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ФЕНОТИПИЧЕСКИЕ И ФУНКЦИОНАЛЬНЫЕ СВОЙСТВА ГЕНЕРИРУЕМЫХ ДЕНДРИТНЫХ КЛЕТОК ЧЕЛОВЕКА ПОСЛЕ ОБРАБОТКИ ЦИТОТОКСИЧЕСКИМИ ЛЕКТИНАМИ B. SUBTILIS B-7025

Рассмотрены фенотипические и функциональные свойства генерируемых дендритных клеток человека после обработки цитотоксическими лектинами B. subtilis B-7025.

Ключевые слова: дендритные клетки, цитотоксические лектины, B. subtilis B-*7025, фенотипические и функциональные свойства.*

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THE PHENOTYPIC AND FUNCTIONAL PROPERTIES OF THE GENERATED HUMAN DENDRITIC CELLS AFTER TREATMENT WITH CYTOTOXIC LECTINS B. SUBTILIS B-7025

The article describes the phenotypic and functional properties of human dendritic cells generated after treatment with cytotoxic lectins B. subtilis B-7025.

Keywords: dendritic cells, cytotoxic lectins, B. subtilis B-*7025, phenotypic and functional properties.*

UDC УДК 591.33:57.086.13

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VITRIFICATION OF PRONUCLEAR-STAGE MOUSE EMBRYOS USING DIFFERENT CRYOPROTECTANTS AND THEIR SUBSEQUENT DEVELOPMENT IN VITRO WITH OR WITHOUT OVIDUCT EPITHELIAL CELLS TO THE BLASTOCYST STAGE

The results of experimental researches on vitrification of PN mice embryos on solid surface with use of different cryoprotectants and evaluation their viability and subsequent development in vitro after vitrified-warmed are resulted in this article.

Key words: vitrification, pronuclear stage mouse embryos, oviduct epithelial cells.

Introduction. Cryopreservation of mouse embryos is a cost-effective approach for the maintenance of scientifically important stocks, stains, and line [15]. Vitrification as an ultra rapid cooling technique has been used in cryopreservation of embryo at low temperatures by creating a completely vitreous (glass-like) state [19]. Improvements in ice formation have been made by selecting the most appropriate cryoprotectants (CPAs) and using a mixture of CPAs, macromolecules and nonpermeating agents [14].

Ethylene glycol, a permeating compound, has an important function in stabilizing the cellular membrane during freezing, though it also has some harmful effects on embryo development [18]. In the use of PVP for 2-cell stage mouse embryos, Kim C.G. *[et al.]* [10] reported that addition of PVP macromolecules significantly reduced the incidence of zona and cellular damage, but exposure of embryos to PVP was deleterious to their subsequent development.

Cryopreservation causes morphological and biochemical alterations including damages to membranes and mitochondrial [4], cytoplasm disorganizations [1], and DNA fragmentation [6], which in turn may decrease development and implantation capabilities of embryos. An alternative way of improving the developmental ability of embryos at the pronuclear stage after cryopreservation may be their *in vitro* cultivition to avoid the stresses caused by inappropriate vitrification conditions by the prevention of stresses that arise during vitrification. Several culture systems have been used for improve in vitro embryo development and most of them include somatic cells such as granulosa cells [16],oviductal cells [21], uterine cells, Vero cells [11], as the co-culture support [7]. Based on these observations and our recent finding that co-culture with oviduct epithelial cells enhances the in vitro development of the embryos [8], it seems that improvement in the post warming culture conditions by using oviduct epithelial cells can affect the embryo development and the quality of resulting blastocysts.

The purpose of this study was to evaluate the effects of cryoprotectant treatment of the pronuclear stage on the developmental ability of vitrified mouse zygotes, its developmental ability to the blastocyst stage. We compared various components vitrification solutions to find a more suitable composition for vitrification of PN embryos. Finally, in a series of experiments we investigated the the developmental competence of vitrified-warmed pronuclear-stage mouse zygotes in co-culture system using oviduct epithelial cells.

