



Università degli Studi di Perugia  
Dipartimento di Chimica, Biologia e Biotecnologie  
Corso di Laurea Triennale in Biotecnologie

Tesi di laurea  
**I microRNA sierici come marker di gravidanza precoce e  
benessere fetale nel bufalo mediterraneo**

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Anno Accademico 2017-2018



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# Presentazione dell'output di ricerca in formato Smart Poster

DEPARTAMENTO DI CHIMICA, BIOLOGIA E BIOTECNOLOGIE UNIVERSITÀ DEGLI STUDI DI PERUGIA  
TESI TRIENNALE IN BIOTECNOLOGIE - ANNO ACCADEMICO 2017 / 2018

## SERUM MICRORNAs IN BUFFALO COWS: POTENTIAL BIOMARKERS OF PREGNANCY

**Abstract:** In recent decades, interest in buffalo breeding has largely increased worldwide due to focusing the attention on this species as a dairy species instead. Problems related to long calving intervals, late puberty and seasonal anestrus hamper reproductive efficiency in this species. Early pregnancy diagnosis is important to shorten the calving interval and increase lifetime production on dairy animals. MicroRNAs have recently emerged as key molecules in facilitation of various species even though in buffalo, few previous studies have investigated miRNAs. The aim of this research was to identify the best miRNAs indicators in serum among miR-101, miR-205b, miR200c, and miR200b4. Consequently, assess the expression levels of miR-101, miR-200b, miR-201a, miR-205b, miR-171, miR-201 and miR-202 involved in buffalo pregnancy-maturation events and pregnancy. Interestingly, we found that all the miRNAs analyzed at 45 days after artificial insemination were differentially expressed among pregnant and non-pregnant buffaloes.

**TESTATA**  
Michela Caporin

**RELATORE**  
di Daniela Zampini

**COORDINATORE**  
di Daniela Zampini  
di Paola Cappuccini

**BACKGROUND**  
Reproductive efficiency is the primary factor affecting productivity. Failure to become pregnant leads to a negative impact on reproductive performances in the dairy industry and causes important economic losses ...

**Animation - MicroRNAs** (by Katerina Panek)  
*Bit.ly/ser-michela-caporin2018*  
Biogenesis and functions of microRNAs within the cell. MicroRNAs are small RNA molecules that regulate gene expression and have a big impact on many biological processes.

**EXPERIMENTAL PROCEDURE**  
Animal selection and study design. Fifteen lactating Italian Mediterranean buffalo cows were included in the present study ...  
Serum sample collection and preparation. Blood was withdrawn at D0, D15 and D40 from AI and C buffaloes ...  
Computational prediction of miRNAs and pathway enrichment analysis. Kyoto Encyclopedia of Genes and Genomes was used to identify the genes involved in buffalo pregnancy-related oocyte maturation ...  
Total RNA isolation, reverse transcription, and qPCR amplification. Total RNA, including miRNAs, was extracted ...

**RESULTS**  
Analysis of stability of candidate reference miRNAs ...  
MicroRNA detection and differential expression analysis  
A D15 miR-452, miR-452-5p, miR-200b are differentially expressed between P and NP groups. While a D40 in P groups all miRNAs are upregulated, differentially expressed between P and NP groups. While a D40 in P groups all miRNAs are upregulated ...

**DISCUSSION**  
Data suggest that there could exist a relationship between miRNAs investigated and pregnancy outcome in buffaloes. In conclusion, this study may be considered a first step for gaining further insight into miRNAs biological function in buffalo reproduction. However, miRNA-mediated regulation of the various transcripts in pregnancy is not entirely clear and further investigation is required to explore the functional implications of miRNAs in a successful pregnancy.

