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Estimation the Time Period for Human Spermatozoa Activity in Vitro Under the Same Conditions

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Abstract. Twenty one semen samples from the young men, their ages ranged between 18-35 were taken, divided into three groups depending on the activity of sperm motility. The time period (per hours) of sperm activity was monitored microscopically in vitro. Semen samples were taken from the males after not ejaculating for three days respectively . In the first 30 minutes, took equal volumes of semen for all samples and kept at 37 °C and examined each sample separately. The samples which contain oligoasthenozoospermia, high rates of pyospermia and higher numbers of abnormally shaped sperm (more than 40%), were neglected so as to avoid some of the possible causes of the dead of spermatozoa early. In the current study, the results showed significant differences ($p< 0.05$) in the remaining time period for spermatozoa in vitro under the same conditions, where the lowest remained period in vitro was recorded about six-hour while the largest remained period was recorded about 11 hours, followed by about 10.5 hours after ejaculation. Moreover, it was found that there is a direct correlation between the increasing remained time period in vitro for spermatozoa and the percentage of active spermatozoa after ejaculation. However, results have shown that 15-30% of the total activity of spermatozoa decreases after two-three hours from ejaculation and the death rate reaches about 80% after six hours and about 95% after nine-hour from ejaculation for most samples.

Keywords: Spermatozoa, Human, In vitro.

INTRODUCTION

Spermatozoa can be defined as matured motile cells, which are spermatogenesis products. The average healthy man produces a range of (20 – 240) million sperms daily [1] the spermatogenesis represents a chain of the cellular events, supporting the daily sperm production [2]. One of the most significant sperm cell characteristics is their motility that is necessary for ensuring the male fertility. In fact, there are 2 motility types, which are: hyper-activated motility, observed in the spermatozoa at the fertilization site and activated motility, which is observed in ejaculated spermatozoa. The two motility types need a sufficient energy supply in a form of the ATP, which is utilized by flagellar dynein-ATPase. There has been a long-standing dispute about which metabolic pathway, OXPHOS or glycolysis, is involved in the sperm motility energy production [3-4]. In the human beings, the spermatogenesis process generates from 20 million to 240 million sperms daily and it is dependent upon the tight cellular metabolism regulation. In addition to that, several selective mechanisms in the epididymides and testes play a role in ensuring that there are high quality sperms ready for the ejaculation [5].

Generally, the geographic and racial variations may have a role in specifying the characteristics of the semen, where, several researches showed ethnic differences concerning the parameters of the semen. For instance, a

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national study of the Chinese men that 7%, 79%, 7%, 46%, 52%, and 10% of men had values below the criteria of the World Health Organization (WHO) for sperm concentration, rapid progressive motility, total sperm count, sperm viability, sperm progressive motility, and normal morphology, respectively [6]. Throughout the ejaculation, the semen is generated from a concentrated spermatozoa suspension, stored in paired epididymides, diluted by, and mixed with fluid secretions from accessory sex organs. It's emitted in a number of the boluses. Comparison of the semen volumes of pre- vasectomy and post-vasectomy revealed that approximately 90% of the semen volume comprises secretions from accessory organs [7], mostly, the seminal vesicles and the prostate, with small contributions from epididymides and bulbourethral (Cowper's) glands.

There are several evidences of the fact that the semen specimens' quality differs according to the way the ejaculate has been produced. The ejaculates that are produced by the masturbation and gathered in containers in a room near the lab may be of a lower quality compared to the ones that have been recovered from the non-spermicidal condoms that are utilized throughout the intercourse at home [8]. Such difference could be reflecting a different sexual arousal form, due to the fact that the time that is spent to produce a sample by the masturbation—reflecting the seminal emission extent prior to the ejaculation affects the quality of the semen too [9].

Under specified collection conditions, the quality of the semen is dependent upon the factors that can't be altered usually, like the production of the sperm by testes, recent (in particular, febrile) illness, accessory organ secretions, and other factors, like the time of abstention, which must be recorded and considered in the interpretation of results. The accessory sex gland activities, as well as their fluids, play a role in the dilution of concentrated spermatozoa at the ejaculation [10]. the concentration of the sperm isn't a direct testicular sperm output measure, due to the fact that it is impacted by other reproductive organ functioning; nonetheless, the total amount of the ejaculated sperm (i.e. Semen volume multiplied by sperm concentration) is. For instance, sperm concentrations in the semen from the old and young men could be equal, however, the total numbers of the sperm can be different, due to the fact that the seminal fluid volume as well as the total sperm output are decreased with the age, at least in some populations [11].

