Accessory Sex Gland Function in Normal Young (20–25 Years) and Middle-aged (50–55 Years) Men

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A comparison of various parameters of prostatic and vesicular secretory function was made between the seminal plasma of young (20–25 years; n = 23) and middle-aged (50–55 years; n = 19) male volunteers. These parameters included prostatic acid phosphatase, zinc, citric acid, spermine, spermidine, putrescine (prostatic origin), fructose, and prostaglandin E (vesicular origin), in addition to protein and testosterone. Spermatozoa were counted and monitored for abnormalities. The concentration in the ejaculate of the majority of the parameters investigated did not change with age, although the total contribution to the ejaculate from the prostate and seminal vesicles was reduced significantly in the older men. The concentration of three constituents was significantly altered in the older age group: putrescine ($P < 0.001$) and prostaglandin E ($P < 0.01$) were reduced, while zinc levels were elevated ($P < 0.05$). These changes are discussed in relation to possible disturbances of prostate function and pathology in the middle-aged man.

Key words: semen, age, prostate, seminal vesicle, sperm.

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Few studies have been published on changes in semen quality with age in the human male. The age-related increase in pathologic changes in the human accessory sex glands, particularly the prostate (Berry et al, 1984), justifies such investigations, since alterations in the various constituents of the reproductive fluids may reveal changes before they are manifested clinically. To facilitate this study, semen was collected from healthy volunteers from two age groups, 20 to 25 and 50 to 55 years. These semen samples were analyzed for prostatic acid phosphatase, zinc, citric acid, putrescine, spermidine, and spermine. In addition, protein, testosterone, sperm concentration, sperm morphology, and the seminal vesicle secretory products, fructose and prostaglandin E, were assessed.

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Materials and Methods

Semen samples were provided by medical students (n = 23) aged 20 to 25 years and physicians (n = 19) aged 50 to 55 years. The subjects were healthy and had no history of genital disease. Two of the men in the older age group had been vasectomized, but since their seminal plasma values fell within the confidence limits of their age group, the data were included in estimates of the mean. Semen was collected by *masturbatio condomata* at home after 3 to 7 days of abstinence, and the ejaculate was frozen immediately in the condoms at −20 C. Within 2 days they were transported frozen to the laboratory and transferred to −70 C. The collection period spanned 14 days. The samples then were thawed, transferred to tubes, and allowed to liquefy for 10 to 15 minutes at room temperature. An aliquot was then removed for sperm count, and the rest was centrifuged at 1500 × g for 10 minutes to remove the remaining spermatozoa. A smear was prepared from a portion of the sperm pellet, stained, and assessed for sperm morphology. Aliquots of the seminal plasma were then pipetted out to the various assays on ice, and stored at −70 C until the day of assay. The condoms used in the study were RFSU Profil (Stockholm, Sweden) without any chemical addition.

The methods used for the determination of prostatic acid phosphatase, citric acid, zinc, and fructose have been described previously (Rui et al, 1984). Protein was assessed by the method of Lowry et al (1951). Prostaglandin E was determined using a radioimmunoassay kit (Travenol Laboratories Inc., MA, at No. CA-501). Testosterone was

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assessed using a radioimmunoassay procedure described elsewhere (Purvis et al, 1974).

