

# Impact of embryo production environment on the bovine blastocyst RNA expression

AGM 2011



UNIVERSITÉ  
LAVAL



**CRSNG**  
**NSERC**

## 2 questions were asked and addressed

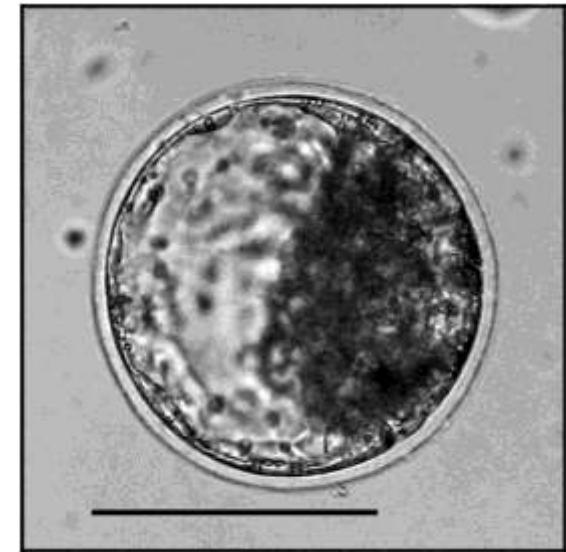
- 1) Can we compare data between teams if we consider using a common IVP protocol?
  - Hypothesis: By sharing biological extracts (BSA, serum, semen,...), data can be repeated.
- 2) What are the impacts of the routinely used production systems?
  - Hypothesis: The different phenotypes observed between production systems will translate into divergent gene expression.

# Can we compare data between teams if we consider using a common IVP protocol?

- IVM: TCM-199 with 10% serum
- IVC: mSOF with BSA

- Sharing biological compounds

- Semen
- BSA
- Serum



- Using a defined blastocyst stage/ morphology

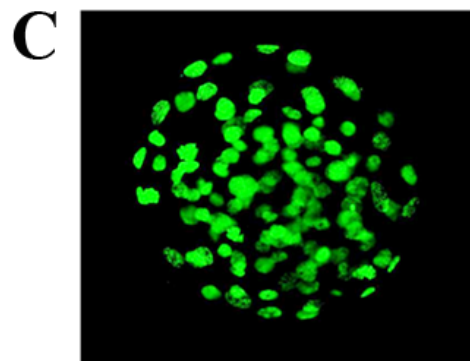
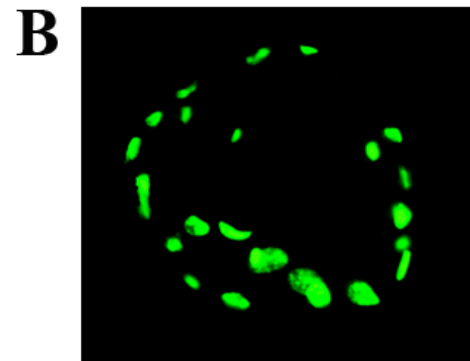
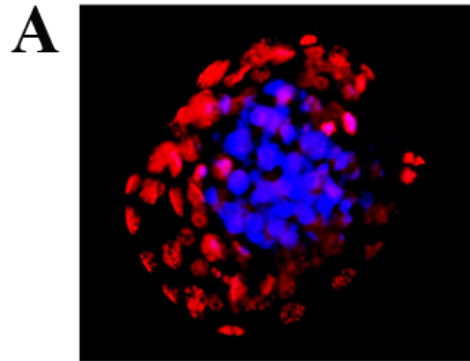
# Phenotypic characterization

Site	n	Efficacy			Gender proportion		
		Day 4 cleavage rate (%)	Day 4 8-cells rate (%)	Day 7 blastocyst rate (%)	n	Male (%)	Female (%)
<b>A</b>	<b>2309</b>	<b>73 ± 7</b>	<b>43 ± 9</b>	<b>20 ± 3*</b>	<b>46</b>	<b>24 (52)</b>	<b>22 (48)</b>
<b>B</b>	<b>1894</b>	<b>N/A</b>	<b>N/A</b>	<b>30 ± 8*</b>	<b>477</b>	<b>245 (52)</b>	<b>222 (48)</b>