As the epididymides are not entirely emptied by one ejaculation [12], some of the spermatozoa remain from the previous ejaculation time, and that affects the range of age and the spermatozoa quality in the ejaculate [13,14], although The extent of that influence is hard to ascertain and it's rarely taken under consideration. Due to the variation in information about how long spermatozoa time outside the body (in vitro), as well as its lack of availability of this type of studies in particular for Arab peoples, the current study aimed to study the period time of spermatozoa outside the body (in vitro) for a group of youth in Basra Governorate / Iraq.

MATERIAL AND METHODS

The study was carried out in the laboratories of Basra University - College of Education - Department of Biology - Al-Qurna, so, samples were obtained from medical examination laboratories. 21 semen samples from the young men, their ages ranged between 18-35 were taken, divided into three groups depending on the activity of sperm motility. The time period per hours(h) (1h,3h,6h,9h until sperms death completely) was monitored microscopically in vitro, as follows: Semen samples were taken from the males after not ejaculating for three days respectively, in the first 30 minutes, took equal volumes of semen for each sample and kept at 37 °C and examined each sample separately. As well as, was prepared a wet preparation for the assessment of the sperm motility, microscopic appearance, and dilution that is needed to assess the number of the sperm. Moreover, it was assessing the vitality of the sperm (in the case where the motile cells' percentage is low), and make semen smears for the assessment of the sperm morphology. In the first hour, the most suitable samples for the study were selected. Where, in the current study the samples which contain oligoasthenozoospermia, higher numbers of abnormally shaped sperm (more than 40%) figure (1) and high rates of pyospermia (more than 10 of leukocytes in the high-power field (HPF)), figure (2), under a microscope were neglected so as to avoid some of the possible causes of the dead of spermatozoa early as well as, it was determined the ratio of motile spermatozoa to non-motile. The samples were examined in the first hour by taking 0.1 ml of semen using an automatic sterile pipette and placed it on the glass slide and then covered with the glass cover then left for one minute to settle, then the samples were placed under the microscope at the low-power field (LPF) then at the high-power field and (HPF) for examination. Fig.1 and Fig.2 (Photograph by the author).

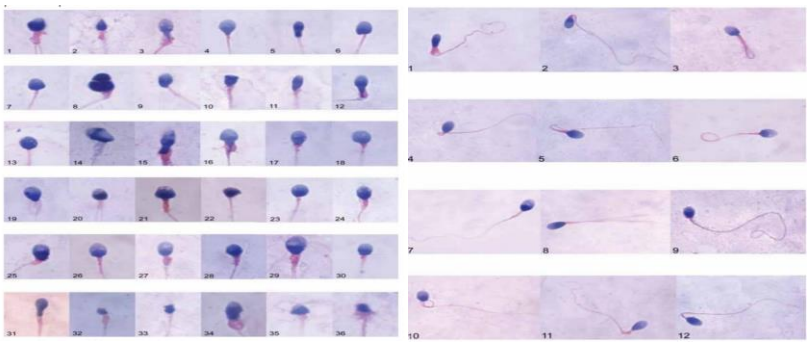

FIGURE 1. Some of the abnormal forms of human spermatozoa.

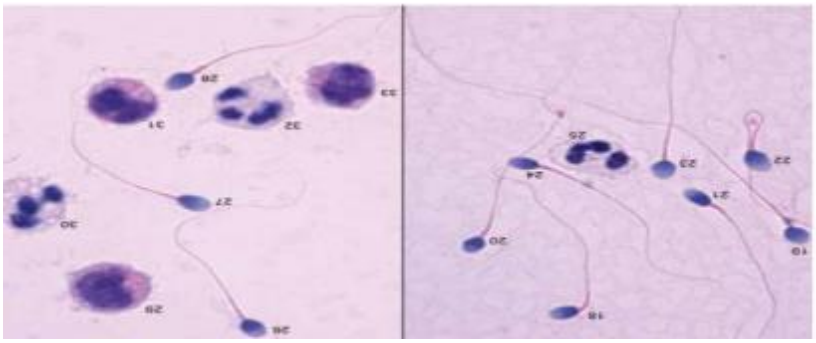

FIGURE 2. Leukocytes in a semen sample.

Calculating the Ratio of Pyospermia

The pyospermia ratio was calculated numerically under the microscope, where this examination was done under the high-power field (HPF). The process was done in the first hour of ejaculation in order to neglect samples that contain a high percentage of pyospermia (more than 10 of leukocytes under HPF).

