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The Effect of Mechanical Vibration on Human Sperm Activity in Vitro

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It is well known that sperm is a unique cell in that it has a function to be done by itself outside the body and this function is essential for species' continuity thus sperm by its power and intact structure has to reach the ova and perform the fertilization and this journey is affected by the chemical and physical factors that might increase or decrease its ability to move or fertilize or even to survive. The aim of this study is to find the effect of vibration that is a vigorous movement with high frequency for 20min on whole seminal fluid samples as an external physical factor. 40 fresh seminal fluid samples were selected. 1ml of each semen samples was placed in the bottom of conical tube; the tube was exposure to vibration waves by using a special shaker designed for this purpose for 20 min. This shaker consist of a M540 DC motor equipped with PWM controller to control the rotational speed from 5-2400 rpm. Semen analysis was done before and after subject vibration. A significant increase ($P<0.05$) was found in percentage of sperm active directed motility (grade A) with a non-significant increase in sluggish motility and a non-significant decrease in percentage of immotile sperms percentage. No significant changes were founded regarded sperm morphology and count. It was concluded that vibrating seminal sample for 20min increases the overall sperms activity with significant increase in percentage of highly active directed sperms.

Keywords: Vibration, sperm activation in vitro, sperm motility.

Due to the wide use of in vitro fertilization procedures (IVF), sperm preparation processes in vitro that include centrifuge, pipate aspiration , shaking , and the thermal changes that include heating or cooling in addition to the photo-effects of room lighting cause several drawbacks with manipulation semen[1] and the cause of these side effects is still unknown[2] but animal studies on in-vitro prepared samples of ova and in turn fertilize ova; suggested spontaneous activation of metaphase II in female oocytes during their retrieval from oviduct and during their in vitro culture in rat germ cells[3, 4]; in addition, to poor or mal-developmental competence of rat zygotes to blastocyst stage embryos in vitro[5].

In many cases, handling sperm are performed consecutively that may cause adverse effects that are cumulative at the end of the entire procedure. In addition to the media used and different pH, osmolality, light and temperature. Semen undergoes multiple pipettings and centrifugation in order to remove seminal/ epididymal fluid[6].

Vibration is the oscillatory motion of various bodies. All bodies with mass elements and elasticity are capable of vibration; hence, most machines and structures including the human body experience vibration to some degree. Forced vibration is caused by the action of external forces. If the frequency of excitation coincides with the natural frequency of the system, resonance occurs. The result is large oscillations within the structure of creating potentially harmful stress. Continues vibration is uncommon force to affect semen samples in vitro although the human body and genitalia as well are sometimes being vibrated as a part of some types of jobs or machines[7]. In physiotherapy; whole body vibration has been introduced as a training method to improve muscle power and strength[8]. A pineal vibrator could be used to induce ejaculation in cases of impotence due to spinal cord injury[9].

Sperm motility is a primary function of sperms and this ability is gained in the epididymis as a part of sperm maturation and defects in sperm activity or called asthenospermia could be slow, non-directed, or immotile but viable according to the severity of ma-function[10]. And ART is an important treatment of this problem by using single sperm selected to be injected in an ova (ISCI) or activation of the whole seminal fluid using washing and activation media to be added to the ovum (IVF) or injected in the female vagina (IUI). The outcome of ART is directly affected by the sperm activity and the quality of activity thus many studies were performed to find the activation methods that could be used to activate sperms like heat[11]., laser irradiation in vitro[12]., or improving the quality of seminal fluid[13].

Vibrations influence the body in many different ways. The response to a vibration exposure is primarily dependent on the frequency, amplitude, and duration of exposure. Other factors are important when vibrating human body; include the direction of vibration input, location and mass of different body segments, level of fatigue and the presence of external support (9). Thus, when vibrations are attenuated in the body, its energy is absorbed by the tissue and organs that had medical significant. For example: vibrating muscles lead to both voluntary and involuntary contractions, and can cause local muscle fatigue, particularly when the vibration is at the resonant-frequency level. Furthermore, it may cause reflex contractions, which will reduce motor performance capabilities (9). Vibration affects living cells and bacterial growth was found to be affected as the growth of many types of bacteria including (E. coli, Staphylococcus aureus, Bacillus subtilis, and Pseudomonas aeruginosa), and as vibration accelerated there will be changes in the growth curve of the organism that include reducing lag time and increasing growth rate in addition to Oxygen consumption of the bacteria (15), but the mechanism was unclear. Addition of O2 to the cultures enhanced this effect, but removal of O2 did not negate the effect[14].

In this study; the effects of vibration will be studied on samples of human whole seminal fluid.

