Analysis of sheep SERPINA1 gene expression in milk during lactation

Cinzia Marchitelli
Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria
Animal Production Research Centre
cinzia.marchitelli@entecra.it

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Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

Talk outline

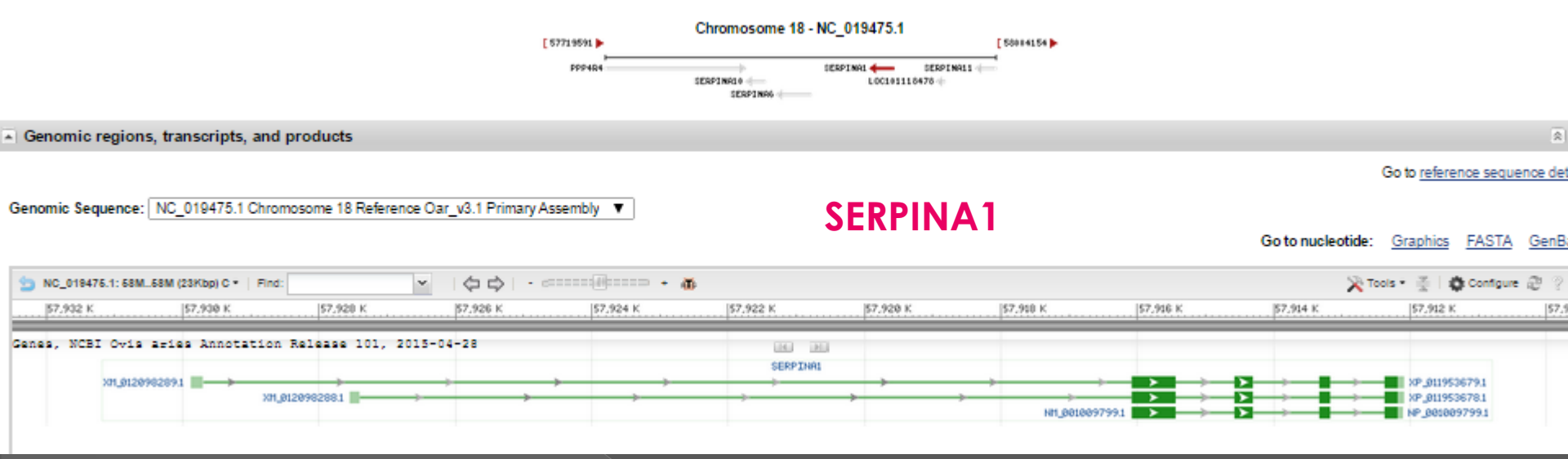
Overview and related work

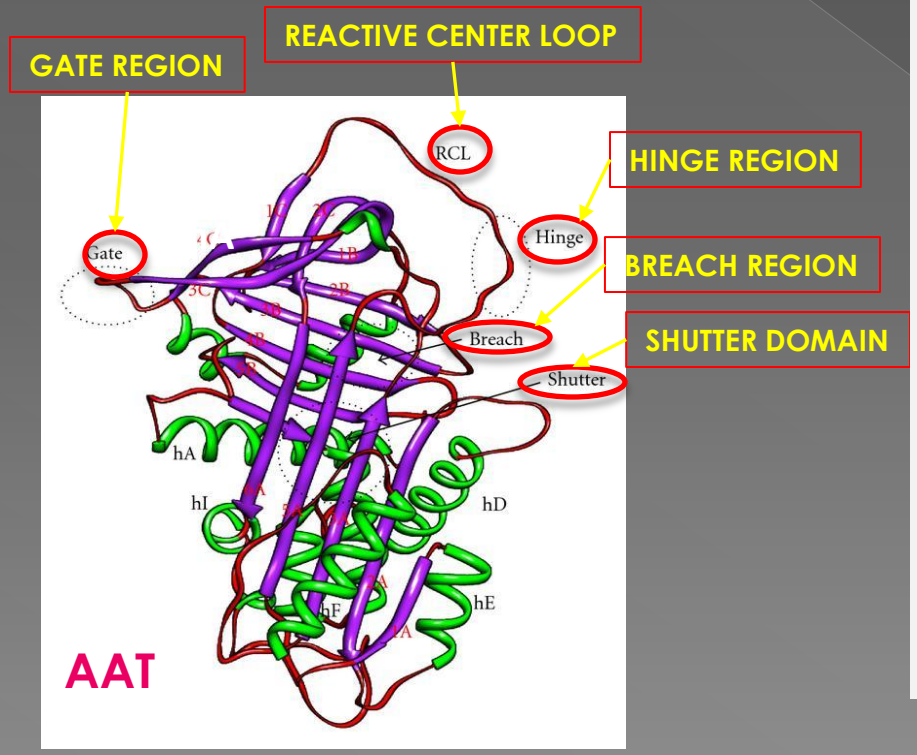
Our research

- Characterization ovine SERPINA1 cDNA and gene
- Differential expression of sheep SERPINA1 gene during lactation

Conclusion

Future work





✓**Expression:** liver, muscle, mammary gland, macrophages and neutrophil

✓ **Biological processes:** blood coagulation, apoptosis, reproduction, fibrinolysis and inflamamtory responses

✓ **Primary fucntion:** inactivation of neutrophil elastase

References

Review Article ISRN Immunology 2012, Article ID 354365

An Emerging Role for Serine Protease Inhibitors in T Lymphocyte Immunity and Beyond

Philip G. Ashton-Rickardt

Available online at www.sciencedirect.com ScienceDirect

ELSEVIER

The International Journal of Biochemistry & Cell Biology 39 (2007) 1165–1176

IJBCB

www.elsevier.com/locate/biocel

α1-Antitrypsin regulates CD14 expression and soluble CD14 levels in human monocytes *in vitro*

Izabela M. Nita [a], Danielius Serapinas [a,b], Sabina M. Janciauskiene [a,*]

[a] Department of Clinical Sciences, Wallenberg Laboratory, University Hospital Malmö, Lund University, SE-20502 Malmö, Sweden
[b] Department of Pulmonology and Immunology, Kaunas University of Medicine, Kaunas, Lithuania

