
I Klinika Ginekologii Operacyjnej. Instytut Położnictwa i Chorób Kobięcych.
Kierownik: prof. dr hab. n. med. Henryk Zrubek

Marian SEMCZUK

An Evaluation of the Generative and Hormonal Functions of the Male Gonad in Chronic Alcoholics

Ocena czynności generatywnej i hormonalnej gonady mężczyzn—przewlekłych alkoholików

Оценка генеративной и гормональной функции мужской гонады хронических алкоголиков

Ethyl alcohol which is introduced into the organism penetrates through to the organs and body fluids on a diffusive basis, depending on its dose, concentration, total water content in the organism and the tissue hydration. Alcohol which is introduced into the organism in excess is a toxic substance and in very high concentrations can even cause protein denaturation and directly damage many cells of the organs. Damage to the organ function can be caused by a direct action of alcohol and the products of its transformation (15, 16) or it can be a consequence of intrasystemic disturbances caused by alcohol (16, 17).

First, ethyl alcohol damages parenchymatous organs, among these the male gonad (15, 16, 17). Up-to-date experimental research indicates to a negative influence of ethyl alcohol on the structure and function of the male gonad (12, 13). A reduced biological value of the semen of male chronic alcoholics has been confirmed (1, 11, 15).

The present research was undertaken to determine to what degree long-lasting ethyl alcohol consumption leads to the injury of the spermatogenesis and hormonal function of the male gonad and if there is an interdependence between the damage of the two related gonad functions. The evidence of the generative function was the morphological picture of the semen and the substructure of the male reproductive cell, while the hormonal function of the testicles was evaluated on the basis of the tests on fructose and citric acid contents in the seminal plasma and testosterone in the blood plasma.

MATERIAL AND METHODS

The investigation was carried out on 25 men who habitually consumed ethyl alcohol for ever 15 years (average 18.9 years) and on 40 healthy fertile men who were the control group.

The age of the male chronic alcoholics ranged between 38—50 years, on the average 40 years. The age of the men in the control group ranged between 28—52 years, on the average 32 years.

Deviations in the somatic state symptoms, known in chronic alcoholism, were found in some of the patients. And thus, degeneration of the parenchymatous organs, inflammations of the throat and of the alimentary tract were confirmed by additional tests. No distinct changes in the genital organs were found in any of the patients clinically examined. All the examined men stated that their sexual development was normal. The obtained data were analysed statistically by using Student's *t* test.

The semen, which was obtained through masturbation, was collected twice for examination from each patient. The break between the examinations ranged from 3 weeks to 2 months. The shortest sexual abstinence period was 5—6 days. There were no diabetics among the examined men.

The basic morphological examination of the semen was carried out by standard methods. In the preparations stained with hematoxylin and eosin, spermatozoa of abnormal shape were differentiated. The method of preparing the material for the observation of spermatozoa under an electron microscope was described earlier (10). The seminal plasma fructose level was determined by Roe's method and that of citric acid — by Camp and Former's method (5). Testosterone in the blood serum was determined by the double antibody radioimmunological method using Serono's kit. The obtained numerical data were statistically analysed by using Student's *t* test.

RESULTS

The number of spermatozoa in 1 ml of the semen and the percentage content of motile spermatozoa were significantly lower in the group of chronic alcoholics in comparison with those found in the control group ($P < 0.001$). The mean content of abnormal spermatozoa in the semen of the men in the control group was 19.0%, which was by 38.44% lower than that in the respective examined group and was a distinctly significant statistical difference ($P < 0.001$). The abnormal shape of the spermatozoa concerned its head, neck and tail or individual structural parts of a given spermatozoon. Spermatozoa with abnormally shaped heads were most frequently noted with predominant candle-like form among them. Spermatozoa with double heads were seldom observed, heads broken off from the rest of the spermatozoon being more frequent. Abnormalities in the shape of the neck and tail of the spermatozoa were also more frequently observed in the semen of male chronic alcoholics in comparison with that of healthy men ($P < 0.001$).