- Ampia condivisione / diffusione (via email e snail-mail)
- Accesso tramite smartphone / tablet
- Esposizione ad alti numeri di fruitori in luoghi fisici diversi dalla Rete
- Utilizzazione come allegato a riviste e journal sia cartacei che elettronici
- Aggiornamento dei contenuti dalla Rete in modo continuo
- Facile archiviazione e miglioramento ergonomico per l'utilizzatore
- Impiego come materiale pubblicitario per l'attività scientifica e didattica
- Utilizzazione come arredo (aule / laboratorio/ufficio)

# Limitazioni nella performance riproduttiva

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- Raggiungimento ritardato della pubertà
  - Stagionalità della riproduzione
  - Scarsa manifestazione dell'estro
  - Ridotta fertilità
  - Bassi tassi di concepimento
  - Perdita di gravidanze
-

# L'importanza della diagnosi di gravidanza

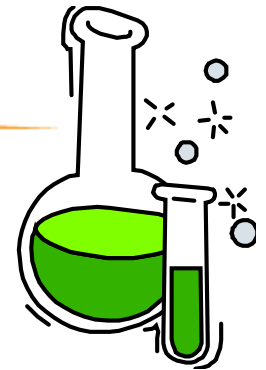
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- ❑ Evitare una gestazione a rischio
  - ❑ Ridurre l'interparto
  - ❑ Adeguamento del regime alimentare
  - ❑ Aspetto economico:
    - crescente interesse nell'allevamento del bufalo
    - protocollo fecondativo con basso tasso di mortalità embrionale
-

# Espressione dei microRNA circolanti: procedura sperimentale

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- Selezione dei microRNA
  - Selezione del campione oggetto di studio
  - Estrazione dell'RNA e quantificazione dei microRNA
  - Retrotrascrizione
  - qPCR
  - Scelta del migliore reference gene
  - Analisi dei dati di qPCR
-

# Selezione dei microRNA circolanti

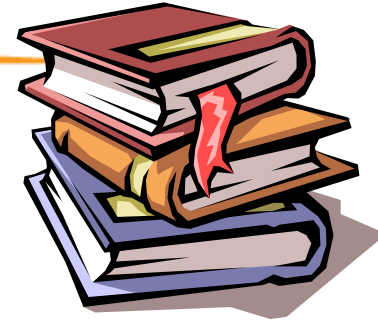
## Scelta del miglior Reference Gene

miR 191

miR 25-3p

SNORD44

SNORD48



## Livelli di espressione

miR 103

miR 200b

miR 301a

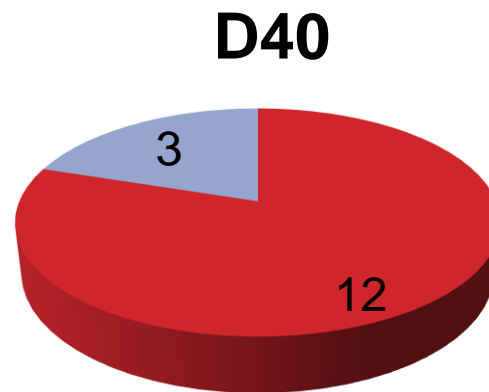
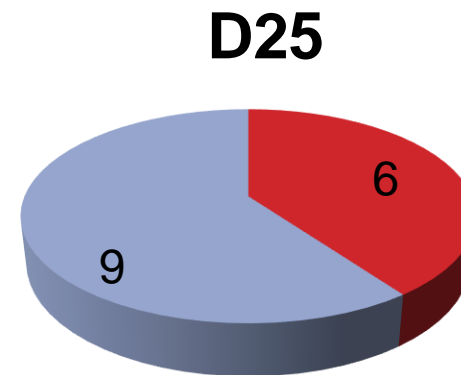
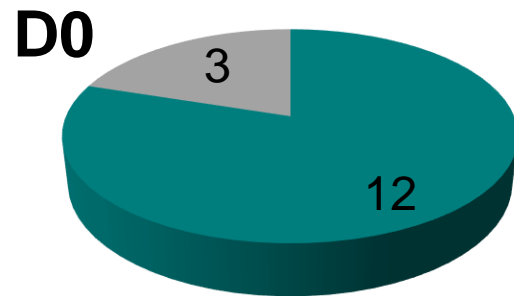
miR 423-5p





miR 375

miR 451

miR 452

# Selezione del campione



Bufale inseminate   
Controllo   
Gravide   
Non gravide 

# Selezione dei microRNA circolanti

**KEGG GENOME**

Collection of KEGG organisms which are the organisms with known complete genome sequence.