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Mechanisms of Macromolecular Protease Inhibitors

Christopher J. Farady [a, b] and Charles S. Craik *[a, b]

AJRI 2001, 45: 266-272

Immunoregulatory Activity, Biochemistry, and Phylogeny of Ovine Uterine Serpin

MORGAN R. PELTIER AND PETER J. HANSEN

DIABETES, VOL. 56, MAY 2007

Original Article

α1-Antitrypsin Protects β-Cells From Apoptosis

Bin Zhang,[1] Yuanqing Lu,[1] Martha Campbell-Thompson,[2] Terry Spencer,[3] Clive Wasserfall, Mark Atkinson,[2] and Sihong Song[1]

α-1 Antitrypsin regulates human neutrophil chemotaxis induced by soluble immune complexes and IL-8

mer P. Reeves,[1] Paula Meleady,[2] Michael Henry,[2]
más P. Carroll,[1] Claire Condron,[1] Sanjay H. Chotirmall,[1]
,[2] Shane J. O'Neill,[1] and Noel G. McElvaney[1]

PERGAMON

The International Journal of Biochemistry & Cell Biology 35 (2003) 1536–1547

IJBCB

www.elsevier.com/locate/ijbcb

Review

Serpins: structure, function and molecular evolution

Diana van Gent, Paul Sharp, Kevin Morgan, Noor Kalsheker*

Division of Clinical Chemistry, Institute of Genetics, Queen's Medical Centre, University of Nottingham, NG7 2UH Nottingham, UK

Received 30 December 2002; accepted 14 March 2003

Abstract

The superfamily of serine proteinase inhibitors (serpins) are involved in a number of fundamental biological processes such as blood coagulation, complement activation, fibrinolysis, angiogenesis, inflammation and tumor suppression and are

Role of AAT in human milk

By Lindberg et al 1982

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6–7	>14 days
Protein (g/liter)	89 ± 8.6 (8)	53 ± 15 (8)	22 ± 1.9 (8)		20 ± 0.8 (8)		11 ± 1.3 (8)
α_1-AT (%)[1]	16.1 ± 1.5 (21)	8.3 ± 1.1 (35)	4.2 ± 0.6 (42)	3.1 ± 0.2 (36)	2.7 ± 0.2 (28)	1.9 ± 0.2 (13)	1.0 ± 0.3 (11)
α_1-Activity (%)[1]	13 ± 1 (21)	7 ± 1 (34)	3 ± 0 (40)	26 ± 2 (36)	22 ± 4 (26)	19 ± 4 (14)	2.4 ± 0.5 (8)
α_1-AT (g/liter) (100% = 1.3 g/liter)	0.21	0.11	0.055	0.04	0.035	0.025	0.013

of endogenous proteases

- Regulaiton of proteolitic activity in mammary gland
- Inactivation of macrophages proteases
- Increase of survival of other milk proteins via partial inhibition of proteolisys

Bioactive Proteins in Human Milk: Mechanisms of Action

Bo Lönnerdal, PhD

Human milk contains a multitude of bioactive proteins, with very diverse functions. Some of these proteins are involved in the synthesis and expression of milk, but the majority appears to have evolved to provide physiological

α_1-Antitrypsin may represent a unique bioactivity in human milk. This protease inhibitor is present in relatively high concentration in human milk, particularly during early lactation.[38] We have shown that this protein is present in intact form in significant quantities in the stool of breast-fed infants and is thus, during infancy, not indicative of protein-losing enteropathy.[39] It binds tightly to trypsin and it is thus possible that α_1-antitrypsin can help to limit protein digestion during early infancy when α_1-antitrypsin concentrations in milk are high and secretion of proteolytic enzymes is immature. In this capacity, α_1-antitrypsin may facilitate the action of other bioactive proteins.[38]

AAT: bioactivity in human milk

AAT: protect other milk protein from proteolysis

α_1-Antitrypsin and antichymotrypsin in human milk: origin, concentrations, and stability[1–3]

Winyoo Chowanadisai and Bo Lönnerdal

SERPINA1 and AAT in farm animal

Biochem. J. (1994) **303**, 383–390 (Printed in Great Britain)

Characterization of two serpins from bovine plasma and milk

Søren CHRISTENSEN and Lars SOTTRUP-JENSEN*
Department of Molecular Biology, Bldg. 130, University of Aarhus, DK-8000 Århus C, Denmark

Biochem. J. (1991) **273**, 685–690 (Printed in Great Britain)

Isolation and characterization of sheep α_1-proteinase inhibitor

Rohitbhai MISTRY,* Phillip D. SNASHALL,* Nicholas TOTTY,† Abraham GUZ* and Teresa D. TETLEY*‡
* Department of Medicine, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF, and
† Ludwig Institute for Cancer Research, Courtaulds Building, 91 Riding House Street, London W1P 8BT, U.K.

