




Gelsolin expression in sheep mammary gland





Francesco Napolitano^a, Giovanni Annicchiarico^b, Gennaro Catillo^a, Francesco Grandoni^a, Bianca Moioli^a,
Alessandra Crisà^a and Cinzia Marchitelli^a

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^a Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - CRA-PCM, Animal Production Research Centre, Monterotondo, Italy;

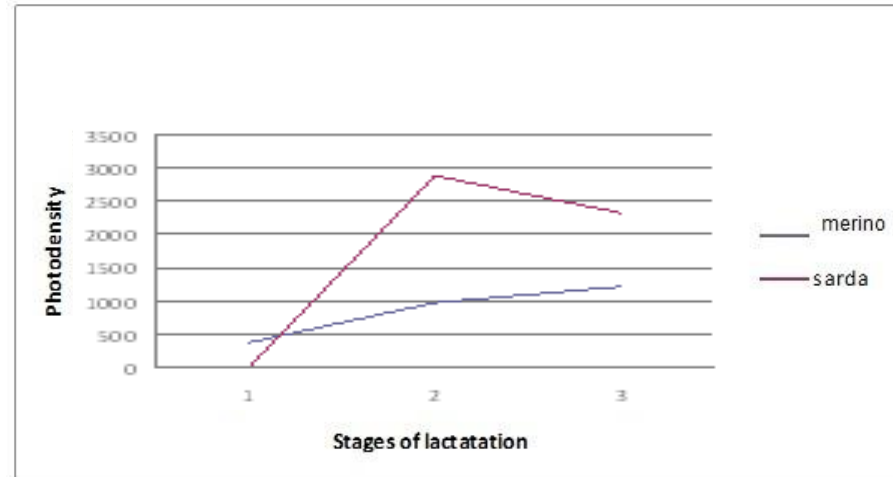
^b Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - CRA-ZOE, Research Unit for Non-intensive Animal Husbandry, Foggia, Italy.



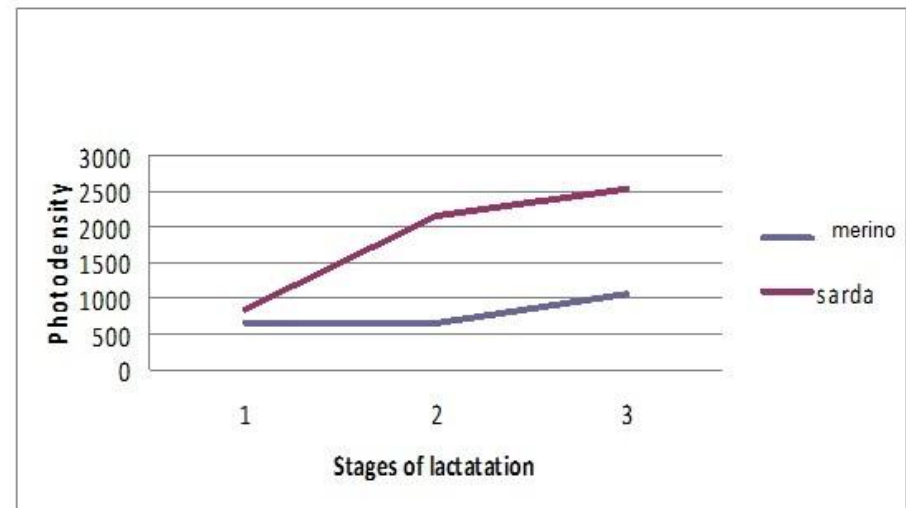
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- The group of proteomics in the project GENZOOT had tried to produce a proteomic map of the sheep mammary gland, reflecting the protein variation occurring during lactation, with the purpose to identify the responsible mechanisms for lactation performances.
 - We had chosen:
 - two sheep breeds: Sarda (Milk-type) and Gentile (Merino-type)
 - three representative stages of lactation:
 - early (one week after lambing)
 - mid (weaning of the lamb)
 - late (before the dry-off)


Proteins differentially expressed during lactation in dairy sheep

Serpin A1




Gelsolin isoform b





In mammalian three main Gelsolin *Isoforms* have been characterized:

- Plasma Gelsolin or *Isoform a*, which is a 782-aminoacid secretory protein;
 - Cytoplasmic Gelsolin or *Isoform b*, which is a 731-amino acid cytosolic protein;
 - Gelsolin-3 or *Isoform c*, which is a 742-amino acid protein localized primarily in the central nervous system.
- 

All *Isoforms* arise by alternative splicing from the same gene.

