Stage-Synchronization of the Seminiferous Tubules after Vitamin A Replacement in Vitamin A Deficient Golden Hamsters

Yasushi HIROTA, Masamichi KUROHMARU, Mitsuharu MATSUMOTO¹⁾, and Yoshihiro HAYASHI

Department of Veterinary Anatomy, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113 and ¹⁾Department of Veterinary Anatomy, Faculty of Agriculture, Kagoshima University, Kagoshima 890, Japan

(Received 16 September 1993/Accepted 13 December 1993)

ABSTRACT. The effect of vitamin A deficiency and vitamin A replacement on spermatogenesis in golden hamsters was studied using a light microscope. Male golden hamsters were fed a vitamin A deficient (VAD) diet from 3 weeks of age. Hamsters with a VAD diet reached maximum body weight at about 13 weeks. After 17 weeks, the body weight of the hamsters began to decrease. When their body weight decreased to 70 g, only Sertoli cells, spermatogonia, and a few spermatocytes were present within the seminiferous tubules. Administering retinol acetate (vitamin A) combined with a conventional diet to the VAD hamsters induced a reinitiation of spermatogenesis with stage-synchronization. At 9, 10, and 11 weeks after vitamin A replacement, the testes with active spermatogenesis possessed only a few successive stages of the seminiferous epithelial cycle.—**KEY WORDS:** golden hamster, stage synchronization, testis, vitamin A deficiency.

J. Vet. Med. Sci. 56(3): 439–442, 1994

Vitamin A is a very important nutrient for maintaining mammalian spermatogenesis [5, 7]. It is well known that spermatogenesis ceases in vitamin A deficient (VAD) rats. Although the seminiferous tubules of VAD rats contain only Sertoli cells, spermatogonia, and a few spermatocytes [8, 11, 13], vitamin A replacement in VAD rats produces a reinitiation of spermatogenesis [4].

Recently, it was found that spermatogenesis synchronously reinitiated after treating VAD rats with a high dose of vitamin A. This peculiar phenomenon is called “stage-synchronization” [9]. The normal rat testis with active spermatogenesis contains 14 stages of the seminiferous epithelial cycle, whereas the testis that reinitiates spermatogenesis after vitamin A replacement includes only a few successive stages of the cycle. An explanation of this phenomenon may be that the cell cycle of type A spermatogonia ceases at the G2 phase in the testis of VAD rats and that these type A spermatogonia reinitiate to differentiate synchronously [6]. Since testes enriched in seminiferous tubules at specific stages are easily obtained, this interesting model was thought to be useful for studying stage-specific proteins [10] and stage-dependent variations of Sertoli cells.

Some reports confirm the cessation of spermatogenesis in VAD rats [4, 9] and mice [12], but few attempts have been documented in VAD hamsters [3]. Moreover, neither a detailed morphological study nor an experiment on stage-synchronization has been carried out in VAD hamsters. In particular, an investigation on stage-synchronization using a different kind of species may yield new information, since the data on stage-synchronization has come from almost exclusively one species (rat). Therefore, in the present study, we investigated the morphological characteristics of regressed tubules in VAD golden hamsters and stage-synchronization after vitamin A replacement.

MATERIALS AND METHODS

Animals: The golden hamsters used in this study were maintained as a closed colony in our laboratory. Twelve male hamsters were fed a vitamin A deficient diet (Funabashi-Farm, Funabashi, Japan) and water *ad libitum* from 3 weeks of age. It was demonstrated by HPLC that the VAD diet contained no retinol. Seven of these animals were used for testicular observation at the following ages: (1) about 13 weeks when the body weight (BW) reached a maximum (approximately 130 g), (2) about 19 weeks when the BW decreased to 100 g, and (3) about 22 weeks when the BW decreased to 70 g. After their BW declined to 70 g, the other 5 animals were treated with 2 mg of retinol acetate (Sigma, St. Louis, MO, U.S.A.) i.p. and then fed a conventional diet. Retinol acetate was dissolved in 40 μ l ethanol (Wako Pure Chem., Osaka, Japan) and mixed with 80 μ l saline solution. These animals were used for testicular observation at 9 to 11 weeks after retinol acetate administration.

In order to obtain the percentage of each stage of the seminiferous epithelial cycle in normal hamsters, 3 animals were fed only a conventional diet and sacrificed at 22 weeks of age (about 130 g in BW).

Light microscopy: Each animal was anesthetized by sodium pentobarbital and then perfused with Bouin's fixative through the left ventricle after briefly washing with 0.9% saline. The specimens were sliced into slabs and immersed in the same fixative for 2–3 hr. They were then dehydrated in a graded series of ethanols, infiltrated in xylene, and embedded in paraffin. Sections of 4 μ m were stained with periodic acid Schiff (PAS)-hematoxylin and observed with a light microscope. More than 300 tubular cross sections per animal were classified into each stage according to Clermont [1].

