EAAP 2016
67th Annual Meeting of the European Federation of Animal Science
Belfast UK, 29 Aug – 2 Sept 2016

Effects of heat stress on the transcriptomic profile of blood cells in lactating dairy goats

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G2R Grup Recerca Remugants

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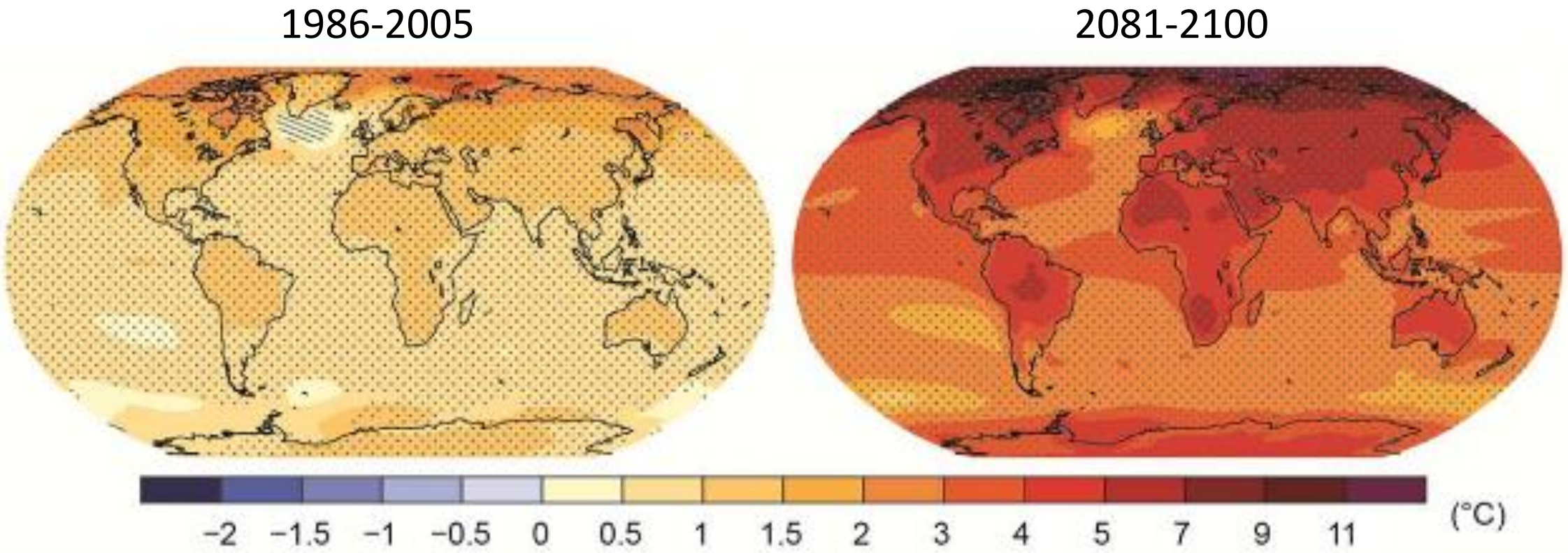
Change in average surface temperature



IPCC (2013)

Heat Stress effects in ruminants

Literature based mostly on phenotypic records

Responses of dairy goats to heat stress (HS)

- **Respiratory Rate** (+300%)
- **Rectal Temperature** (+0.58ºC)

- **Water consumption** (+202%)
- **Dry matter intake** (-21%)
- **Body weight** (-125 g/d)