# Phenotypic characterization

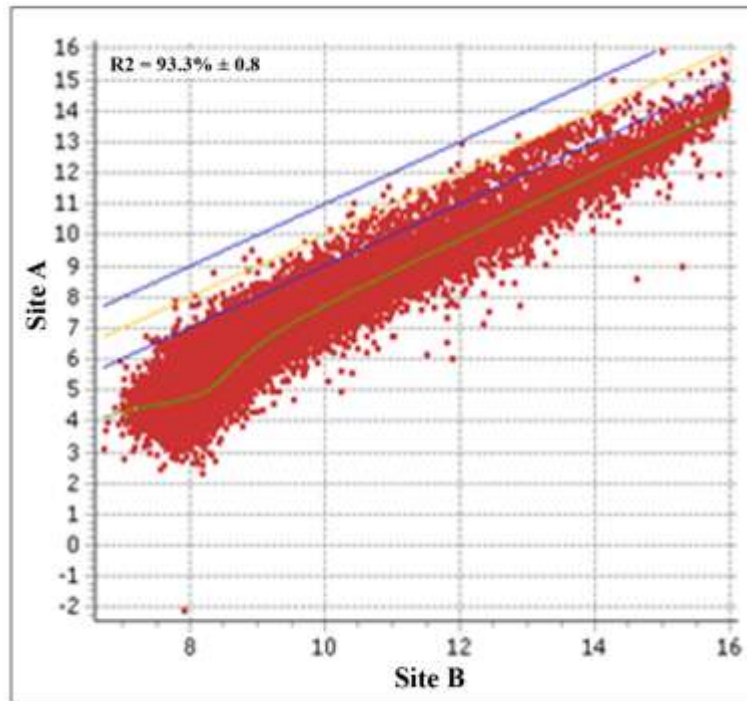
Site	n	Total cell count	Inner cell mass (ICM)	Trophoblast cells (TE)	ICM/TE ratio	ICM %	TUNEL positive cell count	TUNEL positive cells (%)
A	44	111 ± 23	30 ± 9	81 ± 17	1:2.8 ± 0.6	27 ± 5	17 ± 8*	16 ± 9**
B	37	115 ± 25	33 ± 10	82 ± 19	1:2.6 ± 0.8	29 ± 5	11 ± 7*	9 ± 6**

