The bovine colostrum miRNome and its potential implications for the neonate.

Ilke Van Hese – EAAP annual meeting 2019
26th August, 2019

GHENT UNIVERSITY

fwo Research Foundation Flanders
Opening new horizons

ILVO

What is Colostrum?

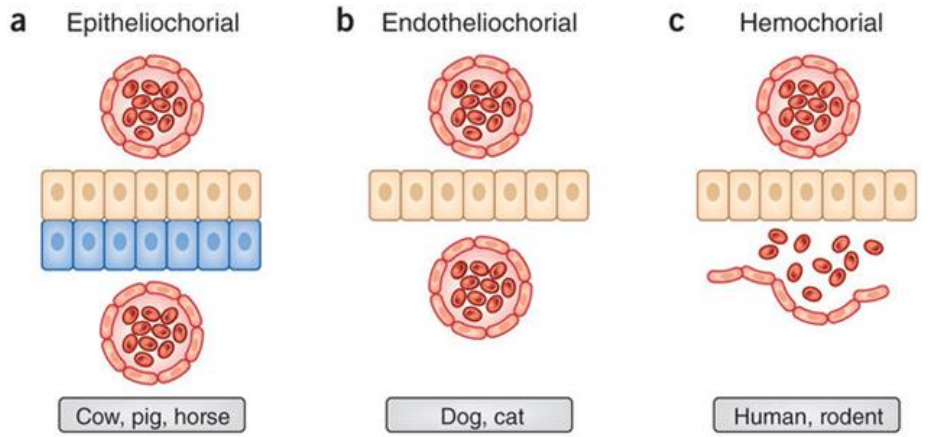
= The first secretion from the mammary glands after giving birth, rich in antibodies (Oxford dictionary)

	Colostrum	Milk
Fat (%)	6.7	4.0
Total protein (%)	14-14.92	3.10
IgG (g/L)	32-76	0.06-0.8
Minerals, Vitamins (%)	1.11	0.74

(Meganck et al., 2012)

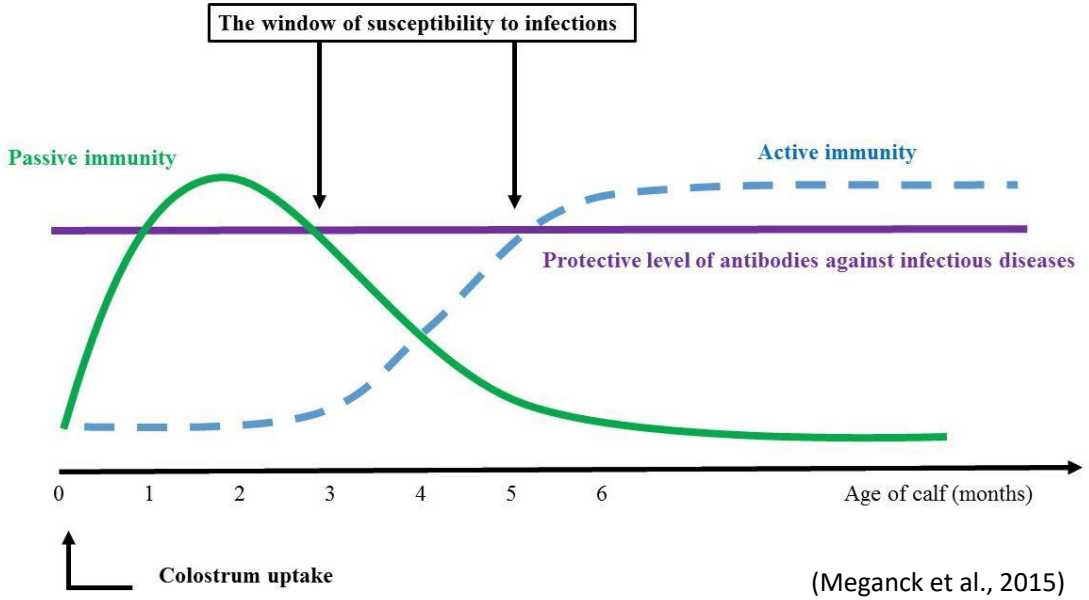
Importance of Colostrum

Specific structure bovine placenta → Calves are born without circulating antibodies



(PrabhuDas et al., 2015)

