Sperm Motility Under Conditions of Weightlessness

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ABSTRACT: The aim of this study was to determine the differences in motility of frozen and thawed bull spermatozoa under conditions of weightlessness compared with ground conditions. The tests were performed within a series of scientific and technologic experiments under microgravity using sounding rockets in the Technologische Experimente unter Schwerelosigkeit (TEXUS) program launched in Kiruna, North Sweden. Using a computerized sperm motility analyzer, significant differences were found in sperm motility under microgravity compared with sperm under gravitational conditions on earth. Computer analysis showed alterations in straight line and curvilinear velocity, as well as in linearity values. The amount of progressively motile spermatozoa, including all spermatozoa with a velocity > 20 μm/second, increased significantly from 24% ± 9.5% in the reference test to 49% ± 7.6% in the microgravity test. In conclusion, there is strong evidence that gravity influences sperm motility.

Key words: sperm motility, weightlessness.

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Several studies have shown that some processes take place differently and more favorably under zero gravity than under terrestrial gravitational conditions. Consequently, certain properties can only be achieved under these conditions. In this study, the motility of frozen and thawed bull spermatozoa was investigated under conditions of weightlessness and compared with motility under normal conditions on earth. Thus, the influence of gravity on functional processes of higher biologic systems was studied.

For many reasons, the spermatozoon is an ideal model for examination of physiologic functions. So far, it is still unknown to what extent and in which manner biologic processes like cell differentiation, transportation, and development are influenced by gravity. Therefore, investigations performed under conditions of zero gravity should contribute to the understanding of the influence of gravity on molecular mechanisms involved in basic biologic functions. In this investigation, the effect of gravity on sperm movement characteristics was clearly demonstrated. As a result of the great attention during recent years to the objective analysis of sperm motility, it has become increasingly apparent that progressively motile spermatozoa and their movement characteristics are of biologic and, hence, clinical importance.

Materials and Methods

Material

Semen samples were obtained from bulls of the animal breeding and insemination station in Grub, Germany. Ejaculates were collected using an artificial vagina. Samples showing at least 80% sperm motility were diluted to a concentration of 40 to 50 × 10^6/ml and frozen in 0.5-ml straws according to the method of Steinbach and Foote (1967). Frozen samples were thawed and maintained at 39°C until examination.

Experimental Design and Mission Performance

Experiments were carried out as part of the Technologische Experimente unter Schwerelosigkeit (technological experiments under microgravity; TEXUS) program in November, 1988 (TEXUS 19) and in May, 1990 (TEXUS 26) within a series of other scientific and technologic experiments under microgravity conditions using sounding rockets (Skylark VII, British Aerospace Corporation). The TEXUS program, which is dedicated to the preparation of scientific experiments on board Spacelab, was initiated in 1976. TEXUS encompasses an unmanned German government rocket program that ensures weightlessness conditions for about 360 seconds and transports a payload of about 300 kg to an altitude of 250 km. The payload is recovered by parachute and returned to the launch site by helicopter. The launch campaign is carried out in Kiruna, North Sweden, in cooperation with the European Space Agency (ESA) and the Swedish Space Corporation (SSC).

