[Frontiers in Bioscience E4, 1111-1126, January 1, 2012]

Endocrine relationships between rank - related behavior and antler growth in deer

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1. ABSTRACT

In this review, we analyze endocrine aspects of the relationships between antlerogenesis and rank-related behavior. The explanation of these relationships has been based on the presumption that the antler growth is regulated by hormones modulated by agonistic behavior. Originally, we assumed that these relationships are primarily testosterone dependent. In the eighties, it was reported that the insulin-like growth factor 1 (IGF-1) is the antler-stimulating hormone. This hypothesis was supposed to replace an earlier theory that the antler-stimulating hormones are either androgens or their derivatives. Here, we present historical and recent views on these issues. In particular, we analyze the arguments in favor and against the role of testosterone and IGF-1 in antler growth and present a comparison of the results obtained across some deer species. In this context, we review and discuss experiments with castration of various deer species and analyze data from papers dealing with *in vivo* studies. We conclude that testosterone and not IGF-1 is the main antler stimulating and regulating hormone, and that concentrations of testosterone may be modified by social behavior.

2. INTRODUCTION

There are a number of reviews focused directly or indirectly on the neuroendocrine regulation of the antler cycle (1-6). It is not our intention to present another one. The present review is based on our investigations lasting over 30 years which were focused on the relationship between dominance rank - related behavior, antler cycle timing and antler growth in deer studied in several species. With the exception of a few details, most behavioral aspects are reviewed elsewhere (7). Here we discuss possibilities of endocrine control of the relationship between dominance rank - related behavior, antler cycle timing as well as antler growth. We concentrate predominantly on the role of supposedly "antler stimulating hormones" such as testosterone and IGF-1. Several decades ago Goss demonstrated (8) that growing antlers were more sensitive to estrogen than testosterone. As reviewed by Riggs *et al.* (9), in males and females the effects of testosterone on the skeleton are indirect, occurring after its local conversion to estrogens by aromatase (6). Higher testosterone and lower 17 beta estradiol concentrations found in plasma compared to antler bone or antler velvet, may indicate a partial conversion of systemic androgens

into estrogens in the tissues of growing antlers (10). In summary, when we discuss testosterone, we do not extend the discussion into possible effects of other androgens (11), estrogens (6) or the possibility that estrogens may be converted locally from testosterone by aromatase (3, 4, 10, 12-14).

In a study on captive red deer *Cervus elaphus* we demonstrated that males of higher rank cast their antlers first and also tended to shed the velvet earlier than subordinate ones (15, 16). In subsequent studies performed on the same species we found evidence that the social position and the related agonistic activity of males during the velvet period influence antler weight and length and the number of points. These studies have suggested that the antler size is a consequence of the previous social position and not vice versa (16, 17). Later we presented evidence that in fallow deer *Dama dama* the changes in behavior, which were related to rank, modified antler growth. Males gaining a higher rank through fighting other males exhibited enhanced growth of that part of the antler that was just growing. This situation changed, if the male lost his position (18). Detailed descriptions of the behavioral aspects of all these relationships are presented elsewhere (7).

We also attempted to explain possible endocrine mechanisms responsible for these results. Some time ago it was believed that correlations between social dominance and levels of hormones, modulated mainly by agonistic behavior exist, and that the changes of hormone levels associated with agonistic interactions are crucial and long lasting (19). Dominant animals were expected to have generally lower pituitary/adrenocortical activities than submissive animals living with them. Males with a dominant position usually tended to have elevated androgen levels (20). Conversely subordinate status seemed to be associated with lower androgen secretion and increased levels of glucocorticoids (21, 22). Therefore, since the very beginning of our investigations, we assumed that the mechanism of the relationship between rank position and antler cycle timing lies in presumably elevated levels of testosterone in dominant males and decreased concentrations in subordinate individuals (15, 16).

Testosterone was a candidate hormone for several reasons. First, we believed testosterone was involved in the regulation and development of antlers (3, 15, 16, 23). Secondly, testosterone promotes the development of secondary sexual characteristics across many species of the animal kingdom (24, 25). Thirdly, as mentioned above, testosterone concentrations can be modified throughout behavior due to a feedback. However, the first prediction, that testosterone is involved in the regulation and development of antlers, was not that clear.

3. ANTLER DEVELOPMENT

3.1. Antler growth and testosterone

Antler development is a dynamic multi-factorial process reflecting changes in the environment. No wonder, generations of deer biologist have attempted to answer the question of what is the main antler growth hormone (3). As shown in many studies, antler development is invariably associated with an increase of testosterone concentrations across cervid species, such as white-tailed deer, *Odocoileus virginianus* (26-29), Columbian black-tailed deer, *Odocoileus hemionus columbianus* (30), roe deer, *Capreolus capreolus* (31-33), red deer (34, 35), axis deer, *Axis axis* (36, 37), fallow deer (38-42), rusa deer, *Cervus* (*Rusa*) *timorensis* (43), Eld's deer, *Cervus eldi thamin* (44), and pudu, *Pudu puda* (45). All these reports provide good arguments in favor of accepting testosterone as the hormone supporting antler growth. On the other hand, it has been also accepted that increasing seasonal levels of testosterone cause cessation of antler growth by mineralization of the antlers, shedding of the velvet, and the attachment of the dead antler to the pedicle, as observed across various deer species (3, 46, 47). However, a considerable increase in testosterone concentration which causes the mineralization of antlers and the shedding of velvet occurs only after the cessation of antler growth and the completion of antler bone development (3, 48). The antler casting is generally associated with a rapid decrease of seasonal levels of testosterone (3, 8, 46). Nevertheless, already in the classical experiments with castrated white-tailed deer, Aub and Wislocki (46, 49) stressed the importance of testosterone in antler growth induction. By giving testosterone to their males, which had never had antlers as a result of castration as fawns, they induced antler growth. Similarly, administration of testosterone to ovariectomized female deer, or a blockade of ovarial function of a doe with an antiestrogen, caused them to grow antlers (46, 49, 50). In roe deer, low levels of testosterone initiated not only growth of the pedicles but also a subsequent growth of antlers (51). On the other hand, high testosterone levels prevented any growth of pedicles on the same deer. Later on (52) a small amount of androgens given to male sika deer in food during the velvet period stimulated their antler growth. In the velvet antlers of white-tailed deer, Bubenik *et al.* (53) localized immunohistologically testosterone in the prochondral blastema layer, i. e. in the preosseous cartilaginous zone responsible for cartilage matrix synthesis but not in the ossification zone. The authors suggested that the potential importance of this hormone is in the bone matrix synthesis and not in ossification. At that time it was already accepted that in humans a low concentration of testosterone can stimulate bone growth, whereas larger doses can be inhibitory (54). That inspired Brown *et al.* (55) to perform a study in white-tailed deer. They determined the relationship between serum androgen concentrations and changes of relative bony density in the antlers and long bones of male deer sampled twice a week during the antler growth period. Circulating androgen concentrations increased over the entire antler-growth period, as did the relative bone mass (RBM) coefficients of the antler. Brown and co-workers found positive correlations between increasing androgen concentrations and increasing antler RBM and negative correlations between androgens and decreasing RBM of the metacarpus. The antler RBM coefficients continued to increase after polishing of antlers, but metacarpus RBM did not change after velvet shedding. Two castrated deer were injected

