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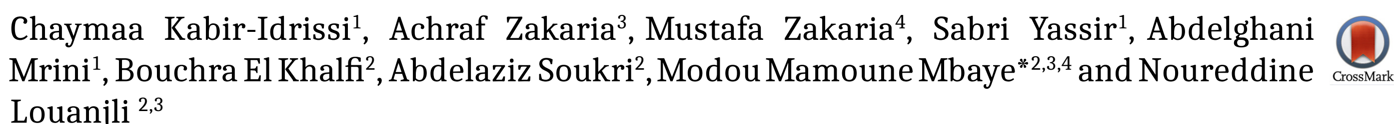
The Influence of Incubation Temperatures 25°C versus 36°C on Sperm Survival after Improvement



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ABSTRACT

Just like in women, the causes of infertility in men are many and varied. To remedy this, several couples resort to medically assisted procreation (PAM). However, the latter comes up against the problem of the sperm quality of the donor. For this, subnormal semen samples are treated with specific chemical agents before artificial insemination to improve the fertilizing power of the sperm. Because sperm health plays a decisive role in male fertility, survival is correlated with the fertilizing power of sperm. It is in this perspective that our study is part of our study consisting in following the survival of the sperm after improvement and incubation at 25 °C versus 36 °C in order to find the optimal temperature of incubation for an apparent increase. Poor results from in vitro fertilization (-20%). The results of the effect of incubating samples at 25 °C and 36 °C on the mobility and vitality of human spermatozoa which were followed in different incubation times (1,2, 3 and 4h) showed that 'after 1 hour of incubation the mobility and vitality at 25 °C dropped considerably compared to that of the sample at 36°C. After 2 hours of incubation time, we note that the two samples drop drastically but still with the samples at 36°C which have kept the best mobility and vitality. This same phenomenon was also noted at 2 and 3 hours of incubation at 25 and 36 °C. It should be remembered that the most favorable temperature was 36°C because the highest fertilization rate was noted at this temperature.

1. Introduction

Infertility is one of the new scourges of the 21st century, and more and more couples are affected every year. Difficulties in achieving pregnancy used to be attributed to women. But today, male infertility alone or not is present in more than 50 % of couples' infertilities Comhaire *et al.*, 1986). The main causes of decreased sperm survival can be due either to the presence of flagellar alteration (Cosson *et al.*, 2004) or to secondary necrozoospermia, which can be due to genital infection (Mbaye *et al.*, 2019), oxidative stress (Bansal et al., 2011), the presence of anti-sperm antibodies (Dimitrova *et al.*, 2017), impaired ATP production (Folgerø *et al.*, 1993), toxic exposure (Hernández-Ochoa *et al.*, 2005) or abnormalities in epididymal sperm transport (El-Taieb *et al.*, 2009).

Infertility in men is manifested in 61% of cases by quantitative (spermogram) and/or qualitative (decreased sperm motility, vitality, and morphology) abnormalities (Elezaj, S. (2020). Qualitative abnormalities, including sperm survival, on the chances of conceiving in *in vitro fertilization (IVF)* or naturally (Lewis *et al.*, 2013). They play an essential role in the penetration of the spermatozoon into the cumulus oophorus (Hong *et al.*, 2004) and in the processes involved in fertilization (Auger *et al.*, 2000). It is in this perspective

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that our study consists of determining the influence of the incubation temperature (25 °C versus 36 °C) on the survival or even the fertility of the spermatozoa after enhancement to find the optimal incubation temperature for an apparent increase in the poor results of in vitro fertilization (-20%).

2. Material and Methods

2.1 Collection of human semen

The samples used in all the experiments of this work were collected at the Laboratory of Medical Analyzes, Reproductive Biology, Labomac, Casablanca, Morocco. We established two study subgroups: 25 samples from male volunteers diagnosed with normozoospermia (concentration ≥ 20 x 106 / ml; progressive motility ≥ 32%). Informed consent was obtained from all included patients before using their semen in this study. Samples were collected by masturbation after 3-4 days of abstinence in sterile, labeled containers. For the liquefaction step, the samples were stored at 37 ° C until examination. We checked at an interval of 10 minutes until liquefaction was done. Microscopic analysis was performed following World Health Organization standards and guidelines.