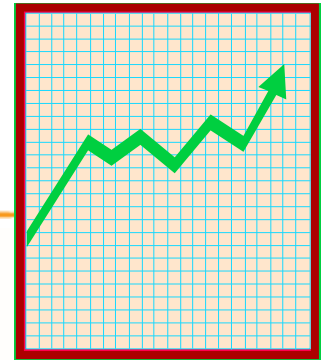
Category	Genome	Identifiers	Gene catalog	Annotation
Organisms	Complete genomes	T0 numbers / three- or four-letter organism codes	GENES	KDALA
	Draft genomes	T1 numbers	DGENES	KAAS
	EST datasets	T2 numbers	EGENES	KAAS
Metagenomes	MGENOME	T3 numbers	MGENES	KAAS
Pangenomes		Genetic species names	GENES	KDALA
Viruses	VGENOME	RefSeq identifiers	VGENES	None

miR 103  
miR 200b  
miR 301a  
miR 423-5p  
miR 375  
miR 451  
miR 452

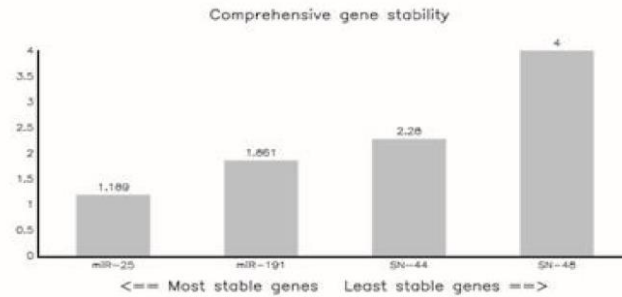




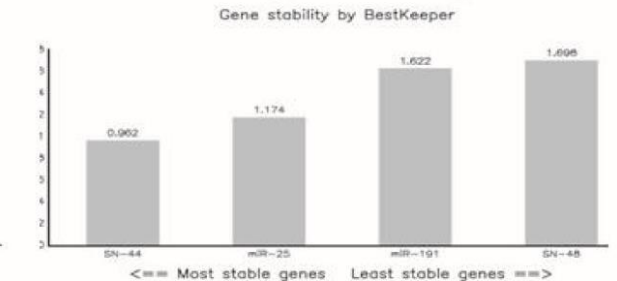
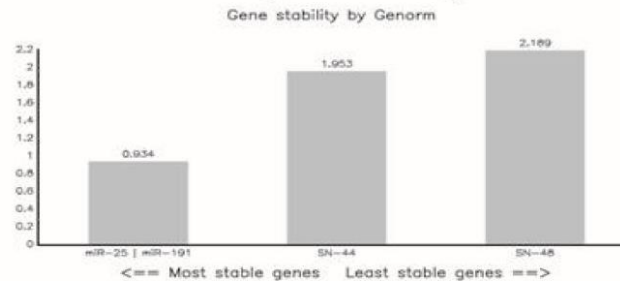
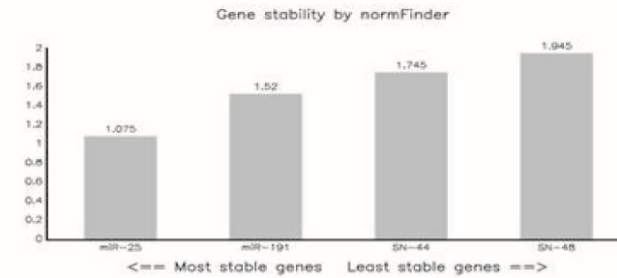
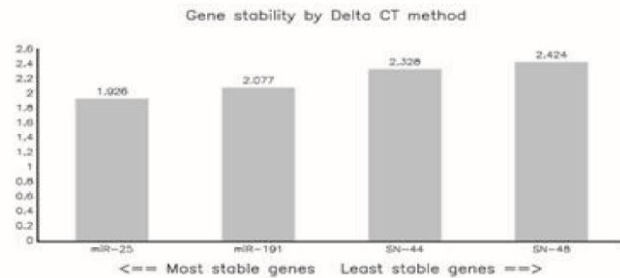
# Risultati: scelta del reference gene



