The Effects of the Presence of the Hydrochlorofluorocarbon (HCFC)-123 Containing Fire Extinguisher in Laboratories upon Sperm Motility and Embryo Development

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Abstract

This study was conducted to investigate *in vitro* effects of HCFC-123 on sperms and embryos due to the possibility of gas leakage from fire extinguishers and *in vivo* studies reporting prenatal toxicity and implantation failure with some compounds of halogenated hydrocarbon family. This study was designed in two study groups; first investigating embryo toxicity in rat embryos and the second, studying possible spermatotoxic effects on human sperm with sperm survival test. The study was conducted in two experiment environments. One of the systems has the presence of HCFC-123 containing fire extinguisher and the second system is without fire extinguisher. As results, fertilization rates were similar in HCFC-123 containing fire extinguisher group and HCFC-123 free group (57%, 60% respectively). Cleavage rate was 45% in HCFC-123 containing fire extinguisher group and found as 50% in HCFC-123 free group ($p>0.05$). HCFC-123 containing fire extinguisher did not have harmful affects upon sperm motility. In conclusion, HCFC-123 containing fire extinguishers can be used safely in IVF laboratories.

Key words: embryo toxicity, fire extinguishers, halogenated hydrocarbons, HCFC-123, in vitro fertilization

INTRODUCTION

Hydrochlorofluorocarbons (HCFCs) are derived for the replacement of halon in gas discharge type. Halon was the propellant used in fire extinguishers in 70s and 80s, and because of its known detrimental effects upon the ozone layer, halon is being replaced by other halogenated hydrocarbons and chlorofluorocarbon alternatives. Halon is still being used in some applications particularly in aviations for onboard engine fires. In most other applications, however, halon is being phased out and replaced with less ozone sensitive compounds such as HCFC-123 [1, 2].

Halogenated hydrocarbons (HHCs) are extensively used in laboratories and house life beside industrial field. It was reported that neuroepithelial cell death occurred in developing neural tube of all embryos which were exposed 16 hours and more to embryo toxic levels of these solutions [3]. Moreover, previously reported that they have potent prenatal toxic effects in a mouse study. The results showed that living fetus number significantly decreased in dose dependent manner. Significant intrauterine growth retardation was detected in living fetuses until 18th day of gestation and cleft palate anomaly rate was increased in dose dependent manner [4]. The studies with HHCs in the aspect of reproductive toxicity showed that HHCs did not affect reproductive performance and they were not gonadotoxic [5, 6]. For instance, trifluoroiodomethane (CF(3)I), a halon alternative, did not cause any toxic effect until 2 % density. But with this amount of compound birth weight of rat fetuses decreased although this difference was not statistically significant [6]. Beside decreased fetal weight, there is also a study reporting decreased implantation rate in HHC environment [7]. So it was encountered from the literature review that there are few animal and human studies investigating the potential harmful effects of HHCs and these indicate that HHCs, for example 1,1-Dichloro-2,2,2-Trifluoroethane (HCFC-123) generally showed no severe toxicity including reproductive system [8]. Although HCFC-123 is highly volatile; any small amounts of HCFC-123 discharged from a leaky cylinder would rise to the upper regions of the in vitro fertilization (IVF) Lab, HCFC-123 containing fire extinguisher would not pose toxicity problems to IVF Lab's staffs. However, some aerosols does not have toxic effects on human or fetus, they may cause toxicity to the pre-implanted embryos and gametes in IVF Labs. Therefore, it is significant to consider if there are any toxic effects of the presence of the HCFC-123 containing fire extinguisher in IVF Lab on embryos and gametes. Current research shows that indoor air quality can present the high potential risk in IVF laboratory and improves embryo development and IVF results. For this reason, chemical air contaminant's impacts micro-environments of IVF laboratories are very important for embryologist.

It can be supposed that HHCs can negatively affect the environmental factors from the point of the results of aforementioned studies. If a developing embryo is affected from a harmful environment; although it may seem to be a good quality embryo morphologically, these toxic conditions, changed environmental content and temperature can impair its quality at molecular level and impede implantation. It is difficult to ascertain a sole criterion to detect the possible toxicity caused by HHC gas. But it is expected that these harmful environmental changes would affect fertilization of oocyte and cleavage of fertilized oocytes. For this reason it is a

necessity to control possible toxic effects of chemical materials and especially volatile gases in IVF laboratories, where a non-toxic environment is crucial.

