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# Relationship Between 13-cis-retinoic Acid or Retinol Acetate and Ratio of Spermatogenesis, Diameter of Semniferous Tubules and Number of Leydig Cells in *Gerbillus cheesemani*

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## Abstract

The results of the present study showed that administration of 13-cis-retinoic acid or retinol acetate for 6 weeks reduced the diameter of the seminiferous tubules in the testes of *Gerbillus chessemani*. Also, a decrease in the Leydig cell count was observed. However, the ratio of spermatogenesis was decreased only by 13-cis-retinoic acid administration. On the other hand the ratio of spermatogenesis, diameter of seminiferous tubules and number of Leydig cells were nearly normal in the experimental animals after 12 week of withdrawal treatment of drugs.

## Introduction

Vitamin A is well-known for its importance in general growth, differentiation of epithelial tissue, visual function and reproduction. Vitamin A and various synthetic analogs received much attention because they inhibit carcinogenesis in a number of experimental tumor systems (Hill and Grubbs, 1982; Sadek and Hayat 1996). Degeneration of the seminiferous tubules and reduction of the germinal epithelium have been observed in cases of vitamin A deficiency in rat and rhesus monkeys (O'Toole *et al*., 1974). Testicular atrophy accompanied by spermatogenesis has been described in retinoic treated hamsters (Longnecker *et al*., 1983) rats (Thompson *et al*., 1963) and toads (Sadek and Sabry 1986).

The testis has two major functions, androgen production and spermatogenesis. In mammals spermatogenesis is totally dependent upon testosterone, but exactly what level of testosterone is produced by the Leydig cells and how it acts upon the Sertoli and peritubular cells of the seminiferous tubule to drive spermatogenesis, is virtually unknown (Sharpe, 1986) one form of regulation of testosterone production is to control Leydig cell division, growth differentiation and or death (Ewing and Zirkin, 1983).

The present study was undertaken to examine possible biological relationships between diameter of seminiferous tubules, ratio of spermatogenesis and number of Leydig cells in male Gerbillus cheesemani and the effect of 13-cisretinoic acid or retinal acetate on this relationship.

## Materials and Methods

**Experimental animals:** Adult male gerbils (*Gerbillus cheeesemarn*) weighing 30-50 g, were obtained from a regular supplier in AL-wafra area, 85 Km. South of Kuwait city. The animals were housed in individual cages under controlled lighting conditions (14 hr light and 10 hr dark) and temperatures of 20-22°C. They had access to Dixon laboratory chow and water *ad libitum*.

**Seasonal variation in spermatogenic cycle:** At the beginning of each month over a one year period the testes of 3 mature wild caught animals were fixed in Bouin's fluid, embedded in paraplast and sectioned at 5 μm. The sections were stained with haematoxylin, counter-stained with eosin to detect seasonal changes (if any) in the spermatogenic cycle.

The animals were divided into 4 groups each of 20 gerbils and treated as follows:

Group I : Control
Group II : Animals injected intra peritoneally with 6 mg 13-cis-retinoic acid (RA) dissolved in olive oil/50 g body weight, three times/week for 6 weeks
Group III : Animals injected intra peritoneally with 6 mg retinol acetate (RT) dissolved in olive oil/50 g body weight, three times/week for 6 weeks
Group IV : Animals injected intra peritoneally with 0.2 ml olive oil/50 g body weight, three times/week for 6 weeks

Vitamin A in the form of 13-cis-retinoic acid, or retinol acetate and olive oil, were purchased from Sigma Chemical Company (St. Louis, MO, USA). After six weeks, the testes of 10 animals from each group were examined histologically. The remaining animals were left for a further twelve weeks without treatment and examined.

**Morphometric analysis:** The testicular tissue from 3 of 10 animals in each group were selected for morphometric analysis (Kerr *et al*., 1987). The ratio of spermatogenesis (% of active seminiferous tubules) was calculated by counting the number of active seminiferous tubules compared to the total number of tubules observed in the section (Kerr and Sharpe, 1989). Mean tubular diameter was measured on 20 seminiferous tubules per section using an ocular micrometer (Pinon-Lataillade *et al*., 1988). The numbers of Leydig cells in 3 random fields per section were

counted using a Zeiss projection microscope and the number of cells per unit area calculated.

**Statistical analysis:** The relationships between ratio of spermatogenesis, seminiferous tubule diameter and Leydig cell count were analyzed using one way analysis of variance (ANOVA). The level of significance was set at 5 percent. Data are expressed as means ± SEM.

