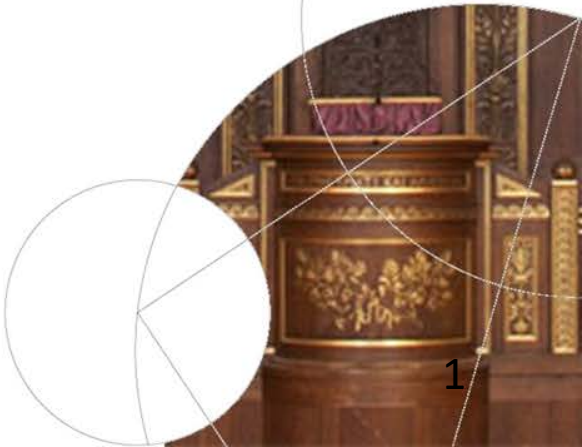
RNA-Seq transcriptomics analyses to identify biomarkers for *in vitro* production of embryos in cows

G. Mazzoni, S.M.Salleh , K.Freude, H.S. Pedersen , L. Stroebech, H.Callesen, P. Hyttel and H.N. Kadarmideen

GIFT project: **G**enomic **I**mprovement of **F**ertilization **T**raits in Danish and Brazilian Cattle

Website**: www.gift.ku.dk**



Background

Increase **genetic improvement** in cow and the **animal breeding process**

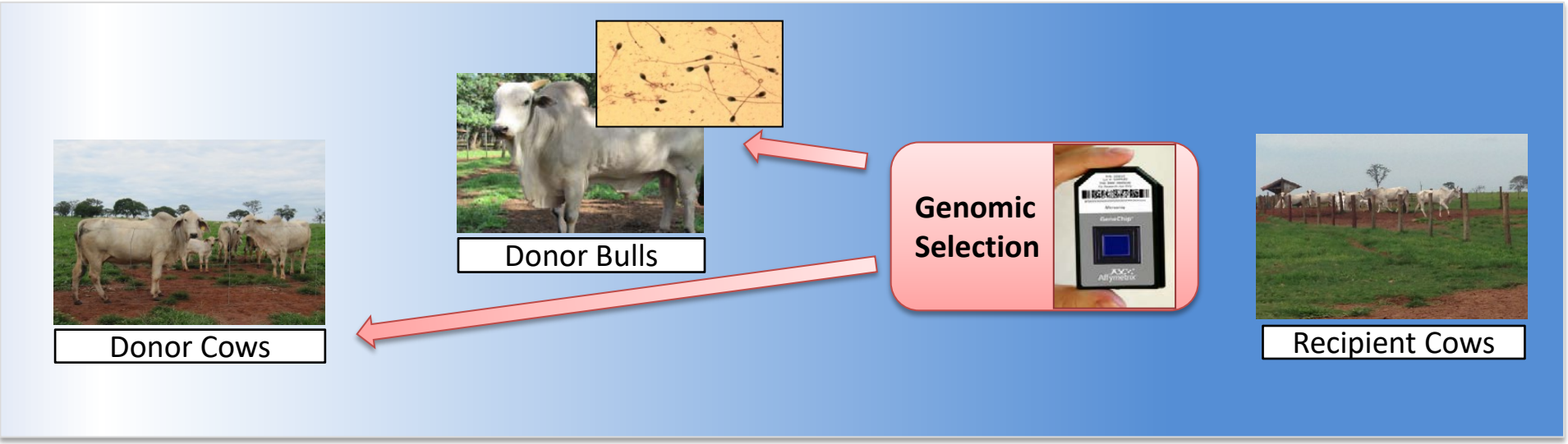
- ***In vitro* production (IVP)**

Production of numerous high genetic merit embryos

- **Genomic Selection (GS)**

Increase the accuracy of the Estimated Breeding Value (EBV), reduce the costs.

Combining IVP and GS



Kadarmideen, HN., et al. 2015. Animal Reproduction 12 (3): 389-396.

