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MILK MICRORNA COMPOSITION DEPENDS ON DAIRY COW BREED

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CONTEXT

Mother's reflect

Direct link between microRNAs expression in the mammary gland and their level in milk
Le Guillou *et al.*, 2012; Laubier *et al.*, 2015; Alsaweed *et al.*, 2016

Healthy food

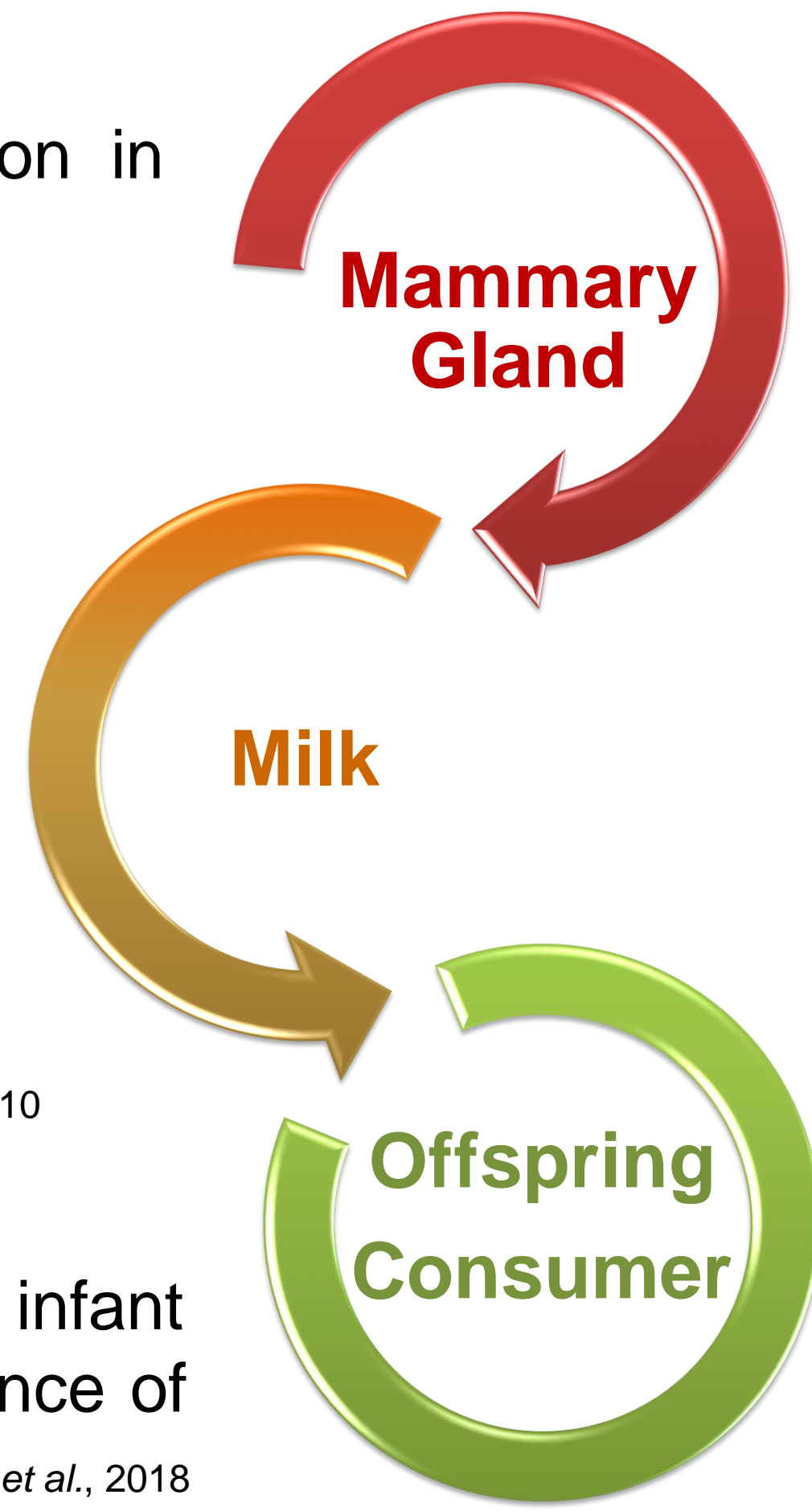
Milk is a source of nutrients for neonate and adult
Milk composition influences consumer health in both short and long terms
Kongerslev Thorning *et al.*, 2016; Mosca *et al.*, 2017

