IMPACT OF THE MARE COLOSTRAL IMMUNOGLOBULINS ON THE EXPRESSION OF *TLR3*, *TLR4* AND *TLR7* IN FOALS.

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NEWBORN FOAL'S IMMUNITY

PRENATAL PERIOD

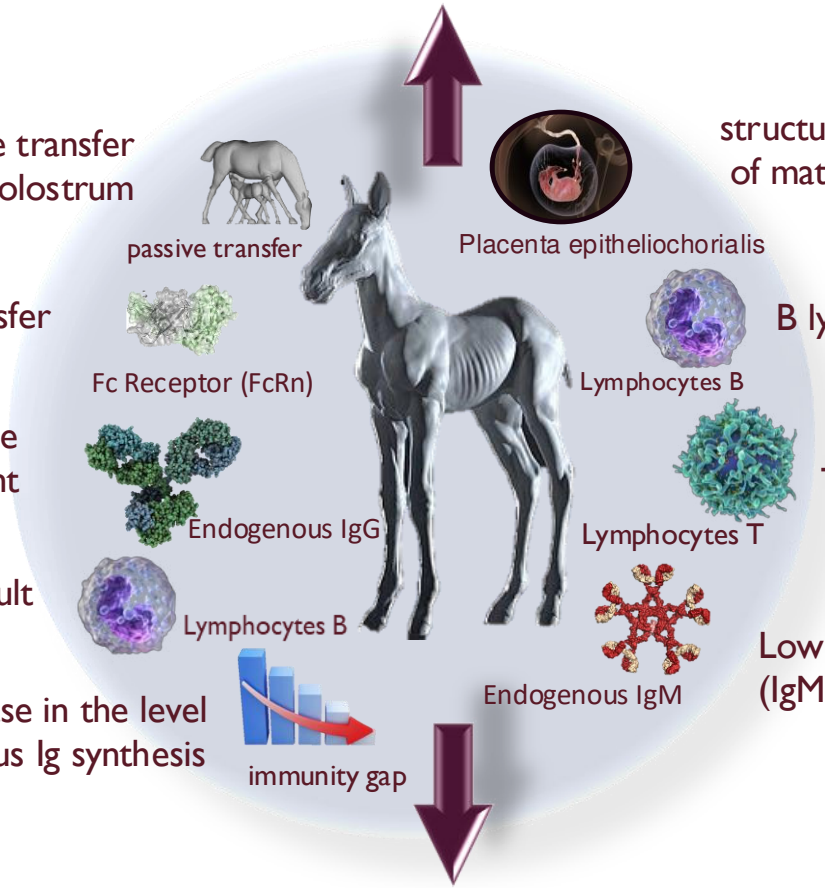
specific immunity conditioned by passive transfer of Ig from colostrum

FcRn receptor-dependent passive transfer

B lymphocytes mainly produce IgG1, IgG3, while endogenous IgG2, IgG4 are absent

30% of the number of B cells found in adult

low immunity zone (6-8 week) - caused by a decrease in the level of maternal Ig and a slow process of endogenous Ig synthesis