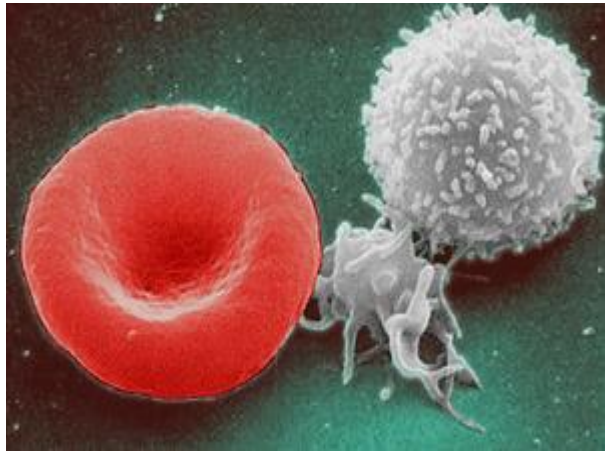
- **Milk yield** (-4 to -10%)
- **Milk quality** (–12.5% CP)

- **Slower Immune response** when the udder is challenged with LPS

Hamzaoui et al. (2012, 2013)
Love (2015)

Objectives

Evaluate the **transcriptomic response** and the metabolic pathways affected on **immune system** of **heat stressed** lactating dairy goats.



Experimental design

- Murciano-Granadina dairy goats in mid-lactation (n = 8)

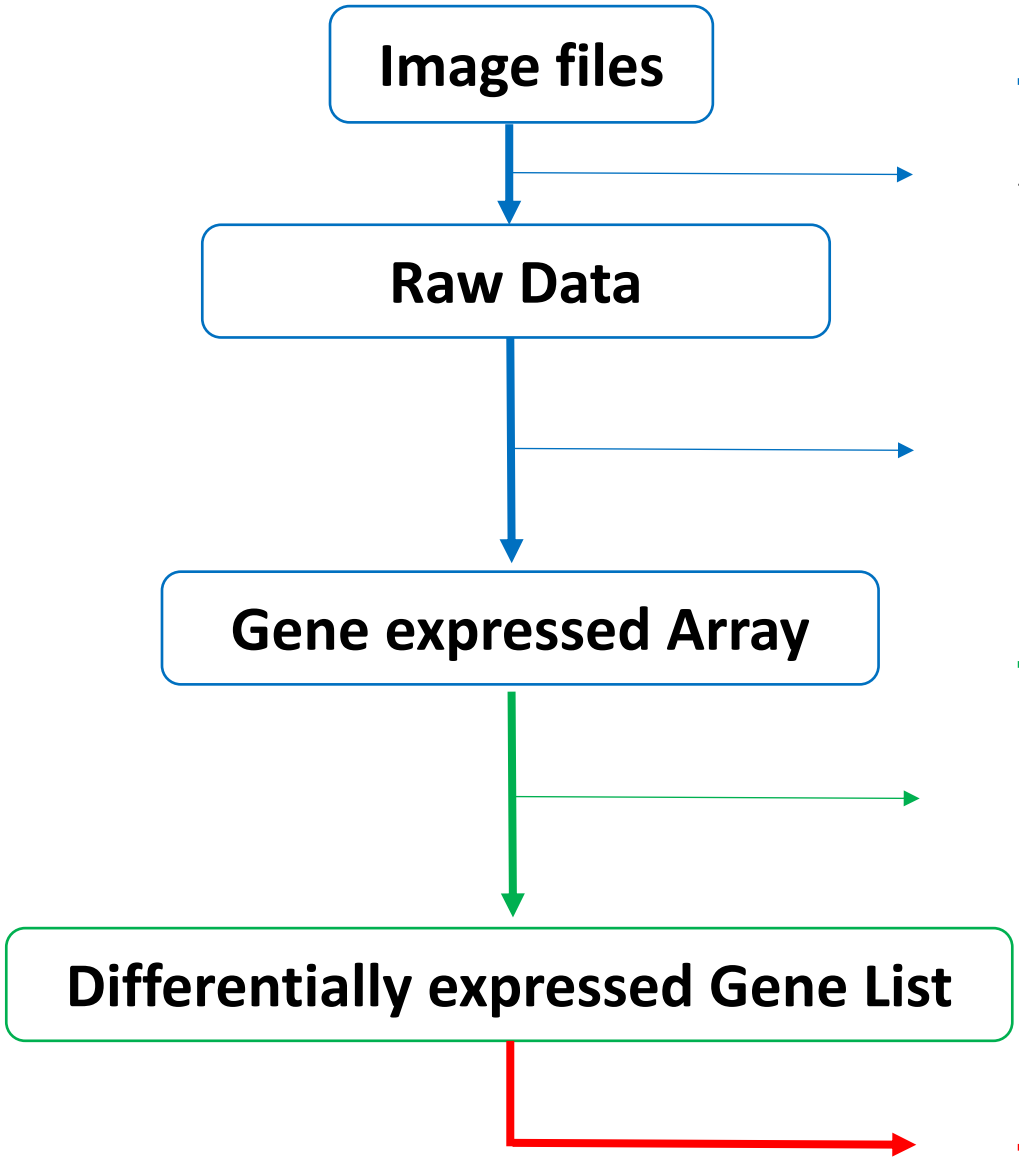
- Climatic chamber:
  - **TN: 15 to 20°C**
  - **HS: 37°C-12h and 30°C-12h**