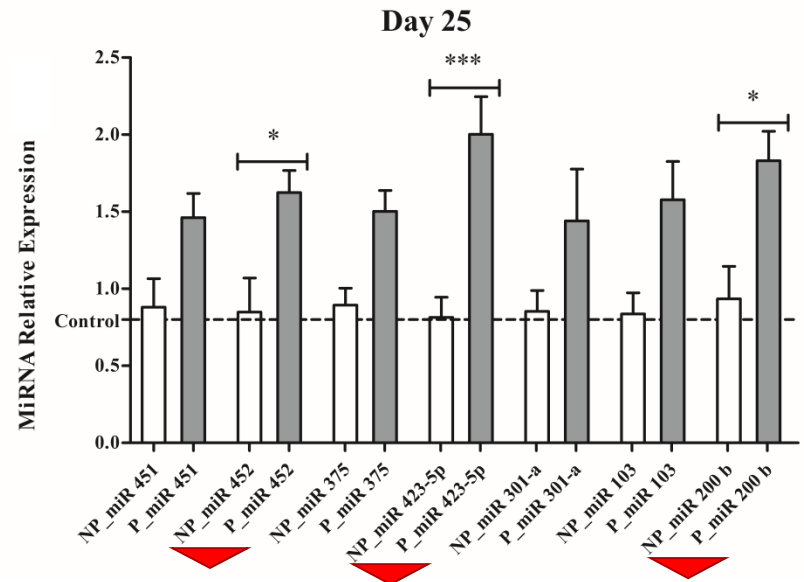
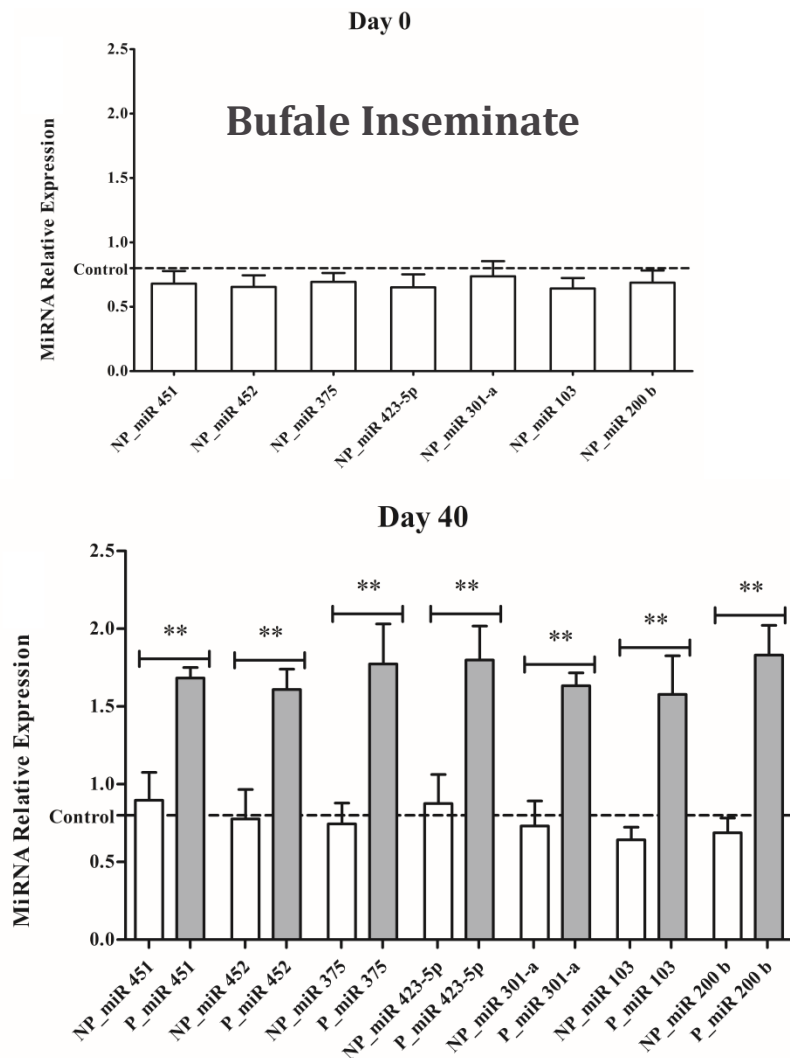
## Stability values of candidate reference miRNAs



Genes Geomean of ranking values  
miR-25 1.19  
miR-191 1.86  
SN-44 2.28  
SN-48 4.00



