

Alcohol consumption and quality of embryos obtained in programmes of *in vitro* fertilization

Artur Wdowiak¹, Magdalena Sulima², Monika Sadowska¹, Grzegorz Bakalczuk², Iwona Bojar³

¹ Diagnostic Techniques Unit, Faculty of Nursing and Health Sciences, Medical University, Lublin, Poland

² Department of Obstetrics, Gynaecology and Obstetrical-Gynaecological Nursing Faculty of Nursing and Health Sciences, Medical University, Lublin, Poland

³ Department for Health Problems of Ageing, Institute of Rural Health, Lublin, Poland

Wdowiak A, Sulima M, Sadowska M, Bakalczuk G, Bojar I. Alcohol consumption and quality of embryos obtained in programmes of *in vitro* fertilization. *Ann Agric Environ Med.* 2014; 21(2): 450–453. doi: 10.5604/1232-1966.1108623

Abstract

Introduction. Infertility is defined as a state when a couple fails to conceive a pregnancy after one year of regular intercourse without the use of contraception. Alcohol consumption is one of the main stimulants which negatively affect the female and male reproductive system.

Objective. The objective of the study was analysis of the effect of alcohol consumption by the examined women on the quality of embryos obtained during *in vitro* fertilization programmes.

Material and methods. The study covered 54 women who received treatment due to infertility. The database and statistical analyses were performed using computer software STATISTICA 7.1 (StatSoft, Poland).

Results. The study showed that 42.59% from among 100% of the women in the study consumed alcohol. In the group of women who consumed alcohol, class A embryos constituted 4.35%, class B embryos – 86.96%, while embryos of class C – 8.69%. A statistically significant difference was observed between the classes of embryos and alcohol consumption by the women examined ($p=0.001$). In addition, a statistically significant relationship was found between the amount of alcohol consumed and the classes of embryos ($p=0.005$). A significantly larger number of class B embryos came from women who consumed more than 25 grams of ethyl alcohol daily (72.72%), compared to those who consumed alcohol sporadically (44.44%), or those who abstained entirely from alcohol (30.00%).

Conclusions. Alcohol consumption causes the development of poorer quality embryos. Significantly more embryos of class B came from oocytes of women who consumed alcohol, compared to class A. An active campaign against alcohol consumption should be carried out among women at reproductive age to safeguard their fertility and future motherhood.

Key words

infertility, embryos, alcohol

INTRODUCTION

Today, despite the existing prevention programmes and easy access to adequate treatment, infertility still remains an important medical problem. Infertility is defined as a state when a couple is incapable of or unsuccessful in achieving pregnancy, despite having regular, unprotected sex for at least a year [1, 2, 3].

An increasing prevalence of the problems related with reproduction in recent years is associated with the style of life, including the consumption of alcohol. Alcohol consumption by women at reproductive age leads to the occurrence of abnormalities with respect to reproductive health, including maturation disorders, difficulties with becoming pregnant, complications in the course of pregnancy and foetal development disorders [4, 5, 6, 7].

The objective of the study was analysis of the effect of alcohol consumption by the women examined on the quality of embryos obtained in the programmes of *in vitro* fertilization.

MATERIALS AND METHOD

The presented study was conducted in the Non-Public Health Care Unit ‘Ovum Reproduction and Andrology’ in Lublin, and covered women treated due to infertility. The research instrument was a questionnaire form independently completed by the respondents who had been informed concerning the objective of the study and its total anonymity. A reservation was also made that the data for coding the questionnaires will be used exclusively for the identification of medical records.

A total number of 60 questionnaires were distributed, and no interferences were observed while carrying out the study. Fifty-four correctly completed questionnaire forms were qualified for statistical analysis. Women with chronic and metabolic diseases and obesity were excluded from the study group. Each questionnaire form qualified for statistical analysis was supplemented by an embryo quality sheet, for which data was collected from medical records identified based on the codes placed on the questionnaires by respondents. Morphological assessment of the embryos was performed by means of an inverted microscope (Olympus CKX41) with mounted digital camera (ARTCAM-500MI). At the first stage (16–20 hours after micromanipulation), an evaluation of pronuclei was performed, and unfertilized cells were rejected. After the subsequent 24 hours, embryos were evaluated, considering the properties associated with