Ovis aries gelsolin isoform b mRNA, complete cds

GenBank: JF412292.1

LOCUS JF412292 2373 bp mRNA linear MAM 05-APR-2011

DEFINITION Ovis aries gelsolin isoform b (GSN) mRNA, complete cds.

VERSION JF412292.1 GI:327346103

SOURCE Ovis aries (sheep)

ORGANISM *Ovis aries*

REFERENCE bases 1 to 2373

AUTHORS Grandoni,F., Moioli,B. and Napolitano,F.

TITLE Identification of the gelsolin transcript variant 2 from sheep milk

JOURNAL Submitted (18-FEB-2011) C.R.A., Animal Production Research Centre, Via Salaria, 31, Monterotondo, Roma 00015, Italy

Ovis aries gelsolin isoform b gene, exons 12 and 13

GenBank: JX914593.1

LOCUS JX914593 2381 bp DNA linear MAM 19-NOV-2012

DEFINITION Ovis aries gelsolin isoform b (GSN) gene, exons 12, 13 and partial cds.

VERSION JX914593.1 GI:414148022

SOURCE Ovis aries (sheep)

ORGANISM Ovis aries

REFERENCE bases 1 to 2381

AUTHORS Grandoni,F., Moioli,B. and Napolitano,F.

JOURNAL Submitted (10-OCT-2012) C.R.A., Animal Production Research Centre, via Salaria 31, Monterotondo, Roma 00015, Italy

SNPs detected on DNA of the *GSN* gene relative to the JX914593 sequence.

SNP name	Position in accession	Location	AA change
g. 524	g. 524 A>G	intron 11	
g. 566	g. 566 C>T	exon 12	
g. 602	g. 602 C>T	exon 12	
g. 772	g. 772 C>G	intron 12	
g. 2003	g. 2003 C>T	intron 12	
g. 2060	g. 2060 A>G	intron 12	
g. 2103	g. 2103 G>A	intron 12	
g. 2116	g. 2116 A>G	intron 12	
g. 2217	g. 2217 T>C	exon 13	



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Identification of *Ovis aries* Gelsolin isoform b, a candidate gene for milk quality



Francesco Napolitano^{a,*}, Giovanni Annicchiarico^b, Gennaro Catillo^a,
Alessandra Crisà^a, Francesco Grandoni^a, Cinzia Marchitelli^a, Bianca Moio^{li}^a

^a Consiglio per la Ricerca e la sperimentazione in Agricoltura – CRA-PCM, Animal Production Research Centre, via Salaria 31, 00015 Monterotondo, Italy

^b Consiglio per la Ricerca e la sperimentazione in Agricoltura – CRA-ZOE, Research Unit for Intensive Wood Production, via Napoli km 12, 71020 Foggia, Italy