Samples were measured in a specially developed, air-tight and temperature constant (39°C) chamber (Strömberg-Mika, Bad Feilnbach, Germany), which was part of the TEXUS module (Fig. 1). Thawed spermatozoa were observed under a negative phase-contrast microscope (objective, Nikon ELWD 20x; ocular, Nikon
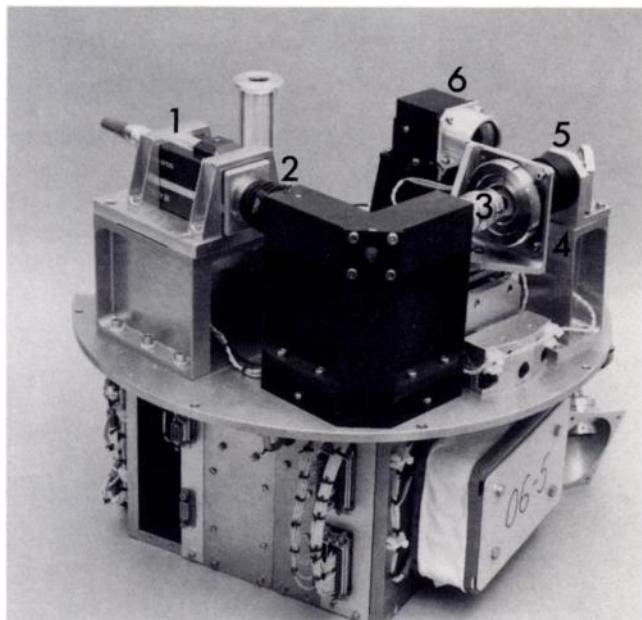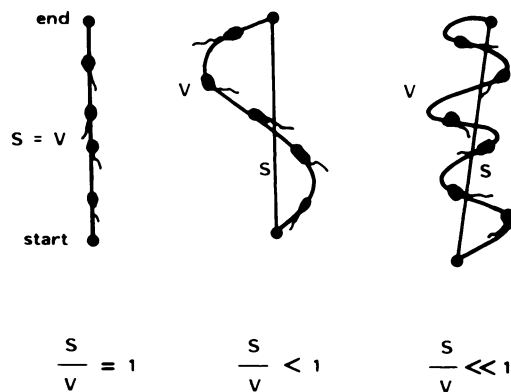
FIG. 2. Examples of motion patterns of spermatozoa. Motility characteristics were derived from video acquisition and processing of the sperm track. V = curvilinear velocity (μm/sec); S = straight line velocity (μm/sec), showing different moving patterns within groups of progressively motile spermatozoa. Progressively motile spermatozoa are split into three groups in accordance with their S/V ratios: S/V = 0.9 to 1.0 = linear forward; S/V = 0.8 to 0.9 = not linear forward; S/V = 0.0 to 0.8 = other movement pattern.

FIG. 1. The TEXUS experiment module. The module shown in the figure was developed by MBB-ERNO (Bremen, Germany). It was equipped with (1) a camera system (CCD); (2) ocular (Nikon CFW 15×); (3) objective (Nikon ELWD 20×); (4) chamber; (5) condenser, filter, deflecting mirror; and (6) lamp casing.

CFW 15x; Nikon Corporation, Tokyo, Japan). About 40 minutes before launching, samples were integrated into the payload. After lift-off, semen cells in the chamber could be monitored directly on line with a television camera. The observer on the ground was able to influence and to control experimental parameters via telecommand channels so that sharpness of focus could be adjusted. Another channel allowed 12 (TEXUS 19) and 17 (TEXUS 26) different fields of vision during the microgravity phase. The television pictures were recorded on the ground. The actual gravitational force during the time of observation was $< 10^{-4}$ g.

Video tapes were evaluated using a computerized semen motility analyzer (SM-CMA; Strömberg-Mika, Bad Feilnbach, Germany), which enabled the measurement of total and progressive sperm motility, as well as determination of sperm velocity (Katz et al, 1985, 1987; Knuth et al, 1987; Mortimer et al, 1988). The frame rate for motility analysis was 25 Hz. The time between two successive video frames was 40 msec; the actual observation time for each spermatozoon was 600 msec. Only those cells that could be detected in eight successive frames were evaluated. Curvilinear velocity was calculated from the sum of the straight line distances between all points along the track. Straight line velocity was calculated from the straight line distances between the first and last point of the track. In addition, within the group of progressively motile spermatozoa, three classifications could be distinguished, characterizing the quality of motion (Auger and Dadoune, 1988) by calculation of the linearity values (ratio of straight line to curvilinear, S/V; Fig. 2).

To compare the data obtained during the flight of TEXUS 19, the motility of another aliquot of the same ejaculate was examined under identical conditions on the ground. In the TEXUS 26 experiment, the reference test was carried out immediately before the

launch using the same sample that was to be observed under microgravity. The advantage of using the same sample was the elimination of variations within the measurements normally found in different aliquots of the same ejaculate. In TEXUS 19, a total of 761 and 698 spermatozoa were evaluated under 1 g and zero g, respectively. By increasing the fields of vision, a total of 1154 and 1090 spermatozoa could be observed in TEXUS 26. The Wilcoxon signed rank test was used for analysis of significance.