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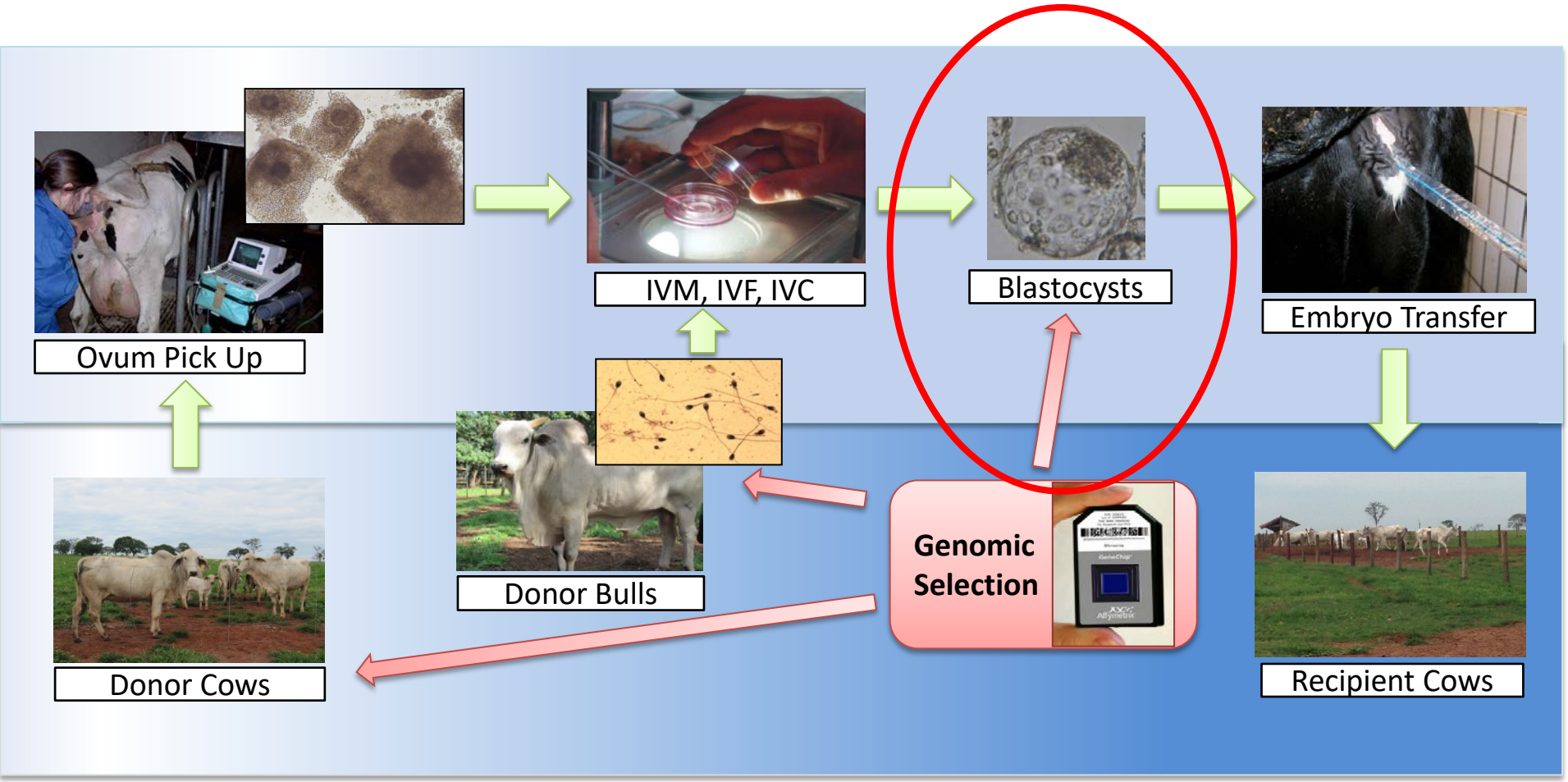
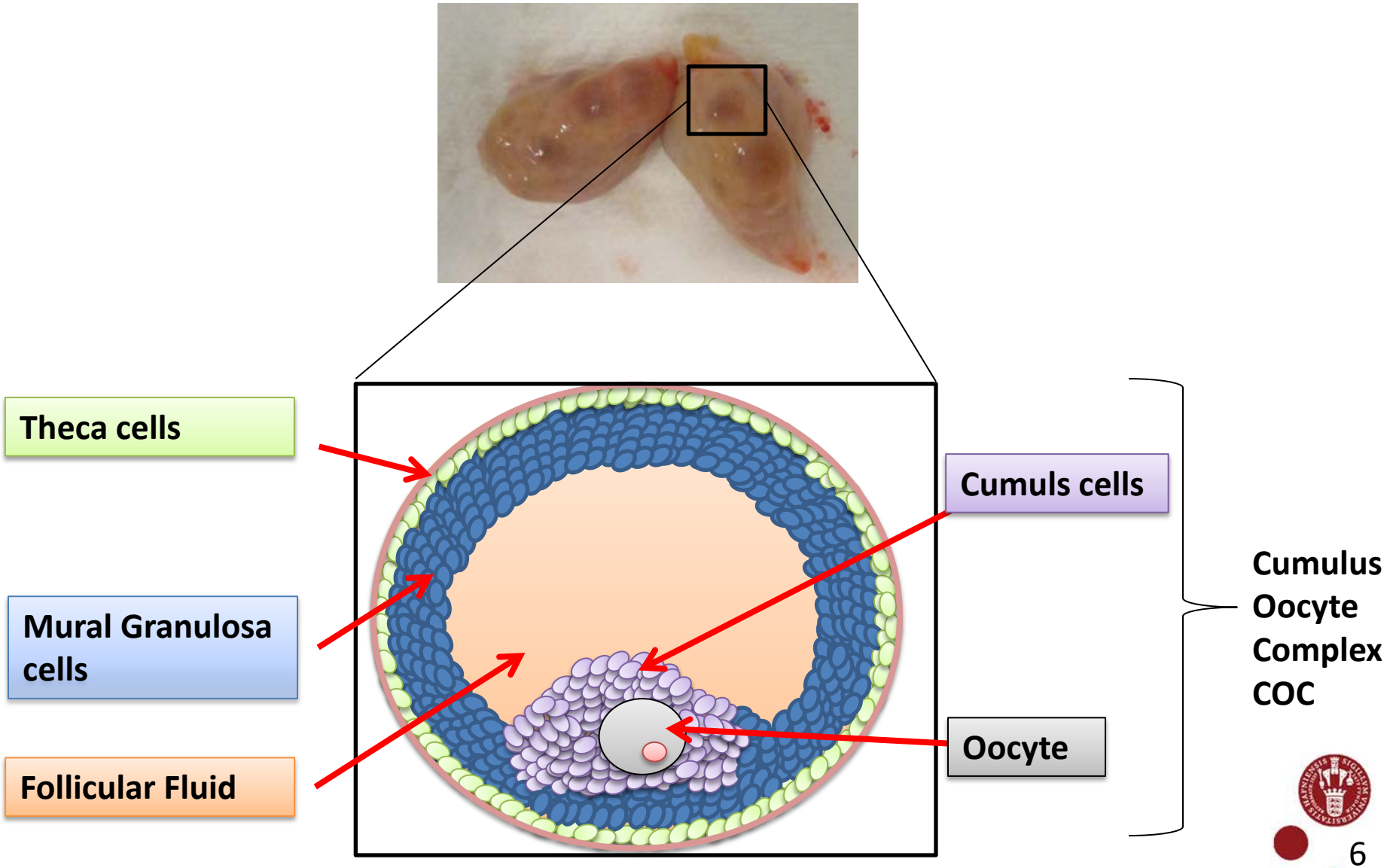


Kadarmideen, HN., et al. 2015. Animal Reproduction 12 (3): 389-396.

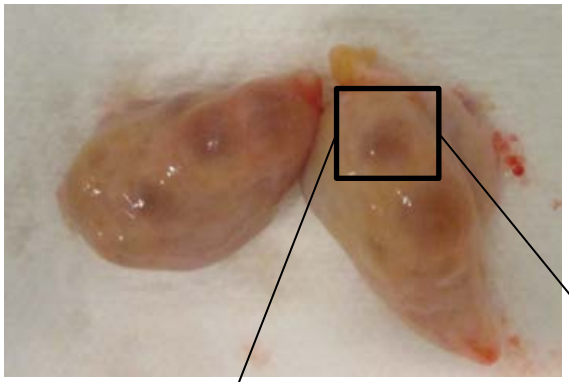
Objectives

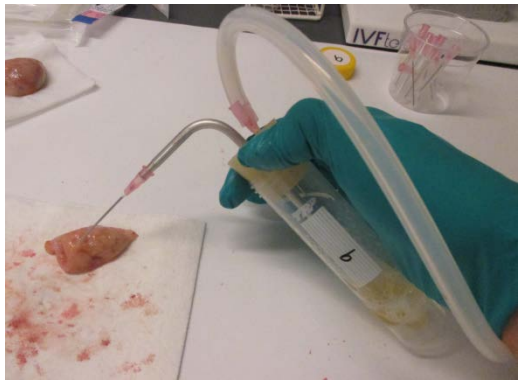
- analyze bull genetic effect in IVP performances
- identify granulosa cell biomarkers for IVP traits in donor cows (GS strategies)
- deliver a better understanding of the mechanisms behind IVP
- **make GS-IVP possible in large scale**

Experimental procedure

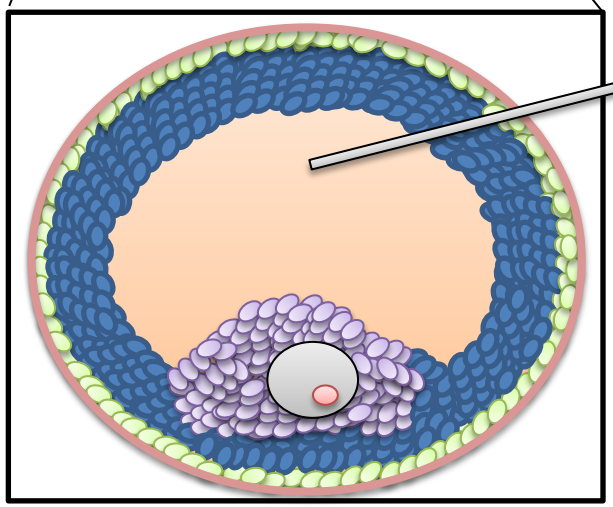


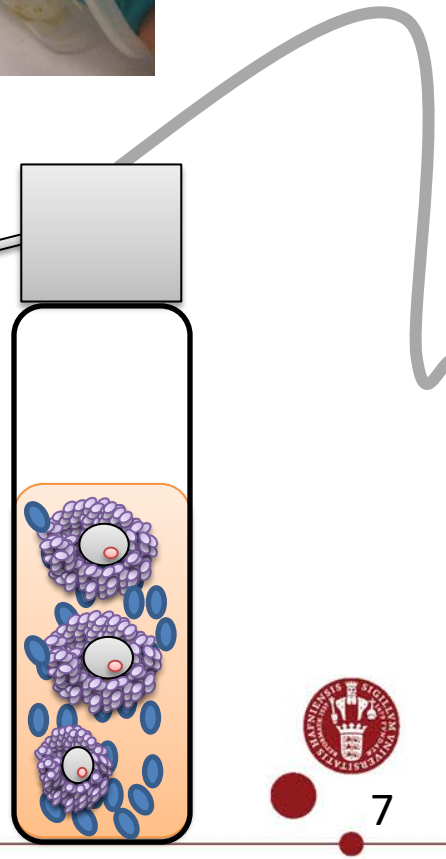
Sampling: follicular aspiration of oocytes