Putrescine, spermidine, and spermine were determined by a high pressure liquid chromatography system (Laboratory Data Control, USA) using a procedure essentially like that of Seiler and Knödgen (1978). Briefly, 20-μl aliquots were mixed with 180 μl of 1.6-diaminohexane (internal standard; 20 nmol dissolved in water) and deproteinized with 200 μl of 0.4 M perchloric acid on ice for 20 minutes, followed by centrifugation at 2000 × g for 30 minutes. An aliquot (25 μl) of the supernatant was diluted with 175 μl of water and mixed with 100 mg of $NaHCO_3$ and 0.4 ml of dansyl chloride (0.11 M in acetone). The reaction mixture was allowed to stand overnight in the dark at room temperature. Excess dansyl chloride was removed by addition of 0.1 ml of L-proline (1.3 M in water) followed by a further 30 minutes of incubation in the dark. The contents of the tubes then were dried at 65 C under a stream of air, dissolved in 0.1 ml of water, and extracted with 0.5 ml of toluene. An aliquot (300 μl) of the toluene phase was evaporated to dryness and dissolved in a final volume of 600 μl methanol. Twenty microliters were applied to the column (250 × 4.6 mm ID, Supelcosil LC-18, particle size 5μ, Supelco Inc.) by an autosampler, and the separation of the dansyl derivatives was obtained using a linear methanol/water gradient changing from 65% to 100% methanol (Ratburn Chemicals Ltd., HPLC grade) in 23 minutes at a flow rate of 1 ml/min. A fluorescence detector with an excitation filter of 340 nm and an emission filter of 460 nm was used, and standards containing 100 pmoles spermine, 50 pmoles of 1.6-diaminehexane, 10 pmoles of spermidine, and 10 pmoles of putrescine per 20-μl injection volume were run after every seventh sample.

Statistics

Results were analyzed by Student's *t* test after logarithmic transformation to normalize the data and by Spearman rank correlation analysis.

Results

When expressed as total amount per ejaculate, the levels of prostatic acid phosphatase, spermine, spermidine, putrescine, protein, fructose, testosterone and prostaglandin E were significantly lower in the older age group (Table 1). A significantly ($P < 0.05$) lower ejaculate volume also was noted in the middle-aged men (3.5 vs. 4.7 ml). Of the above components, only the total amounts of putrescine and prostaglandin E were reduced relatively more than the ejaculate volume (78% and 62% lower, respectively, than that in the young age group). The concentration of zinc, on the other hand, was slightly higher ($P < 0.05$) in the older subjects, although the total amount of zinc per ejaculate was the same in the two age groups. This was also the case with another prostatic product, citric acid. Scatter diagrams of ejaculate volumes, and total content of prostaglandin E and putrescine

TABLE 1. Concentrations and Total Amounts of Various Seminal Plasma Constituents in Ejaculates from Young and Middle-aged Men

Seminal Plasma Constituent	Unit of Measure	20–25 Year Olds	50–55 Year Olds	*P*-Value
Citric acid	(μmoles)	139 (110–175)*	126 (107–148)	NS
	(mM)	26.9 (25.8–33.9)	35.6 (29.5–43.0)	NS
PAP†	(IU)	2860 (2120–3850)	1760 (1320–2240)	< 0.01
	(IU/ml)	608 (484–763)	497 (347–712)	NS
Zinc	(μmoles)	11.3 (8.88–14.5)	11.0 (9.16–13.2)	NS
	(mM)	2.41 (2.03–2.86)	3.12 (2.44–3.98)	< 0.05
Spermine	(μmoles)	10.8 (8.34–14.0)	7.76 (5.60–10.8)	< 0.05
	(mM)	2.29 (1.81–2.91)	2.20 (1.57–3.08)	NS
Spermidine	(nmoles)	1180 (929–1500)	905 (692–1180)	< 0.05
	(μM)	251 (214–295)	256 (193–339)	NS
Putrescine	(nmoles)	529 (277–1010)	114 (67.0– 194)	< 0.001
	(μM)	113 (60.8–208)	32.3 (19.9–52.4)	< 0.001
Testosterone	(pmoles)	4.60 (3.68–5.76)	3.51 (3.04–4.06)	< 0.05
	(nM)	0.98 (0.87–1.10)	0.99 (0.86–1.14)	NS
Protein	(mg)	207 (166–258)	159 (139–188)	< 0.05
	(mg/ml)	44.0 (40.9–47.4)	45.0 (41.3–49.0)	NS
Fructose	(μmoles)	49.3 (34.6–70.2)	29.2 (17.7–48.0)	< 0.05
	(mM)	10.5 (8.11–13.5)	8.26 (5.13–13.3)	NS
PGE‡	(nmoles)	121 (68.8–214)	45.7 (28.6–72.9)	< 0.001
	(μM)	25.7 (16.1–40.9)	12.9 (8.94–18.7)	< 0.01

*Geometric mean and 95% confidence limits ( ).
†PAP = Prostatic acid phosphatase.
‡PGE = Prostaglandin E.