- TMR diet ad libitum (17% CP; 1.3 Mcal ENL) according to requirements (INRA, 2007)

Blood samples and Microarrays

- **Blood samples:** d 35 (n=8)
- **RNA extraction:** RiboPure-Blood Kit
- **RNA integrity:** Agilent Bioanalyzer 2100
- **24K Affymetrix GeneChip** Bovine Genome Array
- **GeneChip 3’IVT Express Kit**

Gene expression Data Analysis



DATA PREPARATION:

Affy package (Bioconductor)

Background correction
Normalization (log_2)
Remove absent probe sets

STATISTICS:

Student's t-test
Benjamini and Hochberg corrected (FDR=0.05)
Fold Change threshold >/1/

PATHWAY ENRICHMENT ANALYSIS:

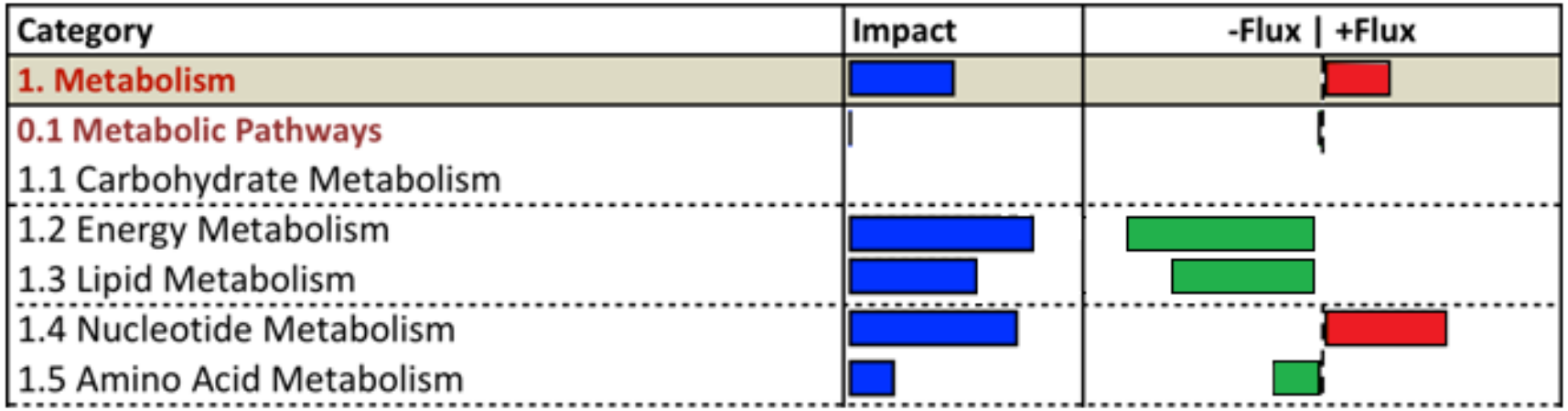
Pathway enrichment analysis

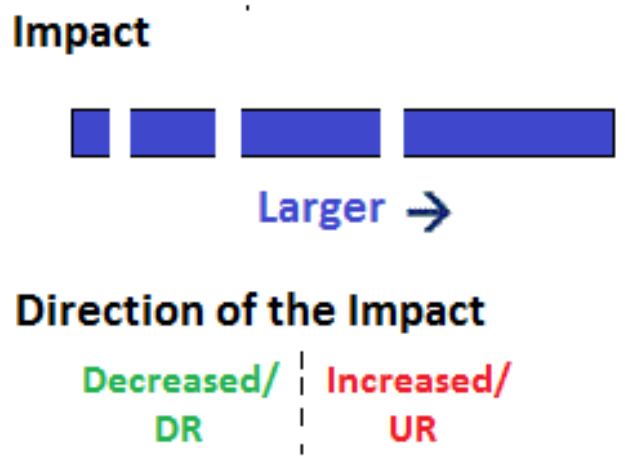
DINAMIC IMPACT APPROACH (DIA):

