Effect of 19-Norandrostenololylaurate on Serum Testosterone Concentration, Libido, and Closure of Distal Radial Growth Plate in Colts

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Koskinen E. and T. Katila: Effect of 19-norandro stenololylaurate on serum testosterone concentration, libido, and closure of distal radial growth plate in colts. Acta vet. scand. 1997, 38, 59-67. – The long-term effect of anabolic steroid was investigated in 3 experiments. In experiment I, 500 mg of 19-norandrostenololylaurate was given to 5 colts and a dose of 100 mg to another 5 colts every 3rd week. Six colts served as untreated controls. The animals were 12–16 months old at the start, and 24 months at the end of treatment. In experiment II, a dose of 1 mg/kg was given every 3rd week to 4 colts and 0.3 mg/kg every week to another 4 colts. Six colts served as controls. The colts were treated from 7 months to 12 months of age. In experiment III, 1 mg/kg of steroid was given every 3rd week to 3 foals between 3 and 8 months of age. Three foals were used as controls. Libido and hCG-induced serum testosterone concentrations were studied after the cessation of treatments, up to 3 or 4 years of age. Closure of the right distal radial growth plate was determined between 21 and 36 months of age.

Treated colts had lower testosterone levels 4.5 months after cessation of treatment in experiment I (p<0.001) and experiment II (p<0.05) when compared with the corresponding control groups. Two years after treatments in experiment I, hCG-induced testosterone levels were higher in treated colts than in untreated controls, but the difference was not statistically significant. The treatments had no effect on libido. The anabolic steroid treatment did not cause premature closure of epiphyseal growth plates in any of the experiments, but closure appeared to be delayed. It was concluded that anabolic steroids have long term effects on reproduction. Their influence on serum testosterone can last for years after cessation of treatment, and they can delay the closure of growth plates which can cause increased susceptibility to cartilage injury during exercise.

stallion; anabolic; steroid; hCG; epiphysis.

Introduction

Treatment with anabolic steroid causes male-like behavior in mares (*Maher et al.* 1983). Testosterone has been used to increase libido in the stallion. However, administration of high doses decreases serum testosterone levels and libido. Exogenous androgens suppress gonadotropin release from the pituitary, which leads to decreased endogenous testosterone production. During and shortly after cessation of anabolic steroid treatment, libido might therefore be decreased because of low endogenous testosterone concentrations (*Davis* 1981, *Garcia et al.* 1987).

In stallions, no controlled trials have been carried out to investigate the long-term effects of

anabolic treatment on libido or serum testoste-
rone concentrations. Usually, animals have
been castrated shortly after treatment (*Carson
& Thompson* 1979, *Squires et al.* 1981 and
1982, *Garcia et al.* 1987). In one study only,
stallions were followed for 3 months after ces-
sation of treatment. Libido was not affected by
testosterone propionate treatment or cessation
of treatment (*Squires et al.* 1981).

In man, reduced plasma testosterone concentra-
tions have been detected after anabolic steroid
treatment (*Blanchard* 1985), but in stallions
testosterone concentrations were not decreased
shortly after treatment with anabolic steroid
(*Carson & Thompson* 1979, *Garcia et al.*
1987). Single testosterone measurements, how-
ever, may fail to reveal differences because tes-
tosterone secretion is episodic in nature. There
were 3-fold differences between testosterone
concentrations over a 24 h sampling period in
stallions (*Ganjam* 1979). Frequent blood sam-
pling is required for dependable serum testoste-
rone determination, but this is very laborious.
Serum testosterone concentrations rise 20- to
30-fold after hCG administration (*Silberzahn et
al.* 1988) or GnRH (*Clay et al.* 1988) reaching
peak levels 2 to 3 days after injection (*Silber-
zahn et al.* 1988). This facilitates reliable single
testosterone measurements which give indica-
tion of tes tosterone production by the testes.

It is commonly claimed that large quantities of
testosterone or anabolic steroids early in life
hasten closure of the epiphyseal plates and thus
prevent further growth in length of bones (*To-
bin* 1978, *Davis* 1981). However, no controlled
studies supporting this claim have been re-
ported. The opposite has, in fact, been observed
(*Herring* 1981, *Saastamoinen & Koskinen*
1993). The aim of the present study was to ex-
amine the long-term effect of an anabolic ster-
oid on serum testosterone levels, sexual arousal
and closure of the distal radial growth plate in
sexually non-mature colts.

