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Can frozen sperm samples withstand being sent to space? Considering the creation of a sperm bank outside Earth

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Abstract

The aim of the research is to investigate the effects of microgravity exposure on the motility and vitality of human sperm. The likelihood of human reproduction in space relies heavily on the conditions in which human sperm may act under altered gravity conditions. A first step before longer duration experiments in microgravity conditions are conducted is to model the alterations that frozen human sperm may suffer, and their validation in short-term parabolic flights. It is unknown if microgravity has a negative effect on frozen sperm samples. Our hypothesis is that frozen samples can withstand different gravity conditions without significant alterations.

In order to test this hypothesis, 15 sperm samples from healthy donors were divided in two fractions (microgravity vs ground conditions) and frozen and stored in liquid nitrogen until the day of the experiment. A specific container as payload carrying the frozen sperm samples in vapors of liquid nitrogen was located in the aircraft cockpit, with no manual operation during the parabolas. A total of three parabolic flights (5 samples/flight) were completed during 2018-19 with 20 parabolas conducted in each flight. The parabolic flights allowed for 5 to 8 seconds of microgravity periods, using a CAP10B aerobatic plane operated by Barcelona-Sabadell Aeroclub, with UPC BarcelonaTech and Women's Health Dexeus, a leading center in human assisted reproduction, in charge of the research. The CAP10B aircraft has successfully proven in the last decade to perform optimal parabolas for both education and research purposes.

After thawing, sperm motility was evaluated by using a Makler® counting chamber and SCA®CASA System as a computerized semen motility analyzer. Sperm vitality was also evaluated by using Eosin-nigrosin staining. The study was approved by the Ethical Board of Hospital Universitari Dexeus, Barcelona (Spain).

Comparing mean values between control group (Earth) and the study group (microgravity) no significant statistical differences were found, in any of the parameters analyzed: motile sperm concentration (10^6/ml); progressive a+b motility (%), velocity (μm/s), straight line velocity (μm/s), linearity index (%) and vitality (%). Limitations of this parabolic flight are a short period of microgravity and hypergravity peaks before and after the parabolas.

In conclusion, these are the first experimental results published while exposing human frozen sperm to microgravity in a controlled parabolic flight experiment. More in-flight short-term and long-term studies are needed to verify the viability of transporting human sperm samples outside Earth, and to continue advancing the possibility of human reproduction in space.

Keywords: human reproduction, microgravity, parabolic flight, human sperm, aerobatic aircraft, space.

1. Introduction

It has been described that lack of gravity affects the molecular and cellular structures as well as certain circuits or functional systems. The cell membrane, the cytoskeleton, the cytoplasm and the nucleus of the cells are sensitive to gravitational changes.

The effects of simulated microgravity have been studied in protozoa, bacteria, plants and animals. It has been seen that microgravity affects certain enzymatic reactions and that it produces important changes in the formation of lipid structures affecting the ion channel and the cell membrane signaling system [1].

In relation to the reproductive system, mouse studies have shown that simulated microgravity affects the development of the testes lasting the fetal stage [2] as well as alterations in the physiology of the testicular cells causing the testicular function of adults. It has been seen that the consequences occur both in cell proliferation and differentiation, germ cell survival and degree of apoptosis, and in the secretion of sex hormones by the testes.

Also in the mouse, oocyte maturation has been affected in simulated microgravity conditions, with changes in the structure and organization of the metaphase plate as well as other phenomena such as an increase in cytoplasmic blebs [3]. Also at the embryonic level, it has been observed that mouse embryos under microgravity conditions activate oxidative processes causing cellular changes and development capacity [4, 5].

In humans, the effects of simulated microgravity on the cardiovascular system [6], the musculoskeletal system [7], the nervous system [8] and the endocrine system [9] among others have been described. The biological affection of cardiomyogenesis proces has been widely described [10]. These authors find important morphological changes and also differences in the patterns of gene expression. The effects of changes in gravity on human reproduction are not known in detail and the possible involvement of human, fresh or cryopreserved gametes and embryos should be investigated in depth. Some preliminary results have been published in fresh male gametes [11], but more specific studies are needed to know the different levels of involvement and their extent.

The UPC Universitat Politècnica de Catalunya is a pioneer and a university of reference in conducting experiments on parabolic flights in a single-engine aircraft. A number of studies have been published dealing with human physiology exposure to microgravity in these particular parabolic flights [6, 12, 13, 14, 15, 16]; DEXEUS is a reference institution in Obstetrics, Gynecology and Reproduction and a pioneer in the application of Assisted Human Reproduction Techniques and Cryobiology. [17, 18, 19, 20, 21, 22, 23].

2. Hypothesis

Our hypothesis is that frozen samples can withstand different gravity conditions without significant alterations.

We present a pioneering work involving frozen human sperm exposed to microgravity. Preliminary results have recently been presented as the first results reported [16]. The study was conducted in short-duration parabolic flights using a CAP10B plane, including 20 parabolas of up to 8 seconds, providing results which initially confirm the hypothesis. Frozen samples were used, and a pre-test and post-test was conducted after exposure to microgravity.

3. Material and methods

3.1.- Parabolic flights with aerobatic aircraft

Parabolic flights have been conducted for a long time as a way of performing short-time duration experiments and technical demonstrations [24, 25]. Aircraft parabolic flights provide up to 23 seconds of reduced gravity and are used for conducting short investigations in Physical and Life Sciences, both for senior researchers and for international student experimentation and motivation, and public outreach.

We report on a research conducted in Sabadell Airport (Barcelona, Spain) with single-engine aerobatic aircraft such as the Mudry CAP10B (Figure 1), achieving up to 8.5 seconds of reduced gravity [12, 15].

Figure 1: Mudry CAP10B aerobatic aircraft used for the research parabolic flights.

