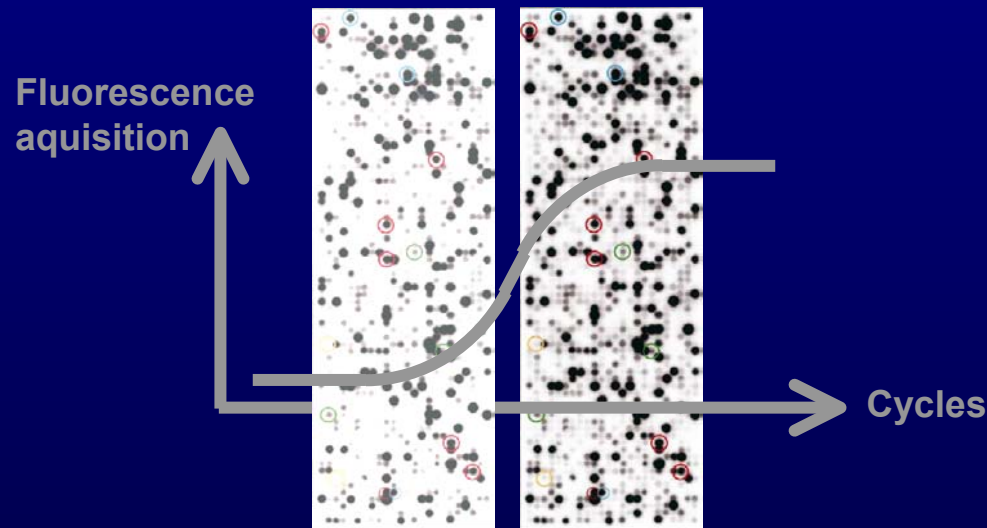


# Using real-time quantitative RT-PCR to validate a transcriptomics analysis advancing embryo-maternal communication



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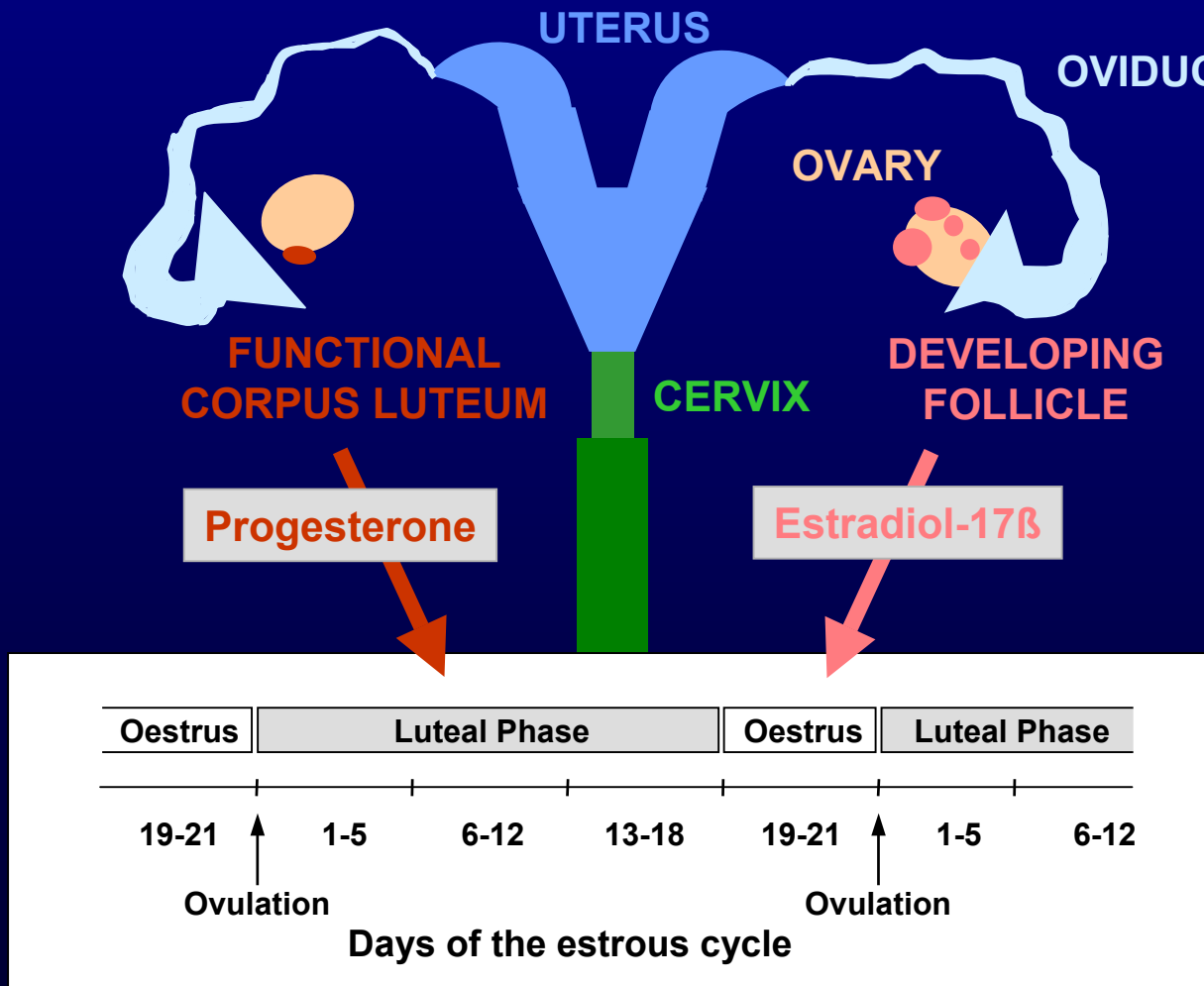
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# The role of the oviduct in reproduction