structure of the placenta prevents transfer of maternal Ig to the foal

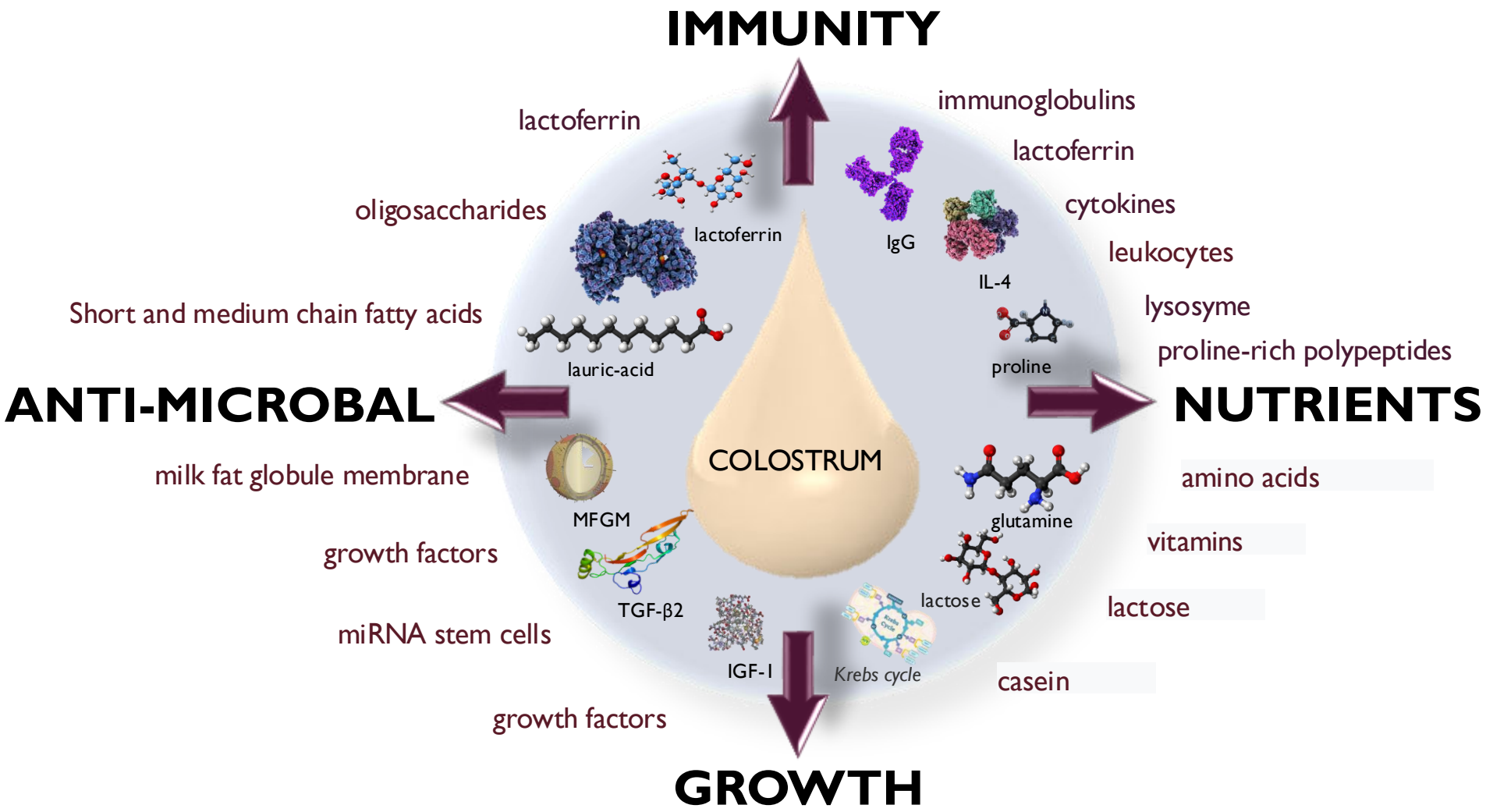
B lymphocytes are present in the bone marrow from 90 d.p

T lymphocytes appear in the fetal thymus from 80 d.p.

