NUMBER OF IMPLANTED EMBRYOS AFTER PRE-CONCEPTIONAL CHRONIC SOCIAL CONSUMPTION OF ALCOHOL IN FEMALE RATS

NÚMERO DE EMBRIÕES IMPLANTADOS APÓS CONSUMO SOCIAL CRÔNICO DE ÁLCOOL NO PERÍODO PRÉ CONCEPTIVO EM RATAS

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Abstract

Alcoholism is an abusive ingestion or exposure to alcoholic beverages creating habit and causing mental and body injury. Alcohol is considered the most commonly used drug in the world, regardless of this usage be frequent or sporadic ("social usage"). Among the diseases associated with the use of alcohol is infertility. The objective of this work was to evaluate possible effects of consumption of alcohol on the embryonic implantation in rats. 12 Wistar rats with a mean age of 110 days received, twice per week, water-alcohol solution 20%, during 60 days. Completed the period, females were randomly separated into groups of three and placed in the presence of a male, for mating. On the 10th day after the detection of cervical cap, these animals were sacrificed and necropsied to withdrawal of the uterus and count of fixed embryos. The same procedure was performed with control animals. The experimental protocols performed are in accordance with the established by the Brazilian College of Animal Experimentation-COBEA and were approved by the Ethics in Animal Experimentation of FUNVIC (EAEC Protocol 023/2012). In general, there was a decrease in the mean number of embryos implanted in female consumers of alcohol when compared to females. This decrease was significant ($p = 0.0117$; test not paired) when comparing the treated and control groups. The results corroborate findings in literature and reaffirm the detrimental effect on the fertility of females, and this effect is related to a commitment in the recruitment, follicular or ovulation rate or even about the conditions for uterine bailout of deploying embryonic.

Keywords: Alcoholism. Embryonic implantation. Fertility.

Resumo

O alcoolismo consiste numa ingestão abusiva ou prolongada de bebidas alcoólicas, a ponto de criar hábito, dependência ou vício causando transtornos mentais e corporais. O álcool é considerado a droga mais utilizada no mundo, independentemente de esse uso ser frequente ou esporádico (“uso social”). Dentre as doenças associadas ao uso do álcool está a infertilidade. Este trabalho teve como objetivo avaliar possíveis efeitos do consumo social de álcool sobre a implantação embrionária em ratas. Doze ratas Wistar com idade média de 110 dias receberam, duas vezes por semana, solução hidroalcoólica 20% à vontade, durante 60 dias. Completado o período, foram separadas aleatoriamente em grupos de três e colocadas na presença de um macho, para acasalamento. No décimo dia após a detecção do tampão cervical, esses animais foram sacrificados e necropsiados para retirada do útero e contagem dos embriões fixados. O mesmo procedimento foi realizado com animais controle. Os protocolos experimentais realizados estão em concordância com o estabelecido pelo Colégio Brasileiro de Experimentação Animal-COBEA e foram aprovados pelo Comitê de Ética em Experimentação Animal da FUNVIC (Protocolo CEEA 023/2012). De maneira geral houve diminuição do número médio de embriões implantados nas fêmeas consumidoras sociais de álcool quando comparadas às fêmeas-controle. Essa diminuição foi significativa ($p = 0,0117$; teste não pareado) quando comparados os grupos tratado e controle. Os resultados corroboram achados de literatura e reafirmam o efeito prejudicial sobre a fertilidade de fêmeas, podendo esse efeito estar relacionado a um comprometimento no recrutamento folicular ou na taxa de ovulação ou mesmo sobre as condições uterinas para viabilização da implantação embrionária.

Palvras-chave: Alcoolismo. Implantação embrionária. Fertilidade.

Introduction

Alcohol is a psychoactive substance with dependence-producing properties that has been widely used in many cultures for centuries. The harmful use of alcohol causes a large disease, social and economic burden in societies.[1] In 2012, about 3.3 million deaths, or 5.9% of all global deaths, were attributable to alcohol consumption.[1]

It is known that there are significant sex differences in the proportion of global deaths attributable to alcohol, for example, in 2012, 7.6% of deaths among males and 4.0% of deaths among females were attributable to alcohol.[2]

Much has been written about the correlation of male alcoholism and fertility impairment and little is known about this issue in relation to women.