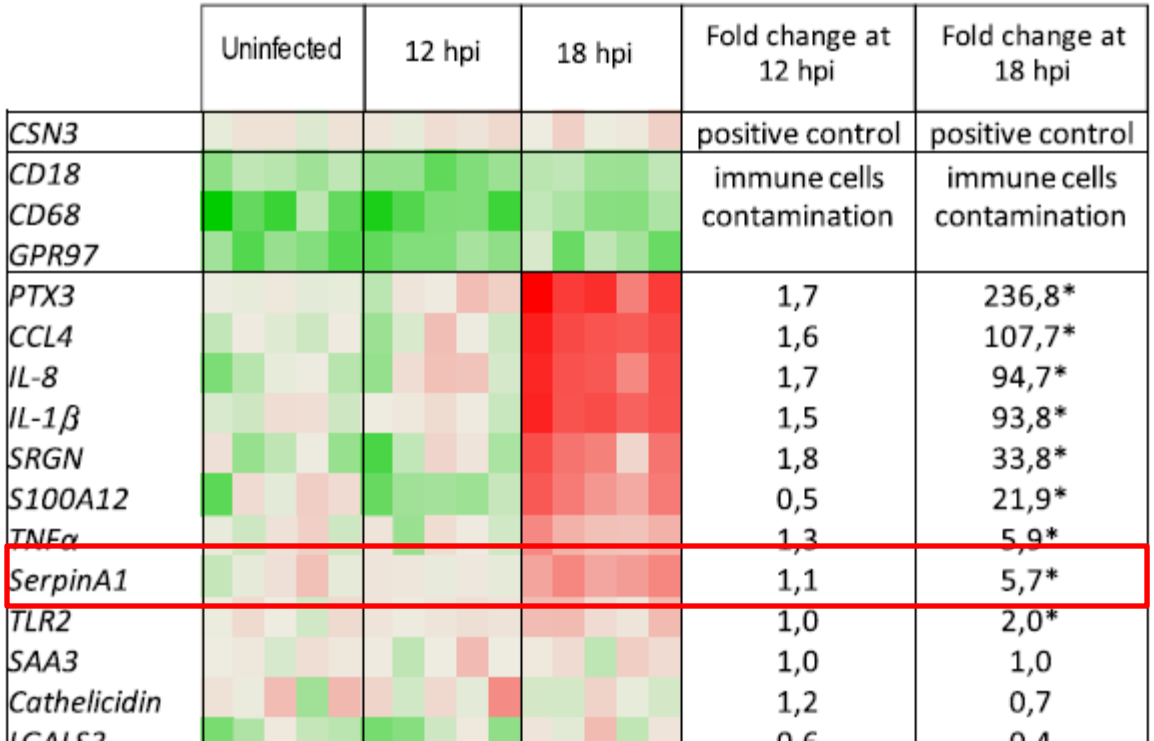
Brenaut *et al. Veterinary Research* 2014, **45**:16
http://www.veterinaryresearch.org/content/45/1/16

VETERINARY RESEARCH

RESEARCH Open Access

Contribution of mammary epithelial cells to the immune response during early stages of a bacterial infection to *Staphylococcus aureus*

Pauline Brenaut[1], Lucas Lefèvre[1,2], Andrea Rau[1], Denis Laloë[1], Giuliano Pisoni[3], Paolo Moroni[3,4], Claudia Bevilacqua[1,2] and Patrice Martin[1,2*]



	Uninfected	12 hpi	18 hpi	Fold change at 12 hpi	Fold change at 18 hpi
CSN3				positive control	positive control
CD18				immune cells contamination	immune cells contamination
CD68					
GPR97					
PTX3				1,7	236,8*
CCL4				1,6	107,7*
IL-8				1,7	94,7*
IL-1β				1,5	93,8*
SRGN				1,8	33,8*
S100A12				0,5	21,9*
TNFα				1,3	5,9*
SerpinA1				1,1	5,7*
TLR2				1,0	2,0*
SAA3				1,0	1,0
Cathelicidin				1,2	0,7
LCALS3				0,6	0,4

Beecher *et al. BMC Genetics* 2010, **11**:99
http://www.biomedcentral.com/1471-2156/11/99

BMC Genetics

RESEARCH ARTICLE **Open Access**

Livestock Science 149 (2012) 224–231

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Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Differentially expressed mammary proteins during lactation in dairy sheep

Federica Signorelli, Giulia Francesca Cifuni, Maria Miarelli *

Agricultural Research Council, Animal Production Research Centre, Via Salaria 31, 00015 Monterotondo, Rome, Italy

218.5 1934 1171.8

Table 2
Protein photodensity (mean ± standard error) at each lactation stage.

Protein	Spot*	Kegg category	Early		Mid		Late	
			mean	std err	mean	std err	mean	std err
Transketolase	1	Carbohydrate metabolism	955.5	455.5	9072.5	671.5	5535	943
Phosphogluconate dehydrogenase	2	Carbohydrate metabolism	3177.5	777.5	27000	9000	16250	2250
Cytosolic NADP-isocitrate dehydrogenase	3	Carbohydrate metabolism	2963	1003	8645.5	450.5	3492	897
Similar to dihydrolipoammide dehydrogenase isoform 2	4	Carbohydrate metabolism	773	428	1759.5	89.5	360.25	310.25
Galactose mutarotase	5	Carbohydrate metabolism	1326.5	606.5	5772.5	1522.5	2439	71
Fatty acid synthase	6	Lipid metabolism	336	286	2105.5	783.5	855.5	443.5
Fatty acid-binding protein	7	Lipid metabolism	30270	25401	97266.5	4438.5	37305	9560
Methylene tetrahydrofolate dehydrogenase1	8	Metabolism of vitamins and cofactors	568.5	431.5	2026.5	621.5	520	10
Transferrin	9	Metabolism of vitamins and cofactors	2667.5	1802.5	8637.5	3862.5	7625.5	4124.5
Eukaryotic translation elongation factor 2	10	Genetic information processing	50	0	619.5	1.5	2719.25	165.25
Protein disulphide isomerase A6	11	Genetic information processing	1203	935	281.5	231.5	1061.5	693.5
Prohibitin	12	Genetic information processing	2599	5	5886	707	3118	210
Serpin1	13	Genetic information processing	218.5	168.5	1934	955	1771.75	544.75

d
d

arthy[2],

e within Holstein-

SCS (log$_e$ SCC *100)
3.16 (11.38)
0.01 (0.01)
-0.01 (0.01)
-0.01 (0.01)
0.01 (0.01)
-0.01 (0.01)
0.7 (0.61)
0.65 (0.62)

0.1; * = P < 0.05;

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- Differential expression of sheep SERPINA1 gene during lactationSecond experiment

Conclusion

Future work

OPEN ACCESS Freely available online

PLOS ONE

Splicing Variants of *SERPINA1* Gene in Ovine Milk: Characterization of cDNA and Identification of Polymorphisms

Cinzia Marchitelli*, Alessandra Crisà, Elisa Mostarda, Francesco Napolitano, Bianca Moioli

Consiglio per la Ricerca e la sperimentazione in Agricoltura - CRA, PCM, Animal Production Research Centre, Monterotondo, Italy

Characterization of cDNA and gene

RNA extraction from milk somatic cells

4 SARDA
3 GENTILE DI PUGLIA
3 COMISANA

RNA extraction from 11 tissues

1 SARDA
1 GENTILE DI PUGLIA

Gene sequencing

4 SARDA
3 GENTILE DI PUGLIA
3 COMISANA

Table 1. List of primers used to amplify and to sequence the ovine *SERPINA1* cDNA and gene.