Results

In TEXUS 19, no differences in quantitative sperm motility (which refers to velocity) were detectable under conditions of weightlessness when compared with motility under gravitational conditions on earth. Total motility and the percentage of spermatozoa with progressive motility (velocity > 20 μm/sec) were nearly identical under 1 g and microgravity conditions (Fig. 3). However, computer analyses showed significant alterations in motility patterns, which signify changes in path shape and are expressed as linearity value. The amount of linear forwardly motile spermatozoa (linearity value > 0.9) rose significantly (Fig. 4) from 42% ± 9% in the reference test on the ground to 73% ± 8% in the microgravity test ($P < 0.005$). On the other hand, there were no significant differences in straight line or curvilinear velocity. For both samples, there were only small deviations between velocity values under conditions of weightlessness and values for the reference sample on earth (Table 1). But these small differences were sufficient to shift the S/V ratio so that the portion of linearly moving spermatozoa rose significantly.

In TEXUS 26, total motility was 71.4% ± 7.2% in the reference test and 79% ± 6.2% under microgravity condi-
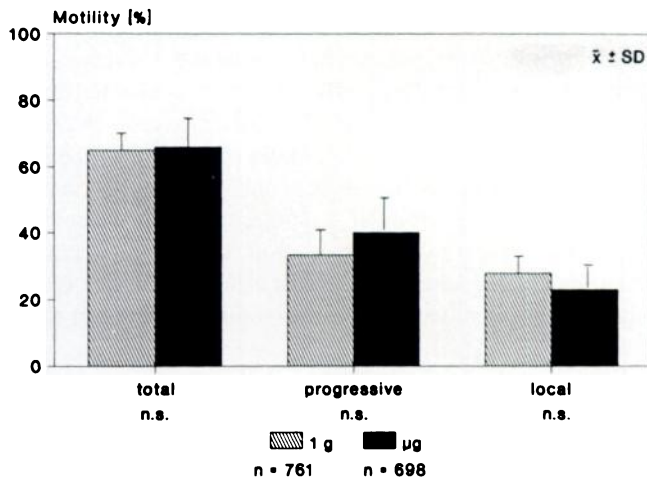
Motility [%]

FIG. 3. Comparison of quantitative motility on the ground (1 g) and under conditions of weightlessness (microgravity) in TEXUS 19. Spermatozoa are classified into three groups according to their velocity values: (a) total motility = all spermatozoa that move at a velocity >10 μm/sec; (b) progressive motility = all spermatozoa that move forward at a velocity > 20 μm/sec; (c) local motility = all spermatozoa that move within a range of 10 μm/sec and 20 μm/sec. n.s. = not significant.

tions ($P < 0.005$). A statistically high significant difference could be observed in the group of progressively motile spermatozoa (Fig. 5). Progressive motility increased from 23.9% ± 9.5% at ground conditions to 49% ± 7.6% (p < 0.005) during flight. Curvilinear velocity values within this group also rose significantly, from 28.8 μm/sec ± 2.5 μm/sec on earth to 31.9 μm/sec ± 3.2 μm/sec in space ($P < 0.01$; Table 1). At the same time, local motility decreased from 47.5% ± 9.1% in the reference test to 30% ± 6.5% in
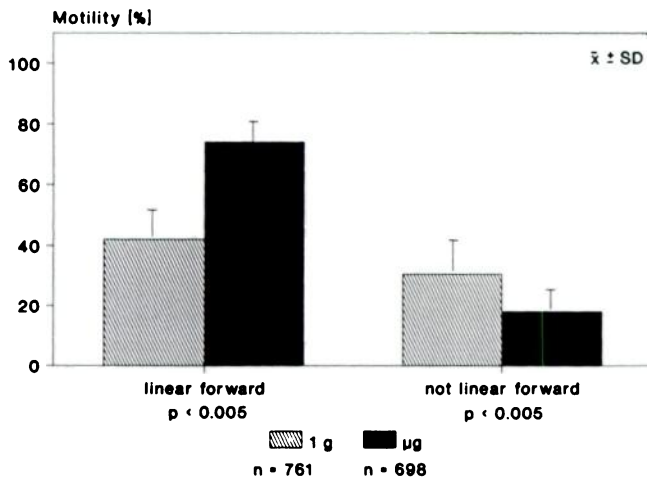
Motility [%]

FIG. 4. Motion patterns of progressively motile spermatozoa on the ground compared with those under conditions of weightlessness in TEXUS 19. Path shape is characterized and expressed by means of the S/V ratios = linearity values. These are defined as shown in Figure 2.