The effects of drinking are more detrimental for females than for male.[3] The metabolization of alcohol and other psychoactive substances occurs more slowly in women than in men, and they become more susceptible to damage associated with consumption ingesting even lower levels of alcohol for a further short period.[4]

Women with chronic problems of alcohol intake, who continue to drink without moderation during pregnancy may show a high rate of fetal abnormalities such as slurred speech and incoordination, mental performance and motor and sensory discrimination equally affected.[5]

Alcohol consumption during pregnancy can also promote the development of fetal malformations, low weight and psychomotor retardation, special characteristics of an imperfect training, as well as tremors and malnutrition, because alcohol acts in embryogenesis and metabolism of the fetus causing disorders in the CNS, growth retardation, facial changes and associated malformations.[5,6]

Embryonic development begins at fertilization, when the male and female pronuclei unite to form the zigoto.[7] After fertilization, the first mitotic divisions give rise to the morula, with approximately 16 blastomeres. This process occurs within the first four to five days in rats.[7,8]

Soon after comes a cavity between the blastomeres, the blastocele, and cell mass differentiates into embrioblasto, which give rise to the embryo and some extra-embryonic tissues, and the trophoblast, thin layer of outer cells to embrioblasto and give rise the part of the placenta. These changes take place soon after the arrival of the blastocyst to the uterus.[8]

With the advent of blastocyst to the uterus, the zona pellucida and trophoblast disappears adjacent embryoblast adheres to the epithelium of the endometrium, leading to the implantation process. The trophoblast differentiates into cytotrophoblast and syncytiotrophoblast. The syncytiotrophoblast starts a process of "digging" on the endometrium allowing the embryo to lodge further.[9]

The process of implantation of blastocyst involves maternal endogenous hormone regulation, held during the progesterone phase, but it depends on many other local factors, PEinduzidos the pre-embryo itself that somehow not well established, it seems to be able to regulate both the transport as its implementation in útero.[9] In the rat, the attachment of the embryo occurs on the fifth day.

However, questions concerning the dose and duration of ethanol exposure required to cause alterations in fertility remain unresolved, though experiments on laboratory animals can be helpful to elucidate this.

The aim of this study was to evaluate the effects of the alcohol intake twice a week in pre-conception period on the number of implanted embryos in Wistar females.

Methods

Animals

Mature Wistar female rats from our own colony were maintained under automatically controlled temperature (24 to 25°C) and a 12 h light/12 h dark cycle. Groups of six animals each were kept in plastic cages, and were fed *ad libitum* with a commercial rat chow diet (Nuvilab®, CR-1 complete food, from Parana, Brazil). The daily amount of food consumed by the animals was determined by the daily offered chow less the amount remaining food. Females were around 110 days old (average body weight 210 g) at the start of the alcohol intake.

Ethanol treatment

Twelve mature female Wistar rats were treated twice a week with 20% (w/v) ethanol in drinking water. Animals were maintained on treatment for 60 days. Controls (n=6) received drinking water. The body weights were recorded weekly throughout the treatment. The amount of daily liquid intake was determined by volume differences between the offered and remaining volume.

Mating and implantation analyze

Immediately after ethanol treatment period, treated and control females were caged with adult

male (3♀:1♂), isolated previously. Mating was confirmed by the presence of vaginal plug. Ten days after plug detection the females are killed by deeply anesthesia. The uterus was removed and washed in phosphate buffered saline (PBS) and the implanted embryos are counted and photographed.

Statistics

Analysis were expressed as mean and standard deviation of implanted embryos and weight of the ovaries. The no paired *t* test ($p<0.05$) was used to determine significant differences between alcoholic and control groups.

Results

Analysis revealed lower number of implanted embryos in right uterine horn ($p=0.0031$), left uterine horn ($p=0.0383$), and both sides ($p=0.0117$), compared to the control group (Table 1, Figure 1).

There were no significant differences ($p>0.05$) in weight in the ovaries between alcoholic and control groups on all sites, in right ovaries ($p=0.1146$), left ovaries ($p=0.3633$), and both sides ($p=0.1880$) (Table 2).