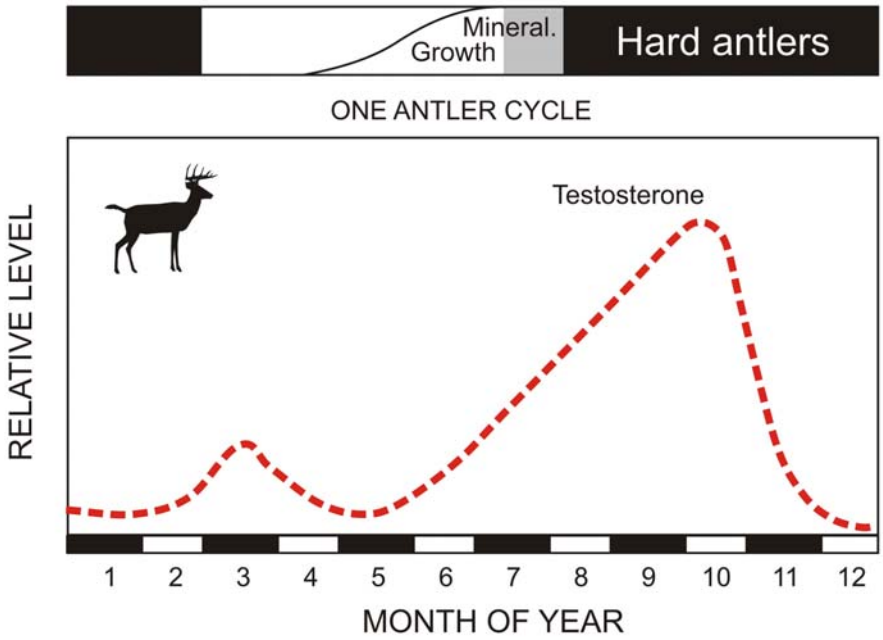

Figure 1. Stylized time course of hormonal levels during the antler cycle of a white-tailed deer (*Odocoileus virginianus*). Adapted with permission from Bubenik (3).

subcutaneously with 1g of testosterone and sampled every other day. Similar but smaller changes occurred in RBM values of the metacarpus and developing antler in castrated deer injected with testosterone. Their experiments on white-tailed deer were in agreement with earlier results published in roe deer by Tachezy (51). In both species the authors indicated that low serum concentrations of testosterone can stimulate bone growth, while higher levels will cause inhibition (51, 55).

Based on these data it was hypothesized that new antler growth may be initiated by a short reactivation of reproduction and hence resulting in a testosterone pulse (Fig.1) (3, 15, 56, 57). This suggestion was later supported by studies showing a short-termed peak of testosterone in the period of antler regrowth in several deer species such as roe deer (32, 33, 58), white-tailed deer (27, 59, 60), wapiti (61), red deer (62-64), fallow deer (38, 41), black-tailed deer (30), etc.

In summary, we interpreted a link between rank - related behavior and antler casting as follows: We would expect that the effect of testosterone on the initiation of antler regrowth and consequent antler casting is modulated by behavior and thus is being involved in the formation of actual hormone levels. The short reactivation of reproduction and hence testosterone pulse (Figure 1), which probably triggers antler regrowth (3, 15, 57), occurs during the period of lowest seasonal levels of testosterone. The more dominant males have earlier, higher and more frequent testosterone pulses during low seasonal concentrations of testosterone. This testosterone range corresponds to the Tachezy's (51) and Brown *et al.*'s (55) 'low amount' of androgens. A new antler bone growth may thus be initiated more vigorously and the antler casting in species like red deer (Group A type casting in (16)) may occur earlier (15, 16). Also in deer males such as seen in white-tailed deer (Group B type of casting in (16)), new antler growth of dominants may start earlier even though antler casting had occurred later. More about the different group types of casting is presented elsewhere (16).

To explain the relationship between rank - related behavior and antler cleaning seems to be less complicated because antler polishing occurs in the time of seasonally elevated testosterone concentrations. The stimulatory effects of social interactions among dominant males probably elevate the levels of testosterone, while the interactions elevate glucocorticoids and depress testosterone levels in subordinates (16). As a result, antler cleaning may occur earlier in dominants and later in subordinates. Many authors have suggested that antler cleaning dates are fully dependent on age, such as it happened in red deer (65-67) and in other cervids (68). With the exception of fallow deer (69), spike-antlered deer polished antlers later than fork antlered males in red deer (65, 67, 70, 71), white-tailed deer (68, 72, 73), and moose *Alces alces* (74). However, both the earliest and the latest cleaning dates were also observed among yearlings in fallow deer (75, 76), in white-tailed deer (73), moose (74), and in our red deer herd (77). In contrast to some of the above mentioned reports, the alpha males were not usually the oldest ones in our study population (78). And, indeed, the antler casting/cleaning times of individual males appeared to be dependent primarily on their social status and the influence of age was of secondary importance (15). Hence we concluded that for the above alternating results this variation is due to different opportunities for social

