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Testosterone reaction after testicular biopsies – further investigation in the normogonad and cryptorchid rat model

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Abstract From the follow-up of patients undergoing extensive testicular sperm extraction, the question of a consecutive decrease in testosterone levels arose. Further investigation was performed in the normogonad and cryptorchid Sprague-Dawley rat model. From groups A ($n = 40$ normogonad rats) and B ($n = 40$ cryptorchid rats), eight animals were taken and the following surgical interventions were performed: general anaesthesia (A-0, B-0), scrotal exploration (A-1, B-1), one testicular biopsy with a 2-mm tunica incision (A-2, B-2), two biopsies with a 4-mm incision (A-3, B-3), and four biopsies with a 2-mm incision (A-4, B-4). Standardised testosterone control was performed 1 day before as well as 1, 7 and 14 days after surgery. No specific testosterone reaction was found in A-0, an insignificant decrease in A-1 (3.8 nmol/l), a significant decrease in A-2 (6.27 nmol/l), in A-3 (12.96 nmol/l) and in A-4 (11.88 nmol/l). In the B rats, even in B-3 and B-4 no significant decrease was measured. The testosterone reaction correlated to the *amount* of tissue that was extracted, but not to the *number* of biopsies. The cryptorchid rats did *not* react in a more sensitive manner.

Key words Testicular sperm extraction · Male infertility · Testosterone · Rat

Introduction

The development of new fertilisation techniques in the treatment of male infertility [9] has had a great impact on andrology. New indications for testicular surgery such as sperm retrieval by means of testicular biopsies in azoospermic males have arisen together with the modification of known operative procedures and the development of completely new techniques [12, 13]. Monitoring and investigation of short-term complications and long-term effects is certainly one of the tasks of the testicular surgeon. Multiple biopsies may be a cause of testicular fibrosis [10, 11]. In a former follow-up study of patients suffering from non-obstructive azoospermia who underwent extensive testicular biopsies to retrieve sperm for intracytoplasmic sperm injection (TESE-ICSI), the question of a consecutive decrease in testosterone levels in the blood arose [6].

In this study, further investigation of the testosterone reaction to multiple testicular biopsies was performed in the normogonad and cryptorchid Sprague-Dawley rat model. The main objectives were to investigate whether the same type and degree of testosterone reaction after TESE occurred in normogonad and cryptorchid rat populations, whether the postoperative testosterone reaction was only correlated to the amount of extracted tissue or also to the number of biopsies needed to extract such an amount and finally whether general anaesthesia and scrotal exploration alone can trigger testosterone reactions.

Materials and methods

Animal population

The animal population used for the investigation consisted of $n = 80$ Sprague-Dawley rats. They were 68–70 days old at the time of surgery. Their mean weight at this point was 300 g. Two separate groups were formed: *group A* containing $n = 40$ normogonad rats and *group B* containing $n = 40$ cryptorchid rats.

Induction of cryptorchidism

Cryptorchidism was induced in the group B rats 2 days after birth by a hormonal treatment, whereby 0.5 ml estradiol was injected subcutaneously [5]. This treatment resulted in reduced to low-normal testicular volume and localisation of the testes in the high scrotum, but never intra-abdominally.

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Surgery and anaesthesia

Anaesthesia was performed by intra-muscular injection of 0.1 ml Rompun (2%) and 0.3 ml Ketanest. Several types of surgical interventions were subsequently carried out on the normogonad group A rats. Eight animals underwent general anaesthesia only (A-0), eight animals scrotal exploration (A-1), eight animals one testicular biopsy per testis with a 2-mm incision (A-2), eight animals four biopsies per testis with a 2-mm incision (A-3) and eight animals two biopsies per testis with a 4-mm incision (A-4). The testicular biopsies were performed in analogy to the TESE technique. After the incision of the tunica albuginea, the testicular tissue was worked out by slight pressure on the testis and carefully removed with the help of scissors (''no-touch'' technique). The cryptorchid group B rats also underwent general anaesthesia only ($n = 8$, B-0), scrotal exploration ($n = 8$, B-1), one testicular biopsy with a 2-mm incision ($n = 8$, B-2), four biopsies with a 2-mm incision ($n = 8$, B-3) and two biopsies with a 4-mm incision ($n = 8$, B-4).

Testosterone measurements

Testosterone measurements were performed 1 day before (T_0) and 1 (T_1), 7 (T_7) and 14 days (T_{14}) after surgery. The blood samples were taken from veins in the rat tail. For this purpose, the rats were fixated in a special cage and the tail was warmed up in a water bath for 5 min. One of the superficial veins was punctured with a three-gauge needle and 2 ml blood was extracted. All blood samples were taken between 8.00 a.m. and 9.00 a.m.