## Stressing the role of local regulatory mechanisms





# Embryo-maternal communication

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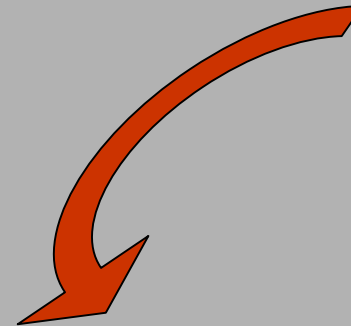
*In vivo* and *in vitro*  
cell culture models

Proteomics

Transcriptomics

Morphological  
Phenotyping

Candidate gene  
approach

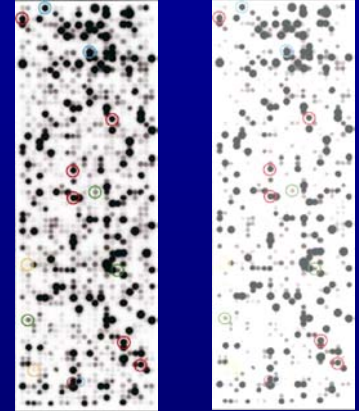




# Transcriptomic analysis

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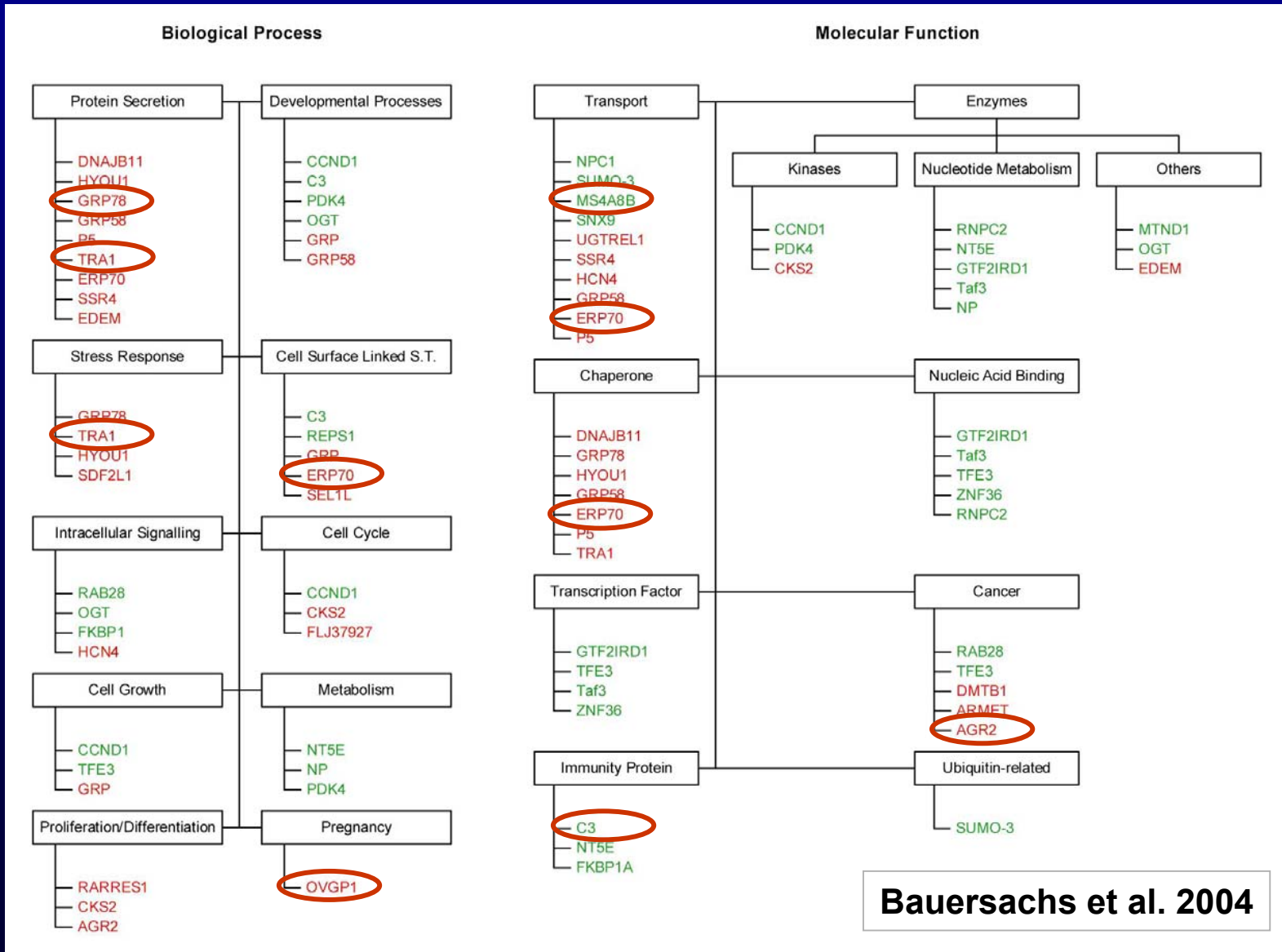
## Identification of transcriptomic changes



- **Suppression Subtractive Hybridisation (SSH) to obtain subtracted cDNA libraries**
- **cDNA array hybridisation**
- **Determination of transcriptomic changes in gene expression profiles**
- **Identification of differentially expressed cDNAs by sequencing**



# Preceding Results Transcriptomics



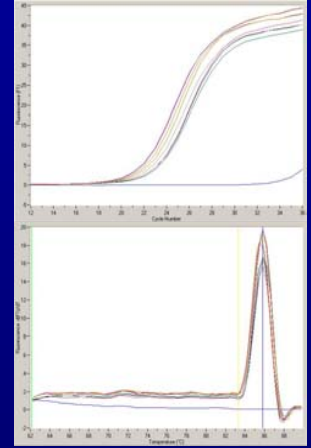


# Subsequent Gene quantification

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## Using real-time RT-PCR

- **Reverse Transcription  
RNA quantification**
- **Primer design  
Multi-species and intron- (exon-) spanning**
- **Quantification  
Second derivative maximum (SDM)  
High temperature fluorescence acquisition**
- **Melting curve analysis  
Specificity of Amplicon**