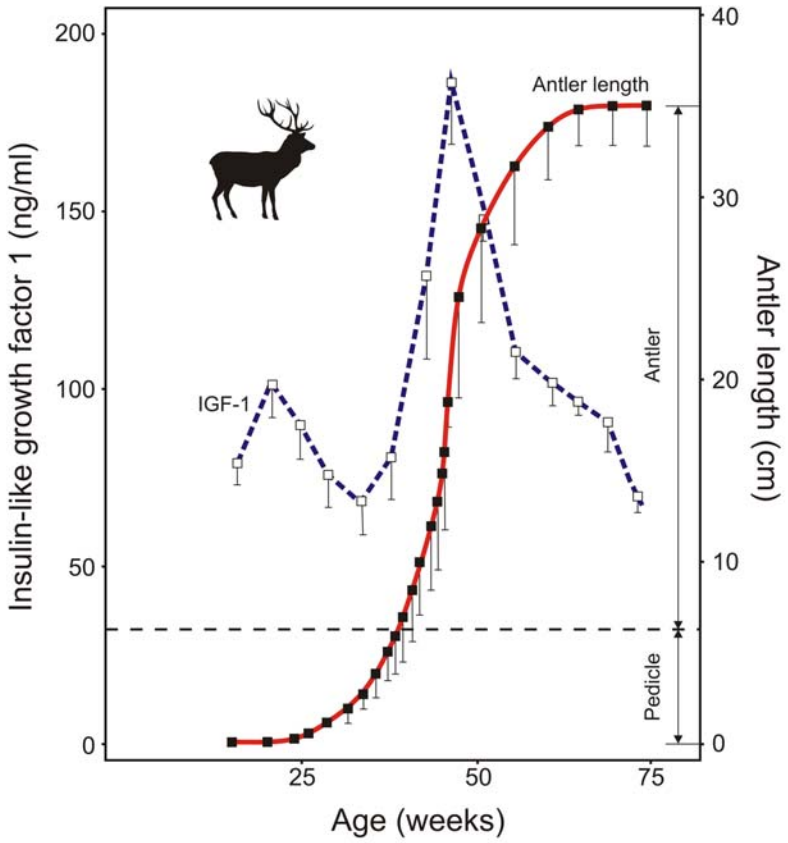

Figure 2. The relationships between insulin-like growth factor 1, antler length and calendar time in red deer (*Cervus elaphus*) yearlings. Adapted with permission from Suttie *et al.* (34).

grouping. It brings a differential social stimulation of the process, as well as diverse opportunity to be stressed (16).

In addition, the relationship between rank - related behavior and antler growth seemed logical. The physiological consequence of the male's behavior on his antler growth may have acted since the beginning of the velvet period. The more dominant a male is, the higher the seasonally attained levels of androgens within the actual physiological range and the greater the enhancement of antler formation (16).

3.2. Antler growth and IGF-1.

In the mid-1980s Suttie *et al.* (34) compared the seasonal variations of hormones with the progress of antler growth in red deer (Figure 2). Based on their data, they concluded that the insulin-like growth factor 1 (IGF-1) is the antler-stimulating hormone. In the subsequent study they aimed to answer the question whether IGF-1 acts on antler growth through the general blood circulation or if it is of local origin. They completely cut growing antlers and observed a significant elevation of plasma levels of IGF-1 in the non-antlered stags compared with normal antlered stags during the antler growth period. Therefore they concluded that the growing antler is a target organ for IGF-1 and that the prevention of antler growth removed a population of IGF-1 receptors (79). A similar increase of IGF-1 concentrations corresponding to the progress of antler growth was also reported by Schams *et al.* (80) in roe deer, and Reyes *et al* (45) in pudu.

The hypothesis that IGF-1 is an antler-stimulating hormone displaced an earlier notion which suggested that the antler-stimulating hormones are androgens, particularly testosterone or its derivatives (3, 4, 15, 53, 55). In 1992 Suttie *et al.* (81) stated that „The male can be considered almost a functional castrate for the first few weeks of velvet antler growth."

3.3. Antler development and castration

The principles of the effect of castration were first described by Aristotle (8, 57) and confirmed in many modern experiments performed since the 1930s and 1940s, either in Europe (82) or North America (46). More recently, several laboratories focused on the evidence indicating that testosterone does not play any significant role in the stimulation of antler growth. They neutered male deer either by administration of androgen receptor blocker or by surgical castration.

To elucidate the participation of testosterone in the formation and maturation of growing antlers, the influence of antiandrogen cyproterone acetate (CA) on the antlers was studied in several species. In a pioneering study

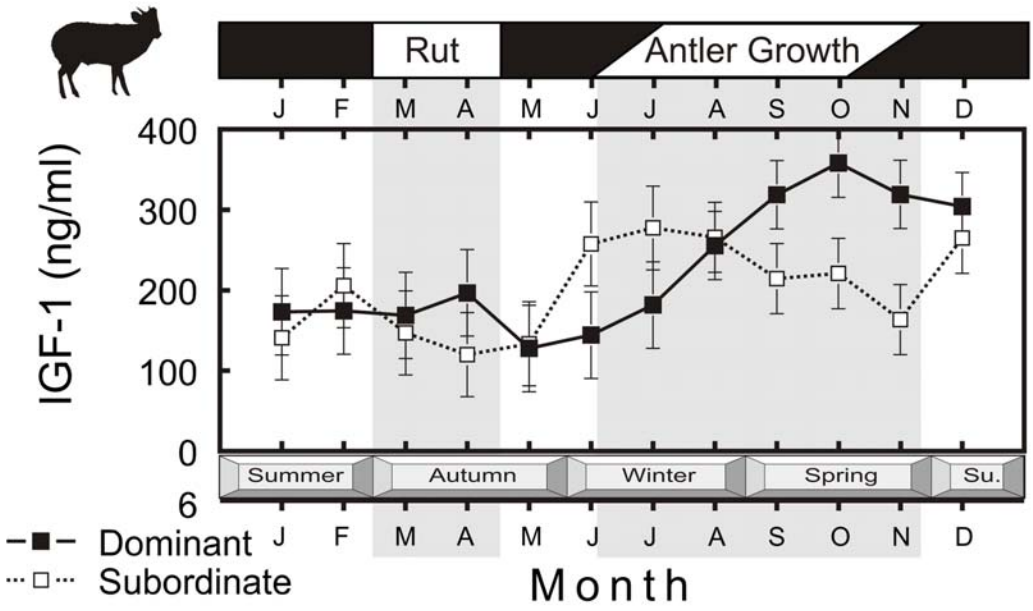

Figure 3. Adjusted means (± S.E.) of seasonal levels of IGF-1 and testosterone according to rank of pudu (*Pudu puda*) males. Shaded areas represent the time of the rut and the velvet antler period. Adapted with permission from Bartoš *et al.* (94).