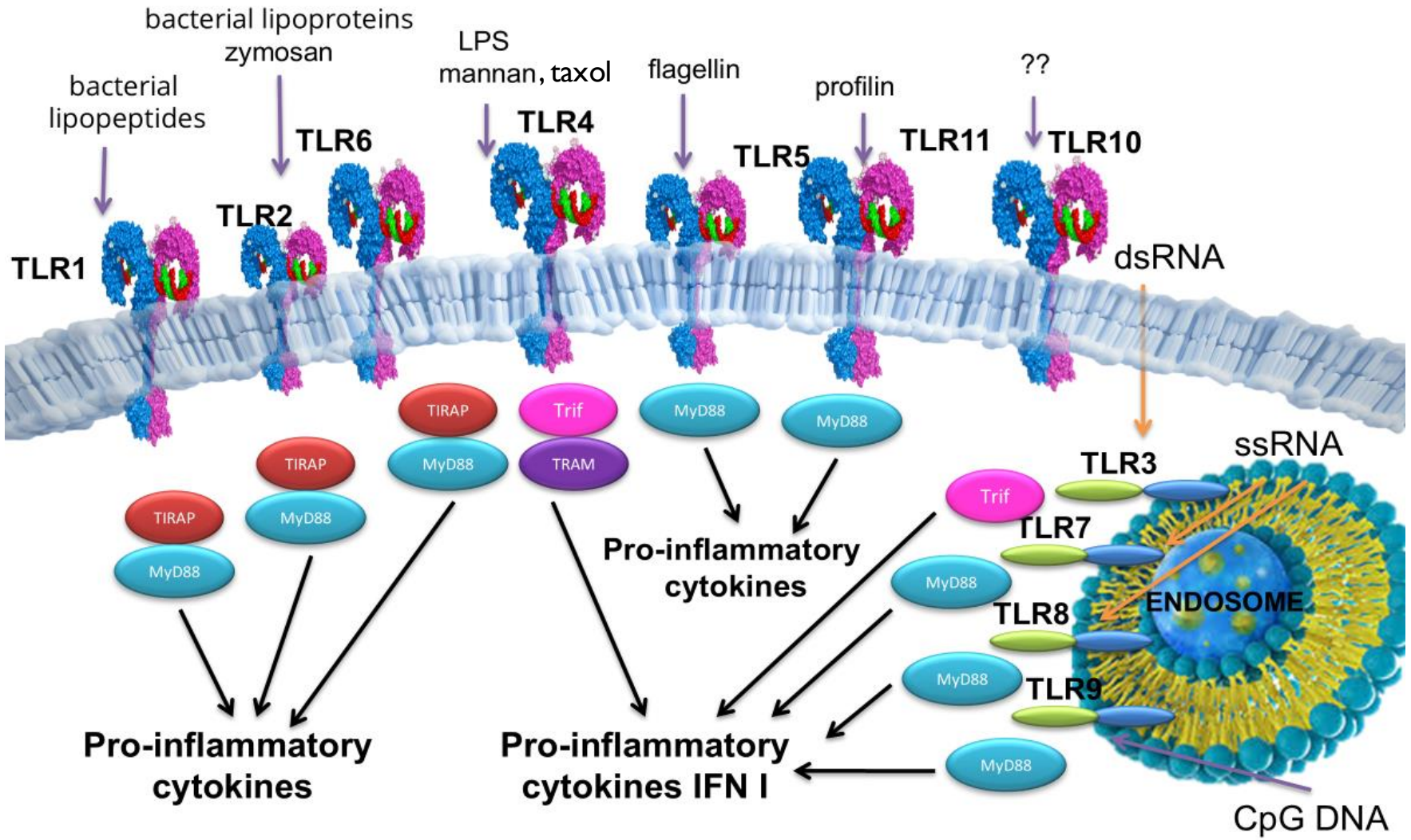
Low level of endogenous immunoglobulin synthesis (IgM and IgG1)

POSTNATAL PERIOD

ROLE OF COLOSTRUM



TLR TYPES AND RECOGNITION OF PATHOGEN-ASSOCIATED MOLECULAR PATTERNS (PAMPS)



HYPOTHESIS OF THE STUDY

Gene expression for Toll-like receptors TLR-3, TLR-4, and TLR-7 in foals is dependent on the quality of colostrum especyal, the concentration of immunoglobulins in the secretions of the mammary gland

AIM OF THE STUDY

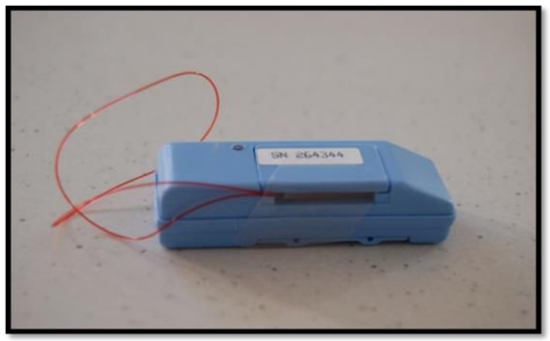
Assess the concentration of Ig in the mammary gland secretions (colostrum and milk) of mares and correlate this parameter with the level of expression of genes related to Toll-like receptors in suckling foals

ANIMALS AND EXPERIMENTAL DESIGN

- 25 foals and mares Polish Pony (Konik, Polish Konik) breed.
- The foals were born in two consecutive spring seasons (February and March)
- All animals were clinically healthy during the experimental period.

