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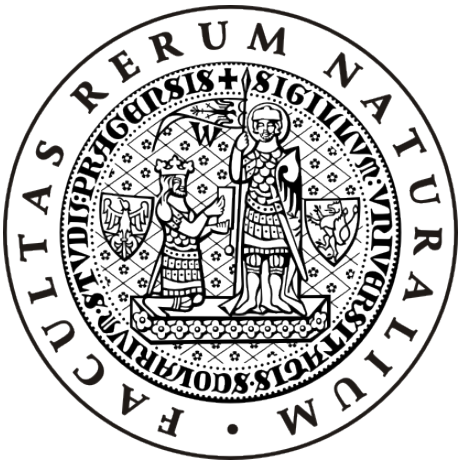

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Hormonal Aspects of Antler Growth Regulation

Doctoral thesis

Supervisor: Prof. Ing. Luděk Bartoš, DrSc.

Prague, 2011

Declaration:

Hereby I declare that I compiled this thesis independently, using only the listed literature and resources. I also declare that the presented work was not used to acquire any other academic degree.

Prague, 30.5.2011 Erika Kužmová

Antlers, such a beautiful and fascinating burden ...

Acknowledgements

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Contents

Abstract

Deer antlers are the only mammalian organ that completely regenerates and therefore they became an object of rising interest as a potential model for bone growth and development. In recent years, it has been confirmed that annual regeneration of the antler is initiated from the stem cell niche localised in the pedicle periosteum. Antlers grow to the length at the tip. Only a little is known about endocrine stimulation of antler growth and some discrepancy has arisen between *in vivo* and *in vitro* studies over the decades. As the secondary sexual character, the antler cycle timing and growth are linked to seasonal levels of testosterone. Since the levels are at their minimum during the antler growth phase, according to many mainly *in vitro* studies, insulin-like growth factor-1 (IGF-1) tends to be accepted as the "antler stimulating hormone".

Since the conclusion about the role of IGF-1 was contradictory to previous opinions and also in contrast with our own experience, we aimed to verify the role of IGF-1 *in vitro*. Our experiments were based on existing *in vivo* studies demonstrating the importance of testosterone, even in its low levels, and on the hypothesis that testosterone should be the "antler stimulating hormone". We performed *in vitro* experiments on cells derived from the growing antler tips of the red deer (*Cervus elaphus*) at various antler growth stages. Within *in vitro* cultivations we studied the effects of different factors such as antler sampling day, male individuality, passaging, concentration of foetal calf serum (FCS) and length of the experiment on the intensity of the antler cell proliferation. We found that all these factors not only significantly influenced the cell proliferation, but depending on these factors the intensity of proliferative response of cells from different individuals or under hormonal treatments was significantly changed. Next we studied the effects of various hormonal treatments as testosterone, IGF-1 and estradiol, as well as effect of antisteroids Cyproterone acetate, Flutamide and ICI 182,780, on antler cell proliferation. None of the treatments caused consistent proliferative response. However, testosterone and, partially, estradiol stimulated the proliferation in several cases. On the other hand, the stimulating effect of IGF-1 was not confirmed in our experiments, as IGF-1 either did not affect the antler cell proliferation or even inhibited it in some cases. We isolated STRO-1 positive mesenchymal stem cells from the mixed antler cell cultures but we could not perform hormonal experiments with the cells, as we were unable to obtain sufficient amounts of the positive cells for our experiments. Despite this, our results suggest that the sex steroids are mitogenic for antler cells *in vitro* and might play an important role in the stimulation of antler growth.

Our results are in accordance with many physiological and behavioural studies. They support the inevitable role of testosterone in the antler re-growth phase and suggest that the primary cultures may better represent the *in vivo* conditions and processes that occur in regenerating antlers.

