Statistical analysis

The data on spermatogenesis were analysed in cross tabulated form by using odds ratios with 95% confidence intervals (CIA software). Confidence intervals excluding the value of 1 were considered to be significant. When analysing testicular features we used two way analysis of covariance. We used covariate structure and adjustment of means to eliminate the confounding effect, when necessary. Post-hoc comparisons were made with Sheffe's test. The data were analysed in logarithmic and square root transformed form, but the results were identical with those based on crude data. The analysis was carried out with Statistica for Windows (version 5.0) on a 486 PC.

Results

Necropsy series

There were no significant differences in the causes of death between the two series. The interval between death and necropsy was slightly longer in the 1991 series, in which the men were also slightly younger and had a significantly higher mean body mass index compared with those in the 1981 series. No significant differences were observed between the occupations of men in 1981 and 1991.

Changes in spermatogenesis score and testicular morphometric characteristics

Normal spermatogenesis was found in 41.7% (220) of all the 528 men. In 1981, 56.4% (148) of the men showed normal spermatogenesis, whereas in 1991 the incidence had decreased by more than a half (29.5% percentage points) to 26.9% (71) (odds ratio 3.5; 2.5 to 5.1; table 3). Simultaneously, the incidence of partial spermatogenic arrest increased from 31.4% (83) to 48.5% (128) (2.1; 1.4 to 2.9) and that of complete spermatogenic arrest from 8.0% (21) to 20.1% (53) (2.9; 1.7 to 5.0). There were 15 cases of the Sertoli cell only syndrome and eight cases of fibrotic testicles. No significant change was observed, however, in their incidences between 1981 and 1991.

Testicular weight showed a significant decline from an adjusted mean of 18.9 g in 1981 to 17.8 g in 1991 (table 4). The adjusted mean diameter of seminiferous tubules and morphometrically measured percentage area of seminiferous tubules were also smaller in 1991, with a corresponding increase in the percentage area of fibrotic tissue in the 1991 series.

Risk factors for testicular disorders

Alcohol—The proportion of men with reported moderate and heavy drinking did not change significantly between 1981 and 1991. Disorders of spermatogenesis increased significantly between 1981 and 1991 among both moderate and heavy drinkers (table 5). In both series normal spermatogenesis was less common in heavy drinkers than in moderate drinkers in corresponding series. Testicular weight showed a slight decrease in both consumption groups in both series (table 6).

Drugs—Of the men with interview data available, 122/260 (46.9%) used sedative or tranquillising drugs, whereas 20 (7.7%) were users of antihypertensive medication. The proportion of men using these drugs, however, did not change from 1981 to 1991.

Table 3 Comparison of status of spermatogenesis (numbers (percentages)) between series (1981 *v* 1991) among all subjects

Status of spermatogenesis	1981 (n=264)	1991 (n=264)	Odds ratio (95% confidence interval)*
Normal	149 (56)	71 (27)	0.3 (0.2 to 0.4)†
Partial arrest	83 (31)	128 (49)	2.1 (1.4 to 2.9)†
Complete arrest	21 (8)	53 (20)	2.9 (1.7 to 5.0)†
Sertoli cell only syndrome	9 (3)	6 (2)	1.5 (0.5 to 4.3)
Fibrosis	2 (1)	6 (2)	3.0 (0.6 to 15.2)

*For difference in comparison between series; 95% confidence intervals excluding 1 are considered to be significant.
†Significant difference in comparison between series.

Smoking—There were 184 (70.8%) reported smokers among the 260 men for whom data were available. Smoking was slightly more common in 1991, but no difference was observed in the status of spermatogenesis between smokers and non-smokers.

Body mass index—There was a significant increase in the mean body mass index of the men between 1981 and 1991. Body mass index, however, showed no statistical association with the occurrence of testicular disorders, although it was a significant covariate for testicular weight, diameter of seminiferous tubules, and testicular morphology.

Pooled risks—Disorders of spermatogenesis were more common in men with pooled risk factors for altered spermatogenesis (heavy drinking and smoking and use of tranquillisers) than in men with none of the mentioned risk factors (odds ratio 3.2). This difference, however, was not significant because of the small numbers of cases in both groups.

