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Effects of the androgen preparation "Testoenatum" on reproductive function in males of Atlantic salmon and several species of whitefish

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Abstract

Atlantic salmon, *Salmo salar*, and several species of whitefish, *Coregonus nasus, C. peled, C. lavaretus baeri*, males were treated with a long-acting medical androgen preparation – "Testoenatum". This preparation contains 24 mg of testosterone enanthate and 111 mg of testosterone propionate in 1 ml peach oil solution. Multiple (2-5) or single intraperitoneal injections of a standard dose (0.1 ml/kg fish body weight) were used. Treatment performed shortly before the beginning or within the breeding season resulted in a significant stimulation of spermiation, improvement of sperm parameters and extention of the gonad functional maturity period for 1-4 months. Low water temperatures (0.5-1.0°C) seem to be essential to obtain a long-lasting effect after one injection. The histology of the pituitary gland suggests that the gonadotropic cells were activated through a positive feedback mechanism. Testoenatum suppressed Sertoli cells' phagocytary activity.

Keywords: *Salmo salar*, whitefish, sperm, testosterone, functional maturity period, Sertoli cells.

Effets d'une préparation androgène le « Testoenatum » sur la fonction reproductrice chez les mâles du saumon atlantique et diverses espèces de corégones.

Résumé

Le saumon atlantique, *Salmo salar*, et plusieurs espèces de corégones mâles, *Coregonus nasus, C. peled, C. lavaretus baeri*, ont été traités avec une préparation d'androgènes, à effet prolongé, le « Testoenatum ». Cette préparation contient 24 mg de testostérone-énanthate et 111 mg de testostérone-propionate dans une solution huileuse de 1 ml. Des injections péritonéales multiples (2 à 5) ou uniques ont été effectuées avec des doses standard de 0,1 ml.kg^{-1} du poids vif. Les traitements effectués peu avant ou au cours de la saison de reproduction, provoquent une stimulation notable de la spermiation et l'amélioration des paramètres caractéristiques du sperme, et également l'allongement de la période de maturité fonctionnelle des gonades de 1 à 4 mois. Des basses températures semblent être nécessaires pour obtenir un effet durable suite à une injection. L'étude histologique de la glande pituitaire suggère que les cellules gonadotropes seraient activées grâce à un processus de feedback. Le Testoenatum supprimerait l'activité phagocytaire des cellules de Sertoli.

Mots-clés : *Salmo salar*, corégones, sperme, testostérone, maturité sexuelle, cellules de Sertoli.

INTRODUCTION

Most temperate and cold water teleosts exhibit a short breeding season. Fish culture, however, needs good quality gametes throughout the year. Several approaches to obtain ripe germ cells outside the natural breeding season are employed, including hormonal treatments. The male sex steroid hormones – androgens – are involved in the control of spermatogenesis and spermiation (Billard *et al.*, 1982, 1990) and are effective in modifying the period of functional testis maturity. Androgens may be administered using four basic methods:

(1) treatment via the water (Borg, 1981; Hunsinger and Howell, 1991). This kind of hormone administration is not always acceptable in aquaculture where large volumes of running water are used,

(2) feeding of fish with a hormone-containing diet (Weber and Lee, 1985). The use of this technique seems to be restricted due to the fact that not all food is consumed and due to loss of hormone which is washed away. Furthermore, many fishes stop feeding during the pre-spawning and spawning periods, or lose their appetite soon after sex steroid administration,

(3) implantation of sustained release systems such as Silastic capsules, (Billard *et al.*, 1982; Lee *et al.*, 1992) and

(4) injecting of fish with hormones (Donaldson and Hunter, 1983).

Methods 3 and 4 avoid the main flaws of the first two methods and give the possibility to reduce expenditure of preparation as well as dosage accurately. A single injection is less injurious and easier to administer than a tube implantation, but the brevity of effects of a single injection often presents a significant problem, even if long-acting preparations are used. For example, by a single injection of Durandron Forte 250, Juario *et al.* (1980) were able to maintain mature males of milkfish, *Chanos chanos* (Forsskål), in good running condition for 7 days only. Shehadeh *et al.* (1973) were forced to repeat injections of grey mullet, *Mugil cephalus* with 17α-methyltestosterone on alternate days to prevent milt resorption for 42 days. Frequent injections are not only laborious but also result in increased stress and often mortality (Lam, 1982). The obtaining of prolonged effects after a minimal number of injections was the main purpose of the present investigation. Hormone half-life in fish blood depends on water temperature and increases greatly when the temperature drops. Thus, the combination of a long-acting preparation and low temperature was expected to be particularly effective. For this reason, autumn- and winter-spawning salmon and whitefishes were injected with a long-acting androgen preparation and the effects on reproduction were studied.

MATERIALS AND METHODS

Fish

Males of Atlantic salmon, *Salmo salar*, peled, *Coregonus peled*, broad whitefish, *Coregonus nasus*, volkhov whitefish, *Coregonus lavaretus baeri*, were used (*table* 1). The experiments were carried out in 1980-1992 close to Saint-Petersburg, Russia under natural photoperiod and ambient temperatures.

Maturing 1-year-old dwarf males of the Atlantic salmon were selected between September and October from the Neva River salmon stock reared at the Research station "Chikino" (experiment 1) and at the Neva hatchery (experiments 2 and 3) by the presence of nuptial colours and running milt. The fish were kept in plastic tanks (2 × 2 m) with through-flowing river water. They were fed daily with commercial salmon dry foods. Water temperature at "Chikino"

Table 1. – Characterization of steroid treatments used.