Primer name	Sequence 5'->3'	Region
cDNA FWD	CAGAAGCTCCTTCCTCCTGC	I exon
cDNA REV	TTTAATGCCATGGAGGGAAGA	V exon
5'UTR FWD	TGCAGAGCCCTGGGTAAGA	5'UTR
5'UTR REV	CCGTATTTAAGCACTGGACCC	5'UTR
5'UTR FWD Int	AAAGCTTGGTGAGCAGGTGT	5'UTR
5'UTR REV Int	CCGTGCACCACAACTAGAGT	5'UTR
II intron FWD	GCTGGGGTTCTCCAAGGAC	II exon
II intron REV	GTTTGCTCATTCACGTGGAAGTC	III exon
III intron FWD	GACTTCCACGTGAATGAGCAAAC	III exon
III intron REV	CCAGTCACCCAGGACAGTTTTC	IV exon
IV intron FWD	GAAAACTGTCCTGGGTGAACTGG	IV exon
3'UTR FWD	TCTTCCCTCCTCCATGGCATTAAA	V exon
3'UTR REV	TCCAAGAGATAGTGAAGGACAGG	3'UTR
3'UTR FWD Int	TGTGGTCTCTGGCTGGAAAC	3'UTR
3'UTR REV Int	TGGGTAATAATCGTGTCATTAATGG	3'UTR

Primers cDNA FWD, 5' UTR FWD, 5' UTR REV and 3' UTR REV were designed using the bovine transcript ENSBTAT00000045193. Primers II intron FWD, II intron REV, III intron FWD, III intron REV, IV intron FWD, IV intron REV, 3' UTR FWD were designed using the ovine mRNA sequence (NM_001009799).

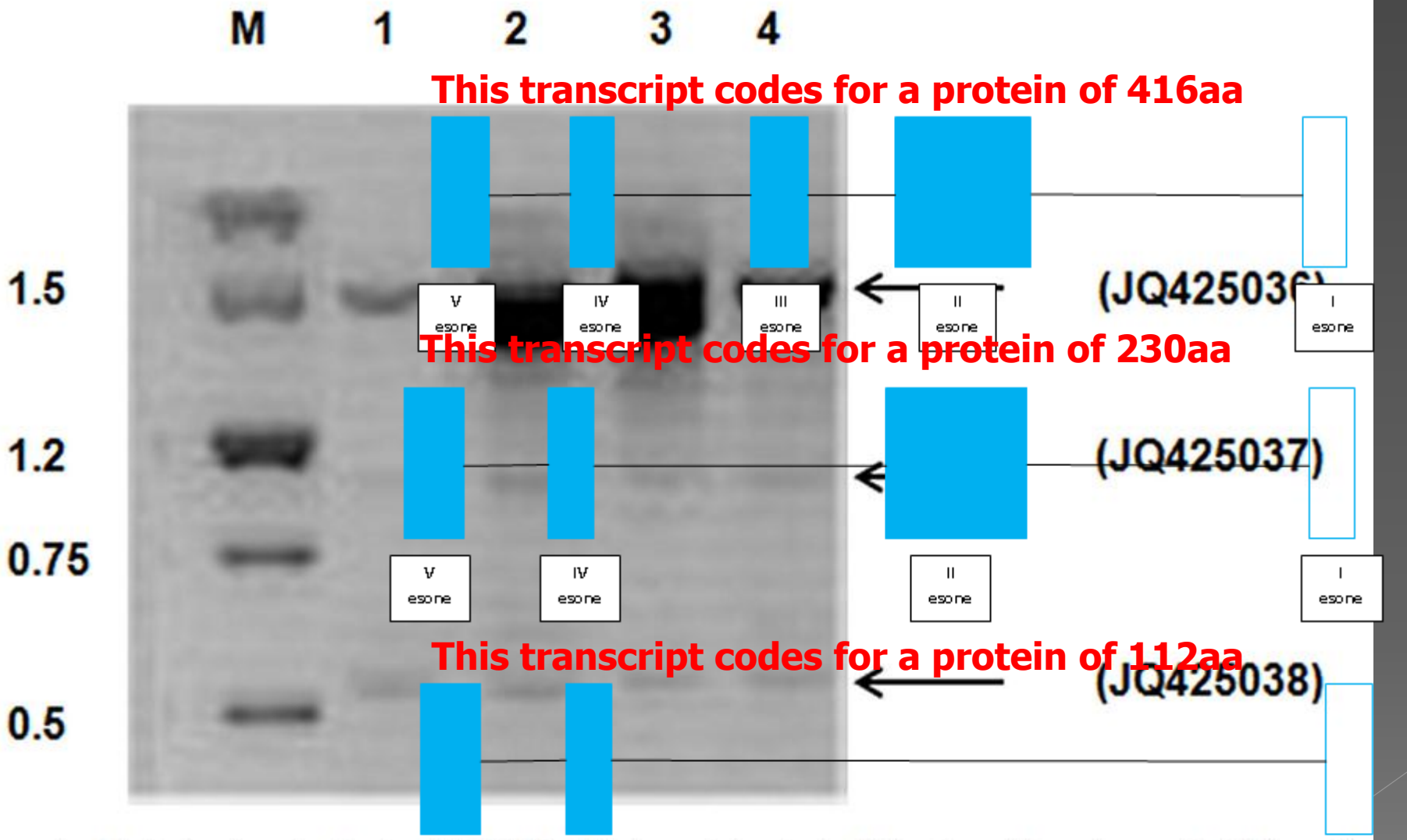


Figure 1. Gel electrophoresis of ovin *INA1* ranscript variants. Molecular weight markers on the left (lane cDNA from mammary gland Sarda (lane Gentile lia (lane 2) breeds, and cDNA from milk cells of Sarda (lane 3) Gentile di Puglia (lane 4) breeds. The three identified splicing variants are indicated by arrows on right side of pictograph.