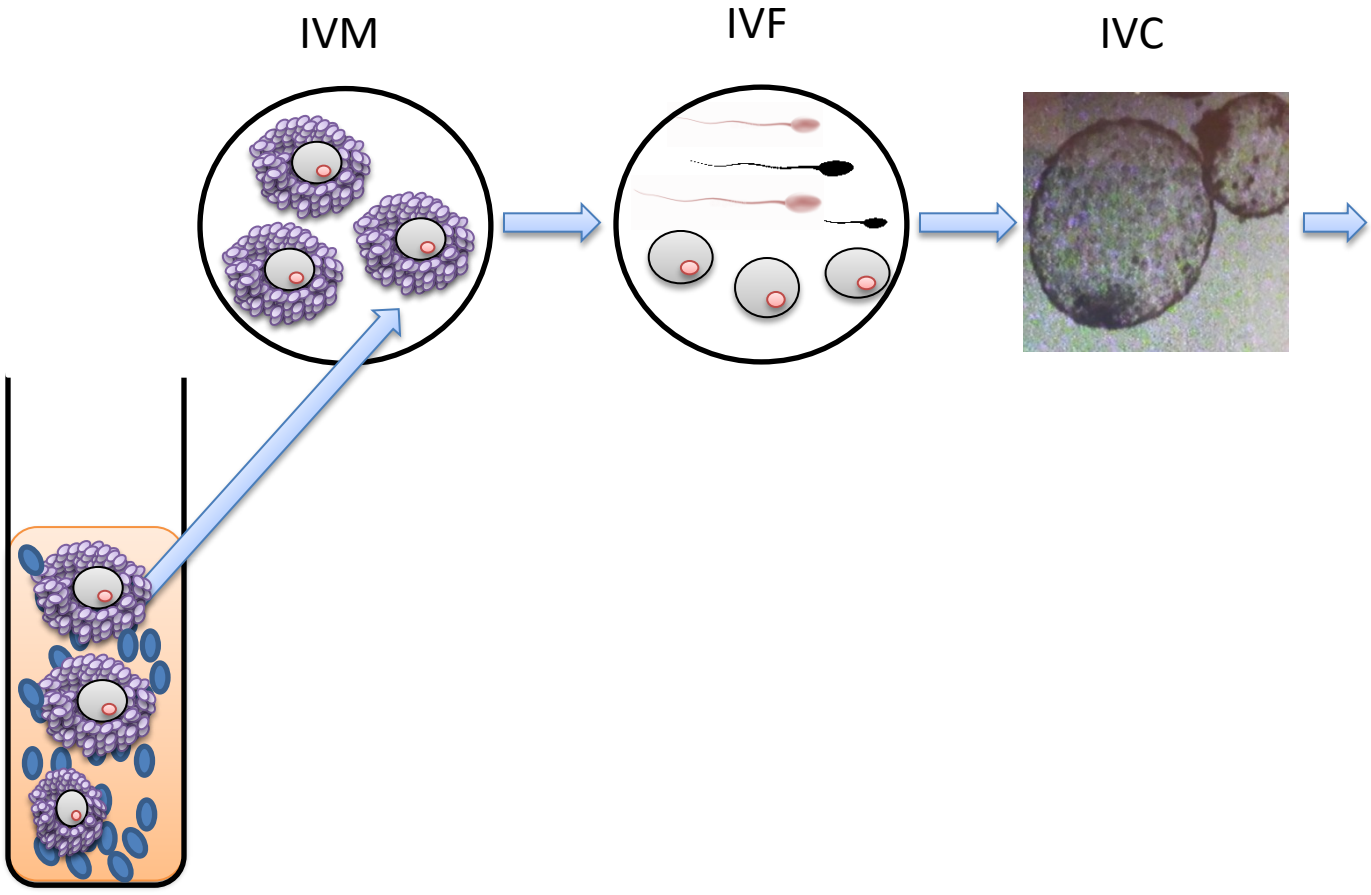


Dataset:
-34 Holstein cows
-No heifers
-all in the luteal phase of the estrous cycle





Experimental Design



IVP parameters:
-Blastocyst Rate
-Kinetic
-Morphology

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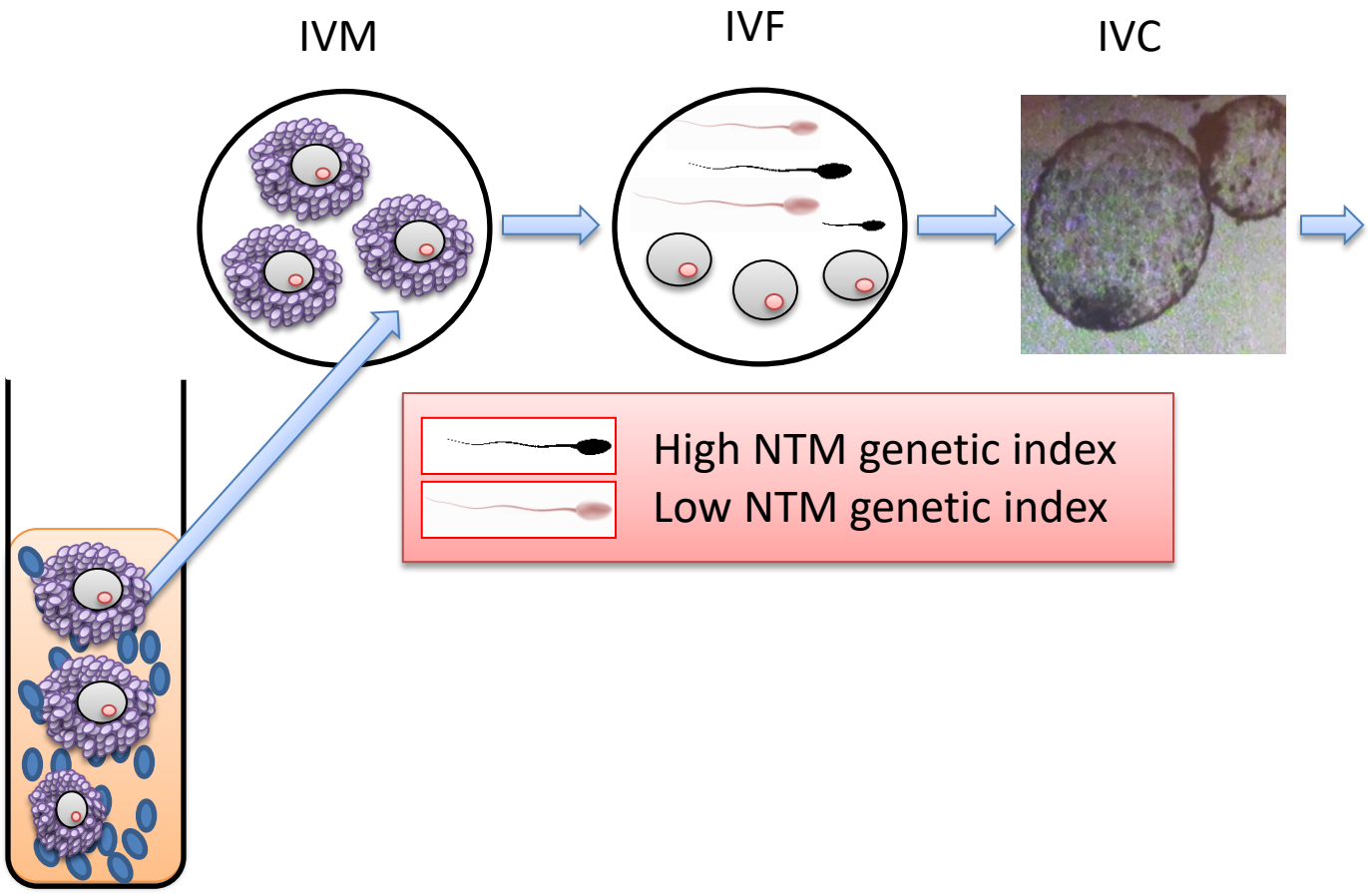

