Genomics of fertility in cattle reproduction

Dawit Tesfaye

- Institute of Animal Science
- Animal Breeding and Husbandry group
- University of Bonn, Germany

Fertility

Breeding for production

- Suboptimal ovarian cyclicity (Opsomer et al. 2002)
- Behavioural expression of oestrus (Van Eerdenburg 2006)
- Success rate of AI (Jorritsma & Jorritsma 2000; Royal et al. 2000)
- Embryo survival (Sheldon et al. 2006)

Good fertility is essential

- Procreation next generation
- Profitability (Milk / Meat yield)
- Breeding progress
- Welfare and sustainability of animal production

Royal et al. 2000; Roxstrom et al. 2001; Beerda et al. 2008

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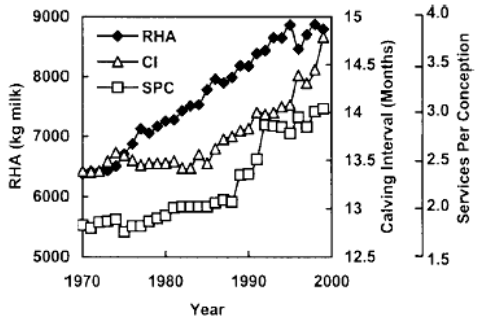
Milk yield and fertility



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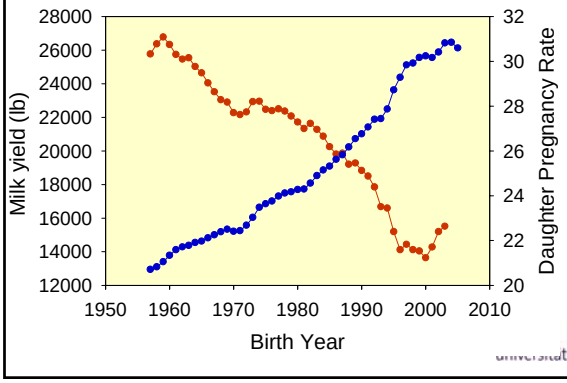
Trends in Milk Yield and Daughter Pregnancy Rate for US Holsteins



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Decline in fertility

- Longer calving intervals
- Higher replacement rates

Profitability | Animal welfare | Sustainability

Reduce the cost of production

Minimize labour imput and cost of getting cows pregnant

Improving fertility with additional management ? Genetic improvement

Beerda et al. 2008

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Genetics of fertility

- Low heritability of fertility
- Understanding the genetic regulation of underlying processes will improve the possibilities for breeders to increase the genetic merit for fertility.

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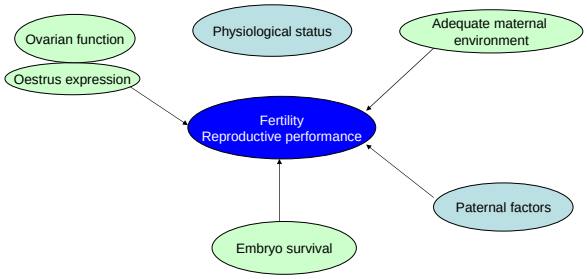
Reproductive performance (Fertility)



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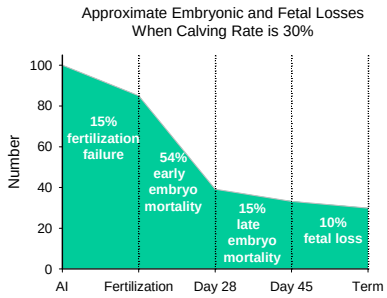
Reproductive Losses



Hansen 2007

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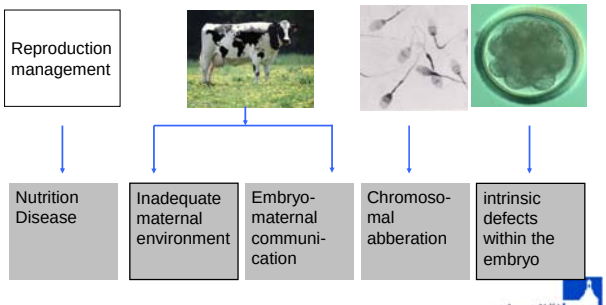
Factors contributing to embryo survival