Abstrakt

Parohy jeleňov sú jediným kompletne sa regenerujúcim orgánom u cicavcov a záujem vedcov o ich využitie ako modelu rastu a vývoja kostí stúpa. V posledných rokoch sa ukázalo, že regenerácia parohov je iniciovaná z kmeňových buniek lokalizovaných v okostici pučnice. Následný rast parohu do dĺžky však prebieha v rastovom vrcholčeku. Len málo sa vie o endokrinnej stimulácii rastu parohu a už dlhé roky existuje nesúlad medzi *in vivo* a *in vitro* štúdiami. Ako druhotný sexuálny znak sú parohy úzko späté so sezónnymi hladinami cirkulujúceho testosterónu. Keďže sú jeho hladiny najnižšie práve v čase rastu parohov a mnohé *in vitro* štúdie poukazujú na stimulačný efekt inzulínu podobného rastového faktoru (IGF-1), viacerí odborníci sa prikláňajú k názoru že IGF-1 je "hormón stimulujúci rast parohov".

Tento záver je ale v rozpore s výsledkami *in vivo* štúdií, ktoré ukazujú nevyhnutnosť testosterónu pre rast parohov aj v jeho nízkych koncentráciách, a taktiež s predchádzajúcim názorom, že testosterón by mal byť "hormón stimulujúci rast parohov". Zamerali sme sa teda na overenie účinkov IGF-1 na parožné bunky. Uskutočnili sme sériu *in vitro* experimentov na parožných bunkách izolovaných z viacerých štádií rastových vrcholčekov parohov jeleňa európskeho (*Cervus elaphus*). Počas *in vitro* kultivácií sme sledovali vplyv rôznych faktorov ako sú deň odberu tkaniva, individualita jedincov, pasážovanie, koncentrácia bovinného séra a dĺžka experimentu na intenzitu proliferácie parožných buniek. Zistili sme, že všetky tieto faktory signifikantne ovplyvnili proliferáciu buniek a dokonca sa vplyvom týchto faktorov menila intenzita proliferačnej odpovede buniek z jednotlivých jedincov, alebo na sledované hormóny. Bunky primárnych kultúr, kultivované v 10% bovinnom sére odobraté na 15. deň od zhodenia parožia proliferovali najintenzívnejšie. Ďalej sme sledovali účinky rôznych hormónov ako testosterónu, IGF-1 a estradiolu, ako aj účinok antisteroidov Cyproterón acetátu, Flutamidu a ICI 182,780 na proliferáciu parožných buniek. Žiadny z hormónov nevyvolával u buniek jednotnú proliferačnú odpoveď, hoci testosterón a čiastočne aj estradiol v niekoľkých prípadoch proliferáciu stimulovali. Naše experimenty však nepotvrdili stimulujúci účinok IGF-1. IGF-1 buď nemalo žiadny účinok, alebo proliferáciu vo viacerých prípadoch inhibovalo. Zo zmiešaných parožných bunkových kultúr sa nám podarilo izolovať STRO-1 pozitívne mezenchymálne kmeňové bunky. Žiaľ, pre hormonálne experimenty sa nám nepodarilo izolovať dostatočné množstvo týchto buniek. Napriek tejto skutočnosti, naše experimenty ukazujú, že pohlavné steroidy majú mitogénny vplyv na parožné bunky *in vitro*, a teda by mohli hrať dôležitú úlohu v stimulácii rastu parožia.

Výsledky, ktoré sme získali, sú v zhode s výsledkami mnohých iných fyziologických a behaviorálnych štúdií. Podporujú úlohu testosterónu vo fáze rastu parožia a ukazujú, že primárne kultúry pravdepodobne lepšie reprezentujú *in vivo* podmienky a procesy prebiehajúce v regenerujúcich sa parohoch.