doi: 10.1371/journal.pone.0073020.g001

Expression of SERPINA1 trasncripts in tissues

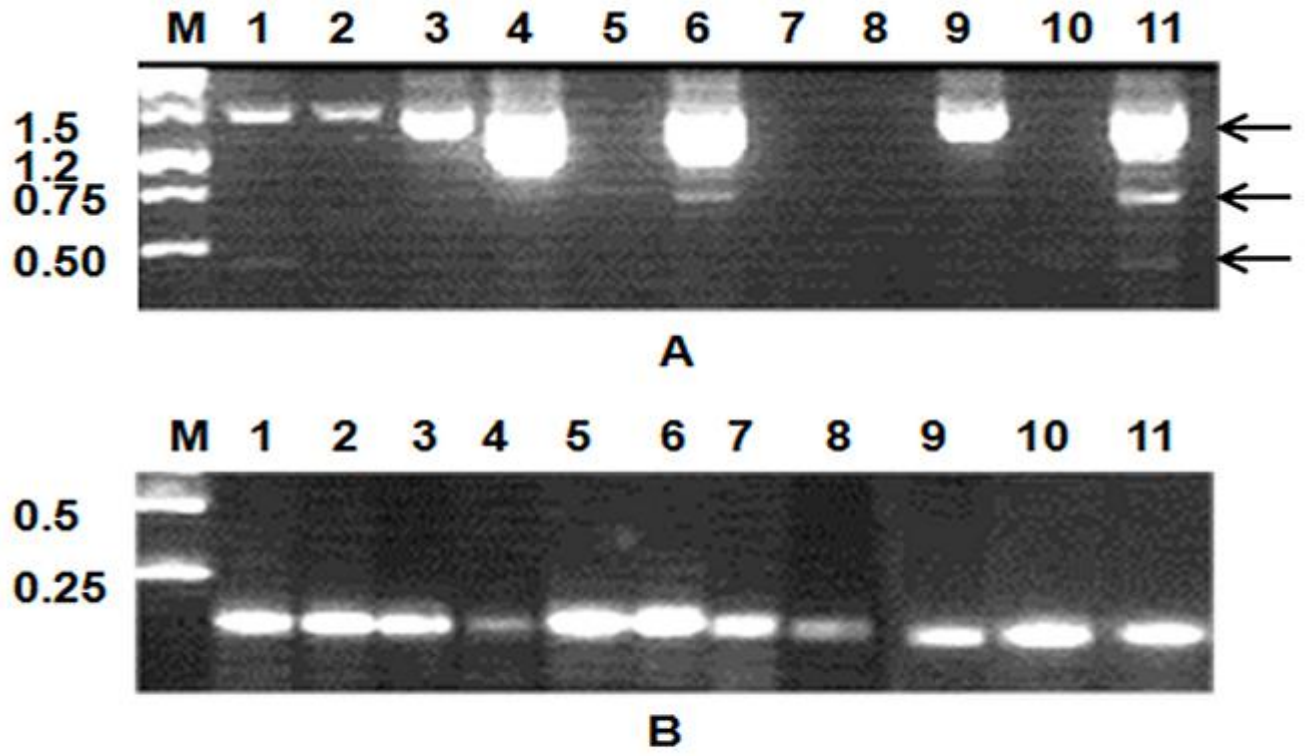
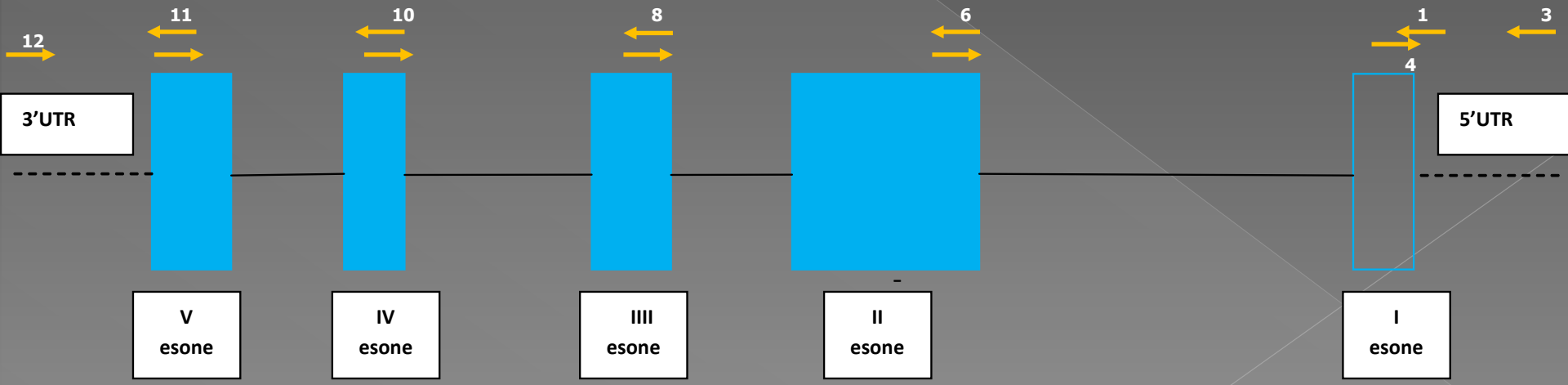


Figure 2. Representative results of *SERPINA1* gene expression in different ovine tissues. A) Expression of *SERPINA1* transcript variants (indicated by arrows on right) and B) expression of ATPB5 control gene in various tissues. Lanes represent molecular weight marker (M) spleen (1), *semitendinosus* muscle (2), longissimus dorsi muscle (3), mammary gland (4), brain (5), cerebellum (6), rumen (7), bladder (8), adrenal (9), uterus (10) and liver (11)..