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Experimental Design

IVM

IVF

IVC

IVP parameters:
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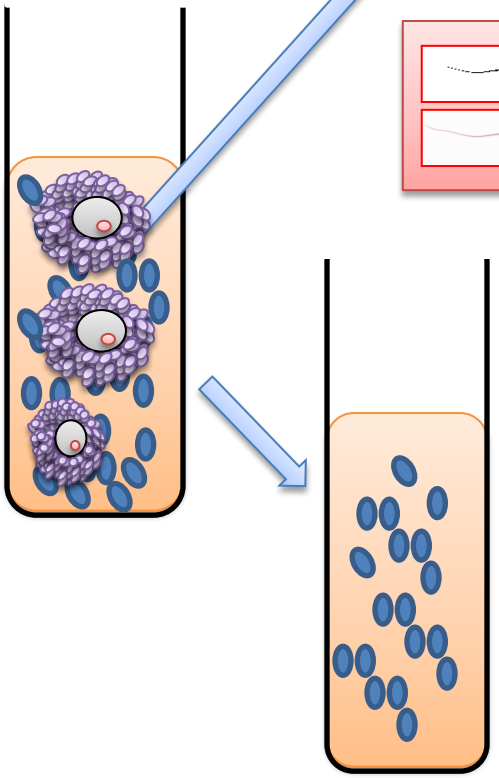
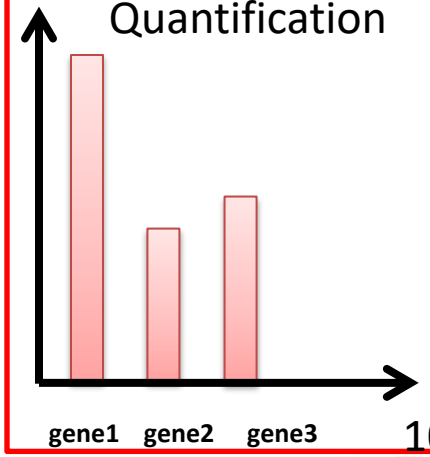


High NTM genetic index
Low NTM genetic index

RNA Extraction

Illumina RNA-Sequencing

Gene Expression Quantification



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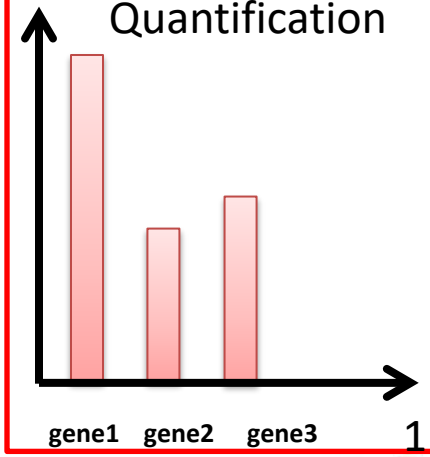
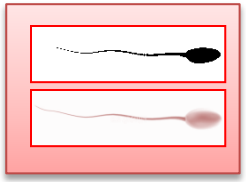
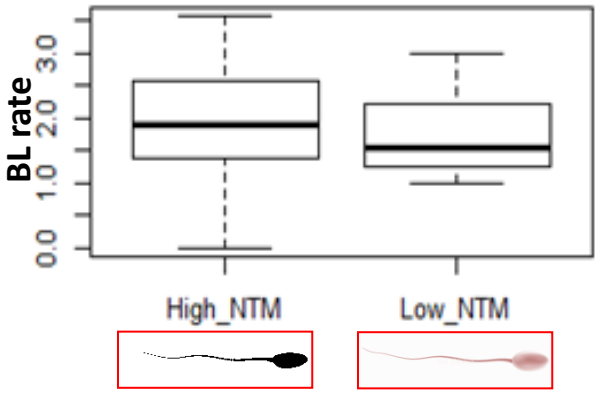


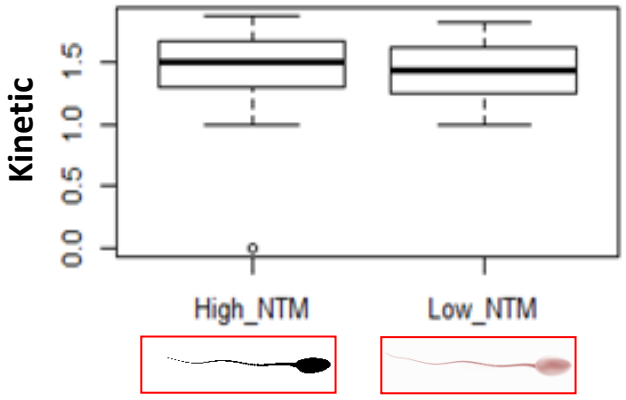
Semen effect (Bull genetic index)

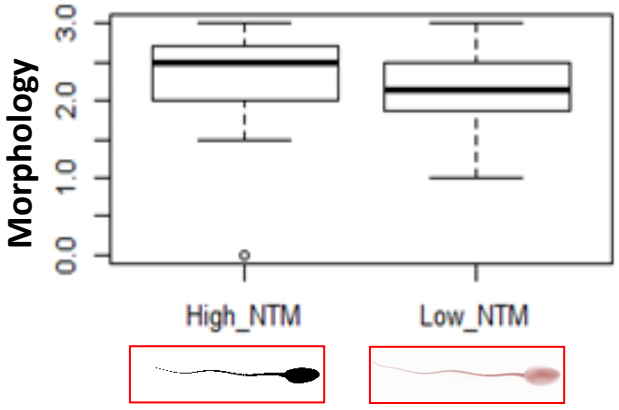


Linear Model:

$$IVP\ trait = slaughtering\ date + age\ at\ slaughter + \textbf{bull index}$$







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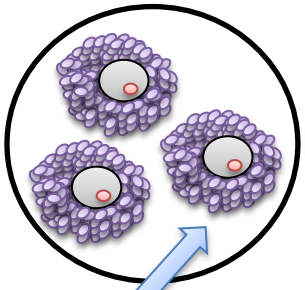

