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FEATURES OF STRUCTURE AND DYNAMICS IN DISORDERS OF FERTILE PROPERTIES IN EJACULATE DEPENDING ON TYPE OF ALCOHOL DRINKS CONSUMED

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Abstract

Infertility in marriage is rather vital not only in our country, but abroad as well. According to the WHO data approximately 10% of married couples have trouble conceiving. The data concerning male sterility, its rate and severity are numerous and contradictory in scientific reports in our country and abroad.

Harmful effect of abusing alcohol being spread amid male population, including men at reproductive age is of particular interest for research within the studies of male sterility.

Due to the scanty information and the lack of precise data on etiology and pathogenesis of fertility in men consuming alcohol this research was aimed at studying the features in changes of sperm counts and the rate in spermatozoa DNA fragmentation depending on type and amount of alcohol consumed.

The article contains the data of studying ejaculate fertile properties and features of spermatozoa DNA fragmentation in 110 men being divided into three groups depending on type and amount of alcohol consumed.

According to the obtained data the degree of spermatogenesis disorder and tendency to increase level of spermatozoa DNA fragmentation depends on type, rate and amount of alcohol consumed. Thus, the low level in consumption of alcoholic drinks was accompanied by variations of admissible values in spermogram. The most evident changes in ejaculate fertile properties were being observed at middle and high risk in consumption of beer and alcohol mixed, as teratozoospermia has been revealed as a result not only from the ethanol effect but from the impact of components free alcohol character. The revealed changes require performing further researches for studying mechanisms of disorders in male reproductive function.

Keywords: *alcohol, male infertility, spermogram, DNA fragmentation, fertility.*

Introduction

Infertility in marriage is rather vital not only in our country, but abroad as well. According to the WHO data approximately 10% of married couples have trouble conceiving. The data concerning male sterility, its rate and severity are numerous and contradictory in scientific reports in our country and abroad [*Kudlay E, 2007*].

According to the state statistical data over Ukraine occurrence of male infertility is 4-5 times less than female one [*Gorpinchenko I, Nikitin O, 2010; Gorpinchenko I, 2012; Yuzko O et al., 2012*]. The causes of female infertility and methods for its management are detailed in the reports of Ministry of Health, but the data about the causes of male sterility and methods for their management are scanty [*Gorpinchenko I, 2012*].

Some authors [*Abubakirov A, 2009; Baikoshkareva S et al., 2009*] report that due to the lack of precise diagnostic criteria for male sterility one may say about 25% male sterility with unclear etiology, but the other authors [*Gleicher N, Barad D, 2006; Bozhedomov V et al., 2009*] allege that idiopathic infertility cases form 30-75%, it may be explained by the lack of information on etiology and pathogenesis in male infertility disorders.

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Harmful effect of abusing alcohol being spread amid male population, including men at reproductive age is of particular interest for research within the studies of male sterility [*Budnik A et al., 2016*].

Currently toxic effect of alcohol on cellular and subcellular structures that causes disorders for key systems (cardiovascular, nervous, digestive and so on), variety of dysregulator effects, distortion and prolapse of functions in all organs was proved [*Perfilyeva S, 2007; Ostroumova O, 2013*]. However there is no common opinion concerning mechanisms in disorders of male reproductive function under alcohol effect.

It should be noted that there are not numerous references considering changes of functions in male urogenital system at abusing alcohol.

The points of view on this problems and obtained data are very contradictory and different.

The complicacy in interpretation of data obtained within researches on the rate and degree of evidence in disorders of reproductive function may be explained by the lack of trials, when types of alcohol such as beer, spirits and mixed alcohol drinks should be taken in account, which explains different approaches in assessment of such pathology and low therapeutic efficiency.

The above-mentioned facts require studying male reproductive system taking into account the type of alcoholic drinks and to assess their effect in disorders of ejaculate fertile properties.

Present research aimed to study the features in changes of spermiologic values and degree of spermatozoa DNA fragmentation depending on type and amount of alcohol consumed.