On the 320th day of pregnancy:

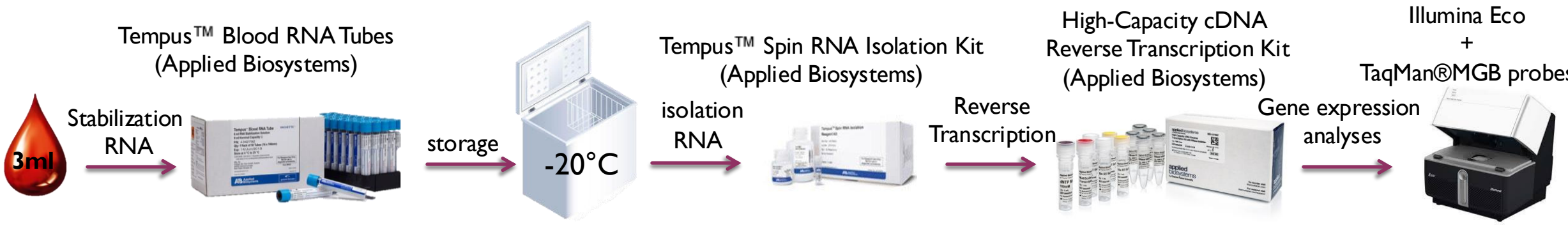
- birthing alarms (*Abfohlsystem, Jan Wolters, Mühlen, Steinfeld, German*) were placed in the labia
- mares were moved to box stalls inside a stable lit with natural light.



SCHEME OF COLLECTING MATERIAL FOR RESEARCH

Time after delivery	before the first suckling	24 h	3 d	5 d	10 d	20 d	30 d
Blood (foal)							
Colostum/milk							

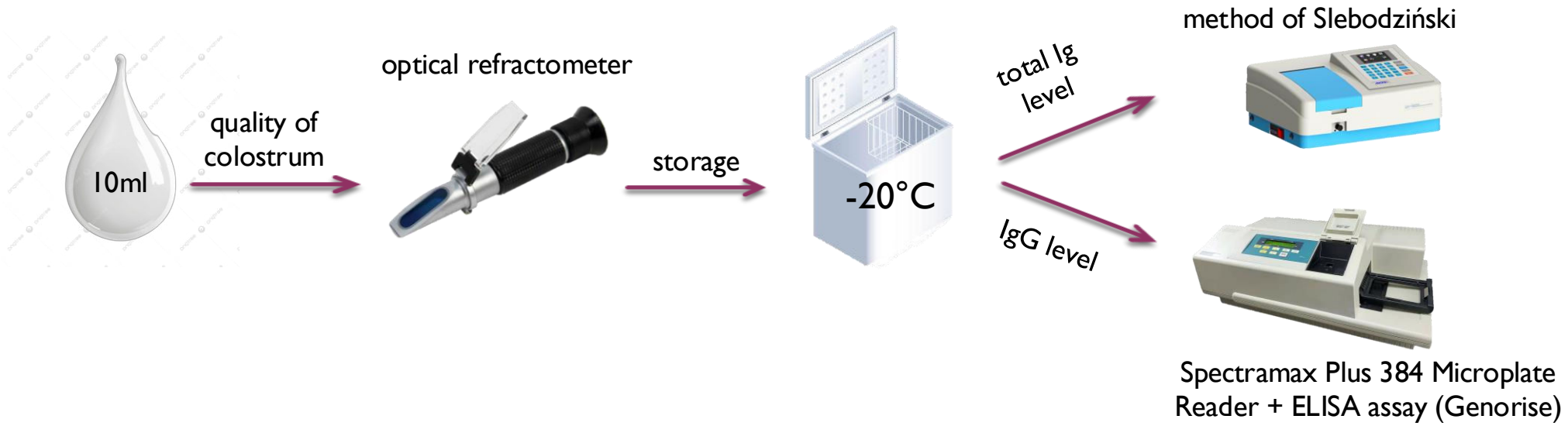
METHODS - GENE EXPRESSION ANALYSES



Probes used for amplification of *TLR* genes and housekeeping genes.