DEG List + Fold Change + adj p-value

DIA + KEGG

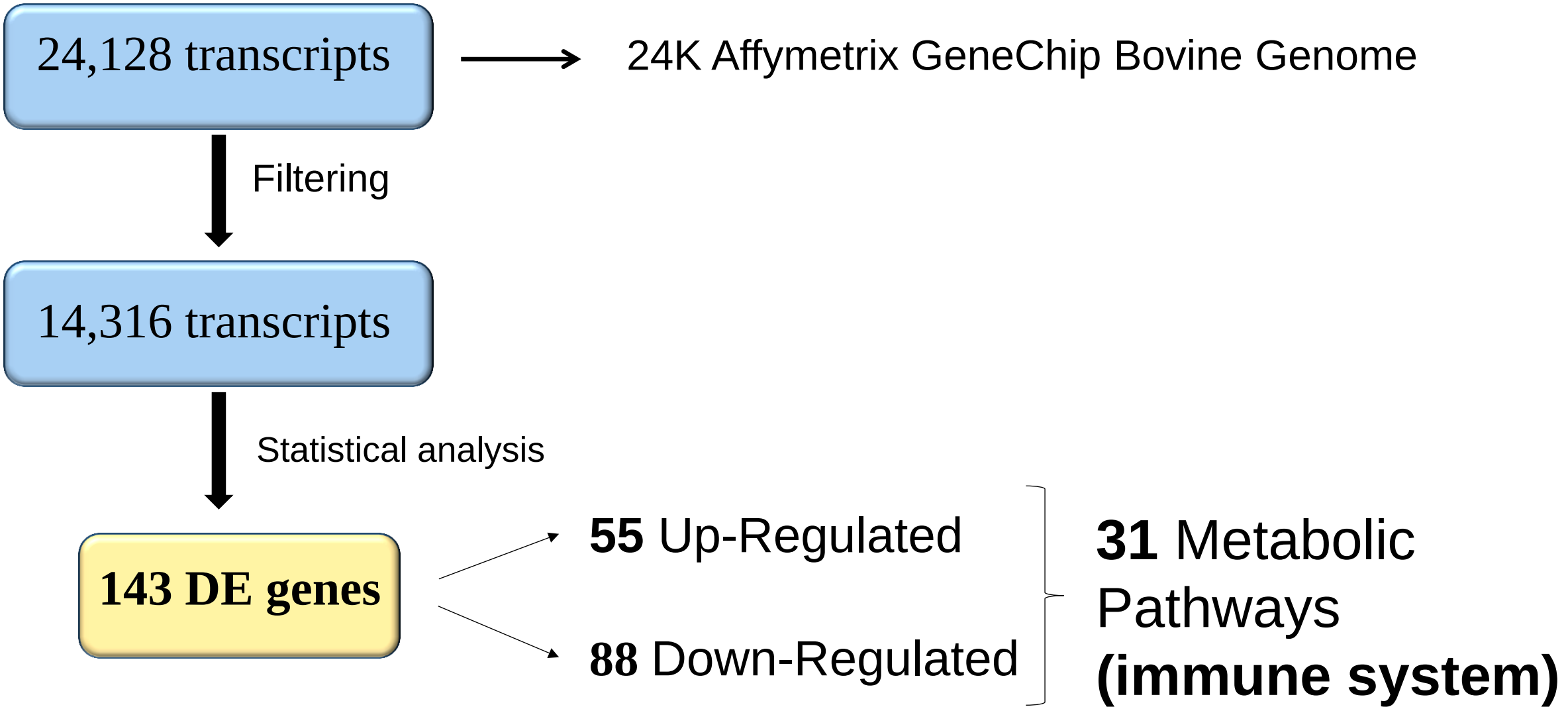
>= 4 genes/pathway
>20% coverage





Bionaz et al. (2012)

Results

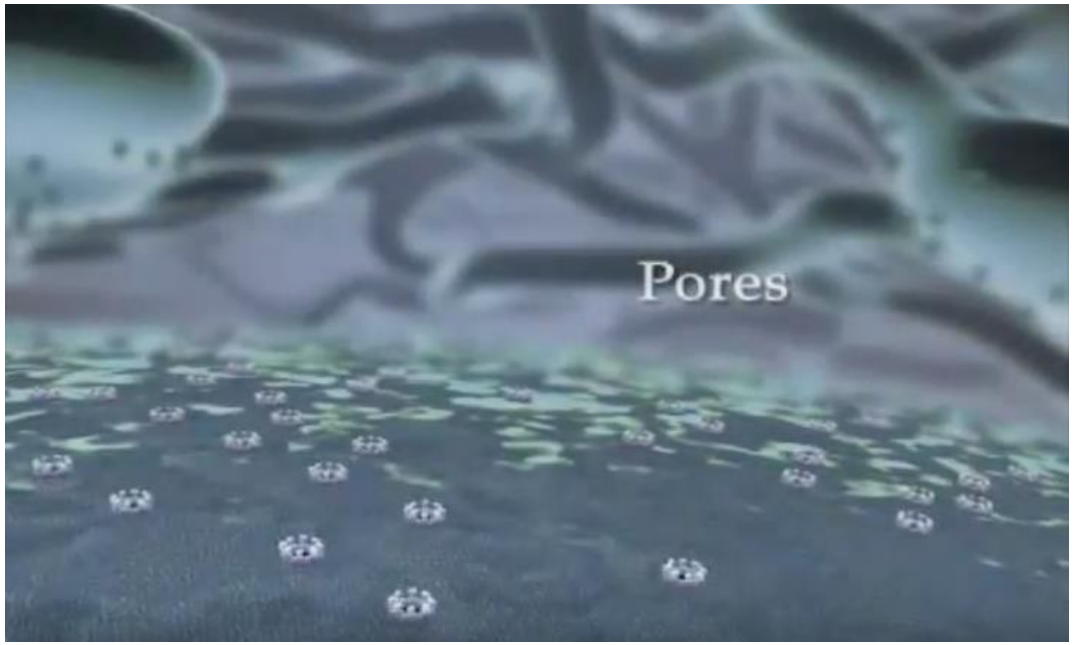

24,128 transcripts
24K Affymetrix GeneChip Bovine Genome
Filtering
14,316 transcripts
Statistical analysis
143 DE genes
55 Up-Regulated
88 Down-Regulated
31 Metabolic Pathways
(immune system)