# Cellular stainings

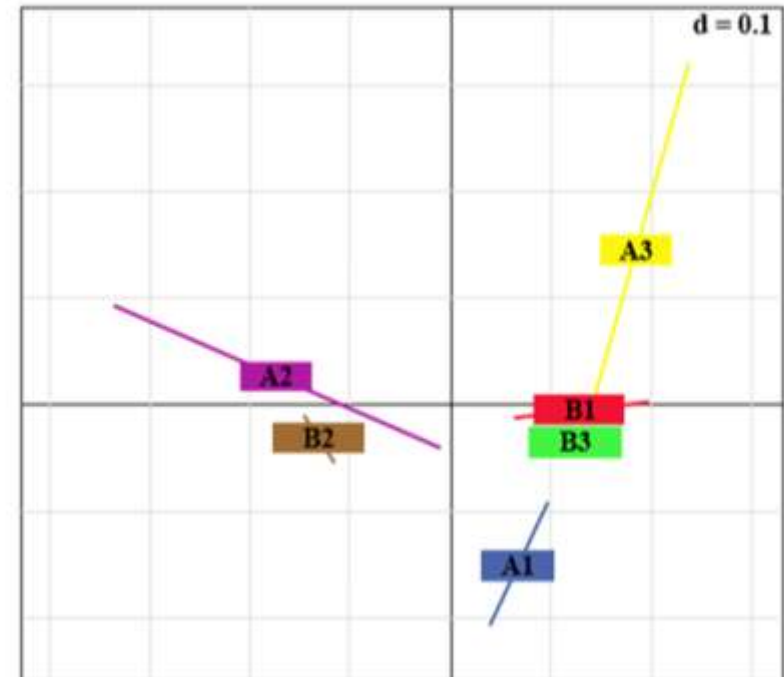


# Overall similarities at the gene expression level

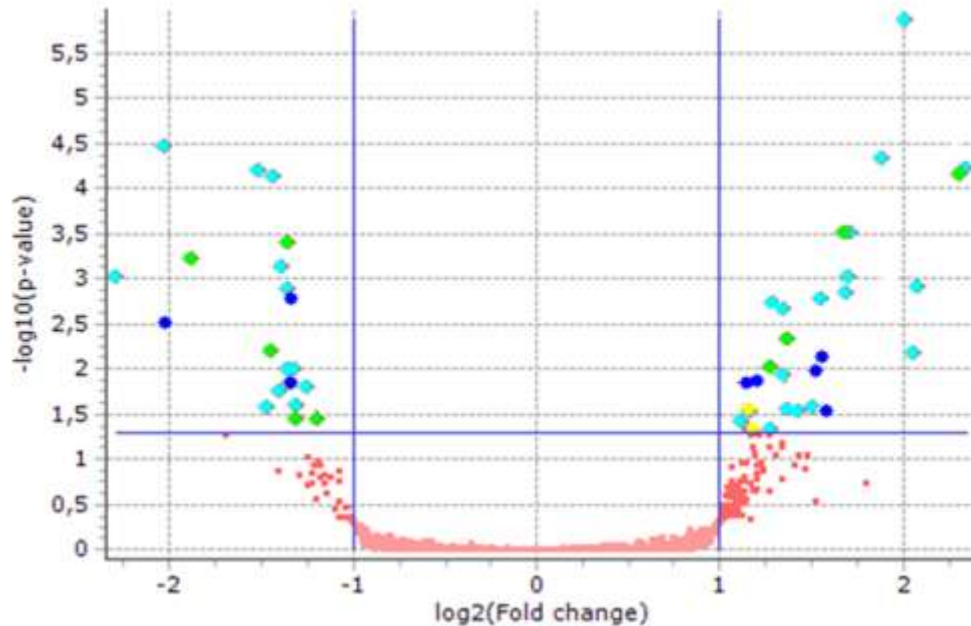
A



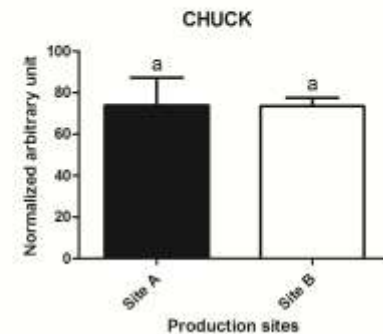
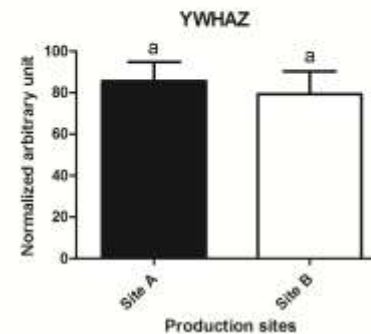
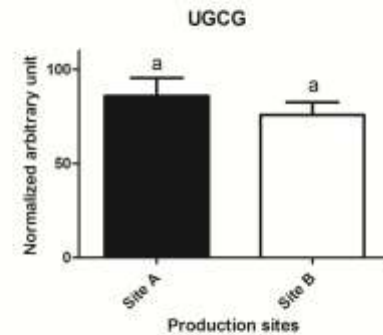
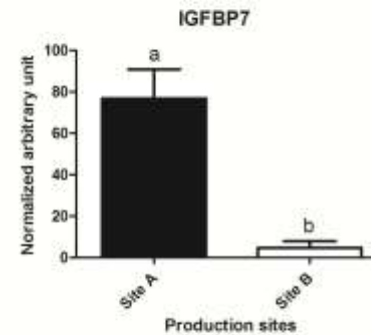
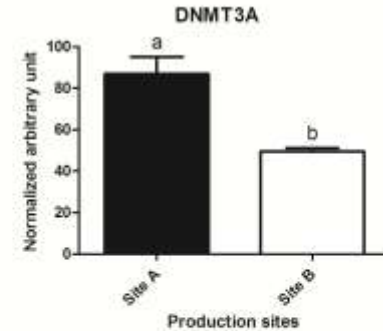
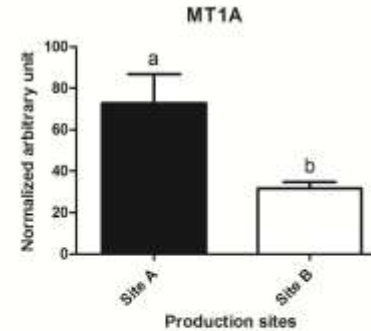
B



# Comparison of gene expression



43 genes were found to be differentially expressed



# Conclusions

- 1) Can we compare data between teams if we consider using a common IVP protocol?
- Results are similar but not identical.
- We observed an impact of production site on blastocyst rate and % of TUNEL positive cells.
- Little difference were observed at the gene expression level.
- The phenotype was reflected at the gene expression level.
- The data is indicative of laboratory setup and personnel experience.
- The limited impact is indicative of technology transfer potential between groups.