Exp. location no.		Species	Age (yr)	Mean body wt. (g)	Number of fish		Injections of Testoenatum T (0.1 ml/kg b.w.)	
					Intact	T-treated	No.	Date
1	Res. Station "Chikino"	*Salmo salar*	1+	28 ± 1	120[1]	120[1]	5	30/11-80 24/12-80 19/01-81 14/02-81 5/03-81
2	Neva hatchery	*Salmo salar*	1+	48 ± 1	50	50	1	1/12-89
3	Neva hatchery	*Salmo salar*	1+	45 ± 1	25	25	1	30/03-90
4	Res. Station "Ropsha"	*Coregonus nasus*	4+	1 250 ± 24	25	25	2	16/10-81 5/11-81
5	Res. Station "Ropsha"	*Coregonus peled*	2+	434 ± 10	31	20	2	27/10-82 16/11-82
6	Res. Station "Ropsha"	*Coregonus peled*	2+	404 ± 14	25	25	1	19/10-86
7	Res. Station "Ropsha"	*Coregonus peled*	3+	650 ± 12	25	25	1	5/12-81
8	Volkhov hatchery	*Coregonus lavaretus baeri*	5+	822 ± 27	10	10	1	15/10-91

(1) These groups were subdivided into two subgroups with 60 unstripped and 60 stripped males.

dropped from 7 to less than 1°C between October and November, stayed at the low level from December to early March and then increased to 4 and 15°C in April and May, respectively. Water temperature at the Neva hatchery dropped from 10-12 to less than 1°C between October and mid-December, remained low, until late March – early April and increased to 10-15 and 14-19°C during May and June, respectively.

Maturing 4-year-old males of broad whitefish (experiment 4) and 2,3-year-old males of peled (experiments 5, 6, 7) were selected in October from commercial pond-reared brood stocks at the Research station "Ropsha". They were kept in tiled tanks (2×4 m) with through-flowing spring water. Water temperature dropped during the experiments from 5 to 0.5-1°C between late October and January. The fish were not fed.

Maturing 5-year-old males of volkhov whitefish (experiment 8) were netted in early October during their upstream migration from the Ladoga Lake into the Volkhov River. The fish were kept at the Volkhov hatchery in plastic tanks (2×2 m) with through-flowing river water. Temperature dropped from 10 to 5-6°C towards the end of October. The fish were not fed.

Treatments

In all cases fish were divided into two groups: controls (intact) and Testoenatum-treated (T-treated). Number of males, body weights and number of injections are shown in *table* 1. The long-acting medical preparation Testoenatum, containing 111 mg of testosterone propionate and 24 mg of testosterone enantate in 1 ml of peach oil (Mashkovsky, 1984) was injected intraperitoneally. These fat-soluble ethers of testosterone do not have androgenous activities by themselves, but rather they hydrolyze gradually in the blood of animals and turn into testosterone (Rogozkin, 1988). Dose-dependent responses of fish reproductive system to T have been found previously (Murza, unpubl. data). In the present study only a dosage of 0.1 ml/kg body weight injection, which seemed to be optimal, was used. Since preliminary tests had shown no effect of injections of peach oil alone, control males were not injected and are referred to as intact.

In experiment 1 half of the intact and T-treated salmon were hand stripping five times during November-February.

Males were marked according to their experimental category as well as individually. Fin-clipping (cutting of either upper or lower part of caudal fin, or by the partial cutting of left or right ventral fins) was used for category marking. Individual marking was performed by hypodermic injections of active dichlortriazine (M-procionic) dyes ventrally as described in Melnikova and Savostjanova (1968).

Measurement of sperm parameters

Sperm were taken several times from each male by stripping. The quota of spermiating (running) specimens in groups, the amounts of sperm collected from each male per stripping as well as over the breeding season were determined. Sperm concentration was estimated using spectrophotometry at 410 nm (Billard *et al.*, 1971). The duration of sperm motility (sec) after fresh water activation and the degree of such sperm motility (quota of mobile sperm cells according to 5-degree scale of conditional units, c.u.) were determined as recommended by Kazakov (1981).

Histology and cytometry

8-10 intact and T-treated males were sampled periodically. The gonads and the pituitary glands were removed. The gonadosomatic index (GSI) was calculated as the ratio of gonad weight to body weight $\times$ 100. The testis and pituitary glands were fixed in Bouin's solution, dehydrated in ethanol, and embedded in Paraplast. Sections of testis were cut at 5 μm and stained with Heidenhein's hematoxylin, whereas the sections of pituitaries were stained with Azan and PAF (Romeis, 1954). The functional state of germ and Sertoli cells was studied. The pituitary gonadotropic (GTH) cells were identified on the basis of their localization and tinctorial properties, whereas their functional state was determined according to their cytomorphological features (Holmes and Ball, 1974; Benjamin, 1979). Measurement of cell height, nucleus and nucleoli volumes of 50 GTH cells were performed on each male.

Blood testosterone measurement

The blood samples were taken from the caudal vein. Serum was separated from clotted blood by centrifugation and then stored at -20°C until use. The levels of testosterone (ng/ml) were measured by radioimmunoassay (RIA) as described by Chaikovsky *et al.* (1983). Cross-reactivity against 11-ketotestosterone was 1% (Christoforov and Murza, 1988). The antiserum did not cross-react with testosterone propionate and testosterone enanthate.