microRNAs

Affect functions such as immunity, growth, development or cell proliferation
Kosaka *et al.*, 2010
Present in large quantities in milk
Weber *et al.*, 2010

Impact

microRNAs are transmitted from mother to infant
Potential action across species and influence of milk effects on consumer health
Title *et al.*, 2015; Manca *et al.*, 2018



Factors for microRNA composition variation

- Nutrition
- Breeds
- Lactation stage

Does milk microRNA composition (miRNome) vary according to the genetic background?

Comparison of milk miRNomes in Normande vs. Holstein dairy cows

EXPERIMENTAL MATERIAL

Primiparous cows raised together in the experimental farm



Normande (N)
n = 8



Two breeds with contrasted lactation performances

Dairy milk production over 5 weeks (kg/day)

N: 18,6
H: 23,3
p = 0.006

Protein content (g/kg)

N: 33,1
H: 31,4
p = 0.102

Fat content (g/kg)

N: 39,1
H: 35,9
p = 0.093

Urea (mg/cl)

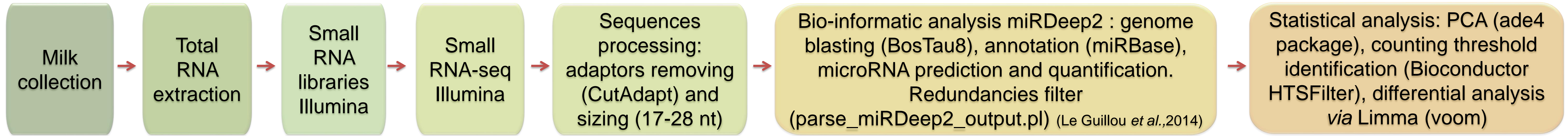
N: 118,8
H: 156,3
p = 0.063



Holstein (H)
n = 9

Mann & Whitney analysis

METHODS



RESULTS

The list of microRNAs in milk is common to both breeds

with variable abundancies, and consists of 957 annotated and 1,422 predicted microRNAs