on white-tailed deer yearlings (23), the growth period of antlers, after CA application, was delayed by three months, in comparison to intact controls. The CA treatment, introduced in the second half of the normal antler growth, resulted in an incomplete mineralization of the antler beam, delayed velvet shedding and a diagonal sequestration of the top portion of the antlers. We observed an incomplete formation and mineralization of the Haversian systems. The antler structure of the tips was similar to that observed in white-tailed castrates (23). Schams *et al.* (83) then reported that CA treatment did not inhibit antler growth in intact roe bucks. It only delayed the velvet shedding until after the end of the treatment period. When CA was applied outside of the season of antler casting, CA treatment did not prevent antler growth nor the attainment of a species-specific antler shape in intact fallow bucks (84, 85) and red deer stags (86). In all these studies antler growth was nearly normal and of species-specific shape. Similarly, the morphology and histological structure of antlers grown after surgical castration of adult fallow deer bucks also remained comparable to intact individuals. Only the velvet was not shed (87). The general believe of various researchers was that antler morphogenesis proved to be non-androgen dependent (84). On the other hand, non-species specific shape of antlers were described in fallow deer castrates (42), white-tailed castrates (88), white-tailed deer after CA (23), in hypogonadic white-tailed deer (89, 90), and presumably hypogonadic California mule deer *Odocoileus hemionus californicus* (91), etc. Also the formation of Haversian systems in the growing antlers was substantially affected by CA (92). Despite these findings some authors still insisted that species-specific antler growth can occur without testosterone stimulation (84, 86, 87). More recently, Kierdorf *et al.* (93) discussed in detail that the antlers of fallow deer castrates show histological signs of immaturity due to the lack of androgens. The overall antler growth, attainment or not of a species-specific shape of the antlers, and the question of the maturation of antler bone, is rather complicated process. The discussion how individual stages of these processes are influenced by androgens is beyond the topic of this review.

3.4. Antler development and IGF-1 in different deer species

The concept that velvet antler growth can occur without testosterone stimulation during the period of velvet growth challenged our speculation about the hormonal base of the relationships between rank - related behavior and the antler development (3, 4, 15, 16). Therefore, we focused first of all on finding the possibility of a link between rank - related behavior and IGF-1. In a study on pudu (94), the analysis revealed that from September to November (the second part of the antler growing period and the time of establishing territories) the IGF-1 levels of dominant males were significantly higher than those of subordinate males. This finding supported the concept that IGF-1 is the antler stimulation hormone. Concurrently, however, these results induced two kinds of doubts. The first doubt was associated with very little evidence about the possible relationship between IGF-1 levels and dominance. At the same time when we performed our study on pudu, Sapolsky & Spencer (95) reported that IGF-1 was suppressed in their socially subordinate baboons. Though, as far as we know, since that time no other study which would show lower concentrations of IGF-1 in subordinate individuals has been published. The second doubt was even more serious.

As mentioned earlier, we determined an increase of IGF-1 concentrations corresponding to the progress of antler growth in pudu (45). When we looked at the increase of IGF-1 from the point of view of the male

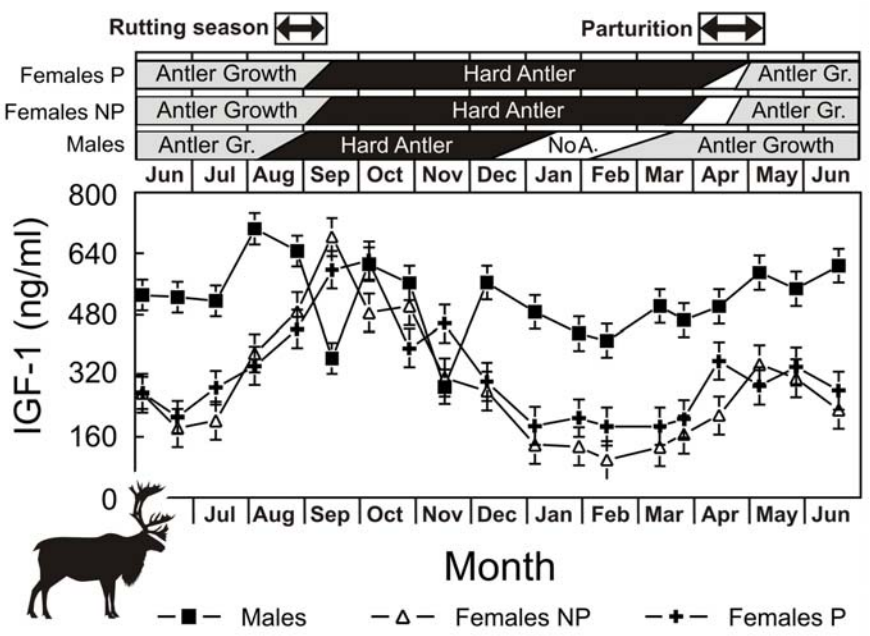

Figure 4. Adjusted means (± S.E.) of seasonal levels of IGF-1 in male and pregnant and non-pregnant female reindeer (*Rangifer tarandus*). Adapted with permission from Bubenik *et al.* (96).

dominance, the increase of IGF-1 corresponded with the progress of antler growth in dominant but not subordinate males (94). Still all males produced antlers (Figure 3). More than that, analyzing hormonal profiles in reindeer males and females, we found that a trend of elevating IGF-1 was associated with growing antlers in males but not females (Figure 4), either pregnant or non-pregnant (96).

4. *IN VITRO* CULTIVATION OF ANTLER TISSUES IN RELATION TO IGF-1 AND TESTOSTERONE

The role of IGF-1 and androgens, particularly testosterone, in the proliferation of antlerogenic cells *in vitro* is still not satisfactorily elucidated and remains controversial. In antler cell culture experiments, IGF-1 was found to stimulate the proliferation of antlerogenic cells from the antler tip (97, 98) and played a role in the regulation of antler growth (6). Similarly, Li *et al* (99) showed that IGF-1 stimulated the proliferation of antlerogenic cells from the antler pedicle in various ossification stages. On the other hand, in our recent study IGF-1 did not have any effect or even inhibited proliferation of cells obtained from the antler tip (100).

Reports on testosterone also vary greatly. Sadighi *et al.* (101) did not observe any stimulating effect of testosterone on antler cells proliferation. Moreover they showed that testosterone at certain levels suppressed the mitogenic effects of IGF-1 on the antler-tip cells. Identically, in the experiments of Li *et al.* (99) testosterone alone did not show any mitogenic effects, but in the presence of IGF-1it increased proliferation at certain ossification stages of the pedicle. On the other hand, Price *et al.* (2) stated that testosterone will induce the proliferation of cells cultured from antlers depending on the stage of antler growth and the stage of cell differentiation. Similarly, in the study of Rolf *et al.* (102) sex hormones (testosterone, dihydrotestosterone) stimulated the proliferations even in high concentrations. Finally, our experiments also showed proliferative effect of testosterone (100).

