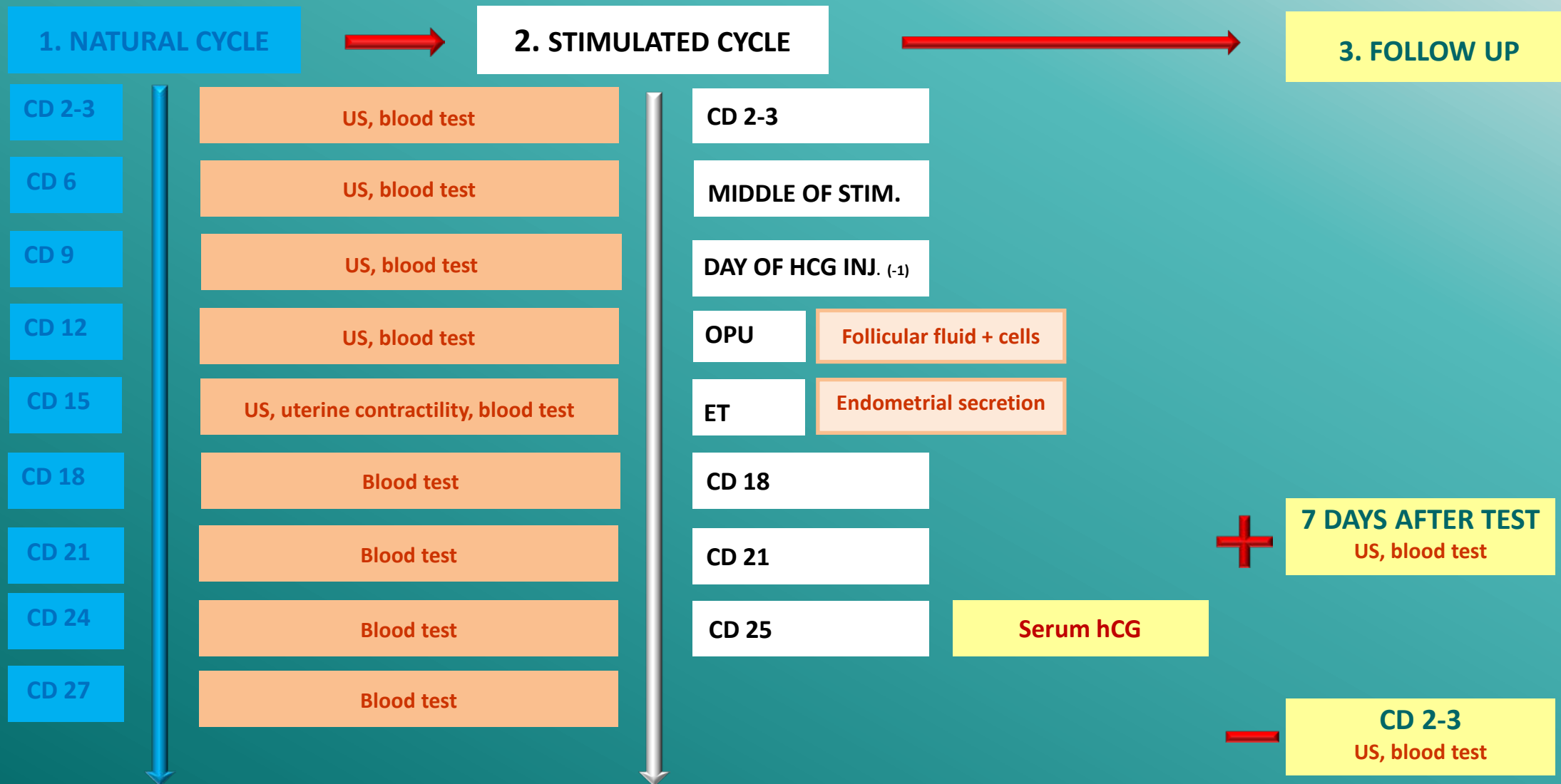
Inhibin A and B

BMPS



OUTCOME MEASURES

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AGENDA

STUDY BACKGROUND

OBJECTIVE

DESIGN

INTERVENTION

POPULATION

OUTCOME MEASURES

TIMELINES

NESTED STUDY

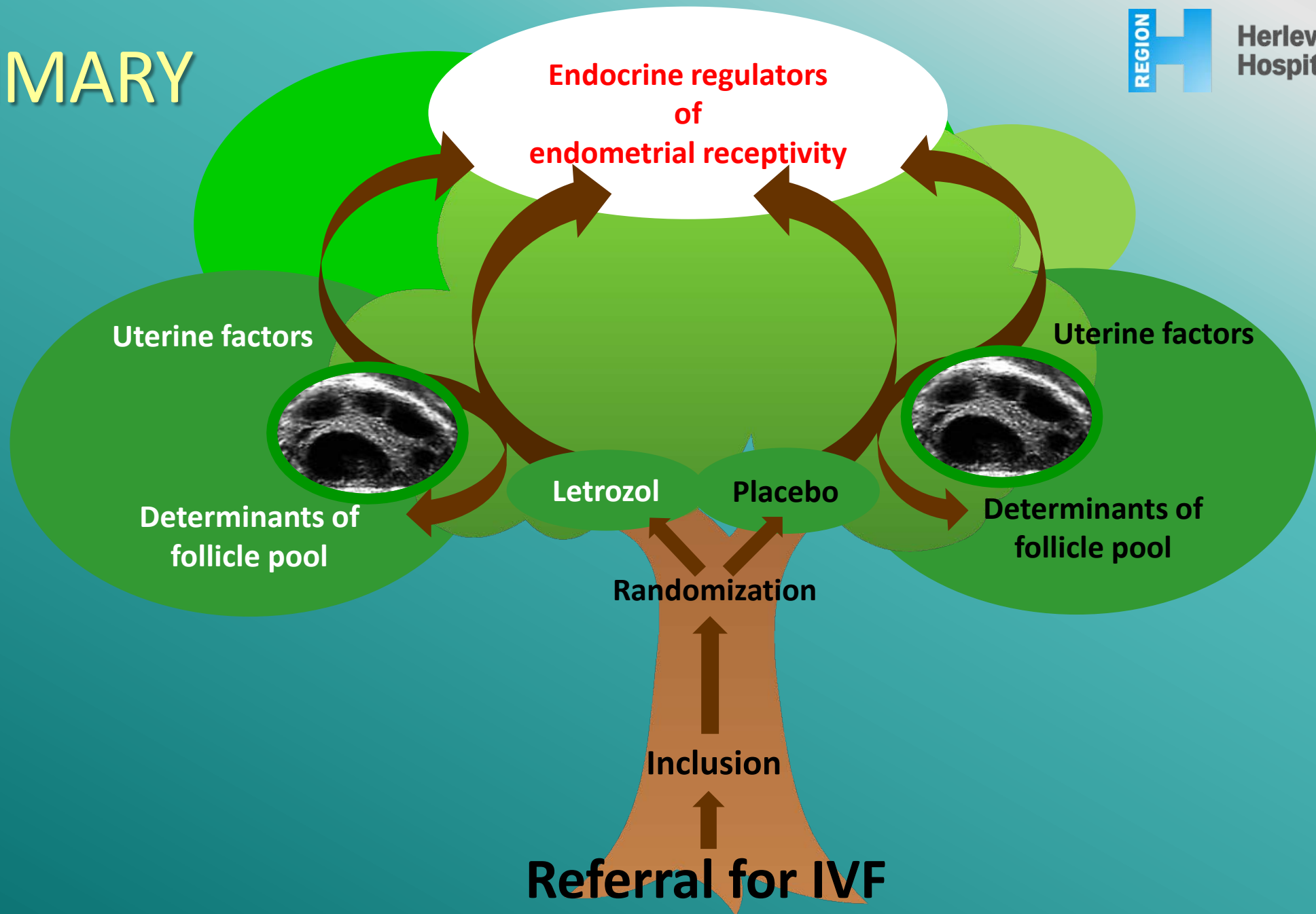
BACKGROUND

OBJECTIVE

OUTCOME MEASURES

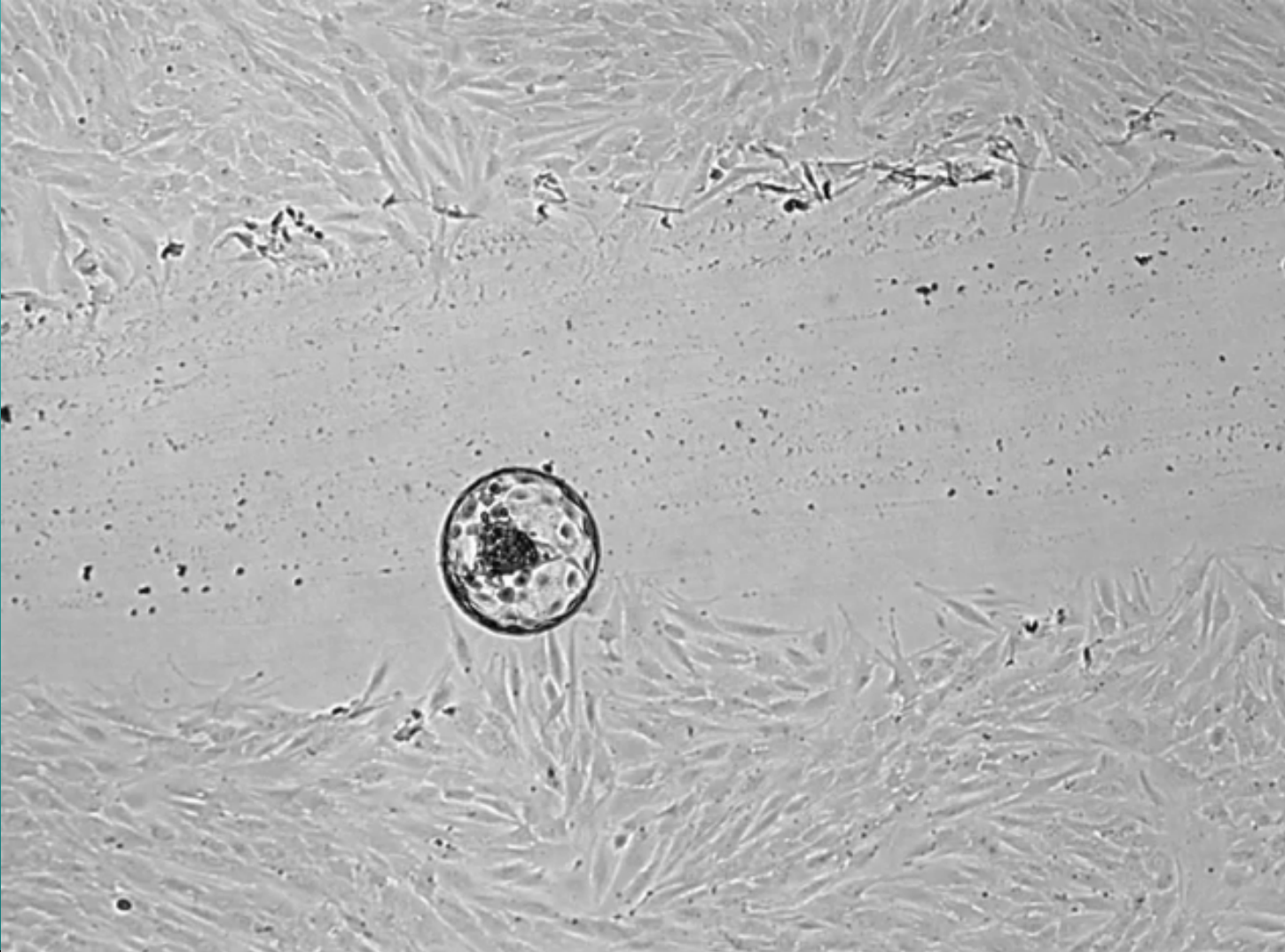
SUMMARY

SUMMARY



The active endometrium in implantation

from Weimar et al, PLoS 2012 - with
courtesy from Nick S. Macklon



Thank you for the attention

Agnieszka K. Warzecha, Nick S. Macklon, Leif J. Bungum, Claus Yding Andersen, Sven O. Skouby

Research Nurse Hanne Udengaard and Laboratory Consultant Marie Louise Grøndahl

THE RIOT PROJECT - RESULTS

RIOT A

PUBLICATION A/1

Can the impact of ovarian stimulation on late follicular phase sex steroid levels (and hence endometrial receptivity) be mitigated by co-treatment with aromatase inhibitors during ovarian stimulation?

PUBLICATION A/2

Does co-treatment with aromatase inhibitors 'normalize' the endocrinology of the luteal phase?

PUBLICATION A/3

Is the gene expression in cumulus and granulosa cells after ovarian stimulation affected by suppression of supra-physiological estradiol with AI?

RIOT B

PUBLICATION B/1

How do specific autocrine, paracrine and endocrine factors act as gate keepers to cyclic follicle recruitment?

Are these factors altered by ovarian stimulation, and can this effect be modulated by AI?

PUBLICATION B/2

Does suppression of supra-physiological estradiol with AI reduce uterine contractility and the disruption of cytokine markers of endometrial receptivity caused by ovarian stimulation with exogenous gonadotropin?

PUBLICATION B/3

Is the gene expression in cumulus and granulosa cells after ovarian stimulation affected by suppression of supra-physiological estradiol with AI?