Gen	Full nameof the gene	Access number GenBank	Taqman assay ID	Dye
TLR3	Toll-like receptor 3	NC_009170.2	Ec03467747_m1	FAM
TLR4	Toll-like receptor 4	NC_009168.2	Ec03468993_m1	FAM
TLR7	Toll-like receptor 7	NC_009175.2	Ec03467530_m1	VIC
SDHA	succinate dehydrogenase complex subunit A	XM_001490889	Ec03470479_m1	VIC
HPRT	Hypoxantine phosphoribosyl transferase	AY372182.1	Ec03470217_m1	VIC

METHODS - MAMMARY GLAND SECRETIONS ANALYSIS



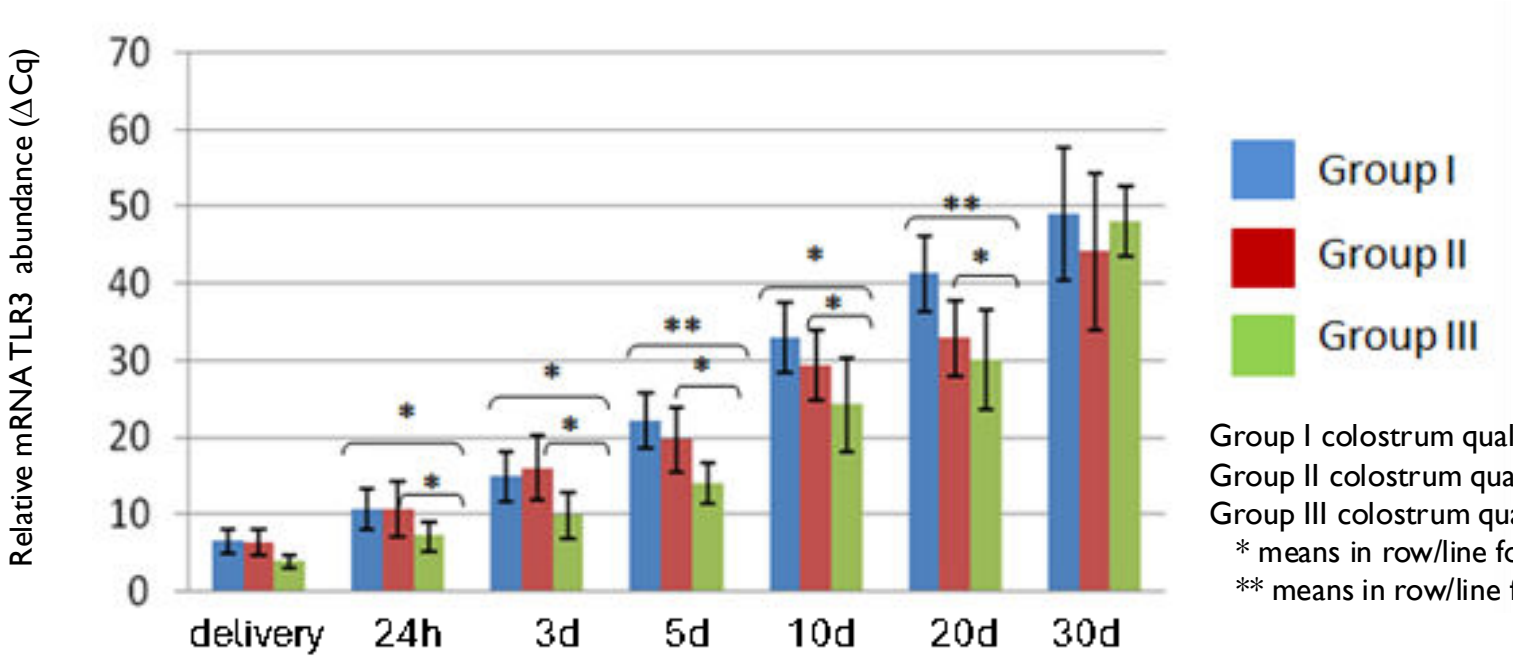
All the foals were divided into 3 groups based on the quality of the collected colostrum:

Group I - (n = 8) foals the ingested concentration of Ig amounting to ≤55 g/l.