Statistical analysis

Student's *t*-test was used in comparisons. Differences between means were considered statistically significant at $p<0.05$.

RESULTS

Experiment 1

Effects of five times T treatment within the breeding season on Atlantic salmon dwarf males.

Table 2. – Changes in gonadosomatic index (GSI, %) in intact and T-treated Atlantic salmon dwarf males during experiment 1.

Date	Groups	Subgroups	
		Unstripped	Stripped
24 Oct. 1980	Intact (initial condition)	5.3 ± 0.4	–
6 Dec. 1980	Intact	3.1 ± 0.4	2.4 ± 0.3
24 Feb. 1981	Intact	2.3 ± 0.2	1.1 ± 0.2[a]
	T-treated	4.0 ± 0.3**	2.3 ± 0.2**[a]
30 April 1981	Intact	1.2 ± 0.3	0.3 ± 0.01
	T-treated	2.4 ± 0.2**	2.8 ± 0.2**

$n = 10$ in all subgroups. Means ± SEM. ** Significantly different from corresponding intact fish. a = Significantly different from corresponding unstripped fish ($p < 0.01$).

Almost all intact males had testis in functioning order from October to February-March, but sperm cells from these fish became unviable as early as in December or early January (*fig.* 1 A). The gradual regression of testis and reduction of GSI values took place in unstripped as well as stripped specimens during December-May (*table* 2). The changes were especially obvious in spring when the water temperature began to rise. They coincided with an inactive state of GTH cells (*fig.* 2 A), dropping blood testosterone levels (*fig.* 1 B) and increasing phagocytary activity of Sertoli cells (*fig.* 3). The majority of intact males had developed a silvery body coloration at the end of the experiment.

On the contrary, T-treated males continued to produce a large amount of high-quality sperm with the maximal (5 c.u.) motility and normal concentration of sperm cells until April (*fig.* 1 A). The total volume of sperm obtained from each T-treated male over the time of experiment was about three times higher than in intact fish. Testis regression and GSI decrease were retarded greatly (*table* 2). The phagocytary activity of Sertoli cells was suppressed (*fig.* 3). The GTH cells of T-treated males seemed to be more active than those in intact fish (*fig.* 2 A). GTH cell height, nuclei (*fig.* 2 B) and nucleoli volumes were significantly ($p < 0.01$) larger in T-treated than in intact males. In T-treated fish also a dramatic increase in blood testosterone level (*fig.* 1 B), a cessation of feeding as well as a considerable body depletion, changes of liver condition and enlargement of the bile-bladder took place. All of the T-treated males maintained their bright parr coloration until May-June. Many fish from this group died of fungal diseases during April-June when the water temperature began to rise. No significant influence of stripping on duration of functional maturity period and on blood testosterone were found.

Experiment 2

Effects of one time T treatment in December on Atlantic salmon dwarf males.

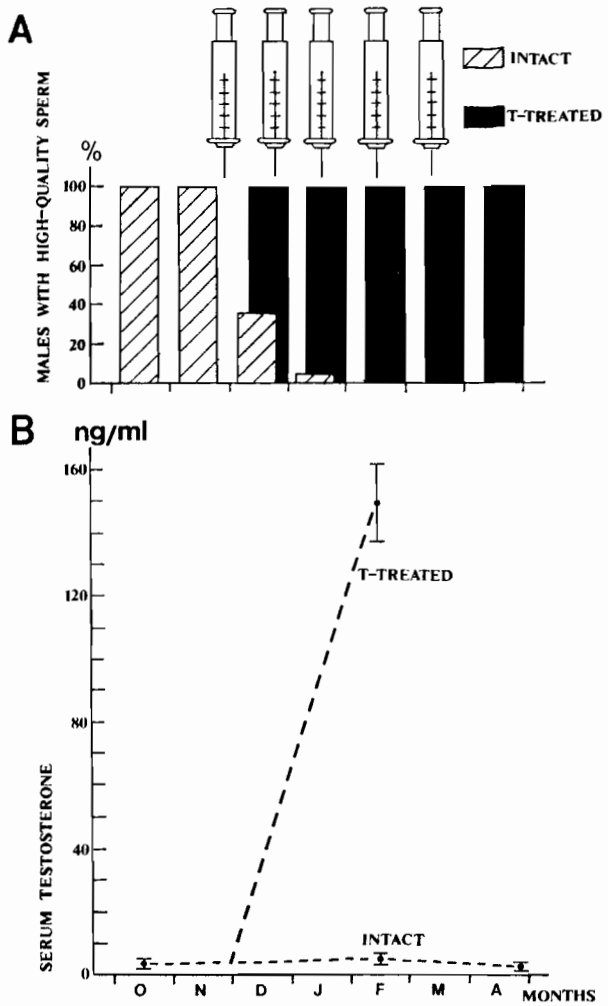

Figure 1. – Experiment 1. (A) High-quality sperm production by intact and T-treated salmon males. Summarized data from both unstripped and stripped males within each group are presented. Initial number was 120 in each group, 10 fish from each group were sacrificed in February. Syringes show the times of steroid injections. (B) Serum testosterone levels in intact and T-treated fish. $n = 10$ for each point. Means ± SEMs. The T-treated group is significantly different from all other groups. Testosterone levels in intact fish in February and April are significantly different ($p < 0.01$).