Material and methods

Totally 110 men aged from 20 to 55 years have been included in investigation who underwent examination on planning child birth. All men have presented an agreement in written form for participation in the studies. The agreement has been approved by Institutional Committee on Bioethics and corresponded to the bases of Helsinki Declaration, as well as approved by the Committee on Bioethics at State Institution "Zaporozhye Medical Academy of Postgraduate Education of Ministry of Health of Ukraine", and corresponded to ethic, moral and legal requirements of the Order No 281 of Ministry of Health of Ukraine from 01.11.2000.

Examined men have been divided into three groups. The first group (control) included 17 men who didn't consume any alcohol and had 1-2 children aged from 1 to 5 years. The second group (comparison) consisted of 27 patients who consumed, but didn't abuse all types of alcohol (1-2 doses of alcohol once for 1-3 months). The third group was composed of 66 patients abusing alcohol (6 and more alcohol units or 22 and more doses a week). Depending on type of alcohol this group was divided into 3 subgroups: IIIa consisted of 13 patients abusing strong alcohol, the second group (IIIb) included 27 patients abusing beer, and the third one IIIc (joint group) included 26 patients abusing both beer and strong drinks.

All men underwent a complex examination including: interview, spermiological analysis, assessment of spermatozoa DNA fragmentation level, and statistical analysis of the obtained data.

Alcohol consumption screening AUDIT questionnaire has been conducted [*Babor T, 2001; Babor T et al., 2001*]. The following risks in alcohol consumption have been assessed according to WHO recommendations: high (6 or more doses a day or more than 42 doses a week), intermediate (not more than 5 doses a day or 22-41 doses a week) and low (not more than 3-4 doses a day or less than 22 doses a week) [*WHO, 2000; Allen J, Litten R, 2001*].

Spermiological analysis was performed over standard technique recommended by WHO. During ejaculate analysis volume, color, consistency, pH, concentration of spermatozoa in 1 *ml* ejaculate and their total amount, degree of motility and microscopic investigation of stained specimen have been assessed [*WHO, 1999*].

To assess spermatozoids DNA fragmentation level technique Sperm Chromatin Dispertion test (RF patent No 2373288) has been used. Spermatozoa DNA fragmentation level up to 30% corresponded to standard values, higher values – than 30% of calculated 500 spermatozoa.

Statistical analysis of obtained data was performed using computer programs set STATISTICA (StatSoft Statistic v.7.0.). Statistical significance of compared indices with distribution different from standard, assessed by Kolmogorov-Smirnov test, has been established using Wald-Wolfowitz runs test at the significance level of 0.05. The data under consideration are presented as median (Me)

and interquartile scope (RQ), presenting difference between meanings of 75 and 25 percentiles (RQ=75% UQ – 25% LQ), where UQ is upper quartile and LQ is lower quartile.

Results

The first (control) group was characterized by ejaculate fertility preserved accordingly to the standard values, recommended by WHO (Table).

In II group ejaculate volume has been decreased at average by 5.5%, dilution time has been increased by 20%, viscosity has been increased by 33.3% in comparison with the control group values.

While examining native and stained specimen under microscope in II group patients active and not motile spermatozoa decreased by 12.3% on average and fixed forms increased by 90.9% on average compared to control group values. Dynamic kinesisgram was the same as initial data.

Concentration of spermatozoa in 1 *ml* and their total amount in ejaculate of II group patients has been decreased by 24.7% on average and 6.1% compared to the values of control group. Pathologic forms increased by 109.1% secondary to decreasing standard forms of spermatozoa by 30.8% concerning values of control group; teratozoospermia index was in conformity with admissible values.

As a result of conducted vinvestigation of DNA spermatozoa fragmentation in men of II group this value increased by 50% regarding control group, however, the amount of fragmentary spermatozoa was at average 15% that corresponded to admissible values.

Thus, the changes of values revealed in spermogram in patients of II group differed from the values of control group, that is clinically significant, but they did not exceed limits of values recommended by WHO, which certifies about preserved ejaculate fertile properties.

The changes of ejaculate physical properties in men of IIIa group as compared with values of both control and II group have been observed, but they didn't exceed admissible values recommended by WHO. While studying spectrogram data decreasing amount of active and not motile spermatozoa was noted by 22.5% on average and 11.5% respectively to I and II groups. The amount of fixed spermatozoa has considerably increased on average by 134.4% and 23.8% comparatively to values in both control and II groups; dyskinetic spermatozoa invisible in other groups have been revealed. The reliable decrease of spermatozoa mobility has been observed in dynamics concerning I (control) and II groups.