Some of the above-mentioned studies were performed in the serum-free conditions (103), which could be regarded as physiologically optimal, while others used serum in various concentrations (97), because it is difficult to keep the tissue growing without it. Both approaches have supporters as well as critics. Clearly, this could be one of the major reasons of the discrepancy between the studies. Even the presence of serum during the precultivation and passaging (103) could be important as this could influence later proliferation response of cell cultures (104).

In conclusion, the antler cell culture experiments have been performed under widely variable conditions. Cells were obtained from pedicle (98, 105) or antler (101-103, 106), from different stages of antler development and growth, cultivated either as primary cultures (106) or after two passages (101-103, 105), grown in medium containing fetal calf serum (98, 101-103, 105, 106) or partially cultured in serum free conditions (98, 101, 103, 105).

As stressed by Borenstein *et al.* (107) obvious limitation of a "narrative review", such as that in the previous paragraph, is the subjectivity inherent in this approach. Different authors might use different criteria for deciding which studies have relevance and which do not. To overcome this, we applied meta-analysis using Comprehensive Meta-Analysis software (Biostat,

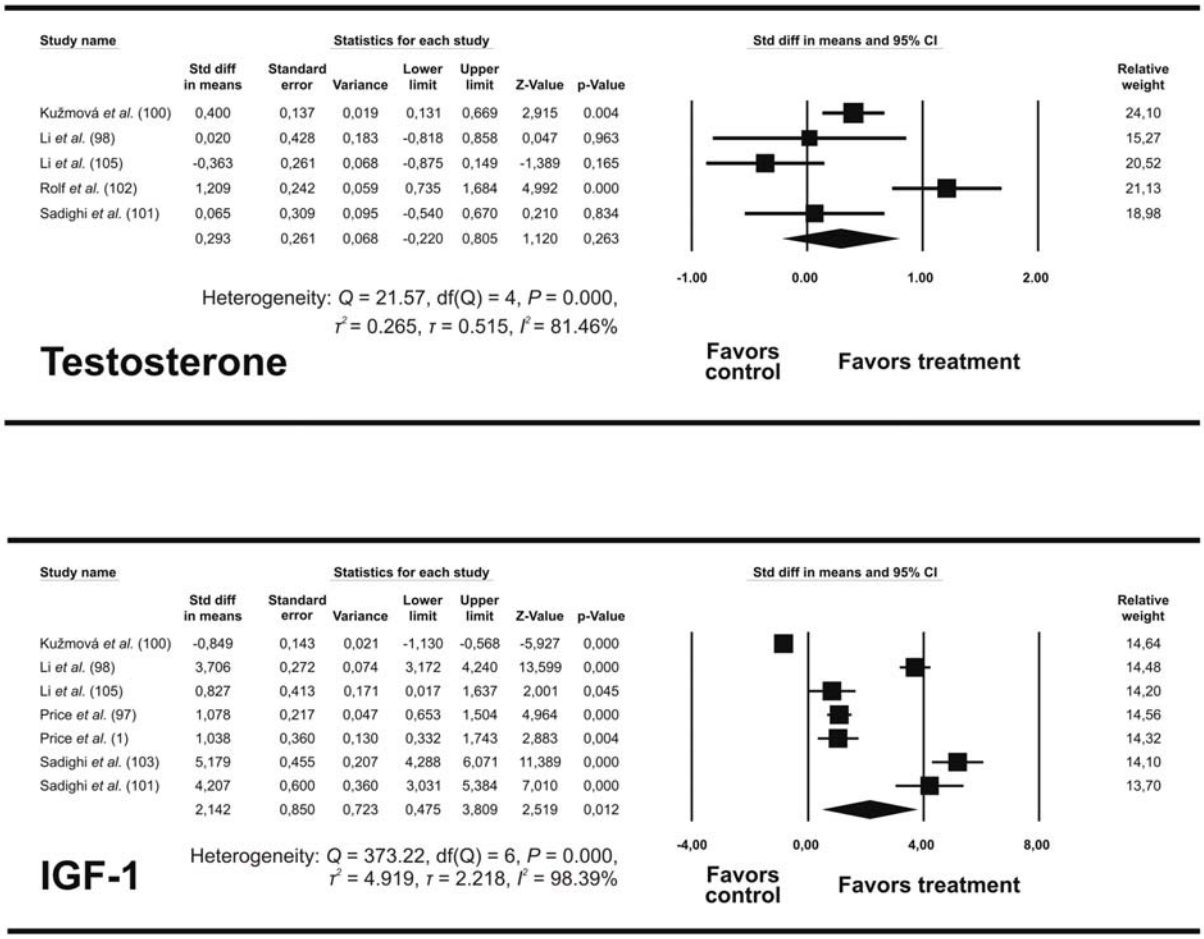

Figure 5. Forest plots showing meta-analysis of studies reporting possible effect of testosterone (top) and IGF-1 (bottom) on antler tissue proliferation *in vitro* expressed as the means with standard deviations and sample size between treated and control groups. Mean differences are shown with 95 per cent confidence intervals. The size of the marker indicates the weight of the study. The summary effect is displayed by the diamond.

Englewood, NJ). Our goal was to analyse all studies refering to the effect of either testosterone or IGF-1 on proliferation of antler cells *in vitro* in order to see if there is a common robust trend of the effect. The studies focussed on an interaction between the two hormones were excluded. For testosterone we collected five studies. Each study was comprised of several experiments which entered the analysis as subgroups. There were 16 subgroups in (100), 4 in (98), 5 in (105), 26 in (102), and 8 in (101). For comparing the studies investigating the effect of IGF-1, 7 studies were available with the number of subgroups 12 in (100), 32 in (98), 3 in (105), 15 in (97), 3 in (1), 20 in (103), and 6 in (101). In the analysis, the subgroups were always combined within the study. All the studies were based on comparing the means with standard deviations and sample size between treated and control groups. Assuming that the true effect size varies from study to study, and the summary effect is our estimate of the mean of the distribution of effect sizes, we used the random-effects model (107). We applied the Q statistic test, Tau-squared, Tau, and inconsistency index (I^2) to estimate the heterogeneity (108) of individual studies contributing to the pooled estimate (107). The results of the meta-analysis are shown in Figure 5 (top for testosterone and bottom for IGF-1). Studies investigating the effect of testosterone reported much more variable and contradicting results either within or between studies than those of IGF-1. The overall summary showed a significant trend for IGF-1 (favouring the IGF-1 treatment in comparison to control) and not for testosterone studies. As such it would somewhat contradict the *in vivo* studies. On the other side, all measures of heterogeneity are very high in both meta-analyses with I^2 suggesting inconsistency across the findings of the studies reaching over 80% of total variation across studies due to heterogeneity for testosterone and nearly 100% of that for IGF-1. This calls the overall summary results in question and further analysis is therefore required (107). The main difficulty here is that 'antlerogenic cells' refers to an ill-defined, mixed population of cells taken from the tip of growing antlers. It is probably a mixture of mesenchymal cells, chondroprogenitors, chondrocytes and possibly even osteoprogenitors and osteoblasts. There is no reason to assume all these cells show the same reaction to IGF-1 and testosterone. Moreover, we have recently reported that for