Intact males produced a large amount of high-quality sperm until December-January only, whereas T-treated males did so until April-May (*fig.* 4 A). T-treatment resulted in a remarkable activation of the pituitary GTH cells, suppression of Sertoli cells phagocytary activity as well as retardation of decline in GSI (*fig.* 4 B). About 80% of intact males had developed a silver coloration at the end of experiment, but none of the T-treated males underwent silvering. Cessation of spermiation (*fig.* 4 A), fall of sperm quality and lowering of blood testosterone levels (*fig.* 4 B) in T-treated fish coincided with the spring rise in water temperature in April-May. The principal differences towards experiment 1 was a reduced elevation of

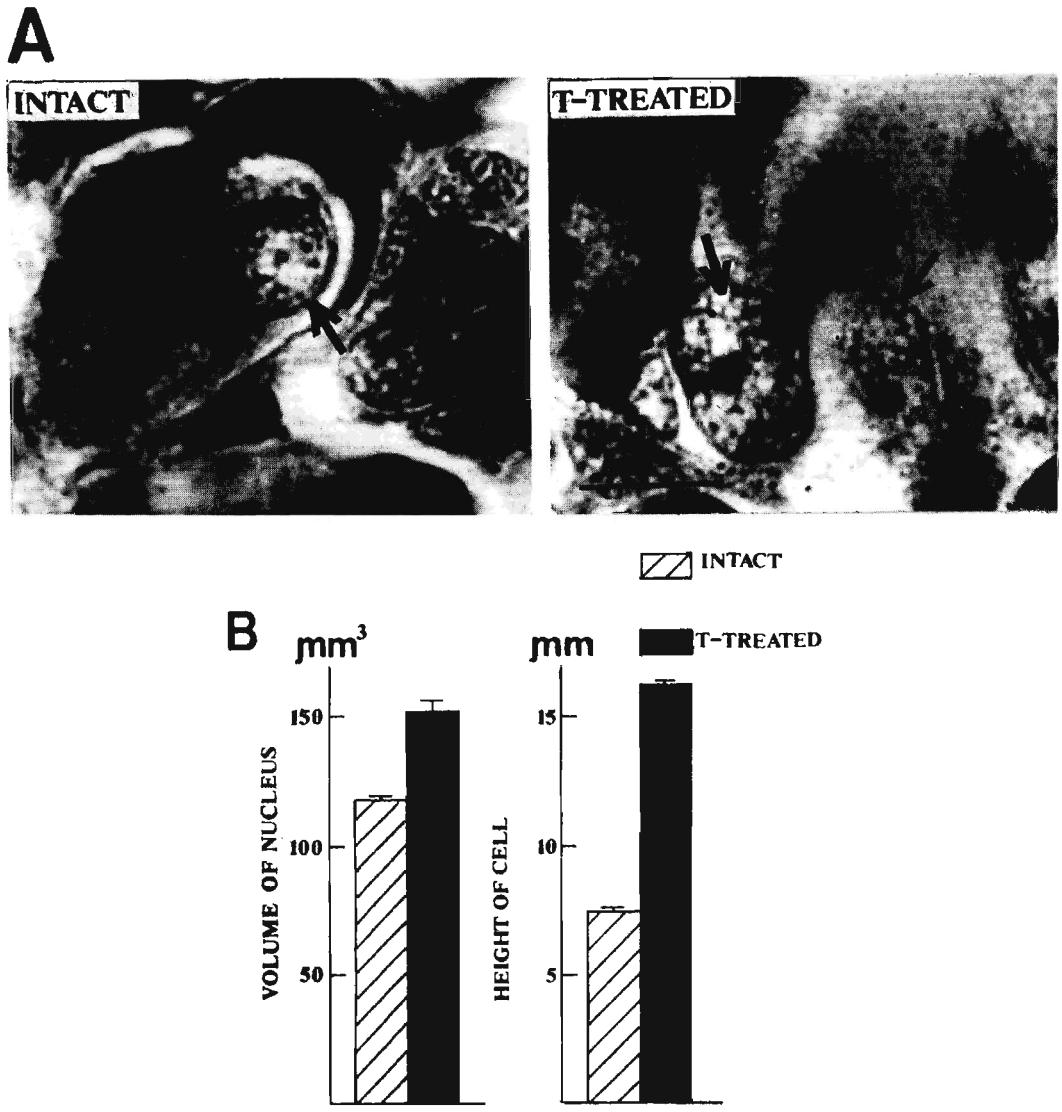

Figure 2. – Experiment 1. (A) Light micrograph of salmon pituitary glands in February. Scale bar represents 5 μm. Arrows show the nuclei of gonadotropic (GTH) cells in intact and T-treated males. The GTH cells, their nuclei and nucleoli are enlarged in T-treated fish. (B) GTH cell heights and nuclear volumes in intact and T-treated salmon males in February. Means ± SEMs. Differences between groups are significant ($p < 0.01$).

blood testosterone level in T-treated males (*fig.* 4B) and absence of mortality due to fungal diseases.

Experiment 3

Effects of one time T treatment in late March on Atlantic salmon dwarf males.