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Overall goal genomics of fertility

- Understanding of the underlying mechanisms of fertility
- Improved understanding of genetic variation
- Provide tools to enhance fertility
  - improve efficiency
  - improve sustainability
  - Welfare

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Specific objectives

- Understanding genetic regulation of mechanisms
  - Oestrus behaviour (ID-L)
  - Embryo developmental competence and endometrium receptivity (Bonn/MTT)

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Part I

Genomics of oestrus behaviour (ID-L)

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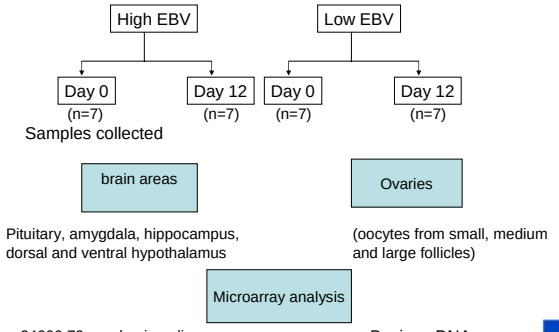
- Identify genes related to oestrus behaviour
- 28 HF heifers with high or low EBV for fertility
- Studied during several cycles post partum:
  - Oestrus behaviour
  - Milk progesterone
  - Follicle and CL development
- Collection of brain tissues and oavaries (oocytes) on day 0 or day 12 of cycle
- Relate expression profile to reproductive characteristics

Large-scale gene expression studies

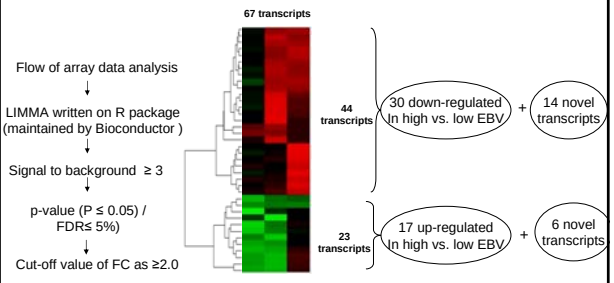


Results (Brain area)

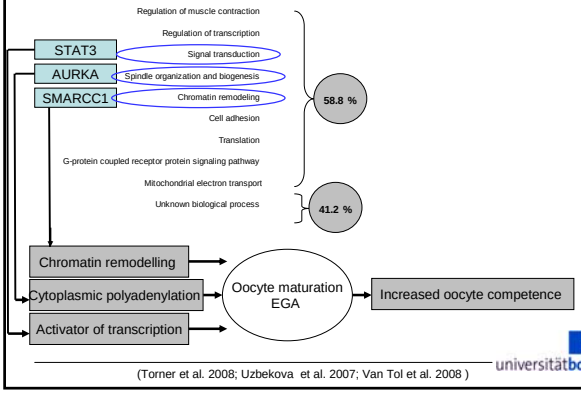
27 genes found differentially expressed in the pituitary of day 0 compared to day 12 cows

Gene name	log_2 fold change
Upregulated (mRNA)	
Bt natriuretic peptide precursor C (NPPC)	2.13
Bt similar to hCG2042429 (LOC524240)	1.55
Bt 5-hydroxytryptamine (serotonin) receptor 2B (HTR2B)	1.44
Bt similar to sulfatase 2, transcript variant 2 (SULF2)	1.40
Bt progesterone receptor (PGR)	1.28
Bt sperm associated antigen 17 (SPAG17)	1.26
Bt PDZ and LIM domain 3 (PDLIM3)	1.24
Bt similar to la related protein (LARP1)	1.21
Bt chromosome 17 open reading frame 48 ortholog (C19H17ORF48)	1.19
Bt misc_RNA (LOC514211)	1.15
Bt lysozyme 1 (LYZ1)	1.14
Bt fracture callus 1 homolog (rat) (FXC1)	1.14
Bt similar to rotatin (RTTN)	1.13
Bt C-type lectin domain family 14, member A (CLEC14A)	1.11
Bt similar to kazrin (LOC510718)	1.05
Bt chromosome 1 open reading frame 181 ortholog (H3C1ORF181)	1.02
Bt similar to zinc finger protein ZNF-U69274 (ZBTB11)	1.02
Down regulated (mRNA)	
Bt similar to CPEB2 protein (CPEB2)	-1.04
Bt follicle stimulating hormone, beta polypeptide (FSHB)	-3.34