doi: 10.1371/journal.pone.0073020.g002

Gene characterization

Amplicone	primers	N° primer	Lunghezza bp	Sequenziamento
Ex1-Ex5	cDNAFWD-cDNARWD	1-2	9000	no
5'UTR	5'UTRFWD-5'UTRRWD	3-4	2009	si
Ex1-Intr1-Ex2	cDNAFWD-I IntronRWD	1-5	5000	No completato
Ex2-Intr2-Ex3	II IntronFWD-II IntronRWD	6-7	1495	si
Ex3-Intr3-Ex4	III IntronFWD-III IntronRWD	8-9	1278	si
Ex4-Intr4-Ex5	IV IntronFWD-cDNARWD	10-2	1132	si
3'UTR	3'UTRFWD-3'UTRRWD	11-12	2082	si



SNP identified in SERPINA1 gene

Region	n°	synonimous	non synonimous
5'UTR	24	-	-
I exon	4	4	0
II exon	13	4	9
II intron	11	-	-
III exon	3	0	3
III intron	2	-	-
IV exon	6	2	4
IV intron	10	-	-
V exon	9	2	7
3'UTR	15	-	-
TOTAL	97	12	23

Remarks 1

- Isolation of 3 different transcripts
- Long transcript codes for a complete AAT
- Alternative splicing is not influenced by SNPs identified in intron regions
- Upstream region to II exon could influence the trasncription of SERPINA1 cDNA
- The ovine *SERPINA1 gene exon and intron organization is* similar to human and bovine
- 12 of 23 non synonimous aa change could affect AAT function
- None aa change is identified in RCL region important for inhibitory action

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Is there a differential expression in milk of SERPINA1 gene during lactation?

Materials and Methods

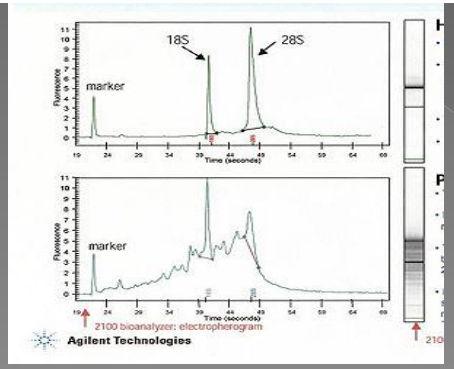
1. RNA extraction from a total of 71 milk samples 60, 90, 120 d

RNeasy Lipid Tissue Mini kit
QIAGEN

RAZZA	STADIO DELLA LATTAZIONE		
	60 GIORNI	90 GIORNI	120 GIORNI
SARDA	14	13	13
GENTILE DI PUGLIA	12	10	4

2.RNA quality check: Agilent 2100 Bioanalyzer

High quality vs degraded RNA



3. RT-PCR and qPCR

4.qPCR

Gene	Primers	nM	T.a	Amplicon cDNA/DNA
SERPINA1	5'AGGGATCACCGAGGAAACAG3' 5'GTTGCTCATTCACGTGGAAG3	300 200	58	112/900
ATP5B	5'TTTGGACTCCACGTCTCGCATC3' 5'TCCTGGAGGGATTTGTAGTCCTG3'	200 200	61	108/NO
EIF2B2	5'CCGTTCCCATTATGCTCAACTCCAG3 5'TCCGTGTCCCTTCCAGTTCCAC3'	200 100	61	81/NO
SDHA	5'ACGATTACTCCAAGCCCATCCAG3' 5'AACGTAGGAGAGCGTGTGCTTC3'	200 100	61	80/80
POLR2A	5' AATGGAAGCATGTCAATGAGGACTCTC3' 5' CACAGGCAGCACAGTGACGATC3'	100 100	61	164/164
UXT	5'TGTGGCCCTTGGATATGGTT3' 5'GGTTGTCGCTGAGCTCTGTG3'	300 300	58	101/NO
RPS9	5'CCTCGACCAAGAGCTGAAG3' 5'CCTCCAGACCTCACGTTTGTTC3'	200 200	58	54/250
MRPS15	5'GCAGCTTATGAGCAAGGTCGT3' 5'GCTCATCAGCAGATAGCGCTT3'	300 300	58	151/2100

a. Evaluation of the optimal set of reference genes

b. SERPINA expression analyses

Research

Highly accessed | Open Access

Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes

Jo Vandesompele, Katleen De Preter, Filip Pattyn, Bruce Poppe, Nadine Van Roy, Anne De Paepe and Frank Speleman*

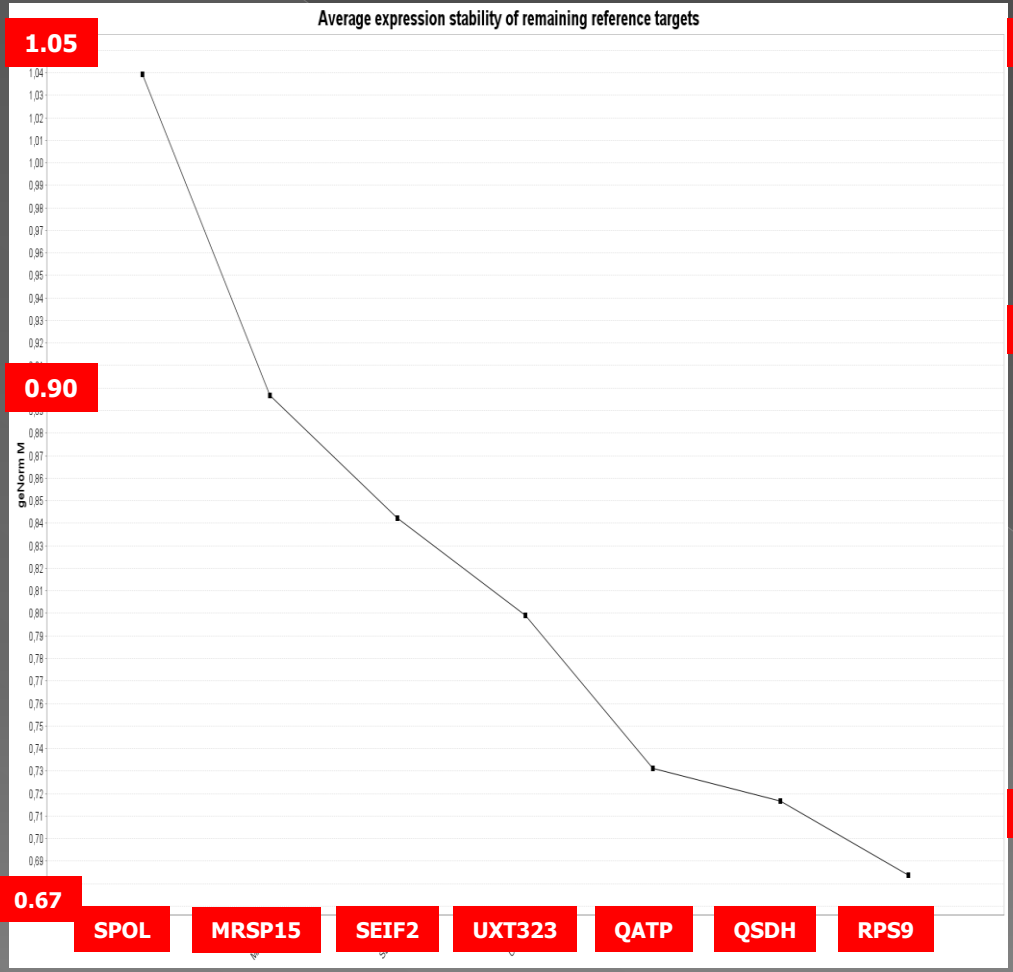
* Corresponding author: Frank Speleman franki.speleman@rug.ac.be ▾ Author Affiliations