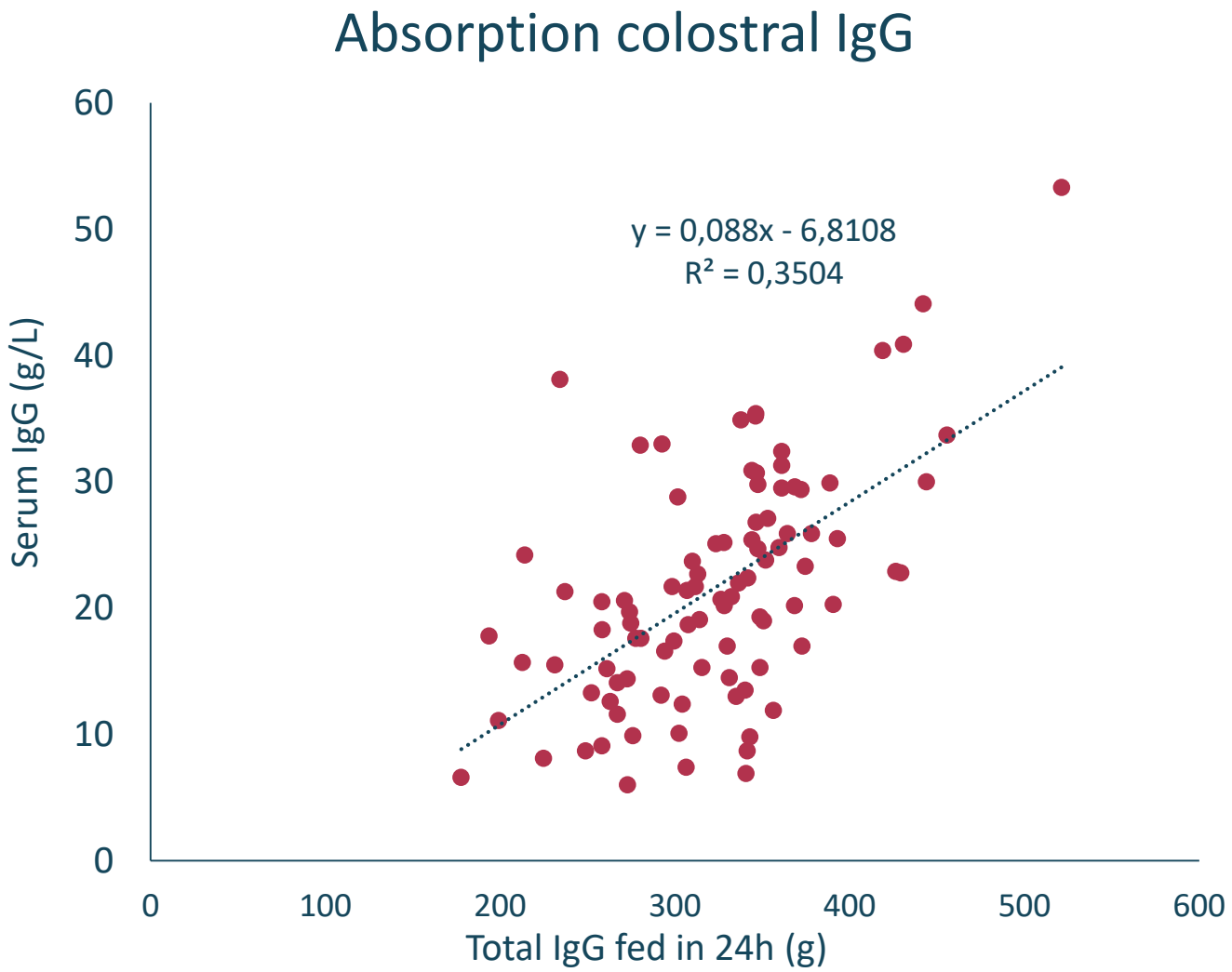
Colostrum supplies neonatal calf with antibodies → Transfer of passive immunity



(Meganck et al., 2015)

ILVO

Individual differences in IgG absorption



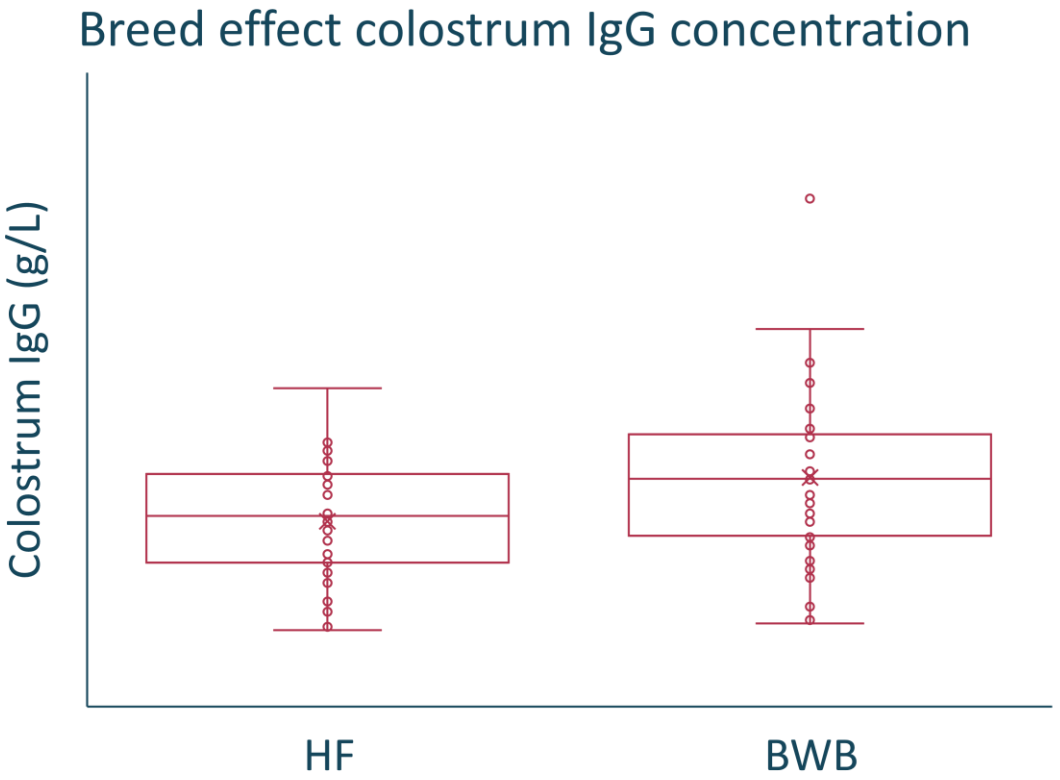
Samples collected from 94 calves (50 HF and 44 BB)