Address for correspondence: Artur Wdowiak, Diagnostic Techniques Unit, Faculty of Nursing and Health Sciences, Medical University, Lublin, Poland
e-mail: wdowiakartur@gmail.com

Received: 05 April 2013; accepted: 02 May 2013



embryo's implantation capability, such as pace of division, degree of fragmentation, presence of a single nucleolus per blastomere, the same size of blastomeres and symmetry in their positioning. Embryos showing the best properties were classified into Class A, possessing the highest reproductive potential. Embryos showing slight deviations in the degree of fragmentation (10–25%), symmetry and division pace were placed in Class B. Considerable and big abnormalities in the structure of embryos were the cause for classifying them into Classes C and D, respectively. The presence of one or more single nucleolus per blastomere resulted in upgrading the embryo class by one position, while the observation of two nucleoli in one blastomere resulted in downgrading the embryo class by 2 positions.

The respondents' age ranged within 25–39. The most numerous group constituted women aged 35–39 (44.44%; n=24), followed by women aged 30–34 (37.04%; n=20), and those aged 25–29 (n=10) – 18.52%. As many as 62.96% of the women examined were urban inhabitants (n=34), whereas 37.04% (n=20) lived in the rural areas. No women in the study group had elementary education level. The majority of respondents possessed university education – 66.67% of women (n=36), followed by secondary school education – 25.92% (n=14), and secondary vocational education level – 7.41% (n=4). Women with obesity and chronic metabolic diseases were not qualified into the study group.

The results of the study obtained were subjected to statistical analysis. The values of the parameters analyzed were determined by means of frequency and percentage. For uncorrelated nominal variables, in order to investigate differences between the classes compared, χ^2 goodness of fit test was applied. The relationships between the values examined were analyzed by means of the χ^2 test for independence. The p values $p < 0.05$ were considered statistically significant. The database and statistical analysis were performed based on the computer software STATISTICA 7.1 (StatSoft).

RESULTS

Figure 1 presents the classes of embryos obtained from respondents during infertility treatment by the IVF-ET method. The ABCD classification reflects the quality of individual classes, where A means the best embryo, while D – the poorest quality embryo.

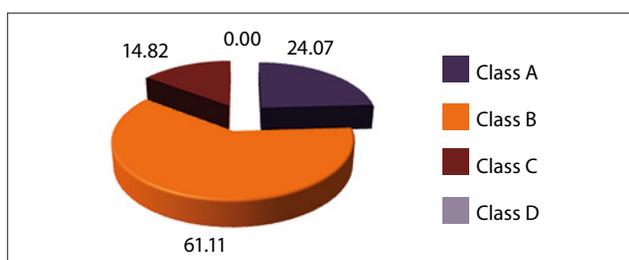


Figure 1. Respondents' distribution with consideration of classes of embryos

The studies performed indicated that the greatest number of embryos were obtained in Class B (61.11%; n=33), followed by Class A (24.07%; n=13), and Class C – 14.28% (n=8). No embryos of Class D were obtained. Figure 2 presents the structure of respondents according to the duration of infertility treatment.

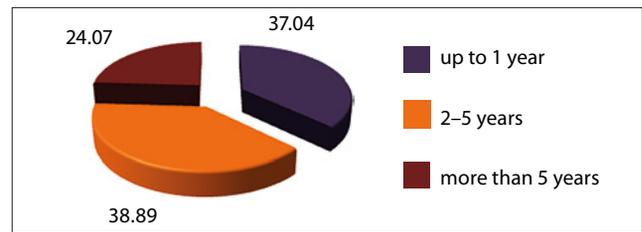


Figure 2. Respondents' distribution according to duration of infertility treatment

Based on the results of the study, it was noted that for 37.04% of respondents (n=20) the duration of treatment was up to 1 year, while for 38.89% (n=21) of the women the period of treatment remained within the range from 2–5 years, and for 13 women (24.07%) this period was over 5 years.

Table 1 presents the relationship between the consumption of alcohol by the examined women and class of embryos. The conducted study showed that 42.59% (n=23) of women consumed alcohol, while 57.41% (n=31) did not. In the group of women who consumed alcohol, class A embryos constituted 4.35%, class B embryos – 86.96%, whereas class C embryos – 8.69%. In the group of women who did not consume alcohol, embryos of class A constituted 41.94%, those of class B – 38.71%, and class C embryos – 19.35%. A statistically significant relationship was observed between the classes of embryos and alcohol consumption by the women in the study ($p=0.001$). A significantly larger number of class B embryos (86.96%) came from the oocytes of women who consumed alcohol, compared to class A (4.35%).

