



Superovulation and Follicular aging theme

THE EFFECT OF SUPERSTIMULATION TREATMENT AND FOLLICULAR AGING ON GENE EXPRESSION OF GRANULOSA CELLS

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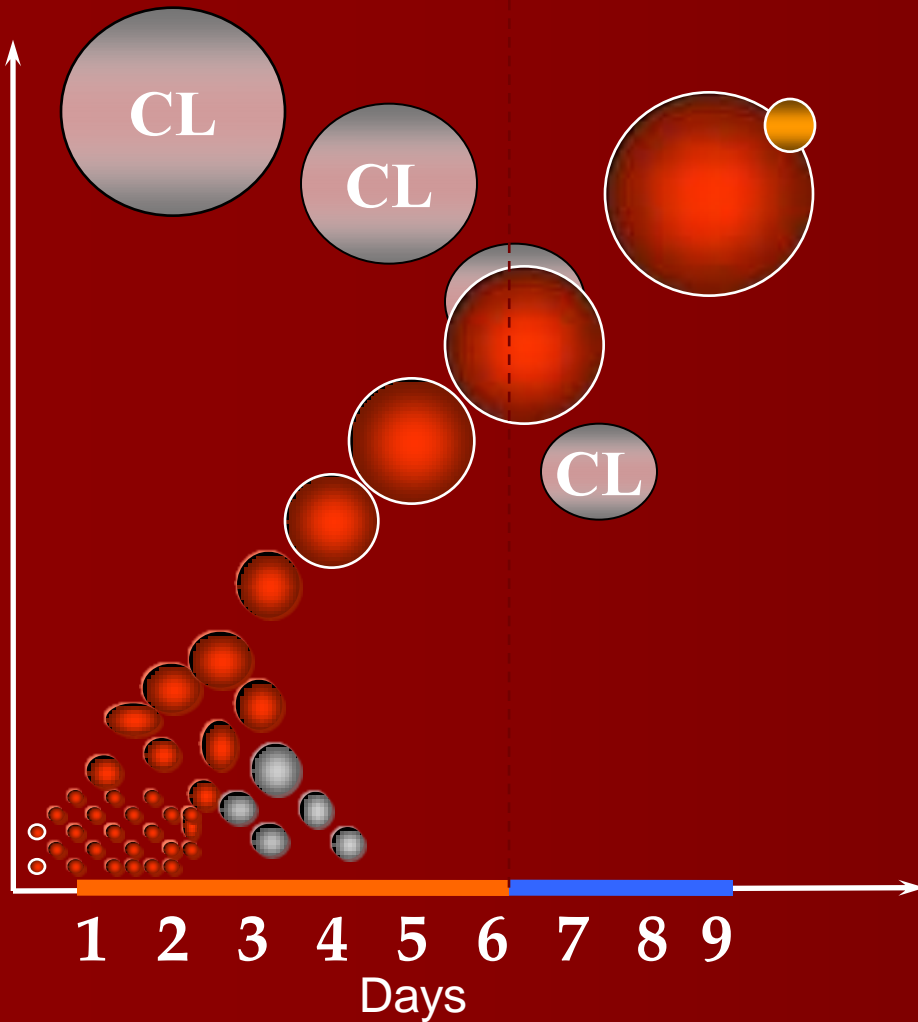
Gregg P. Adams, Marc-Andre Sirard, Muhammad I. R.
Khan, Jaswant Singh



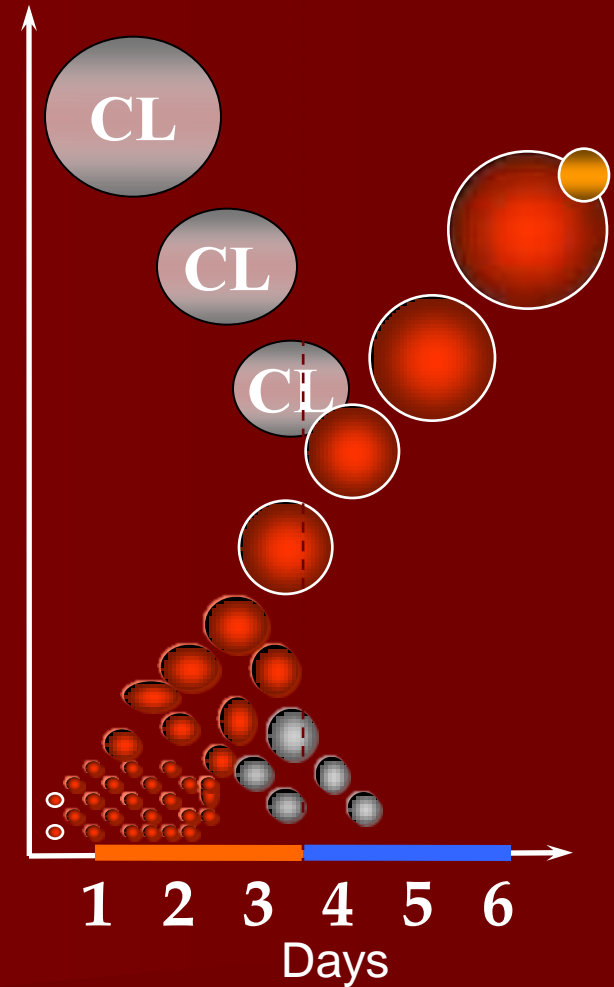
Background

- **Follicles develop in a wave-like pattern**
- **Estrous cycle is composed of 2 or 3 follicular waves**

