

## FAILURE OF INDUCING SPERMATOGENESIS WITH FSH, LH AND TESTOSTERONE IN VITAMIN A DEFICIENT RAT

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### Abstract

Male Fischer rats 22 days of age were fed with vitamin A-free diet until becoming vitamin A deficiency. Ten rats were left as vitamin A deficient control group (A<sup>-</sup>). Forty-eight rats were transferred to diet supplemented with retinoic acid (A, 6 mg/kg of diet) and further divided into 4 groups to be injected daily for 14 days with saline (0.1 ml), FSH (0.1 mg) + LH (0.1 mg), FSH (0.1 mg) + testosterone proprionate (TP, 0.5 mg), and with vitamin A (50 µg). One day after the last injection, final body weight was recorded and the animals were sacrificed by decapitation. Testis, thyroid, adrenal, seminal vesicle and prostate were dissected and weighed. Testes were also prepared for histological examination. It was found that retinoic acid alone or with vitamin A treated could similary induce body growth in vitamin A deficient rats. Retinoic acid with or without injections of FSH + LH or FSH + TP could equally and slightly improves the cytologic condition of the seminiferous tubules over the vitamin A deficient level. However, vitamin A injections for 14 days could further improve the condition of the seminiferous tubules. It is concluded that short-term or long-term treatments with hormone necessary for spermatogenesis without supplementation of vitamin A could not cause full spermatogenesis in rats with testicular damage due to vitamin A deficiency. However, with or without supplementation of such hormones, vitamin A could induce full spermatogenesis.

Vitamin A deficiency has been shown to decrease growth rate in experimental animals (1-2), associated with impairment of testicular development and function (3-6). Long-term treatments of hormones



necessary for spermatogenesis from low to high doses either in single or in combinations has been tried without success (5,7-9). Since long-term treatments of peptide hormone could induce antibody formation and neutralized the effect of hormone injected (10), it is of interest to find out whether short-term treatments of FSH in combination with LH or testosterone propionate (TP) would cause sperm production in vitamin A deficient rats.

### METHODS

Male Fischer rats 22 days of age, previously maintained on standard laboratory diet (Zeiglig Gold Coin Mills), were transferred to vitamin A-free diet (2) except for a group of 10 rats, served as control were maintained on the same diet but with vitamin A(A<sup>+</sup>) added (retinyl acetate, 4 mg/kg of diet). They were housed at room temperature of 25° to 30°C with natural light of approximately 13 hours daily. They became vitamin A deficient rats within 5 weeks by having the weight gain not more than 3 grams per day for three consecutive days. Ten of these rats were left as vitamin A deficient controls(A<sup>-</sup>). The rest were transferred to maintain on comparable diet but supplemented with retinoic acid (A<sup>+</sup>, 6 mg/kg of diet) and divided into 4 groups to be subcutaneously injected for 14 days with FSH + LH (ICN Pharmaceuticals), FSH + testosterone propionate (TP, Schering, in Mazola corn oil), retinyl acetate (vitamin A in normal saline) and with normal saline starting 10 days after the onset of body weight plateau or one week after retinoic acid supplementation. The daily doses of FSH, LH, TP, vitamin A and saline were 0.1 mg, 0.1 mg, 0.5 mg, 50 µg and 0.1 ml, respectively. The injection volume was 0.1 ml. All rats were received the diet and tap water ad libitum. Sacrifice was made by decapitation one day after the last injection, between 0830 and 1030 hr, and the animals were 74 to 78 days old. Testis, thyroid, adrenal, seminal vesicle (with seminal fluid drained) and prostate were dis-

sected and weighed. Testes were prepared for routine histological examination.

## RESULTS

### BODY WEIGHT

As shown in Table 1, the final body weight of vitamin A deficient rat (A<sup>-</sup>) was much lower than that of normal control (122 g vs 218 g,  $P < 0.001$ ). When supplemented with retinoic acid with or without vitamin A or hormone injections to the vitamin A deficient rat, their weight gain was similar to that of rat fed on diet with vitamin A added (normal rat). Thus the final body weight of retinoic acid supplemented rat was significantly higher than that of vitamin A deficient rat ( $P < 0.001$ , Table 1) but not as high as that of normal control.

### TESTICULAR WEIGHT

The testicular weight of vitamin A deficient rat was only 35% of that in normal rat ( $P < 0.001$ ). Retinoic acid supplementation with or without saline, vitamin A or hormone treatments could not cause significant changes of testicular weight from vitamin A deficient level.

### SEMINAL VESICULAR AND VENTRAL PROSTATIC WEIGHT

Both vesicular and ventral prostatic weight of vitamin A deficient rat supplemented or nonsupplemented with retinoic acid and with or without FSH + LH, vitamin A or saline injections were in most cases, significantly lower than that of normal control. However, in the case of FSH + TP treatments, ventral prostatic weight was slightly higher and the seminal vesicular weight was significantly higher than that of normal control ( $P < 0.001$ , Table 1).



Table 1. Effect of vitamin A deficiency ( $A^-$ ), retinoic acid ( $\hat{A}$ ) supplementation with or without gonadotrophin (FSH, LH), testosterone (TP) and retinyl acetate (Vit. A) treatments on final body weight, weights of testis, seminal vesicle and ventral prostate of male rats.