Table 1. Consumption of alcohol and class of embryos

Consumption of alcohol	N/%	Class of embryo A	Class of embryo B	Class of embryo C	Total
Yes	N	1	20	2	23
	%	4.35%	86.96%	8.69%	100.00%
No	N	13	12	6	31
	%	41.94%	38.71%	19.35%	100.00%
Total	N	14	32	8	54
	%	25.93%	59.26%	14.81%	100.00%

$\chi^2=13.39$ $p=0.001$

Table 2 presents the distribution of individual classes of embryos according to the amount of alcohol consumed by the women examined. Based on the results of the study it was found that in the group of women who consumed up to 25 grams of ethyl alcohol daily, class A embryos constituted 15.38%, class B embryos – 69.24%, while embryos of class C – 15.38%. In the group of women who consumed more than 25 grams of ethyl alcohol daily, class A embryos constituted 4.55%, class B embryos – 72.72%, and class C embryos – 22.73%. Among the respondents who consumed alcohol sporadically, class A embryos constituted 44.44%, followed by those of class B – 44.44%, and class C – 11.12%. In addition, the study analysis confirmed that in women who abstained from alcohol, embryos of class A constituted 70.00%, those of class B – 30.00%, whereas class C embryos were not found. A statistically significant relationship was found between the amount of alcohol consumed and class of embryos ($p=0.005$). A significantly larger number of class B embryos came from women who consumed more than 25 grams of ethyl alcohol

Tabela 2. Amount of alcohol consumed and class of embryos

Amount of alcohol consumed	N/ %	Class of embryo A	Class of embryo B	Class of embryo C	Total
up to 25 grams of ethyl alcohol daily	N	2	9	2	16
	%	15.38%	69.24%	15.38%	100.00%
more than 25 grams of ethyl alcohol daily	N	1	16	5	19
	%	4.55%	72.72%	22.73%	100.00%
sporadically	N	4	4	1	9
	%	44.44%	44.44%	11.12%	100.00%
at all	N	7	3	0	10
	%	70.00%	30.00%	0.00%	100.00%
Total	N	14	32	8	54
	%	25.93%	59.26%	14.81%	100.00%

Chi²=18.28 p=0.005

daily (72.72%), followed by those who consumed alcohol sporadically (44.44%) and those who abstained entirely from alcohol (30.00%).

DISCUSSION

Alcohol may be the cause of many negative effects during the reproductive process [8,9,10]. The consumption of alcohol causes hypogonadism in males and females. This state deteriorates with an increase in the amount and duration of alcohol consumption. Ethyl alcohol shows a genotoxic effect, and negatively affects the hormone system (hypothalamic-pituitary-gonadal axis) disturbing the homeostasis of the body. In males, apart from reduction in the content of spermatozoa in the ejaculate, and an increase in the percentage of abnormal sperm in the form of a thick neck, double tail and absence of head, changes are noted on the submicroscopic level, such as abnormally shaped nucleus, acrosomal abnormalities, presence of ring-shaped structures and chromatin condensations. In addition, alcohol causes an inhibition of testosterone biosynthesis in the testes [11,12,13].

According to the relevant literature, the consumption of alcohol by a woman is associated with the occurrence of infertility due to the lack of ovulation and infertility related with endometriosis. Sioda after Wilsnack et al. [14] reported that the highest infertility rate (30%) was observed in the group of women who consumed at least 90 ml of ethanol daily. The studies carried out by Jensen et al. [15] in a group of 430 married couples showed that the consumption by a woman of a moderate amount of alcohol (5 or less drinks weekly) considerably decreased the fertility of the woman. In turn, in the studies conducted by Eggert et al. [16] among 7,393 women aged 18–28, a relationship was found between high consumption of alcohol and increased risk of examinations for infertility. In males, after the consumption of more than 40 grams of alcohol daily, an impairment of spermatogenesis was observed, while in those who drank more than 80 grams, spermatogenesis inhibition occurred more often [17]. Limitation or total elimination of the consumption of alcohol by males and females may increase the chances for success in assisted reproduction – IVF procedures (*in vitro* fertilisation) and GIFT (gamete intrafallopian transfer) [14]. Based on the presented study, it was confirmed that the

