

SABRE
 CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING
Expression profiles of genes regulating dairy cow fertility

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Life history theory, milk↑ = ↓fertility?

Reproduction has its cost



Different life histories compete for resources, fitness is maximized by adjusting resource allocation

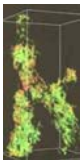


Caenorhabditis elegans: genes involved in the insulin/IGF-like signaling pathway affect both life span and fecundity, but the relationship can be uncoupled..



Genetic improvement to restore fertility

Increasingly important with decreasing profit margins
 h² fertility traits ~ 3%, but genetics do have impact



Bovine genome: ~3 billion nucleic base pairs in 29 autosomes and 2 sex chromosomes (~16 thousand mitochondrial base pairs)



Genomic selection

Nucleotide sequence variation (micro-satellites, restriction fragment length polymorphism, single nucleotide polymorphisms):

-Candidate gene approach

GnRH (Schneider et al., 2006), leptin (Liefers et al., 2005), bovine luteinizing hormone receptor (Hastings et al., 2006)

- Whole genome scan approach: QTL, SNP, ..

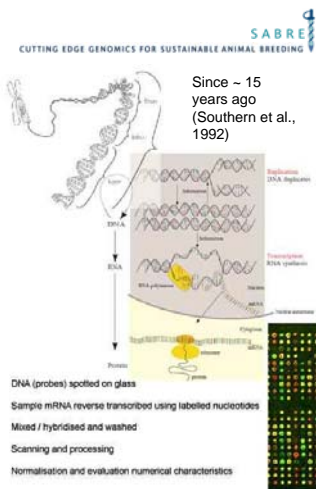


Functional Genomics:

focuses on dynamic aspects such as gene transcription and translation

Effects may be subtle (Niwa et al., 2000)

High-throughput technologies, like microarray-based transcriptome analyses, assess gene function in a genome wide fashion



Gene profiles - dairy cow fertility

Pregnancy (Hashizume et al. 2007): BNC specific bPL, bPRP, PAG): strategic effects on implantation, placentogenesis, fetogenesis and overall progression of pregnancy

Early development (Mamo et al., 2006, Wrenzycki et al., 2004)

Inferior quality IVP embryos: ↓ gene expression, deficiency of the machinery for transcription and translation (Corcoran et al., 2006)

Lasting pregnancy: IVP embryos 50%, for NT ≤15%

NT embryos (Pfister-Genskow et al. 2005) ↓ genes encoding intermediate-filament proteins and heat shock protein 27

↑ genes: compromised membrane formation (nidogen 2) and increased maternal immune responses to over-expressed MHC-I genes



Gene profiles - dairy cow fertility

oocyte maturation (Dalbies-Tran and Mermillod, 2003, Vallee et al., 2005), **non-regressed and regressed corpus luteum tissue** (Casey et al., 2005), **oviduct epithelial cell function** (Bauersachs et al., 2003, Bauersachs et al., 2004), **endometrium during the oestrous cycle** (Bauersachs et al., 2005), **pre-implantation embryonic development** (El-Halawany et al., 2004, Ushizawa et al., 2004, Sirard et al., 2005), **embryo-induced transcriptome changes of the endometrium in the pre- and peri-attachment period** (Ishiwata et al., 2003, Klein et al., 2006)

B. Beerda and R. F. Veerkamp Functional genomics of female reproduction. 8th World Congress on Genetics Applied to Livestock Production, August 13-18, 2006, Belo Horizonte, MG, Brasil, communication 11-10 p133



Summarizing the results ..

Ovaries: **steroid biosynthesis**, **oxygen radical metabolism**, **apoptosis**, **regulation of cell cycles (including DNA replication and repair)**, cell structure and **tissue remodeling**

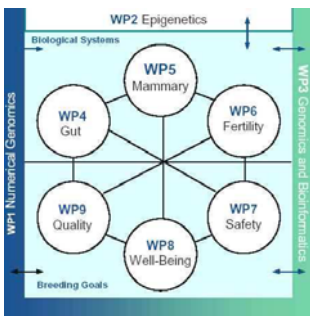
Oviducts: signal transduction pathways, **cell-surface proteins**, **cell-cell interaction proteins**, **immune-related proteins** and those that promote **proliferation**

Endometrium: **immune function**, **regulation of cell cycles**, **apoptosis**, **tissue remodeling**, **intercellular communication**, **cell-cell interaction**, **cell adhesion molecules**, **cell motility**, **intracellular metabolism**, **protein secretion and modification**, and **regulation of gene expression**

Conceptus: cell morphology **proliferation** and migration, **steroid biosynthesis**, **oxygen radical metabolism**, **matrix degradation**, **musculoskeletal growth** and **immune function**



SABRE: [http:// www.sabre-eu.eu/](http://www.sabre-eu.eu/)



WP6. Fine mapping **QTL affecting conception rate** - ARO, Israel, Gene expression profiling **folliculogenesis** -INRA, France, **embryonic developmental competence** - University of Bonn, Germany and MTT Agrifood Research, Finland, **oestrus** - ASG-Wageningen UR, The Netherlands



Genomic regulation of oestrus

Oestrus behaviour - ovulation and timing AI, but $\leq 50\%$ may display standing heat (Roelofs et al., 2005)



Oestrogen actions in the hypothalamus (Pfaff, 2005), **ventromedial hypothalamus**, arcuate nucleus, pre-optic area, paraventricular nucleus and supra-chiasmatic area.