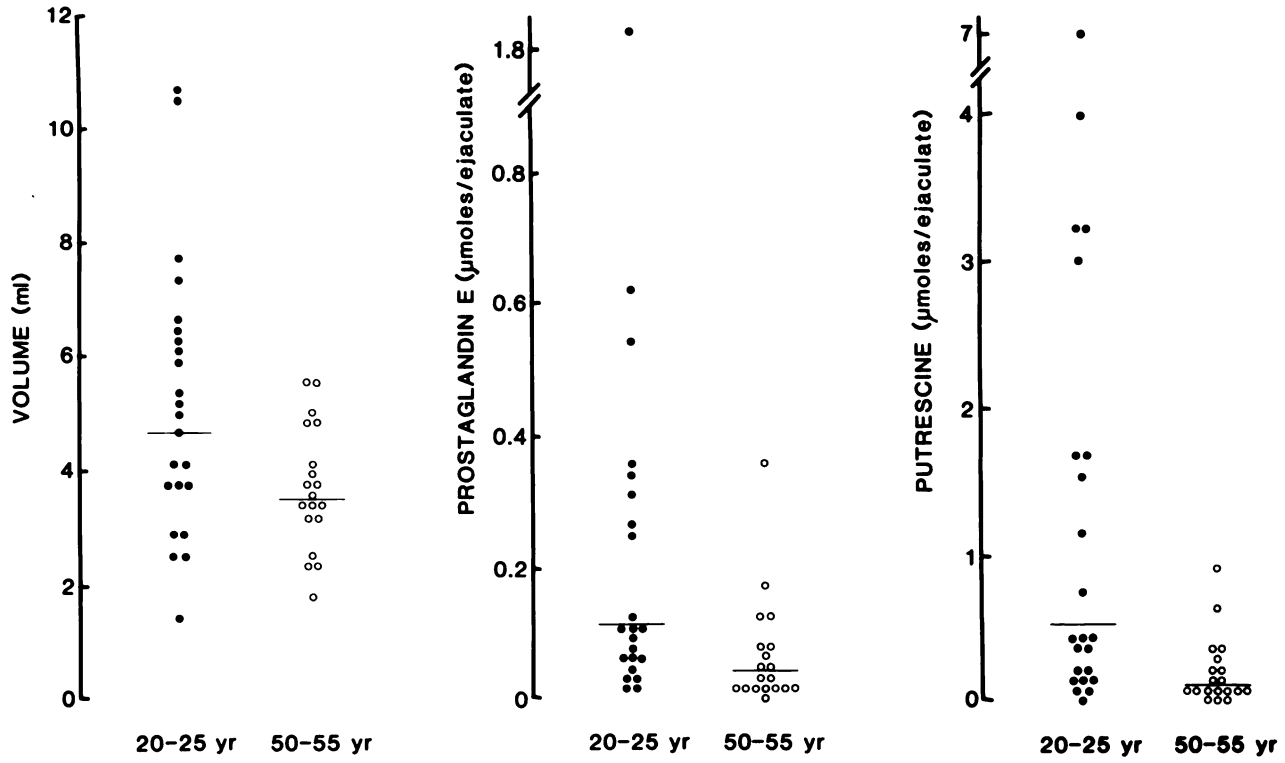

Fig. 1. Scatter diagrams of semen volume and the total amounts of putrescine and prostaglandin E (PGE) in ejaculates from young (20–25 years) and middle-aged (50–55 years) men. Horizontal lines represent geometric means.

are presented in Fig. 1, and of prostatic acid phosphatase and zinc in Fig. 2. Differences between the two age groups were made even more obvious when putrescine was plotted against the corresponding prostatic acid phosphatase activity in the same ejaculates (Fig. 3).