Table 1- Mean values of implanted embryos in alcoholic and control rats

	number of implanted embryos		
groups	**right uterine horn**	**left uterine horn**	**Both sites**
ethanol	3.50	3.67	7.17
control	6.58	6.00	12.58
p*	0.0031	0.0383	0.0117

Table 2- Mean values of weight of the ovaries from alcoholic and control rats

	right ovary	**left ovary**	**both**
ethanol	0.05	0.05	0.11
control	0.06	0.06	0.12
p*	0.1146	0.3633	0.1880

* no paired *t* test (p<0,05) in columns

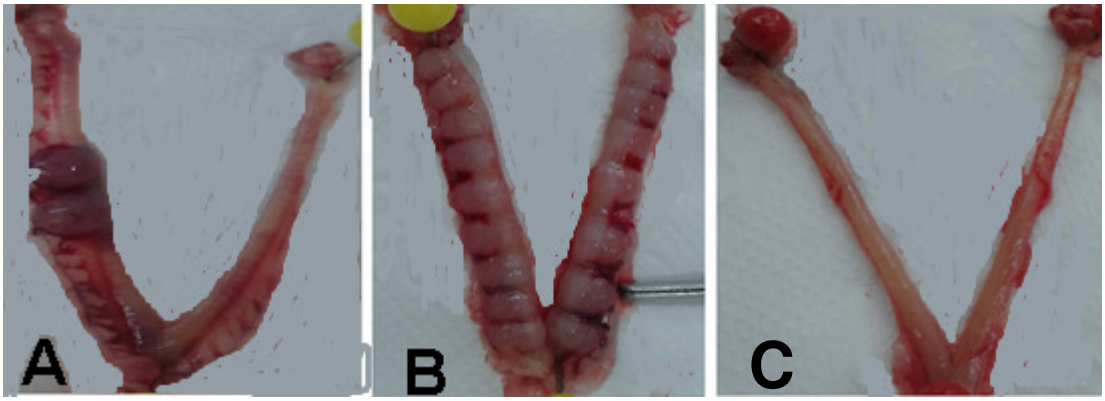

Figure 1- Uteri of rats intoxicated with alcohol solution 20 % twice a week for 60 days. In **A** we observe two embryos implanted in the left uterine horn and none in the right; in **B** nine implanted embryos are observed in the left horn and eight in the right; in **C** implanted embryos are not observed in any of the two uterine horns.

Discussion

In this experiment, the females treated with alcohol had final body weight similar to the control group females. A similar situation was reported by Martinez et al.,[10] Laura et al.[11] and Chuffa et al.[12] in strains of rats voluntary consumer of ethanol, while Emanuele et al.[13] have reported a trend of reducing body weight alcohol. Although there are reports that alcohol administration promotes reduction in ovarian weight,[14, 15] In this study alcohol intake twice a week for 60 days did not affect gonadal relative weight of rats, corroborating the study by Chuffa et al.[12] with strains of spontaneous alcohol-consuming rats.

Gavalier et al.,[14] Hadi et al.,[15] and Cebral et al.[16] reported the absence of corpora lutea in ovaries of females treated with alcohol. Camargo et al.[17] reported a significant reduction in the number of corpora lutea in the ovaries and impaired folliculogenesis in rats treated with alcohol. These results suggest that a similar effect may have occurred with some of the treated rats in this experiment what could explain the absence of deployments and / or reduced number of implanted embryos.

Furthermore, Emanuelle et al.[13] noted that the acute or chronic treatment with alcohol reduces the levels of growth factor similar to insulin (IGF-1) in the bloodstream, thus reducing the neuroendocrine stimulus occurs and consequently rupture of reproductive cyclicity.[13] In two of the rats that did not observe any deployment was not found cervical cap, although they have remained with the male until the day before necropsy. This suggests acyclicity, despite not having been carried out monitoring of the estrous cycle of animals. Grinfeld et al.[18] noted that alcohol consumption before and during pregnancy can increase the embryonic resorption.

Studies on the toxicity of ethanol in women have not been conclusives.[18] There are reports that alcoholism is associated with changes in hormone levels, promoting irregularities in the menstrual cycle, ovulation suppression, reduced fertility and miscarriages.[13,.20]

The increase in alcohol consumption by women of childbearing age associated with the lack of epidemiological data of alcohol consumption by pregnant in Brazil demonstrates the need for such research to assess the extent of the problem. As the alcohol safe dose for each patient was not set, it is recommended to complete abstinence during pregnancy.[17,21]

The literature is extensive as the negative effects and the damage they are subject to the descendants of spontaneous or experimental mothers consuming alcohol, either with high or low frequency, and moderate or high levels. However, it found no experiment in which consumption was stopped on the likely mating, making it difficult to discuss the results.

Conclusion

Alcohol intake, even twice a week, has negative effects on female rat reproduction, negatively interfering with embryo implantation, reducing significantly the number of implanted embryo.

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