Introduction to the Thesis

1 Introduction

Antlers have fascinated people since ancient times and prehistoric antlered deer paintings can be found in many European caves (Fig. 1). This is no wonder as antlers are an extravagance of nature, rivalled by few other biological luxuries as flowers, butterfly wings or peacock tail [1]. However, despite their exceptional growth and regeneration capabilities, little scientific attention has been paid to them. In the second half of the 20th century, highly regarded researchers such as Richard Goss, Zbignew Jaczewski, Anthony Bubenik, George Bubenik, Gerald Lincoln, Robert Brown and many others contributed to the field or even dedicated their lives to antler study. As Richard Goss stated in his outstanding monograph "Deer Antlers: Regeneration, Function, and Evolution", the study of antlers is a rewarding challenge, because in such an unexplored field as this, almost anything one learns is new discovery. Indeed, in the last decades interest in antlers as the only mammalian appendages capable of complete regeneration raised markedly and antlers attract not only zoologists and evolutionary ecologists but also researchers from various biomedical and pharmacological fields. The number of published scientific papers about antlers increases continually and this autumn "The 3rd International Symposium on Antler Science and Product Technology" will be held in China.

Still, almost after 30 years, Goss' words are relevant: "The mechanism by which these "bones of contention" grow and differentiate into such magnificent morphologies is a source of wonder and curiosity."

Figure 1: Sketch of a deer in The Cave of La Pasiega in Spain. By José-Manuel Benito Álvarez.

1.1 Antlers and Their Function

Antlers are a luxurious example of the secondary sexual characters unique to cervids. These cranial bony appendages are typical for males, but can be found also in females of reindeer *Rangifer tarandus*, or initiated in females of other deer species when administrating testosterone [2].

The original function of antlers is not known, and there is some controversy in what function was the primary and which ones were the secondary [1, 3, 4]. While some believe that the antlers developed primarily as weapons [3], paleontological findings indicate that antlers developed first as soft non-mineralised persistent appendages serving more as display and probably scent-dispensing organs than weapons. Only after antlers become mineralised do they serve as weapons in intraspecific male competition [4]. The annual renewal of antlers appears as the compensation of frequent breakages after aggressive encounters or as the adaptation to temperate zones preventing necrosis of frozen ends [5]. The antlers gain also other secondary functions and may be used for many purposes. They enable reindeer to find vegetation underneath the snow [6] or other deer species from the trees [7]. The elaborate palm structure of moose antlers may act as a parabolic reflector and enable moose males to better locate calling females [8]. The abundance of sebaceous glands in the velvet (specialized antler skin) of antlers supports their function as olfactory projectors. As antlers are richly vascularised and almost hot to the touch during the growing velvet period and the branched configuration increases the surface area, they might serve as thermal radiators during summer when males increase their metabolism to fortify themselves for the upcoming rutting period [9].

Regardless of their function, annual antler re-growth represents an incredible nutritional demand for deer and their development is associated with pathogen resistance, thus representing an honest signal of genetic quality [10]. Not surprisingly antlers play a major role in the social life of deer and serve as "social semaphores". They help to establish the rank order, obviate intraspecific conflicts since male combat may cause serious wounds, and not least they serve as intersexual display. Moreover antler size plays a significant role in sexual selection as an indicator of individual quality [11, 12]. As secondary sexual characters, antler growth is closely related to circulating levels of testosterone and both are modulated by other hormones, social position and agonistic behaviour [13, 14].

1.2 Antler Development and Annual Cycle

Antlers grow out from permanent extensions of the frontal bone called pedicles. Pedicles start to evolve during early prenatal development in males but disappear in the later prenatal stages [15]. Later at the time of puberty the males under the influence of testosterone, develop pedicles and start growing primary antlers. In red deer (*Cervus elaphus*), the primary and the later regenerated antlers are cast in spring and antler re-growth starts immediately (Fig. 2). In the next three months the antlers grow and elongate at the most spectacular rate in animal kingdom - up to 1cm per day on average. Moreover if one combines the rates of elongation of the

several tines growing simultaneously on both antlers, the production is as much as 10 cm of new antler material every day in the midseason. Such astonishing growth requires similar growth velocity of nerves and blood vessels and exaggeration of the normal mineral metabolism in the body to mobilise the vast quantities of calcium and phosphorus deposits each year into the regenerating antler [1]. For example, antlers of a 200 kg adult red deer may weight 30 kg [16]. After the rapid growth phase lasting approximately 100 days, antlers remain in velvet until they fully mineralise. In the late summer the velvet shedding begins and antlers are "ready" for the rutting period. Hard bony antlers are cast again in the spring and new antler re-growth follows.