Materials and methods. Animals and embryo collection Eight to twelve weeks old female C57BL/6 mice were superovulated by an intraperitoneally injection of 5IU PMSG (Sigma) followed 48 h later by 5 IU hCG (Pregnyl, Organon, Netherlands). Females were placed with the same strain males immediately after hCG administration. Females were checked up for mating by the presence of vaginal plugs 16 h later. Approximately 19-20 h post-hCG injection oviducts were excised and pronuclear-stage embryos with the rest of cumulus cells were flushed from oviduct ampoules with M2. Zygotes have been collected and transferred into M2 medium supplemented with a hyaluronidase (80 IU/ml) for 3 min. Embryos have been rinsed three times into few M2 medium droplets and classified as pronuclear–stage embryos (PN embryos),

dead/fragmented zygotes and unfertilized eggs. The PN embryos divided to three groups for vitrification and have been kept in CO_2 incubator at 37^0C in M16 medium drops under mineral oil until time they have been used.

Vitrification and warming of embryo All solutions were prepared using M2 as a basic medium. The solid-surface vitrification (SSV) and warming procedure were modified from the method described by Dinnyes *[et al.]* [5]. The two-step vitrification procedure was used in our experiments. In the first step the PN embryos were equilibrated by 15 min incubation in the holding medium M2 with 4% EG and 10% FBS at 37^0C. After equilibration, groups of 25-30 PN embryos were washed three times in M2+10% FBS for 30 sec each in 20-µl droplets of second vitrification solution consisting of different concentration of cryoprotectant agents: Group 1 of 40% EG and 0.5M sucrose; Group 2 of 35% EG with 5% PVP and 0.5M sucrose and Group 3 of 28% EG with 12% DMSO and 0.5M sucrose. PN embryos were sucked into a glass capillary in groups of 20 to 25 with 2 µl droplets of these vitrification solutions and finally dropped onto the cold and dry surface of a piece of aluminum foil (10 × 15 cm2) floating on the surface of liquid nitrogen. Then the vitrified spherical droplets have been placed by using forceps in 1.8 ml cryotubes and were immersed into liquid nitrogen.

Warming was accomplished by direct transfer the droplets into warming 37^0C solution containing 0.5M sucrose + 10% FBS in M2 for 1 min. Afterward, the PN embryos were warmed in three-step into various solutions of sucrose . They were consecutively transferred into the 500µl droplets of M2 medium consisted of 0.2M sucrose at RT for 1 min, 0.1M sucrose for 1 min and 0.05M sucrose for 1 min and finally collected into M2 and kept during 10 min. Vitrified-warmed PN embryos of three groups were transferred into 4-well dishes separately, filled with 1ml DMEM with antibiotics + 10% FBS per a well. Embryos were cultured overnight at 37^0C in the CO_2 incubator to reach 2-cell stage.

Assessment of embryo survival and development after warming Survival rate of post-warming PN embryos were evaluated by observing morphological appearance, including membrane integrity and blastomeres after warming. Embryos with a normal spherical shape and a visible perivitelline space and without any injures in zona pellucida were considered normal and were selected for further investigation.

Two-cell embryos obtained from frozen-warmed PN embryos in different vitrification solution divided to two groups each as Groups+ suggesting embryo co-culture in DMEM with OEC+10% FBS+1% penicillin/streptomycin solution and Groups- suggesting embryo culture in DMEM+10% FBS+1% penicillin/streptomycin solution without OEC. The viability of embryos has being determined every 24 h without replacement a fresh medium by subsequent development to 4-, 8-cell stage, morula and blastocyst during in vitro culture for 72 h.

Oviduct Epithelial Cells Culture (OEC) performance The oviducts from the rabbit females were used as a source of OEC. After PN embryos were flushed the oviducts transferred into Petri dishes with DMEM and 1% penicillin-streptomycin solution + 2% FBS. The oviduct ampoules were squeezed by eye-forceps to get the clamps of epithelial tissue. Concentrated suspension of epithelial cells was sucked throughout 200 µl pipette tip 10 times and squeezed throughout 27g surgical needle 25 times until almost single cell suspension was reached [13]. The cells have been left in the CO_2- incubator at 37^0C for 30 min to let them put down. The upper layer of the medium has been discarded then and fresh DMEM + 20% FBS (just in this step) added to reach a desirable rate of cell proliferation. The cells have been left in the CO_2-incubator at 37^0C for 3 days. After old condition medium was discarded and fresh DMEM + 10% FBS + 1% antibiotic solution were added again the OEC culture was ready to use as a cell feeder substrate for 2-cell embryos developed from thawed PN embryos. All percentage data of this study were performed using MS Exel.