Center for Medical Genetics, Ghent University Hospital 1K5, De Pintelaan 185, B-9000 Ghent, Belgium

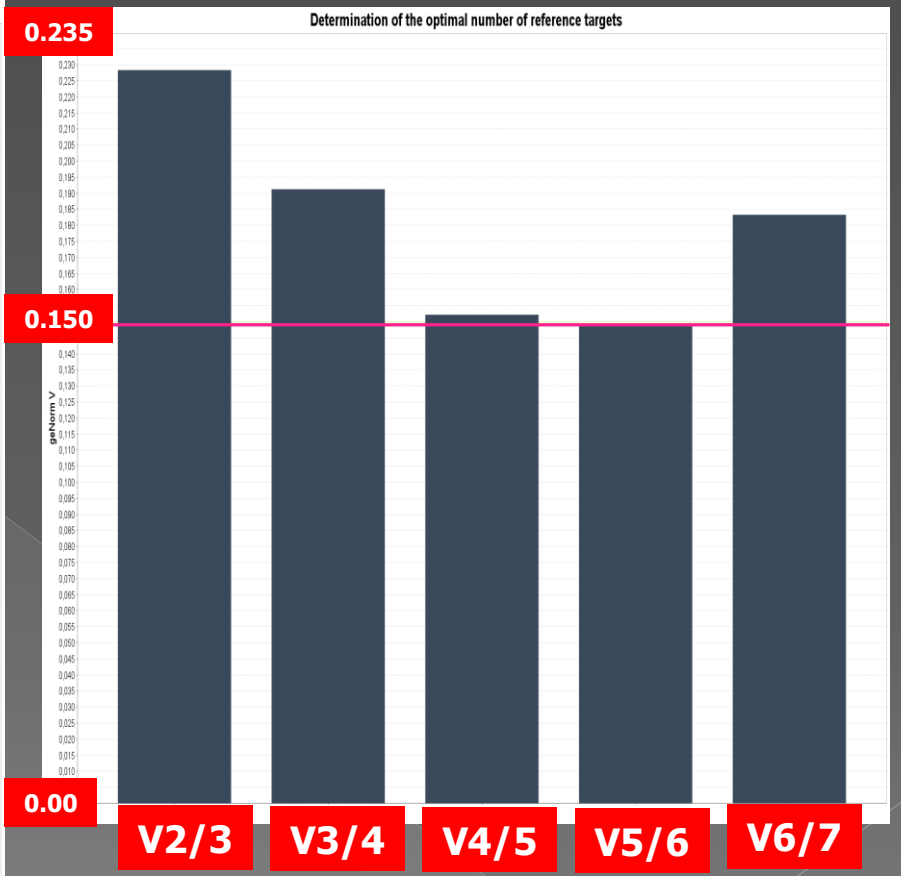
For all author emails, please log on.

Genome Biology 2002, **3**:research0034-research0034.11
doi:10.1186/gb-2002-3-7-research0034