example the individuality of the animal from which the antler tissue was taken, and also various other factors, significantly affected antler cell proliferation (104). However, some of these factors, if not all, were omitted in most of the earlier studies. This may be another reason why no obvious general trend across the studies has been as yet discovered. An alternative possible explanation is that testosterone plays significant role in antler growth, but through an indirect way (acting on another molecule or aromatized to estrogens, etc.) *in vivo*. These systems are not available in an *in vitro* condition and that is why an effect of testosterone could not be expressed so much.

In summary, dispite the fact that the *in vitro* studies have potential to exclude a number of confounding factors that operate *in vivo* and despite the tremendous development of this field, the results of such studies which focused on hormonal regulation of antler growth should be taken with extreme caution (104). In general all important factors affecting antler cell proliferation *in vitro* should be first satisfactorily investigated and the methodology generally standardized. Furthermore, we have not found any link between behavior and IGF-1 concentrations in any of our previous studies except one (94), although we did investigate it. Hence, combination of all factors presented in this section led us to decide not to use the results obtained from antler cell cultivation in the further discussion.

5. ANTLER DEVELOPMENT, IGF-1, TESTOSTERONE AND "DOUBLE CASTRATION"

In the above-mentioned studies working with deer castrates (84, 86, 87), the detection limit of testosterone was about 0.1 ng/ml plasma. Therefore the question of a possible biological function of testosterone at concentrations below or near 0.1 ng/ml has not been addressed. We got our inspiration from Rivest *et al.* (109). They have demonstrated that alterations of various biological processes are triggered by the fluctuation of the day length regardless of the light intensity. If there was any variation in testosterone below the level of 0.1 ng/ml, we postulated that analogically, those androgens still might have been of biological relevance as the sensitivity of androgen receptors is enhanced during the period of low circulating levels. It is also a well known fact that in several biological systems, hormones are effective not only in quantities of ng/ml but also in pg/ml, such is the case of pineal hormone melatonin day-time levels of which are ranging from 10 to 20 pg/ml (110). In order to test this hypothesis, we designed an experiment with the code name "double castration". The aim of that study was to test experimentally the effect of a complete or an almost complete withdrawal of any androgen action on antler growth in fallow deer by comparing surgically castrated fallow bucks with surgical castrates treated with high doses of the CA. High doses of CA were given in order to block the action of androgens produced in the adrenal cortex. We tested the following two hypotheses: (i) If new antler growth is induced by a short-term pulse of testosterone, then such a pulse should be detected in animals producing antlers even below the level of 0.1 ng/ml; (ii) If androgens were required for antler growth, then the CA-treated animals should not produce antlers at all or only a reduced antler growth should be observed, as compared to surgical castrates (41).

We divided twelve yearling fallow deer bucks into two groups of six animals each. The experimental animals (CA group) were injected with high doses of CA while the control bucks (Control) were given a vehicle solution (castor oil) only. Treatments were performed two days before castration, at the day of castration (day 0) and afterwards at two day intervals until day 22, when all of the animals had cast their antlers. Blood samples for hormone analyses were taken at the same time as the treatment and the antlers, if any were produced, were measured. Thereafter CA treatment and blood sampling were continued at weekly intervals. Testosterone was measured by EIA (111) reaching a sensitivity level between 1 and 10 pg/ml plasma.

After surgery, all animals cast their antlers 12 to 22 days post castration. New velvet antler growth was first observed more than one month after casting, around the time when new antlers usually start to develop in intact animals. Antler regrowth occurred in all controls and the antlers produced by these animals were much larger than those of CA-treated castrates. Only four of the six CA-treated castrates initiated antler growth at the same time as the controls, while in the remaining two no regrowth was observed until day 196. At that time, a unilateral (left side) antler formation started in one of these bucks, whereas in the other no antler growth at all occurred until the end of the experiment. Thus we demonstrated that also in fallow deer CA application to surgical castrates has a potential to prevent any antler growth as shown earlier by Bubenik (3) in white tailed deer. The antlers produced by our Controls were much larger than those of CA-treated castrates (Figure 6).

Moreover, we have addressed the question whether the onset of antler regrowth is triggered by a short-term pulse of androgens. In both groups we found a significant temporary increase in testosterone levels around the time of the onset of antler regrowth, the elevation being more pronounced in the control bucks (Figure 6, middle, white arrow) (41). It can be argued, however, that this increase should have occurred at the sampling date preceding the one at which it was found. On the other hand, in this situation one week interval for sampling could have been too long. Thus, our elevated testosterone levels could be in fact already decreasing from the previous peak not detected due to the time interval. As shown in Figure 6, the short-termed testosterone increase putatively initiating antler regrowth may be relative. If the scaling of the graph for the seasonally lowest values is the same as that for maximal seasonal concentrations, the relative increase, if present, would be hardly recognized. This is perhaps, why in fallow deer bucks Asher *et al.* (112) and in red deer yearlings Suttie *et al.* (81) did not find any variation in a testosterone surge during the antler growth initiation. In both studies the sampling was performed so often that the peak should not be missed if it ever existed. The blood

