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ヒト精巣の継続的調査及び臍帯におけるダイオキシン類等化学物質の蓄積・暴露状況調査

財団法人　日本公衆衛生協会

ヒト精巣の継続的調査及び臍帯における
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調査方法

行政解剖実施施設で収集した成人男性の解剖記録から精巣重量の調査を行うとともに、同施設にて保存されていた精巣の病理組織標本を用いて、形態学的に精子形成状況の検討を行った。

- 対象者

＊精巣重量調査

1949～1998 年までに死亡した 20～69 歳までの 13,185 人、および、1999 年末までに収集した 20～69 歳までの 3750 人、合わせて 16,935 人

＊精巣病理調査

1978～1998 年までに死亡した 20～69 歳までの 697 人、追加分 36 人、および 1999 年中に死亡した 102 人、あわせて 835 人

- 判定方法

精巣重量調査では、出生年、死亡年、死亡時年齢、死因、身長、体重、精巣重量、肝臓重量の項目に着目し、統計的解析を行った。

精巣病理調査では、Johnson's score count に基づき判定を行った。

なお、本調査研究において、京都大学医学部および千葉大学医学部の各倫理委員会、また東京都監察医務院の共同研究委員会にてそれぞれ承諾を受けた。

調査結果(別添資料1～3参照)

1997 年のフィンランドの報告(Pajarinen, J et al(別添資料1参照))において、1981 年および 1991 年の精子形成状況を死亡した年で比較した場合、精子形成状況の悪化が認められたとの結果が報告された。昨年度の報告では、精巣重量の変化に関しては、死亡年で比較をしていたため、今回の報告では出生年を基準とした報告を行う。

- 精巣重量(別添資料2参照)

図 8 の結果として、身長はどの年代においても出生年に対して直線的な増加現象を示しているが、精巣重量は必ずしも直線的な増加現象を示していない。図 10 の結果として、ピーク時における精巣重量は 1940～1960 年生まれでは継続的に上昇しているが、それ以降では上昇傾向は留まっている。図 11 の結果として、精巣重量がピークに達する年齢は、経年的に若年化傾向がみられる。

- 病理調査＜精子形成状況＞（別添資料3参照）

  精巣の病理組織学的検討により、精子形成が正常と判定された割合は、1978～1998 年の結果では横ばいであったが、1999 年の結果も同様割合に大きな変動はなかった。また、精子形成不全と判定された割合もほぼ横ばいであった。

- まとめ

  全般的に考察すると、精巣重量の変化は、身長の変化とは必ずしも同じ挙動を示してはおらず、今後も継続した調査の必要性が強く示唆される。また、死因別における解析や多変量解析による更なる調査が必要である。

別添資料１

フィンランドにおける精子形成状態に関する報告

Incidence of disorders of spermatogenesis in middle aged Finnish men, 1981-91: two necropsy series

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Abstract

Objective: To investigate if the incidence of disorders of spermatogenesis and testicular tissue morphology have changed in middle aged Finnish men over 10 years.

Design: Two necropsy series completed in 1981 and in 1991.

Setting: Department of Forensic Medicine, University of Helsinki, Finland.

Subjects: 528 men, aged 35 to 69 years, subjected to medicolegal necropsy.

Main outcome measures: Scoring of spermatogenesis and morphometric analysis of testicular tissue components. Individual risk factors for testicular disorders obtained by postmortem blind interviews with acquaintances.

Results: Normal spermatogenesis was found in 41.7% of the men (mean age 53.1 years). Between 1981 and 1991, the ratio of normal spermatogenesis decreased significantly (odds ratio 3.5; 95% confidence interval 2.5 to 5.1) from 56.4% to 26.9%, with a parallel increase in the incidence of partial and complete spermatogenic arrest (2.1; 1.4 to 2.9 and 2.9; 1.7 to 5.0, respectively). During this period, the size of seminiferous tubules decreased, the amount of fibrotic tissue increased, and the weight of testicles decreased significantly. Alterations in testicular characteristics over time could not be explained by changes in body mass index, smoking, alcohol drinking, or exposure to drugs.