2-wave cycle



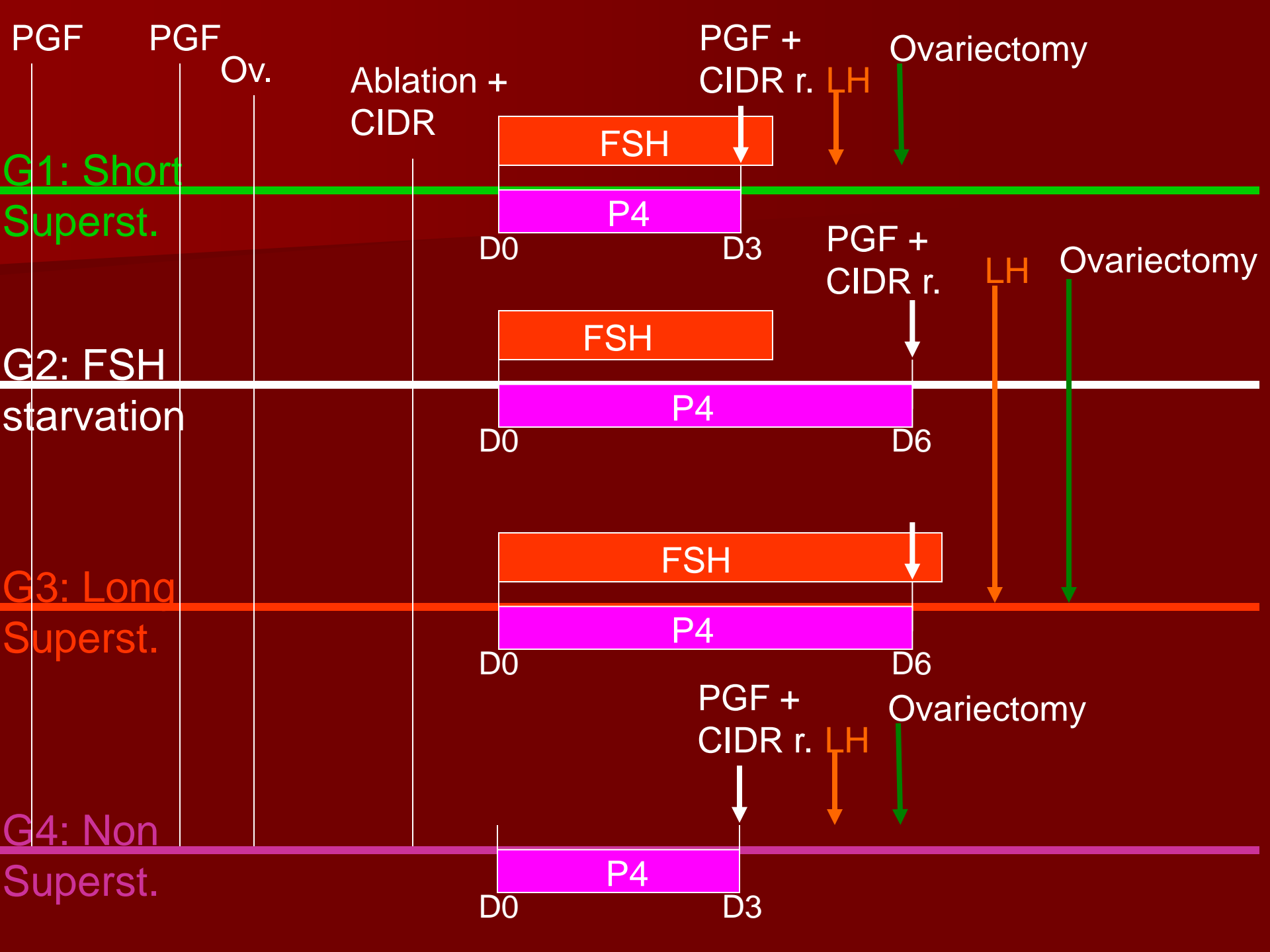
3-wave cycle



■ Is fertility higher in 3-wave animals compared with 2-wave animals??
Contradictory results.

Experiments

1. *Gene expression in follicle obtained after superstimulation treatment*
2. *Follicular aging/FSH starvation*



Previous study

- Short Superstimulation vs Long Superstimulation – differences in the response to superstimulation but no difference in oocyte competence (Blastocyst rate)
- FSH starvation treatment led to a loss of ovulatory capability.

General Objective

To determine the effect of superstimulation treatment and follicular aging on gene expression of granulosa cells.

General Hypothesis

We hypothesize that aged follicles and follicles that undergo superstimulation will have different gene expression than dominant follicles from natural cycle

Samples	G1: Short Super	G2: FSH Starvation	G3: Long Super	G4: Non Super	TOTAL
Whole Foll wall	18	18	18	18	72
Granulosa	18	18	18	18	72
Theca	18	18	18	18	72
Oocytes	18	18	18	18	72
Foll Fluid	18	18	18	18	72
Plasma*	12	12	12	12	48

6 animals/group – 3 animals for microarray and 3 for RT-PCR

3 follicles/animal = 18/sample type/group

*at time of LH treatment and at time of surgery

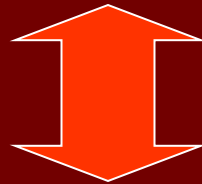
Microarray design

1) Effect of superstimulation

Pool of 3 largest follicles vs. dominant foll

Granulosa cells

Group 1 (Superstimulation)