Fig. 1. A vertical section of a spermatozoon. Its head, neck and central part of the tail are visible. Magn. ca. 58 500 ×



Fig. 2. A fragment of the spermatozoon head. A changed acrosome structure. Magn. ca. 35 000 \times



Fig. 3. A section of a spermatozoon. An obliteration of the acrosome structure, a decreased condensation of nuclear chromatin and vacuoles are visible. Magn. ca. 40 000 ×



Fig. 4. A section of a spermatozoon. Vesicle forms "whorls" are visible. Magn. ca. 41 700X

While evaluating the spermatozoa of the chronic alcoholics under the electron microscope, apart from frequently observed normal shape and substructure of the spermatozoa (10), many abnormalities were noted. A changed irregular shape of the spermatozoon head was often determined by the abnormal shape of its nucleus (Figs. 1, 3). In the spermatozoon nucleus, vacuoles and a changed chromatin condensation were observed more frequently and were more spread than in normal spermatozoa (Figs. 1, 2, 3, 4). In some of the cases a bloated acrosome membrane or its deformation, enlarged subacrosomal and periacrosomal spaces were noted (Figs. 2, 4). Under the cellular membrane in various areas of the head, vesicles or round forms (whorls) formed from annularly or irregularly entwined membranes (Fig. 4), which were single structures unconnected with the cellular membrane, were observed. In the region of the neck, an enlarged cytoplasm (Figs. 1, 3), a break in the continuity of the basal plate, a lack of centriols, a changed electron density of the segment columns, and a disappearance of thick fiber endings of the tail were noted. In the closer sections the observations showed: an irregular arrangement of mitochondria, sometimes a lack of them in a certain section (Fig. 1), a "naked" — deprived of a cytoplasmic sheath — section of the mitochondrial spirals, a break in the axial fibre complex and, sometimes, a disturbed arrangement of its fibers.

In the group of male chronic alcoholics the mean fructose level in the seminal plasma 683.5 $\mu\text{g/ml}$ — (Table 1) appeared to be by 2151.1 $\mu\text{g/ml}$ lower than the values observed in its respective control group, the difference being distinctly significant ($P < 0.001$). In the examined group variations in the seminal plasma fructose content between individual persons ($S = 268.6$, $V = 39.3\%$) were higher than those in the control group ($S = 719.3$, $V = 25.4\%$).

Table 1. Statistical analysis of the results obtained from the examined groups

| | | Blood serum | | Seminal plasma | |
|-------------------------|----------------------|-----------------------------|---------------|-------------------------------|-----------------------------|
| | | Testosterone ng/ml | | Fructose ($\mu\text{g/ml}$) | Citric acid (mg%) |
| Chronic alcoholics | Statistical features | $S = 2.59$ | $V = 48.42\%$ | $S = 268.6$ $V = 39.3\%$ | $S = 107.2$ $V = 54.6\%$ |
| | From—to | 2.15—10.75 | | 144—1150 | 59.6—411.6 |
| | Mean value | 5.36 | | 683.5 | 196.2 |
| Significant differences | | $t_0 = 4.88$ $P < 0.001$ | | $t_0 = 14.56$ $P < 0.001$ | $t_0 = 7.22$ $P < 0.001$ |
| Control group | Mean value | 8.78 | | 3834.6 | 381.9 |
| | From—to | 5.10—15.75 | | 1580—4348 | 262.0—630.0 |
| | Statistical features | $S = 2.76$ | $V = 31.47\%$ | $S = 719.3$ $V = 25.4\%$ | $S = 94.5$ $V = 24.6\%$ |

The mean citric acid content in the semen of the chronic alcoholics, estimated as 196.2 mg%, turned out to be significantly lower than the values in its respective control group ($P < 0.001$). The range of the citric acid levels in the seminal plasma of the men in the examined group (59.6—411.6 mg%) and the values of changeable measurements ($S = 107.2$, $V = 54.6\%$) indicate to a large individual fluctuation in this series of determinations (Table 1).

The mean testosterone level in the blood serum of the men in the examined group (5.36 $\mu\text{g/ml}$), was by 3.42 $\mu\text{g/ml}$ lower than the values in its control group, and the difference was statistically significant ($P < 0.001$). The variations in the blood serum testosterone in the group of the chronic alcoholics appeared to be higher in comparison with the values of healthy men ($S = 2.764$, $V = 31.47\%$), however the differences were slight (Table 1).

DISCUSSION

The present results indicate to a damage of the reproductive and hormonal functions of the gonad in the chronic alcoholics. A distinct decrease in the biological values of the semen was observed as a result of a decreased number of spermatozoa in a volume unit, a decreased content of active spermatozoa, an increase in the amount of abnormally shaped spermatozoa, and the presence of substructural abnormalities in many reproductive cells. The significant fall in the fructose and citric acid levels in the seminal plasma and that of testosterone in the blood serum of the examined men suggests a disturbed hormonal function of the gonad or a dissociation in the axial function of the gonad — hypophysis.