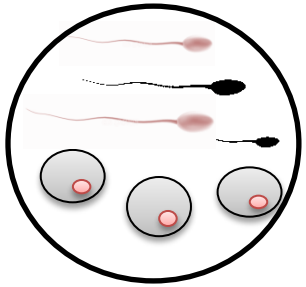

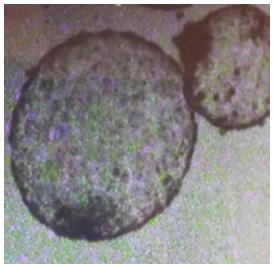

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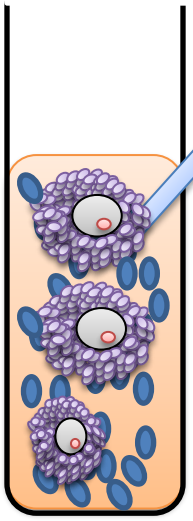

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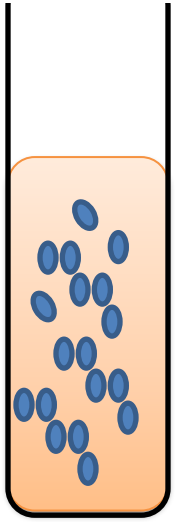

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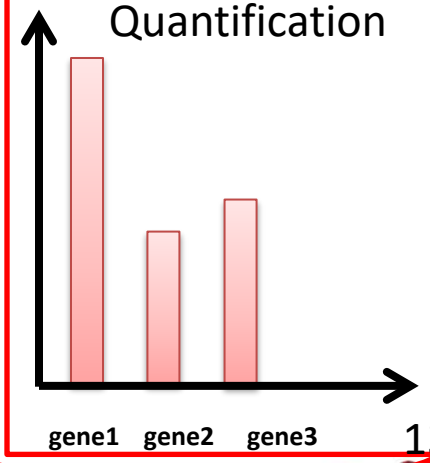
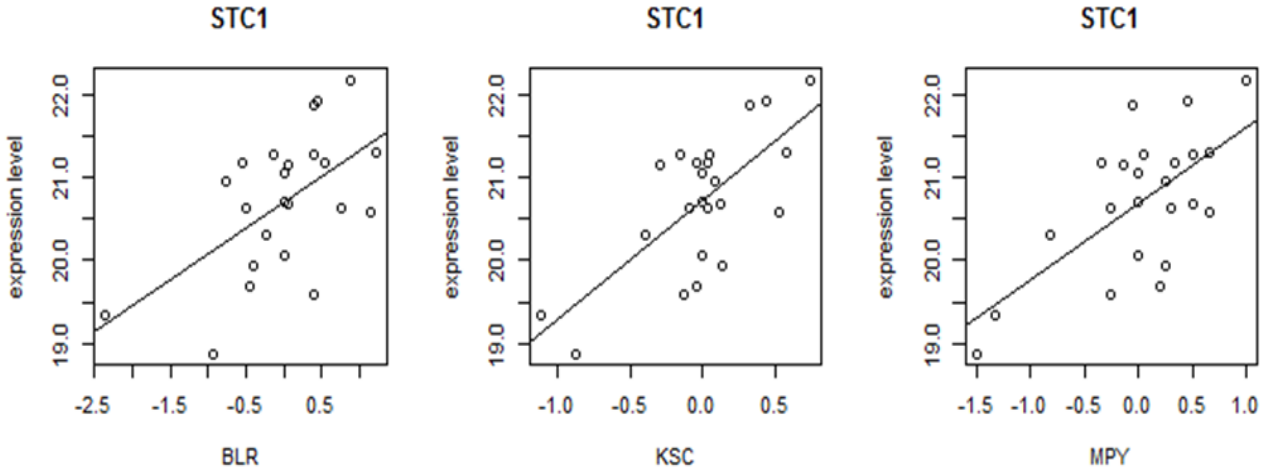


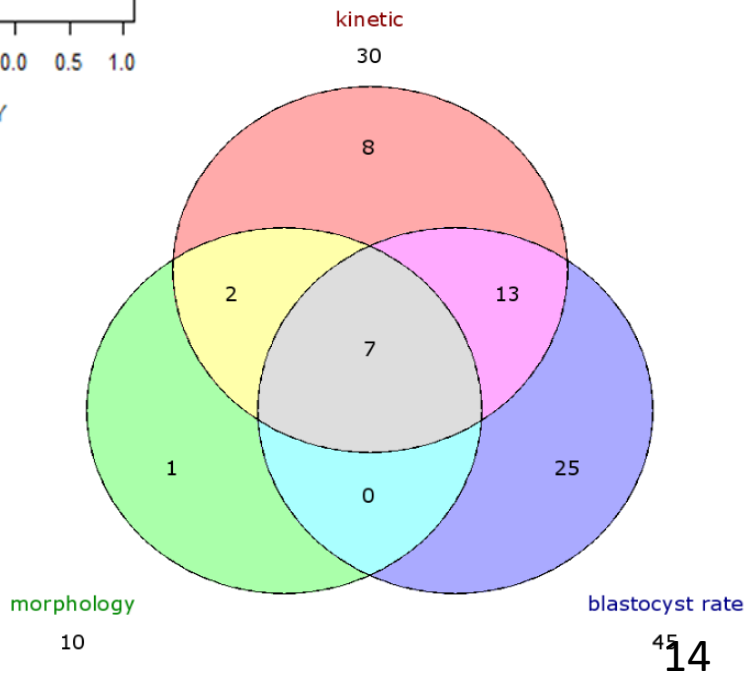
Cow Effect (Biomarker identification)

Linear Model:

Gene counts = slaughtering date + age at slaughter + RIN + $\boldsymbol{IVP\ trait(cleaned)}$



56 significant genes



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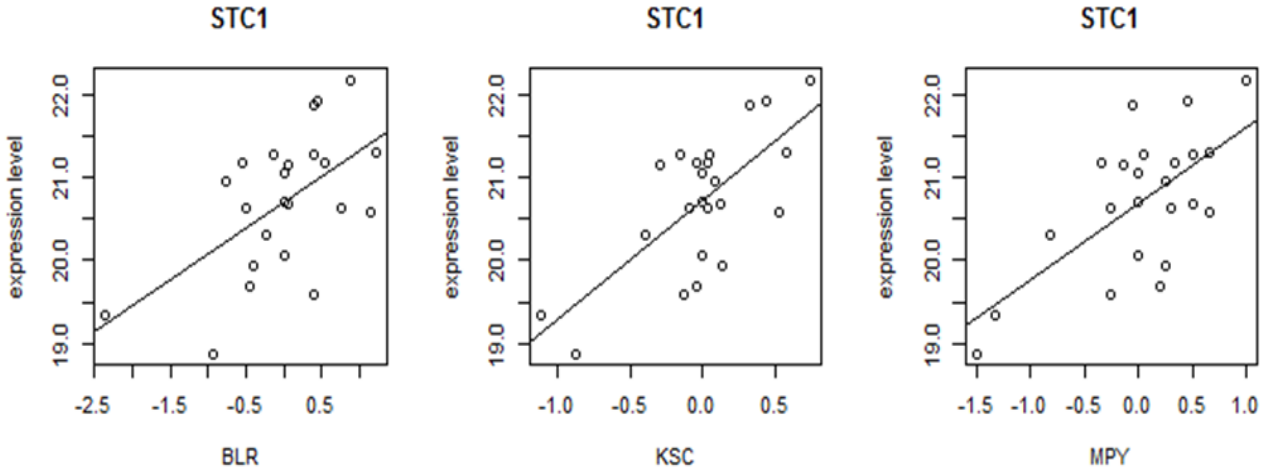