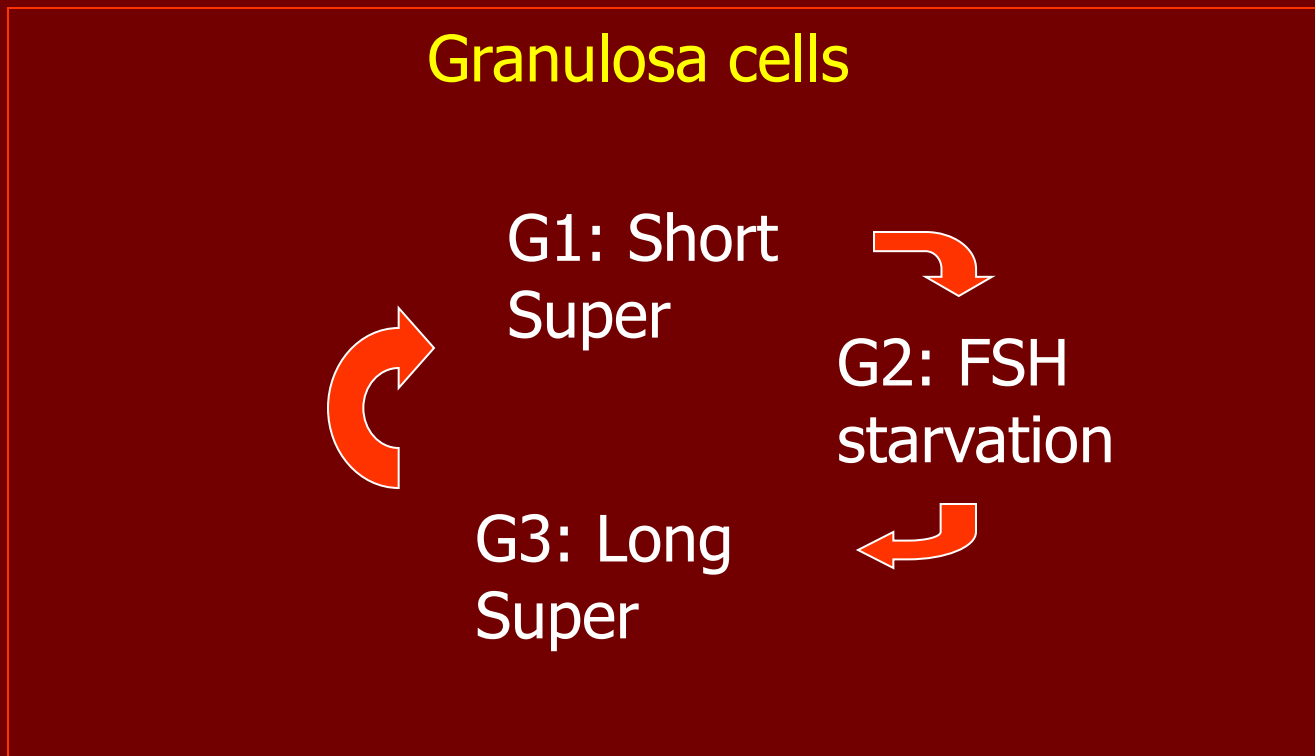


Group 4 (Non Superstimulation)

Microarray design

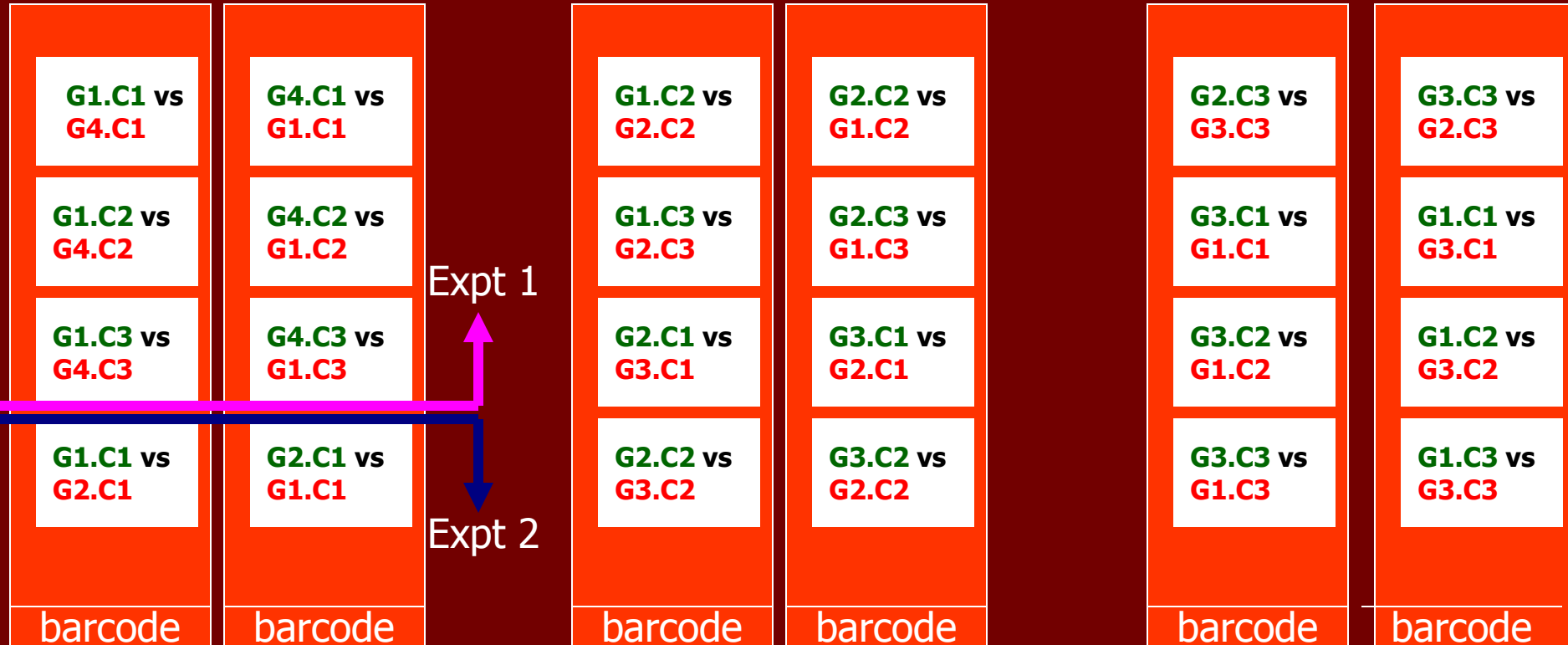
3) Follicular aging – Loop design

Pool of 3 largest follicles



Labeling and Hybridization Design

■ Microarray layout:



Data analysis

- Softwares used:
 - 6.0 Array-Pro Analysis
 - ELMA
 - Gydle
 - FlexArray
 - NIA (PCA analysis)
 - Ingenuity