Top 15 Pathways affected by HS

#	Pathway	Impact	-Flux | +Flux
1	Leukocyte transendothelial migration		
2	Pyrimidine metabolism		
3	Purine metabolism		
4	Cell adhesion molecules (CAMs)		
5	Drug metabolism - cytochrome P450		
6	Tight junction		
7	Fat digestion and absorption		
8	RNA transport		
9	Hematopoietic cell lineage		
10	Adipocytokine signaling pathway		
11	PPAR signaling pathway		
12	Adherens junction		
13	ECM-receptor interaction		
14	Calcium signaling pathway		
15	Arginine and proline metabolism		

1) Down-regulated pathways in HS goats

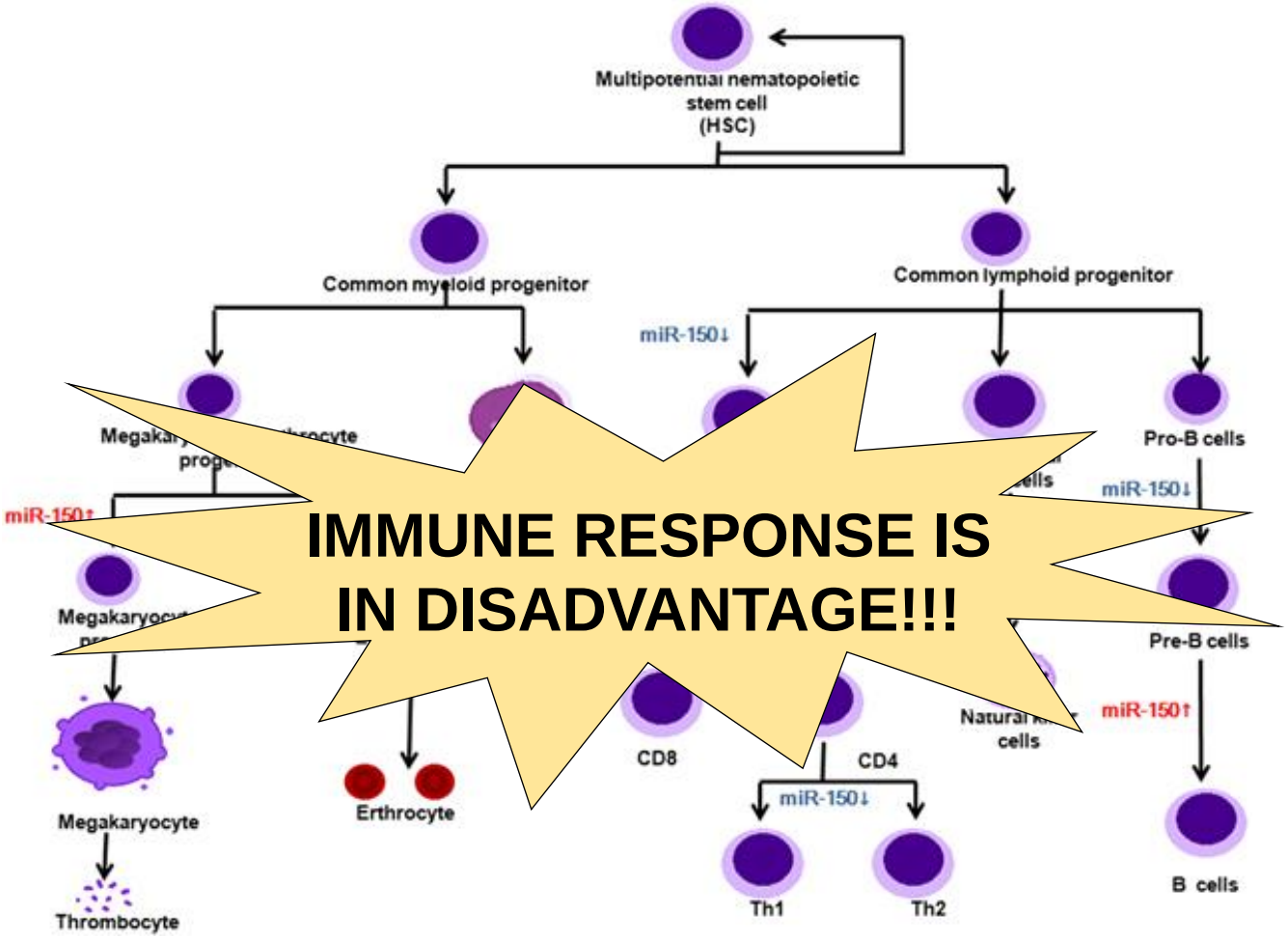
RNA TRANSPORT



Reviewed by Strambio-De-Castillia et al. (2010)