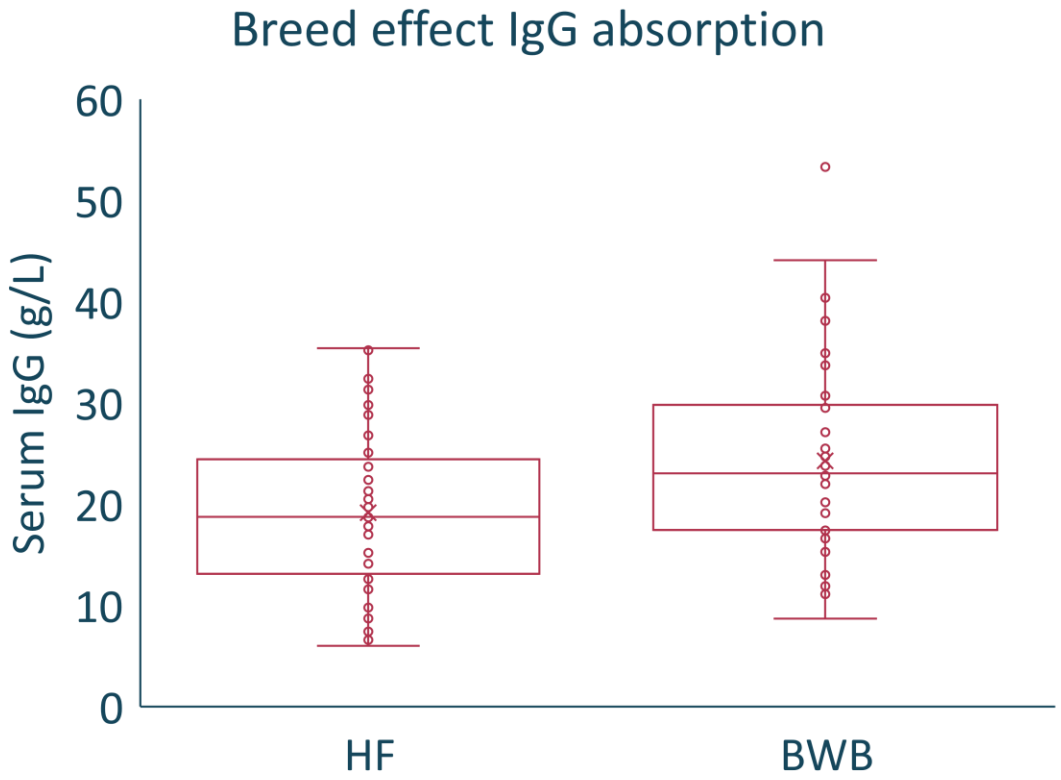
- Mean ± SD serum IgG: 21.5 ± 9.1 g IgG/L
- Mean ± SD total IgG fed: 322.2 ± 61.4 g
- Total IgG fed within 24h has a significant effect on serum IgG level (p-Value <0.001)

(Unpublished results, Van Hese)

How can we explain this variation?