The flight profile results coming from a steady flight profile an introductory pull-up maneuver is performed at increased acceleration (roughly 3-3.5g for these aircraft), pilot reduces thrust and, with throttle or idle engines the airplane follows the parabolic trajectory of a free-flying body. As a consequence, after a short phase of transition, microgravity (0.05g) is obtained for 5-8 seconds.

After the recovery maneuver at increased acceleration (2.5-3g), the airplane flies again horizontally to the ground level for some minutes before introducing the next parabola. During one flight mission typically 10-15 parabolas are performed achieving 5-8 seconds of microgravity/parabola.

A total of 3 parabolic flights with a total of 20 parabolas for each flight and 5 samples in each flight were conducted during 2018-19.

An average flight profile of these parabolic flights can be seen in Figure 2.

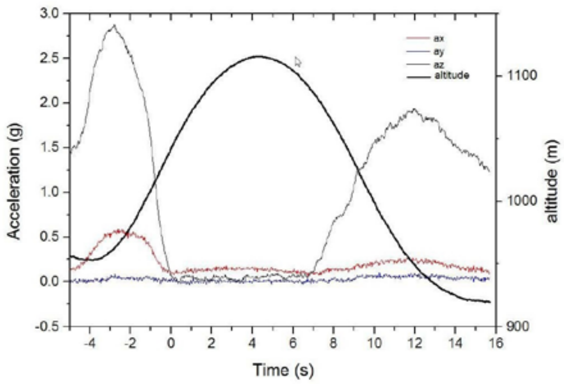

Figure 2: Flight profile of an average parabola performed during the research flight.

Residual acceleration in a parabola to which the samples were exposed are shown in Figure 3. During the hypogravity periods residual acceleration is reduced to less than 0.1g, reaching 0.01g in the vertical axis (az; ground to height).

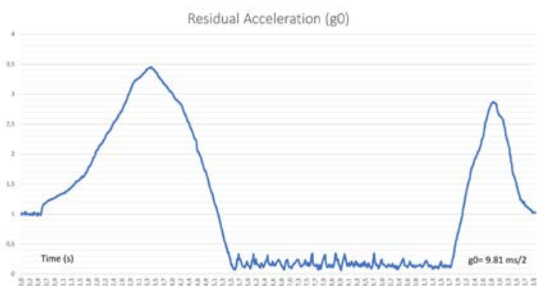

Figure 3: Residual acceleration of a parabola conducted with the CAP10B aircraft in this research.

3.2.- Frozen human sperm samples

A total of fifteen sperm samples from healthy donors were divided in two fractions (microgravity vs ground conditions) and frozen using glycerol as cryoprotector, aliquoted in high security straws and stored in liquid nitrogen until the day of the experiment when they were exposed to µG and 1G conditions. A specific container as payload carrying the frozen sperm samples in vapors of liquid nitrogen was located in the aircraft cockpit, with no manual operation during the parabolas. The container with frozen samples of the study group was located on board the aircraft, strongly attached to the co-pilot seat in front of the aircraft cockpit, whereas samples of the control group were on hold on ground in order to compare the differences.

After thawing, evaluation of sperm concentration and motility using a computerized semen motility analyser as well as vitality (eosin-nigrosin staining) were performed.

The study was approved by the Ethical Board of Hospital Universitari Dexeus, Barcelona (Spain).

4. Results

Comparing mean values between control group (1G) and study group (µG) no significant statistical differences were found in any of the parameters analysed ($p \geq 0.05$):

• Motile Sperm concentration (M/ml) 13.03±12.13 vs 13.72 ± 12.57
(-0.69 95% CI [-2.9;1.52])

• Progressive a+b sperm motility (%) 22.54±12.83 vs 21.83±11.69 (0.03 95% CI [-0.08;0.15])

• Sperm velocity (µm/s) 43.50±9.82 vs 39.88±8.70 (3.62 95% CI [-0.03;7.27])

• Straight line velocity(µm/s) 24.57±8.12 vs 22.23±8.00 (2.34 95% CI [-1.11;5.78])

• Linearity index (%) 65.88±11.30 vs 62.60±10.39 (0.05 95% CI [-0.03;0.13])

• Sperm vitality (%) 44.62±9.34 vs 46.42±10.81 (-0.04 95% CI [-0.13;0.05])

5. Discussion

Minor variations observed were more probably related to heterogeneity of the sperm sample than to the effect of exposure to different gravity conditions.

This study has limitations. Aerobatic parabolic flights offer a limited time of microgravity (5-8 seconds) while larger aircraft provide between 20-25 seconds of microgravity thanks to a more powerful engine. Another limitation to take in consideration is that short periods (1-3 seconds) of hypergravity preceding and following µG cannot been avoided.

This research is not feasible in a Random Position Machine, as the continuous random rotation of the cellulae will significantly affect its motility, so it cannot be considered a model of simulated microgravity for this particular topic. Drop Towers may be considered, but the size of the cylinder containing the sample makes it unpractical to hold. Parabolic flights in larger aircraft may provide further validation for this hypothesis, as are capable of exposing the payload to up to 23 seconds of microgravity with residual acceleration in the range of 0.01 g [24, 25]. Experimentation at the International Space Station would be the best option for further studies as it offers a continuous, permanent exposure to microgravity. It is known that NASA is conducting the experiment Micro-11 [26] with human sperm samples, on the International Space Station, since April 2018, although no results have been published up to date.

6. Conclusions

Although it has to be confirmed with further studies and being conscious that radiation in another important effect to be taken in consideration, the lack of differences observed in the sperm characteristics between frozen samples exposed to microgravity and those maintained in ground conditions open the possibility of considering safely transporting male gametes to space and the possibility of creating a human sperm bank outside Earth.

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