

# IGF - 1 IN BOVINE SERUM AND LEUKOCYTES



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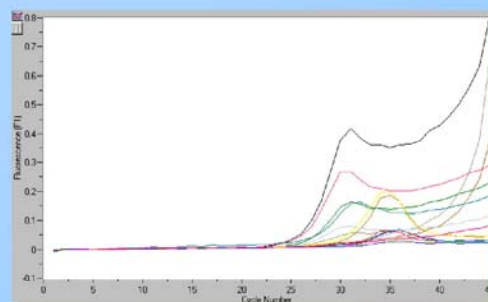
During postnatal growth, IGF-1 stimulates protein synthesis and improves glucose utilization. As regulatory protein, it plays important role in the immune system by modulating immune responses, including lymphocyte activation e.t.c. Its binding proteins and receptors modulate IGF-1 biological activity. The aim of study was to investigate the expression of IGF-1 and IGF-1R in cattle leukocytes in early development.

## Material and Methods

Animal: 55 clinically healthy female Holstein calves in age from 4 to 6 weeks in farms Vorbuse and Rahinge. The samples were collected from V. jugularis into disposable non-heparinized test tubes for IGF-1 testing and EDTA-tubes for mRNA studies. Blood serum was separated by centrifugation at 2000 g for 10 min and was then frozen (-24°C) for analysis. Whole blood samples were stabilized using RNA/DNA Stabilization Reagent for Blood/Bone Marrow (Roche Applied Science) and then frozen (-24°C) for analysis.

IGF-1 in blood serum was measured using RIA (DSL) kit. Roche mRNA Isolation Kit for Blood/Bone Marrow was used for mRNA purification from stabilized blood samples. LightCycler RNA Master SYBR Green I The LightCycler RNA Master SYBR Green I kit (Roche Applied Science) was used for hot start one-step RT-PCR in glass capillaries using the LightCycler Instruments and SYBR Green I dye as the detection format. The suitable genetic sequences were represented in (Pfaffl, 2001; Pfaffl et al., 2002a; Pfaffl et al., 2002b). According to literature data, the IGF-1 primers, for this experiment, were synthesised in TIB MOLBIOL (www.tib-molbiol.com). The PCR protocol has been optimized on the basis of articles (Pfaffl, 2001; Pfaffl et al., 2002a; Pfaffl et al., 2002b) and Roche LightCycler RNA master SYBR Green I metod manual. GAPDH and Ubiquitine genes were used for IGF-1 mRNA relative quantification as housekeeping-genes (Smolkina, Karus, 2004). Data was analyzed by the statistical programs of SYSTAT 10.0 and Microsoft EXCEL.

Segment nr.	Target Temperature (°C)	Incubation time (s)	Temperature Transition rate (°C/s)	Second temperature (°C)	Step size (°C)	Acquisition mode
<b>Reverse transcription</b>						
1	61	1200	20	0	0	None
<b>Denaturation, 1 cycle</b>						
1	95	0	20	0	0	None
<b>Quantification, 50 cycles</b>						
1	95	1	20	0	0	None
2	55	5	20	0	0	None
3	72	13	20	0	0	None
4	82	3	20	0	0	Single
<b>Melting Curve Analysis</b>						
1	95	5	20	0	0	None
2	65	15	20	0	0	None
3	95	0	0.1	0	0	Continuous
<b>Cooling</b>						
1	40	30	20	0	0	None



Sample of IGF-1 Quantification Screen

## Results

1. IGF-1 content in blood serum vary from 0.6 - 287 mg/l.
  2. There was low significant correlation between IGF-1 content in serum and IGF-1 mRNA relative concentration in leukocytes (0.21; P 0.95).
  3. IGF-1R mRNA relative concentration in leukocytes was lower detectable limits.
- Results indicate that leukocytes may contribute to blood serum IGF-1 content.

## References

- \* Pfaffl M. Development and validation of an externally standardised quantitative insulin-like growth factor-1 RT-PCR using LightCycler SYBR Green I Technology. In: S. Meuer, C. Wittwer and K. Nakagawara (eds.): Rapid cycle Real-Time PCR. Berlin: Springer 2001. P. 281-291.
- \* Pfaffl M., Daxenberg A., Hageleit M., Meyer H. H. D. Effects of Synthetic Progestagens on the mRNA Expression of Androgen Receptor, Progesterone Receptor, Oestrogen Receptor  $\alpha$  and  $\beta$ , Insulin-like Growth Factor-1 (IGF-1) and IGF-1 Receptor in Heifer Tissues. J. Vet. Med. 2002. Vol. 49. P. 57-64.
- \* Pfaffl M., Mircheva Georgieva T., Penchev Georgiev I., Ontsouka E., Hageleit M., Blum J. Real-time RT-PCR quantification of the insulin-like growth factor (IGF)-1, IGF-1 receptor, IGF-2, IGF-2 receptor, insulin receptor, growth hormone receptor, IGF-binding proteins 1, 2 and 3 in the bovine species. Domestic Animal Endocrinology 2002. Vol. 22. P. 91-102.
- \* Smolkina Z., Karus A. 2004: IGF-1 and some housekeeping gene candidates for real-time RT-PCR expression studies in cattle. J. Agr. Sci. (Agraarteadus), XV, 1, 38-46.