## Results

The present results revealed no obvious seasonal variation in spermatogenesis in the testes of wild caught animals. The ratio of spermatogenesis was significantly reduced (67.1 ± 2.2%) in animals treated with retinoic acid in comparison with control or olive oil (Table 1). Also, the seminiferous tubules showed a significant decrease in their diameter (75.2 ± 1.8 pm). Moreover, insignificant decrease in the Leydig cell count was recorded (423.2 ± 37.3/1 $mm^2$). Statistical analysis showed that there was insignificant difference in the ratio of spermatogenesis (91.9 ± 2.8%) in the testis of animals treated with retinol acetate in comparison with control (Table 1), but a significant decrease in the diameter of seminiferous tubules was observed (86.9 ± 2.9 μm) compared to control. However, there was insignificant reduction in the number of Leydig cells (456.3 ± 37.4/1 $mm^2$) compared to control. The ratio of spermatogenesis, diameter of seminiferous tubules and number of Leydig cells were nearly normal in the animals of 12 weeks of withdrawal treatment of 13-cis-retinoic acid or retinol acetate (Table 1).

## Discussion

The present results showed that 13-cis-retinoic acid administration for 6 weeks decreased the ratio of spermatogenesis and the diameter of the seminiferous tubules in the testis of the gerbil. However, the testis of retinol acetate treated animals was more or less normal except for a significant difference in the diameter of seminiferous tubules. This can be regarded as compatible with view that retinoic acid causes stop of spermatogenesis (Border and Pitt 1981, 1983). Testicular atrophy accompanied by spermatogenesis has been described in retenoid treated hamsters (Longnecker *et al*., 1983) and rabbets (Sadek *et al*., 1986).

The present data showed that spermatogenesis in animals receiving 13-cis-retinoic acid or retinol acetate for six weeks then left for twelve weeks without treatment appeared almost identical to that in control animals. It is worth mentioning that these-results are confirmed by the study of Tsambaos *et al*. (1979), who used guinea pigs as an experimental model. Also, Sadek and Sabry (1986) observed that spermatogenesis in male toads was shifted by retinoic acid and these changes were found to be reversible after discontinuing treatment.

The association of retinoic treatment of the gerbil with inhibition of spermatogenesis and decrease in number of Leydig cell may reflect a decrease in the testosterone level. Cesstion of spermatogenesis has been reported in the presence of low serum testosterone levels in male rats (Rich and de Kretser 1977) and in male New-Zealand rabbits treated with 13-cis-retinoic acid (Sadek *et al*., 1986). Numerous investigations have attempted to relate specific differences in testicular Leydig cell number with testicular steroidogenic activity under conditions where testosterone production changes experimentally (Gondos *et al*., 1980; Nussdorfer *et al*., 1980).

There is now a general recognition of the existence of on RH receptor sites in the Leydig cells of the rat testis (Clayton *et al*., 1980; Quinn and Payne 1984). Also, long term treatment of maltreats with a superactive analog of on RH has been reported to induce atrophy of the testis, inhibit spermatogenesis, as well as lowering plasma testosterone concentration and elevating plasma. FSH and LH

Table 1: Variations in morphometric parameters in control and treated animals after (a) six weeks of treatment and (b) 12 weeks from treatment withdrawal

| | Group | | | |
|---|---|---|---|---|
| Parameters | Control | Retinoic acid | Retinol acetate | Olive oil |
| | **a) Six weeks of treatment** | | | |
| Ratio of S (%) | 97.8 ± 0.7 | 67.1 ± 2.2* | 91.9 ± 2.8 | 94.4 ± 0.9 |
| Diameter of Seminiferous tubules (μm) | 96.2 ± 2.0 | 75.2 ± 1.8* | 86.9 ± 2.9* | 97.5 ± 2.5 |
| Number of Leydig cells/1 $mm^2$ | 568.6 ± 48.3 | 423.2 ± 37.3 | 456.3 ± 37.4 | 549.2 ± 72.2 |
| | **b) 12 weeks from treatment withdrawal** | | | |
| Ratio of S (%) | 94.8 ± 0.7 | 92.4 ± 3.4 | 91.2 ± 1.9 | 94.3 ± 0.3 |
| Diameter of Seminiferous tubules (μm) | 96.2 ± 2.0 | 92.0 ± 3.0 | 94.0 ± 3.1 | 97.5 ± 3.0 |
| Number of Leydig cells/1 $mm^2$ | 568.6 ± 48.3 | 534.7 ± 98.1 | 564.9 ± 84.0 | 540.0 ± 49.3 |

*Significant from control at $p < 0.05$

concentrations (Belanger *et al*., 1979; Pelletier *et al*., 1978). It has been shown that the level of both FSH and LH were elevated in plasma samples from male New-Zealand rabbits treated with vitamin A acid and plasma testosterone concentration lowered (Sadek *et al*. 1986). This elevation may reflect an increase in the sensitivity of CNS to low circulating androgen (McCullagh, 1932).

Although the damage caused in the testis of the male gerbil by vitamin A treatment can be corrected by withdrawal of the drug for at least twelve weeks, there are a number of important questions to be answered before we may confidently make recommendation regarding dietary retinoids in the prevention of cancer.

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