In late March all intact males produced only small amounts of viscous residual milt. Sperm cells could not be activated by water. The mean blood testosterone level was 6.6 ± 1.0 ng/ml. Injection of T resulted 19 days later (at 18 April) in a significant ($p < 0.01$)increase in blood testosterone levels (25.5 ± 1.3 ng/ml, vs 5.4 ± 0.4 ng/ml in intact fish) and milt production (1.5 ± 0.4 ml, vs 0.2 ml in intact fish). No sign of sperm cells viability was apparent. Active seminal fluid production in lobules of testis was revealed histologically.

Experiment 4

Effects of T treatment twice within the breeding season (October and November) on broad whitefish.

Intact males began to produce milt in mid October and displayed peak spermiation during November. Most stopped releasing milt later and lost their nuptial dress (tubercles or pearl organs on the scales and fin rays). On the contrary, all males injected with T showed well expressed nuptial dress and a significantly increased volume of sperm per stripping in early December, when the observations were completed (*table* 3). The differences between the intact

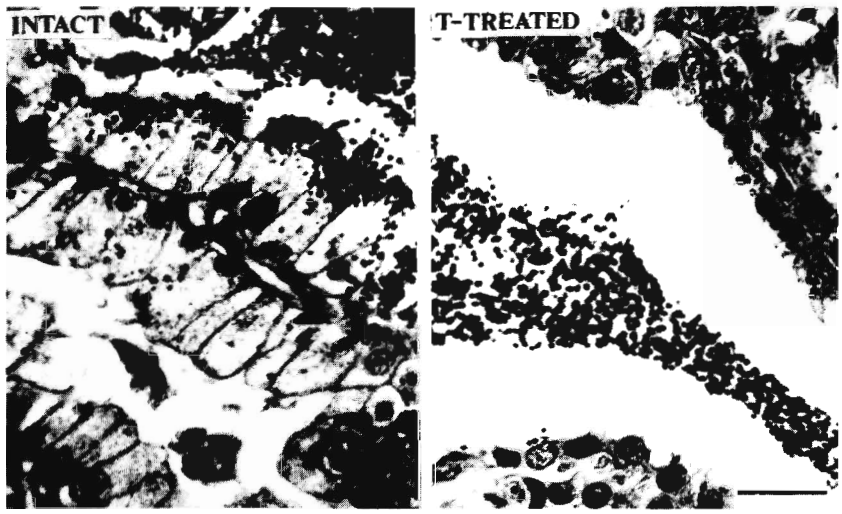

Figure 3. – Experiment 1. Light micrograph of intact and T-treated salmon testis in February. Scale bar represents 35 μm. Sertoli cell phagocytary activity is obvious in intact, but it is suppressed in T-treated fish.

and T-treated males in the other sperm parameters (concentration of sperm cells, degree and duration of their motility) were not significant. Due to the absence of mature broad whitefish females at completion of the experiment, the fertilizing capacity of sperm obtained from T-treated males were checked using eggs obtained from 50 peled females. The average percentage of successfully developing hybrid embryos was as high as 85%.

Experiment 5

Influence of T treatment twice within the breeding season (October-November) on peled males.

The intact males remained in running condition from October or November to late December. The spermiation in these young (2-year-old) first time matured fish was weak and the percentage of running specimens dropped gradually towards the end of the breeding season (*fig.* 5A). The average volume of sperm obtained per stripping (*fig.* 5B) and the total volume of sperm obtained from each intact male over the breeding season did not exceed 0.40±0.04 ml and 1.50±0.11 ml, respectively. The concentration of sperm (*fig.* 5C) and duration of sperm motility (*fig.* 5D) did not show any consistent changes, whereas the degree of sperm motility (*fig.* 5E) showed a tendency to decrease over the period. The differences between average values of the last parameter were significant ($p<0.01$) when the data obtained on 16 November and 10 December were compared. The intact fish lost their nuptial dress gradually.

All T-treated males retained a well-expressed nuptial dress and continued to produce large amounts of acceptable quality milt (*fig.* 5A) over the experiment. The volumes of sperm obtained from each T-treated male per stripping were significantly ($p<0.01$) higher than those obtained from each intact fish in all samples obtained (*fig.* 5B). The concentration of sperm in steroid-treated fish was similar to that in intact fish (*fig.* 5C). The duration of sperm cell motility increased in T-treated males as compared with intact males, but the differences were significant ($p<0.01$) on 26 November and 10 December only (*fig.* 5D). The degree of sperm cell motility was stable over the course of the experiment (*fig.* 5E). The mean values of the sperm parameters were always higher in T-treated males compared with intact ones, but the differences were reliable ($p<0.01$) on 10 December only.

Table 3. – Sperm characteristics of the broad whitefish during experiment 4.

Date	Groups	Vol. per stripping (ml)	Duration of motility (s)	Degree of motility (c.u.)	Concentration (10^9/ml)
Oct. 1982	Intact (initial condition)	0.6±0.1	60.9±3.1		7.9±0.4
Nov. 1982	Intact	1.1±0.2	88.5±7.1	3.8±0.5	6.9±0.4
	T-treated	2.0±0.2**	101.5±7.3	4.1±0.3	6.8±0.6
Dec. 1982	Intact	1.1±0.2	62.9±8.3	3.2±0.3	6.9±0.6
	T-treated	5.2±0.6**	77.8±6.9	4.0±0.6	6.4±0.1

$n=25$ in all groups. Mean±SEM. ** Significantly different from corresponding controls ($p<0.01$). 20% of intact males were unable to release sperm or produced only few drops of a weakly motile (0-2 c.u. degree) sperm at the end of the experiment.