Materials and methods

The animals used were Finnhorse colts raised
for trotting and riding. The Finnhorse is a cold-
blooded breed. Adult horses weigh 500–600
kg. The colts were not trained but light exercise
started at 3 years of age. They were kept on pas-
ture from June to September. During winter
they were in an unheated barn, with free access
to a paddock. Three experiments were carried
out. The first started in autumn, the second at
the start of the following year, and the third in
autumn 2 years later. All colts in the 3 experi-
ments were born in May or June except for 3
born in April, July and August. The anabolic
steroid used was an injectable 19-norandroste-
nololylaurate preparation[1]. The manu facturer
recommends a dose of 100–200 mg every 3rd
week for adult horses, for instance to aid recov-
ery after a severe operation. The recommended
dose corresponds to a dose of 0.2–0.4 mg/kg in
a 500 kg horse. Because it is alleged that much
higher doses of steroids are used for doping
than therapeutical doses, the dose chosen in ex-
periment I was 500 mg for colts weighing
around 400 kg during the treatment period.
Dose in experiments II and III was calculated as
mg/kg of body weight. Treatment periods var-
ied between 5 and 12 months. Treatment proto-
cols in experiments I–III are shown in Table 1.
The examination protocols of the 3 trials are
shown in Table 2. Blood samples were collected
for testosterone determinations during breeding
and nonbreeding seasons. Intramuscular injec-
tion of 5000 IU of hCG (Chorio-Gonadon,
Orion-Farmos Oy, Finland) was used to induce
rises in serum testosterone concentration. A
jugular vein blood sample was drawn on day 3
after hCG injection for testosterone determina-
tion. Serum was separated, frozen, and testoste-

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[1] Laurabolin vet®, Intervet International B.V., 5830
AA Boxmeer, The Netherlands.

Table 1. Number of animals, age at start and end of treatment and dosage during treatment of colts with 19-norandrostenololylaurate in experiments I–III.

Experiment	Number of animals	Age at start of treatment (months)	Age at end of treatment (months)	Dose level	Dosage inter-val
I	6	–	–	untreated	–
	5	12 – 16	24	100 mg	3 weeks
	5	12 – 16	24	500 mg	3 weeks
II	6	–	–	untreated	–
	4	7	12	1 mg/kg	3 weeks
	4	7	12	0.3 mg/kg	1 week
III	3	–	–	untreated	–
	3	3	8	1 mg/kg	3 weeks

Table 2. Time of examination of serum testosterone concentration, libido, and closure of radial distal growth plate in relation to age and cessation of treatment.

Experiment	Testosterone		Libido		Closure og radial distal growth plate	
	Age (months) treatment	Months after treatment	Age (months) treatment	Months after	Age (months)	Months after
I	28.5	4.5	24	0	21	–3[a]
	48	24	36	12	28.5	4.5
					31.5	7.5
					36	12
II	16.5	4.5	24	12	23.5	11.5
					30	18
					33	21
III	18	10	24	16	22	14
	20	12	29	21	24	16
	24	16	36	28	25	17
	28	20	41	33	26	18
	41	33	48	40		
	53	45				

[a] 3 months before the final treatment.

rone determined later using a quantitative radioimmunoassay test (Spectria* Testosterone [125I] Coated Tube Radioimmunoassay, Orion Diagnos tica, Finland). Sensitivity of the test was 0.1 nmol/l and cross-reactivity with 5α-dihydrotestos terone was 4.5%. Intra-assay and interassay coefficients of variation were 5.3% and 5.4%, respectively.

Libido was evaluated as "time to ejaculation" during the breeding season. In experiment III, libido was also evaluated during the nonbreeding season. Evaluations started at 24 months of age. Libido was determined every other day. Each animal was assessed 2–8 times during the semen collection period and the mean value per period was calculated for each colt. "Time to ejaculation" began when a colt entered the breeding hall. Ejaculation into an open ended artificial vagina (Model Krakow, Poland) marked the end of the "time to ejaculation". If a colt failed to ejaculate, the estimated time was not included in the calculation. Failure to ejaculate occurred randomly in all groups.

Closure of the right distal radial growth plate was determined from craniocaudal radiographs between 21 and 36 months of age. Lack of translucency of the growth plate, particularly at plate ends, was considered as closure of the plate. For technical reasons, one radiograph relating to one colt in the 500 mg group in experiment I is lacking.