Major milk microRNAs are the same for both breeds

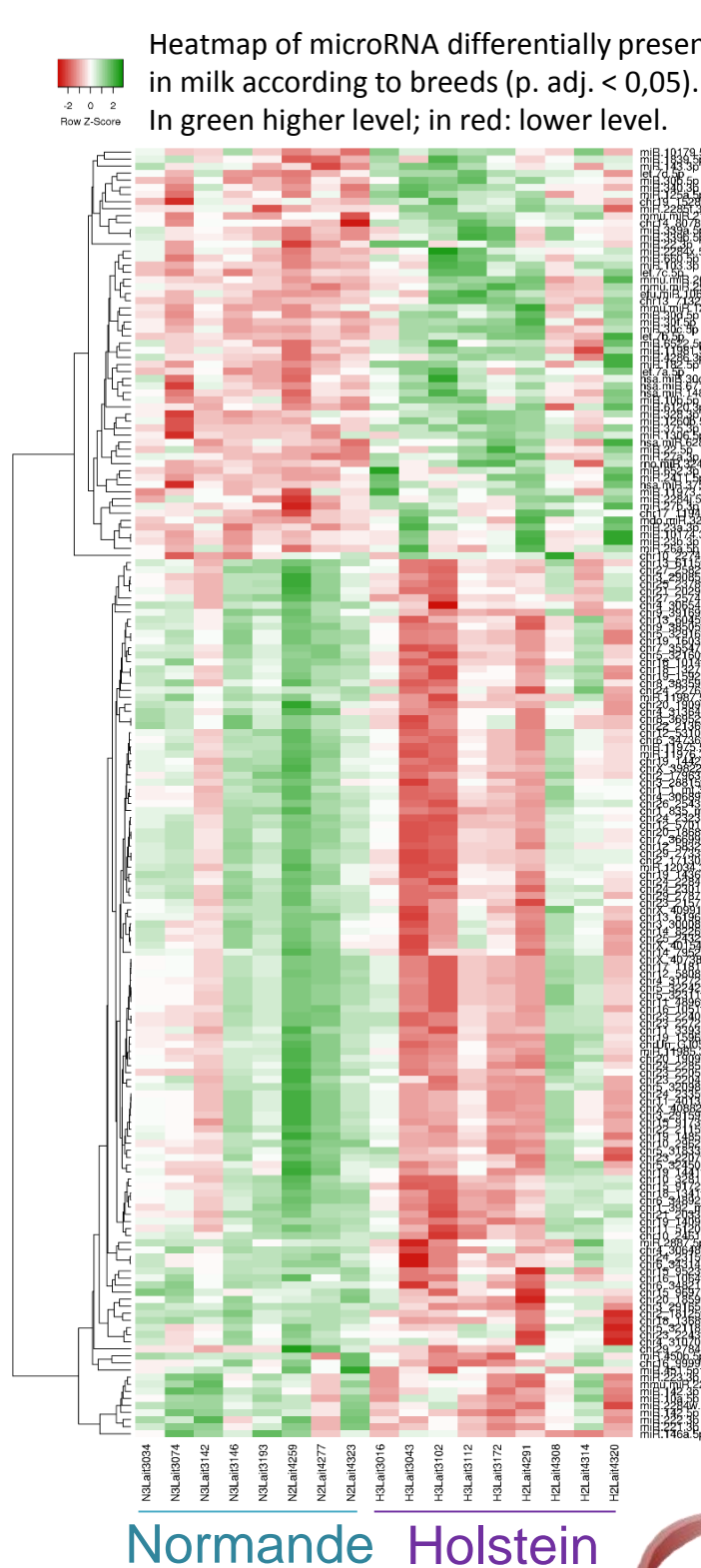
microRNA abundance in Holstein (H) and Normande (N) milk

microRNA	% of reads	Rank	% of reads	Rank
<i>bta-miR-148a-3p</i>	19.4%	1	19.8%	1
<i>bta-miR-30a-5p</i>	14.4%	2	16.4%	2
<i>bta-miR-22-3p</i>	8.1%	3	7.8%	3
<i>bta-miR-26a-5p</i>	4.5%	4	4.2%	4
<i>bta-miR-27b-3p</i>	3.9%	5	3.3%	7
<i>bta-miR-186-5p</i>	3.5%	6	3.1%	9
<i>bta-miR-21-5p</i>	3.2%	7	3.7%	6
<i>bta-miR-181a-5p</i>	3.0%	8	3.1%	8
<i>bta-miR-141-3p</i>	3.0%	9	4.1%	5
<i>bta-let-7a-5p</i>	2.5%	10	2.1%	11
<i>bta-chr10_2883_mt-5p</i>	2.3%	11	2.3%	10
<i>bta-miR-30d-5p</i>	2.2%	12	1.7%	12
<i>bta-chr20_18762_mt-3p</i>	2.1%	13	1.3%	15
<i>bta-miR-92a-3p</i>	1.6%	14	1.4%	13
<i>bta-miR-191-5p</i>	1.6%	15	1.4%	14
<i>bta-miR-375-3p</i>	1.6%	16	0.6%	20
<i>bta-let-7f-5p</i>	1.5%	17	1.3%	16
<i>bta-miR-660-5p</i>	1.2%	18	0.9%	18

> 40 % of milk reads

Milk miRNome differs between breeds

182 milk microRNAs are significantly different between breeds (p<0.05)



58 microRNAs are more abundant in Holstein milk (53 known, 5 predicted), including:

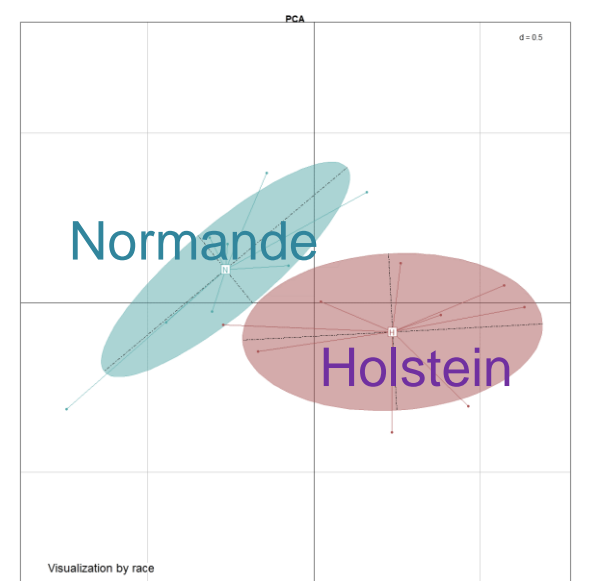
miR-26a-5p, miR-27b-3p, let-7a-5p, let-7c-5p, miR-30d-5p, miR-375-3p, and miR-660-5p

124 microRNAs are more abundant in Normande milk (17 known, 107 predicted), including:

miR-10a-5p, miR-142-3p & -5p, miR-221-3p, miR-222-3p, miR-223-3p & -5p, and miR-451-5p

Genes and pathways targeted by these microRNAs are particularly related to the regulation of milk fat synthesis and metabolism, and protein processing in endoplasmic reticulum

Principal component analysis



Comparison with bovine mammary miRNomes



Holstein cow
Le Guillou *et al.*, PLoS One 2014

12 of the major milk microRNAs (in red in the previous table), of which the 3 most significant, are also the most expressed in Holstein lactating mammary gland

4 microRNAs show a significant enrichment in milk compared to mammary gland

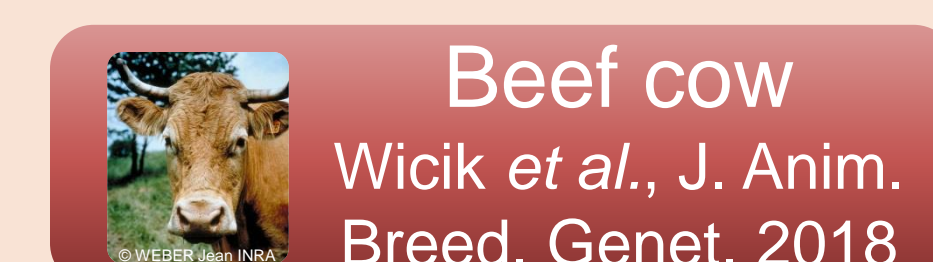
microARN	Milk Rank	Mammary Rank
<i>bta-miR-30d-5p</i>	12	48
<i>bta-miR-191-5p</i>	15	45
<i>bta-miR-375-3p</i>	16	59
<i>bta-miR-660-5p</i>	18	100

Variations according to breed in milk are also observed in mammary gland for 5 microRNAs



Dairy cow
Billa *et al.*, IMGC 2018 Poster

Between Holstein and Montbéliarde breeds: *miR-30c-5p, miR-143-3p* and *miR-340-3p*



Beef cow
Wick *et al.*, J. Anim. Breed. Genet. 2018

Between Holstein and Limousine breeds: *miR-375-3p* and *miR-2285t-3p*

CONCLUSION

This study allowed to evaluate genetic variations of the bovine milk miRNome according to the breeds, highlighting 182 microRNAs with significantly different levels between Holstein and Normande milk.

As milk is a source of nutrients and may influence health, the variation of its miRNome according to genetic variables, such as breeds, may have several impacts on consumers and thus requires particular attention.

MicroRNAs' evaluation in milk opens a field of investigation, as they can be used as biomarkers, for downstream consequences on health, physiological or metabolic status, in animals and humans.