Fig. 1. Light micrographs of testes in conventional, vitamin A deficient, and vitamin A-replaced hamsters. (a, b, c, e: $\times 200$, d: $\times 400$) (a) Cross section of seminiferous tubules of 13-week-old VAD hamsters. Active spermatogenesis is observed. Roman numerals indicate each stage of the seminiferous epithelial cycle. (b) Cross section of seminiferous tubules in VAD hamsters of approximately 100 g in body weight. There are neither spermatozoa nor elongated spermatids within the tubules. (c) Cross section of seminiferous tubules in VAD hamsters of approximately 70 g body weight. (d) Higher magnification of Fig. 1c. Only Sertoli cells (stars), spermatocytes (arrows), and spermatogonia (arrowheads) are detected within the tubule. (e) Cross section of seminiferous tubules at 70 days after vitamin A replacement, showing stage VII.

RESULTS

VAD golden hamsters reached their maximum body weight (approximately 130 g) at about 13 weeks of age. This maximal body weight was the same as for hamsters fed a conventional diet. At 17 weeks of age, the body weight of VAD hamsters began to decline. It decreased to 100 g at 19 weeks, and finally, to 70 g at 22 weeks.

Light microscopy: The seminiferous epithelium of 13-week-old VAD hamsters revealed active spermatogenesis (Fig. 1a). A large number of spermatozoa were detected within the tubular lumen. At 19 weeks (approximately 100 g BW), there were some round spermatids but no elongated spermatids within the seminiferous epithelium (Fig. 1b). At 22 weeks (approximately 70 g BW), only Sertoli cells, spermatogonia, and a few spermatocytes were present (Fig. 1d). The diameter of the seminiferous tubule in VAD hamsters was approximately two-thirds of that in normal hamsters (Fig. 1c). Sertoli cell processes and some vacuoles occupied a relatively large area of the epithelium. A few multinucleate giant cells were also observed. The interstitial region in VAD hamsters was similar in appearance to that of conventional hamsters.

After administration of vitamin A to VAD hamsters, spermatogenesis reinitiated (Fig. 1e). At 9 weeks after vitamin A replacement, a large number of spermatozoa were present within the lumen of seminiferous tubules. Reinitiation of spermatogenesis was observed in more than 91% of the seminiferous tubules. In a few seminiferous tubules without reinitiation of spermatogenesis, only Sertoli cells occupied the epithelium. The percentage of each stage of the seminiferous epithelial cycle in normal hamsters and VAD hamsters treated with retinol acetate is shown in Table 1. Each stage was classified according to Clermont [1]. In the testes of 4 out of the 5 animals treated with retinol acetate, more than 90% of the seminiferous tubules contained only 3 or 4 successive stages of the cycle; the seminiferous tubules of the other animal had 6 successive stages.

DISCUSSION

Male golden hamsters fed a VAD diet from 3 weeks of age reached a maximum body weight at about 13 weeks. Hamsters fed a conventional diet reached a maximum body weight at about 12 weeks. Thus, in the growth curve, only a small difference was found between VAD and conventional hamsters. Additionally, active spermatogenesis was also detected within the seminiferous tubules of both VAD and conventional hamsters during this period. These findings suggest that vitamin A deficiency had little effect on the hamsters until 13 weeks of age. This is probably due to a relatively sufficient vitamin A supply from vitamin A storing cells.

At about 19 weeks of age, when body weight decreased to 100 g, there were neither spermatozoa nor elongated spermatids. In hamsters with the lowest body weight

Scheme of the stages in the seminiferous epithelium after vitamin A replacement

Fig. 2. On the y axis, the duration of the stages of the cycle in a conventional state is plotted according to the control data showed in Table 1. The duration of the cycle is 8.7 days. The time scale for the duration of the stages on the y axis is similar to that used for the interval after vitamin A replacement on the x axis. The vertical lines mark the synchronized stages observed in the seminiferous epithelium after vitamin A replacement.

Table 1. Percentage of each stage of the seminiferous epithelial cycle in testis of golden hamsters after vitamin A replacement

Stage	Normal	Days after vitamin A replacement			
		63	70	70	77
I	13.4	0	0	0	1.7
II	12.4	0	0	0	1.4
III	6.7	0	0.7	0	0
IV	4.3	0	13.4	0.3	7.5
V	7.7	0	20.9	0.2	5.5
VI	11.0	0.5	36.4	35.2	42.5
VII	17.2	73.5	28.6	60.3	40.4
VIII	6.7	18.3	0	3.5	1.0
IX	2.4	5.7	0	0.6	0
X	3.3	0.5	0	0	0
X I	2.4	1.4	0	0	0
X II	7.7	0.2	0	0	0
X III	4.8	0	0	0	0