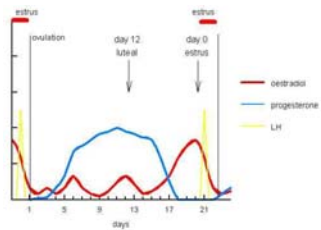
Limbic system, i.e. amygdala, hippocampus, septum and cingulate gyrus,

Stria terminalis and the **ventral tegmental area**



Experimental set up

| High Fertility | Low Fertility | |
|----------------|---------------|---------------|
| n=7 | n=7 | Day 0 oestrus |
| n=7 | n=7 | Day 12 luteal |



Measurements

behaviour

transrectal ultrasonography

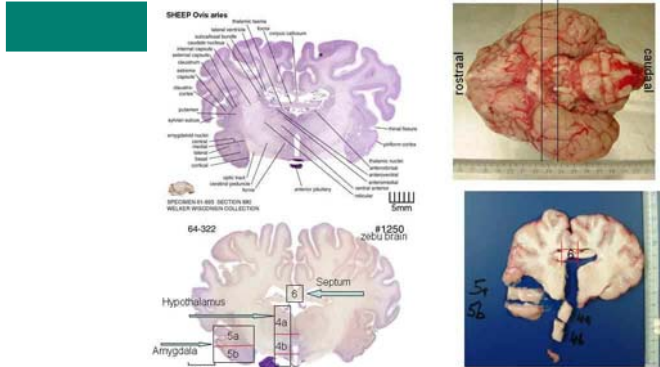
Protocol Van Eerdenburg, 2006

Results PCA analysis

Variance explained - in original dataset of 12 parameters –
 (1) 29% (2) 18%

| | load[1] | load[2] |
|----------------------------|---------|---------|
| genetic merit fertility | 0.04 | -0.82 |
| latency 1e behav oestrus | -0.82 | 0.13 |
| 1e behav score | 0.83 | 0.15 |
| fraction cycles + oestrus | 0.88 | -0.08 |
| mean behav score | 0.93 | -0.07 |
| P4 peak | -0.15 | -0.81 |
| interval between luteal ph | 0.04 | 0.72 |

euthanasia between 77 and 131 DIM



Summary findings

| High Fertility | Low Fertility | |
|---|---------------------------------------|---------------|
| 375* (+) immunoglobulin superfamily proteins (+) protein phosphatase 1 beta (-) tyrosine hydroxylase | 87 (+) gamma 2 actin (+) desmin | Day 0 oestrus |
| 414 (+) SCO-spondin (-) immunoglobulin superfamily proteins (-) promelanin-concentrating hormone | 64 | Day 12 luteal |

* mean individual behavioural oestrus expression scores

Summary findings

- Intra-cellular signalling, cell shape, motility and proliferation (gamma 2 actin, desmin, protein phosphatase 1 beta)
- Cell-cell interactions (immunoglobulin superfamily proteins)
- Catecholamine signalling (tyrosine hydroxylase, SCO-spondin, gamma 2 actin)
- Energy homeostasis (promelanin-concentrating hormone)

Epigenetics

Epigenesis .. alterations in DNA function not DNA sequence, e.g., methylation of cytosine residues - gene silencing (Li, 2002, Jaenisch and Bird, 2003)

Genomic imprinting: differential expression paternal and maternal alleles - tissue specific / in the prenatal period (Moore, 2001) / restricts transcripts (Allegrucci et al., 2005)

Imprinted genes ~ 0.3 % (Miozzo and Simoni, 2002), but major impact on fertility (Fowden et al., 2006).

RNA interference

micro RNAs and short interfering RNAs → micro RNA containing ribonucleoprotein particles (**represses translation of target mRNA**) and RNA-induced silencing complex (increases **degradation of target mRNA**)

RNA interference without transgenic approaches (Hiendleder et al., 2005): knock-out studies in farm animals?

Future applications of RNA interference tools : Lew et al., 2005

Variable copy numbers (CNV)

DNA segments (≥ 1 kb) present as CNV alter gene dosage, interfere coding sequences, long-range gene regulating effects

Human genome : ~5% of duplicated sequences, within and between chromosomes (Sharp et al., 2006)

Redon et al., 2006: 12% of the genome is CNV regions (i.e. more nucleotide content than SNPs), CNV effects on phenotypic variation substantial. (Stranger et al., 2007)



Nutrigenomics

Effects of dietary components on gene expression patterns (transcriptome), chromatin organization (epigenome), protein expression patterns (proteome) and metabolite profiles (metabolome) (Afman and Muller, 2006).

e.g. PPAR α targets 3 to 4 thousand (hepatic) genes (Mandard et al., 2004).

Early work and prospects for nutritional genomics in cattle (Dawson, 2006)... promising, but complex



High throughput technologies

identification candidate genes / mechanisms,
via bioinformatics increasingly
self-explanatory