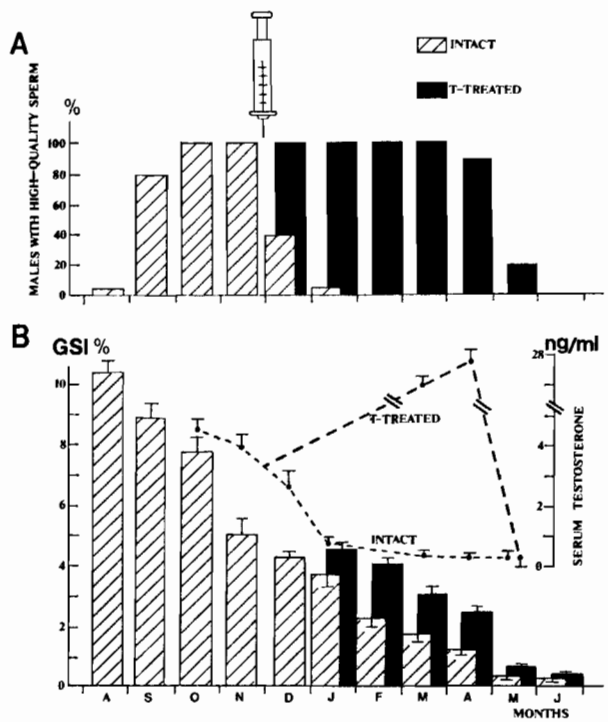

Figure 4. – Experiment 2. 50 intact salmon males were studied in August-December (they are not included in *table* 1). Initial number of intact and T-treated fish was 50 per group at the end of December, 8 fish from each group were sacrificed every month from January. (A) High-quality sperm production by intact and T-treated salmon males. Syringe shows time of injection. (B) GSI and serum testosterone dynamics in intact and T-treated salmon males. $n = 8$-10. Means ± SEMs. Differences between the hormone values in intact and T-treated groups are significant in March and April. Differences in GSI between groups are significant ($p < 0.01$) in January-May.

Experiment 6

Influence of one T treatment at the beginning of breeding season (mid October) on peled. These fish were of the same age (2-year-old) as in previous experiment, but the period of observation lasted until the end of January.

Not all intact males produced a high-quality sperm and the quota of such fish dropped rapidly during December (*fig.* 6A). The average volumes of sperm obtained from each intact male per stripping decreased too (*fig.* 6B).

On the contrary, almost all of T-treated males were able to produce high-quality sperm until completion of the experiment (*fig.* 6A). Average volumes of sperm obtained from each T-treated male per stripping tended to increase from December to January (*fig.* 6B). They were significantly ($p<0.01$) higher in T-treated fish than in intact ones in the samples obtained from 3 December to 22 January (*fig.* 6B). Blood testosterone levels were 5.6±0.17 ng/ml and 22.2±1.1 ng/ml on 25 December and 2.4±0.3 ng/ml and 6.6±0.6 ng/ml on 22 January in intact and T-treated groups respectively. The differences were

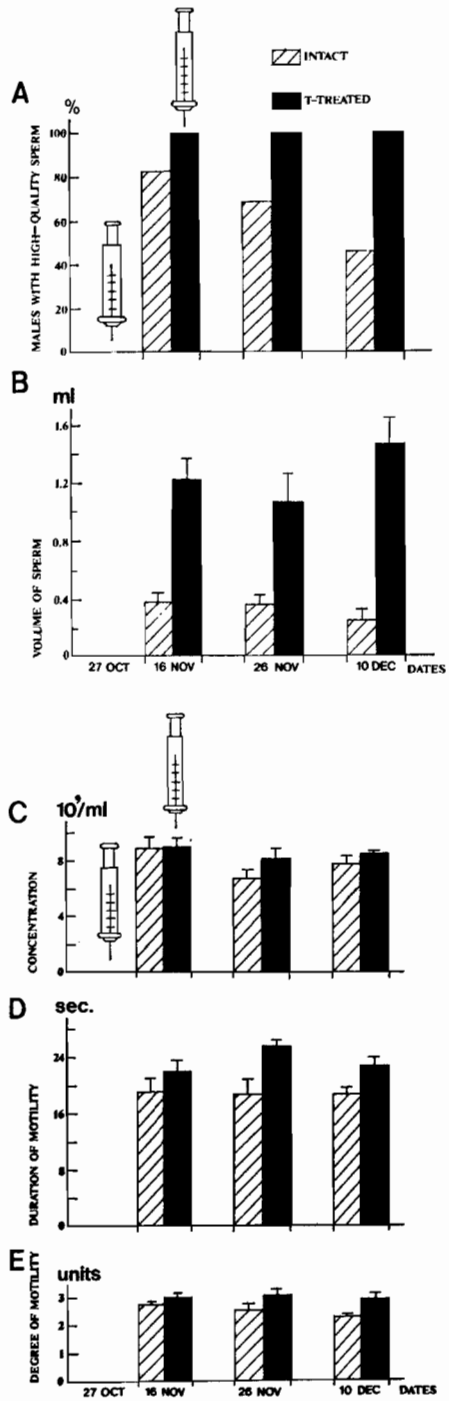

Figure 5. – Experiment 5. Fish 31 intact and 20 T-treated fish per sample were studied. (A) High-quality sperm production by intact and T-treated peled males. Syringes indicate injections. (B) Volumes of sperm obtained from intact and T-treated peled males per stripping. Means ± SEMs. Differences between groups are significant ($p < 0.01$) in all samples. (C, D, E) Sperm concentration, duration and degree of its motility in intact and T-treated peled males. Means ± SEMs. Differences between groups are significant for the duration of sperm motility in November and December samples, whereas they are significant ($p < 0.01$) for the degree of sperm motility in December only.