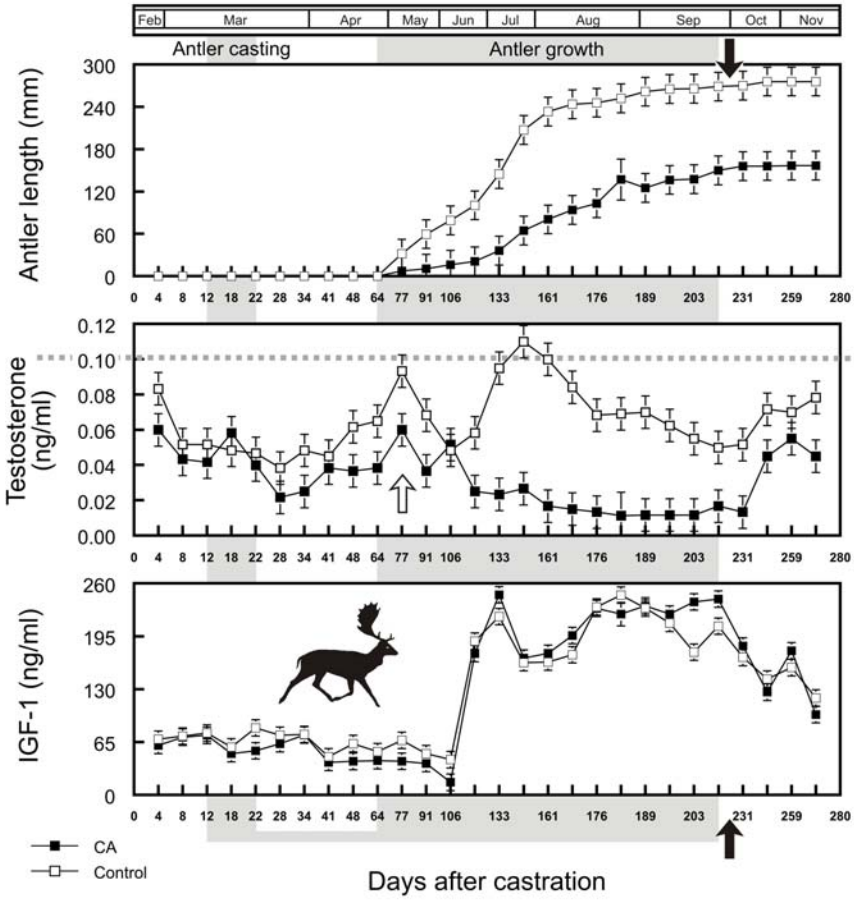

Figure 6. Antler length, testosterone and IGF-1 concentrations (adjusted means ± S.E.) between 4 and 273 days after castration of fallow deer (*Dama dama*) yearlings in Cyproterone acetate (CA) and control groups. (The white arrow indicates a short-termed elevation of testosterone associated with the initiation of antler regrowth; black arrows indicate the time of the last treatment with CA and with castor oil; the dashed horizontal line in the middle frame indicates the level of 0.1 ng/ml.) Adapted with permission from Bartoš *et al.* (41).

samples were withdrawn at intervals of 20 (112) or 30 min for 24 h (81) from each male. However, a temporary increase of testosterone during the initiation of antler growth was indicated in the former study (112). The levels detected below 1 ng/ml suggest that such an increase in the testosterone pattern of the intact bucks G3 and G32, seen in Asher *et al.*'s Figure 2 (112) is not well visible when the highest levels in the same graph reached nearly 6 ng/ml. Because the study was aimed a different way, no wonder this short-termed increase was neither mentioned nor analyzed. Figure 7 is based on the same data originating from our study as Figure 6 (41), with only a two day backward extension before the animals were castrated (not shown in the paper). A variation in testosterone levels is not seen here. This is because testosterone concentrations before and at the time of castration are 10 times higher than after castration. When pooled, the pre-castration data strongly shape the calculation of standard errors during the post-castration period. These make the standard errors much greater than the average values. As the result, when analyzed statistically, no variation in the post-castration period could be significant. In the otherwise existing differences in the post-castration period, the means of the two groups would thus be completely masked. In the "double castration" experiment we also tested if the growth process itself requires low levels of androgens. Antlers produced in that study were much larger and androgen levels were significantly higher in controls than in the CA bucks (Figure 6). Hence, we concluded that in fallow deer a minimum threshold level of androgens, testosterone in particular, is a necessary prerequisite for antler growth to occur. Moreover, within the low range of plasma testosterone concentrations recorded in our experimental animals, we were able to demonstrate an increase in an antler growth rate with increasing testosterone levels (expressed as areas under the curve), i.e. a dose related response of the antlers to testosterone. An increase of antler length between successive sampling sessions correlated with the change over the same period in testosterone (r_s = 0.64, $P < 0.02$) but not IGF-1 (r_s = 0.13, NS) (41).

It is obvious that our experimental design constituted an extremely artificial situation for the bucks. Nevertheless, change in testosterone concentrations was

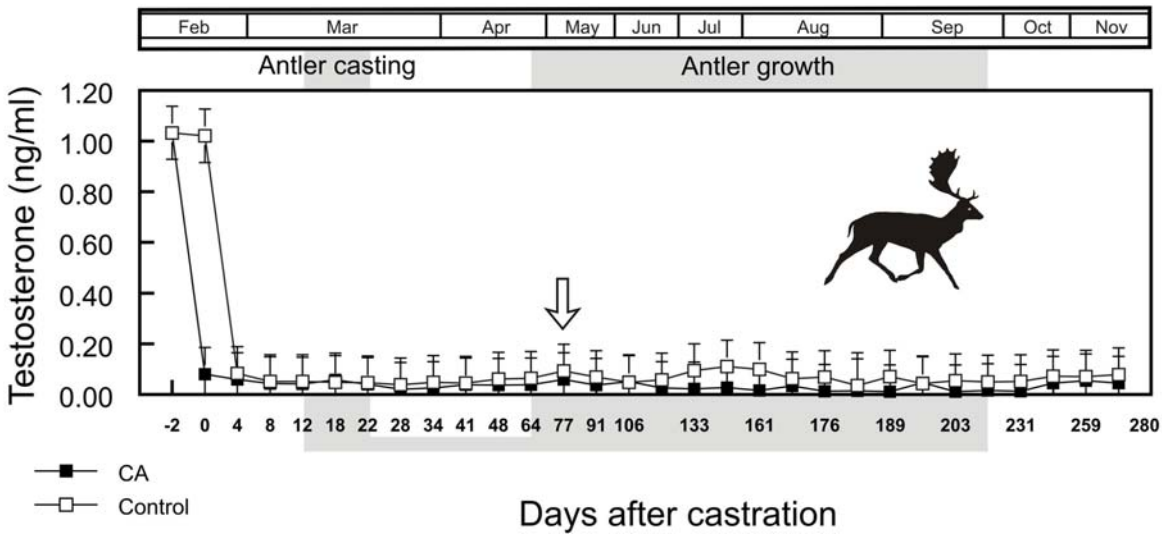

Figure 7. Testosterone concentrations (adjusted means ± S.E.) between -2 and 273 days after castration of fallow deer (*Dama dama*) yearlings in Cyproterone acetate (CA)-treated and control groups. (The white arrow indicates the time after castration. The short-termed elevation of testosterone is associated with the initiation of antler regrowth, as shown in Figure 6.).

positively associated with the rate of antler growth in castrated fallow deer without CA treatment (42) but also with the antler growth in intact red deer (35). (The change in concentration rather than the actual testosterone concentration was used in the analyses because it reflects better the dynamic of the process and helps to avoid overlooking time shifts if there were any.)