# Risultati: livelli di espressione dei microRNA circolanti

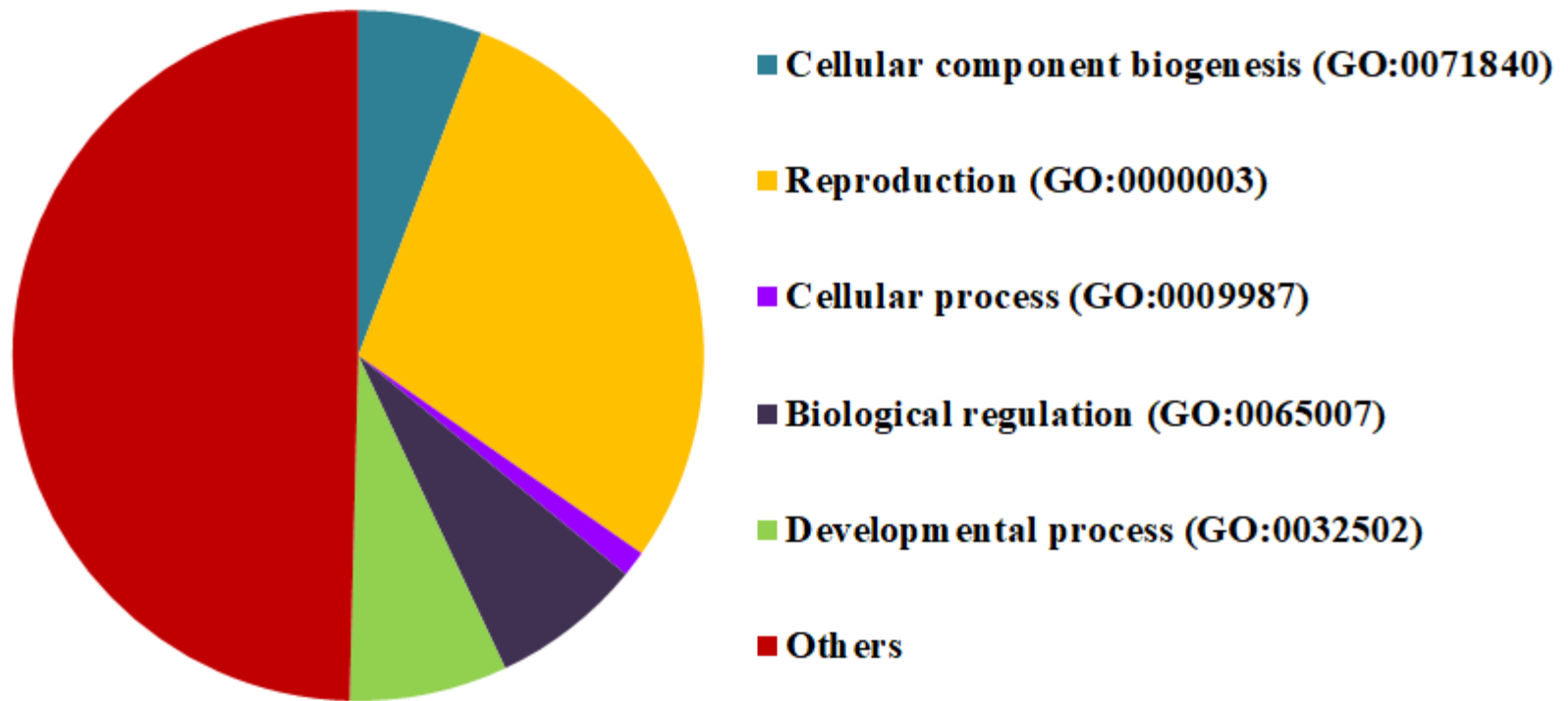


□ Bufale non gravide

■ Bufale gravide

# D25 Functional GO Categories (Panther analysis)

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# Conclusioni

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I risultati della ricerca confermano che

**miR 452, miR 423-5p e miR 200b**

differentemente espressi al **D25** possono essere considerati biomarcatori precoci di gravidanza nel bufalo.

Caratterizzare marcatori precoci di gravidanza non fornisce solamente un supporto clinico, ma potrebbe anche indicare un bersaglio terapeutico



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