Calculating the Activity Ratio of Spermatozoa

This was done by placing a small drop of semen on the glass slide and examined under the low-power field (LPF) and (HPF), where several and different microscopic spaces were examined, then the percentage of motile sperms to non-motile were estimated, as following :

$$\% \text{ sperm active } = \frac{\text{The number of active sperm in sample}}{\text{Total number of sperm in sample}} \times 100 \quad (1)$$

The samples were divided into three groups depending on the motility of sperm in the first hour, as follows:

1. The first group (the percentage of activity is weak): This group included the percentage of active sperms that less than 40 %, where seven samples were selected for this group.
2. The second group (activity rate is average): This group included the percentage of active sperms that range between 40-60%, where seven samples were selected for this group.
3. The third group (activity rate is high): This group included the movement percentage of sperms that exceeded 60%, as well as, seven samples were selected for this group.

The age of sperm in vitro was estimated per hour from the first hour of taking the sample, by observing the movement of sperm for the three groups by calculating the percentage of movement of spermatozoa while recording ratios of movement per hour until the death of the sperm completely. Where the spermatozoa inability to move under the microscope is considered an indication of the death of spermatozoa completely.

Statistical Analysis

Using SPSS statistical package for social sciences, all data were analyzed using ANOVA at a significance level $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of the current study showed significant differences ($p < 0.05$) in the remained time period for spermatozoa in vitro under the same conditions, where the lowest remained time period in vitro was recorded for six-hour while the largest remained time period was recorded about 11 hours, followed by about 10.5 hours after ejaculation . Moreover, it was found that there is a direct correlation between the increasing remained time period in vitro for spermatozoa and the percentage of active spermatozoa after ejaculation. Nonetheless, the results have shown that 15-30% of the total activity of spermatozoa decreases after two-three hours from ejaculation and the death rate reaches about 80% after six-hour and about 95% after nine-hour from ejaculation for most samples, figure (3,4,5).

In the current study, although the taken samples contain less than 10 of pyospermia, the decrease in the remained period rate of some spermatozoa samples in vitro may be due to variation in the percentage of these cells in the semen sample, where leukocyte-dependent damage to spermatozoa depends on the total leukocyte number in the ejaculation and the number of leukocytes relative to the number of spermatozoa in the sample, where leukocytes can impair sperm motility [15-17]. As well as may be due to the non-ejaculating period, especially (the penultimate abstinence period).As the epididymides are not completely emptied by one ejaculation [12], so, some spermatozoa stay from the previous ejaculation time and that can influence the range of age and the spermatozoa quality in the ejaculation [13]. Though, the extent of that influence is hard to ascertain and it's seldom taken under consideration. As well, testicular size can have an effect on the spermatogenic activity level, which affects the morphology of the sperm too [18], thus may be the effect on the motile of sperm**.** Moreover, differences in nutrition and a person's health may also have a role in the different activity of sperm and remains of it outside the body**.**

TABLE 1. Activity percentage and remaining time (h) for spermatozoa in vitro for the first group.

Sample	Time (h)					End Time (h)
	1	3	6	9	12	
1	20	15	10	5	0	9
2	35	20	15	5	0	9
3	35	30	20	15	0	11
4	25	20	10	0	0	8
5	25	15	5	0	0	6
6	25	15	10	5	0	9
7	25	20	10	5	0	9

TABLE 2. Activity percentage and remaining time (h) for spermatozoa in vitro for the second group.

Sample	Time (h)					End Time (h)
	1	3	6	9	12	
1	40	30	15	5	0	9
2	45	35	20	10	0	10
3	45	30	15	5	0	9
4	40	25	15	5	0	9
5	40	25	15	5	0	9
6	40	20	15	5	0	9
7	40	30	20	15	0	11

TABLE 3. Activity percentage and remaining time (h) for spermatozoa in vitro for the third group.

Sample	Time (h)					End Time (h)
	1	3	6	9	12	
1	65	45	25	10	0	10
2	65	55	40	20	0	11
3	60	45	25	5	0	9
4	55	45	35	25	0	10
5	65	50	30	5	0	9
6	75	60	35	10	0	10
7	65	55	30	15	0	11

CONCLUSION

The results showed that 15-30% of the total activity of spermatozoa decreases after two-three hours from ejaculation and the death rate reaches about 80% after six-hour and about 95% after nine-hour from ejaculation for most samples. Moreover, it was found that there is a direct correlation between the increasing remained time period in vitro for spermatozoa and the percentage of active spermatozoa after ejaculation.

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