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ABSTRACT

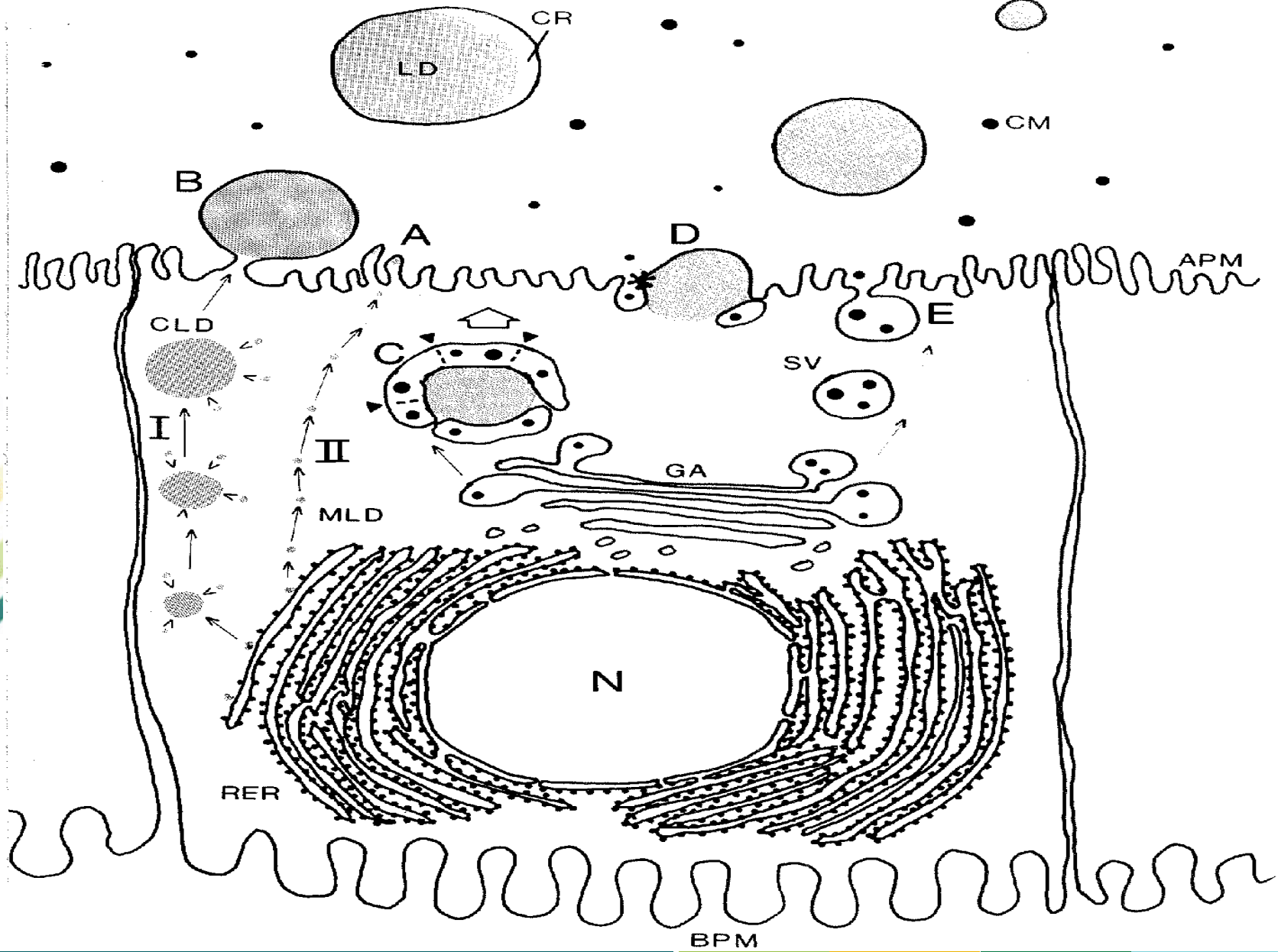
In this report, we describe the identification of the complete ovine gelsolin (*GSN*) isoform b cDNA. We sequenced exons 12 and 13 and parts of introns 11, 12 and 13 in 263 sheep of three breeds. We found 11 novel SNPs, of which 2 were missense mutations. *GSN* is a Ca²⁺-dependent actin-regulatory protein that modulates actin assembly and disassembly. Because actin is involved in the regulation of intracellular lipid metabolism, particularly in the formation of cytoplasmic fat droplets and in the transport of lipid constituents, we tested the hypothesis that different *GSN* genotypes might influence actin activity and, consequently, milk yield and quality. An association analysis was performed between each SNP and the following traits, which were evaluated in 464 recorded lactations of the same sheep: milk yield, fat and protein content, as well as somatic cell score. Significant associations ($P=0.02$) were found between three SNPs in intron 12 and fat content in the Altamura breed. For two of the SNPs, the effect was also noted in the Gentile breed ($P=0.09$). Suggestive associations were also found between the SNPs and other traits, albeit not in all of the breeds. The three SNPs that affected fat content were located in the consensus sequences of binding sites for the following transcription factors: Tal-1 alpha, Evi-1 and Egr-3. Therefore, we hypothesised that the incorrect modulation of actin assembly occurs in one of the *GSN* genotypes, with a particularly evident effect on fat content.


Estimated means, frequency of genotypes, Minor Allele Frequency and significant substitution allelic effect of the *gelsolin*'s SNPs on milk traits.