Statistical analysis

Analysis of variance for repeated measurements and one-way analysis of variance followed by Tukey's test was used to assess treatment effects on serum testosterone concentration and on time to ejaculation. The significance of effects of treatment on closure of growth plates was tested using the chi-squared test.

Results

Experiment I

Four and a half months after the last treatment, when the colts were aged 28.5 months, mean hCG-induced testosterone levels were significantly lower in the treated groups compared with the controls ($p<0.001$). Two years after treatment, when the colts were 48 months old,

testosterone levels were higher in the treated groups than in the control group. However, the variation was large at this time point and the difference was not statistically significant. The changes in serum testosterone concentration from 28 months to 48 months were significantly different between the control group and the 500 mg group ($p<0.05$) (Table 3).

At the time of the final treatment, at age 24 months, time to ejaculation was similar in the 500 mg group (111 sec), 100 mg group (71 sec) and control group (86 sec). One year later, the situation was the same (times 138 sec, 87 sec and 98 sec, respectively).

Table 4 shows closure of the right distal growth plate. Four and a half months after treatment, when the colts were aged 28.5 months, closure of the growth plate had occurred more often in the control group than in the treated groups ($p<0.05$). At age 31.5 months, the difference was similar but no longer significant ($p = 0.089$). At 36 months, the difference was even smaller.

Experiment II

Serum testosterone concentrations were determined 4.5 months after the final treatment, when the colts were aged 16 months. The mean hCG-induced testosterone levels were relatively low in all groups, but lowest in the treated groups ($p<0.05$) (Table 5).

Time to ejaculation was similar in all groups at age 24 months, 12 months after the final treatment: 101 sec in the control group, 95 sec in the 1 mg/kg every 3rd week group and 137 sec in the 0.3 mg/kg every week group.

At 23.5 months of age, no closure of the right distal radial growth plate was observed in any of the groups. At 30 months of age, 5 out of 6 in the control group, 3 out of 4 in the 1 mg/kg every 3rd week group, and 2 out of 4 in the 0.3 mg/kg every week group showed closure of the growth plate (Table 6). The difference between groups was not statistically significant.

Table 3. Mean (SEM) serum testosterone concentration (nmol/l) on day 3 after hCG-injection (5000 IU im) in experiment I.

Age of colts (months)	28.5	48
Months after treatment	4.5	24
Month	October	May
Controls (6 colts)	23.50 (2.78)	7.17 (1.89)
100 mg every 3rd week (5 colts)	12.80 (2.24)*	14.60 (7.23)
500 mg every 3rd week (5 colts)	5.20 (1.59)*	15.40 (5.97)

* Statistically significant difference ($p < 0.05$) in relation to controls.

Table 4. Number of colts with closed epiphyseal plates at different times in experiment I.

Age of colts (months)	28.5	31.5	36
Months after treatment	4.5	7.5	12
Controls (6 colts)	5/6	6/6	6/6
100 mg every 3rd week (5 colts)	2/5	3/5	4/5
500 mg every 3rd week (5 colts)	0/4*[a]	2/5*	3/5

* Statistically significant difference ($p < 0.05$) in relation to controls.
[a] Radiograph for one colt is missing.

Experiment III

Serum testosterone concentrations were determined 6 times between 18 and 53 months of age. The highest concentrations of testosterone were found at age 24 months, during the breeding season. The rest of the measurements were made during the nonbreeding season. Group profiles did not differ significantly between treatments (Table 7).

In both groups, time to ejaculation was short during the breeding season (88 sec at 3 years of age, and 94 sec at 4 years of age in the control

Table 5. Mean (SEM) serum testosterone concentration (nmol/l) on day 3 after hCG-injection (5000 IU im) in experiment II.

Age of colts (months)	16
Months after treatment	4.5
Month	October
Controls (6 colts)	4.50 (0.89)
1 mg/kg every 3rd week (4 colts)	1.25 (0.48)*
0.3 mg/kg every week (4 colts)	1.75 (0.25)*

* Statistically significant difference ($p < 0.05$) in relation to controls.

Table 6. Number of colts with closed epiphyseal plates at different times in experiment II.