2.2 Preparation and processing of semen

After one hour of sperm production, a routine analysis was performed to determine sperm count, motility, and vitality using a 20 µm Makler counting chamber. The density gradient optimization technique carried out the pretreatement of the spermatozoa. Thus, 1 ml of 70 % PureSperm®, 1 ml of 40 % PureSperm® and 1 ml of semen sample were processed, respectively; then, they were added to a 10 ml Falcon tube and centrifuged at 500 rpm for 20 minutes. The sperm fraction remaining at the bottom of the tube for the normozoospermic or asthenozoospermic sample was supplemented with 20 µl of ferticult, and then the whole was divided into two equal aliquots in 10 ml Falcon tubes. The first tube was incubated at 25 ° C and the second at 36 ° C for 1h, 2h, 3h, and 4h (figure 1).

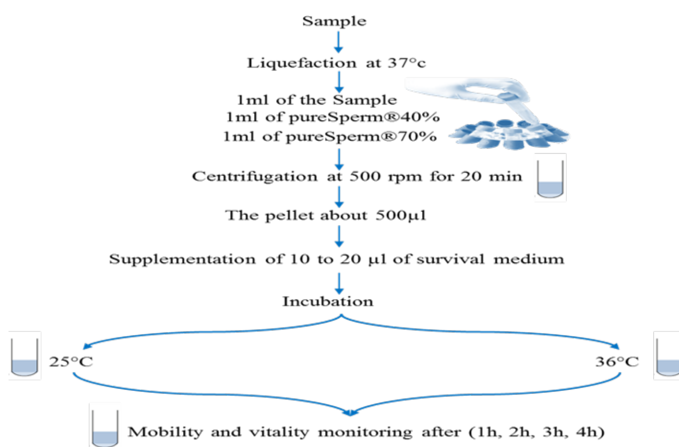

Figure 1: Sample processing treatment methodology.

2.3 Mobility test on CASA

The effect of incubating samples at 25 ° and 36 ° C on the mobility of human spermatozoa was monitored at different incubation times: 1h, 2h, 3h and 4h. The protocol consists of depositing an aliquot of 10 µl of each sample on a Makler counting chamber of 20 µm. The latter is observed at CASA "Computer Assisted Sperm Analysis" immediately after the sample is deposited Auger *et al.,* 2000). Figure 2 shows an example of the images captured and analyzed by CASA showing the different types of spermatozoa observed (Cooper *et al*., 2015) (figure 2).

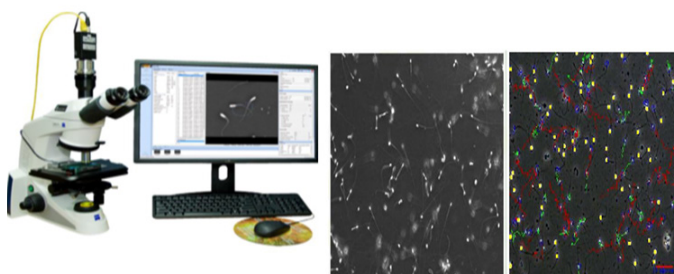

Figure 2: Analysis of sperm by CASA system analyzer software (Cooper *et al*., 2015)

2.4 Vitality test

The effect of incubating samples at 25 ° C and 36 ° C on the vitality of human spermatozoa was monitored at different incubation times: 1 h, 2 h, 3 h, and 4 h. Evaluation of sperm viability was performed with 2% eosin staining (Hirano *et al.,* 2001). From there, we proceeded to the following protocol: A drop of semen and a decline of 2% eosin solution were mixed (Hirano *et al.,* 2001). Subsequently, a smear is performed for each treatment, and 100 spermatozoa are counted on different fields of the smear and the percentage of those who are dead "pink" or alive "white" is evaluated under a white light optical microscope with a magnification x40 in. using a laboratory counter. This test is carried out after incubation at 25 ° and 36 ° C, after 1h, 2h, 3h, 4h.

2.5 Statistical analysis

The data obtained during our experiment were the subject of a statistical study. The results of monitoring the mobility and vitality of human spermatozoa after incubation at 25 and 36 ° C respectively were carried out by the Student's test (t-test). All the graphs and histograms represented in this study were produced using the software: GraphPadPrism7.

3. Result

3.1 Mobility

The results of the effect of the incubation of samples at 25 ° and 36 ° C on the mobility of human sperm which was followed in different incubation times (1,2, 3 and 4h) showed that after 1h incubation mobility at 25 ° C drops significantly from that of the sample at 36 ° C. After 2 hours of incubation time, we note that the two samples drop drastically but still with the sample at 36 ° C which retains the best mobility. This same phenomenon was also noted at 2 and 3 hours of incubation at 25 and 36 ° C (figure 3).