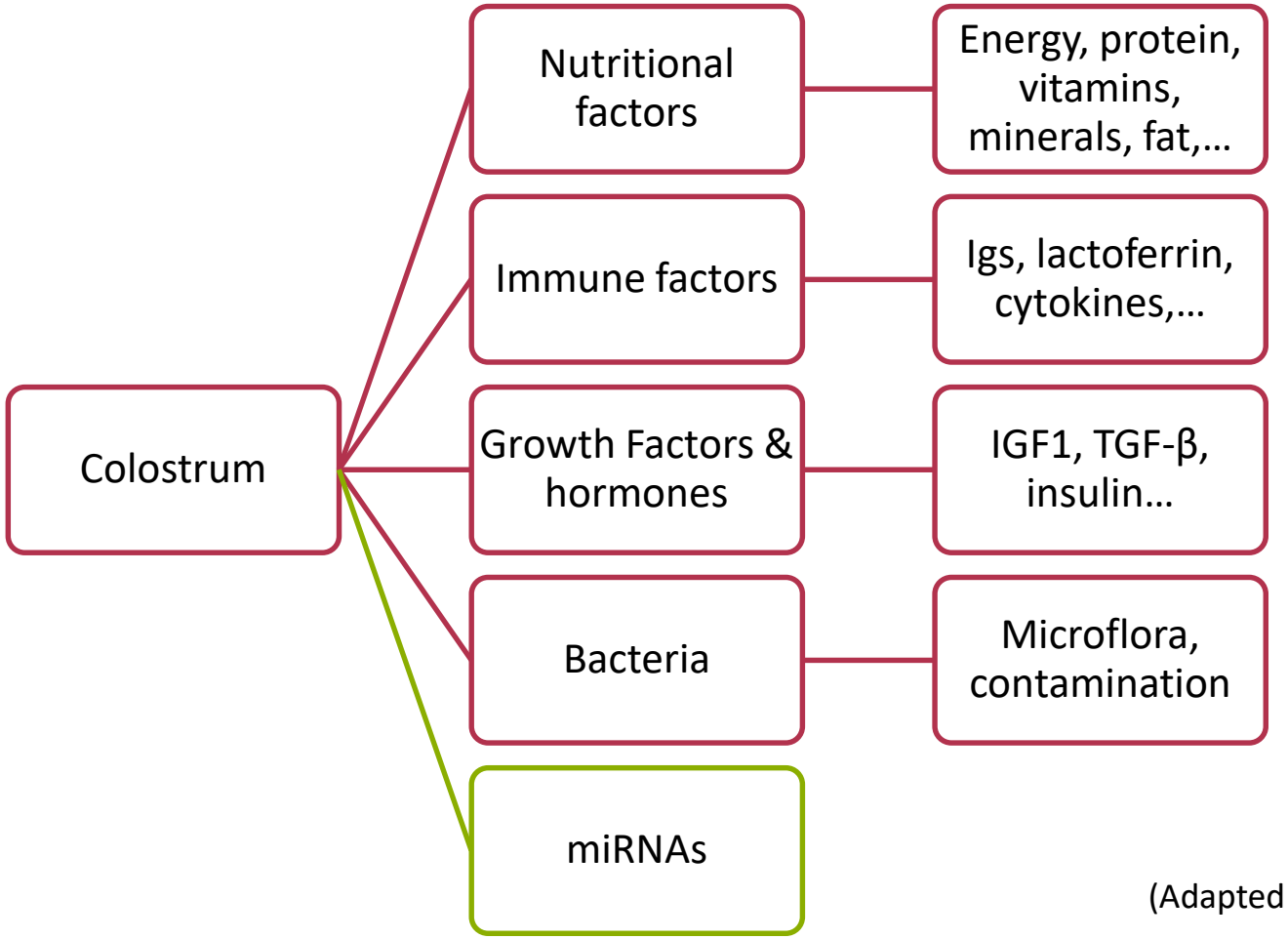
- Breed effect: HF vs BB
  - Breed effect on colostrum IgG concentration is significant (p-value= 0.001)
  - Breed effect on absorption in calves not significant (p-value= 0.803)





Aim of this research

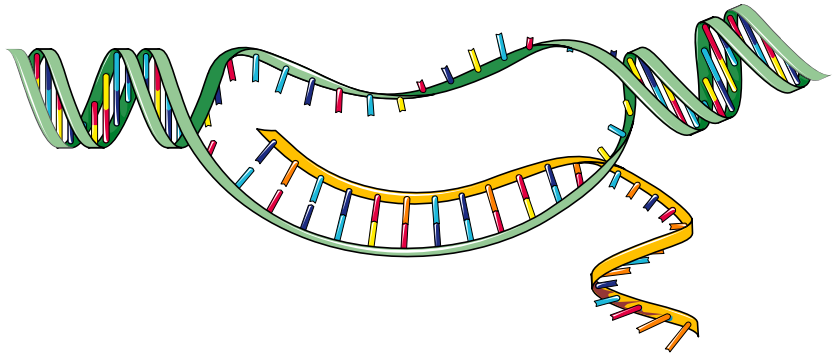
- Can variation in IgG absorption be explained by other components present in colostrum??
- Important source of nutrients and bioactive components:

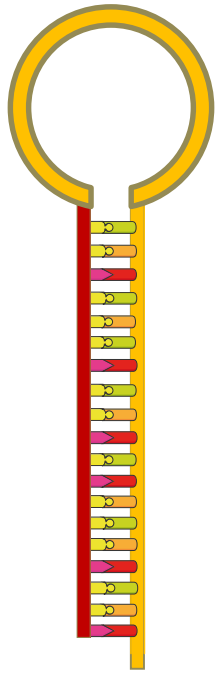


(Adapted from Godhia (2013))