Table 1. *Velocity data from bull spermatozoa on earth and under conditions of weightlessness*

	TEXUS 19		TEXUS 26	
	Microgravity	1 g	Microgravity	1 g
Velocity (S)	29.1	27.5	26.9	23.8
	± 9.4	± 6.2	± 2.8	± 2.5*
Velocity (V)	29.8	30.9	31.9	28.8
	± 9.9	± 7.5	± 3.2	± 2.5†

S = straight line velocity in μm/sec, V = curvilinear velocity in μm/sec.
* $P < 0.005$.
† $P < 0.01$.

the microgravity test ($P < 0.005$; Fig. 5). This implies that 17.5% of the so-called local motile spermatozoa, which are defined as cells with velocity values between 10 μm/sec and 20 μm/sec, could overcome the theoretically fixed velocity borderline of 20 μm/sec. Under the influence of weightlessness, these cells could be classified by computer analysis as progressively motile spermatozoa. Regarding qualitative motility patterns (path shape), the portion of linear forwardly motile spermatozoa with linearity values > 0.9 rose significantly from 30.5% ± 12.8% on the ground to 42.2% ± 16.1% ($P < 0.01$) during flight (Fig. 6). This means there was an increase of linearity of 12% under the influence of weightlessness.

Discussion

Using bull spermatozoa, it was possible for the first time to show the influence of gravity on the motility of biologic
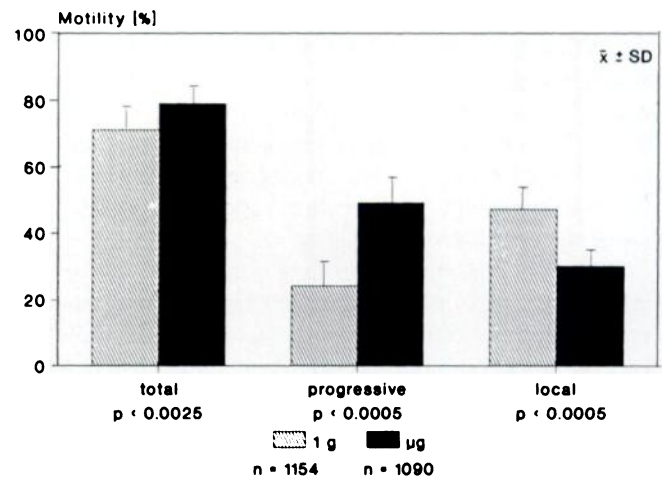
Motility [%]

FIG. 5. Comparison of quantitative motility on the ground (1 g) and under conditions of weightlessness (microgravity) in TEXUS 26. Spermatozoa are classified into three groups according to their velocity values: (a) total motility = all spermatozoa that move at a velocity >10 μm/sec; (b) progressive motility = all spermatozoa that move forward at a velocity >20 μm/sec; (c) local motility = all spermatozoa that move within a range of 10 μm/sec and 20 μm/sec.
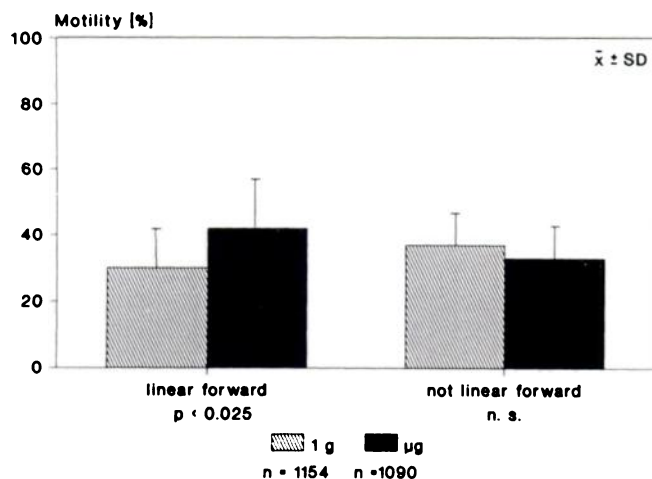
FIG. 6. Motion patterns of progressively motile spermatozoa on the ground compared with those under conditions of weightlessness in TEXUS 26. Path shape is characterized and expressed by means of the S/V ratios = linearity values. These are defined as shown in Figure 2. n.s. = not significant.

systems. In the TEXUS 19 experiment, the first indications of the effects of microgravity were detectable, which were confirmed after the reflight of TEXUS 26. Reasons for the differences in the results found in both experiments can be attributed to the better experimental conditions during the second flight test. The use of the same ejaculate sample for both the reference test under terrestrial conditions and the microgravity test was very advantageous. In the first TEXUS 19 experiment, the reference test was carried out by thawing another sample of the same ejaculate.