highest percentage of class B embryos (72.72%) came from oocytes of women who consumed more than 25 grams of ethyl alcohol daily, whereas the highest percentage of class A embryos (70.00%) – from women who entirely abstained from alcohol. A statistically significant relationship was observed between the amount of alcohol consumed and the class of embryos ($p=0.005$). Significantly more embryos of class B came from women who consumed more than 25 grams of alcohol daily (72.72%), compared to women who consumed alcohol sporadically (44.44%) or abstained entirely from alcohol (30.00%).

Studies conducted by Kolonoff-Cohen et al. [18] in a group of 217 women who had reproductive problems showed that the consumption of alcohol by women a year before the IVF or GIFT attempt resulted in the reduction in the number of egg cells, the consumption of alcohol a month prior to the attempt of *in vitro* fertilization caused an increase in the number of failures in achieving pregnancy, whereas the consumption of alcohol a week before carrying out the procedure increased the risk of occurrence of abortion after the IVF or GIFT attempt. The studies confirmed that a year before the *in vitro* fertilization attempt the women consumed 7.0g/daily of alcohol, on average, a month before 6.0 g/daily of alcohol, a week before – 7g/daily of alcohol, and a day prior to the procedure – 2 g/daily of alcohol. The average duration of the infertile period in the group of women in the study was 4 years. Based on own studies, it was found that 42.59% of women consumed alcohol, while 57.41% of them abstained from alcohol. In the group of women who consumed alcohol, class A embryos constituted 4.35%, followed by class B embryos – 86.96%, and embryos of class C – 8.69%. A statistically significant relationship was noted between the classes of embryos and consumption of alcohol by the women in the study ($p=0.001$). Significantly more embryos of class B (86.96%) came from women who consumed alcohol, compared to class A (4.35%). In addition, the study showed that for 37.04% of respondents the duration of treatment was up to 1 year, for 38.89% of the women examined the period of treatment was within the range from 2–5 years, and for 24.07% – this period was more than 5 years.

Also, studies conducted on animals indicated abnormalities in the development of embryos subjected to the effect of alcohol, caused abnormalities concerning the cleavage, and the embryos obtained might have led to the development of many contagious defects or the occurrence of spontaneous abortion [19, 20, 21, 22, 23, 24].

Considering literature reports and the results of the presented study, it is important to carry out education among couples at reproductive age concerning the unfavourable effects of the consumption of alcohol on the quality of embryos.

CONCLUSIONS

Consumption of alcohol causes the development of embryos of poorer quality. Significantly more class B embryos came from oocytes of women who consumed alcohol, compared to class A.

An active campaign against alcohol consumption should be carried out among women at reproductive age for the sake of their fertility and future motherhood.