Conclusions: The incidence of normal spermatogenesis decreased among middle aged Finnish men from 1981 to 1991, and the incidence of disorders of spermatogenesis and pathological alterations in testicles increased. Deteriorating spermatogenesis may thus be one important factor in the explanation of declining sperm counts observed worldwide.

Introduction

In 1992 Carlsen *et al* reported a significant decrease in the quality of human semen during the past 50 years, including a deterioration in mean semen volume and mean sperm concentration in semen from voluntary sperm donors.[1] Although contradictory and critical results have been published,[2-4] this observation has subsequently been corroborated by several reports additionally suggesting that a similar deterioration may have taken place in the morphology and motility of sperm.[5-8] The reasons for the declining quality of semen and sperm are subjects of current research. Several environmental toxins and chemicals such as alcohol,[9] drugs,[10] industrial solvents,[11] and endogenous and exogenous oestrogen-like compounds[12] have been suggested to alter the function of male reproductive organs via multiple mechanisms, including endocrinological disorders and direct toxic damages on gonadal cells.[13 14]

Thus far, most reports suggesting a deterioration of human semen and sperm during the past decades have analysed the quality of semen from voluntary sperm donors and men attending infertility clinics. Nothing is known, however, about the changes in quality of sperm from middle aged or older men, who may have been exposed to environmental and other risk factors for a longer time than those younger subjects in previous studies. In addition to high selection of subjects in previous studies, which possibly biases the outcome, semen analysis may not relate directly to the severity of testicular lesion at the level of spermatogenesis,[15 16] although it reflects well the overall function of the male reproductive tract. Therefore, to what extent changes in semen quality can be explained by a deterioration of spermatogenesis or some other disorder(s) in male sex organs is undetermined. Alterations in semen quality of donors may even be unrelated to spermatogenesis, reflecting other factors such as changes in the frequency of coitus, or be due to non-toxic exogenous means such as variations in scrotal temperature because, for example, differences in types of clothing. Moreover, it is not known if changes in some known individual risk factors might be associated with declining sperm counts. In this study, we investigated possible changes in incidence of disorders of spermatogenesis and testicular morphology in two necropsy series between 1981 and 1991, comprising middle aged men with no selection as regards spermatogenesis and fertility. Data on exposure to risk factors for testicular disorders were obtained by interviewing close relatives or friends of the deceased.

Subjects and methods

Necropsy series

We examined two necropsy series comprising middle aged men who were subjected to medicolegal necropsy at the Department of Forensic Medicine, University of

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Table 1 Features of necropsy series. Figures are means (SD; 95% confidence intervals) unless stated otherwise

	1981 (n=264)	1991 (n=264)	Difference*
Age (years)	54 0 (9 6; 52 8 to 55.1)	52.2 (9 6, 51.1 to 53.2)	1 8 (0.2 to 3.4)†
Body mass index (kg/m²)	24 1 (4.4; 23 6 to 24.6)	25 8 (4 6; 25 2 to 26.4)	1.7 (0.9 to 2.5)†
No (%) according to cause of death:			
Cardiovascular disease	138 (52)	126 (48)	4.6 (-4.0 to 13.1)
Other disease	40 (15)	39 (15)	0.4 (-5.7 to 6 5)
Intoxication	23 (9)	31 (12)	3.0 (-2.1 to 8.2)
Other violent cause	61 (23)	63 (24)	0.8 (-6.5 to 8.0)
Unknown	2 (1)	5 (2)	1.1 (-0.8 to 3.1)
Interval between death and necropsy (days)	3 5 (1 6; 3.3 to 3.7)	3.8 (2.1; 3.6 to 4.1)	0.3 (-0.0 to 0.6)

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

Helsinki. The capital Helsinki and the surrounding Uusimaa province in southern Finland are the most densely populated parts of Finland, with nearly one fifth (1 million) of the population of the country. About 2500 medicolegal necropsies are performed annually in this area. The first series was collected in 1981 and the second in 1991, both as substudies of a study of changes in the risk factors for sudden and violent death. Both series initially comprised 264 consecutive men aged 35 to 69 years (mean 53.1). More than half of the men (65%) died from disease (table 1), cardiovascular diseases being the most common. One third (33.7%) died violently or due to intoxication, most of them accidentally or by committing suicide. In seven cases (1.3%) the cause of death remained unknown. By covering 42% of all deaths of men aged 65 years or less in this area, this series is the most representative sample of middle aged men that can be obtained.