# What are the impacts of the routinely used production systems?

## The systems used:

OPU: oocytes collected by transvaginal aspiration.

IVM = TCM-199; IVC = mSOF

SOF = oocytes collected on slaughterhouse ovaries.

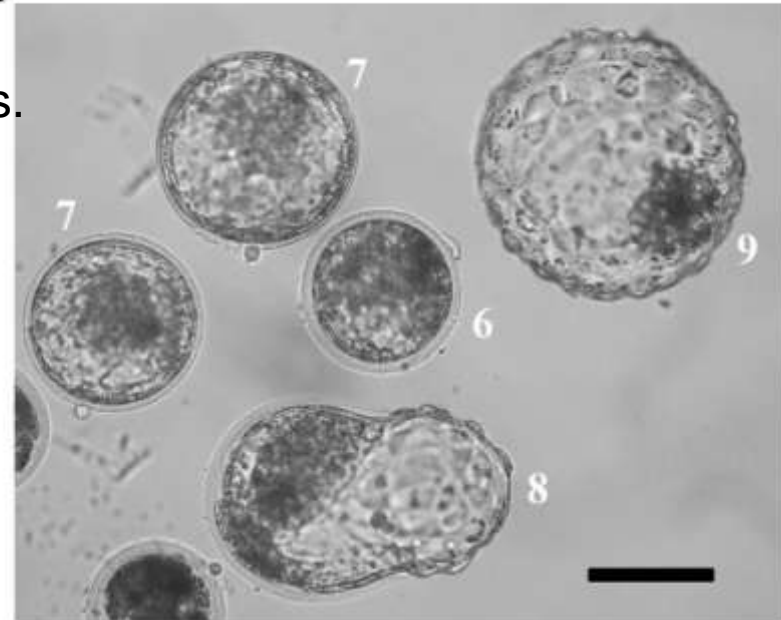
IVM = TCM-199; IVC = mSOF

BRL = oocyte collected on slaughterhouse ovaries.

Co-cultures in B2-BRL system.

- impact of oocyte source
- Impact of *in vitro* environment

A



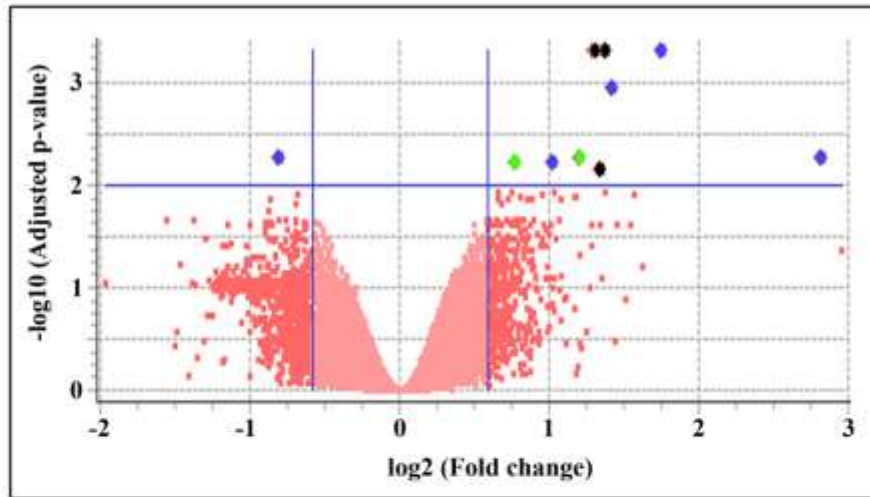
# Performance of production systems

System	n	Day 7	n	Conception	n	Sex
	COCs	blast.	transferred	rate	sexed	ratio
	in	rate	embryos	(%)	blast.	(M/F)
	IVM	(%)				
<i>In vivo</i>	N/A	N/A	887	60	923	51/49
BRL	826	29 ± 6	391	54	802	60/40
SOF	1894	30 ± 8	N/A	N/A	46	52/48
OPU-SOF	1177	55 ± 26	373	57	477	52/48