Array analysis & heatmap of differentially regulated genes



Biological process represented in oocytes recovered from High vs. Low genetic merit cows



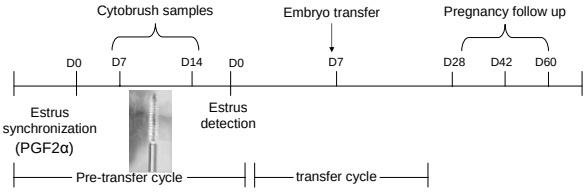
(Torner et al. 2008; Uzbekova et al. 2007; Van Tol et al. 2008)

Part II

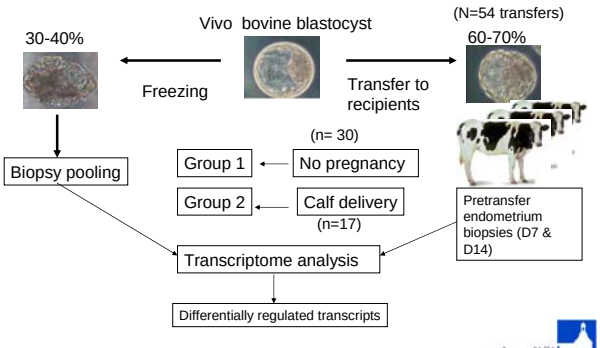
Embryo developmental competence and endometrium receptivity (Bonn/MTT)

Time schedule for synchronization, sampling and embryo transfer

Simmental cyclic heifers (n=54)

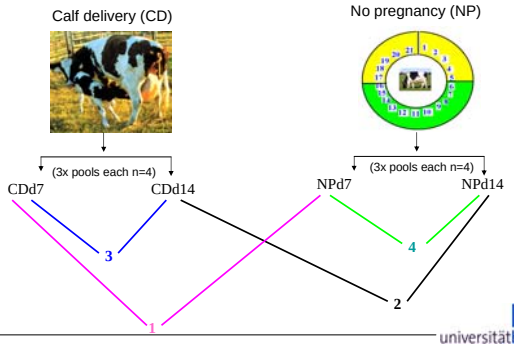


Overall design for phenotyping and array analysis



Endometrium transcriptome analysis

Pregnancy diagnosis and classification of endometrial samples



1. Transcriptome profiling of CDd7 vs NPd7

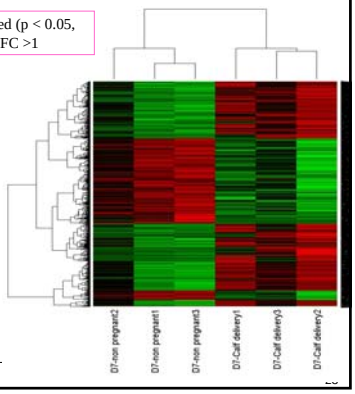
1130 → Differentially expressed ($p < 0.05$, fold change ≥ 2 or logFC >1

626 → Enriched in D7-Calf delivery endometrium

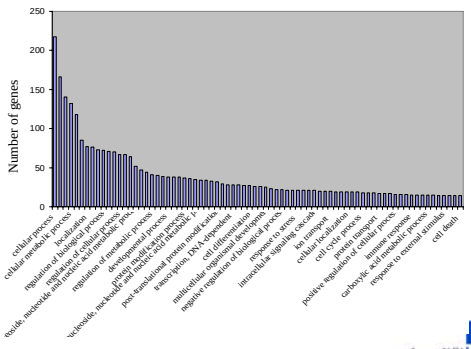
504 → Enriched in D7-Non pregnant endometrium

