Effect of Age Upon the Spermatogenic and Steroidogenic Elements of Rabbit Testes[1] (36577)

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There is a paucity of information documenting the effect of senescence upon the steroidogenic and spermatogenic elements of mammalian testes. We must infer from experiments in man (1, 2) that androgen production declines, from rats and rabbits (3, 4) that testes atrophy and from the bovine (5) that fertility declines with advancing age. Moreover, it is unclear whether the decline in circulating testosterone levels in man coincide with or precede the subsequent decline in libido and fertility associated with advancing age. Therefore, we quantified daily sperm and testosterone production in 6, 12, 24, and 36 month old rabbits to examine the effects of aging upon the spermatogenic and steroidogenic elements of testes. Testosterone secretion decreased significantly ($p < .05$) with age when testes were perfused with an artificial medium containing exogenous gonadotropic hormones. Total daily sperm production reached a peak at 24 months and declined significantly ($p < .05$) by 36 months of age.

Materials and Methods. Testosterone-1, 2-^{3}H was purchased from New England Nuclear Corporation, Boston, and radiochemical purity was determined to be in excess of 98%. Lutenizing hormone (Ovine, NIH-LH-S16) and follicle stimulating hormone (Ovine, NIH-FSH-S7) were gifts from the National Institute of Arthritis and Metabolic Diseases. Bovine albumin powder fraction V was purchased from the Armour Pharmaceutical Company, Chicago. Crystalline potassium penicillin and dihydrostreptomycin sulfate were obtained from E. R. Squibb and Sons, New York.

Four month old New Zealand White rabbits were purchased from Redwood Game Farms, Salt Lake City, Utah, at intervals of 6, 12, 24, and 36 months and housed individually in an air-conditioned and light-controlled (10:14 LD) room. Water and Evergreen pellets were provided *ad libitum*. Animals were treated for external parasites, upper respiratory infections, and inner ear infections as required, and only those animals in apparent good health were used for experimentation. The mortality rate was minimal through 2 years but approached 50% between 2 and 3 years of age.

Animals were weighed and anesthetized with sodium pentobarbitol. One testis was removed, chilled immediately to 4° in 0.25 *M* sucrose in preparation for *in vitro* perfusion. The contralateral testis and epididymis was removed and chilled immediately to 4° in 0.143 *M* KCl. The epididymis of the latter testis was removed, trimmed of adhering tissue and separated into head–body and tail. Each section of the epididymis and a portion of tunic-free testicular tissue was weighed and minced in saline to quantify gonadal and extragonadal sperm reserves (6).

Testes were perfused as described by VanDemark and Ewing (7) and Ewing and Eik-Nes (8) with three exceptions. The perfusion procedure was modified by replacing the New Brunswick peristaltic pump with a Harvard Sigma motor peristaltic pump, by substituting silastic for rubber tubing, and by

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use of an artificial media rather than defibrinated rabbits blood. The artificial media (9) contained Krebs–Ringer bicarbonate buffer (pH 7.4), bovine albumin powder, fraction V, (3%, w/v), glucose (1 mg/ml), crystalline penicillin and dihydrostreptomycin and washed bovine erythrocytes added to a hematocrit of 25%.

Testosterone present in the venous effluent of perfused rabbit testes was extracted, isolated and quantified via gas–liquid chromatography (GLC) equipped with electron capture detection as originally described by Brownie *et al.* (10) and modified by Kirschner and Coffman (11). Mass spectra of authentic testosterone heptafluorobutyrate and that isolated from testicular venous effluent were obtained with the prototype of the LKB-9000 gas chromatograph-mass spectrometer (12, 13). The mass spectra of material quantified via GLC was similar to authentic testosterone heptafluorobutyrate. Radioactivity measurements of testosterone-1,2-^{3}H used to assess recovery through the testosterone assay method was accomplished in a toluene scintillation fluid (14) in a Packard Tri-Carb (Model 3365) liquid scintillation spectrometer.

The experiment was arranged as a completely randomized design. Analyses of variance were calculated for each criterion (15) and Duncan's Multiple range test (16) was used to determine the significance of individual means when significant treatment effects were observed.

Results. Results in previous experiments showed that testes exhibit a low basal testosterone secretion when perfused with an artificial medium (17) and that addition of a gonadotropin (GTH) mixture so that the artificial media contained 4.0 μg FSH and 8.0 μg ICSH/ml resulted in maximum testosterone secretion in 6–12 month old rabbits. Based on this information we used perfused rabbit testes to determine the maximum rate of testosterone secretion by testes of different aged rabbits in the absence or presence of GTH. The amount of testosterone secreted during the first 2 hr of perfusion did not differ significantly with age when testes were perfused with an artificial medium in the absence of gonadotropin (Fig. 1). In contrast, in the presence of GTH, testosterone secretion averaged (fifth and sixth hours) 0.83, 0.73, 0.65, and 0.45, μg/hr in testes from 6, 12, 24, and 36 month old rabbits, respectively. Analysis of variance indicated that the rate of testosterone secretion varied significantly ($p < .05$) with age during the fifth and sixth hours of perfusion. Comparison of individual treatment means (16) showed that there was no significant difference ($p > .15$) between the rate of testosterone secretion in testes from 6, 12, and 24 month old rabbits, but that there was a significant ($p < .05$) difference between the testes of 6 and 36 month old rabbits. Thus, perfused testes from 36 month old rabbits failed to secrete as much testosterone as testes from 6 month old rabbits when maximally stimulated by exogenous GTH.