Preliminaries Results

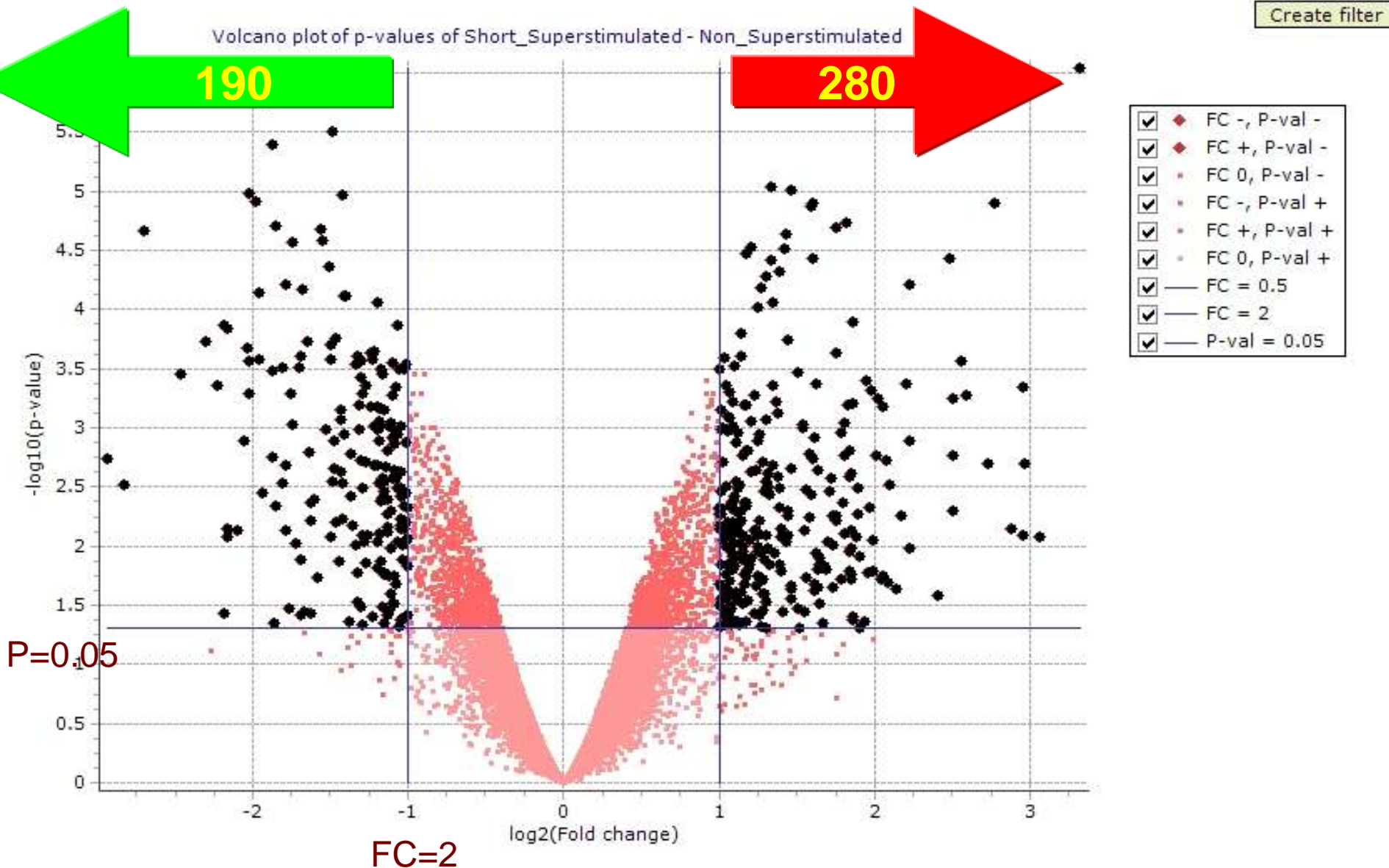
- Expt 1 (Super vs. Non):

470 total genes

- FDR:

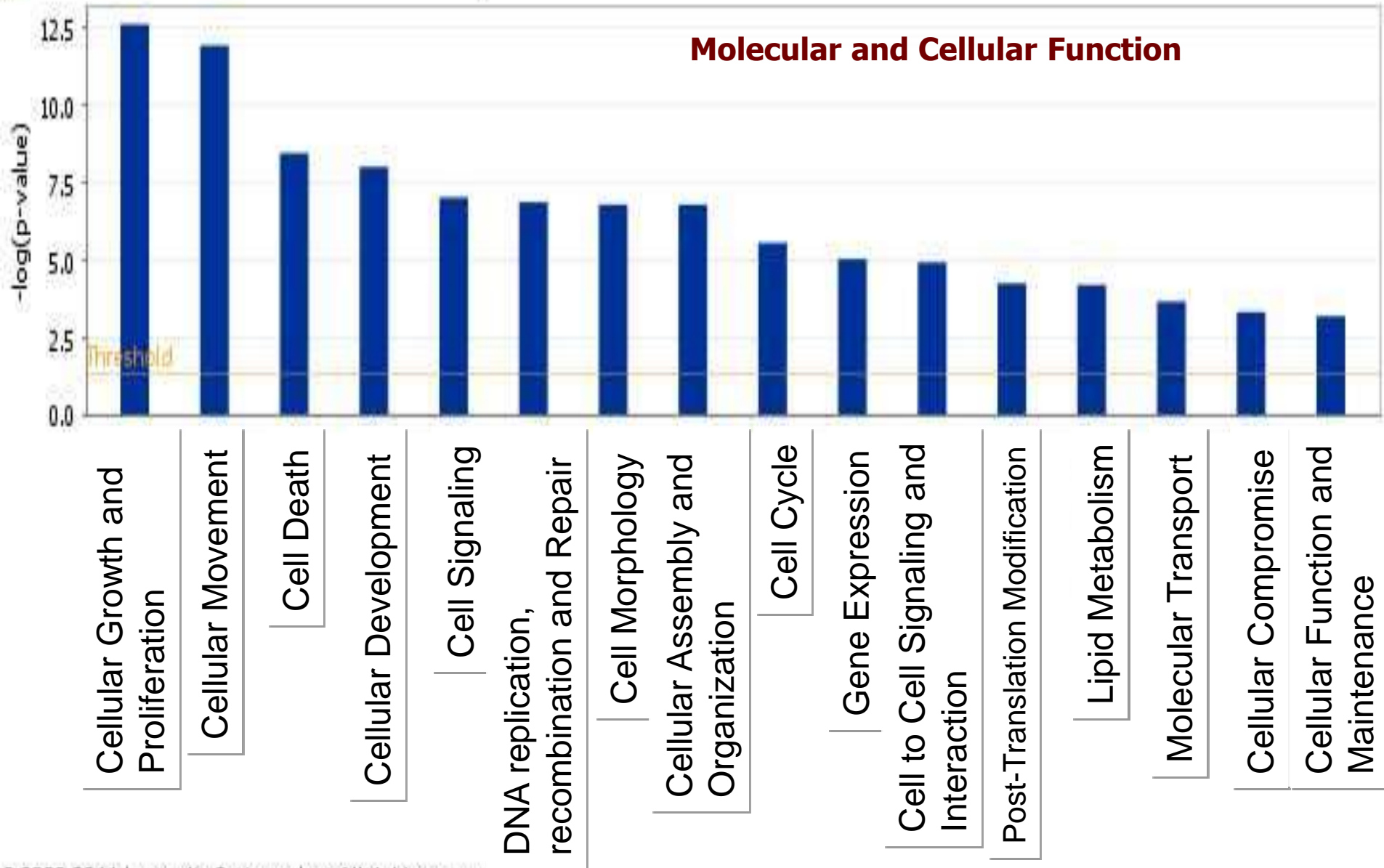
1. FC=2; P=0.05
2. FC=2; P=0.1
3. FC=1.5; P=0.1