MATERIAL AND METHODS

Samples and methods

Forty samples were selected depending on sample's macro and micro-features of the seminal fluid of patients consulting the Infertility Unit at the High Institute of Infertility diagnosis & ART's at Al Nahrain University in the period from November 2015 to April 2016. Semen samples collected in sterile containers in private room after at least 72h free of any sexual act. Any sample with abnormal color, prolong liquefaction time, sever oligospermia (less than 5mlion sperm /ml), high levels of WBC, was not included in this study. Standard seminal analysis was done according to WHO 1999[15,16]. The parameters of seminal fluid studied in this study are sperm concentration, sperm motility (%) and morphologically normal sperm (%). Motility of sperms was classified according to the quality and direction into: A class which mains that the sperm speed is high and directed in linear line and move along the microscopic examination field. While B class motility means that sperms are sluggish and the direction of motility is not linear and remain in the examination field unlike C class that include moving sperms in their spots while D class include immotile sperms that could be alive or dead. 1ml of each semen samples was placed in the bottom of conical tube; the tube was exposure to vibration waves for 20 minutes in a variable rotational speed shaker fig.(1) a and b incubated in 37C. Semen analysis was done

before and after vibration. Data were collected and analyzed using SPSS (Statistical program for social studies, Version 17, Illinois; USA) for more information details see[16-19]

RESULTS

A significant increase (P<0.05) was found in percentage of sperm motility grade A before and after vibration in addition to increase in class B motility that was not significant (P>0.05) and also decreases in class C and D that were not significant also. No significant changes were found regarding sperm morphology and count.

DISCUSSION

In this study; an increase in the overall sperms activity after vibrating the seminal sample and the significant increase was in grade A with a non-significant increase in class B and these increases were associated with decrease in both class C and D. These findings disagreed with studies that showed the effects of sperm samples shaking (17), centrifuging [1], or repeated pipating[20].

The secret may be in the type of moving the sample because vigorous vibration for 20min is unusual situation affecting seminal samples during sperm preparing or examination because of high frequency. It is well documented that mechanical vibration have a specific frequency and modulation affect growth and multiplications of living organisms which in turn affected by the chemical and physical composition of environmental medium and initial states of water, nutrients, and organisms[21].

It is well known that vibration of affected bone, joint, or muscle is commonly used to rapid and augments the regeneration process in tissues at cellular level as "physiotherapy". In bone tissue for example; vibration leads to murine osteoblastic cells respond to vibration signals of varying magnitude and frequency induced

Table 1. Certain parameters of standard seminal fluid examination before and after 20 min Vibration at 37C

	Certain sperm function parameters	Before Vibration	After 20 min. Vibration	P – Value
	Count (sperm/ml)	42.82 ± 19.52	42.25 ± 19.15	P > 0.05
Motility (%)	Grade A (%)	3.37 ± 5.92	7.12 ± 9.66	P < 0.05
Motility(%)	Grade B (%)	39.12 ± 16.44	41.25 ± 16.86	P > 0.05
Motility(%)	Grade C (%)	23.37 ± 11.62	21.25 ± 11.07	P > 0.05
Motility(%)	Grade D (%)	34.37 ± 19.38	31 ± 20.19	P > 0.05
	Morphology (%)	34 ± 11.72	38.87 ± 14.60	P > 0.05

Values are expressed as Means ± SD.
Patients No. = 40.

Fig. 1. Mechanical shaker used

frequency dependent increase in nitric oxide (NO) and decrease in prostaglandin E2 (PGE2) secretion from cells immediately after loading, indicating the activation of bone formation pathways[22]. without changing the cell viability[23].

Sperms motility is affected by the structure of the sperm flagella which called primary asthenospermia. While secondary asthenospermia occurs due to the presence of white blood cells in semen[24]. and/or morphologically abnormal sperms in the semen[25]. as these abnormal cells cause liberation of factors that increase the oxidative stress in seminal plasma[26].

Thus no clear chemical explanation could be suggested to explain the activation occurs in this study because vibrating abnormal cells in seminal plasma could enhance liberating these harmful factors that decrease the motility. But the explanation could be physical as high frequency movements cause activation of the structure of sperm flagella or mitochondria that supplied energy.

Theses controversy showed that Nobody knows how cells coordinate the complex activity inside them. But vibrations of the cell membrane could act as a pulse that synchronises the lot, as suggested by Sepehr Ehsani at the University of Toronto in Canada His idea is that vibrations in the cell membrane act as a kind of cellular pacemaker, providing a background pulse rate for synchronising activities[27]. Shaking will effect the vicosity of the semen and accordingley sperm velocity will increase. Shaking and centrifugation for small duration and acceleration are negligible because it doesn't have any effect on the cellular membarn for the vital components of specimens. On other hand higher rates of acceleration and velocity will also be of no advantage for sperm motility and it has side effect and consider harmful for it.

According to this study; vibrating seminal samples for 20min increase the percentage of active sperms and this study showed the need for further understanding of characters of seminal fluid and sperm physiology.

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