Spearman rank correlation analysis on the pooled data (n = 42) revealed highly significant correlations ($P < 0.001$) among the acknowledged prostate secretory products acid phosphatase, zinc and critic acid, but no correlation between spermine, which is also of prostatic origin, and any of the above parameters (Table 2). On the other hand, spermine was strongly correlated with its precursor, spermidine ($P < 0.001$), but neither spermine nor spermidine was correlated to putrescine. When correlation analyses were performed separately on the two age groups, both spermidine and spermine were strongly correlated with citric acid ($P < 0.001$) and $P < 0.01$, respectively) in the 50 to 55 year age group (Table 3), whereas no such correlation was seen in the younger group (Table 4).

No correlation was observed between the seminal vesicle secretory products fructose and prostaglandin E (Table 2). Sperm concentration was positively correlated to the percentage of normal spermatozoa ($P < 0.01$), and the percentage of normal spermatozoa was correlated with testosterone concentration, although only at the 5% significance level.

Table 5 shows that neither total sperm production nor sperm concentrations were significantly altered with age, and no differences were found in sperm morphology between the two age groups.

Discussion

The present study compares secretory functions of the reproductive tract in young and middle-aged men and reveals a major reduction in the levels of certain secretory parameters that appear to be superimposed on a generalized decrease in accessory sex gland function with age.

Significant differences were observed in the total ejaculate content of prostatic acid phosphatase and the polyamines. A decline in the acid phosphatase

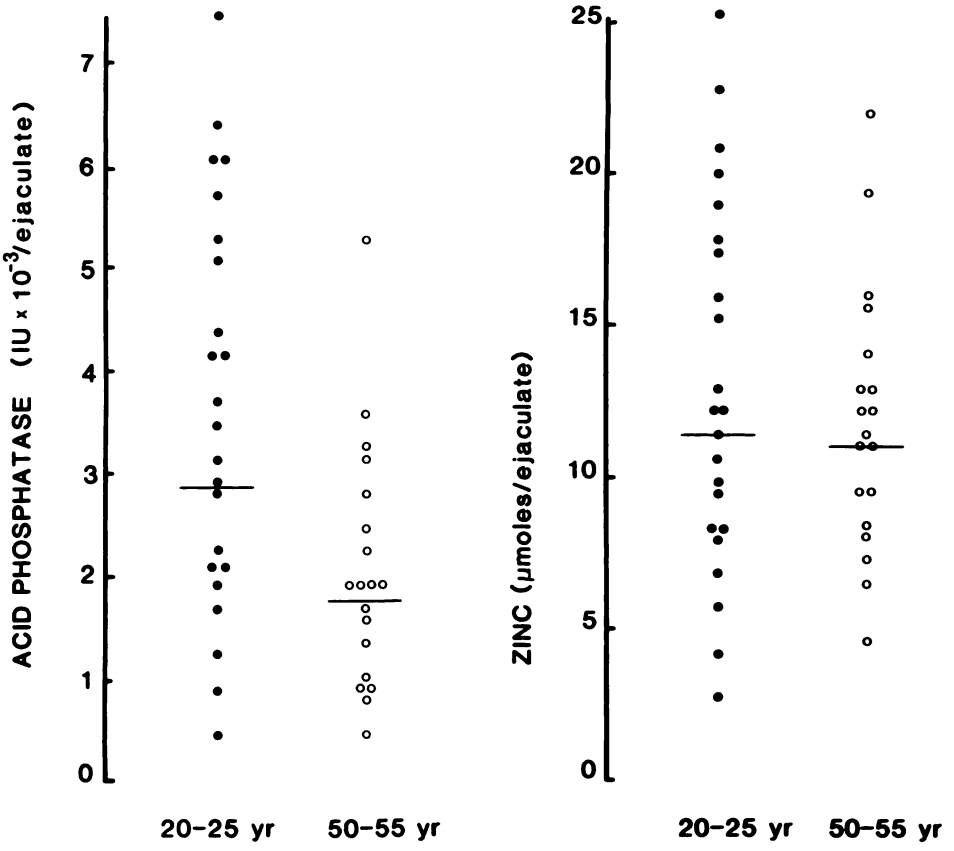

Fig. 2. Scatter diagrams of total ejaculate contents of prostatic acid phosphatase and zinc in seminal plasma from young and middle-aged men. Acid phosphatase activity is expressed in international units (IU), which correspond to the amount of enzyme yielding 1 μmol of p-nitrophenol/min.