Results and discussion. Effect of the different vitrification solutions on survival rate of PN mouse embryos. Rapid vitrification procedures need much higher concentrations of cryoprotectants than conventional slow freezing. Different CPs have different toxicity, penetration rate, and transition temperature [17]. The combination of different CPs can result to increase viscosity, transition temperature and reduce the level of toxicity. In the present study we examined various components to find a more suitable composition for vitrification of PN embryos.

One hour after warming, survival of embryos was assessed by observing the intactness of blastomeres and zona pellucida. Embryos with an abnormal appearance, dark or a wrinkled cytoplasm membrane and blebs in the intracellular zone, were considered as damaged during vitrification. As it is shown in Table 1, high survival rate of warmed PN embryos with normal morphology being observed in all groups: 92% (115 out of 125) vitrified with 40% EG in Group SSV 1; 90.8% (218 out of 240) vitrified with 35% EG + 5% PVP in Group SSV 2 and 96.7% (232 out of 240) vitrified with 28% EG + 12% DMSO in Group SSV 3 at droplet vitrification.

Table 1. **Effect of different cryopreservation agents on survival rate and development vitrified-warmed pronuclear stage mouse embryos to the 2-cell stage**

Index /Groups with different vitrification solution	SSV-1 40%EG	SSV-2 35%EG+5% PVP	SSV-3 28%EG+12%DMSO
No. of vitrified PN embryos	125	240	240
No. of warmed and survived zygotes	115	218	232
Survival rate (%)	92.0	90.8	96.7
No. of cleavaged zygotes (2-cell embryos)	46	94	122
Cleavage rate (%)	40.0	43.1	52.6

Developmental stage and morphological quality of vitrified-warmed embryos are the primary parameters affecting their survival rate. About 40% PN embryos of vitrified in the solution with 40% EG were developed to 2-cell stage after warming, what is less than the 2-cell cleavage rate in group vitrified with 35% EG + 5% PVP (43.1%, 94 out of 218) and significantly lower than vitrified in solution with EG partial replaced with DMSO (52.6%, 122 out of 232).

The loss of viability can be influenced by the type and concentration of cryoprotectant, the cryoconservation protocol, developmental stage of the embryo. In our study we frozen PN mouse embryos by drop vitrification, where the vitrification solution contained as cryoprotectant EG and combination EG with PVP or DMSO. Such cryoprotectants as EG, PVP or DMSO were successfully used in many studies on cryopreservation of embryos in

different stage and of different animal species. In particular, our research results are consistent with Levent Keskintepe [9] studies demonstrating that post-warming survival rates and development to blastocyst of pronuclear embryos cryopreserved in the presence of DMSO were higher ($P<0.05$) than those cryopreserved with either PG or EG. Such improved survival was demonstrated, when rabbit oocytes were vitrified in combination with EG and DMSO as cryoprotectants [3]. Liebermann *[et al.]* [12] noted that vitrification of pronuclear stage human embryos with ethylene glycol and DMSO was a good alternative to vitrification with 1,2-propanediol and DMSO.

Further development PN embryos exposed in solutions with different cryopreservation agents and co-cultured with OEC. The post-warming survival rates of embryos vitrified in different cryoprotectants combination from first experiment, were used for testing effect of culture media DMEM and culture system OEC for *in vitro* developments of embryos. As shown in Table, there were some differences in developmental rates at 4-cell and 8-cell stages between both experimental groups at different culture conditions.

The rates of development to 4-cell stage were higher in both SSV 3 group cultured in medium in and without OEC compared to another experimental groups. The survival rate of 4- and 8-cell embryos in the SSV 2 (-) group with 35% EG and 5% PVP were lower than the vitrified-warmed embryos in the SSV 2 (+) group were co-cultured into feeder epithelial cells monolayer. Hypothetically, the supporting effect of the OEC could reduce the possible toxicity of the PVP in SSV 2 and to be found for increased number of the compacted morula and blastocysts for next 24 h.