The living conditions for the rats were kept as stable as possible. Four rats were together in one cage at all times. Any change of room or cage was strictly avoided. The rats lived in a constant day-night rhythm and were fed and cleaned by the same person at the same time of the day during the whole experimental period.

Statistical analysis

Statistical analysis was performed by determination of the standard deviations. The significance was determined by Wilcoxon's rank-sum test with a significant P-value of < 0.05.

Results

Testosterone reaction in the normogonad rats (group A)

Two weeks after surgery (T_{14}) the rats were examined to determine their testosterone values. In group A-0, which contained normogonad rats undergoing general anaesthesia only ($n = 8$), it was seen that four rats had a decrease in testosterone concentration in the blood, another three rats an increase, and the remaining one had no change. In the group A-1 rats, which underwent additional scrotal exploration, a decrease was found in five animals and an increase in the other three. Of the normogonad animals undergoing testicular biopsies with one biopsy per testis (A-2) five rats showed a decrease in testosterone levels, two had constant levels and one rat showed an increase. In the group with four 2-mm biopsies per testis (A-3) and also in the group with two 4-mm biospies per testis (A-4), a decrease in testosterone was found in all the rats (Fig. 1).

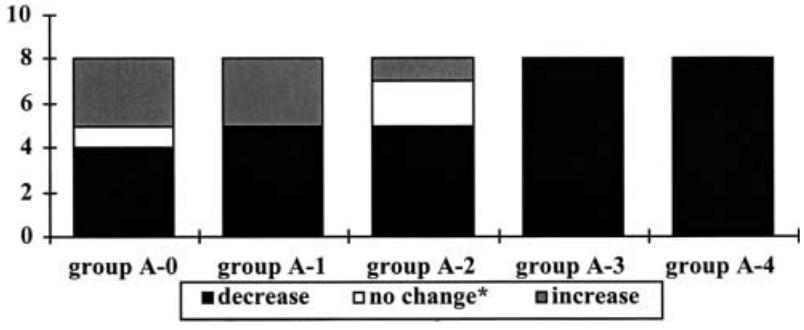
Mean initial testosterone concentration in the blood was 9.72 in group A-0, 12.52 in group A-1, 11.1 in group A-2, 15.13 in group A-3, and 13.74 in group A-4. Two weeks after surgery, the mean levels had increased to 9.95 in group A-0 and decreased to 8.72 in group A-1, to 4.83 in group A-2, to 2.17 in group A-3, and to 1.86 in group A-4 (Fig. 2a). In most cases, this decrease was already measurable at day 1 after surgery (T_1 value), and it did not recover during the 2-week investigation period after surgery (Fig. 2b).

Thus the mean decrease in testosterone levels in the blood showed the same decrease in both groups with extensive biopsies (A-3 and A-4) without any significant difference (12.96 nmol/l vs. 11.88 nmol/l). The mean decrease was less in the rats of group A-2 undergoing only one biopsy per testis (6.27 nmol/l), while the smallest decrease (3.8 nmol/l) was measured after scrotal exploration (A-1). After anaesthesia alone, no decrease at all was detectable. In group A-0 a slight increase was even recorded (Fig. 3).

Testosterone reaction in the cryptorchid rats (group B)

The initial testosterone blood levels were much lower in the cryptorchid than in the normogonad rats. The average preoperative concentration was 1.39 nmol/l in group B compared to 12.44 nmol/l in group A (Fig. 4).

Fig. 1 Number of rats with decrease or increase in testosterone concentrations after surgery ($T_0 \rightarrow T_{14}$): in both groups undergoing extensive biopsies (A-3, A-4), a decrease was seen in all the animals, whereas after general anaesthesia only, scrotal exploration and even after one biopsy per testis, no general trend in the testosterone reaction was found. *Difference between T_0 and T_{14} < 1 nmol/l