concentration in prostatic fluid with increasing age has been reported by others (Kirk, 1949, Grayhack and Kropp, 1964). In the present study, this decrease in prostatic acid phosphatase was not accompanied by declines in citric acid or zinc, which remained at the same total level in the older age group. It is interesting that Grayhack and Kropp (1964) noted an increase in the concentration of citric acid in prostatic fluid during middle age (51–60 years). The human prostate is a multilobulated structure (Tisell and Salander, 1975) where the different lobes may contribute different components to prostatic secretions, as has been demonstrated in the rat (Gerhardt et al, 1983). Therefore, such results could be explained by a selective decline in the function of certain areas or lobes of the gland, while the function of other regions may be maintained. The similarities in zinc and citric acid concentrations could be explained by the observation by Arver (1982) that citrate is the major zinc-binding component of low molecular weight in human semen. Furthermore, zinc appears to exert an inhibitory effect on citrate metabolism in the rat prostate (Costello and Franklin, 1981).

Although these prostatic parameters appeared to alter differently with age, a clear correlation nevertheless existed between prostatic acid phosphatase, zinc, and citric acid in both age groups, emphasizing their common prostatic origin (Rui et al, 1984).

Putrescine and the polyamines spermidine and spermine in seminal plasma share a prostatic origin (Mann and Lutwak-Mann, 1981) but were not correlated to other prostate parameters in the seminal plasma of the younger men. The same finding was reported earlier concerning the semen of young men during frequent ejaculation (Rui et al, 1984). The reasons for this are unknown, but may be due to the fact that the polyamines not only are secretory products of the gland but also intracellular components believed to influence growth and protein synthesis (Heston et al, 1982; Herr and Kleinert, 1984).

The most striking difference observed between the two age groups was the low putrescine levels in the older subjects. The implications of this finding are uncertain, but lower tissue levels of putrescine have been reported to be associated with benign prostatic hyperplasia when compared with normal prostate glands (Dunzendorfer et al, 1981). Current views favor the concept that the pathologic changes of benign prostatic hyperplasia are initiated in early middle age, and develop to the extent that 50% of men between the ages of 51 and 60 years already exhibit significant alterations in their prostate morphology (Berry et al, 1984). The fact that the men in the older age group in the present study secreted smaller quantities of putrescine into the ejaculate than the young may imply that the prostate was undergoing early hyperplastic changes in this age group. When the putrescine data were plotted against the corresponding prostatic acid phosphatase values from the same ejaculate, it was possible to further differentiate the older age population from the younger age group. Such mathematical manipulation may provide a criterion for a better evaluation and selection of individuals with changes in their semen parameters that can be attributed to pathologic or age-associated disturbances.

In addition to these parameters of prostate function, the total amounts of protein, testosterone, and the seminal vesicle products fructose and prostaglandin E were reduced, indicating a general, but mild, decline in the function of the aging male reproductive system. There also was a 26% decrease in ejaculate volume parallel to this reduction in secretory products, so that the concentrations of most of them were not altered. This volume decrease in the middle-aged men is in close agreement with the 30% reduction in semen volume observed by Homonnai et al (1982). The total glandular contribution to the ejaculate apparently decreases with age, but since there appears to be a parallel decline in the quantity of the secretory product as well as the volume of the fluid vehicle, the actual quality of the ejaculate remains relatively unchanged.