# Material and Methods

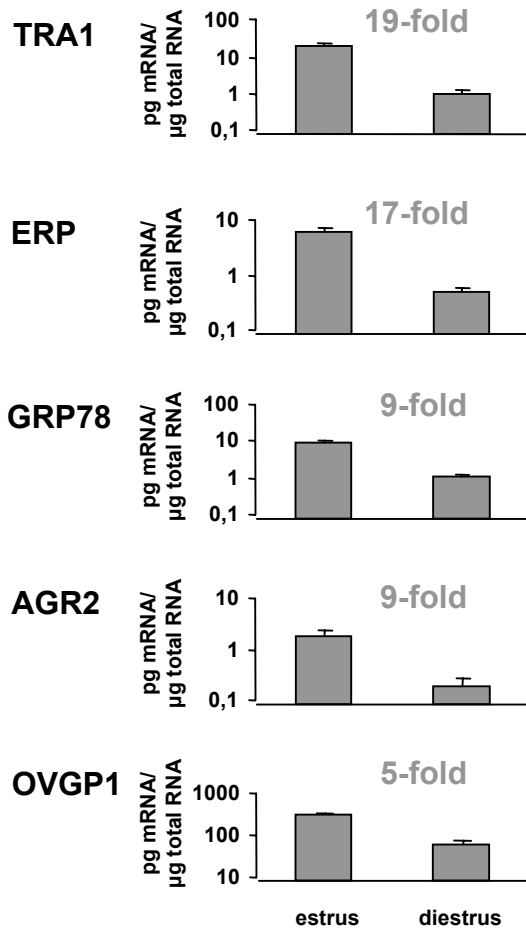
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- **Animal treatment**
  - Cycle synchronization of 6 heifers**
  - Slaughtered at day 0 or day 12 of the estrous cycle**
  
- **Tissue preparation**
  - Isolation of oviduct epithelial cells**
  - Directly frozen in liquid nitrogen**
  - within 10 min after death**
  - Extraction of total RNA**
  - Reverse transcription**
  - Real-time PCR of selected genes**