Group II - (n = 9) foals that ingested colostrum concentration of Ig ranging between 56 and 70 g/l.

Group III - (n = 8) foals that ingested colostrum concentration of Ig ranging exceeding 70 g/l.

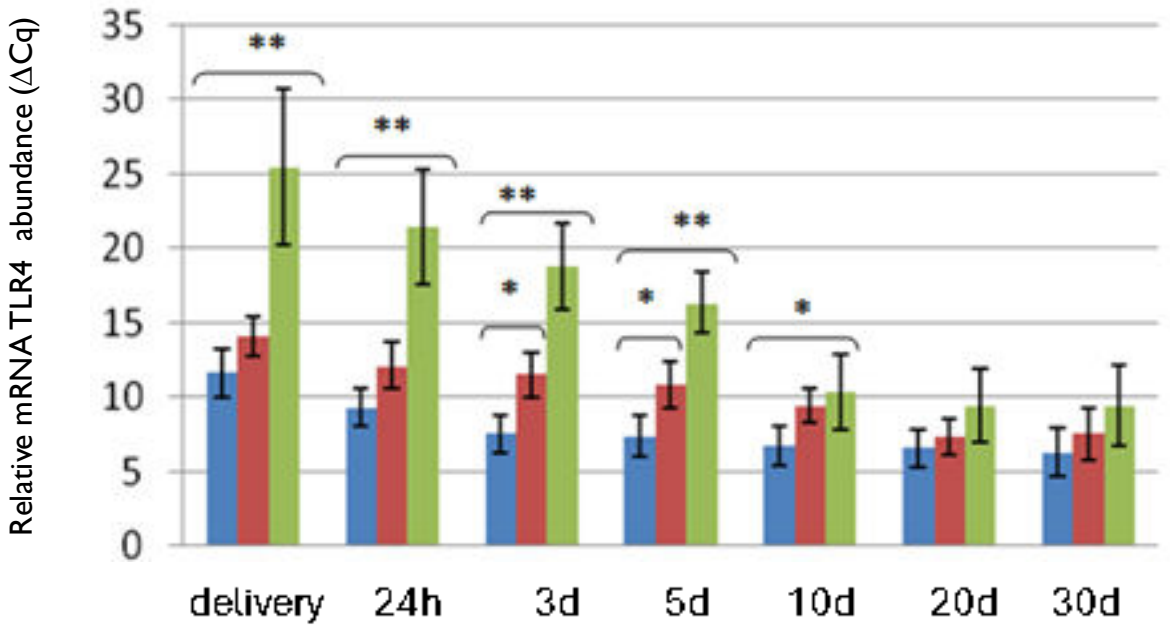
RESULTS – EXPRESSION OF TLR3 MRNA



Group I colostrum quality poor -concentration of Ig <55 g/l
Group II colostrum quality good - concentration of Ig 56-70 g/l
Group III colostrum quality very good -concentration of Ig >70 g/l
* means in row/line for receptor show significant differences (p<0,05)
** means in row/line for receptor show highly significant statistical differences (p<0,01)

Effects of colostrum Ig concentration on relative messenger RNA (mRNA) abundance of f toll-like receptors of foal up to 30 days life. Relative mRNA abundance (ΔCq) = Cq target gene–Cq housekeeping gene; higher ΔCq values indicated lower mRNA abundance. Values were presented as means±sd.