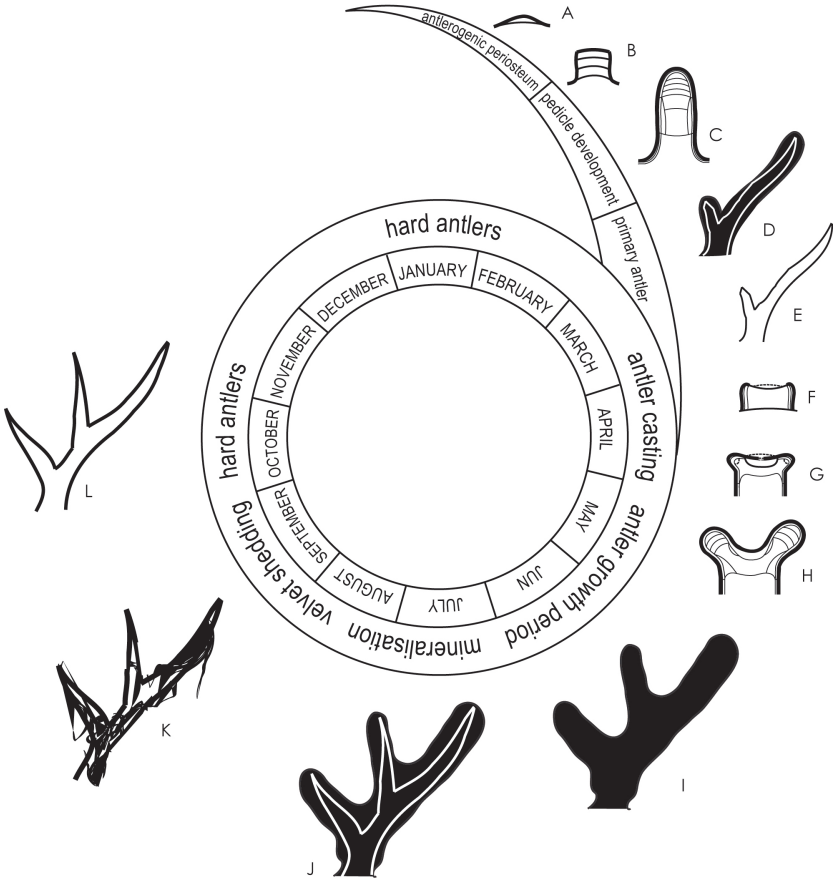

Figure 2: Development of the pedicle from the antlerogenic periosteum, the primary antler and the annual antler cycle. (A) antlerogenic periosteum as a thickening of the periosteum of the frontal bone, (B) development of the pedicle, (C) development of the primary antler, (D) primary antler in velvet, usually unbranched, (E) mineralised primary antler, (F) pedicle on the antler casting day "the stage of oil lamp bowl", (G) formation of the growth centres "the millstone-like structure", (H) formation of the main beam and the brow tine "small saddle stage", (I) branched velvet antler, (J) fully mineralised velvet antler, (K) velvet shedding, (L) hard antler. A, B, C, F, G, H adapted from Price et al. [16]

1.3 Pedicle and Antler Origin

Both pedicles and antlers are derived from so called "antlerogenic periosteum" overlying the frontal bone. The origin of antlerogenic periosteum has not been experimentally shown yet, but is likely to be neural-crest-derived as are other skull bones [17]. Even more, due to its remarkable capacity for self-differentiation and the fact that the cells contain abundant glycogen, the antlerogenic periosteum resembles a piece of post-natally retained embryonic tissue as noticed by Li and Suttie [15]. However, the expression of embryonic stem cell markers in antlerogenic periosteum has not been studied so far [18]. A transplantation of the antlerogenic periosteum onto the foreleg or forehead causes a pedicle and antler development at these sides [15]. On the other hand, recently it has been demonstrated that transplantation of pedicle periosteum cannot initiate antler development and its function is restricted to antler regeneration [19].