# Comparisons at the gene expression level

Impact of  
oocyte source

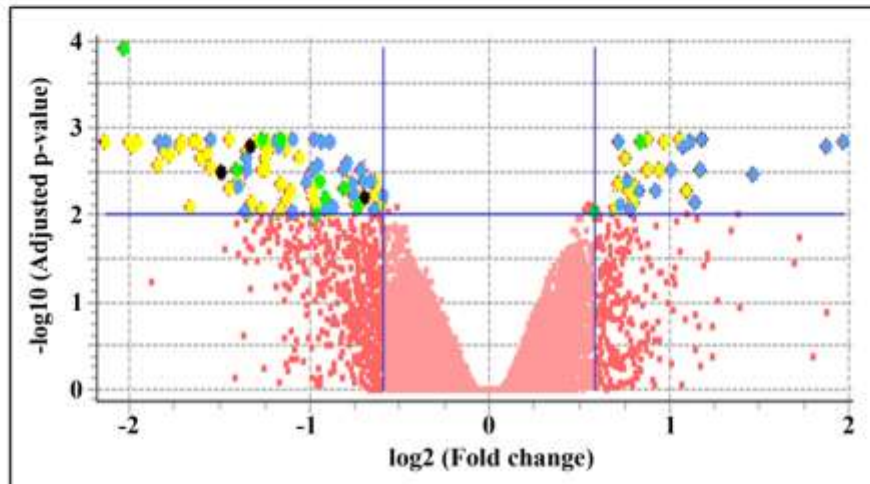
**A**



5 reference genes  
2 pseudogenes  
3 alternate splicing

Impact of  
*in vitro*  
environment

**B**

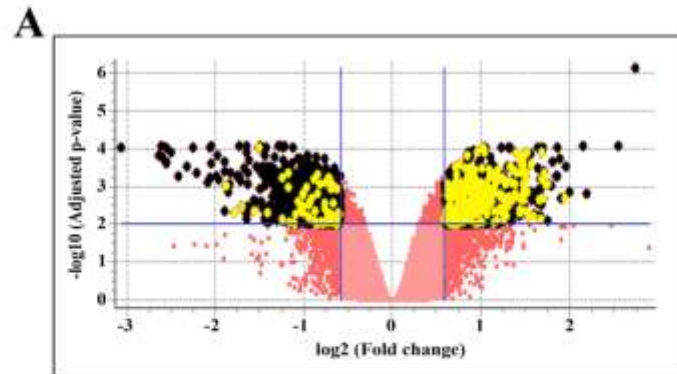


48 reference genes  
7 pseudogenes  
3 alternate splicing  
64 uncharacterized

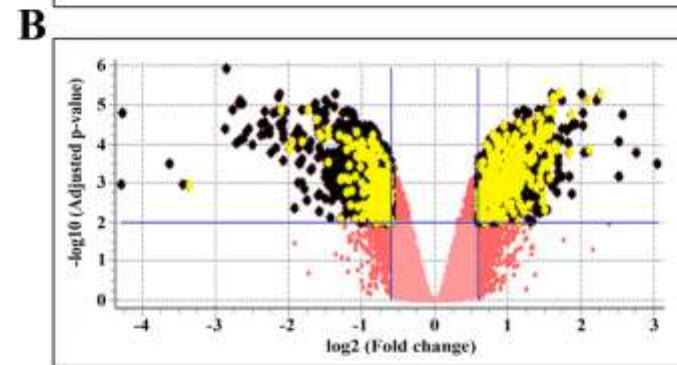