Age of colts (months)	23.5	30	33
Months after treatment	11.5	18	21
Controls (6 colts)	0/6	5/6	6/6
1 mg/kg every 3rd week (4 colts)	0/4	3/4	4/4
0.3 mg/kg every week (4 colts)	0/4	2/4	4/4

Table 7. Mean (SEM) serum testosterone concentration (nmol/l) on day 3 after hCG-injection (5000 IU im) in experiment III.

Age of colts (months)	18	20	24	28.5	41	53
Months after treatment	10	12	16	20.5	33	45
Month	Nov	Jan	May	Oct	Oct	Oct
Controls (3 colts)	3.33	3.33	4.33	2.00	1.67	0.33
(SEM)	(0.33)	(1.32)	(1.67)	(0.58)	(0.33)	(0.33)
1mg/kg every 3rd week (3 colts)	2.67	2.33	5.33	2.67	2.33	0.66
(SEM)	(0.33)	(0.33)	(0.33)	(1.67)	(0.33)	(0.33)

Table 8. Number of colts with closed epiphyseal plates at different times in experiment III.

Age of colts (months)	22	24	25	26
Months after treatment	14	16	17	18
Controls (3 colts)	0/3	2/3	3/3	3/3
1 mg/kg every 3rd week (3 colts)	0/3	0/3	2/3	3/3

group, and 132 sec and 88 sec in the treated group at the same time points) and long during the nonbreeding season (208 sec at 2 years of age, and 178 sec at 3 years of age in the control group, and 232 sec and 186 sec in the treated group at the same time points). At 2 years of age, the time was long also during the breeding season, 228 sec in the control group, and 382 sec in the treated group. There were no statistically significant differences between the groups.

Closure of the right distal radial growth plate occurred slightly earlier in the control group than in the treated group (2 out of 3 and 0 out of 3, respectively, at 24 months of age and 3 out of 3 and 2 out of 3, respectively, at 25 months of age) but the difference was not statistically significant (Table 8).

Discussion

The animals in the 3 experiments were treated at different ages, during different years, and examined after different time intervals. Comparisons between the experiments are therefore impossible. Discussion of the results focuses on differences within each experiment.

Serum testosterone concentrations follow a seasonal pattern in stallions. Values are low from August to December and high from March to June (*Burns et al.* 1984, *Clay et al.* 1988). The results of experiment III confirm these findings. Testosterone concentrations were higher during the breeding season at 2 years of age than during the nonbreeding season at 2, 3 and 4 years of age. In control animals in October at 2 years of age testosterone concentration was high (23.5 nmol/l) in experiment I, but low (2.0 nmol/l) in experiment III. The social status of a colt in relation to other males (harem male vs. bachelor male) can markedly affect serum testosterone concentrations (*McDonnel & Murray,* 1995). The social status was not examined in the present study. Testosterone measurements done in different years may not be comparable. It is unknown what effect variations in weather, nutrition and management might have on hormone levels.

Four and a half months after the final treatment in experiment I and II testosterone levels were much lower in colts injected with 500 mg every 3rd week or 1 mg/kg every 3rd week, respectively, than in controls. It is not known when testosterone levels started to decrease, because testosterone levels were not measured during steroid treatments. Other studies in which anabolic steroids have been given to stallions have shown no decreases in testosterone levels during courses of treatment. *Carson & Thompson* (1979) gave monthly injections of 1.1 mg/kg of boldenone undecylenate for 6 months to colts, starting at ages 15 to 17 months, i.e. at ages similar to those in our experiment I. Testosterone values were higher in the treated group than in the control group when testosterone was determined at two-week intervals (*Carson & Thompson* 1979). *Garcia et al.* (1987) administered stanozolol or boldenone undecylenate, every 3rd week or every week, to 2- to 16-year-old stallions. Anabolic steroids did not decrease testosterone levels. The highest levels in fact occurred in the group that had received 0.55 mg/kg boldenone undecylenate every week. Testosterone was determined in every week blood samples without hCG administration.

Two years after treatments, hCG-induced testosterone levels were much higher in colts treated in experiment I than in untreated controls. It is not known whether testosterone levels remained permanently increased. In experiment III, serum testosterone levels were similar in the treated and control groups at all times. However, these animals were examined for the first time as late as 10 months after cessation of treatment. Either there were no effects or they had disappeared by the time of the first examination. No "rebound" phenomenon was found in these colts, which may indicate that there was no effect at all.