Primary antler growth and annual antler re-growth are initiated from a stem cell niche localised in the pedicle periosteum [20–22]. Progenitor cells isolated from pedicle periosteum as well as from the growing antler tip express markers of undifferentiated cells and differentiate along osteogenic, chondrogenic and with antler tissue unrelated adipogenic lineages *in vitro* [21]. Developmental signalling pathways involved in the control of skeletal development and regeneration in other vertebrates were also shown to be involved in antler regeneration [17].

1.4 Process of Regeneration and Structure of the Growing Antler Tip

As mentioned above, antler re-growth starts by activation of the stem cell niche localized in the pedicle periosteum [18, 21]. Prior to antler casting, these cells form a swollen rim around the distal pedicle [23]. At this place the osteoclast activity is the most intense and antler casting is initiated. After antler casting the exposed casting surface of the pedicle is rapidly covered by a migrating epidermis. The wound healing and formation of the antler bud and future growth centres occur very rapidly [1]. The morphological stages of the initial antler regeneration are nicely described in Chinese [23]. Immediately after antler casting, blood is retained in the depressed central top of the casting surface resembling a bowl. This stage is called "the stage of oil lamp bowl". The early wound healing stage occurs one or two days after antler casting, when the blood dries and a scab is formed. This stage is called "tiger eye stage". Once the diameter of the scab becomes smaller a "millstone-like structure with an axle", the scab, located in the centre is created. Finally, formation of the main beam and the brow tine is called "small saddle stage" and after the bez tine is created, the structure is called "the stage of silver ingot" [23].

Antler growth occurs at the antler tip. The growing tip is divided into zones [16] (Fig. 3). Under the velvet, the fibrous perichondrium is localised. This is followed by an intensively proliferating progenitor cell layer of reserve mesenchyme responsible for growth in length. Cells isolated from the mesenchymal zone have extended life span *in vitro* since they can be grown for over 80 passages and for

up to 10 months in culture before they stop dividing [24]. Under the mesenchymal zone, the prechondroblastic zone is situated. Cells in these zones start to arrange into longitudinal columns and are richly vascularised. Further proximally, the chondrocytes undergo maturation and the cartilage matrix is mineralized. During the special form of endochondral ossification, the mineralized cartilage is resorbed and completely replaced by bone [18].

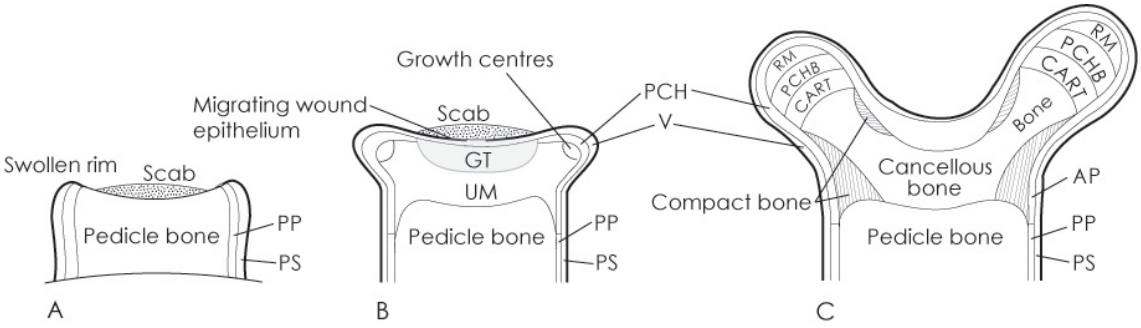

Figure 3: Antler growing tip development and zones. (A) pedicle on the antler casting day and swollen rim presence around the edge, (B) formation of the growth centres and of the scab, approximately 10 days after hard antler casting, (C) formation of the main beam and the brow tine, approximately 30 days after hard antler casting. PS-pedicle skin, PP-pedicle periosteum, PCH-perichondrium, V-velvet, GT-'granulation' tissue, UM- undifferentiated mesenchyme, AP-antlerogenic periosteum, RM-reserve mesenchyme, PCHB-prechondroblasts, CART-cartilage. Adapted from Price et al. [16].