Colostrum: beyond IgGs

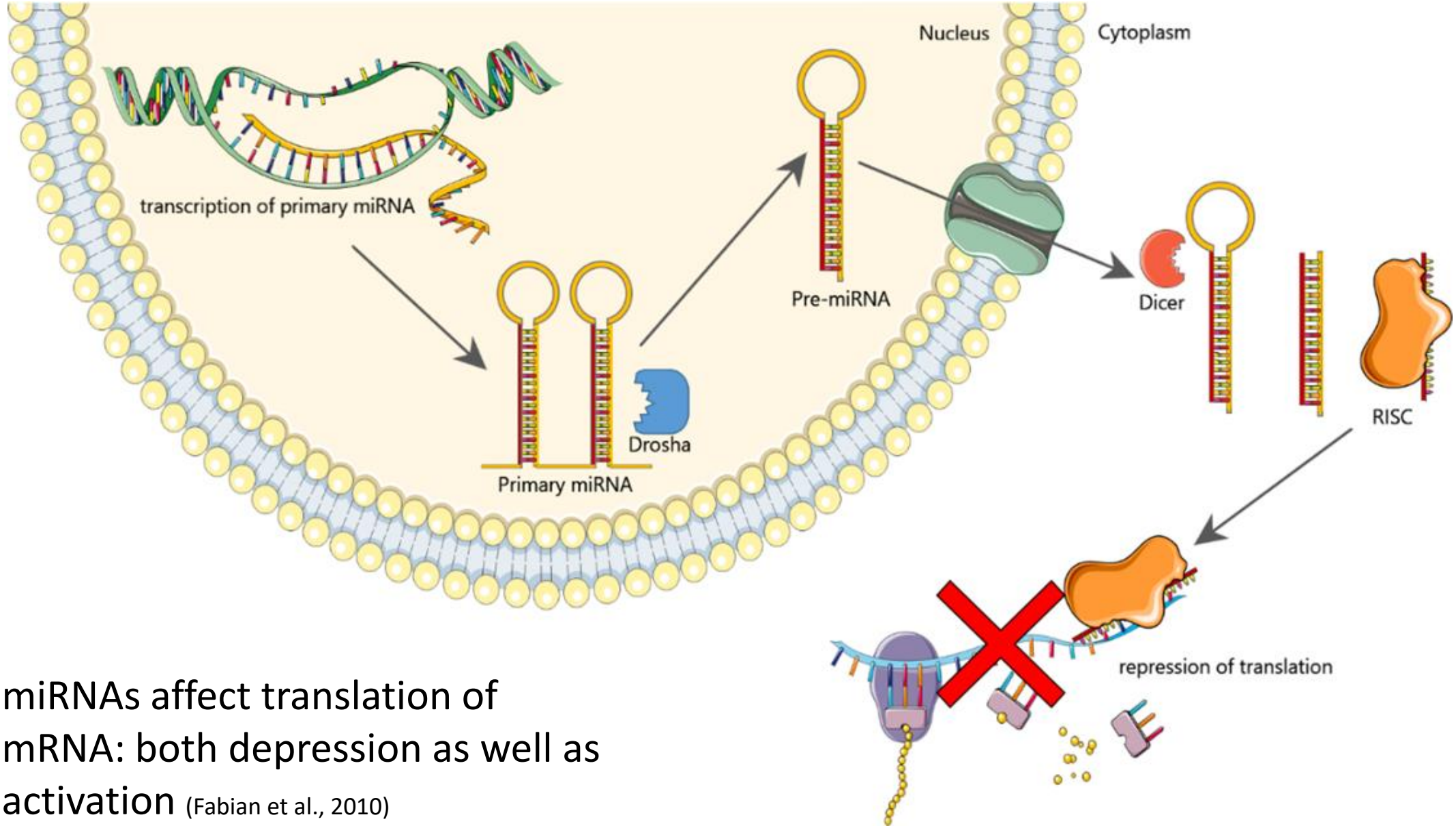
- What are miRNAs?
  - short, noncoding RNA molecules: 21-25 nts
  - Produced in the nucleus
  - Regulate gene expression
  - Post transcription





ILVO

miRNAs: Biogenesis and function



- miRNAs affect translation of mRNA: both depression as well as activation (Fabian et al., 2010)

Adapted from Lodish et al., (2008). This figure was created using Servier Medical Art templates, licensed under a Creative Commons Attribution 3,0 Unported License; https://smart.servier.com

ILVO

miRNAs: Biogenesis and function

- Almost **49,000** mature miRNAs have been discovered in **271** species: **1025** mature miRNAs in the bovine species (miRBase, release 22)
- Conservation among different species
  - E.g.: *let-7* family detected in arthropods, nematodes, fish, birds and mammals
- One miRNA can regulate different mRNAs
- Involved in a variety of biological processes
  - Cell development
  - Cell differentiation
  - Immune system
- Alterations in miRNA expression linked to cancer

O'Brien et al., 2018; He and Hannon, 2004; Dong et al., 2013

Summary of total number of microRNAs (miRNAs) detected in milk, colostrum and the mammary gland