Preliminaries Results



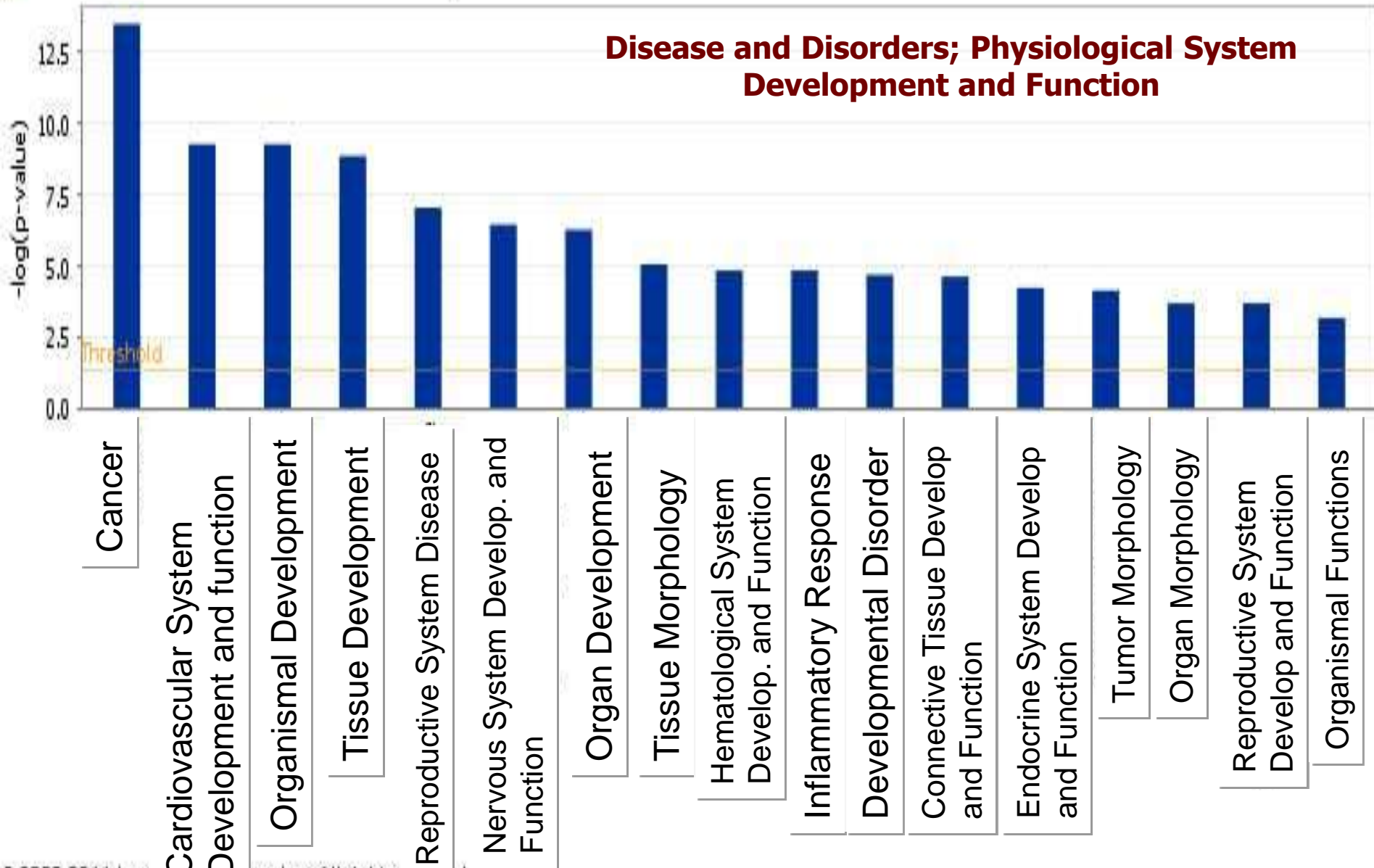
Ingenuity

■ super_vs_non_loess_median - 2011-05-27 11:18 AM



Ingenuity

■ super_vs_non_loess_median - 2011-05-27 11:18 AM



Top Genes (up and down regulated)