Methods

Testicles were weighed at necropsy, and the middle section of one testis per man was fixed in 10% buffered formalin solution or in Bouin's solution. Histological sections (5 μm) were prepared after samples were dehydrated and embedded in paraffin and were then visualised by Herovic staining.

Spermatogenesis score

In each slide spermatogenesis was scored in 25 randomly chosen cross sections of seminiferous tubules by one member of the study group (JP) with a light microscope at a magnification of ×200. The analysis was performed without knowledge of interview data or other features and series of the subjects. Each sample was allocated to one of the following groups.

Normal spermatogenesis—Germinal epithelium was normal in most tubules. Sertoli cells, spermatogonia, spermatocytes, spermatids, and spermatozoa were all apparent in the tubules with the epithelium appearing thick and condensed. Men showing modest hypospermatogenesis, characterised by a thin germinal epithelium with diffusely reduced cell quantity at all stages of spermatogenesis, were included in this group.

Spermatogenic arrest—All tubules contained at least Sertoli cells and spermatogonia. No mature spermatozoa were observed, suggesting an arrest in the normal spermatogenesis. Complete spermatogenic arrest was defined as a state in which all tubules exhibited arrest of spermatogenesis, whereas for partial spermatogenic arrest some of the seminiferous tubules (> 5%) showed normal spermatogenesis.

The Sertoli cell only syndrome—A complete absence of germinal cells was observed. Only Sertoli cells remained in most tubules, and in some areas tubules were obliterated by fibrosis. In some cases with complete spermatogenic arrest some of the tubules showed only Sertoli cells, but these cases were grouped on the basis of the main features of the section.

Fibrotic tubules—All seminiferous tubules were totally obliterated by fibrotic tissue with both germinal cells and Sertoli cells missing.

Morphometric analysis of testicular tissue components

Measurement of the percentage area covered by fibrotic tissue and Leydig cells was performed with a light microscope equipped with an ocular grid with 100 defined elements. The number of these elements situated on each of the testicular tissue components was then calculated for one of three randomly chosen cross sections of testicular tissue from each man at a magnification of ×40. An average percentage value for tissue components was calculated. Mean diameters of seminiferous tubules were measured in a proportioned segment of line on the microscope view on a computer screen. Ten randomly chosen circular cross sections of seminiferous tubules were measured, and an average diameter was calculated.

Scoring of spermatogenesis, morphometric analysis of areas of tissue components of testis, and measurement of the diameter of seminiferous tubules were performed blind to interview data.

Interview study and risk factors for testicular disorders

To evaluate the role of various individual risk factors for altered spermatogenesis a relative or a close acquaintance of the subject was contacted and interviewed (table 2). The interview comprised a structured set of more than 50 detailed questions on occupation, use of medications, and smoking and drinking habits.[17]

A complete forensic toxicological examination was performed for all men, including a determination of blood and urine alcohol concentration at the time of necropsy and analysis of the presence of drugs and chemicals in blood, stomach, and liver.

Table 2 Details of risk factors for testicular disorders obtained through interviews with acquaintances in two necropsy series. Figures are numbers (percentages) of subjects

Series	1981 (n=264)	1991 (n=264)	Difference*
Acquaintances interviewed	151 (57)	109 (41)	15.9 (7.5 to 24 3)†
Use of drugs:			
Antihypertensive drugs	15 (10)	5 (5)	5.4 (-0.8 to 11.5)
Sedatives or tranquillisers	68 (45)	54 (50)	4.5 (-7.8 to 16.8)
Other	42 (28)	32 (29)	1.5 (-9.6 to 12.7)
Smoking	103 (68)	81 (74)	6.1 (-5.0 to 17.2)
Use of alcohol:			
Low or moderate drinking	80 (53)	52 (48)	5 3 (-7.0 to 17.6)
Heavy drinking	71 (47)	57 (52)	5 3 (-7.0 to 17.6)

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