Annotated = 1103

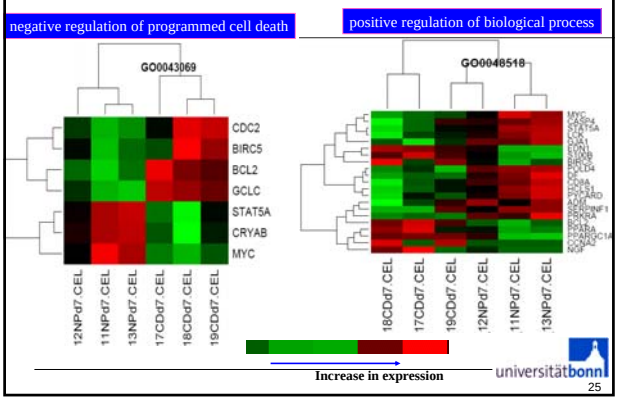
Not annotated = 27



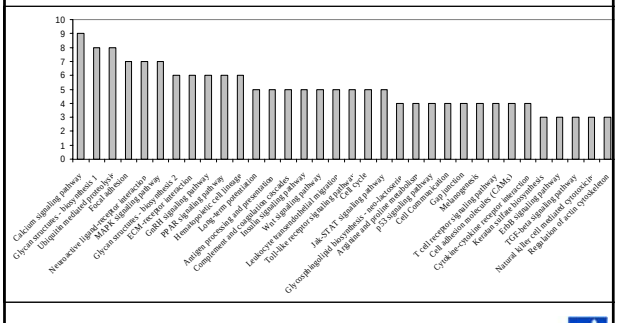
Classification of differentially expressed genes according to their biological process



Expression of genes from the same biological process



Involvement of differentially regulated genes in some Pathways

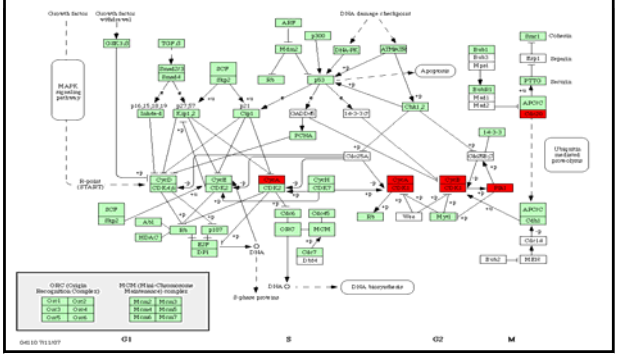


Involvment of differnetially regulated genes in some Pathways

Pathway	Molecules
Synaptic Long Term Potentiation	PPP1CC, PRKCQ, PPP1R3C, PPP1CB, ITPR1, CREB3L4, CAMK2D, PRKCI, PPP1R10, PRKAG2, MRAS, PLCB1, CAMK2B
Insulin Receptor Signaling	PPP1CC, SGK1, PPP1R3C, PPP1CB, EIF4E, EIF4EBP1, SCNN1G, PRKCI, PPP1R10, PDE3B, PTPN1, PRKAG2, MRAS
ERK/MAPK Signaling	PPP1CC, PLA2G10, PPP1R3C, ITGA2, PPP1CB, CREB3L4, EIF4E, ELF1, EIF4EBP1, MYC, PRKCI, PPP1R10, PRKAG2, MRAS, RPS6KA5 , ELK3
Chemokine Signaling	PPP1CC, CAMK2D, MRAS, PPP1CB, PLCB1, MAPK13, MAPK12, CAMK2B
Apoptosis Signaling	GAS2, ROCK1, PRKCQ, MRAS, IKBKE, CDC2, DFFA, BCL2
FGF Signaling	FGF2, RPS6KA5, MAPK13, ITPR1, CREB3L4, MAPK12
Tight Junction Signaling	MYLK, TIAM1, F2RL2, PRKCI, CLDN23, CLDN5, JAM3, PRKAG2, JAM2, ARHGEF2, PVRL1, CLDN7
BMP signaling pathway	MRAS, MAPK6, PRKAG2, BMPR2, MAPK13, MAPK12, AXL
JAK/Stat Signaling	STAT5A, PIAS2, SOCS2, PTPN1, MRAS
PPAR Signaling	NR2F1, PPARA, STAT5A, IL18, MRAS, IKBKE, PPARGC1A
G-Protein Coupled Receptor Signaling	CAMK2D, PDE3B, MRAS, PRKAG2, PLCB1, ADORA2B, IKBKE, CREB3L4,AGTR1, CAMK2B