UP Regulated

- NTS
- S100B*
- IGFBP1
- GFPT2
- SLC39A8
- COBLL1*
- FOS
- LUM
- RCAN1*
- THBS1

Down Regulated

- FES
- NRP1*
- ANKRD43
- CNIH3
- MGST2
- ANGPT2
- LTF
- TRIB2*
- SYNE1
- MXRA8

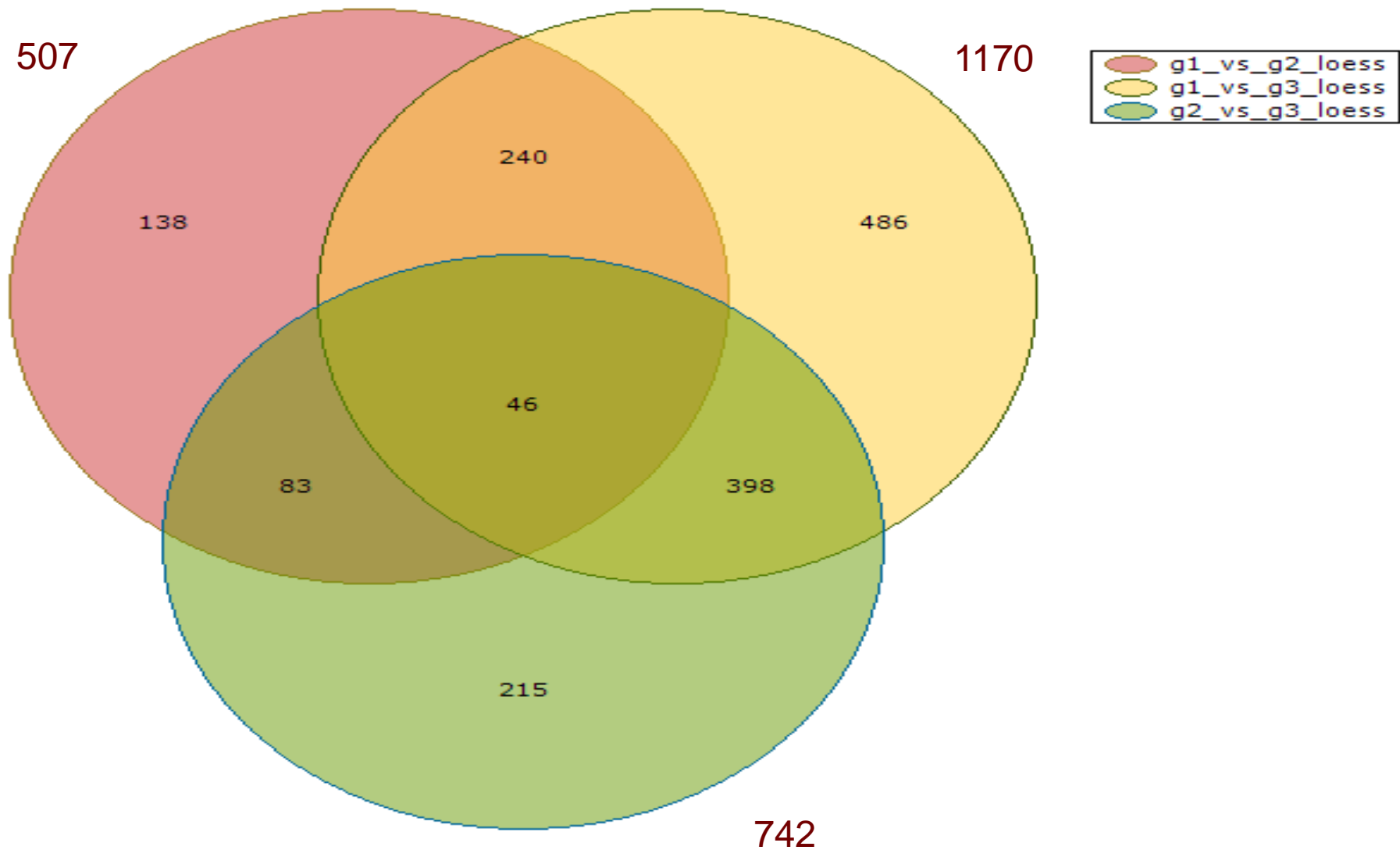
Preliminaries Results

- Expt 2 (Follicular aging):
 1. Short Super vs FSH Starvation - **507** total genes
 2. Short Super vs Long Super: **1170** total genes
 3. FSH Starvation vs Long Super: **742** total genes

Preliminaries Results

■ Expt 2:

Venn diagram

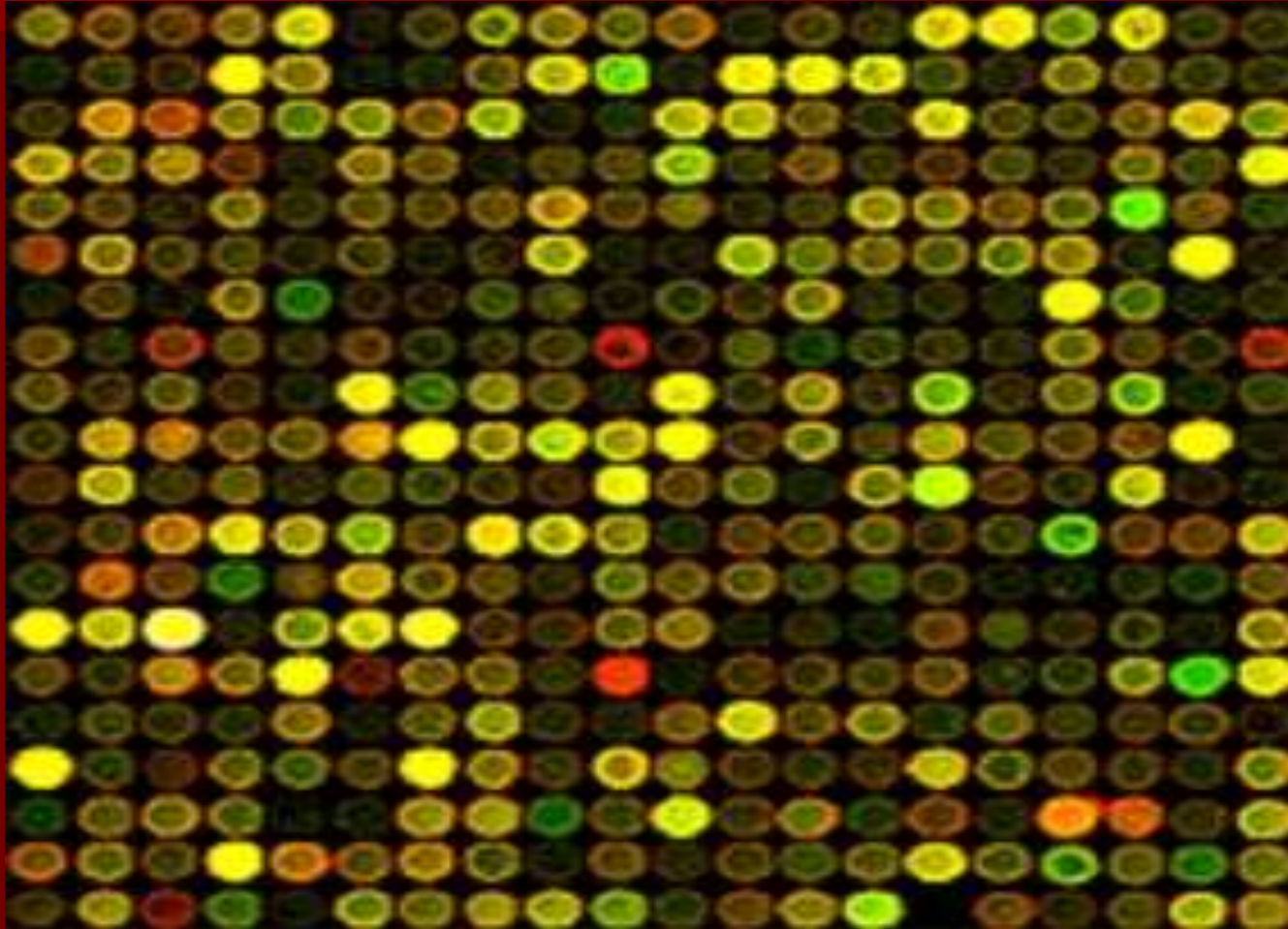


Any Questions??