1.5 Hormonal Regulation of the Antler Cycle

Since antlers play an important role in the social interactions of deer during the breeding time, their cycle is linked to the seasonal fluctuation of sex hormones which is regulated by changing day length [24]. Though regeneration of antlers is a complex process regulated by environmental and systemic factors, they show endogenous rhythms [1]. The function of other hormones as $1.25(OH)_2D_3$, thyroid hormones, cortisol and prolactin associated to the antler cycle are only poorly understood [16].

Generally, it is accepted that sex steroids, particularly testosterone, are required for pedicle and primary antler development and are the most important for the timing of the annual events in the antler cycle. While high levels of testosterone cause antler mineralization and velvet shedding in the late summer, their rapid decline below distinct threshold values during springtime cause antler casting [25, 26]. Castration of a calf prevents pedicle and primary antler development. Castration during the hard antler period causes a drop in testosterone levels and premature antler casting. Castration during the velvet period will delay velvet shedding and prevent full mineralisation of the antlers [16]. There is evidence that antlers of castrates are in fact benign tumours [18] and roe deer will react more massively than the other species by developing so called "peruke" [27].

During antler re-growth, systemic levels of testosterone are at their minimum and deer males are considered as almost "functional castrates" [24, 28]. Hence many authors have assigned only a minor role to testosterone in this phase, but this has been a matter of controversy over the decades [13, 29, 30]. Based on several *in vivo* and *in vitro* studies [28, 31–37] IGF-1 has become widely accepted as the "antler stimulating hormone" [24, 38]. However there exists sufficient evidence speaking for the need of low concentrations of testosterone for the stimulation of the antler regeneration [13]. Nonetheless still only a little is known about the endocrine stimulation of the early stages of antler re-growth, about the activation of the antler progenitor cells in the pedicle periosteum and their proliferation and differentiation into an antler bud [17].

1.6 Why to Study Antlers

Deer antlers are remarkable creations of nature offering an opportunity to study basal mechanisms of behavioural regulation of an honest secondary sexual character directly representing the hormonal background of the owner. Furthermore, they provide a unique model for studying developmental processes and complete regeneration of a complex bony organ in mammals [17]. As antler regeneration does not depend on innervation or direct contact between wound epithelium and mesenchymal tissue, they moreover demonstrate that the regeneration of a large bony appendage in mammals can be achieved by a different process as is the epimorphic regeneration in lower vertebrates [18]. Understanding of the underlying mechanisms may provide information to design therapeutic strategies for the diseased or damaged human tissues and help to elucidate why regeneration is limited in other mammals [16]. Recent findings of stem cell based origin of antler regeneration make antler regeneration even more relevant as a model for human bone, nerve and vascular regeneration [18, 21, 39–43]. It is worth mentioning that in addition to the rapid growth the antler innervating neurons show other remarkable characteristics as an amazing neuron survival after repeated axotomy and the ability to re-enter the growth/regeneration stage every year after more than 8 months of denervation [43].

The potential biomedical applications of antlers are far-reaching. Recently, establishment of a new stem cell line from antlerogenic cells and successful xenoimplantation of these cells has been reported [44, 45]. Antler bone has been used as a suitable scaffold material for bone tissue-engineering and bone reconstruction [46]. Last but not least, deer antler velvet is a promising farmacological product which has been used by Oriental cultures for thousands of years. Antler velvet has long been used as a traditional medicine for relieving pain, to combat aging, increase energy, stimulate muscle growth or enhance sexuality. It is believed to have anti-inflammatory, anti-cancer, immune stimulative and pro-growth effects, but not all of them have been experimentally proved yet [47, 48].