2) Down-regulated pathways in HS goats

HEMATOPOIETIC CELL LINEAGE



- Immune cells have ≠ life time.
- Have to be constantly replaced
- Slower creation and differenciation

Seita and Weissman (2011)

3) Down-regulated pathways in HS goats

LEUKOCYTE TRANSENDOTHELIAL MIGRATION

By interaction among **Cell Adhesion Molecules** and **Extracellular Matrix**

Step 1 Rolling → Selectins

Step 2 Adhesion → Integrins and Ig family

↓

Ca^{2+} SIGNALING

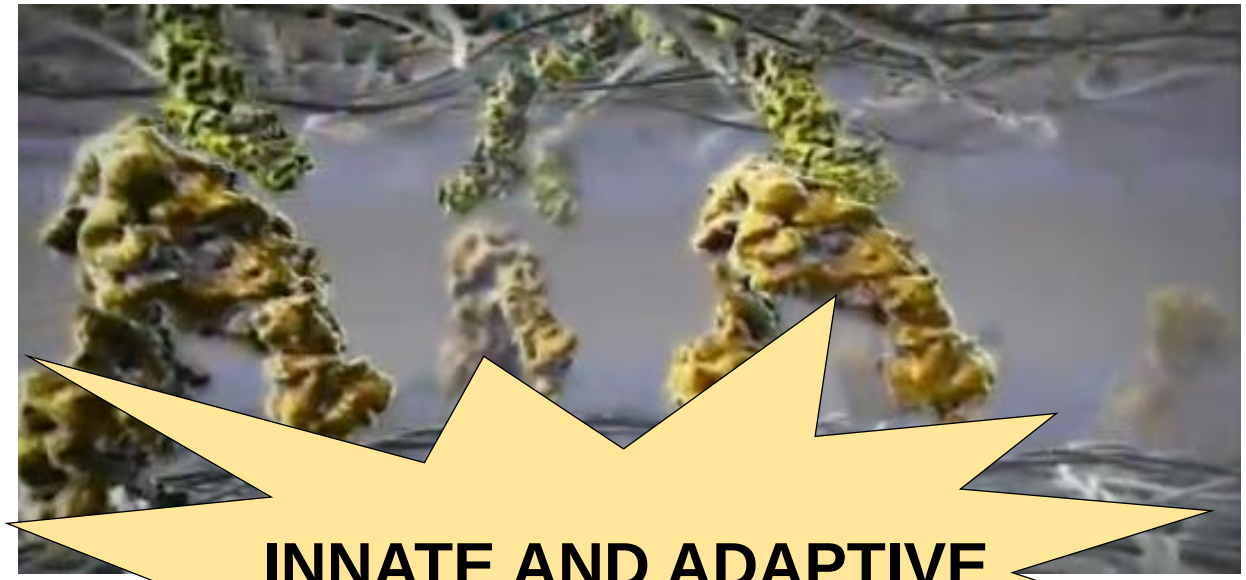
↓

Step 3 Extravasation

Reviewed by Etzioni (1996); Muller et al. (2011)

3) Down-regulated pathways in HS goats

LEUKOCYTE TRANSENDOTHELIAL MIGRATION



INNATE AND ADAPTIVE RESPONSE!!!