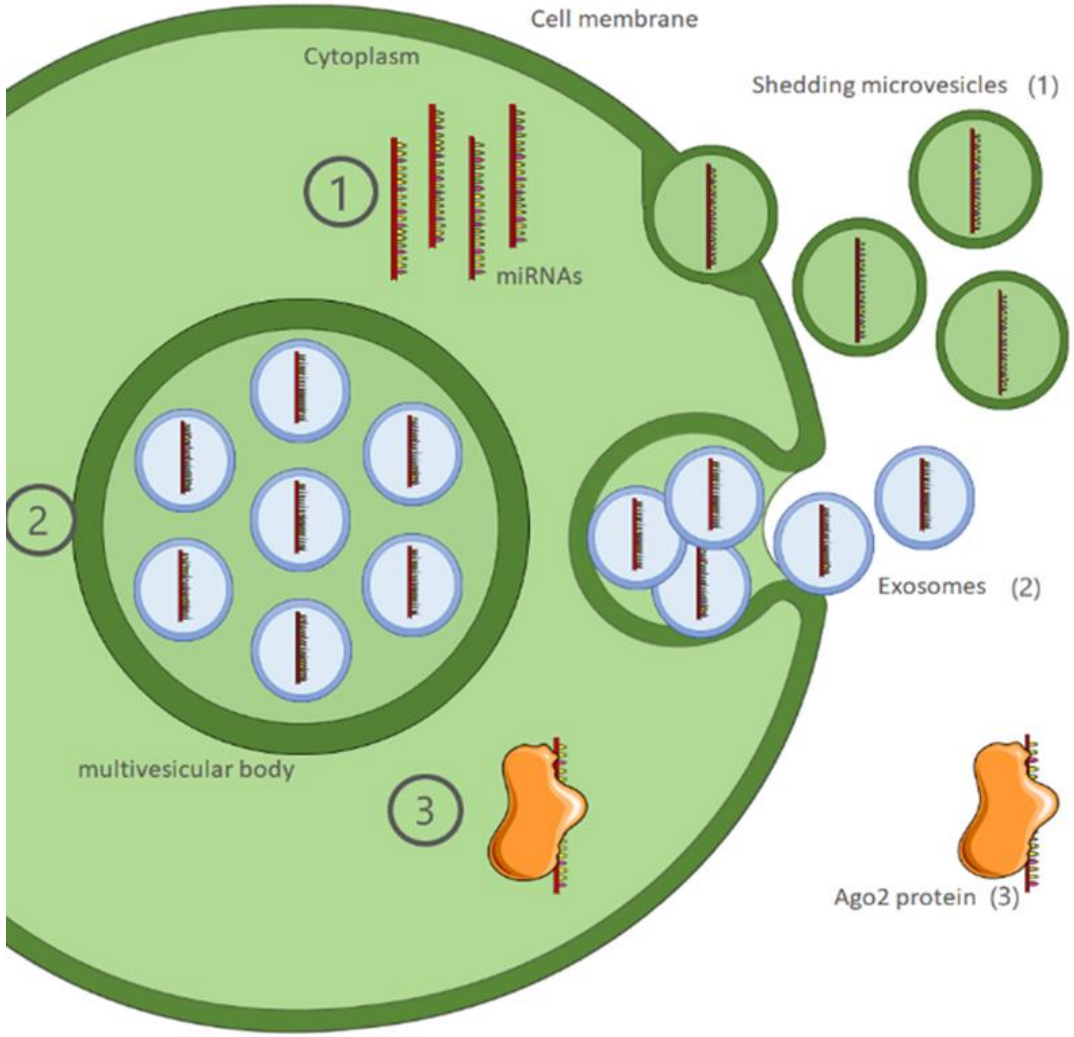
Number	Matrix	Sample amount	Species	Technique	Extraction method	Reference
386	Skimmed colostrum	300 µL	Human	Quantitative real-time PCR	miRNeasy kit (Qiagen)	(Weber et al., 2010)
395	Mammary gland day 1 postpartum	unknown	Murine (rat)	NGS[1]	Trizol reagent (Invitrogen)	(Zhang et al., 2014)
400	Mammary gland day 7 postpartum	unknown	Murine (rat)	NGS	Trizol reagent (Invitrogen)	(Zhang et al., 2014)
242	Milk fat	24-78 mL	Human	Microarray analysis	miRCURY RNA Isolation-Biofluids kit (Ambion)	(Alsaweed et al., 2016)
921	Mammary gland	unknown	Bovine	Microarray analysis	miRNeasy mini kit (Qiagen)	(Li et al., 2012b)
491	Milk exosomes	unknown	Porcine	NGS (Solexa sequencing)	Trizol reagent (Invitrogen)	(Chen et al., 2014)
100	Colostrum whey	2.5-5.5 mL	Bovine	Microarray analysis	miRNeasy mini kit (Qiagen)	(Izumi et al., 2012)
53	Mature milk whey	3.5-6.5 mL	Bovine	Microarray analysis	miRNeasy mini kit (Qiagen)	(Izumi et al., 2012)
230	Colostrum	100 mL	Bovine	NGS (Solexa sequencing)	Trizol LS Reagent (Invitrogen)	(Chen et al., 2010)
213	Mature milk	100 mL	Bovine	NGS (Solexa sequencing)	Trizol LS Reagent (Invitrogen)	(Chen et al., 2010)
303	Mammary gland peak lactation	unknown	Caprine	NGS (Solexa sequencing)	Trizol Reagent (Invitrogen)	(Li et al., 2012a)
337	Mammary gland dry period	unknown	Caprine	NGS (Solexa sequencing)	Trizol reagent (Invitrogen)	(Li et al., 2012a)

[1]NGS = next generation sequencing
[2]DE = differentially expressed

Van Hese et al., submitted

ILVO

miRNAs in colostrum



- miRNA expression is higher in colostrum than in milk
- Colostrum is especially rich in immune-related miRNAs
- Stored in microvesicles or exosomes
- Stable under harsh conditions
  - Rnase treatment
  - Acidic environment (~GIT)
  - Freeze-thaw cycles

Chen et al., 2010; Kosaka et al., 2010; Hata et al., 2010; Izumi et al., 2012; Sun et al., 2013

ILVO

Limitations previous research

- miRNA expression of first milking colostrum?
- Are they maternal signaling molecules?
- Do miRNA act locally in the gut or are they absorbed?
- Which function do they have? Do they contribute in transfer of passive immunity?