The lack of correlation between the seminal vesicle secretory products fructose and prostaglandin E has been reported earlier in apparently healthy men and men in infertile marriages (Bygdeman and Eliasson, 1969; Conte et al, 1979), and indicates differential control of the synthesis of these two parameters. Indeed, Skakkebaek et al (1976) reported no increase in the seminal concentration of prostaglandin E during androgen treatment of hypogonadal men, where-

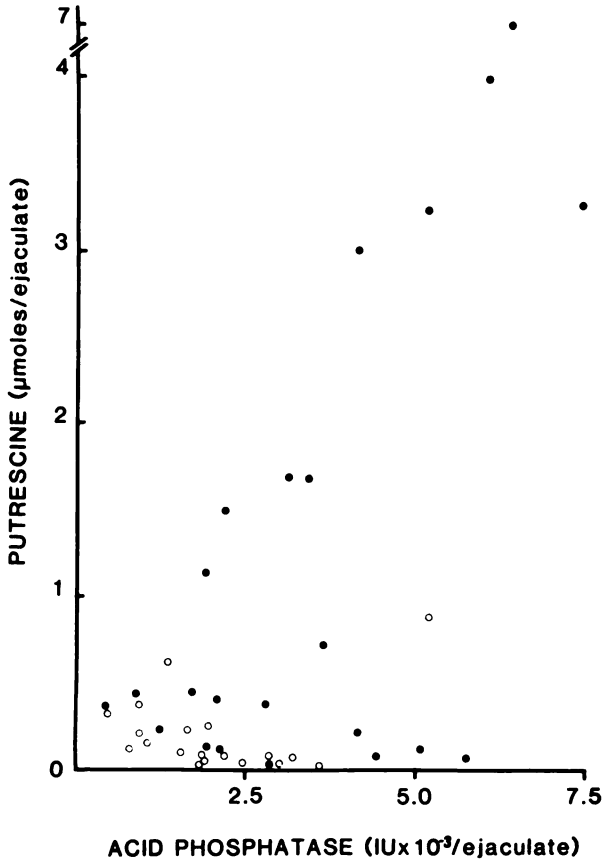

Fig. 3. Total ejaculate content of putrescine plotted against respective prostatic acid phosphatase (PAP) levels in semen from men aged 20–25 years (●) and 50–55 years (○).

as seminal plasma fructose has long been recognized as an androgen-dependent parameter (Mann, 1967).

Johnson et al (1984) reported a decrease in sperm production with age in man, as assessed from histologic studies on testes from autopsies. The present study could not confirm this statistically, in spite of the 25% difference between the means of the two groups. Studies on larger populations may be necessary before a clear reduction in sperm production can be demonstrated satisfactorily, at least in men of this age group. On the other hand, Schwartz et al (1981) found no change in semen volume, sperm concentration, or total sperm number in ejaculates from men of different ages, but reported a slight reduction in the percentage of normal spermatozoa. No such reduction could be detected in the present study, not even between the various categories of sperm abnormalities.

TABLE 2. Spearman Rank Correlation Analysis Carried Out on the Concentrations of Various Seminal Plasma Constituents in Ejaculates from Young (20–25-year-old) and Middle-aged (50–55-year-old) Men*

Seminal Plasma Constituents	Citric Acid*	PAP	Zinc	Spermine	Spermidine	Putrescine	Testosterone	Protein	Fructose	PGE	Sperm
PAP*	0.52 †										
Zinc	0.77 †	0.68 †									
Spermine	0.27 NS	0.15 NS	0.08 NS								
Spermidine	0.40 ‡	0.25 NS	0.22 NS	0.88 †							
Putrescine	−0.04 NS	— NS	−0.14 NS	0.02 NS	0.04 NS						
Testosterone	0.04 NS	0.20 NS	0.05 NS	0.21 NS	0.30 NS	0.26 NS					
Protein	0.03 NS	0.15 NS	0.12 NS	−0.22 NS	−0.13 NS	−0.14 NS	−0.05 NS				
Fructose	−0.06 NS	−0.06 NS	— NS	−0.37 §	−0.31 §	−0.02 NS	−0.06 NS	0.20 NS			
PGE	−0.39 §	−0.29 NS	−0.34 §	−0.20 NS	−0.14 NS	0.35 §	0.17 NS	0.11 NS	0.10 NS		
Sperm	−0.20 NS	0.16 NS	0.03 NS	0.21 NS	0.19 NS	−0.03 NS	−0.05 NS	0.02 NS	−0.20 NS	−0.28 NS	
Percent Norm	−0.12 NS	0.15 NS	— NS	0.03 NS	0.02 NS	0.36 §	0.35 §	0.09 NS	−0.14 NS	0.20 NS	0.41 ‡