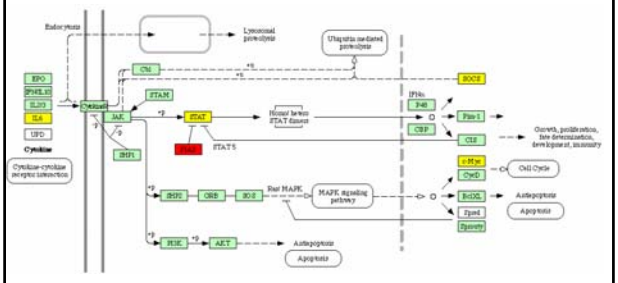
Pathways

Cell cycle



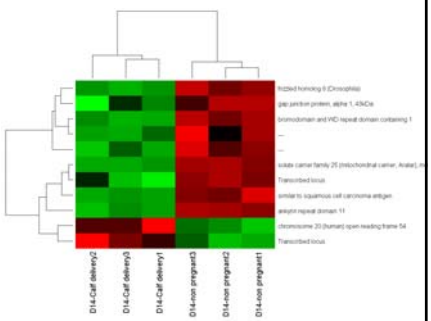
Transcriptome profiling of CDd7 vs NPsd7

JAK-STAT signaling pathway



2. Transcriptioanl profiling between CDd14 and NPd14

14 transcripts were differentially expressed between CDd14 and NPd14 of which 1 and 13 were up and down regulated respectively, in CDd14 compared to NPd14 at p < . 0.0005, fold change > 2, FDR < 27 %



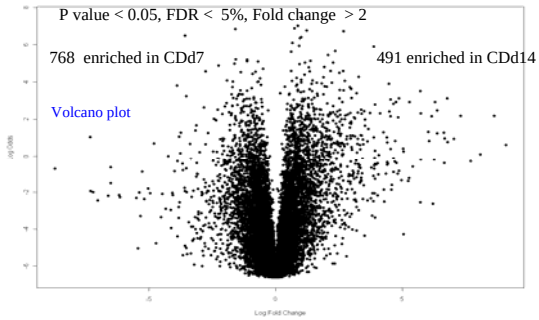
2. Transcriptioanl dynamics between CDd7 and CDd14

P value < 0.05, FDR < 5%, Fold change > 2

768 enriched in CDd7

491 enriched in CDd14

Volcano plot



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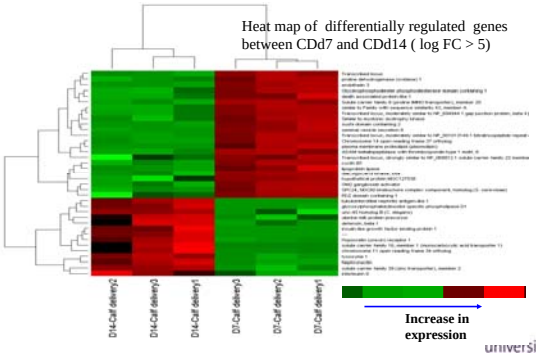
3. Transcriptioanl dynamics between CDd7 and CDd14

Heat map of differentially regulated genes between CDd7 and CDd14 (log FC > 5)



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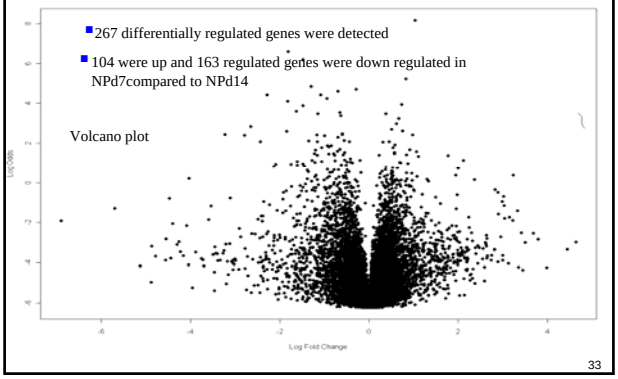
4. Transcriptioanl dynamics between NPd7 and NPd14

- 267 differentially regulated genes were detected
- 104 were up and 163 regulated genes were down regulated in NPd7compared to NPd14