The morphological examination of the semen belongs to the oldest evaluation methods of the generative function of the testicles (14). Simmonds (14) observed hypo- and even azoospermia in the semen of chronic alcoholics.

Doepfmer and Hinkers (1), in *in vitro* examination, while using an increasing concentration of alcohol in a solution of physiological salt, observed a significant decrease in the vitality of spermatozoa, motility, a reduction in number and some morphological changes in the spermatozoa. Żukow stated that with the advancement of clinical symptoms of chronic alcoholism, changes in the morphological structure of the spermatozoa increase and variations concerning their activity and number are observed (17). This was partly confirmed in my earlier investigations (11).

Observations, performed by research workers (1, 11), on the spermatozoa of chronic alcoholics, under a light microscope, permitted to differentiate normal and abnormal shapes of the cells and to determine their percentage content in the semen. The observations of these cells under the electron microscope enable an accurate differentiation of the male gamete substructure, the physiological maturity of which is connected with the attainment of a proper substructure. Many of substructural abnormalities observed in this study such as: a changed nucleus shape, a decondensation of its chromatin, abnormalities of forms called "whorls", in the region of the head may be considered the factors which reduce biological value of the semen of chronic alcoholics. Similar substructural abnormalities were observed by other research workers in the spermatozoa of infertile men (6, 7, 9).

Earlier works of many authors drew attention to the dependence between the androgen function of the organism and the fructose and citric acid contents in the semen (2, 3). Some information in the literature undermines the above opinion (2).

An excess of androgens causes an accumulation of fructose and citric acid in the male additional sexual glands. „Fructose test" according to M a n determines a dependence between the fructose level in the semen and testosterone production (3). A similar citric acid test determines testosterone production in Leydig's cells (2). A dependence between testosterone concentration in the peripheral blood and a citric acid content in the semen has been shown (2). According to Eliasson (3) the determination of the fructose level in the seminal plasma has a higher value as an index of the androgen function than the determination of the testosterone level. On the basis of animal examinations H a r d i n g et al. (4) suggests a certain dependence between the production of fructose and that of citric acid in the semen, suggesting their mutual influence.

In male chronic alcoholics a decrease in the fructose level in the semen was observed (1, 8, 17). While in the course of an acute ethanol poisoning the changes in the fructose concentration in the semen are insignificant (1), distinct disturbances in the synthesis of the compound are observed in men constantly exposed to alcohol action (8, 17). A progressive reduction of the fructose content in the seminal plasma was observed in case of long-lasting alcohol intoxication (8).

An impairment in the generative and hormonal functions of the gonad in chronic alcoholics may be the result of a direct damage of the spermatogenic epithelium and of the parenchymatous gland by alcohol, which was confirmed by my earlier experimental studies (12, 13). While on the other hand, the reduction in the gonad androgen function, confirmed in the experimental studies (13), and the present reduction in the

testosterone level in the blood serum of chronic alcoholics may also have a certain inhibiting influence on the normal course of spermatogenesis and spermiogenesis. It seems that ethyl alcohol has also an inhibiting influence on the maturation process of spermatozoa within the periphery of the gonad. I often observed abnormalities within the periphery of the male reproductive cells, which, in turn, determine their reduced biological maturity. The decreased fructose and citric acid levels in the semen may be due to a testicular androgene deficiency, while at the same time — one should consider the possibility of a direct toxic impairment of the seminal vesicles and the prostate gland — the organs directly responsible for the production of the metabolites discussed.