Determination of HK more stable

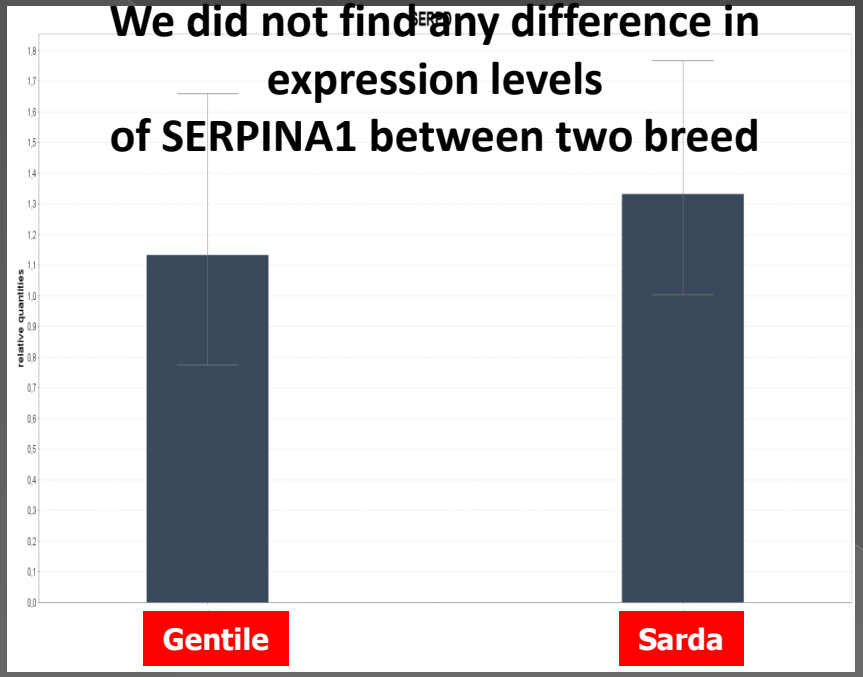


Determination of the optimal number of HK



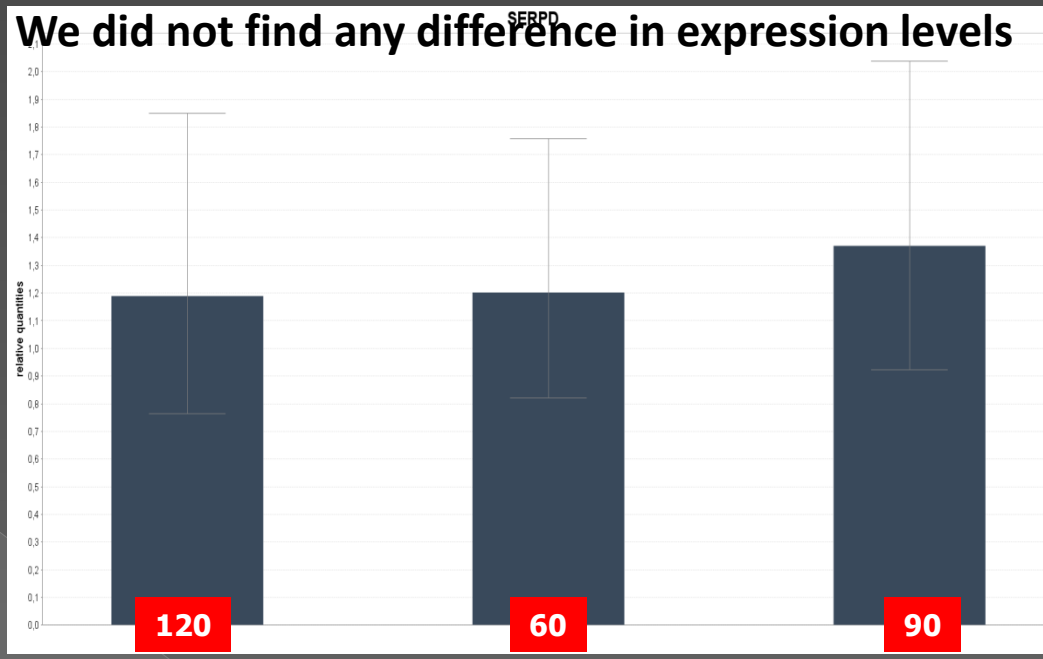
Results 1

We did not find any difference in expression levels of SERPINA1 between two breed



Results 2

We did not find any difference in expression levels



Razza		Gentile	Sarda
	N	26	40
	μ ± SE	1.64 ± 0.04	1.69 ± 0.05

Giorni		60	90	120
	N	26	23	17
	μ ± SE	1.65 ± 0.07	1.70 ± 0.04	1.64 ± 0.07

Razza		Gentile	Sarda	Gentile	Sarda	Gentile	Sarda
Giorni	N	12	14	10	13	4	13
60	μ ± SE	1.67 ± 0.08	1.63 ± 0.07				
90	μ ± SE			1.60 ± 0.07	1.80 ± 0.10		
120	μ ± SE					1.66 ± 0.04	1.63 ± 0.06

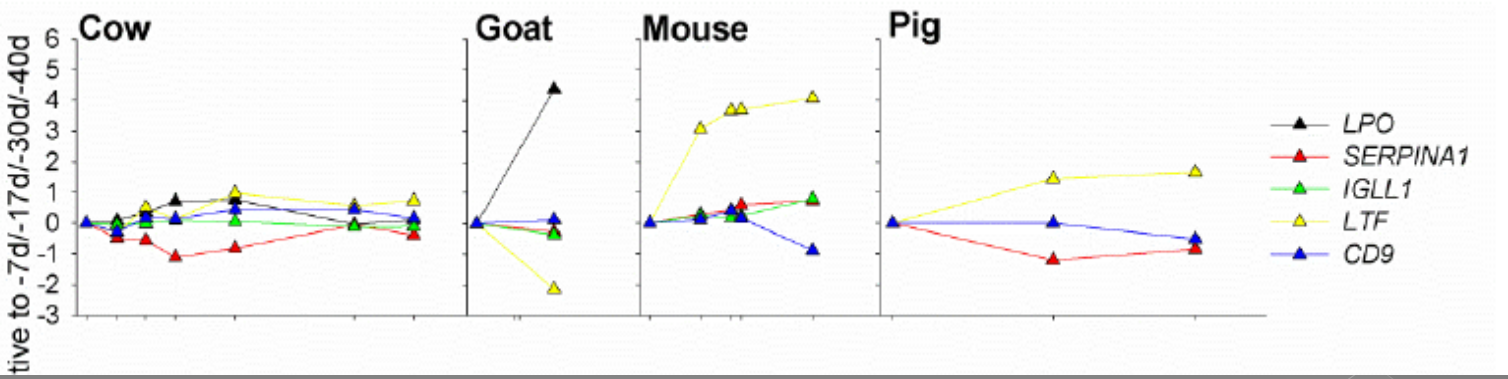
Analysis of data by a repeated measure analysis In GLM procedure of SAS software

Remarks 1

- C
- In
- V

Milk Protein Synthesis in the Lactating Mammary Gland: Insights from Transcriptomics Analyses

Massimo Bionaz, Walter Hurley and Juan Loor



Could we consider SERPINA1 a biomarker for infiammation?

Is there a differential expression of SERPINA1 in different blood cell

Immune response

Which is expression of SERPINA1 during peripartum?

Does SERPINA1

Regulate expression of other immune genes as CD62L and IL8

Acknowledgement

DESIGN and REALIZATION of experiments

Dr.ssa Alessandra Crisà

Dr. Francesco Napolitano

Dr.ssa Bianca Moiloli

Dr.ssa Elisa Mostarda

Sig. Francesco Grandoni

Care and management of animal
CRA-ZOE staff

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