					Trait							
					N - Obs	Lactation d	Milk gr	Fat %	Protein %	Somatic cell 10 ³		
Breed					$\mu \pm SE$	$\mu \pm SE$	$\mu \pm SE$	$\mu \pm SE$	$\mu \pm SE$	$\mu \pm SE$		
Altamura					155 - 266	143 ± 4 ^b	47,438 ± 1,981 ^b	8.37 ± 0.09 ^b	6.03 ± 0.05 ^b	1,108.5 ± 142.2 ^a		
Gentile					62 - 111	111 ± 5 ^c	36,904 ± 2,638 ^c	9.12 ± 0.12 ^a	6.47 ± 0.07 ^a	640.0 ± 189.4 ^b		
Sarda					46 - 87	239 ± 6 ^a	110,289 ± 3,376 ^a	8.09 ± 0.18 ^b	5.83 ± 0.10 ^b	1,200.7 ± 242.4 ^{ab}		

SNP	Genotype			Breed	MAF, %	Substitution allelic effect / P-value								
g. 2003 C>T	95	CC	52	CT	7	TT	Altamura	21	- 0.23	0.02			303.4	0.08
	58	CC	4	CT	0	TT	Gentile	3	- 0.59	0.09				
	26	CC	20	CT	0	TT	Sarda	22						
g. 2060 A>G	94	AA	53	AG	7	GG	Altamura	22	- 0.23	0.02			303.7	0.08
	58	AA	4	AG	0	GG	Gentile	3	- 0.58	0.09				
	33	AA	13	AG	0	GG	Sarda	14						
g. 2103 G>A	41	GG	81	GA	32	AA	Altamura	53	0.21	0.01				
	40	GG	19	GA	3	AA	Gentile	20			- 0.25	0.006	- 520.0	0.04
	26	GG	19	GA	1	AA	Sarda	23	- 12,968	0.005				

Within a column means without a common superscript differ ($P < 0.01$).





What guides this apical migration is not known with certainty but it is probable that cytoskeleton elements are involved.

Actin has been localized, by immunofluorescence microscopy (Franke et al., 1980; Amato e Loizzi, 1981), to the cytoplasmic surface along basal regions of budding lipid droplets.



Actin has been identified rather recently (Heid and Keenan, 2005) as a constituent of the

Milk Fat Globule Membrane

and is an active participant in the

Milk Fat Secretory Process.





There are many proteins associated with Actin filaments:

- proteins that inhibit the polymerization of the filaments
- others that cut the filaments
- still others that cover them as a cap to prevent the growth.



Gelsolin is a Actin filament severing and capping protein that is implicated in Actin remodelling.

In response to increased Ca^{2+} and H^+ levels, Gelsolin is stimulated to sever Actin filaments after which it remains attached to the barbed end of the filament as a cap.