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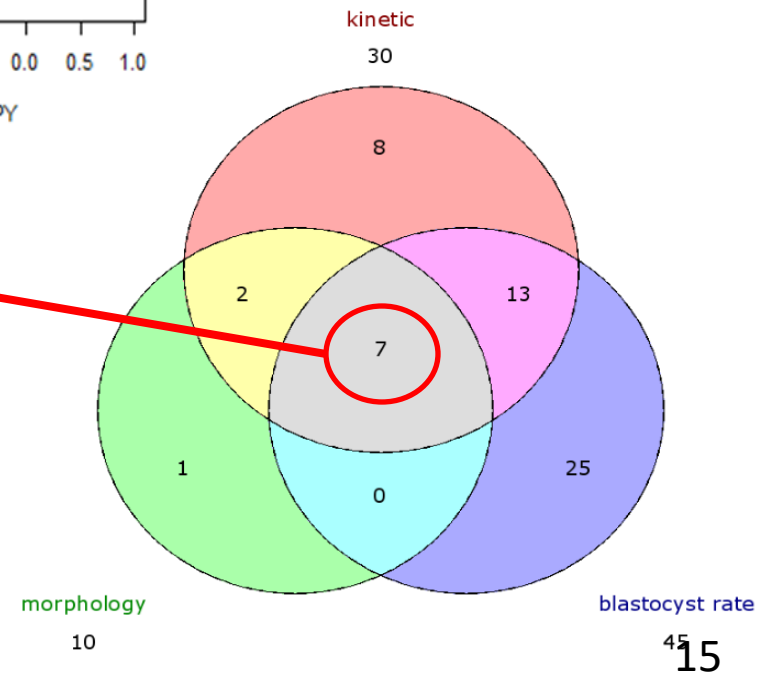

Genes involved in:
-follicle **development**
-follicle **atresia**

STC1, OAT1
patented as biomarkers
in human

BEX2
HEY2
Mx1
RGN
STC1
TNFAIP6
TXNDC11

Functional analysis: follicle atresia

In animals with good performances in IVP we identified:

Ingenuity
Pathway
Analysis

Inhibition	Activation
CELL GROWTH & PROLIFERATION CELL DEVELOPMENT	CELL DEATH -Apoptosis -Necrosis

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Gene **S**et **E**nrichment **A**nalysis	-Progesterone mediated oocyte maturation -Cytokine-cytokine receptor interaction -Oocyte Meiosis -Cell cycle Control	-TP53 signaling pathways -Interferon signaling

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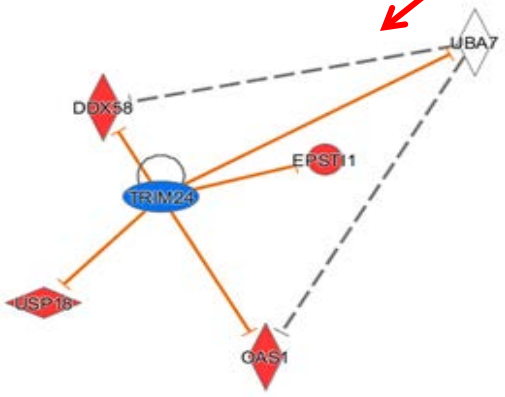
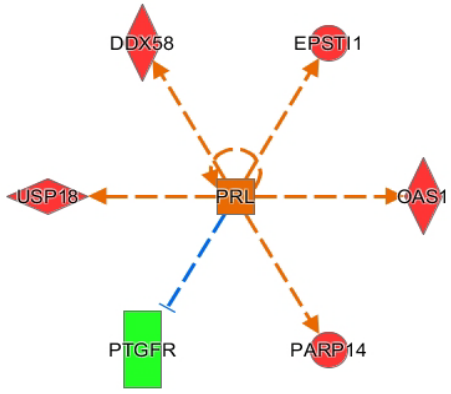
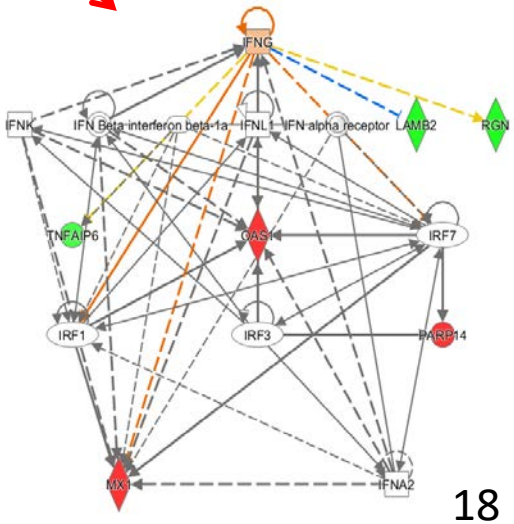
Ingenuity **P**athway **A**nalysis

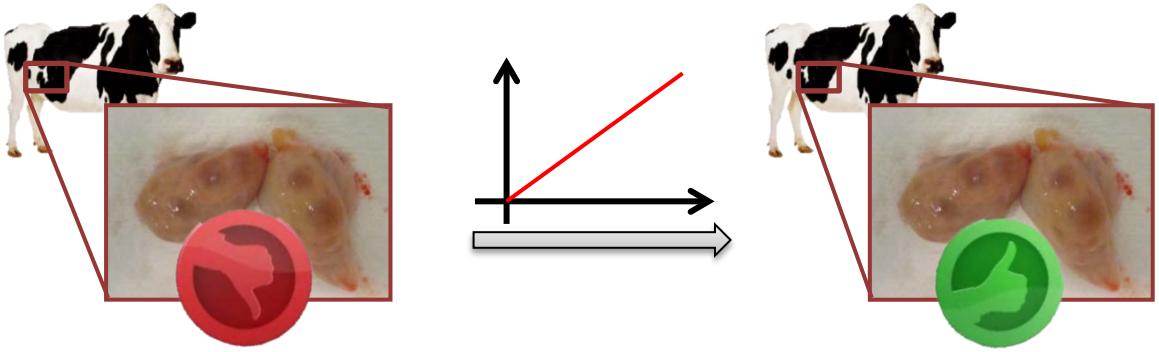
(Upstream Regulators)