In the testis after vitamin A replacement, more than 90% of the seminiferous tubules contained only 3 or 4 successive stages of the cycle. Each stage was classified according to Clermont [1]. (normal hamsters; n=3, VAD hamsters treated with retinol acetate; n=4)

(approximately 70 g), only Sertoli cells, spermatogonia, and a few spermatocytes were present within the seminiferous tubules as reported in VAD rats [5, 7, 11]. In VAD rats, the number of spermatids and spermatocytes decreased to a great degree within 12 days after the onset of weight loss [8]. In VAD hamsters, the number of spermatids and spermatocytes markedly decreased at about 2 weeks and 5 weeks, respectively, after weight loss began. After initiating a VAD diet for 3-week-old rats, it took about 10 weeks until the vitamin A deficient state was completely induced; in this study, it took about 19 weeks to induce VAD in hamsters. Thus, golden hamsters showed a relatively strong resistance to vitamin A deficiency. We suppose that hamsters may have a higher capacity for storing vitamin A in comparison with rats [9] and mice [12].

As shown in Table 1, only a few successive stages of the cycle were present at 9, 10, and 11 weeks after vitamin A replacement. Therefore, the occurrence of stage-synchronization was definitely demonstrated in VAD hamsters treated with vitamin A (Fig. 2). This result is very similar to those in VAD rats and mice [9, 12].

In conclusion, spermatogenesis was severely interrupted in VAD hamsters. After vitamin A replacement, spermatogenesis was reinitiated in a stage-synchronized way. This suggests that the stage-synchronized testes of golden hamsters are a useful model for analysis of stage specific proteins and stage-dependent variations of Sertoli cells.

ACKNOWLEDGEMENTS. The authors wish to thank Mr. Iwao Tsugiyama (Department of Veterinary Anatomy, The University of Tokyo) for his expert care of the animals and thank Dr. Ann Ford for her critical revision of the English presentation of our paper. This work was supported in part by a Grant-in-Aid for Scientific Fund (No. 04660285) from the Ministry, Science and Culture, Japan.

REFERENCES

1. Clermont, Y. 1954. Cycle de l'épithélium seminal et mode de renouvellement des spermatogonies chez le hamster. *Rev. Can. Biol.* 13: 208-245.
2. Clermont, Y. and Trott, M. 1969. Duration of the cycle of the seminiferous epithelium on the mouse and hamster determined by means of ^3H -thymidine and radioautography. *Fertil. Steril.* 20: 805-817.
3. De Rooij, D. G., Van Dissel-Emiliani, F. M. F., and Van Pelt, A. M. M. 1989. Regulation of Spermatogonial proliferation. Regulation of testicular function signaling molecules and cell-cell communication. *Ann. New York Acad. Sci.* 564: 140-153.
4. Huang, H. F. S. and Hembree, W. C. 1979. Spermatogenic response to vitamin A in vitamin A deficient rats. *Biol. Reprod.* 21: 891-904.
5. Howell, J. McC., Thompson, J. N., and Pitt, G. A. J. 1963. Histology of the lesions produced in the reproductive tract of animals fed a diet deficient in vitamin A alcohol but containing vitamin A acid. I. The male rat. *J. Reprod. Fertil.* 5: 159-167.
6. Ismail, N., Morales, C., and Clermont, Y. 1990. Role of spermatogonia in the stage-synchronization of the seminiferous epithelium in vitamin A-deficient rats. *Am. J. Anat.* 188: 57-63.
7. Mason, K. E. 1933. Differences in testes injury and repair after vitamin A deficiency, vitamin E deficiency and inanition. *Am. J. Anat.* 52: 153-239.
8. Mitranond, V., Sobhon, P., Tosukhowong, P., and Chindaduangrat, W. 1979. Cytological changes in the testes of vitamin A-deficient rats. I. Quantitation of germinal cells in the seminiferous tubules. *Acta Anat.* 103: 159-168.
9. Morales, C. and Griswold, M. D. 1987. Retinol-induced stage synchronization in seminiferous tubules of the rat. *Endocrinology* 121: 432-434.
10. Morales, C. and Griswold, M. D. 1991. Variations in the level of transferrin and SGP-2 mRNAs in Sertoli cells of vitamin A-deficient rats. *Cell Tissue Res.* 263: 125-130.
11. Thompson, J. N., Howell, J. McC., and Pitt, G. A. J. 1964. Vitamin A and reproduction in rats. *Proc. R. Soc. Lond., B* 159: 510-535.
12. Van Pelt, A. M. M. and De Rooij, D. G. 1990. Synchronization of the seminiferous epithelium after vitamin A replacement in vitamin A-deficient mice. *Biol. Reprod.* 43: 363-367.
13. Unni, E., Rao, M. R. S., and Ganguly, J. 1983. Histological and ultrastructural studies on the effect of vitamin A depletion and subsequent repletion with vitamin A on germ cells and Sertoli cells in rat testis. *Indian J. Exp. Biol.* 21: 180-192.