*N = 42, except for sperm concentration and the % of spermatozoa with normal morphology, where n = 40. PAP = prostatic acid phosphatase; PGE = prostaglandin E; % NORM = percentage of spermatozoa with normal morphology.
† $P < 0.001$.
‡ $P < 0.01$.
§ $P < 0.05$.

TABLE 3. Spearman Rank Correlation Analysis Carried Out on Ejaculate Volume and Selected Seminal Plasma Constituents in Middle-aged Men (50–55 years)*

Seminal Plasma Constituents	Volume	Citric Acid	PAP	Zinc	Spermine	Spermidine
Citric acid	−0.50 †					
PAP	−0.71 ‡	0.71 ‡				
Zinc	−0.66 §	0.73 ‡	0.87 ‡			
Spermine	−0.16 NS	0.60 §	0.31 NS	0.24 NS		
Spermidine	−0.33 NS	0.80 ‡	0.41 NS	0.42 NS	0.87 ‡	
PGE	0.57 †	−0.59 §	−0.43 NS	−0.31 NS	−0.45 NS	−0.59 §

*See Table 2 for details.
† $P < 0.05$.
§ $P < 0.01$.
‡ $P < 0.001$.

TABLE 4. Spearman Rank Correlation Analysis Carried Out on Ejaculate Volume and Selected Seminal Plasma Constituents in Young Men (20–25 years)*

Seminal Plasma Constituents	Volume	Citric Acid	PAP	Zinc	Spermine	Spermidine
Citric acid	0.01 NS					
PAP	−0.08 NS	0.55 †				
Zinc	−0.12 NS	0.75 ‡	0.77 ‡			
Spermine	−0.36 NS	0.11 NS	−0.11 NS	−0.02 NS		
Spermidine	−0.23 NS	0.15 NS	0.01 NS	0.08 NS	0.81 ‡	
PGE	0.22 NS	0.05 NS	−0.30 NS	−0.17 NS	0.09 NS	0.34 NS

*See Table 2 for details.
† $P < 0.01$,
‡ $P < 0.001$.

TABLE 5. Concentration, Total Number and Distribution of Abnormalities of Sperm Cells in Ejaculates from Young and Middle-aged Men

	20–25 years	50–55 years	Significance Level
Concentration (10^6/ml)	76.3 (51.2–114)*	73.1 (51.9–103)	NS
Total 10^6/ejaculate)	359 (256–503)	269 (192–377)	NS
Normal (%)	67.7 (65.6–70.9)	63.2 (57.2–69.9)	NS
Tail abnormality (%)	12.9 (11.0–15.0)	15.6 (11.9–20.5)	NS
Amorphous head (%)	4.04 (3.31–4.94)	3.35 (2.75–4.07)	NS
Immature (%)	3.59 (2.94–4.37)	3.26 (2.53–4.21)	NS
Absent head (%)	5.00 (3.86–6.46)	5.53 (4.27–716)	NS
Microcephalic (%)	3.16 (2.68–3.73)	3.00 (2.60–3.46)	NS

*Geometric mean and 95% confidence limits ( ).

In summary, the present study has two major findings: 1) There are no dramatic changes in the general function of the male reproductive tract between the young and the middle-aged. The quality of the semen as reflected in the concentration of its various substituents appears to be unchanged. 2) There is, however, a more marked reduction in certain secretory components, for example putrescine, which may reflect the initial stages in the development of pathologic processes such as prostatic hyperplasia.