6. DISCUSSION

Because we wanted to keep the time context of this review, we reviewed mostly literature of the period in which the discussed studies were published. Hence, it is a rightful claim to ask how our conclusions agree with the recent views and scientific evidence. In general, our conclusions are in full accord with recent reviews on physiology of bone formation in humans and/or laboratory rodents (113, 114). It has been repeatedly shown that the direct effects of androgen on the skeleton are complex and both stimulation and inhibition of bone formation were observed *in vivo* (115, 116). Androgens increase bone mass in specific skeletal compartments through effects on bone cells, enhancing the activity of bone-forming cells, the osteoblasts, but inhibiting that of bone resorbing cells, the osteoclasts (117). Androgens can stimulate the skeleton not only through direct activation of the androgen receptor, but also indirectly, after aromatization to estrogens and subsequent activation of estrogen receptors (12-14). As Callevaert *et al.* (13) reviewed, in peripheral tissues including bone, testosterone can be irreversibly converted to the more potent dihydrotestosterone. In addition, testosterone can be converted to 17 beta estradiol and subsequently activate estrogen receptors. Therefore, androgens might activate both, the androgen and the estrogen receptors, depending on the relative activities of the responsible enzymes. These enzymes are all expressed in bone tissues, thus suggesting that the local hormone synthesis might be important (13).

Similarly to our experiments on castrated fallow or intact red deer (35, 41), studies with male mice also concluded that androgens (118) and estrogens (119) enhance skeletal growth independently of either systemic or local IGF-I production.

IGF-1 is recognized as an important regulator of bone formation. Various *in vitro* studies reported that IGF-1 regulates proliferation and differentiation of bone cells (120-122). *In vivo*, IGF-1 was shown to regulate growth and density of bones (123, 124). However as demonstrated by Ciarmatori *et al.* (125) the effect of IGF-1 on proliferation and differentiation of chondrocytes on the cellular level is mediated by at least four signaling pathways which are progressively activated and inactivated as chondrocytes differentiate. This clearly shows that the anabolic effect of IGF-1 is complex and cell stage-specific. Besides, the systemic effect of IGF-1 is regulated by its binding proteins which have both a stimulating and inhibiting effect on osteoblast function (126). Since antlers are bones (46, 51, 127), we may ask again the question why should the regulation of antlers evolve differently from that of other bones? Therefore, in the view of the presented data, we concluded that it is basically testosterone (possibly in an interaction with other steroids) and not IGF-1 which is primarily responsible for the intensity of antler growth in deer males (35).

At the same time it must be stressed that we are not maintaining that testosterone is the only hormone solely regulating antler growth. There is no doubt IGF-1 is an important hormone involved in regulation of body growth (128, 129). Clearly, it is also somehow involved in regulating antler growth, directly or indirectly, either as such or in an interaction with testosterone (35, 130), as may be other steroid and peptide hormones involved in bone growth and modeling (2, 6) which should be further investigated.

Perhaps, this review may be found biased in favor of the literature dealing with the role of testosterone in antlerogenesis. Part of the bias is based on an imbalance of the previous debate in the literature. On one hand, studies involved in the debate after the pioneering article of Suttie *et al.* (34), still endorsing or at least admitting that testosterone is an antler stimulating hormone, usually also studied IGF-1 effect on antler tissues *in vivo* (35, 41, 80, 94, 96). On the other hand, the introduction of IGF-1 as a possible antler stimulating hormone has not been confronted with the minimal variations of testosterone levels nor with an earlier arguments favoring testosterone (34, 79).

Nevertheless, once we accept that testosterone is primarily responsible for the intensity of antler growth, we can explain the **e**ndocrine aspect of the relationship between rank - related behavior and antler growth in deer. In a recent study on red deer we discovered that small changes in social conditions can profoundly affect the relationship between their rank and testosterone levels. Adding much younger and weaker sparring partners into the experimental group of adult males altered the agonistic behavior of the adults. Adult males targeted preferentially their attacks on individuals much lower in the hierarchy. Experimental male deer with a higher social rank had lower levels of testosterone when they were in a group of adults. After an addition of young conspecifics, it was just the opposite. Stress from competition with equally strong group members was reflected in cortisol concentrations. In a situation when adults were alone, they had elevated cortisol concentrations. These concentrations declined after the youngsters were added. Thus, changing the social environment of adult red deer males resulted in a change of the relationship between rank and testosterone and also cortisol concentrations despite the fact that the rank position of the adults itself did not change (131). This was reflected in an alteration of their antler development (unpublished).

7. PERSPECTIVE

A further investigation thus should focus on understanding the social relationships among male deer during the period of antler growth. Especially, we should concentrate on facts how to record most objectively the social structure of a male deer group. This should be done in order to foster further analyses linking the antler development to concentrations of hormones. It will also bring a better understanding of the role of antlers in mate selection (7).

8. ACKNOWLEDGEMENT

This review was supported by the Czech Science Foundation (grant number 523/08/0808); and the Ministry of Agriculture of the Czech Republic (grant number MZe 0002701404).

We would like to thank (in alphabetic order) Bob Brown, Jitka Bartošová, Bára Fričová, Dominika Formanová-Vaňková, Adam Dušek, Marek Fiala, Klaus Fischer, Jana Holečková, late Jaromír Hyánek, Petr Janovský, Eva Jozífková, Uwe Kierdorf, Vratislav Kšáda, Radim Kotrba, Jitka Lancingerová, Soběslav Losos, Karl V. Miller, David Osborne, José Panamá, Jan Pluháček, Eugenia Reyes, Pavel Šustr, Jimmy Suttie, Dieter Schams, Jiří Šiler, late Ludmila Švecová, Milan Tománek, Václav Perner, and late Dalibor Zaorálek.

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Key Words: Deer, Antlers, Antler Growth, Antler Casting, Antler Cleaning, Hierarchy, Rank, Review

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