We found no difference between groups in respect to libido, as judged by time to ejaculation.

This finding is in agreement with the findings of *Squires et al.* (1981, 1982). It is logical that time to ejaculation is long at 2 years of age and during the nonbreeding season. Individual variations in time to ejaculation are large, and statistically significant differences are therefore difficult to demonstrate, especially in young animals without sexual experience.

Closure of radial distal growth plates has been reported to take place in Thoroughbred and Standardbred horses at 25-28 and 28-31 months of age, respectively (*Dietz et al.* 1988). Two control colts in our experiment III had closed growth plates as early as at 24 months, and one control colt in our experiment II as late as at 30 months of age. Nutrition and management could explain this difference between colts born in different years. The results of our experiments indicate that anabolic steroid treatment did not cause premature closure of epiphyseal growth plates. It was observed that colts treated with anabolic steroids in fact exhibited open growth plates more often than the controls. If anabolic steroids are given at an early age, or treatment is stopped shortly before the critical time for closure of the growth plates, serum testosterone levels may be low, and delay closure. This observation is important since colts treated with anabolic steroids before or during training will be more susceptible to cartilage injury.

In conclusion, anabolic steroid treatment has long-term effects on reproduction. It can influence serum testosterone concentration for years after cessation of treatment. Further, steroid treatment can delay the closure of growth plates. Colts treated with anabolic steroids may suffer cartilage injury, if intensive training is started soon after or during the treatment. Although some effects of anabolic steroid treatment can be reversible, we advice that anabolic steroids are not used in order to improve performance.

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Sammanfattning

*Inverkan av 19-norandrostenololylaurat på serum-
koncentrationen av testosteron, libido och tillslut-
ningen av den distala tillväxtzonen i radius hos unga
hingstföl.*

Tre experiment utfördes där inverkan av den anabola
steroiden 19-norandrostenololylaurat på serum tes-
tosteron, libido och tillslutningen av den distala
tillväxtzonen i radius undersöktes hos hingstföl av
olika ålder. I det första experimentet erhöll fyra
hingsföl 500 mg och likaså fyra hingstföl 100 mg var
tredje vecka. Djuren var 12–16 månader gamla i
början och 24 månader gamla i slutet av experimen-
tet. Sex djur tjänstgjorde som kontroller. I det andra
experimentet behandlades fyra hingstföl med 1
mg/kg var tredje vecka och ytterligare fyra hingstföl
med 0.3 mg/kg varje vecka. Dessa djur var 7 månader
gamla i början och 12 månader gamla i slutet av ex-
perimentet. Sex hingstföl i samma ålder ingick som
kontrollgrupp. I det tredje experimentet gavs 1 mg/kg
var tredje vecka till tre föl som var 3–8 månader
gamla.
Efter att behandlingarna avslutats följdes djurens li-
bido och HcG-inducerade koncentration av serum
testosteron ända tills djuren uppnått en ålder mellan
3 och 4 år. Då djuren var mellan 22 och 36 månader
gamla fastställdes huruvida radius distala tillväxtzon
tillslutits. De behandlade fölen hade lägre testosteron
nivåer 4.5 månader efter avslutad behandling i ex-
periment I (p<0.001) och II (p<0.05) än kontroll-
djuren. Två år efter behandlingarna i experiment I var
de hCG-inducerade serum testosteron nivåerna
mycket högre hos de behandlade hingstfölen än de
obehandlade hingstfölen. Behandlingen påverkade
inte hingstarnas libido. Ej heller tydde något av ex-

perimenten på att behandlingen skulle leda till att de epifysiala tillväxtzonerna tillsluts prematurt, snarare tvärtom. Fyra och en halv månad efter avslutad behandling i experiment I hade endast en liten del av hingstfölen tillslutna tillväxtzoner (0/4 hingstar i 500 mg gruppen och 2/5 i 100 mg gruppen). De flesta hingstarna i den obehandlade gruppen hade däremot tillslutna tillväxtzoner (5/6)(p<0.05). Sammanfattningsvis kan konstateras att anabola steroiden har en långvarig inverkan på reproduktionen. Deras inverkan på serum testosteron kan kvarstå i flera år efter avslutad behandling och de kan fördröja tillslutningen av epifysiala tillväxtzonerna, vilket kan leda till ökad risk av brosskador under träning.