Total number of spermatids and spermatozoa per paired testes increased linearly between 6 and 24 months of age and declined significantly ($p < .01$) by 36 months of age (Table I). This reduction was due in large part to a reduction in the number of spermatids and spermatozoa per gram of testis (Table I). These changes, accompanied by similar changes in sperm numbers in the head–

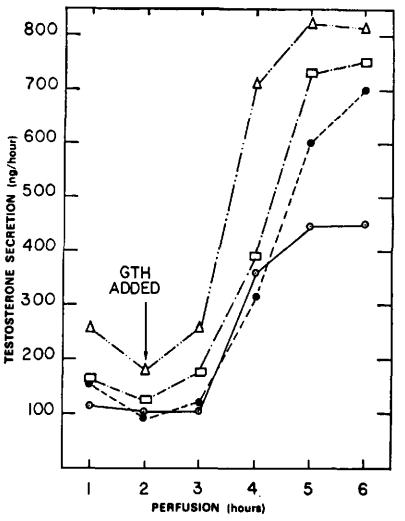

FIG. 1. Testosterone secretion by perfused rabbit testes. (△—••) 6 month old rabbits ($n = 9$); (□—) 12 month old rabbits ($n = 7$); (● - -) 24 month old rabbits ($n = 9$); and (⊙—) 36 month old rabbits ($n = 7$).

TABLE I. The Effect of Aging on Spermatid and Spermatozoa Numbers (10^6) Present in Rabbit Testes and Epididymides.

	No. of spermatids[a] and spermatozoa; age (months):			
	6 ($n = 10$)	12 ($n = 10$)	24 ($n = 10$)	36 ($n = 7$)
Paired testes[b]	355 ± 11	582 ± 76	743 ± 77	397 ± 41
Gram of testes[c]	86 ± 6	114 ± 15	134 ± 13	88 ± 8
Head and body of the epididymides[c]	61 ± 20	134 ± 21	154 ± 20	48 ± 11
Tail of epididymides[b]	226 ± 28	696 ± 42	527 ± 63	268 ± 111

[a] Each value represents the mean ± standard error of mean.
[b] Statistical significance: $p < .05$; [c] $p < .01$.

body and tail of the epididymides (Table I) suggest that daily sperm production declines between 24 and 36 months in rabbits. These findings were substantiated when total daily sperm production was calculated to be 103 ± 10, 170 ± 22, 217 ± 20, and 115 ± 17 10^6 spermatozoa/day for 6, 12, 24, and 36 month old rabbits, respectively. Analysis of variance indicated that daily sperm production increased through 24 and decreased significantly ($p < .05$) by 36 months.

Discussion. Previous experiments (1, 2) failed to elucidate whether decreased testosterone concentration in peripheral blood of man with advancing age was due to decreased gonadotropic hormone secretion, decrease in testicular androgen secretion in response to GTH, or to increased testosterone metabolism and excretion. It is impossible to ascertain which mechanism is acting by monitoring peripheral testosterone levels in intact males because of the interaction between the anterior pituitary gland and the testes, the production of adrenal androgens, peripheral conversion of blood borne precursors to androgens and to the potential alteration in androgen metabolism with advancing age. We chose to isolate rabbit testes from the milieu of different aged rabbits in a perfusion apparatus, to perfuse with artificial medium essentially devoid of GTH and to challenge with sufficient quantities of exogenous GTH to stimulate maximum testosterone secretion. The results demonstrate that aged rabbit testes (36 months) fail to secrete as much testosterone as that of young (6 months) rabbits when maximally stimulated with GTH. We realize that testosterone production by testes perfused with artificial medium containing exogenous GTH's may not reflect testosterone production *in vivo*. However, it does reflect the relative difference in maximum testosterone secretion between perfused testes from rabbits of different ages. It is impossible to say whether this difference was due to decreased numbers of Leydig cells, altered population of Leydig cell types, or to refractorines of Leydig cells of 36 month old rabbits to exogenous GTH.

Total daily sperm production was quantified by determining the numbers of spermatids and spermatozoa in homogenates of rabbit testes. This technique allowed us to determine potential sperm production of rabbits, thus circumventing an experimental problem associated with reduced libido or increased epididymal sperm resorption with advancing age.

Daily sperm production of male rabbits increased through 24 months and declined between 24 and 36 months of age. Since testosterone is required to maintain spermatogenesis in numerous species (18), it was of interest to assess the temporal relationship between daily sperm production and testosterone secretion by perfused testes maximally stimulated with exogenous GTH. The fact that sperm production increased through 24 months of age indicates that testosterone secretion is adequate to maintain spermatogenesis. The decreased daily sperm production coincident with reduced testosterone secretion by perfused testes between 24 and 36 months is suggestive of a cause effect relationship. However, whether or not impaired testosterone production causes reduced

sperm production in aged rabbits awaits experiments demonstrating: (a) the minimum 24 hr testosterone production *in vivo* required to maintain spermatogenesis in rabbits; and (6) the 24 hr testosterone production *in vivo* in aged rabbits. These two experiments are now feasible since Goodwin (19) recently described a technique for estimating *in vivo* testosterone production rates in unanesthetized, unrestrained male rabbits.

Summary. Spermatozoa and testosterone production by testes of 6, 12, 24, and 36 month old rabbits were quantified. Testosterone secretion decreased significantly with age when testes were perfused with an artificial medium containing exogenous gonadotropic hormones. Total daily sperm production reached a peak at 24 months and declined significantly by 36 months of age. The fact that sperm production increases through 24 months of age indicates that testosterone secretion is adequate to maintain spermatogenesis. The decreased daily sperm production coincident with reduced testosterone secretion by perfused testes between 24 and 36 months is suggestive of a cause effect relationship.

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