Diet and treatment	No. of rats	Final body weight g	Testis g	Seminal vesicle mg	Ventral prostate mg
$A^{+*}$	10	$218 \pm 9.8^{**}$	$2.21 \pm 0.23$	$286.3 \pm 52.0$	$119.1 \pm 34.7$
$A^-$	10	$112 \pm 6.2^{1,2}$	$0.77 \pm 0.05^3$	$204.3 \pm 26.3$	$117.6 \pm 13.6$
$\hat{A}$ +Saline	11	$156 \pm 10.0$	$0.85 \pm 0.03$	$184.3 \pm 18.1$	$103.7 \pm 26.9$
$\hat{A}$ +FSH+LH	9	$153 \pm 9.1$	$0.84 \pm 0.04$	$147.6 \pm 29.1$	$90.0 \pm 19.3$
$\hat{A}$ +FSH+TP	10	$155 \pm 8.0$	$0.71 \pm 0.04$	$476.1 \pm 15.7^4$	$251.1 \pm 18.6$
$\hat{A}$ +Vit. A	8	$146 \pm 9.8$	$0.80 \pm 0.06$	$180.6 \pm 38.6$	$86.1 \pm 20.5$

\*Diet with retinyl acetate (Vit. A) added  
 \*\*Mean  $\pm$  S.E.

1. Final body weight of vitamin A deficient rat ( $A^-$ ) was significantly lower than that of normal rat ( $A^+$ ) ( $P < 0.001$ ).
2. Final body weight of  $A^-$  was significantly lower than rats receiving retinoic acid ( $\hat{A}$ ) with or without FSH+LH, FSH+TP or vitamin A injections.
3. Testicular weight of  $A^-$  with or without retinoic acid supplement was much lower than that of  $A^+$  ( $P < 0.001$ ).
4. Seminal vesicular weight significantly increased ( $P < 0.001$ ) in  $\hat{A}$ +FSH+TP group when compared with that of all other groups.

## HISTOLOGY OF THE TESTIS

It is obvious that vitamin A deficient rat had much smaller seminiferous tubules with wider lumen when compared to those of normal rat. Only one to two layers of lining cells were found; most of them were Sertoli cells and only few spermatogonia. Leydig's cells were also seen with pyknotic nuclei. In contrast, all stages of germinal cells were found in normal tubules with healthy Leydig's cells. Retinoic acid supplementation could only slightly increase spermatogonia



number forming two layers of epithelium while the Leydig's cells were practically unchanged. The injection of FSH + LH or FSH + TP for 14 days could not change histology of the tubules when compared to vitamin A deficient rat supplemented with retinoic acid alone. However, somewhat active appearance as evinced by a slight increase in the size of nuclei and cells of Leydig's cells after injection with FSH + LH. Reverse appearance of Leydig's cells was observed when treated with FSH + TP. Vitamin A treatments could make the histology of testis appeared healthier than that observed in the rat supplemented with retinoic acid alone as two to three layers of slightly enlarged spermatogonia were found in the seminiferous tubules and the Leydig's cells appeared to be similar to those in rats injected with FSH + LH.

#### DISCUSSION

The results of this study support those of the previous investigations (5,9-11) that vitamin A deficiency results in testicular atrophy leading to sterility. Supplementations of vitamin A for a short period of 14 days following sterility due to vitamin A deficiency could slightly improve the condition of the gonad. Furthermore, injections of FSH + LH or FSH + TP, also for 14 days, could not further stimulate spermatogenesis beyond what is seen in vitamin A deficient rat supplemented with retinoic acid. Thus the unresponsiveness of the seminiferous tubule to prolonged treatments with exogenous hormones necessary for spermatogenesis of the previous studies (5,9) as well as short treatments in this study may be due to the severe damage of the gonad as the results of vitamin A deficiency rather than the neutralizing effect of the antibodies formed by chronic injections of peptide hormones (10). It is interesting however that completion of spermatogenesis along with the rising of FSH, LH and testosterone was observed in vitamin A deficient rats treated with vitamin A for 30 to 60 days (12-13), and a period of 48 to 53 days is needed for sperma-



togonium to complete differentiation and be released as mature spermatozoon (14). Thus, the completion of spermatogenesis is likely impossible without vitamin A.

Although the vitamin A deficiency could cause permanent damage to the testis, the results of this study as well as those of Mayer and Traunt (7) indicate that seminal vesicle and ventral prostate decrease their activity under vitamin A deficient condition but could be very well under stimulatory effect of testosterone. It is likely that vitamin A deficiency could cause temporary effect on these two accessory sexual organs.

Vitamin A deficiency results in degenerative changes of various epithelia (3,15-16), the pituitary gland, a differentiated epithelial tissue could also be affected so that pituitary hormone production as well as secretion should be impaired. Thus the reduction of gonadotrophin production and secretion leading to further damage of the gonad is possible. Likewise, growth hormone synthesis and release could be well decreased resulting in growth retardation. The indirect effect of vitamin A deficiency on growth through pituitary-thyroid axis and through pituitary-gonadal axis could as well be considered since growth hormone production and secretion are under the control of thyroid hormones (17-21). In addition, orchidectomy could reduce pituitary growth hormone and the supplements of testosterone achieve the opposite effect (22-26). Study on pituitary cytology as well as FSH, LH, and growth hormone assay to verify the statements above in connection with the present study should be carried out.



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