Our laboratory has one way entrance/exit pathway and because of safety measures few number of HCFC based HCFC-123 containing fire extinguishers were placed at easily reachable parts. Due to the loss of their interior pressure, they are checked for every 6 months and their pressures are adjusted. This loss of interior pressure is thought to be due to gas leakage. Experimental animal studies researched the exposure of severe aerosol impairs on embryo development, but the effects of the presence of the HCFC-123 containing fire extinguishers in humans IVF laboratories haven't been investigated. For this reason, in this study the effects of the presence of the HCFC-123 containing fire extinguisher on sperm motility and embryo development were investigated in an experimental environment.

MATERIALS AND METHODS

Since different aim, our methods are not like ordinary as aerosol concentrations, etc. to analytical methodology, which are obligatory for habitual *in vivo* toxicity studies. We were examined that the effects of presence of the HCFC-123 containing fire extinguisher in the *in vitro* environment. The study was designed in two separate study groups; first investigating embryo toxicity in rat embryos and the second studying possible spermatotoxic effects on human semen samples with sperm survival test.

Experiment environments

The study was conducted in two experiment environments. One of the systems is HCFC-123 containing fire extinguisher and the second system is without fire extinguisher.

The HCFC-123 containing experiment environment includes 4 different components.

1. Closed system: It is isolated cartoon box in 70x70x70 cm dimensions including HCFC-123 containing portable fire extinguisher and modular mini incubator.
2. HCFC-123 containing portable fire extinguisher placed inside the closed system.
3. Modular mini incubator (Modular Incubator Chamber, 21-MIC101): Oocytes and developing embryos were preserved in this mini incubator throughout the experiment and its atmosphere was supplied by gases from mixture tube.
4. Mixture tube: It is located outside the closed system and connected to mini incubator via a pipe system. It supplies constant gas content including 6 % CO_2, 5 % O_2 and 89 % N_2.

The temperature inside the closed system was fixed to 37 °C and this was kept with time and temperature adjusted radiators inside the room. Temperature checks were done with digital thermometer.

Study group I:

The experiment was carried out on sexually mature 3.5 months-old 4 female Wistar albino rats and one male Wistar albino rat weighing 180-200 grams in HCFC-123 exposure group. High dose (50 IU) recombinant FSH (Puregon®, Organon) injected intraperitoneally to all female rats to achieve controlled ovarian hyper stimulation and 48 hours after that 50 IU hCG (Pregnyl®, Organon) applied intraperitoneally for super ovulation. Female rats were sacrificed by using cervical dislocation method, 17-21 hours after hCG injection. Fallopian tubes of rats were taken into gamete culture medium, MOPS (G-MOPS®, Vitrolife AB, Göteborg, Sweden); thinned and widened ampullary regions were cut with 26 gauge injector needle under dissection microscope. Retrieved oocytes cumulus corona complexes (OCCC) were washed out with MOPS and left in 700 μl of early embryo development medium (G-1:3®, Vitrolife AB, Göteborg, Sweden) that was equilibrated overnight in presence of the HCFC-123 containing fire extinguisher environment. The caudal epididymes of one male rat were cut with 26 gauge injector needle inside MOPS medium under dissection microscope. In vitro early embryo development medium was added and centrifuged at 300 g. Deep pellet was taken and it was waited in incubator for swim-up for about 1.5 hours until the time of OCCC insemination. For insemination of MII mature oocytes 7 μl of epididymal sperm added for 700 μl medium and left for fertilization inside the presence of the HCFC-123 containing fire extinguisher environment.

In control group of study I, seven female and 2 male Wistar albino rats were used. The same protocol but in HCFC-123 containing fire extinguisher free environment by using an incubator (Nuve® Jouan EC150) containing 6 % CO_2 in air at 37 °C was applied for these rats.

Oocytes cumulus distribution and fertilization control was done 17-18 hours after insemination. Fertilization ratio was calculated by dividing the number of oocytes with two pronuclei (2PN) and two polar bodies (2PB) to the total number of MII oocytes. The fertilized oocytes were taken into early embryo development medium equilibrated one day before. Cleavage control was done 44 hours after insemination under inverted microscope. Embryos with at least two blastomeres were evaluated as cleavage positive. Embryos with more than five blastomeres were taken into late embryo development medium (G-2:3®, Vitrolife AB, Göteborg, Sweden). Embryos were followed until 68-72 hours after insemination. Cleavage rate was calculated as number of embryos with at least two blastomeres and continuing cleavage/total fertilized oocyte number. The last evaluation of embryos continuing their cleavage and increasing their blastomere number was done and the experiment was ended.