dislocation d'un filament d'actine par la gelsoline

ARP2/3

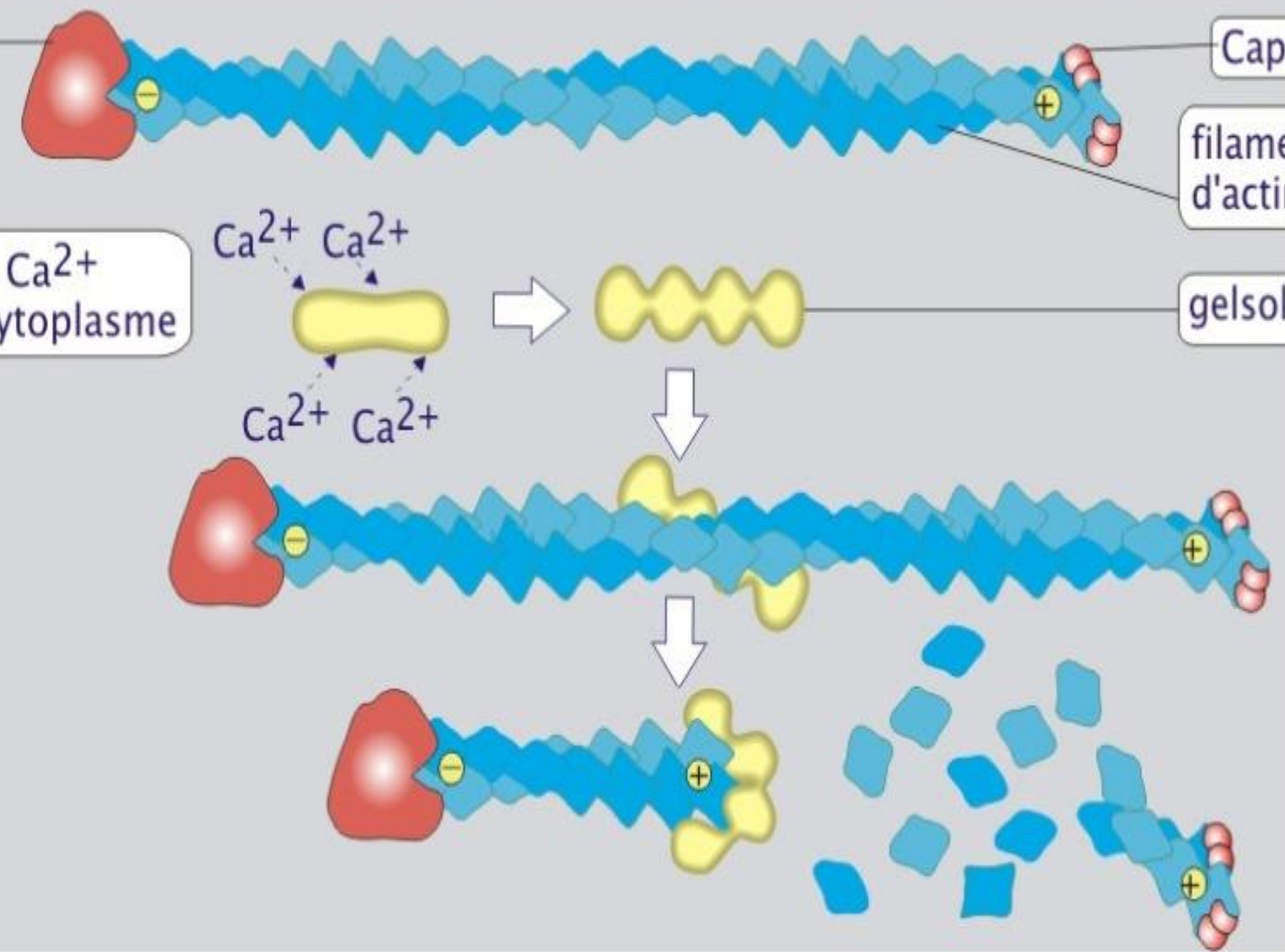
CapZ


filament d'actine

gelsoline

afflux de Ca^{2+}
dans le cytoplasme

Ca^{2+} Ca^{2+}
 Ca^{2+} Ca^{2+}





To estimate the level of expression of the “Gelsolin isoform b” in sheep’s milk, a total of 26 ewes of two breeds (12 Gentile and 14 Sarda) were studied at the Foggia Experimental Station of the CREA.



Three times during lactation (60, 90 and 120 days) individual milk samples were collected for RNA extraction.

Fat, protein, lactose contents and somatic cell count were detected in milk samples.

Estimated means of the level of expression of the 'Gelsolin isoform b' in sheep's milk.

Breed		Gentile	Sarda	
	N	26	40	
	$\mu \pm SE$	1.58 ± 0.07^b	1.91 ± 0.05^a	


Time of control		60 d	90 d	120 d
	N	26	23	17
	$\mu \pm SE$	1.53 ± 0.06^b	1.85 ± 0.06^a	1.86 ± 0.09^a

Within a row without a common subscript differ ($P < 0.001$).


Pearson's correlation coefficients between the traits of milk and the level of expression (RNA) of the 'Gelsolin isoform b'.

Trait	<u>Lactation</u> d	<u>Milk</u> gr	<u>Fat</u> %	<u>Protein</u> %	<u>Lactose</u> %	<u>Somatic cell</u> 10^3
RNA	0.37	0.18	-0.66*	-0.14	-0.39	0.56*

*: $P < 0.05$




Certain cell types, such as polymorphonuclear leukocytes, monocyte/macrophages and metastatic cells, are able to move rapidly through tissues. These movements are mediated by the Actin cytoskeleton (Goldberg M.B., 2001).



Because of its ability to bind several inflammation inducers, plasma Gelsolin isoform might represent a promising approach to prevention of inflammation and secondary injuries. (Osborn T.M. et al., 2007)





Already in 1999 Jevon et al. proposed a mechanism of internalization of *Staphylococcus aureus* occurs via a receptor-mediated pathway, requiring the participation of cytoskeletal elements, principally Actin.



Subsequently, the data obtained with kidney epithelial cells and fibroblasts showed that *S. aureus* internalization requires polymerization of the Actin cytoskeleton. Oviedo-Boyso et al., 2011.



The host cell cytoskeleton plays a key role in the life cycle of viral pathogens.

Garcia-Esposito et al., 2013

propose the Gelsolin as a new factor that can limit HIV-1 infection.



Our findings,
along with recent research in the biomedical field,
may suggest to use of **Gelsolin** as a possible topic for study issues
related to infection and immunity.

Thanks for your attention