Table 2. Comparison of in vitro development of vitrified-warmed 2-cell mouse embryos to blastocysts obtained from vitrified-warmed pronuclear stage embryos in different vitrification solution after culture with or without OEC

Groups		No of embryos developed to				
		2-cell (%)	4-cell (%)	8-cell (%)	Morula (%)	Blastocysts (%)
Groups ($^{+}$) co-culture with OEC monolayer in culture media	SSV1-40% EG	23	19-82.61%	14-60.87%	13-56.52%	12-52.17%
	SSV2-35%+5%PVP	47	40-85.11%	31-65.96%	24-51.06%	24-51.06%
	SSV3-28%EG+12% DMSO	61	52-85.25%	43-70.49%	40-65.57%	40-65.57%
Groups ($^{-}$) Culture media only	SSV1-40% EG	23	20-86.96%	16-69.57%	13-56.52%	12-52.17%
	SSV2-35%+5%PVP	47	39-82.98%	30-63.83%	24-51.06%	21-44.68%
	SSV3-28%EG+12% DMSO	61	54-88.52%	42-68.85%	39-63.93%	36-59.02%

Post-warming development to the morulae stages of embryos vitrified in solution contenting EG and DMSO were higher (i.e. 65.57% into SSV 3(+) and 63.93% into SSV3(-) than embryos frozen in solution with 40% EG (56.52%) and 35% EG with 5% PVP (51.02%), respectively. The developing rate to morulae stage between SSV 1 and SSV 2 groups in both culture systems was similar.

When compared the rate of the development to blastocyst stage were observed some differences between SSV 3 (+) and SSV 3(-) groups (i. e. 65.57% vs 59.02%) and between SSV 2 (+) and SSV 3(-) groups (i. e. 51.06% vs 44.68%). More detailed analyses showed that division rate and ability to create a blastocyst cavity from previous morula stage in SSV 3 group was better when embryos were cultured in presence of OEC (from 65.57 to 65.57%) than without OEC (from 63.93 to 59.02%). At the end of cultivation all morula were developed to the blastocysts stage in culture system containing OEC monolayer.

These observations may be related to a beneficial effect of oviduct cell on the developmental frozen-warmed embryos. The feeder monolayers cells may have more effective biophysical and biochemical properties, which assisted the embryo to continue its development. The features of the oviduct epithelial cells like their changing protein profile, a high proliferative speed, secreting oviduct-specific glycoprotein [2], specific growth factors [20] suggests that monolayer of mammalian oviduct epithelial cells possesses properties that used in co-culture experiments to improve embryonic development of in vitro.

Conclusions. Our present results show that droplet vitrification of pronuclear-stage mouse embryos with using the combination of different CP allow a moderate decrease in CP concentration so as to minimize its toxic and osmotic hazardous effects and it can be successfully cryopreserved for PN mouse embryos. This study shows that PVP, as a macromolecule, could be used for reducing toxicity of an EG-based vitrification solution, but combination of EG and 15% DMSO in a vitrification protocol is advantageous to the the survival and development of PN mouse embryos.

Co-culture with oviduct epithelial cells combined with simple culture media DMEM enhances the in vitro development of the vitrified-warmed mouse embryo. Additionally, embryos generated from our culture system appeared to produce better quality blastocysts.

This study was supported by an NCI Fellowship (project N PO 4A 040 30 "Mechanism of Hsf1 induced apoptosis").

References

1. Ahn H. Characteristics of the cell membrane fluidity, actin fibers, and mitochondrial dysfunctions of frozen-thawed two-cell mouse embryos / H. Ahn, I. Sohn, H. Kwon [et al.] // Molecular Reproduction and Development. – 2002. – Vol. 61 (4). – P. 466–476. Available from: http://onlinelibrary.wiley.com

2. Buhi W. S. Characterization and biological roles of oviduct-specific, oestrogen-depended glycoprotein / W. S. Buhi // Reproduction. – 2002. – Vol. 123. – P. 355–362. Available from: http://www.biochemj.org

3. Cryoloop vitrification of rabbit oocytes / X. Y. Cai, G. A. Chen, Y. Lian, X.Y. Zheng, H.M. Peng // Human Reproduction. – 2005. – Vol. 20(7). – P. 1969–1974. Available from: www.ncbi.nlm.nih.gov/pubmed

4. Characterization of cellular damage in female gametes and embryos after cryopreservation / A. A. Carvalho, L. R. Faustino, S. V. Castro [et al.] // Acta Scientiae Veterinariae. – 2012. – Vol. 40(3). – P. 1046. Available from: www.ufrgs.br/actavet

5. Solid surface vitrification (SSV): an efficient method for oocyte and embryo cryopreservation in cattle, pig and mouse / A. Dinneyes, X. Z. Yang, X. L. Li [et al.] // Cryobiology. – 2001. – Vol. 43. – P. 332–338.