Another important fact to mention is that due to castration antlers develop tumour-like structures permanently covered in velvet. The answer to the question of why antlers develop benign tumours in the absence of sex steroids and appear

resistant to malignant cell transformation might have important implications for cancer biology [18].

1.7 Antler Study and Its Limitations

Study of antlers in general brings some difficulties along the way. The seasonal nature of antler growth limits the number of experiments that can be undertaken each year. The fact that deer is not a "mainstream" organism reduces the commercially available deer specific antibodies for immunohistochemical and immunocytochemical studies and limits the usage of molecular methods. However, the recent announcement of the sequencing of a substantial portion of red deer genome by researchers from New Zealand is a milestone for the deer industry and may bring benefit also to antler research. The utilization of deer as a model organism is however limited not only by difficulties of potential genetic manipulations but also by the demands of extensive *in vivo* experiments. To overcome these limitations, the xenograft approach of deer tissue transplantations into nude mice (Fig. 4) has the potential to become an appropriate tool to study the underlying mechanism of antlerogenesis and organogenesis/regeneration in general, although more research is required to further develope this model [49].

Figure 4: A pedicle-shaped protuberance (arrow) formed from the subcutaneously transplanted antlerogenic periosteum on a nude mouse head (from Li and Suttie [15], with permission of the author).

The more advanced knowledge and methods about bone development and regeneration processes in other model organisms together with our desire for deeper understanding of the underlying mechanisms of antler regeneration draw our attention to signalling pathways and local molecules involved in antler regeneration. Hence less attention is paid to the hormonal regulation and physiological mechanisms of antler re-growth although one of the most important questions yet to be satisfactorily examined is the identification of the "antler stimulating hormone" or "antler growth stimulus". Here there is no unified opinion whether testosterone or

IGF-1 is the main factor responsible for antler growth and the discrepancy is mainly between the *in vivo* and *in vitro* reports. While the majority of the *in vivo* studies support the role of testosterone [13, 29, 30], many of the *in vitro* studies show the mitogenic effect of IGF-1 on antler cells [32–36]. However, there are several issues related to the *in vitro* cultivation of antler cells. First, there are several factors that might modify the proliferative response of the antler cells *in vitro* and which have not been satisfactorily investigated in the existing literature. Second, all *in vitro* hormonal studies were performed on mixed antler cell populations containing different types of progenitor mesenchymal cells, chondro- and osteo-progenitors, chondroblasts, or even osteoblasts.

One way to overcome some of the above mentioned problems would be to use the defined cell populations instead of mixed antler cell populations. Such attempts were for the first time performed by Rolf et al. [21, 22] and resulted in isolation of "pure STRO-1+ mesenchymal stem cell cultures" derived from pedicle periosteum or regenerating antler tip. However, surprisingly high numbers of these cells could be isolated (up to 38% from fallow deer cultures and 16.5% from red deer cultures). One has to face problems connected to maintaining the undifferentiated state of the isolated cells as well as obtaining sufficient amounts for extensive hormonal experiments.

2 Aims of the Study

The presented work deals with the *in vitro* experiments on cells derived from the growing antler tips at various antler growth stages. The aims of this study were:

1. **To investigate factors influencing antler cell proliferation *in vitro*.** Among existing studies, we found out that some factors were not satisfactorily investigated, but might influence the proliferative response of antler cells. The important ones in our opinion were: tissue sampling date, deer individuality and factors of culture conditions such as effect of foetal calf serum concentration, passaging or length of the hormonal treatment.

2. **To examine the proliferative response of mixed antler cell populations to hormonal and growth factor treatments (particularly to testosterone, estradiol and IGF-1) alone or in the co-treatment with antiandrogens cyproterone acetate and flutamide and antiestrogen ICI 182,780 under varying experimental design.**
   If one of the treatments is the "antler stimulating hormone", its mitogenic effect should be present in all culture conditions and identically in cell cultures of all sampling days.