By interaction among **Cell Adhesion Molecules** and **Extracellular Matrix**

Step 1 Rolling → Selectins

Step 2 Adhesion → Integrins and Ig family

Ca^{2+} SIGNALING

Step 3 Extravasation

Reviewed by Etzioni (1996); Muller et al. (2011)

4) Down-regulated pathways in HS goats

1. PPARɣ SIGNALING

F(x): Modulates gene expression of lipid-related genes

(Széles et al., 2007; O'Donell et al., 2014)

2. Ca^{2+} SIGNALING

F(x): Platelet activation and aggregation

(Razell et al., 2013; O'Donell et al., 2014)

3. ARGININE AND PROLINE METABOLISM

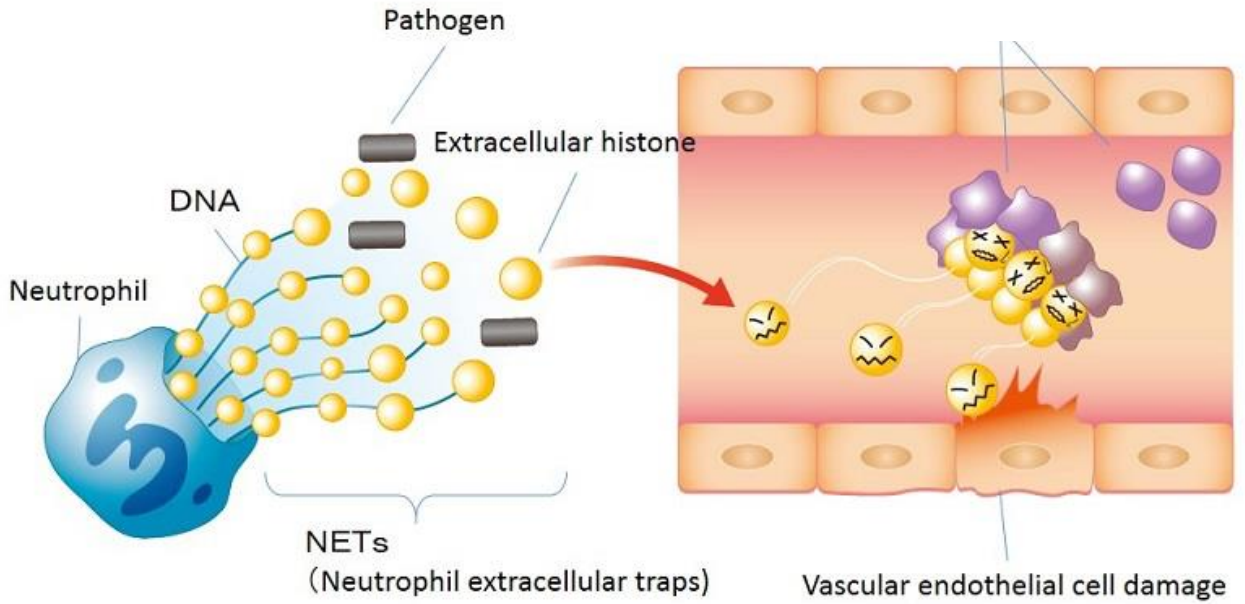
F(x): Decrease inflammatory response
Promote wound healing

(Gallardo-Soler et al., 2008; Munder et al., 2009)

COMPROMISE WOUND HEALING

Up-regulated pathways in HS goats

- **PYRIMIDINE METABOLISM**
- **PURINE METABOLISM**
- **CYTOCHROME p450**



22.2 pg of cytosolic protein/leukocyte (humans)

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

Immune cell fate for extra fuel and wound healing

Hibbs et al. (2016)

Conclusions

1. 143 Differentially expressed genes were found in healthy lactating dairy goats after 5 wk of heat stress exposure.

    - 55 of them were up-regulated in heat stress goats involved in nucleotide catabolism and oxidation.

    - 88 genes were down-regulated in heat stress goats mainly related with RNA transport, leukocyte transendothelial migration and wound healing.

2. Heat stress hinders both innate and adaptive immune responses.

Thank you for your attention