RESULTS – EXPRESSION OF TLR4 MRNA



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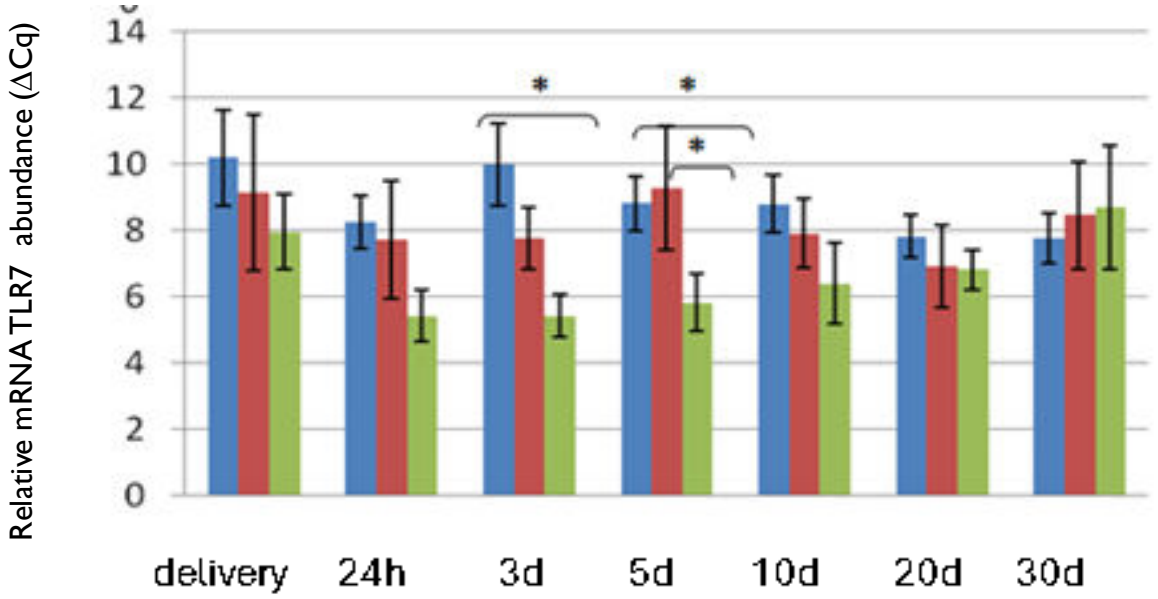
RESULTS – EXPRESSION OF TLR7 MRNA



Group I
Group II
Group III

Group I colostrum quality poor -concentration of Ig <55 g/l
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RESULTS – SPERMAN'S RANK CORRELATION

		TRL4	TRL7	Mammary gland secrecjon Ig concentration
All	TRL3	- 0.54	0.35	-0.51
	TRL4		-0.38	0.43
	TRL7			-0.04
delivery	TRL3	-0.16	0.63	-0.57
	TRL4		-0.34	0.51
	TRL7			-0.02
24h	TRL3	-0.19	0.46	-0.47
	TRL4		-0.39	0.38
	TRL7			-0.03
3d	TRL3	-0.10	0.49	-0.55
	TRL4		-0.47	0.47
	TRL7			-0.07
5d	TRL3	-0.09	0.33	
	TRL4		-0.34	
	TRL7			
10d	TRL3	-0.35	0.57	
	TRL4		-0.19	
	TRL7			
20d	TRL3	-0.58	0.42	
	TRL4		-0.31	
	TRL7			
30d	TRL3	-0.04	0.37	
	TRL4		-0.27	
	TRL7			

Sperman's rank correlation values (r-value) of the expression of TLR3, TLR4, and TLR7 mRNA over the immunoglobulin concentration in mammary gland secretions

Correlation strength for |r|

-0.2< |r| < 0.2 – no relation
0.2 < |r| < 0.4 – weak positive correlation
0.4 < |r |< 0.7 – moderate positive correlation
|r| ≥ 0.7 –strong positive correlation
-0.2< |r| < - 0.4 – weak negative correlation
-0.4< |r| < - 0.7 – moderate negative correlation
|r| ≤ - 0.7 –strong negative correlation

CONCLUSIONS

- Colostrum consumption may put the immune system, in a state of alert that results in an increased response to subsequent infection.

- Different influence of Ig concentration in colostrum on expression level of TLR3, TLR4, and TLR7 in the first few days after birth in foals might indicate that colostrum contributes to the development of the innate immune system.

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