6. Ultrastructure and cell death of in vivo derived and vitrified porcine blastocysts / D. Fabian, J.O. Gjorret, F. Berthelot [et al.] // Molecular Reproduction and Development. – 2005. – Vol. 70 (2). – P. 155–165. Available from: http://onlinelibrary.wiley.com/doi/10.1002/mrd.20129

7. Farha N. Optimal in vitro Culture Conditions for Sustainable Development of Preimplantation Mouse Embryos during Prolonged Culture / N. Farha, V. Marcela // Health and the Environment Journal. – 2010. – Vol. 1 (2). – P. 3–11. Available from: www.hej.kk.usm.my

8. Вплив фідерних клітин яйцепроводів на дозрівання ембріонів овець до трансферабельних стадій / І. І. Гевкан, О. В. Штапенко, А. В. Мадіч [та ін.] // Науково-технічний бюлетень ІТ УААН. – 2005. – № 90. – С. 92–97.

9. Use of cryopreserved pronuclear embryos for the production of transgenic mice / L. Keskintepe, Y. Agca, G. A. Pacholczyk, A. Machnicka, J. K. Critser // Biology of Reproduction. – 2001. – Vol. 65(2). – P. 407–411. Available from: http://www.biolreprod.org/content/65/2/407

10. Effect of the polyvinylpyrrolidone concentration of cryoprotectant on mouse embryo development and production of pups: 7.5 % of PVP is beneficial for in vitro and in vivo development of frozen-thawed mouse embryos / C.G. Kim, H. Yong, G. Lee, J. Cho // Journal of Reproduction and Development. – 2008. Vol. 54(4). – P. 250–253. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18490859

11. Vero cells, but not oviductal cells, increase the hatching frequency and total cell count of mouse blastocyst partly by changing energy substrate concentrations in culture medium / Y. L. Lee, J. S. Xu, S.T. Chan, P.C. Ho, W. S. Yeung // Journal of Assisted Reproduction and

Genetics. – 2001. – Vol. 18(10). – P. 566–574. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11699129

12. Liebermann J. Vitrification of human blastocysts: an update / J. Liebermann, F. Nawroth, V. Isachenko // Reproductive Biomedicine Online. – 2001. – Vol. 19 (4). – P. 105–114. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20034409

13. Клітинні культури і можливості їх використання в ембріональній біотехнології : навч.-метод. посіб. / А. В. Мадіч, В. І. Шеремета, І. І. Гевкан [та ін.]. – К. : АртЕконом, 2012. – 144 с.

14.Massip A. Cryopreservation of embryos of farm animals / A. Massip // Reproduction in Domestic Animals. – 2001. – Vol. 36(2). – P. 49–55. Available from: http://onlinelibrary.wiley.com/doi/10.1046/j.1439-0531.2001.00248

13. Mazur P. Cryopreservation of the germplasm of animals used in biological and medical research: importance, impact, status, and future directions / P. Mazur, S.P. Leibo, G.E. Seidel // Biology of Reproduction. – 2008. – Vol. 78(1). – P. 2–12. Available from: http://www.biolreprod.org/content/78/1/2.full

14. Ultrastructural characteristics of human granulosa cells in a coculture system for in vitro fertilization / S. A. Nottola, R. Heyn, A. Camboni, S. Correr, G. Macchiarelli // Microscopy Research and Technique. – 2006. – Vol. 69(6). – P. 508–516. Available from: http://onlinelibrary.wiley.com/doi/10.1002/jemt.20309

15. Do physical forces contribute to cryodamage? / J. Saragusty, H. Gacitua, I. Rozenboim, A. Arav // Biotechnology and Bioengineerinh. – 2009. – Vol. 104(4). – P. 719–728. Available from: http://onlinelibrary.wiley.com/doi/10.1002/bit.22435

16. Sommerfeld V. Cryopreservation of bovine in vitro produced embryos using ethylene glycol in controlled freezing or vitrification / V. Sommerfeld, H. Niemann // Cryobiology, – 1999. – Vol. 38 (2). – P. 95–105. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10191033

17. Vajta G. Improving cryopreservation systems / G. Vajta, M. Kuwayama // Theriogenology. – 2006. – Vol. 65. – P. 236–244. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16289262