→ Reveal miRNA expression pattern in first milking colostrum

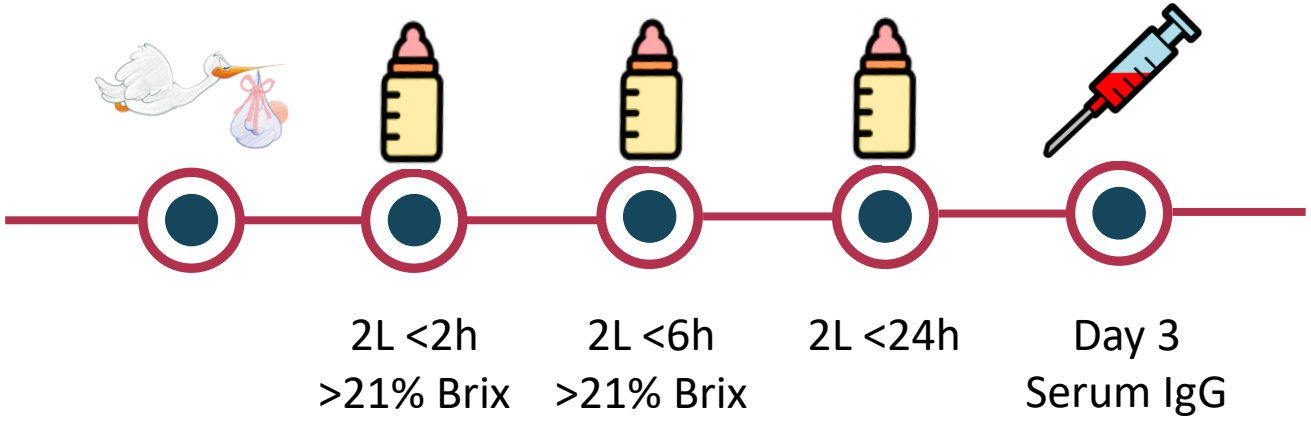
→ Correlate miRNA expression in colostrum with passive immunity in the calf

Present research

- Colostrum samples first milking
  (n= 50 HF and 44 BB)
- Sampling period: December 2017 to June 2019
- RNA extracted from whole colostrum
- Small RNA sequencing currently being performed

Present research

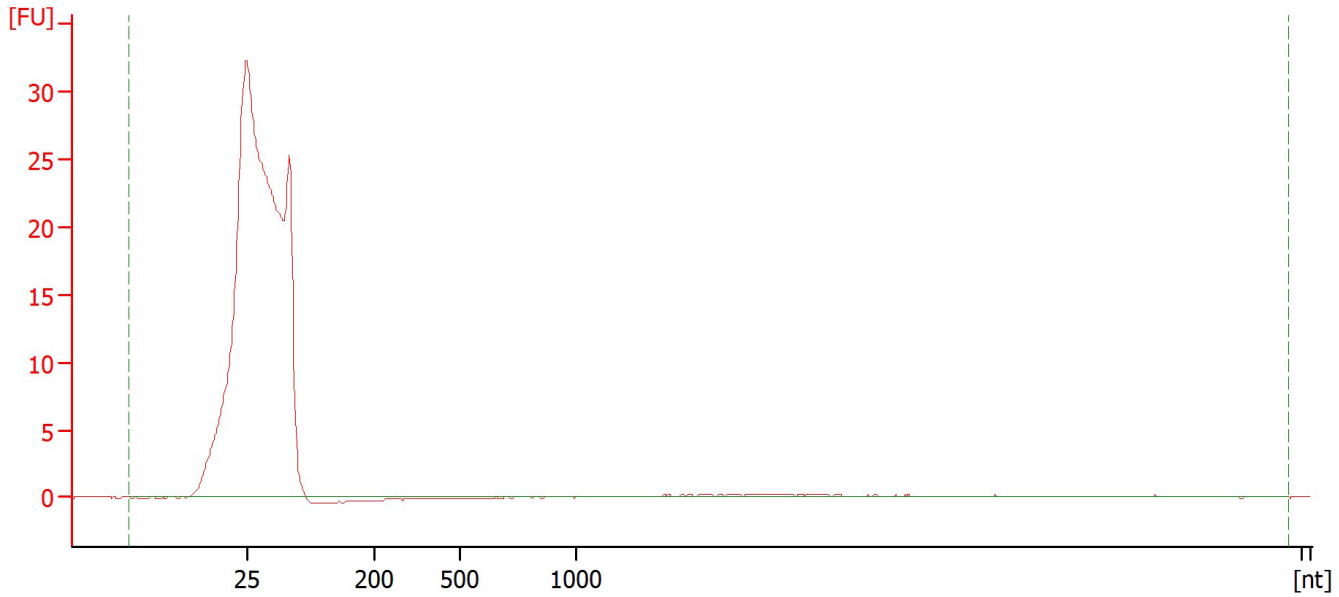
- Colostrum administration



→Test correlation between miRNA expression level OR presence/absence certain miRNAs and serum IgG level calves