Discussion

Declining sperm counts

Carlsen *et al* recently reviewed several reports published from 1938 to 1991 on semen quality of voluntary sperm donors and suggested that sperm counts may have decreased by 42% during the past five decades with a concurrent slight decrease in volume of semen.[1] Although criticism has been directed against statistical methods used in that work[2,3] and controversial reports have been published,[4,18,19] including on the semen quality of Finnish men,[3,20] several results corroborating the observation of Carlsen *et al* have been published in recent years.[6-8] The hypothesis of deteriorating function of the male reproductive tract has thus far been based on findings observed in quality

Table 4 Comparison of status of testicular features between series (1981 *v* 1991) among all subjects. Figures are adjusted means (SD; 95% confidence intervals) unless stated otherwise

Testicular features	1981	1991	Difference*
Testicular weight (g)	18.7 (5.7; 18.0 to 19.4)	18.0 (6.2; 17.2 to 18.7)	0.7 (−0.3 to 1.7)
Adjusted mean	18.9	17.8	1.1 (0.1 to 2.1)†
Seminiferous tubular diameter (μm)	184.9 (25.2; 180.7 to 189.9)	180.9 (26.3; 174.7 to 187.1)	4.0 (−0.4 to 8.4)
Seminiferous epithelium (%)	52.3 (13.1)	47.2 (13.2)	5.1 (2.9 to 7.4)†
Adjusted mean	52.7	47.1	5.6 (3.4 to 7.9)†
Fibrotic tissue (%)	40.8 (12.6; 39.3 to 42.4)	46.8 (13.1; 45.5 to 48.8)	6.0 (3.8 to 8.2)†
Adjusted mean	40.4	47.2	6.8 (4.6 to 9.0)†
Leydig cells (%)	6.9 (3.0; 6.5 to 7.2)	5.7 (3.7; 5.2 to 6.5)	1.2 (0.6 to 1.8)†

*Difference in means (95% confidence interval) between series. 95% Confidence intervals excluding 0 for means considered to be significant.
†Significant difference in comparison between series.

Table 5 Comparison of status of spermatogenesis among moderate and heavy drinkers between series (1981 *v* 1991). Values are numbers (percentages) of subjects

	Moderate drinkers			Heavy drinkers		
Status of spermatogenesis	1981(n=80)	1991(n=52)	Odds ratio (95% confidence interval)*	1981(n=71)	1991(n=57)	Odds ratio (95% confidence interval)*
Normal	62 (78)	16 (31)	0.1 (0.05 to 0.3)†	27 (38)	12 (21)	0.4 (0.2 to 1.0)†
Partial arrest	16 (20)	33 (64)	7.0 (3.2 to 15.3)†	29 (41)	29 (51)	1.5 (0.7 to 3.0)
Complete arrest	2 (3)	3 (6)	2.4 (0.4 to 14.8)	10 (14)	12 (21)	1.6 (0.6 to 4.1)
Sertoli cell only syndrome	—	—	NA	5 (7)	4 (7)	1.0 (0.3 to 3.9)
Fibrosis	—	—	NA	—	—	N/A

NA=not applicable.
*For difference in comparison between series; 95% confidence intervals excluding 1 considered to be significant.
†Significant difference in comparison between series.

of semen and sperm, which reflect well the overall function of male reproductive organs. Meinhard *et al*, however, reported normal spermatogenesis or blockage of seminiferous tubules, a status in which spermatogenesis proceeds normally, in half of 100 infertile men who were oligospermic.[15] Similarly, in a recent study, fine needle cytology indicated normal spermatogenesis in almost one third of 534 azoospermic men.[16]

Thus, semen analysis may, in fact, not be a good guide to the severity of testicular lesion at the level of spermatogenesis, and therefore it is questionable whether or not the declining changes in sperm counts are due to increases in disorders of spermatogenesis or whether other disorders in the male reproductive tract are involved. In the present study, we found that the incidence of normal spermatogenesis has decreased significantly among middle aged men, with a parallel increase in the rate of disorders of spermatogenesis during an interval of 10 years between 1981 and 1991. This finding suggests that the quality and dispatch of spermatogenesis are deteriorating in middle aged men and also confirms earlier presumptions on deteriorating spermatogenesis being the main cause of decreasing sperm counts.