# Comparisons with *in vivo* blastocysts

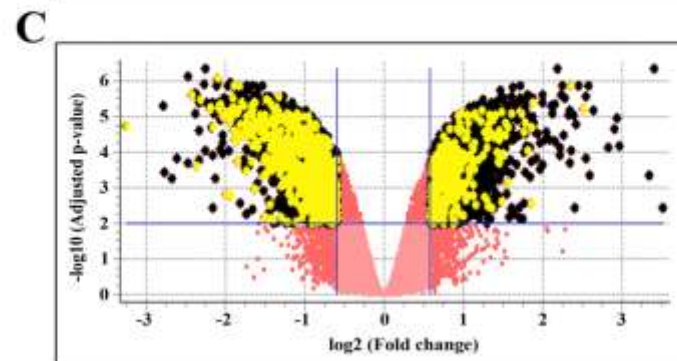
OPU



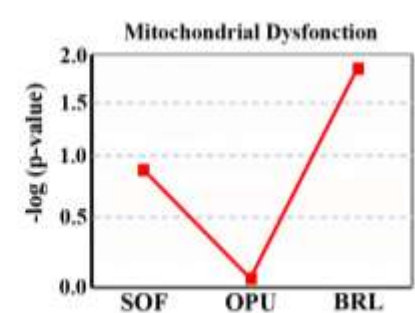
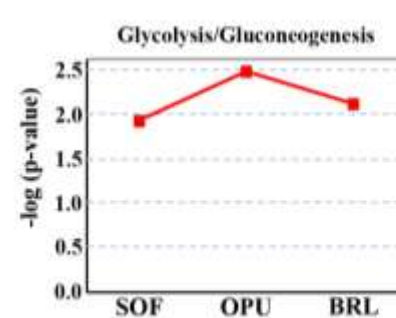
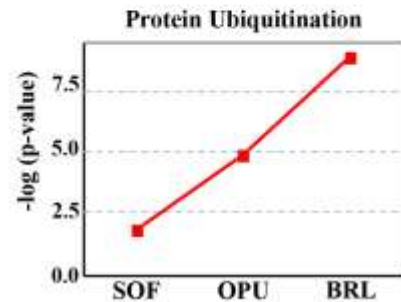
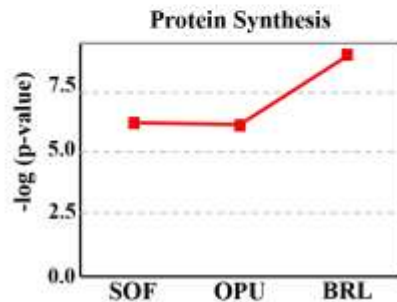
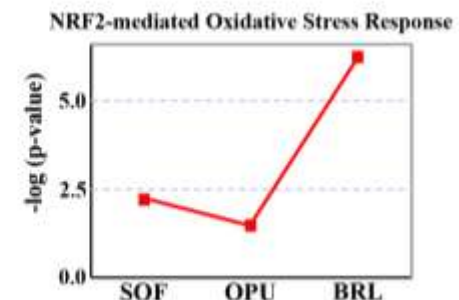
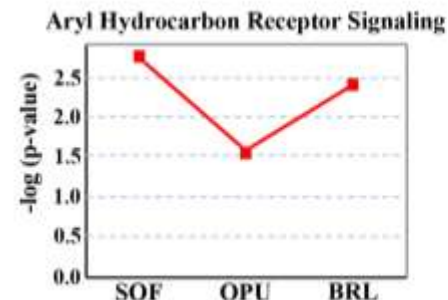
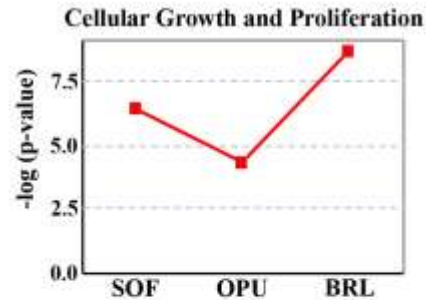
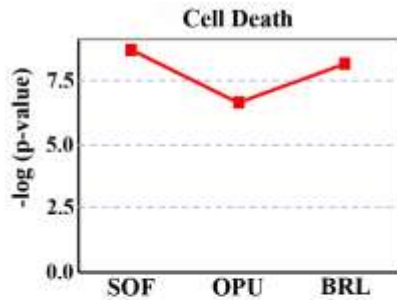
SOF