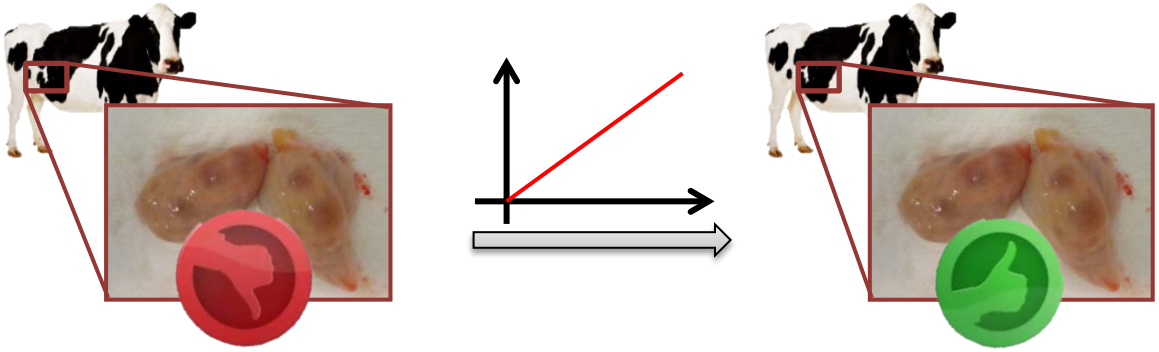
Comparison with follicular expression profiles



Low IVP performances

High IVP performances

Comparison with follicular expression profiles



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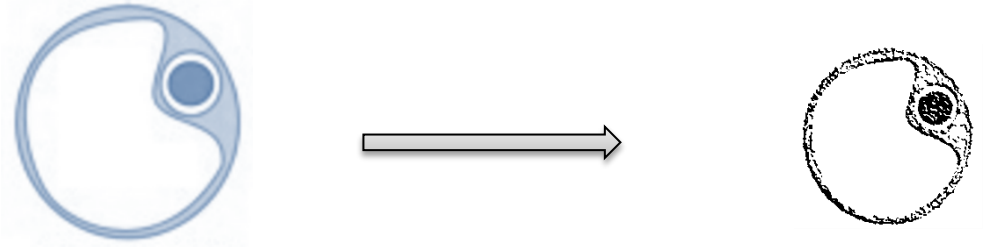
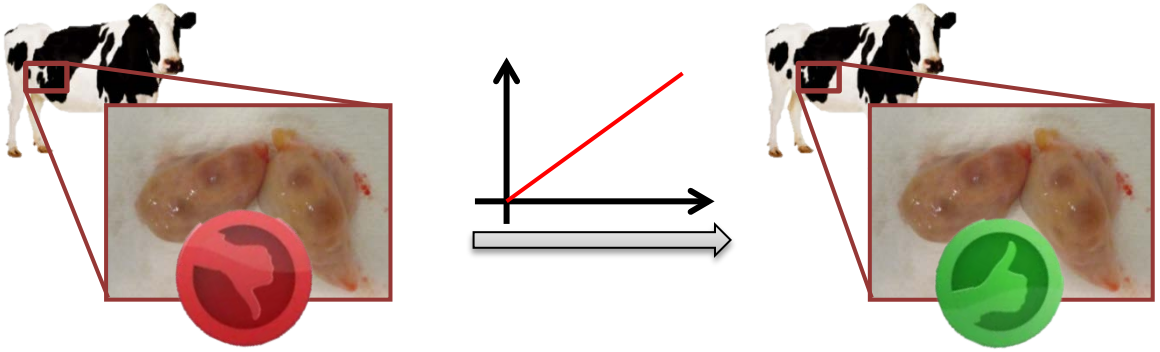
Hatzirodos N. et al 2014 (a)



Healthy

Atretic

Comparison with follicular expression profiles



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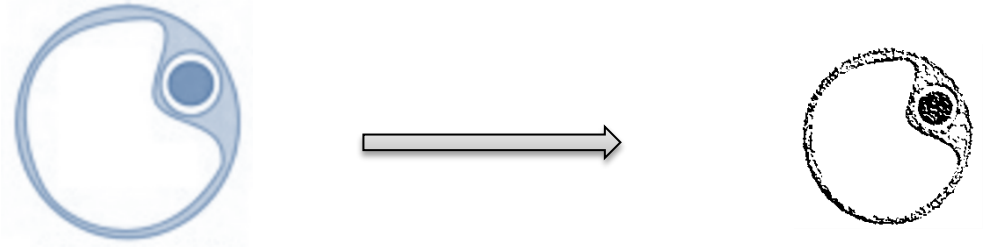

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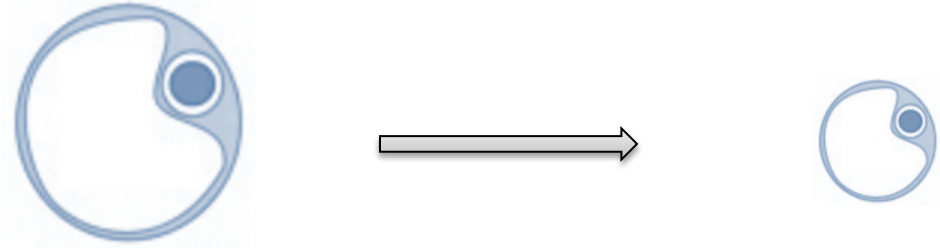

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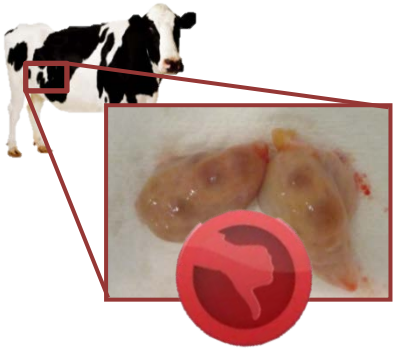

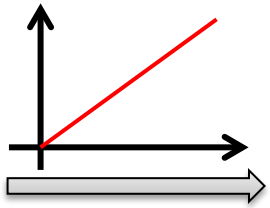

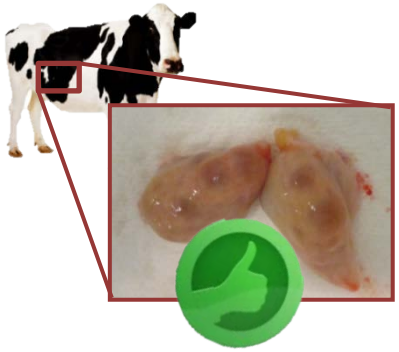

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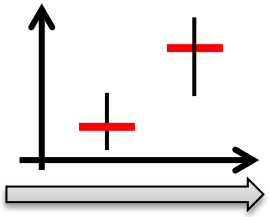

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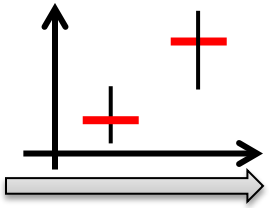
81% of the genes with same trend

Hatzirodos N. et al 2014 (b)



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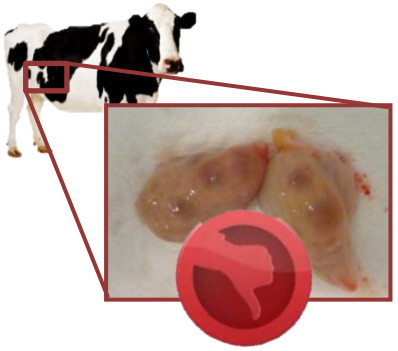