The changes detected in motility characteristics are definitely due to weightlessness, as the results of the experiments demonstrate. This could also be proven by a series of supporting ground experiments in which launching conditions, like acceleration (12 g) and vibration, were simulated. No change in sperm movement properties was detectable in any case. Thus, microgravity conditions favor the motility pattern of spermatozoa and might possibly improve their fertilization capacity.

There are possible explanations for these effects of microgravity on sperm motility characteristics. The enhancement may be due to changes in membrane permeability, energy balance, or both. It also might be that in microgravity, energy supplying mechanisms function more economically and more rapidly. The activity of membrane-bound enzymes like thymidine kinase (E.C. 2.7.1.21) might be influenced by microgravity-induced changes of membrane permeability, causing an effect on DNA synthesis (Tairbekov et al, 1982). The results of other space missions substantiate these assumptions, proving that weightlessness can have an influence at the cellular level.

The Cytos Franco-Soviet experiments made on a unicellular organism, the paramecium, on board Salyut 6 have shown a strong stimulating effect on cell growth rate (Planel et al, 1981). Among other effects, an increase in cytoplasmic hydration, a drop in total protein content, and a change of the electrolytic content were found. This was shown by a decrease in intracellular calcium, probably related to structural changes in the cytoskeleton proteins, especially in their sites for calcium binding, or to modifications of the energetic metabolism connected to ciliary movement (Tixador et al, 1981).

Montgomery et al (1978) reported that embryonal lung cells (WI-38) cultivated under microgravity conditions showed a 20% reduction in glucose consumption compared with cells tested in ground-based experiments. This suggests that energy consumption was lower for cells cultured in the absence of gravity than for cells kept in the ground laboratory. On the basis of these findings and the results of the TEXUS experiments, it would be of great interest to extend these studies to other biologic systems, especially to primate cells.

Acknowledgments

References

Auger J, Dadoune JP. Computerized sperm motility and application of sperm cryopreservation. Arch Androl. 1988;20:103–112.

Katz DF, Davis RO, Delandmeter BA, Overstreet JW. Real-time analysis of sperm motion using automatic video image digitization. Comput Methods Programs Biomed. 1985;21:173–176.

Katz DF, Davis RO. Automatic analysis of human sperm motion. J Androl. 1987;8:170–181.

Knuth UA, Yeung C-H, Nieschlag E. Computerized semen analysis: objective measurement of semen characteristics is biased by subjective parameter setting. Fertil Steril. 1987;48:118–124.

Montgomery POB Jr., Cook JE, Reynolds RC, et al. The response of single human cells to zero gravity. In Vitro. 1978;14:165–173.

Mortimer D, Serres C, Mortimer ST, Jouannet P. Influence of image sampling frequency on the perceived movement characteristics of progressively motile human spermatozoa. Gamete Res. 1988;20:313–327.

Planel H, Tixador R, Nefedov Y, et al. Space flight effects on Paramecium tetraaurelia flown aboard Salyut 6 in Cytos 1 and Cytos M experiments. Adv Space Res. 1981;1:95–100.

Steinbach I, Foote RH. Osmotic pressure and pH effects on survival of frozen bovine spermatoza. J Dairy Sci. 1967;50:205–213.

Tairbekov M, Voronkov LA, Gzova A. Some physical and biochemical features of cells of carrot gall developed in weightlessness. Space Biol Aerospace Med. 1982;16:62–67.

Tixador R, Richoilley G, Templier J, Monrozies E, Maotti M, Planel H. Etudes de la teneur intra et extracellulaire des electrolytes dans les cultures de paramecies realisées pendant un vol spatial. Biochim Biophys Acta. 1981;649:175–178.