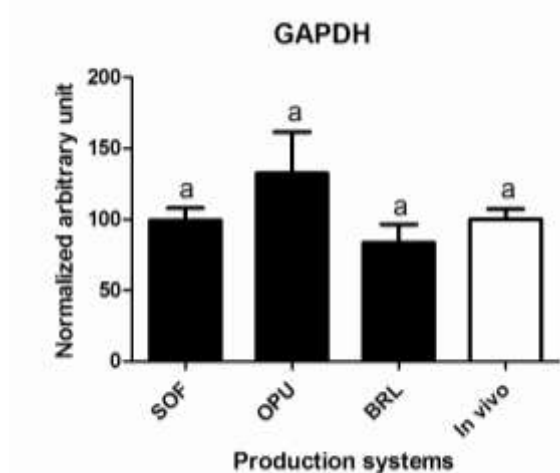
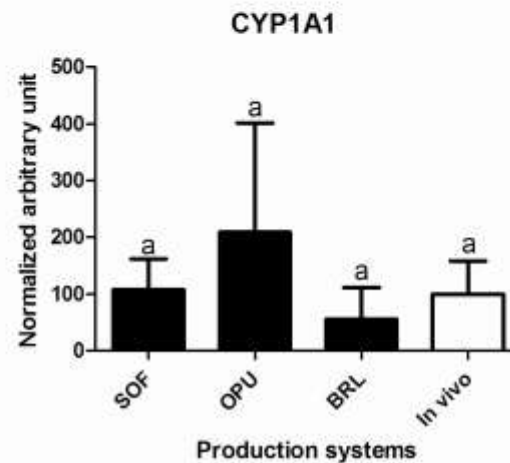
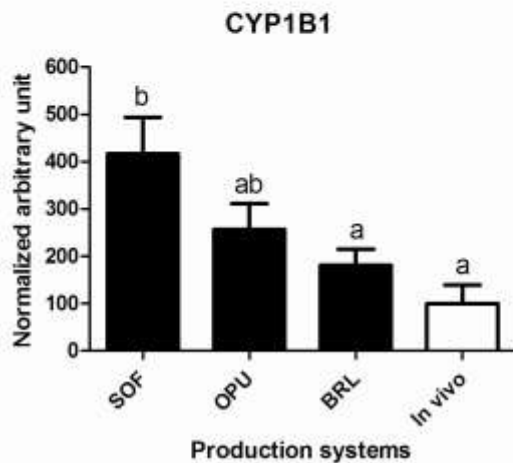
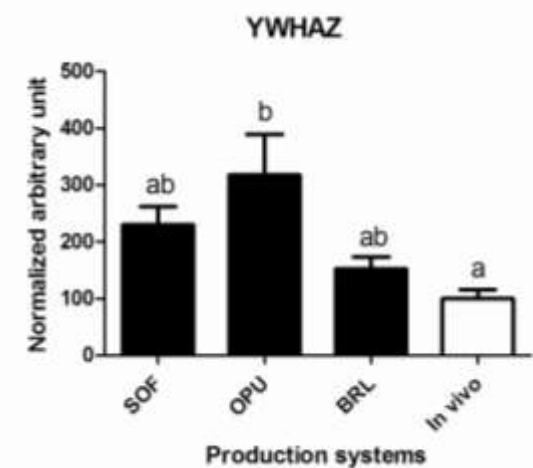
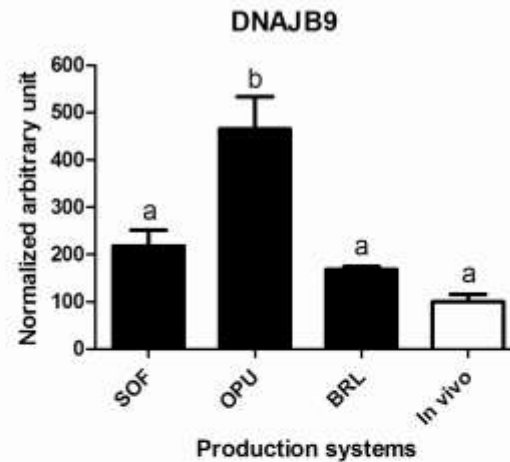
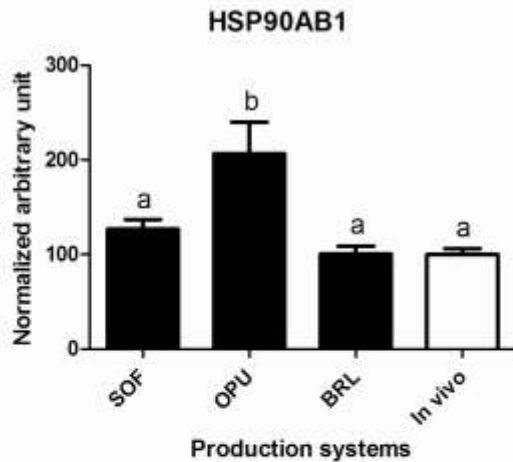
BRL