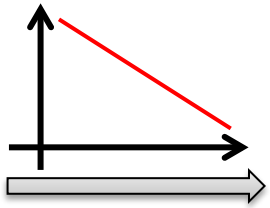

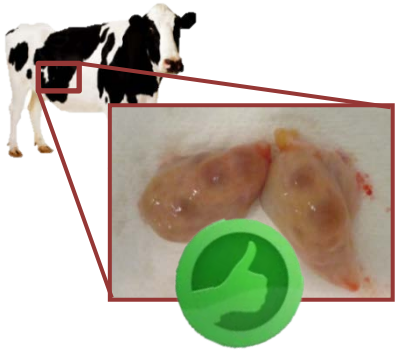

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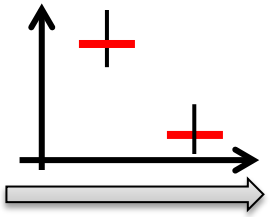

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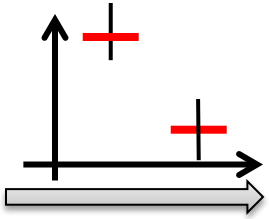

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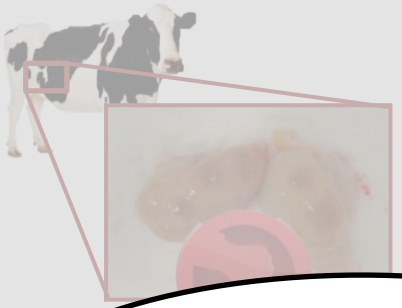
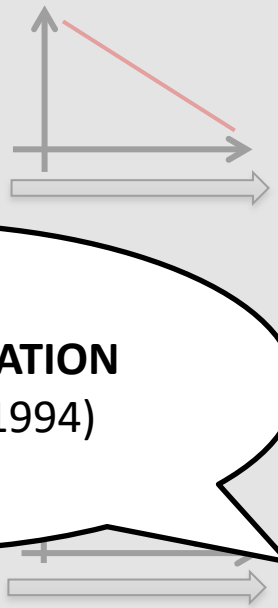
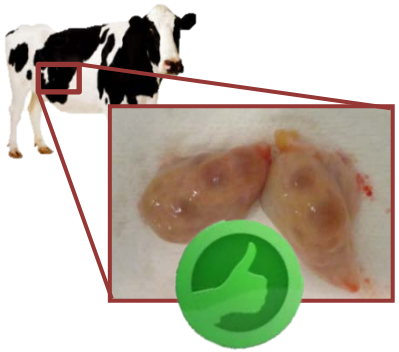


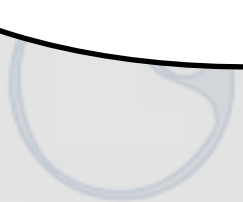




High IVP performances

PSEUDOMATURATION
(Assey R. *et al* 1994)

Hat



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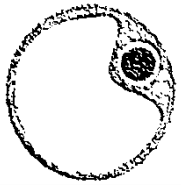


Atretic

81% of the genes with same trend

Hatzirodos N. et al 2014 (b)

Big Antral (10-15 mm)

Small Antral Follicles (8-11mm)

91% of the genes with same trend

Secreted Biomarkers

Network generated with IPA ordered by sub-cellular localization

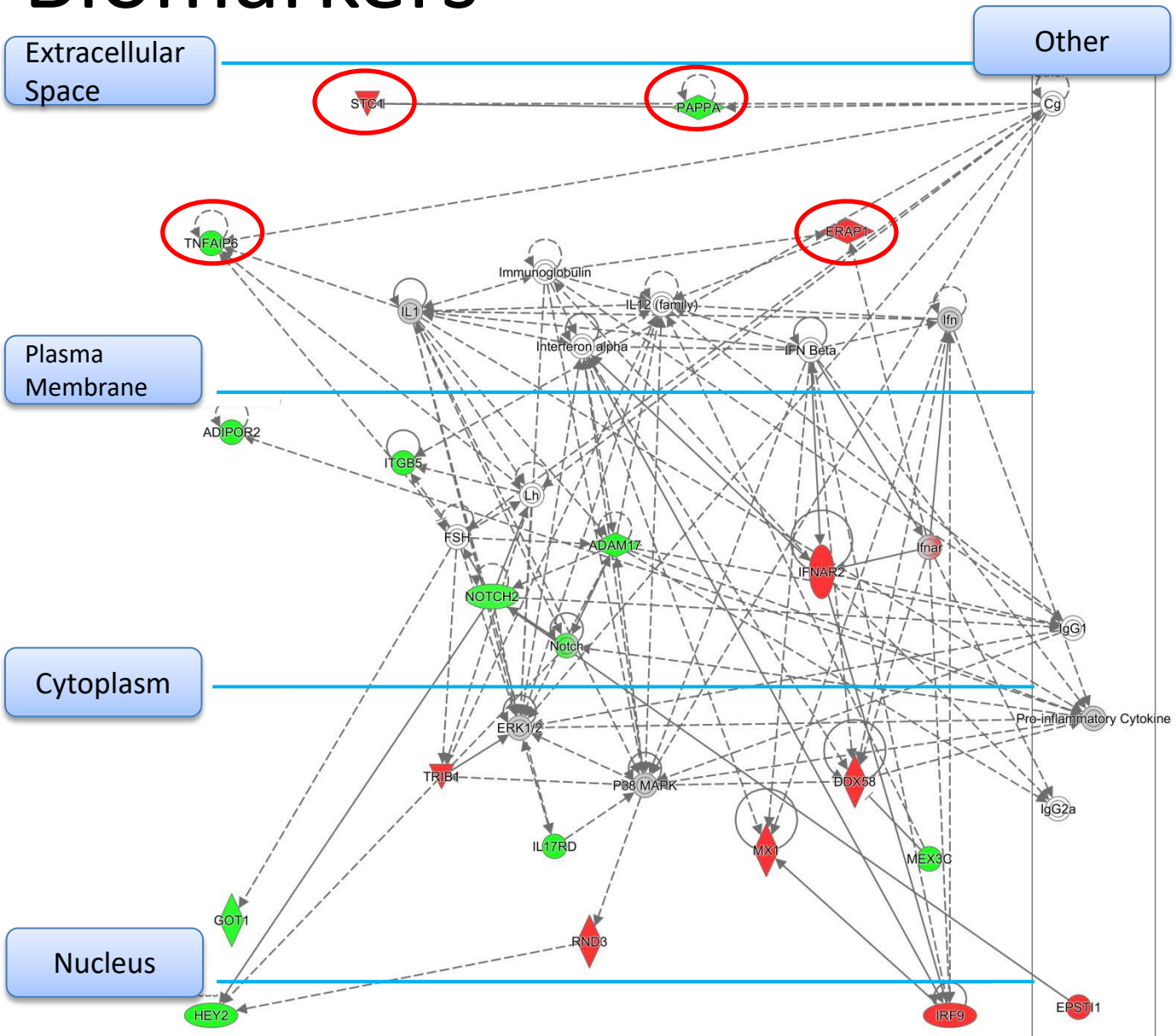
Candidate genes (secreted):

STC1

PAPPA

TNFAIP6

ERAP1



Conclusions

- No effect of the bull genetic index (NTM) on the IVP outcome
- First study that identifies candidate genes for IVP efficiency at single animal level.
- 56 candidate genes (7 in common for all traits).
- Higher number of atretic follicles in cows with good IVP outcome.

Future perspectives

- Real-Time PCR Validation
- Gene Co-expression analysis
- Biomarker Development *(eQTL analysis, test synthetic protein in media, proteomics studies)*

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