REFERENCES

1. Polskie Towarzystwo Ginekologiczne. Rekomendacje dotyczące diagnostyki i leczenia niepłodności- skrót. Ginekol Pol. 2012; 83: 149–154 (in Polish).
2. Mumtaz Z, Shahid U, Levay A. Understanding the impact of gendered roles on the experiences of infertility amongst men and women in Punjab. *Reprod Health*. 2013; 10: 3.
3. Wdowiak A, Lewicka M, Plewka K, Bakalczuk G. Nicotinic and quality of embryos obtained in in-vitro fertilization programmes. *Ann Agric Environ Med*. 2013; 20(1): 82–85.
4. Kot-Leibovich H, Fainsod A. Ethanol induces embryonic malformations by competing for retinaldehyde dehydrogenase activity during vertebrate gastrulation. *Disease Models & Mechanisms*. 2009; 2: 295–305.
5. Moskalewicz J. Problemy zdrowia prokreacyjnego związane z konsumpcją alkoholu. *Alkohol Narkom*. 2007; 20(1): 55–63 (in Polish).
6. Ornoy A, Ergaz Z. Alcohol abuse in pregnant women: effects on the fetus and newborn, mode of action and maternal treatment. *Int. J. Environ. Res. Public Health*. 2010; 7: 364–379.
7. Żuralska R, Kuzepska M, Mziray M, Postróżny D, Muczyn A, Studzińska B, Książek J. Alkohol i ciąża. Wstępne badanie opinii kobiet na temat spożycia alkoholu w okresie ciąży. *Probl Pielęg*. 2011; 19(4): 533–537 (in Polish).
8. Halder D, Park JH, Choi MR, Chai JC, Lee YS, Mandal C, Jung KH, Chai YG. Chronic ethanol exposure increases goosecoid (GSC) expression in human embryonic carcinoma cell differentiation. *J Appl Toxicol*. 2013: 1–7.
9. Zhou FC, Zhao Q, Liu Y, Goodlett CR, Liang T, McClintick JN, Edenberg HJ, Li L. Alteration at early neurulation. *BMC Genomics*. 2011; 12:124.
10. Wojtyła A, Kapka-Skrzypczak L, Diatczyk J, Fronczak A, Paprzycki P. Alcohol-related Developmental Origin of Adult Health- population studies in Poland among mothers and newborns (2010–2012). *Ann Agric Environ Med*. 2012; 19(3): 365–377.
11. Rokicki T. Dojrzwianie komórek jajowych w warunkach *in vitro*. *Ginekol Dypl*. 2008; 9(4): 23–27 (in Polish).
12. Rokicki T. Zachowanie płodności u kobiet. *Ginekol Dypl*. 2008; 10(3): 33–37 (in Polish).
13. Ouko LA, Shantikumar K, Knezovich J, Haycock P, Schnugh DJ, Ramsay M. Effect of alcohol consumption on CpG methylation in the differentially methylated regions of H19 and IG-DMR in male gametes- implications for fetal alcohol spectrum disorders. *Alcohol Clin Exp Res*. 2009; 33(9): 1615–1627.
14. Sioda T. Wpływ alkoholu na prokreację i wczesny okres macierzyństwa. *Pediatr Pol*. 2009; 84(4): 344–361 (in Polish).
15. Jensen TK, Hjollund NK, Henriksen TB, Scheike T, Kolstad H, Giwercman A i wsp. Does moderate alcohol consumption affect fertility? Follow up study among couples planing first pregnancy. *BMJ* 1998; 3: 505–510.
16. Eggert J, Theobald MB, Cramer DW. Effects of alcohol consumption on female fertility during an 18- year period. *Fertil Steril*. 2004; 81: 379–383.
17. Royal College of Obstetricians and Gynaecologists Statement. Wpływ alkoholu na przebieg ciąży. *Med Prakt Ginekol Położ*. 2007; 3 (in Polish).
18. Klonoff-Cohen H, Lam-Kruglick P, Gonzalez C. Effects of maternal and paternal alcohol consumption on the success rates of in vitro fertilization and gamete intrafallopian transfer. *Fertil Steril*. 2003; 79: 330–339.
19. Aronne MP, Guadagnoli T, Fontanek P, Evrard SG, Brusco A. Effects of prenatal ethanol exposure on rat brain radial glia and neuroblast migration. *Experiment Neurol*. 2011; 229: 364–371.
20. Liu Y, Balaraman Y, Wang G, Nephew KP, Zhou FC. Alcohol exposure alters DNA methylation profiles in mouse embryos at early nerulation. *Epigenetics*. 2009; 4(7): 500–511.
21. Mukhopadhyay P, Rezzoug F, Kaikus J, Greene RM, Pisano MM. Alcohol modulates expression of DNA methyltransferases and methyl CpG-/CpG domain- binding proteins in murine embryonic fibroblasts. *Reproduct Toxicol*. 2013; 1: 1–19.
22. Peng Y, Yang PH, Ng SSM, Wong OG, Liu J, He ML, Kung HF, Lin MCM. A critical role of PAX6 in alcohol-induced fetal microcephaly. *Neurobiol Disease*. 2004; 16: 370–376.
23. Tran TD, Kelly SJ. Critical periods for ethanol-induced cell loss in the hippocampal formation. *Neurotoxicol Teratol*. 2003; 25: 519–528.
24. Yelin R, Ben-Haroush S, Kot H, Sharon Z, Frumkin A, Pillemer G, Fainsod A. Ethanol exposure affects gene expression in the embryonic organizer and reduces retinoic acid levels. *Develop Biol*. 2005; 279: 193–204.