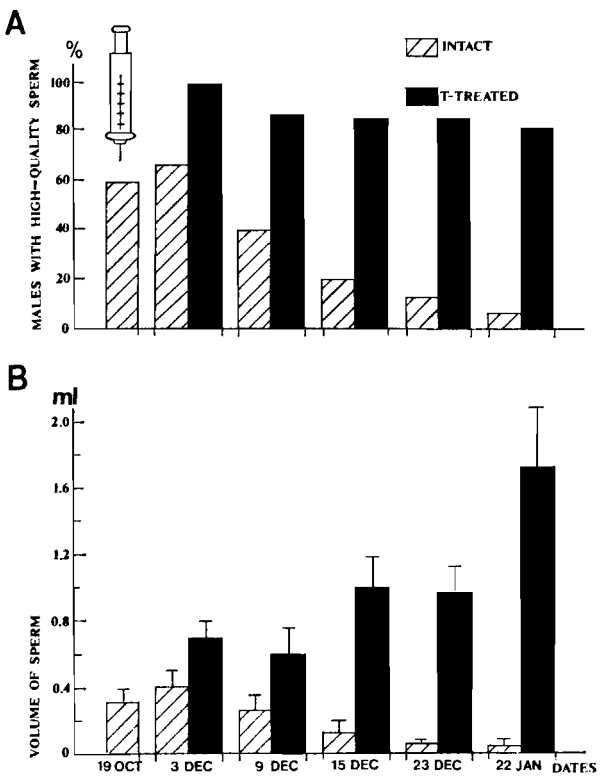

Figure 6. – Experiment 6. (A) High-quality sperm production by intact and T-treated peled males. Initial number of fish was 25 per group, 8 fish from each group were sacrificed 23 December. Syringe indicates injection. (B) Volumes of sperm obtained from intact and T-treated peled males per stripping. Means ± SEMs. Differences between groups are significant ($p<0.01$) in all samples obtained in December-January.

statistically reliable ($p<0.01$) in both cases. GSI tended to decrease from 1.63±0.06% in late December to 1.26±0.07% in late January in intact fish, whereas it remained constant (1.59±0.07%) in T-treated fish. The preservation of well-expressed nuptial dress was also characteristic of T-treated fish.

Increased phagocytary activity of Sertoli cells was found in gonads of intact males but not of T-treated male. Signs of active seminal fluid production were obvious in the testis lobules of T-treated fish. GTH cells seemed to be more active in the steroid-treated group as compared with intact one. GTH cell height and nuclei volume in fish injected with T (28.1±0.4 μm; 208.4±8.6 μm^3) were significantly ($p<0.01$) higher than those in intact ones (23.5±0.2 μm; 120±4 μm^3).

Experiment 7

Effects of one T treatment not a long before the end of breeding season (December) on peled.

Spermiation in intact males was obvious from late November until mid-January. A single injection of T resulted 20 days later in a significant ($p<0.01$) increase of duration and degree of sperm motility and sperm volume, as compared with intact fish (*table* 4). Sperm concentration remained at the same level (about 8×10^9/ml).

Table 4. – Sperm characteristics of the peled during experiment 7.

Date	Groups	Vol. per stripping (ml)	Duration of motility (s)	Degree of motility (c.u.)
5 Dec. 1981	Intact (initial condition)		weak running	
25 Dec. 1981	Intact	0.9±0.02	18.5±0.7	2.7±0.1
	T-treated	1.2±0.01**	24.3±0.9**	3.5±0.1**

$n=25$ in all groups. Mean ± SEM. ** significantly different from corresponding controls ($p<0.01$).

Experiment 8

Effects of one T treatment not long before the beginning of breeding season (mid October) on volkhov whitefish.

A remarkable stimulation of sperm release was revealed after 15 days in T-treated fish (*table* 5). The average volume of sperm obtained per stripping and degree of sperm motility in these males were significantly ($p<0.01$) increased compared with the intact ones, whereas the differences in the duration of sperm motility were not reliable. Well-defined signs of active seminal fluid production found in T-treated fish testis.

High secretory and excretory activity of GTH cells were obvious on the sections of T-treated fish pituitaries, whereas basophilic substance storage was predominant in GTH cells in intact fish. GTH cells height and nuclei volume in males injected with T (25.6±0.5 μm; 174.6±8.1 μm^3) were significantly ($p<0.01$) higher than in the intact ones (22.9±0.3 μm; 106±4 μm^3). T-treated fish were also characterized by significantly ($p<0.01$) higher GSI (2.4±0.1% as compared with 1.5±0.1% in intact fish), significantly higher ($p<0.01$) blood testosterone level (10.3±1.0 ng/ml as compared with 5.5±0.5 ng/ml

Table 5. – Sperm characteristics of the volkhov whitefish during experiment 8.