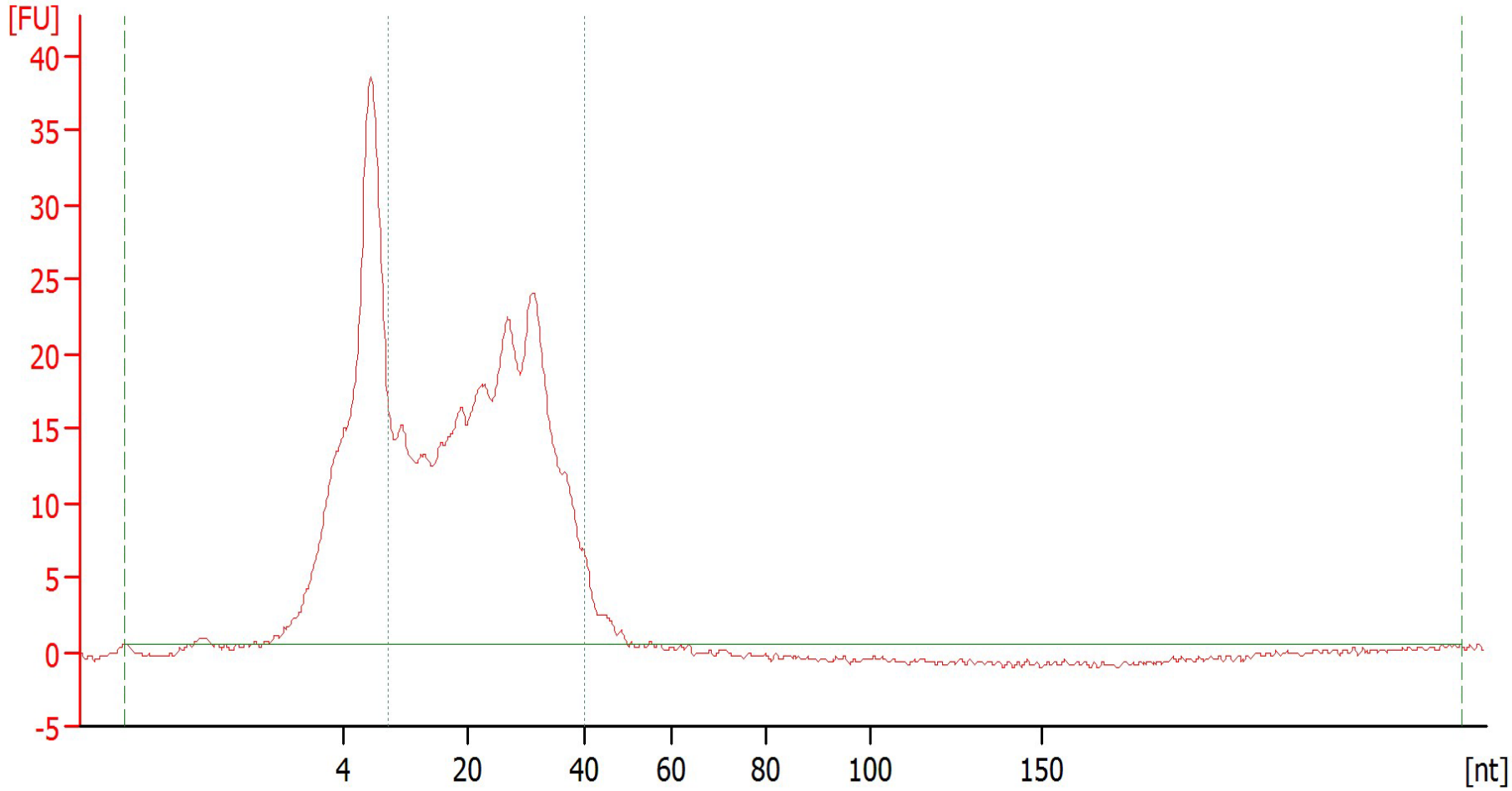
Small RNA sequencing

- Quality control of 9 samples
  - With Agilent 2100 Bioanalyzer
  - Eukaryote Total RNA (RNA 6000 pico chip):



Small RNA sequencing

- Quality control of 9 samples
  - With Agilent 2100 Bioanalyzer
  - Small RNA chip: samples ranging from 42 – 88% miRNAs



Small RNA sequencing

- Library prep
  - Lexogen small RNA kit
  - Dual indexing
- Sequencing
  - Hiseq3000
- Data Analysis
  - miRNA identification and expression analysis
  - Correlation analysis between miRNA abundance and diversity and IgG absorption
  - Target gene prediction for miRNA of interest

Illumina

Wrap up

- Wide variation between calves in uptake of colostral IgGs
- No breed effect on absorption IgGs despite effect on colostrum IgG concentration
- Quality control miRNA
  - Colostrum is possibly rich in miRNAs
  - Small RNAs are most represented; no or only little ribosomal RNA
- To draw conclusion we have to wait for sequencing results

Thank you

GHENT UNIVERSITY

Flanders
is agriculture and fisheries

fwo Research Foundation Flanders
Opening new horizons

Flanders Research Institute for Agriculture, Fisheries and Food
Scheldeweg 68
9090 Melle – Belgium
www.ilvo.vlaanderen.be

Ilke.vanhese@ilvo.vlaanderen.be
T + 32 (0)9 272 26 63

ILVO