Study Group II:

The experiment was carried out on 4 random semen samples taken from the rest of samples for routine semen analysis of infertile males applied to our IVF center, for each group. The amount of progressive motile sperms in first day semen sample was accepted as (+++++). The same analysis was repeated two times with 24 hours interval and evaluated according to basal value. Ones preserving motility >3/4 but less than total were assigned as (++++), ones with >2/4 but <3/4 as (+++), ones with >1/4 but <2/4 as (++), ones with <1/4 as (+) and sample without motile sperm were assigned as (-). The samples were kept at room temperature (24±2°C) inside the isolated closed system including portable HCFC-123 containing fire extinguisher in

one group and in normal laboratory environment in HCFC-123 containing fire extinguisher free group.

Statistical analysis

Statistical analysis was performed using Statistically Package Programmer for Social Sciences (SPSS® 10.0, Chicago, IL, USA). To assess the differences between groups nonparametric Chi-square test was used. The p value was set as higher than 0.05., which means there is no significance.

RESULTS

Our data and results are summarized in Tables 1 and 2. We determined that:

In study group I, when oocytes were grouped according to the presence of fertilization and cleavage, there were no significant differences between the group which includes "the presence of HCFC-123 containing fire extinguisher (HCFC-123 group)" and "HCFC-123 containing fire extinguisher free group (HCFC-123 free group)" as a control.

35 MII mature oocytes were retrieved from 4 female rats in the presence of the HCFC-123 containing fire extinguisher environment group and 28 MII mature oocytes were obtained from 7 female rats in HCFC-123 free environment group. Number of fertilized oocytes was 20/35 and the fertilization rate was 57 % in the presence of the HCFC-123 containing fire extinguisher environment group. In HCFC-123 free group, fertilized oocyte number was 17/28 and the fertilization rate was 60 %, namely the rate was similar with the presence of the HCFC-123 containing fire extinguisher environment group ($p>0.05$). These data were presented in Table 1. Nine embryos were developed in both presence of the HCFC-123 containing fire extinguisher environment group and HCFC-123 free group. Thus as shown in Table 1, the cleavage rate was 45 % in the presence of the HCFC-123 containing fire extinguisher environment group and it was 50 % in HCFC-123 free group. This little difference has no statistical significance ($p>0.05$).

In study group II, the findings of sperm survival test were presented at Table 2. It was found that the presence of the HCFC-123 containing fire extinguisher environment does not create a harmful effect on the sperm survival.

Furthermore, we observed that the sperm motility preservation were indicating better rates at 48 hour control in the presence of the HCFC-123 containing fire extinguisher environment group, than HCFC-123 free group.

DISCUSSION

Halogenated hydrocarbons are halon alternatives in which hydrogen atom of hydrocarbons is substituted with a member of halogen family (like chlorine, flour, brome, iodine). The chlorofluorocarbons and fluorinated organic compounds has relatively less researched than the other halogenated hydrocarbons such as chlorinated and brominated compounds. In particular, per fluorinated (fully fluorinated) compounds have the potential to persist in the environment and PFOS accumulates in eggs, etc [9]. For this reason, HCFC-123 is one of the halogenated hydrocarbons have developed in order to replace chlorofluorocarbons [8] It is crucial to consider the possible toxicity problems resulting from the halon derivatives in *in vitro* laboratories, since the presence of these aerosols are evident due to the fact that the HCFC-123 containing fire extinguishers in the laboratory get empty after 6 months; and previous reports demonstrated that halon and other halon derivatives decreases fetal weights and implantation [7]. The related human and animal researches are focused on *in vivo* effects of halon or halon derivate aerosols [10, 11]. There are no data at literature investigating toxicity in IVF applications. Although there are reports declaring their general non-toxic property for healthy human body; few studies indicated central nervous system depression, decreased body weight, minimal injuries in organogenesis [2-4, 12].