Sperm counts in Finland

Vierula *et al* reported recently that sperm density and total sperm counts of Finnish men have not gone through any changes in the past 28 years,[5] confirming earlier findings on good semen quality of Finnish men.[20] Although we found a significant decrease in the incidence of normal spermatogenesis in the past 10 years, our results do not inevitably disagree with those of Vierula *et al*. By definition, testes expressing partial spermatogenic arrest, a slight and probably reversible disorder which was most commonly found among men in 1991 series, still produce various amounts of mature spermatozoa. Therefore, if semen analysis is performed after a few days of abstinence before sperm donation, men with partial spermatogenic arrest may not differ significantly from those with completely normal spermatogenesis. The subjects in our study, however, were on average 20 years older than those of Vierula *et al*, and thus the results are not fully comparable. In addition, our material came from the densely populated province of Uusimaa, mostly in and around Helsinki, whereas that of Vierula *et al* originated from less densely populated and industrialised communities several hundred kilometres north east of Uusimaa province. Recent work from France has shown a significant decline in sperm counts during an interval of 20 years between 1973 and 1992,[6] possibly suggesting that geographical variations do exist in the abundance and distribution of factors causing disorders of male reproductive tract, as also hypothesised by Vierula *et al*. Two recently published reports also found unchanged sperm counts in the United States over the past few decades, suggesting that deterioration of semen quality is not geographically uniform.[18 19] Also, the fertility of Finnish couples was recently observed to be significantly greater than that of British couples, further supporting this hypothesis.[21]

Disorders of male reproductive tract

In addition to declining sperm counts, the incidences of testicular cancer and specific disorders of male reproductive tract have increased in recent decades.[22-25] Both observations have provoked an active discussion of a possible common aetiology. Several environmental factors are known to cause alterations in the male reproductive tract, such as certain drugs, chemicals,[10 11]

Table 6 Comparison of status of testicular features among moderate and heavy drinkers between series (1981 *v* 1991). Figures are adjusted means (SD; 95% confidence intervals) unless stated otherwise

	Moderate drinkers			Heavy drinkers		
Testicular features	1981(n=80)	1991(n=52)	Difference*	1981(n=71)	1991(n=57)	Difference*
Testicular weight (g)	20.0 (5.9; 18.7 to 21.3)	20.2 (6.0; 18.6 to 21.8)	0.2 (−1.9 to 2.3)	16.9 (4.9; 15.7 to 18.0)	17.2 (4.6; 16.0 to 18.4)	0.3 (−1.4 to 2.0)
Adjusted mean	20.5	19.9	0.6 (−1.5 to 2.7)	17.1	17.0	0.1 (−1.6 to 1.8)
Seminiferous tubular diameter (μm)	190.9 (23.5; 186 to 196)	185 (22; 177 to 193)	5.9 (−2.2 to 14.0)	175 (26; 167 to 184)	176 (30; 165 to 187)	1.0 (−8.8 to 10.8)
Adjusted mean	191.9	181.1	10.8 (2.7 to 18.9)†	176.9	174.9	2.0 (−7.8 to 11.8)
Seminiferous epithelium (%)	55.1 (11.2; 50.7 to 53.9)	50.4 (9.2; 45.4 to 48.9)	4.7 (1.0 to 8.4)†	48.5 (14.5; 45.1 to 51.9)	48.7 (11.0; 45.9 to 51.6)	0.2 (−4.4 to 4.8)
Adjusted mean	56.5	50.3	6.2 (2.5 to 9.9)†	48.6	47.9	0.7 (−3.9 to 5.3)
Fibrotic tissue (%)	38.0 (10.6; 35.7 to 40.3)	43.9 (9.1; 41.5 to 46.4)	5.9 (2.4 to 9.4)†	44.0 (14.2; 40.7 to 47.3)	45.2 (11.3; 42.2 to 48.1)	1.2 (−3.4 to 5.8)
Adjusted mean	36.5	44.2	7.7 (4.2 to 11.2)†	43.9	46.1	2.2 (−2.4 to 6.8)
Leydig cells (%)	6.9 (3.2; 6.2 to 7.6)	5.5 (3.5; 4.7 to 6.6)	1.4 (0.2 to 2.6)	7.5 (2.8; 6.8 to 8.1)	6.1 (3.2; 5.2 to 6.9)	1.4 (0.4 to 2.4)†