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References

Arver S. Studies on zinc and calcium in human seminal plasma. Acta Physiol Scand 1982; 507(suppl):1–21.

Berry SJ, Coffey DS, Walsh PC, Ewing LL. The development of human benign prostatic hyperplasia with age. J Urol 1984; 132: 474–479.

Bygdeman M, Eliasson R. Distribution of prostaglandins, fructose and acid phosphatase in human seminal plasma. Andrologia 1969; 1:135–139.

Conte D, Laguzzi G, Giovenco P, Dondero F, Isidori A. Relationship between seminal PGE and 19-OH-PGE and seminal fructose in man. Prostaglandins 1979; 17:135–139.

Costello LC, Franklin RB. Aconitase activity, citrate oxidation and zinc inhibition in rat ventral prostate. Enzyme 1981;26:281–287.

Dunzendorfer U, Weber W, Russell DH. Polyamines, hydrolases (PAP, LAP, SDH, plasmin), TSH, T_3, T_4, and C-peptide in benign hyperplasia of the prostate. Urol Res 1981; 9:137–142.

Gerhardt PG, Mevåg B, Tveter KJ, Purvis K. A systematic study of biochemical differences between the lobes of the rat prostate. Int J Androl 1983; 6:553–562.

Grayhack JT, Kropp K. Changes with aging in prostatic fluid: Citric acid, acid phosphatase and lactic dehydrogenase concentration in man. Transactions of American Association of Genito-Urinary Surgeons 1964; 56:6–11.

Herr HW, Kleinert EL. Antigrowth effect of polyamine biosynthesis inhibitors on the Dunning R 3327-G prostatic tumor. Prostate 1984; 5:439–444.

Heston WDW, Kadmon D, Lazan DW, Fair WR. Copenhagen rat prostatic tumor ornithine decarboxylase activity (ODC) and the effect of the ODC inhibitor alpha-difluoromethylornithine. Prostate 1982; 3:383–389.

Homonnai ZT, Fainman N, David MP, Paz GF. Semen quality and sex hormone pattern of 29 middle aged men. Andrologia 1982; 14:164–170.

Johnson L, Petty CS, Neaves WB. Influence of age on sperm production and testicular weights in men. J Reprod Fertil 1984; 70:211–218.

Kirk E. The effect of testosterone administration on acid phosphatase concentration of the prostatic exprimate in old men. Urol Cutan Rev 1949; 53:683–689.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193:265–272.

Mann T. Appraisal of endocrine testicular activity by chemical analyses of semen and male accessory secretions. Ciba Foundation Colloquia of Endocrinology 1967; 16:233–248.

Mann T, Lutwak-Mann C. Male reproductive function and semen. New York: Springer-Verlag, 1981.

Purvis K, Illius AW, Haynes NB. Plasma testosterone concentrations in the ram. J Endocrinol 1974; 61:241–253.

Rui H, Gerhardt P, Mevåg B, Thomassen Y, Purvis K. Seminal plasma characteristics during frequent ejaculation. Int J Androl 1984; 7:119–128.

Schwartz D, Mayaux MJ, Spira A, Moscato ML, Jouannet P, Czyglik F, David G. Study of a group of 484 fertile men: II. Relation between age (20–59) and semen characteristics. Int J Androl 1981; 4:450–465.

Seiler N, Knödgen B. Determination of di- and polyamines by high performance liquid chromatographic separation of their 5-dimethyl-aminonaphthalene-1-sulphonyl derivatives. J Chromatogr 1978; 145:29–39.

Skakkebaek NE, Kelly RW, Corker CS. Prostaglandin concentrations in the semen of hypogonadal men during treatment with testosterone. J Reprod Fertil 1976;47:119–121.

Tisell L-E, Salander H. The lobes of the human prostate. Scand J Urol Nephrol 1975; 9:185–191.