Yes, many questions!!

Thank you!



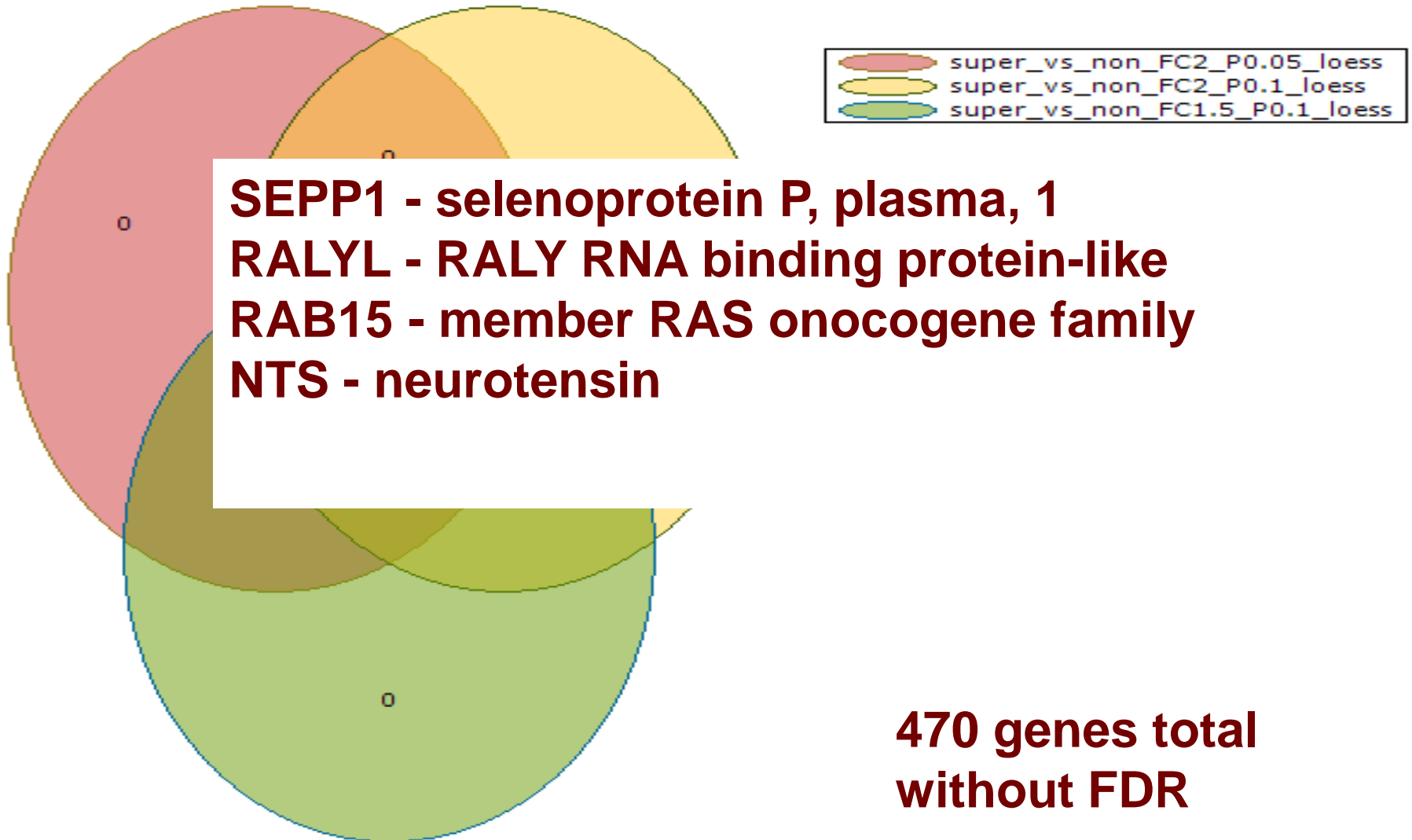
Samples

- Samples: Granulosa, Theca, Oocytes, Whole Follicular wall, Follicular Fluid, Plasma for RIA (at LH treatment and at day of surgery).
- 6 animals/group
- 3 samples/animal = 18/samples/group

Preliminaries Results

■ Expt 1:

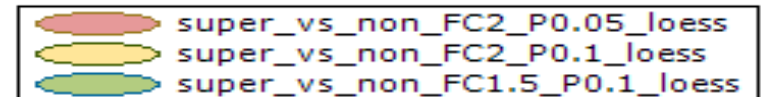
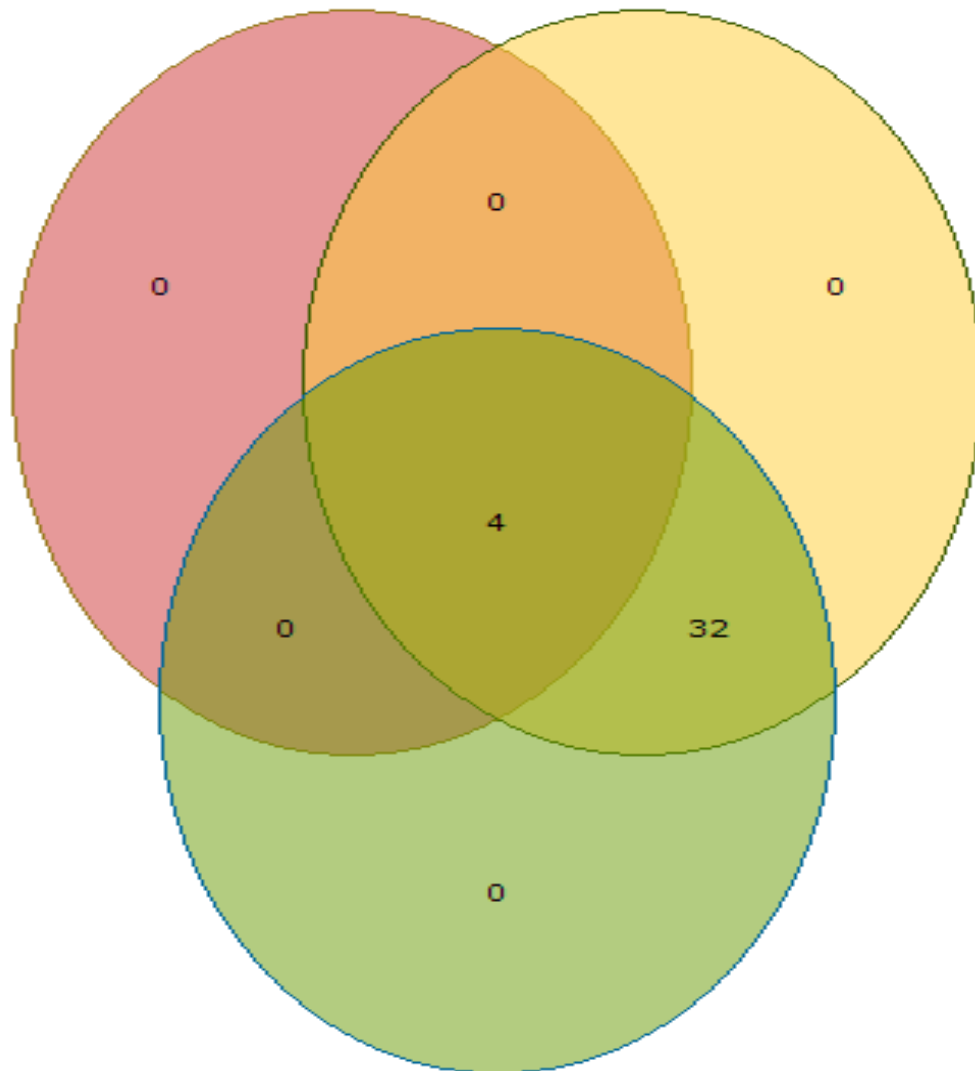
Venn diagram



Preliminaries Results

■ Expt 1:

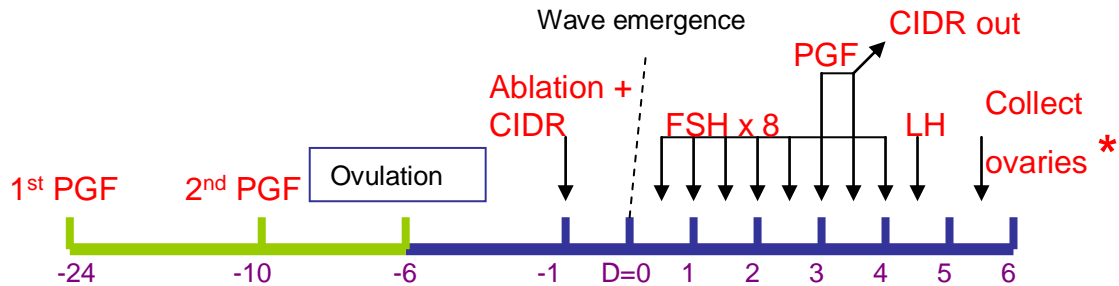
Venn diagram



**470 genes total
without FDR**

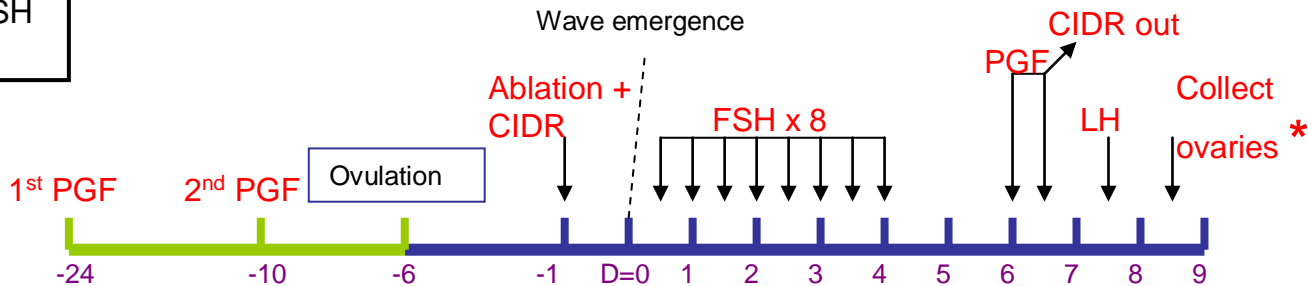
Group 1

Non aged follicle - control



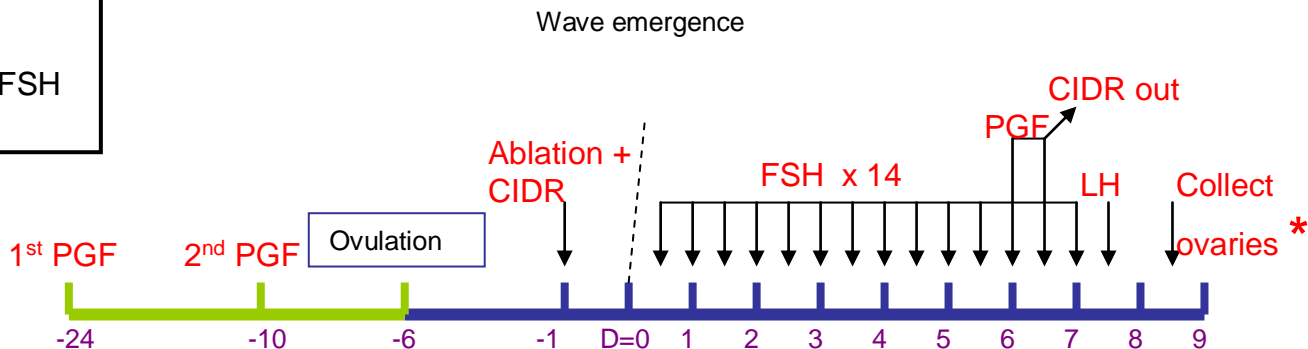
Group 2

Aged follicles with FSH starvation



Group 3

Aged follicle without FSH starvation



Group 4 (control)

Non-superstimulated