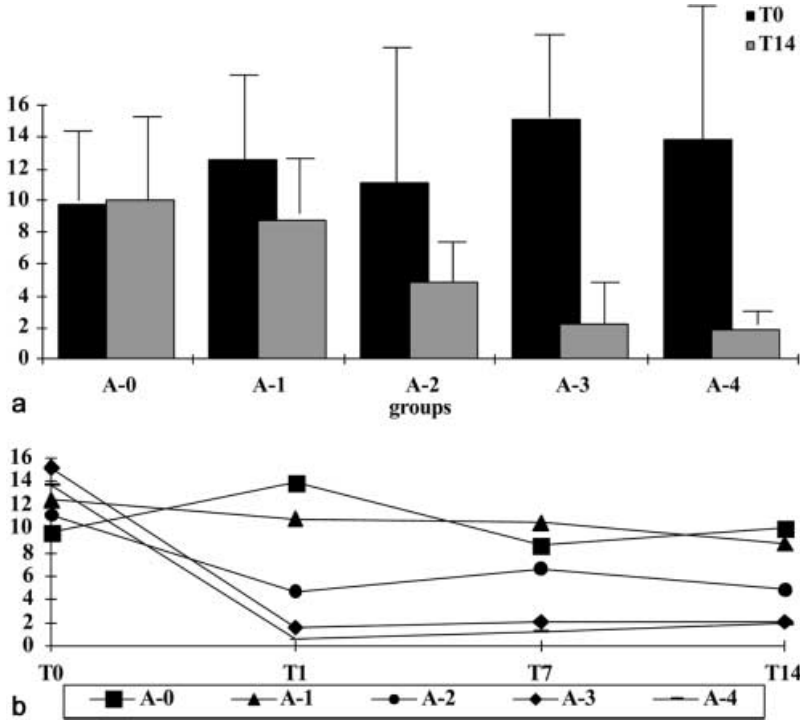


Fig. 2 **a** Mean testosterone concentrations before (T_0) and 14 days after surgery (T_{14}) in the different A groups. **b** Mean testosterone concentrations before surgery (T_0) and 1 (T_1), 7 (T_7) and 14 days (T_{14}) after surgery in the different A groups: the decrease in testosterone concentrations occurred directly after surgery (T_1 value) and did not recover until day 14

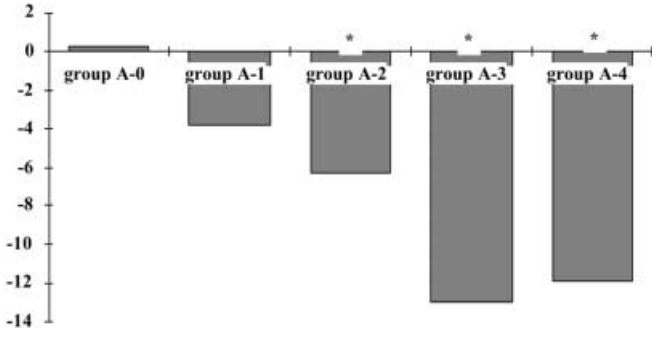


Fig. 3 Mean decrease in testosterone concentrations after surgery ($T_0 \rightarrow T_{14}$) in group A: the decrease was found in groups A-1 to A-4. This decline was most notable in groups A-3 and A-4, without any significant difference between these two groups. Even in A-1 (scrotal exploration only), a certain decrease in testosterone was observed. *Decline in testosterone concentrations was statistically significant

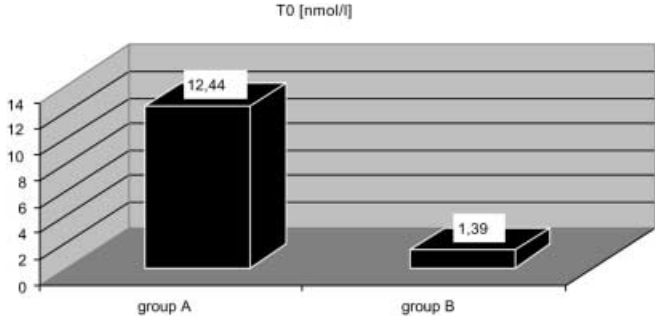


Fig. 4 Mean initial testosterone concentrations before surgery (T_0) in group A (normogonad) compared to group B (cryptorchid): the testosterone levels were significantly lower in group B

In group B, no special post-surgical testosterone reaction and, above all, no general decrease in blood concentrations was found (Fig. 5).

Discussion

Decrease in testosterone concentrations in the blood after surgical trauma has been described in several reports [3, 4, 14]. The level of decrease depended on the extent of the surgical trauma. A postoperative testosterone reaction was also reported after general anaesthesia only [3, 7, 8]. As testosterone plays an essential role in the initiation [1, 16], the maintenance [15, 16] and the re-initiation of spermatogenesis once it has been interrupted [2, 15], the post-surgical testosterone decline should be avoided or at least minimised especially in patients suffering from fertility disorders.