Table 1. FERTILIZATION AND CLEAVAGE RATES IN THE PRESENCE OF THE HCFC- 123 CONTAINING FIRE EXTINGUISHER GROUP AND HCFC- 123 CONTAINING FIRE EXTINGUISHER FREE GROUP

Group	Total Oocyte Number	Fertilized Oocyte Number/ Fertilization Rate(%)	Number of Developing Embryo/ Cleavage Rate(%)	p Value
HCFC-123 group	35	20/ 57%	9/ 45%	>0.05
HCFC-123 free group	28	17/ 60 %	9/ 52 %	>0.05

Table 2. SPERM SURVIVAL TEST RESULTS IN THE PRESENCE OF THE HCFC- 123 CONTAINING FIRE EXTINGUISHER GROUP AND HCFC- 123 CONTAINING FIRE EXTINGUISHER FREE GROUP

Group	Semen sample #	Sperm motility at 24 hour	Sperm motility at 48 hour
HCFC-123 group	Sample #1	++++	+++
HCFC-123 group	Sample #2	+++++	++++
HCFC-123 group	Sample #3	++++	+++
HCFC-123 group	Sample #4	++++	++
HCFC-123 free group	Sample #1	++++	++++
HCFC-123 free group	Sample #2	++	-
HCFC-123 free group	Sample #3	++++	-
HCFC-123 free group	Sample #4	++	-

These findings pose a necessity on the research focusing the toxicity effects in *in vitro* embryos.

This study is conducted for HCFCs effects upon embryo development and sperm motility. Our results showed no difference between HCFC-123 group and HCFC-123 free group ratios with respect to fertilized oocyte number (Fertilization Rate) and number of developing embryos (Cleavage Rate). This *in vitro* finding is not similar to that of *in vivo* reports, which emphasize that *in vivo* embryos or fetus may have affected negatively by HCFC [6, 7]. *In vitro* results could not be predicted by *in vivo* criteria. The fact that an aerosol that is not harmful for the Lab staff does not necessarily mean that it does not pose any harm for the *in vitro* embryos. Furthermore, Malinverno *et al.* [8], demonstrated that exposure to HCFC-123 do not influence reproductive process, based on the results on the subsequent infertility or pup survival or birth gain of the offspring during lactation. However, current researches show that indoor air quality can present high potential risks in IVF laboratory and influence embryo development and IVF results [13]. For this reason, chemical air contaminant's impacts on micro-environments of IVF laboratories are very important for embryologists. In our study, we found that presence of the HCFC-123 containing fire extinguishers did not impair on any of the *in vitro* fertility parameters. As shown in Table 1, fertilization rates were similar in the HCFC-123 containing fire extinguisher environment group and HCFC-123 free group ($p>0.05$). In other words HCFC-123 in portable fire extinguisher does not have harmful effects on fertilization rates. Our data suggests that presence of the HCFC-123 containing fire extinguisher in the IVF Labs has no deleterious effect on in vitro fertilization.

As presented in Table 1, cleavage rates were not different statistically in presence of the HCFC-123 containing fire extinguisher group and HCFC-123 free group ($p>0.05$). There is no study at literature in this issue. This finding suggests that presence of the HCFC-123 containing fire extinguisher in the IVF Labs may not harm pre-implanted embryo development. Because of relative decrease in cleavage rates, this should be evaluated in larger series whether it has any effects on cleavage potency of fertilized oocytes or not. It can be seen that the HCFC-123 containing fire extinguisher environment does not pose any danger on sperm survival when sperm motility indications were evaluated in Table 2. Moreover, there were three cases without motility at 48 hour in control group that was not seen in the presence of the HCFC-123 containing fire group. But this result was originated from the use of the randomized sample, that is to say the samples with severe male factor couples in HCFC-123 free group. There is no study at literature investigating the effects of halogenated hydrocarbons on sperms. Our data suggests that presence of the HCFC-123 containing fire extinguisher in the IVF Labs does not have spermatotoxic effects. But sample size should be increased to conclude.

As Boone et al., have reported, assuring the cleanliness of the laboratory room increases the embryo development on IVF and subsequent pregnancy rate [10]. It is a general concern of the IVF Lab's staffs that the aerosols which are not harmful for humans may have a negative affect on the embryos and gametes. This research will introduce a new approach that can initiate further studies that will focus on the other aerosols which may have significant influences on the embryos and gametes.

CONCLUSION

We finally conclude that although a leakage can be encountered HCFC-123 containing fire extinguishers could be used safely in IVF laboratories. But safety measures and periodic control of these instruments should not be neglected.

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