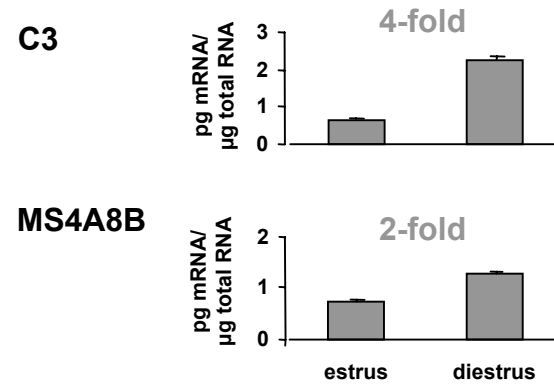


# Results real-time RT-PCR

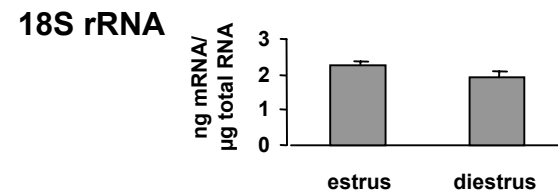
## Upregulated genes at estrus



## Upregulated genes at diestrus



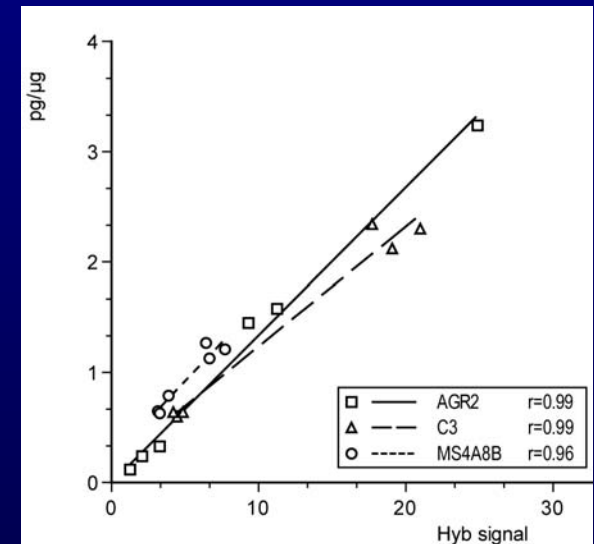
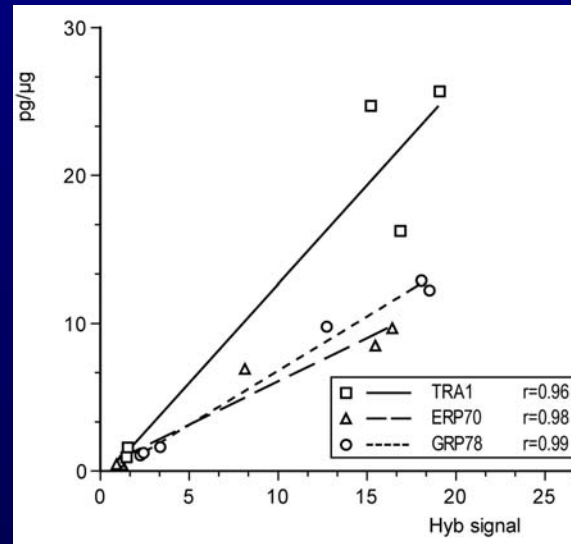
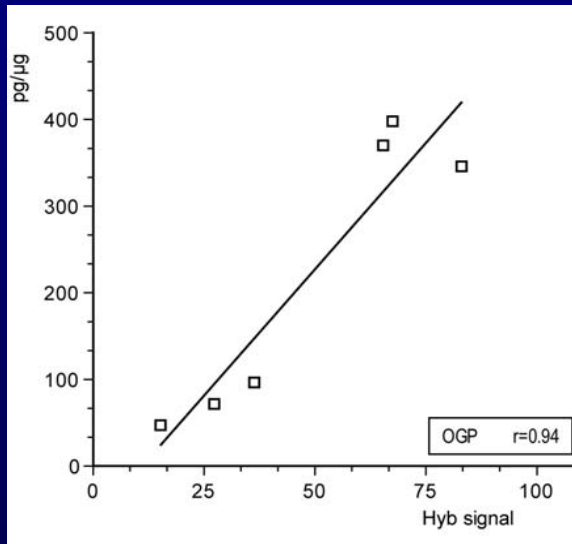
## Unregulated genes

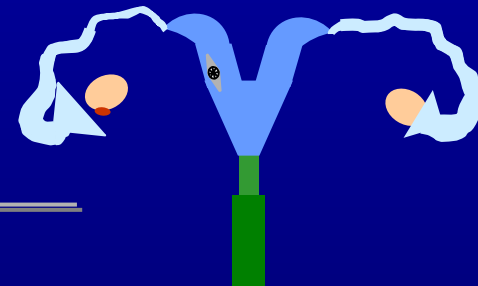




# Results

## Comparison of Micro Array Hybridization / Real-time-RT-PCR





## Conclusions

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- **Differential gene expression was found when comparing estrous and diestrous oviducts**
- **Regulated genes of different functional classes and biological processes are involved in oviduct differentiation**
- **SSH and micro array technique allow screening and identification of differentially expressed genes**
- **Real-time RT-PCR is the most adequate technique to exactly quantify candidate genes**
- **Transcriptomic and candidate gene approach can complement one another in advancing the identification of embryo-maternal signalling mechanisms**