In the present study, a decrease in testosterone concentrations was found after extensive testicular biopsies in the rat model. This post-surgical testosterone decline was clearly correlated to the amount of extracted testicular tissue. This was demonstrated by a mean decrease of 12.96 nmol/l in A-3 and 11.88 nmol/l in A-4 rats, which had the largest amount of tissue extracted, vs. a decline of 6.27 nmol/l only in A-2 rats, which underwent only one biopsy per testis with a 2-mm incision.

The nearly identical degree of post-surgical testosterone decline in groups A-3 and A-4 suggested that the amount of extracted tissue is the decisive factor – no matter whether this amount of tissue is taken in one or two large biopsies or in multiple small-sized biopsies

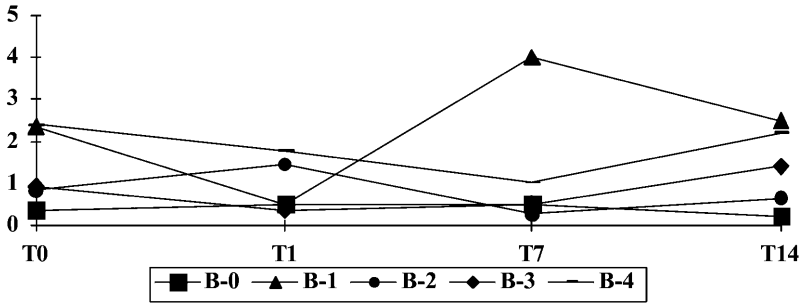


Fig. 5 Mean testosterone concentrations before (T_0) and 1 (T_1), 7 (T_7) and 14 days (T_{14}) after surgery in the B groups, showing an indifferent development

from different locations. A larger trauma, as was created by several incisions in multiple biopsies, did not seem to increase the testosterone reaction. In the present study, general anaesthesia alone did not create any testosterone reaction. In contrast, the scrotal exploration caused a decline with borderline significance.

As a clinical consequence, it can be said that *multiple* testicular biopsies do not have to be avoided due to the fear of a strong testosterone reaction. In contrast, the *amount* of extracted tissue should be minimised. Furthermore, the hormonal reaction after scrotal exploration shows that even this slight intervention can trigger a post-surgical testosterone reaction and may cause a relevant trauma on the testis. This underlines the fact that the indication for testicular biopsies in order to extract spermatozoa has to be considered carefully.

Even though the mean initial testosterone level was much lower in the cryptorchid (1.39 nmol/l) than in the normogonad (12.44 nmol/l) rats, no relevant postoperative decline was found in the cryptorchid rats. Even after extensive biopsies in groups B-3 and B-4 with two 4-mm incisions and four 2-mm incisions, respectively, a slight decrease was measured at day 1 after surgery (T_1 value), which recovered and reached the initial level (or even higher) 2 weeks later. These data do not point to any increased risk of a decline in testosterone concentrations in the blood after testicular biopsies in hormonal borderline patients or in patients with testicular deficiency. Of course, we cannot completely exclude the possibility that the low initial hormone levels in the cryptorchid rats as well as the hormonal induction of cryptorchidism itself might have influenced the postoperative testosterone reaction in this study. Similar problems occur with any other model involving the induction of cryptorchidism or testicular damage. Alternative *surgical* induction of cryptorchidism by intra-abdominal placement of the testes represents, in our opinion, an even stronger intervention with a possible effect on the results. Especially the location of the testes in the (high) scrotum after hormonal treatment and the low to low-normal size of the testes represents the ideal model of a cryptorchid male (with fertility disorders). However, the conclusions drawn from the testosterone curves of the group A and B rats are not directly comparable. As the initial blood concentrations are very low in the group B rats, these testosterone levels possibly derive from the adrenals only.

Clinically speaking, the data in the present study do not joint to a higher risk of testosterone decrease after TESE in patients with low preoperative levels. Nor does a TESE technique with multiple biopsies from different locations seem to cause problems with testosterone levels. The important factor is the amount of testicular tissue which is extracted. This underlines the need for guidelines regarding the maximal limit for tissue extraction.

Conclusions

The decrease in testosterone levels in the blood after testicular biopsies correlated to the amount of extracted tissue, but not to the number of biopsies (when the total amount of extracted tissue remained unchanged). General anaesthesia alone did not create any decrease in testosterone concentrations. In contrast, scrotal exploration created a decline in testosterone concentrations, which was significantly lower than the decrease after tissue extraction.

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