3. **To isolate mesenchymal stem cells out of mixed antler cell cultures and to perform hormonal experiments with "pure" antler cell populations.**
   If antler renewal is caused by re-activation of stem cells in the pedicle periosteum and the antler growth is localised to the growing antler tip, where the progenitors proliferate, then the hormonal experiments performed on such cells would be of high significance in answering the question of "antler stimulating hormone".

3 Conclusions

Our experiments confirmed the significant effect of factors antler sampling day, male individuality, passaging, foetal calf serum concentration and length of the experiment (hormonal treatment) on the antler cell proliferation *in vitro*. The cultivation factors, mainly passage, also significantly influenced the number of stem cells obtained from the mixed antler cell cultures.

The proliferative response of antler cells to hormonal treatments varied significantly with respect to all the factors. We observed significant difference in the proliferative response of antler cells between two examined concentrations of foetal calf serum. In the high concentration, the responses were more intense and for some treatments even opposite to the ones in the low concentration. The same goes for primary versus passaged cultures. As for the sampling day, the cells sampled on the 15th day after antler casting proliferated the most intensively. In our experiments we did not observe any consistent effect of the treatments. Generally, testosterone stimulated or did not show any effect on the antler cell proliferation. In contrast, IGF-1 did not stimulate or even inhibited the antler cell proliferation. Antisteroidal treatments and estradiol showed no general trend. Unfortunately we could not perform hormonal experiments on the stem cells isolated from the mixed antler cell cultures, since we were unable to obtain sufficient amounts of positive cells or to expand their numbers while keeping the undifferentiated potential. Despite this fact, our findings suggest that sex steroids play an important role in stimulation of antler growth but their effect seems to be time- and antler-stage dependent. In addition, we could not confirm the mitogenic effect of IGF-1 reported by the previous *in vitro* studies [32–34]. Our results are in accordance with many physiological and behavioural *in vivo* studies and support the role of testosterone in the antler re-growth phase [13]. Furthermore, our results suggest that the primary cultures may better represent *in vivo* conditions and processes that occur in regenerating antlers.

In conclusion, we believe that testosterone might be the "antler growth stimulus", but since there are many factors influencing antler cell proliferation *in vitro* and antler regeneration/re-growth study is in its beginning, there is a need for further and more detailed experiments that could confirm this hypothesis.

4 Summary of Papers

The thesis consists of four papers. Each of them is presented in the following separate chapter.

Paper I
Effect of different factors on proliferation of antler cells, cultured *in vitro*. Erika Kužmová, Luděk Bartoš, Radim Kotrba, George A. Bubenik (2011) PLoS ONE 6(3): e18053. doi:10.1371/journal.pone.0018053

Paper II
Factors affecting the number of STRO-1+ stem cells derived from regenerating antler and pedicle cells of red and fallow deer. Erika Kužmová, Radim Kotrba, Hans J. Rolf, Luděk Bartoš, Günter K. Wiese, Jutta Schulz, George A. Bubenik (2011) Animal Production Science 51 (4) pp. S35-39.

Paper III
The effect of testosterone and IGF-1 on antler cell proliferation *in vitro*. Erika Kužmová, Luděk Bartoš, Radim Kotrba, Hans J. Rolf, George A. Bubenik. *Submitted to an international journal.*

Paper IV
Endocrine relationships between rank-related behavior and antler growth in deer with a focus on *in vivo* studies. Luděk Bartoš, George A. Bubenik, Erika Kužmová (2011) Frontiers in Bioscience. *In press.*

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PAPER I

Effect of Different Factors on Proliferation of Antler Cells, Cultured in Vitro

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PAPER II

Factors Affecting the Number of STRO-1+ Stem Cells Derived from Regenerating Antler and Pedicle Cells of Red and Fallow Deer

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PAPER III

The Effect of Testosterone and IGF-1 on Antler Cell Proliferation in Vitro.

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PAPER IV

Endocrine Relationships between Rank-Related Behavior and Antler Growth in Deer with a Focus on in Vivo Studies

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