

## **A) Evolution of seasonally dependent gene expression in European roe deer (*Capreolus capreolus*)**

Growth factors are involved in the regulation of testicular growth and involution of seasonal breeders. Therefore, we studied the seasonal expression of several growth factors in roe deer: aFGF, bFGF, IGF-1, IGF-2, and TGF- $\alpha$ . Total RNA from testis tissue was extracted monthly and analysed using quantitative RT-PCR. Localisation of mRNAs was examined by *in situ*-hybridisation.

Levels of expression differed by more than three orders of magnitude. Expression also showed different seasonal patterns. IGF-1, IGF-2 and bFGF were maximally transcribed during testis recrudescence in spring. In contrast, the mRNA amount of aFGF reached its maximum between July (breeding season) and January. TGF- $\alpha$  mRNA-levels were very low and showed poor seasonal variation.

Each growth factor showed its own typical expression localisation in testicular tissues and cell types. The results suggest the specific role of different growth factors in the paracrine control of spermatogenesis and its seasonal regulation.

more Publications (before 2003):

Wagener A, Blottner S, Fickel J (2000): Detection of growth factors in the testis of roe deer (*Capreolus capreolus*). ANIMAL REPROD. SCI. 64, 65-75.

Wagener A, Blottner S, Einspanier R, Fickel J (1999): Testicular growth factor expression in seasonal and non-seasonal ruminants. Reproduction in Domestic Animals - Physiology, Pathology, Biotechnology (Eds. Rath, D. & Meinecke, B). Proceedings of the 32<sup>nd</sup> Conference on Physiology and Pathology of Reproduction and of the 24<sup>th</sup> Joint Meeting on Veterinary and Human Medicine, 34(1), 18.

Göritz F, Hildebrandt TB, Broich A, Hermes R, Lengwinat T, Jewgenow K, Fickel J, Reinsch A, Wagener A, Lange A, Gilles M, Blottner S, Hofmann RR (1999): Experimental investigation in embryonic diapause and seasonality of spermatogenesis in European roe deer (*Capreolus capreolus*). Proceedings of the European Roe deer Meeting, 7-10.

Wagener A, Blottner S, Einspanier R, Fickel J (1998): Investigation of growth factors gene expression in the testis of ruminants. ADV. ETHOL. 33, 75.

Wagener A, Fickel J, Gehrke J, Einspanier R, Jewgenow K (1998): Bovine oocyte quality in relation to follicular wall apoptosis and expression of growth factors - preliminary results. J. REPROD. FERTIL. SUPPL. 22 (72a), 28.

## **B) Molecular mechanisms of larger herbivore adaptation to tannin-rich plants**

Many dicotyledonous plants produce substances (e.g. tannins) whose consumption may have negative consequences for herbivores. Some ruminants avoid their effects by producing salivary proteins which bind and thus eventually neutralise these plant substances. Research is focused on identification, isolation and characterization of these salivary proteins and includes protein-chemical (2D-electrophoresis, tannin binding assay) and molecular biological methods (cloning, sequencing).

In unadapted herbivore species plant secondary metabolites such as tannins can reduce food digestibility and thus diminish growth rate and health status (antinutritive action). Some ruminant species feed on tannin containing plants but do not suffer from antinutritive consequences. The

production of tannin binding salivary proteins is one possible adaptation mechanism to neutralise the effects of tannins.

Saliva of several ruminant species was investigated for the presence of tannin binding proteins. Salivary proteins of browsers such as roe deer, reindeer (*Rangifer tarandus*) fallow deer (*Cervus dama*) and musk ox (*Ovibos moschatus*) bound hydrolysable tannins (tannic acid) twice as effective as those from grazers such as cow (*Bos taurus*) and moufflon (*Ovis orientalis*). The same was seen comparing the binding capacity to condensed tannins (quebracho), except for moufflon salivary proteins which bound as effective as browser salivary proteins.

Analysis of trichloroacetic acid (TCA)-soluble salivary proteins of roe deer and moos (*Alves alves*) revealed a prolin content of more than 35%, in musk ox it was 29%. No prolin-rich proteins (PRP) were found in cattle and fallow deer saliva indicating the existence of other compounds in fallow deer saliva to deal with tannins. Direct comparison of cattle and roe deer TCA-soluble salivary proteins showed a 10-25 times higher tannin binding capacity of the latter. Tannin binding capacities of the proteins from musk ox and fallow deer saliva was higher than those from cattle but lower than those from roe deer.

Roe deer TCA-soluble salivary proteins were glycosylated and could be grouped into five groups according to their molecular weights and isoelectric points (MW 15-50 kD, IP 4.0-8.2). N-terminal sequences were obtained from 15 of these proteins. Amino acid sequences were used for primer design to isolate roe deer specific PRP mRNAs. Feature of these transcripts was a C-terminal sequnz of PPPEEQPE<sup>E</sup>/<sub>Q</sub>SPD E<sup>E</sup>/<sub>D</sub>SPSE. Search for homologies with other mammalian PRPs did not yield any sensible similarities. Results are the prerequisite for further research aimed at understanding the evolutionary basis for the interactions between plants and larger herbivores.

#### more Publications (before 2003)

- Gehrke J, Fickel J, Lechner-Doll M, Hermes R, Flach EJ, Hatt JM, Clauss M (2002): Tannin-binding salivary proteins in three captive rhinoceros species. Proceedings of the Joint Nutrition Symposium [American Academy of Veterinary Nutrition (AAVN), American College of Veterinary Nutrition (ACVN), Comparative Nutrition Society (CNS), European College of Veterinary and Comparative Nutrition (ECVCN), European Society of Veterinary and Comparative Nutrition (ESVCN), and the European Zoo Nutrition Research Group (EZNRG)], 130.
- Clauss M, Gehrke J, Fickel J, Lechner-Doll M, Flach EJ, Dierenfeld ES, Hatt JM (2002): Induction of salivary tannin-binding proteins in captive black rhinoceros (*Diceros bicornis*). Proceedings of the Joint Nutrition Symposium, 131.
- Gehrke J, Fickel J, Lechner-Doll M, Lason K, Clauss M (2002). Salivary tannin-binding proteins are not affected by mid-term feeding history in captive roe deer (*Capreolus capreolus*). Proceedings of the Joint Nutrition Symposium, 132.
- Fickel J, Pitra C, Joest BA, Hofmann RR (1999): A novel method to evaluate the relative tannin-binding capacities of salivary proteins. COMP. BIOCHEM. PHYSIOL. C 122, 225-229.
- Gehrke J, Fickel J (1998): Investigation of tannin binding proteins in ruminant saliva. ADV. ETHOL. 33, 101.
- Fickel J, Göritz F, Joest BA, Hildebrandt T, Hofmann RR (1998): Analysis of parotid and mixed saliva in roe deer (*Capreolus capreolus*, L.). J. COMP. PHYSIOL. B 168, 257-264.