8 Summary

Seasonal spermatogenesis in roe deer:
Seasonal changes of histomorphometric parameters in roe deer testis in relation to the immunohistochemical localisation of growth factors TGFβ1 and 3, aFGF and VEGF

One of the most exciting problems in spermatogenesis regulation is the clarification of mechanisms of growth factor activity. Little is known about paracrine and autocrine testicular regulation systems in *seasonal* breeders as they are studied to a much lesser extent than non-seasonal breeders, although their physiological seasonal up and down regulation is an excellent model for investigating general mechanisms of spermatogenesis. The time-specific and cell-type-specific pattern of the occurrence of growth factor during the course of a year could reflect their functional role in the complex interaction between germinative and somatic cells of testis parenchyma.

The actions of paracrine regulators are very specific and adapted to their environment. Thus the possibility to study them under artificial conditions, such as cell cultures or in laboratory animal model are restricted. Our roe deer are an excellent, realistic model as they are not domesticated and show a natural, strict seasonality, with a short rutting season in July and August. They were kept in outdoor enclosures under the influence of a natural photoperiod.

In order to effectively assess the mechanisms involved in spermatogenesis, basic knowledge on tissue structure and its seasonal changes are requiered. The aim of the present study was therefore to characterise roe buck spermatogenesis and to record seasonal changes in testis parenchyma quantitatively throughout the course of the year. In addition selected growth factors, known from previous studies to show seasonal patterns of RNA-expression, were detected immunohistochemically. These growth factors are the transforming growth factor beta 1 (TGFβ1), the transforming growth factor beta 3 (TGFβ3), the acidic fibroblast growth factor (aFGF) and the vascular endothelial growth factor (VEGF).

Every two months three roe bucks were castrated and samples taken for histology, electron microscopy and molecular biological investigations. The stages of spermatogenesis were characterised and seasonal changes in the composition of testis parenchyma examined by histomorphometry using a computer aided image analysis system. To demonstrate the exact localisation and quantity of Leydig cells and blood vessels, they were specifically marked by immunohistochemistry. Antibodies against the growth factors were tested for their specificity by western blot analysis and were then used in indirect immunohistochemistry.

Staging and histomorphometry

- The seminiferous epithelium cycle of the roe buck can be divided into 8 stages comparable to other ruminantia. These stages are only recognizable just before the rutting season in June, because the cell population within the tubuli seminiferi is subjected to distinct seasonal changes. There is little evidence for a dynamic equilibrium of mitosis, meiosis, spermiogenesis and spermiation.
- The mitotic activity of seminiferous epithelium reached its maximum before the rut. During rutting season only the meiotic activity is at its maximum and provides a large number of spermatids. The proliferation of other germinative cell types has already terminated and stages of spermatogenesis can be observed no longer.
- A quantitative evaluation of individual cell types within the testis is much more useful for the investigation of spermatogenesis in a seasonal breeding species than staging the seminiferous epithelium cycle.
- The results suggest that seasonal changes in testis parenchyma are caused by proliferative processes within the dynamic population of germinative cells and by functional rearrangement within a constant population of somatic cells.
- A simple mathematical model is consistent with the theory that the number of Sertoli cells and the number of interstitial cells remain constant throughout the year.
- The specific marker used to identify the Leydig cells depends on the level of differentiation of the cells. I therefore suggest that “seasonal variations” within Leydig cell population are most likely caused by dedifferentiation and redifferentiation of identical cells and not by proliferation and apoptosis.

Immunohistochemical detection of growth factors

- The seasonally determined mRNA expression patterns of the investigated GFs are reflected by the partially season and stage-specific localisation of the proteins.
- A comparison of the knowledge gained by histomorphometry with the data on the localisation of proteins and specific mRNA expression patterns suggests functions of these growth factors. TGFβ1 could be participating in apoptosis within the tubuli seminiferi. TGFβ3 seems to affect meiotic processes. aFGF might be involved in communication between Sertoli cell and spermatid, and VEGF could play a role within the testis parenchyma that goes beyond its previously suggested angiogenic effects.

My results suggest priorities for future studies. Concerning the seasonal changes within the interstitial compartment future approaches should target the specific evidence of proliferation and apoptosis. To gain a deeper insight into growth factor function it is necessary to analyse the receptor localisation and density for each cell type and season.