Spermatozoa concentration for 1*ml* in men of this group has been decreased on average by 49.5% and 32.8%, and total spermatozoa amount in ejaculate has been decreased by 34.7% and 30.5% in regard to values in control and second groups, respectively.

Microscopic investigation of stained specimen in patients of IIIa group has shown decreasing standard spermatozoa amount on average by 44.9% and 20.4%, as well as increasing amount of pathologic forms by 186.4% and 36.9% comparatively with values in control and II groups. As for II group increasing mixed defects of spermatozoa by 42.8% has been revealed, though teratozoospermia index hasn't exceeded admissible values recommended by WHO.

The level of spermatozoa DNA fragmentation was on average 27% that exceeded by 170% and 80% corresponding to the values in I and II groups, but didn't exceed the limits of 30% admissible level.

Thus, revealed changes for the values in males of IIIa group suggested teratozoospermia and slight dyskinesis assuming non-significant decrease but keeping ejaculate fertile properties.

While investigating spermatozoa motility in IIIb group men reliable amount of active and not motile spermatozoa decreasedon average by 23.6% comparatively control group and slight decrease concerning II group (12.8%) and IIIa group (1.4%). Increasing fixed spermatozoa on average by 127.3% and 19% respectively to the values in control and II groups has been observed.

Amount of dyskinetic spermatozoa in IIIb group has been increased on average by 25% comparatively to the values for IIIa group, while spermatozoa motility in dynamics was decreased on average by 93.2% corresponding to values in control and II groups, though motility was practically unchanged to the values in IIIa group.

Concentration values of spermatozoa in 1 *ml* ejaculate for men from IIIb group have been decreased on average by 38.4% and 18.2% respectively to control and II groups. But these values have been increased by 21.9% comparatively to

Table 1

The main values of spermogram and DNA fragmentation level in men depending on type and amount of alcohol consumed
Me (75% UQ – 25% LQ = RQ)

Values, units		I group (n = 17)	II group (n = 27)	III group		
				IIIa (n = 13)	IIIb (n = 27)	IIIc (n = 26)
Ejaculate volume (*ml*)		3.6 (4-3=1)	3.4 (4.8-2.3=2.5)	3.6 (5-3=2)	3.7 (4-2.8=1.2)	3.1 (3.8-2.3=1.5)
Dilution (*min*)		25 (35-20=15)	20 (30-15=15)	30 (50-25=25)	23 (40-20=20)	30* (69-20=49)
Viscosity (*sm*)		0.3 (0.4-0.3=0.1)	0.4 (0.8-0.3=0.5)	1 (1.7-0.4=1.3)	0.6 (1.2-0.4=0.8)	1**;**** (2.6-0.5=2.1)
pH		7.8 (8.0-7.8=0.2)	7.8* (7.8-7.4=0.4)	7.8* (8-7.6=0.4)	7.8* (8.2-7.4=0.8)	7.8 (8-7.6=0.4)
Spermatozoa active and not mobile, %		89 (91-88=3)	78* (80-76=4)	69*;** (72-64=8)	68* (74-55=19)	62.5*;** (68-53=15)
Dyskinesis, %		0 (0-0=0)	0* (2-0=2)	4*;** (9-2=7)	5* (9-2=7)	7*;** (9-4=5)
Spermatozoa fixed, %		11 (12-9=3)	21* (23-19=4)	26* (31-23=8)	25*;** (36-22=14)	30.5*;** (38-26=12)
Kinesisgram 2 hours later	Spermatozoa active and not mobile, %	90 (90-88=2)	78* (81-73=8)	62*;** (68-55=13)	62*;** (67-43=24)	52.5*;** (60-40=20)
	Dyskinesis, %	0 (0-0=0)	0 (3-0=3)	4*;** (14-4=10)	6* (12-2=10)	9.5*;** (14-5=9)
	Spermatozoa fixed, %	10 (12-10=2)	21* (25-19=6)	31* (35-27=8)	30* (41-27=14)	35.5*;** (46-30=16)
Spermatozoa concentrationon, (×*10⁶/ml*)		95 (108-79=29)	71.5 (112-62.5=49.5)	48*;** (59-29.5=29.5)	58.5 (103-24.5=78.5)	44.25*;**;**** (76.5-22=54.5)
Total spermatozoa amount in ejaculate, (×10⁶)		285 (390-231=159)	267.7 (393.3-166.6=226.7)	186 (224-88.5=135.5)	264 (328-63.2=264.8)	125.5*;** (234-79.2=154.8)
Standard spermatozoa (%)		78 (80-75=5)	54* (60-49=11)	43* (46-28=18)	30*;** (38-27=11)	29*;** (39-19=20)
Total amount of pathologic spermatozoids (%)		22 (25-20=5)	46* (51-40=11)	63*;** (72-56=16)	70*;** (73-62=11)	71*;** (81-61=20)
Juvenile spermatozoa (%)		2 (2-2=0)	3 (6-2=4)	3 (6-2=4)	3 (6-2=4)	3 (5-2=3)
Spermatozoa defects mixed (%)		0 (0-0=0)	7* (8-5=3)	10*;** (19-9=10)	17*;** (21-13=8)	19*;** (30-16=14)
Teratozoospermia index		-	1.2 (1.24-1.15=0.09)	1.27 (1.4-1.22=0.18)	1.33** (1.41-1.25=0.16)	1.42** (1.53-1.35=0.18)
Spermatozoa DNA fragmentation, %		10 (13-8=5)	15 (26.2-8=18.2)	27* (36.8-17.6=19.2)	19.8* (29-14=15)	30* (48.6-17=31.6)