Volcano plot



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Networks

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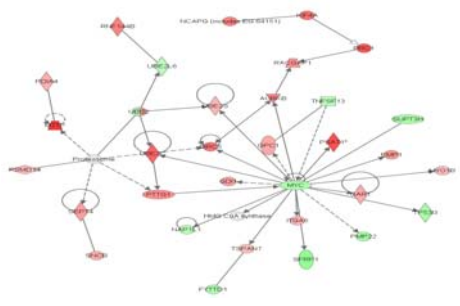
Cell cycle network



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Embryo biopsy transcriptome analysis

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Embryo biopsy transcriptome analysis

Biopsies Calf delivery (3x pools each n=4 biopsies)

Biopsies No pregnancy (3x pools each n=4 biopsies)

RNA isolation and amplification

Labelling and hybridization on bovine cDNA array (2000 clones)

Data analysis

Differentially expressed transcripts

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Embryo biopsy transcriptome analysis

Array analysis & heatmap of differentially regulated genes

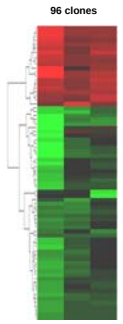
Flow of array data analysis

LIMMA written on R package (maintained by Bioconductor)

Signal to background ≥ 3

p-value (P ≤ 0.05) / false discovery rate (FDR≤ 5%)

Cut-off value of fold change as ≥2



15 up - regulated In NP vs. CD + 12 novel transcripts

48 up - regulated In CD vs. NP + 21 novel transcripts

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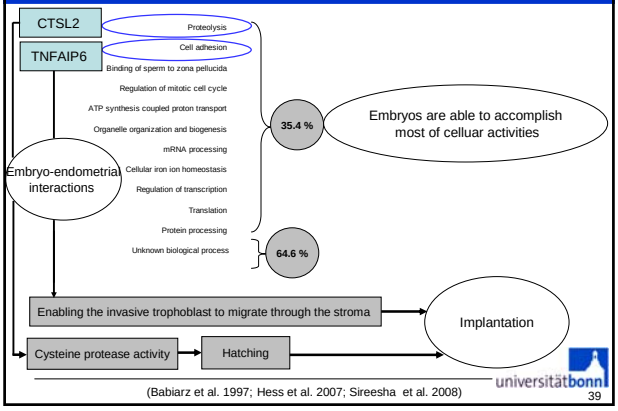
Biological process represented in embryo biopsies resulted calf delivery vs. no pregnancy



(Babiarz et al. 1997; Hess et al. 2007; Sireesha et al. 2008)

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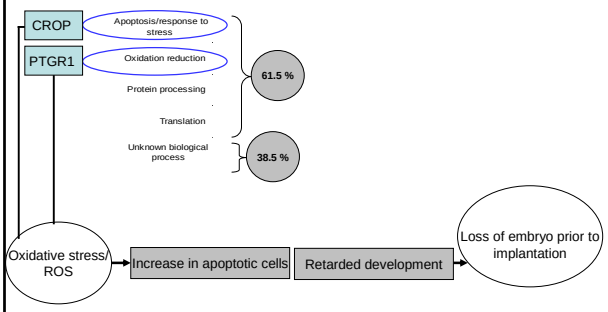
Biological process represented in embryo biopsies resulted no pregnancy vs. calf delivery



(de Castro et al. 2008; Betts and Madan 2008)

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Comparative presentation of candidate genes between in vitro and in vivo biopsies

Genes	Vitro NP	Vitro Res	Vitro CD	Vivo NP	Vivo Res	Vivo CD
Transcription factors						
MSX1	↑					
PTTG1	↑				↑	
CDX2		↓				
HOXB7		↓				
Protein binding and synth.						
KRT8	↓				↓	
RPLPO	↓				↓	
RPL26, P2		↑			↑	
EEFIA1	↑				↑	
ATP5A1	↑	↑				
CD9	↑				↑	
Growth factor						
BMP15		↓		↓	↓	

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Genes	Vitro NP	Vitro Res	Vitro CD	Vivo NP	Vivo Res	Vivo CD
Ion binding						
S100A10				↑	↑	
S100A14, 16				↑	↑	
ANAX2	↑	↑				
Signal transduction/ Oxidative stress						
TXN	↓	↓			↓	
AKR1B1	↑	↑				
TNF	↑			↑		
PLAU		↓				
GPX4	↑					
Hsp		↑			↑	
COX-2		↓		↓		
Tissue specific						
Plac8		↓			↓	

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