*Difference in means (95% confidence interval) between series. 95% Confidence intervals excluding 0 for means considered to be significant.
†Significant difference in comparison between series.

and heavy metals as well as excessive drinking.[26] The role of the increase in environmental oestrogen as a possible common denominator to the adverse effects on male reproductive tracts has achieved much emphasis during recent years.[12,29] The suggested increase of oestrogens or oestrogen-like compounds in past decades may originate from several sources, such as from diet,[30] which has gone through many changes in industrialised countries, or from the increasing use of many organochlorides that act like oestrogens[31] and may accumulate in fat tissue. It has also been hypothesised that fat tissue may convert certain steroids to oestrogens and that increasing body fat content may lead to an increase in the bioavailability of oestrogens through a decrease in the concentration of sex hormone binding globulin. In our work, men in the 1991 series had a significantly higher mean body mass index compared with men 10 years earlier, possibly indicating higher body fat content of men in the latter series. Body mass index, however, showed no association with disorders of spermatogenesis.

The mechanisms of declining sperm counts induced by oestrogen or toxins may entail a disturbance in prepubertal multiplication of Sertoli cells, possibly inducing a decrease in their number. Sertoli cells are involved in the formation of the blood-testis barrier and are known to provide nutritional and mechanical support to spermatogenic cells, thereby sustaining normal spermatogenesis.[32] In the present study, small seminiferous tubules were associated with disorders of spermatogenesis, corroborating our own earlier findings[9] and suggesting a disturbance in the function of Sertoli cells or a decrease in their number as a possible aetiological mechanism for declining sperm counts.

Risk factors for altered spermatogenesis

Excessive use of alcohol is often associated with disorders of spermatogenesis and testicular damage.[33,34] In the present study, we analysed time dependent changes of spermatogenesis over a 10 year period among moderate and heavy drinkers and examined changes in smoking and use of drugs to evaluate the effect of possible changes in the exposure to these individual risk factors. The time differences in spermatogenesis scores were of greater extent among moderate drinkers than among heavy drinkers. This observation is likely to exclude increasing alcohol consumption in Finland between 1981 and 1991[35] as an explanatory factor to widespread deterioration of spermatogenesis. In 1991 moderate drinkers showed incidences of scores of spermatogenesis almost equal to those among heavy drinkers 10 years earlier. Similarly, there was a highly significant increase in the amount of fibrotic tissue in testes of men with moderate drinking habits, whereas no such time dependent change was observed among heavy drinkers. These results suggest that heavy drinkers showing normal spermatogenesis and testicular morphology might be more tolerant to toxic stress originating from the environment. We have also recently reported a possible involvement of a genetic component in the development of alcohol induced disorders of spermatogenesis.[36]

There were more smokers in the 1991 series compared with 1981. A large number of studies have produced controversial results on the effects of smoking on semen and sperm.[37-41] In the present study, no association was observed between smoking and status of spermatogenesis. Additionally, there were no significant changes in the ratio of men who used drugs between 1981 and 1991.

Key messages

- Several recent reports have suggested a significant decrease in human sperm counts over the past few decades
- We used middle aged subjects, with no biasing selection as regards fertility or status of spermatogenesis, to evaluate changes in the incidences of disorders of spermatogenesis from 1981 to 1991
- Normal spermatogenesis was observed significantly less often in the 1991 series than in 1981, whereas the incidences of disorders of spermatogenesis increased significantly during that time
- Changes in the status of spermatogenesis coexisted with decreased testicular weight, smaller seminiferous tubules, and increased fibrosis of testicular tissue
- Alterations in spermatogenesis could not be explained by a change in individual risk factors between the series, such as smoking, drinking, or use of medication, thus challenging further research to illuminate specific reasons for deteriorating spermatogenesis and declining sperm counts

Conclusions

In conclusion, we report a deterioration in male reproductive function at the level of spermatogenesis among middle aged Finnish men between 1981 and 1991. This finding supports several previous observations on declining sperm counts, suggesting that both events might be explained by common genotoxic factors that affect spermatogenesis. Declining spermatogenesis was not explained by different exposure to drugs or incidence of smoking or alcohol consumption, and thus more research is clearly needed to evaluate the roles of different factors for declining sperm counts and deteriorating spermatogenesis.

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