Notes:** * – statistically significant difference compared to control group (p<0.05), ** – statistically significant difference compared to II group (p<0.05), *** – statistically significant difference compared to IIIa group (p<0.05), * – statistically significant difference compared to IIIb group (p<0.05).*

IIIa group. Total amount of spermatozoa in males ejaculate of this group was slightly different from values in control and II groups (amount decreased by 7.4% and 1.4% respectively), but it was increased by 41.9% comparatively to IIIa group.

The amount of pathologic spermatozoa has been increased compared to analogous values in control (by 218.2%), II (by 52.2%) and IIIa groups (by 11.1%) secondary to decreasing standard spermatozoa on average by 61.5%, 44.4% and 30.2%, respectively.

Reliable increasing mixed spermatozoa defects were noted for males in IIIb group relatively to control and II groups, though teratozoospermia index corresponded to admissible values.

The level of spermatozoa DNA fragmentation was on average 19%, that is, it was increased respectively by 98% and 32% against values in I and II groups, but it was decreased by 26.7% comparatively values in IIIa group, though this index didn't exceed admissible values.

Thus, the changes revealed in spermogram of men in IIIb group display slight dyskinesis, astenozoospermia but pronounced teratozoospermia, inherent to decreasing moderately pronounced ejaculate fertile properties relatively to preceding groups.

While investigating ejaculate physical properties-in men of IIIc group deviations from standard values-recommended by WHO have not been observed but differences relatively to all groups examined earlier have been revealed. When investigating under microscope the main values of spermogram decreasing active and not motile spermatozoa have been observed on average by 29.8%, 19.9%, 9.4%, 8.1% and increasing amount of fixed forms by 177.3%, 45.2%, 17.3%, 22% respectively analogous values in control, II, IIIa and IIIb groups.

Increasing dyskinetic forms in IIIc group was observed on average by 75% and 40% compared to IIIa and IIIb groups, respectively.

While investigating spermatozoa motility in dynamics it decreased by 82.6% compared to values in control and II groups and relatively to values in IIIa and IIIb groups by 74.5% and 156.6%, respectively.

Decreasing spermatozoa concentration in 1 *ml* of ejaculate on average by 53.4%, 38.1%, 7.8%, 24.3% and consequently, total amount of spermatozoa in ejaculate by 56%, 53.1%, 32.5% and 52.5% relatively values in control, II, IIIa and IIIb groups have been observed.