# Impacted cellular functions



# Interrogating the AhR pathway

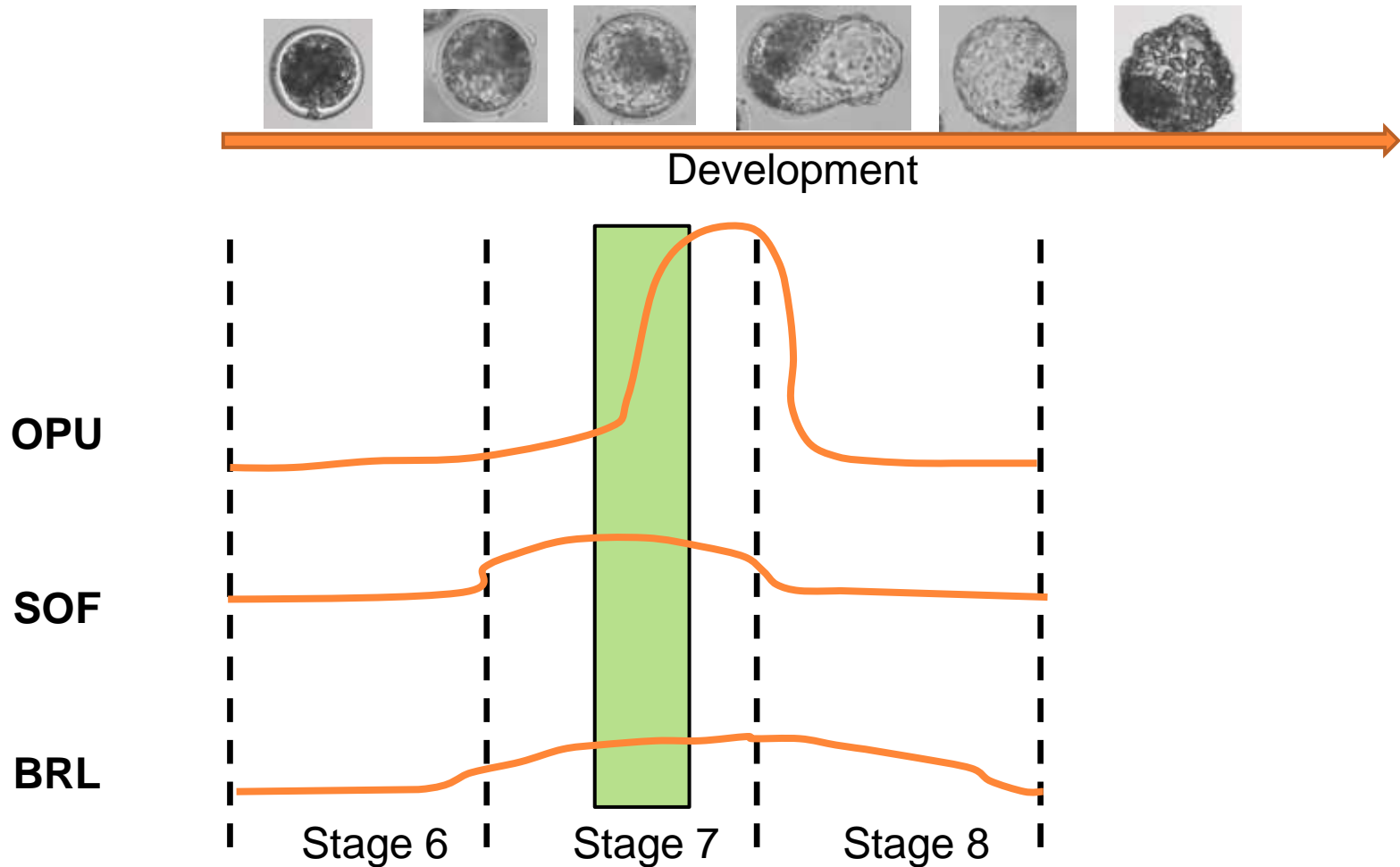


# Conclusions

- 2) What are the impacts of the routinely used production systems?
- Production systems perform differently (BRL = skewed sex ratio; OPU = best blastocyst rate).
- At the gene expression level: the source of oocyte had a marginal impact at the blastocyst stage.
- The *in vitro* environment had a more profound impact.
- Regardless of production systems, the *in vitro* embryos exhibit marked differences in gene expression compared to *in vivo* counterparts.
- Expression of long non-coding RNAs are markedly impacted.

# Conclusions

Embryonic cohorts are heterogeneous and differ across production systems/ treatments



# Acknowledgements

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*Dany Plourde*