Date	Groups	Vol. per stripping (ml)	Duration of motility (s)	Degree of motility (c.u.)
15 Oct. 1991	Intact (initial condition)		no running	
30 Oct. 1991	Intact	0.9±0.1	50.9±4.2	3.0±0
	T-treated	3.3±0.1**	58.0±3.3**	5.0±0

$n=10$ in all groups. Mean ± SEM. ** Significantly different from corresponding controls ($p<0.01$).

in intact fish), as well as by well-expressed nuptial dress at the end of the experiment. The satisfactory fertilizing capacity of sperm obtained from T-treated males was demonstrated by successful fertilization of eggs obtained from 5 volkhov whitefish females. The percentage of developing embryos exceeded 80% and normal hatching of alevins took place.

DISCUSSION

We believe that the main purpose of the experiments was achieved and an optimal regime of androgen treatment was found. A single intraperitoneal injection of 0.1 ml "Testoenatum" per 1 kg of fish body weight 15-20 days before the beginning of natural breeding season stimulates spermiation, milt parameters and extends the period of functional testis maturity by 1-4 months in Atlantic salmon and whitefish. If necessary, a second injection may be given shortly before the spring rise in water temperature. It is well known that steroid glucuronid formation (Fostier *et al.*, 1983) and urine output (Love, 1970) are reduced in fish at low temperature. For this reason low water temperatures are favourable in allowing a longer half-life of injected androgen in fish blood, as is clear when comparing the development of T levels in intact and T-treated males. We were able to maintain significantly increased levels of testosterone in fish for several months under our experimental conditions.

Of course, it is interesting to understand the physiological mechanisms behind the stimulatory and prolonging actions of exogenous testosterone on the male reproductive function. We suggest that this hormone may act on fish testis not only directly, but also indirectly through central feedback mechanisms, because the morphological features of GTH cell activation were obvious in androgen-treated males in our experiments. These findings are in agreement with data obtained by many other investigators who has revealed stimulatory influence of exogenous androgens on brain aromatase activity, pituitary GTH cells' sensitivity to GnRH, as well as on GnRH, GTH and endogenous androgen production in salmonid males (van Overveek and McBride, 1971; Crim and Peter, 1978; Crim and Evans, 1979, 1983; Weil and Marcuzzi, 1990; Mayer *et al.*, 1991; Amano *et al.*, 1994). Furthermore, significant changes in testosterone binding in the brain were found in salmon males injected with "Testoenatum" (Christoforov and Murza, 1991). The progestin 17α,20β-dihydroxy-4-pregnen-3-one (17,20P) was more effective than androgens in stimulating spermation in amago salmon (Ueda *et al.*, 1985). It has also been observed that testosterone administration can lead to increased plasma 17,20P levels in Atlantic salmon (Berglund *et al.*, 1995; Antonopoulou *et al.*, 1995). For these reasons it is possible than androgens exert their effects on spermiation via an increased 17,20P-production perhaps mediated via a positive feedback on the GTH cells.

It seems to be important to avoid over-dosage of injected androgen, especially at elevated water temperature. Fish have a well-developed immune system (Corbel, 1975; Thuvander, 1990) and sex steroids may act as immunodepressants (Pickering and Christie, 1980). By this reasoning, androgens may be responsible for the increase in fungal diseases among maturing salmon males (Murphy, 1981). Immunodepressive effects of androgens may also be involved in the extension of the period of functional gonad maturity. It may be connected with the influence of these hormones on the status of the blood-testis barrier (Raitzina, 1985). Such a barrier system is present in fish (Abraham *et al.*, 1980; Marcaillou and Szöllösi, 1980) and testosterone has been shown to regulate its function in vertebrates (Kevorkov *et al.*, 1983). Specific antigens are presented on the spermatids and spermatozoons surfaces (Secombes *et al.*, 1985; Lou *et al.*, 1989; Beck *et al.*, 1992). High androgen levels might suppress the immune responses, including autoimmunity, and therefore prevent the ripe germ cells from destruction during the natural breeding season. Later, decreasing androgen levels might result in clearance of spent testis from residual sperm cells by a typical autoimmune response (Christoforov and Murza, 1985). Sertoli cells, isolated phagocytes and perhaps other cell-types as well as humoral factors are involved in this phenomenon (Murza *et al.*, 1982; Billard and Takashima, 1983; Murza and Christoforov, 1983). Treatment with androgens might lead to the extension of the period of autoimmune response suppression. Immune responses in fish are temperature-dependent (Corbel, 1975).

Androgen treatment could have an important application, not only in obtaining of ripe germ cells out of natural spawning season, but also in the artificial propagation of salmon and whitefish populations effected by unfavourable environmental conditions. 20-50% of the Atlantic salmon males in the Neva River and Narova River populations in the North-West region of Russia have produced a low-quality milt in some recent years. Deterioration of water quality in the Volkhov River during recent years due to heavy pollution resulted in late and weak spermiation in males of volkhov whitefish used for artificial propagation. A stripping of milt from at least 3-4-males was necessary in many cases to fertilize the eggs of each whitefish female under the Volkhov hatchery condition. In addition, steroid-induced extension of gonad functional maturity may be useful to obtain cross-breeding between species (Murza *et al.*, 1983). However, the possible effects of male androgen treatment on progeny development needs to be fully evaluated before this method can be widely applied to aquaculture.

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