The amount of pathologic spermatozoa in IIIc group reliably increased by 222.7% and 54.3% comparatively analogous to values in control and II groups; insignificant increase for indices in IIIa and IIIb groups has been observed (by 12.7% and 1.4% respectively). The amount of standard spermatozoa has been decreased on average by 62.8%, 46.3%, 32.5% corresponding to the values in control, II and IIIa groups, though slight decrease was observed comparatively values in IIIb group (by 3.3%).

Increasing mixed spermatozoa defects was observed in IIIc group relatively to indices in control, II, IIIa, IIIb groups, though index of teratozoospermia corresponded to admissible values.

Studying spermatozoa DNA fragmentation level in IIIc group revealed the growth of this index by 200%, 100%, 11.1%, 51.5% concerning values in control, II, IIIa, IIIb groups though it was 30% on average that is the upper bound of standard value.

Thus, the changes revealed in spermograms of IIIc group display considerable astenozoospermia and dyskinesis, more pronounced teratozoospermia, than in preceding groups testifies about more pronounced decrease of ejaculate fertile properties.

Discussion

According to WHO data diseases caused by alcohol consumption take the third place over the world, among which special attention should be paid to sexual dysfunction [*Kukurekin Yu et al., 2009*].

In 2010 the World Health Assembly approved resolution on global strategy for reducing harmful alcohol consumption, according to which some countries were recommended to strengthen national measures on Public health concerning problems caused by harmful alcohol consumption. Adoption, implementation and observation of strategic principles will decrease negative consequences of alcohol for health [*WHO, 2015*].

The effect of alcohol on health of population is considerably determined by two separate, but interconnected parameters, such as total amount and pattern of alcohol consumption influencing key systems in body, in this way resulting in dysregulator effects [*WHO, 2015*].

Our research has revealed the north type of alcohol consumption in almost all investigated

males, that is, great amount for short period, when the structure and dynamic in ejaculate fertile properties are different. The tendency to changes in different degrees of manifestations for main ejaculate parameters (concentration, motility and number of morphologically normal spermatozoa) depending on type and amount of alcohol drink consumed has been established.

The most pronounced changes in ejaculate fertile properties have been observed at middle and high risks in beer and strong alcohol consumption when pronounced teratozoospermia connected not only with ethanol effects, but with the effects of available components of not alcoholic character. Revealed disorders indicate a decrease of ejaculate fertile properties physiologically, that had an important prognostic and diagnostic significance concerning assessment of male reproductive system.

One of the important agents of disturbing male fertility is spermatozoa DNA fragmentation, pathologic mechanisms of which haven't been studied yet. Spermatozoa DNA integrity is an internal parameter for spermatozoa damage which is impossible to be revealed through routine spermiologic investigation and which has prognostic significance concerning therapy efficiency or/and choice of appropriate auxiliary method for child reproduction [*Rudneva S et al., 2014*].

Revealed tendency of increasing spermatozoa DNA fragmentation level extends our view concerning disorders of ejaculate fertile properties that is obviously connected with alcohol consumption.

Thus, alcohol consumption in great amounts, especially, consuming beer with mixed strong alcohol and also pattern of consuming alcohol as "great doses for short time" contribute to a considerable damage to ejaculate fertile properties.

Revealed changes require further researches concerning studies on mechanism of male reproductive function disorders.

Conclusion

The low level of alcoholic drink consumption was accompanied by fluctuations of spermatogenesis values in admissible limits of standards recommended by WHO.

The tendency to oligo-astenozoospermia and increasing DNA fragmentation level was inherent to males in all study groups. Mild dyskinesis and teratozoospermia of different degree were observed depending on type of alcoholic drink consumed.

Mild teratozoospermia and dyskinesis were observed in abusing strong alcohol, mild dyskinesis was observed secondary to pronounced teratozoospermia in abusing beer; abundance of mixed alcoholic drinks led to subsequent intensification of oligo-astenoteratozoospermia and dyskinesis aggravating disorders of ejaculate fertile properties.

In combined abusing beer and strong alcohol more pronounced spermatogenesis and increasing spermatozoa DNA fragmentation were observed comparatively preceding groups that may be resulted not only from effects of ethanol, but from components of non-alcoholic character. Revealed disorders assert considerable physiologic decrease of ejaculate fertile properties and tendency to early abortions.

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