18. Temporal effect of human oviductal cell and its derived embryotrophic factors on mouse embryo development / J.S. Xu, T. M. Cheung, S. T. Chan, P. C. Ho, W. S. Yeung // Biology of Reproduction. – 2001. – Vol. 65(5). – P. 1481–1488. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11673265

19. Yeung W. S. B. The oviduct and development of the preimplantation embryo / W. S. B. Yeung, C. K. F. Lee, J. S. Xu // Reproductive Medicine Review. – 2002. – Vol. 10(1). – P. 21–44. Available from: http://dx.doi.org/10.1017/S0962279902000121

Received to editorial board 12.05.15

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ВІТРИФІКАЦІЯ ЕМБРІОНІВ МИШЕЙ НА СТАДІЇ ПРОНУКЛЕУСІВ ПРИ ВИКОРИСТАННІ РІЗНИХ КРІОПРОТЕКТОРІВ ТА ЇХ ЗДАТНІСТЬ ДО ПОДАЛЬШОГО РОЗВИТКУ НА КУЛЬТУРІ КЛІТИН ЯЙЦЕПРОВОДІВ ДО БЛАСТОЦИСТИ

Наведено результати досліджень з кріоконсервування ембріонів мишей на стадії пронуклеусів методом вітрифікації на твердій поверхні із використанням різних кріопротекторів та оцінено їх життєздатність і подальший розвиток in vitro після деконсервування.

Ключові слова: вітрифікація, ембріонів мишей на стадії пронуклеусів, клітини яйцепроводів

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ВИТРИФИКАЦИЯ ЭМБРИОНОВ МЫШЕЙ НА СТАДИИ ПРОНУКЛЕУСОВ ПРИ ИСПОЛЬЗОВАНИИ РАЗЛИЧНЫХ КРИОПРОТЕКТОРОВ И ИХ СПОСОБНОСТЬ К ДАЛЬНЕЙШЕГО РАЗВИТИЯ НА КУЛЬТУРЕ КЛЕТОК ЯЙЦЕПРОВОДОВ К БЛАСТОЦИСТЫ

Приведены результаты исследований по криоконсервировании эмбрионов мышей на стадии пронуклеусов методом витрификации на твердой поверхности с использованием различных криопротекторов и оценена их жизнеспособность и дальнейшее развитие in vitro после деконсервирования.

Ключевые слова: витрификация, эмбрионов мышей на стадии пронуклеусов, клетки яйцепроводов.

УДК 615.9:616.36-099:576.2.24:577.161.3

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DISORDERS OF ANTENATAL AND POSTNATAL DEVELOPMENT IN LONG-TERM ALCOHOL EXPOSED MALE WISTAR RATS' OFFSPRING

It was established increase of CYP2E1 mRNA expression in rats' testes under chronic 15% ethanol solution administration. In addition, study results suggest significant alteration of locomotor and emotional reactivity in offspring of chronically alcohol-treated males as compared with control. Decrease of horizontal, vertical and exploration activities were recorded. Thus, a direct relationship between paternal alcohol exposure and offspring health and behaviour exists.

Keywords: CYP2E1 mRNA expression, rats' testes, ethanol.

It is well-known that chronic alcohol dependence causes testicular abnormalities and sexual dysfunctions both in humans and animals [1, 2]. Studies that included alcohol as a subject of investigation point to alcohol negative effect on all three factors that influence the male reproductive function: hypothalamus-hypophysis-gonads system, endocrine glands, and hormones [3]. The less attention has the paternal effects of alcohol-addicted male on the posterity. Relatively early epidemiological studies indicate an association between lowered birth weight in offspring and paternal alcoholism [4]. Adolescent sons of alcoholics and non-alcoholics were compared on a battery of intellectual, neuropsychological, personality, and behavioral measures. The former group demonstrated certain neuropsychological deficits in perceptual-motor ability, memory, and language processing [5]. Moreover, adoption studies suggest positive associations between hyperactivity and lowered cognitive abilities in offspring and alcoholism in the biological father but not the adoptive father [6].

It is evident that the human data on preconceptional alcohol consumption paternal effects are limited. And, what about the rodents' data, they are insufficient and contradictory due to great diversity of animal species/strains and models of alcoholism [7]. Due to these facts findings from studies in which males consume alcohol chronically are difficult to interpret, and they are potentially confounded. Moreover, multiple causal mechanisms for a paternal effect (including mutagenicity and epigenetic changes) have been suggested, but none seems satisfactory to explain all findings [7].