REFERENCES

1. Doepfmer D., Hinkers H. J.: *J. Zeitschr. f. Haut und Geschlecht*, **5**, 39, 94—107, 1965.
2. Dondero F., Sciarra F., Isidori A.: *Fertil. Steril.* **23**, 2, 168, 1972.
3. Eliasson R.: *Fertil. Steril.* **19**, 3, 334—337, 1968.
4. Harding B. W., Samuels L. T., Mann T.: National Cancer Institute, Monograph. **12**, 253—261, 1963.
5. Kokot F.: *Metody badań laboratoryjnych stosowane w klinice PZWL*, Warszawa 1969, 306.
6. Pedersen H.: *Danisch. Med. Bull.* **21**, 1, 1974.
7. Ross A., Christie S., Edmont P.: *J. Reprod. Fertil.* **32**, 243, 1973.
8. Semczuk M.: *Ann. Univ. Mariae Curie-Skłodowska, Lublin, Sectio D* **24**, 243, 1972.
9. Semczuk M.: *Z. Mikrosk. Anat. Forsch.*, Leipzig, **90**, 1113, 1977.
10. Semczuk M.: *Z. Mikrosk. Anat. Forsch.*, Leipzig, **91**, 31, 1977.
11. Semczuk M., Żrubek H., Czajka R.: *Pol. Tyg. Lek.* **33**, 24, 961, 1968.
12. Semczuk M.: *Ann. Univ. Mariae Curie-Skłodowska, Lublin, Sectio D*, 1977 (in print).
13. Semczuk M.: *Mat. Med. Pol.* 1978 (in print).
14. Simmonds M.: *Dtsch. Arch. Klin. Med.* **61**, 412, 1998.
15. van Thiel D. H., Lester R., Sherings R. J.: *Gastroenterol.* **67**, 118, 1188, 1974.
16. van Thiel D. H., Gavalier J. S., Lester R., Goodman M. D.: *Gastroenterol.* **69**, 326, 1975.
17. Жуков Я. Т.: К вопросу о нарушении сперматогенеза при хроническом алкоголизме. [in:] *Вопросы психоневрологии*. Москва 1965.

STRESZCZENIE

Badania przeprowadzono u 25 mężczyzn, którzy nałogowo spożywali alkohol etylowy ponad 15 lat (średnio 18,9 lat) oraz u 40 zdrowych, płodnych mężczyzn. Średni wiek mężczyzn—przewlekłych alkoholików wynosił 40 lat, zaś zaliczonych do grupy kontrolnej 32 lata. W każdym przypadku wykonywano badanie morfologiczne nasienia oraz przeprowadzano obserwacje plemników w mikroskopie elek-

tronowym. Fruktozę w płazmie nasienia oznaczano metodą Roe'a, kwas cytrynowy — Campa i Formera, a zawartość testosteronu w surowicy krwi oznaczano metodą radioimmunologiczną podwójnych przeciwciał z użyciem kitu, firmy Serono. Nasienie i krew do badania pobierano w tym samym czasie.

U mężczyzn—przewlekłych alkoholików stwierdzono zmniejszenie liczby plemników w jednostce objętości nasienia, zmniejszenie wśród nich zawartości plemników aktywnych, wzrost liczby plemników o nieprawidłowym kształcie oraz obecności nieprawidłowości substrukturalnych w wielu komórkach rozrodczych. Wykazano także istotny spadek poziomu fruktozy i kwasu cytrynowego w płazmie nasiennej oraz testosteronu w surowicy krwi mężczyzn nałogowo spożywających alkohol etylowy. Obecne badania własne wskazują na upośledzenie funkcji rozrodczej i hormonalnej mężczyzn—przewlekłych alkoholików.

РЕЗЮМЕ

Исследования проведено на 25 мужчинах, хронически принимающих более 15 лет (в среднем 18, 9 лет) этиловый алкоголь, а также на 40 здоровых плодовитых мужчинах. Средний возраст мужчин-хронических алкоголиков — 40 лет, а относящихся к контрольной группе — 32 года. В каждом случае производилось морфологическое исследование спермы, а также проводилось наблюдение сперматозоидов в электронном микроскопе. Фруктоза в плазме сперматозоида обозначалась методом Роза, лимонная кислота — Кампа и Формера, а количество тестостерона в сыворотке крови обозначалось радиоиммунологическим методом двойных антител с употреблением замазки фирмы Сероно. Сперма и кровь для исследования брались одновременно.

У мужчин-хронических алкоголиков установлено уменьшение количества сперматозоидов в единице объема спермы, уменьшение среди них количества активных сперматозоидов, увеличение количества сперматозоидов неправильной формы, а также наличие субструктурных аномалий во многих генеративных клетках. Обнаружено также значительное понижение уровня фруктозы и лимонной кислоты в плазме спермы, а также тестостерона в сыворотке крови мужчин хронически принимающих этиловый алкоголь. Данные исследования указывают на ослабление генеративной, гормональной функции мужчин-хронических алкоголиков.