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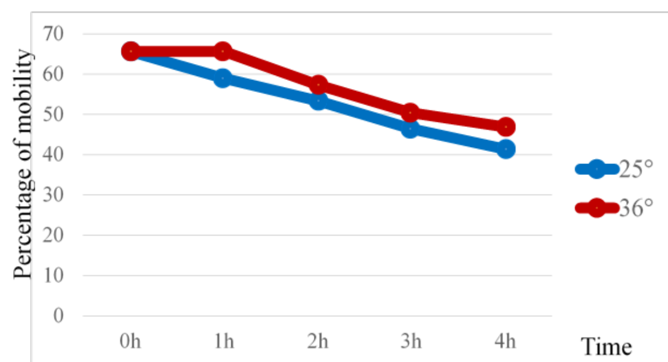

Figure 3: The effect of incubation temperatures of 25 and 36 ° C on sperm mobility after improvement

3.2 Vitality

The results of the effect of incubation of samples at 25 ° and 36 ° C on the vitality of human spermatozoa which was followed in different incubation times (1, 2, 3 and 4 h) showed that after 1 h during incubation the vitality at 25 ° C drops considerably compared to that of the sample at 36 ° C. After 2 hours of incubation time, we note that the two samples drop drastically but still with the sample at 36 ° C which retains the best mobility. This same phenomenon was also noted at 2 and 3 hours of incubation at 25° and 36°C.

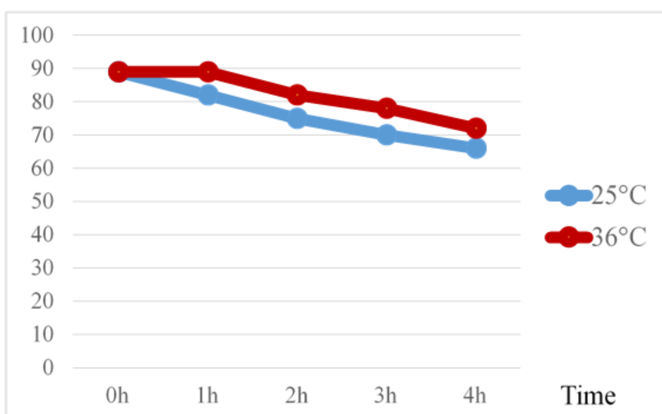

Figure 4: The effect of incubation temperatures of 25°c and 36 ° C on sperm vitality after improvement

4. Discussion

Some medically assisted procreation procedures, such as *in vitro fertilization,* intrauterine insemination, is performed with spermatozoa whose fertility has been improved (Brahiti *et al*., 2016; Liu *et al*., 2004). It is in this context that the Survival Migration Test (TMS) reproduces in vitro the filtration selection steps occurring during the ascent of spermatozoa into the female genital tract. In fact, during this test: non-motile or poorly motile sperm are eliminated; in other words, there is a selection, and at the same time, the seminal plasma (containing factors that inhibit sperm capacitation) is also eliminated. Moreover, it is replaced by a capacitating medium containing in particular energy factors essential for spermatozoa. Also, cellular debris, epithelial cells, bacterial infectious agents are eliminated. In this test, the number of progressively motile sperm selected is an expression of the fertility of the sperm. It is of utmost importance in the management of the infertile couple. It guides the assisted reproduction technique to remember. It is essential before any attempt at assisted reproduction.
The results of the effect of the incubation of samples at 25° and 36 °C on the mobility of human sperm, which was followed in different incubation times (1,2, 3 and 4h) showed that after 1h incubation mobility at 25 °C drops significantly from that of the sample at 36 °C. After 2 hours of incubation time, we note that the two samples drop drastically but still with the sample at 36 °C which retains the best mobility. This same phenomenon was also reported at 2 and 3 hours of incubation at 25 and 36 °C (figure 3).

The results of the effect of incubation of samples at 25 ° and 36 °C on the vitality of human spermatozoa which was followed in different incubation times (1, 2, 3 and 4 h) showed that after 1 h during incubation the vitality at 25 °C drops considerably compared to that of the sample at 36 °C. After 2 hours of incubation time, we note that the two samples drop drastically but still with the sample at 36 ° C which retains the best mobility. This same phenomenon was also noted at 2 and 3 hours of incubation at 25 °C and 36 °C (figure 4).

This decrease in the decline in the mobility of vitality may suggest the explanation of Folgero et al., as although reactive oxygen species (ROS) is one of the factors that damage sperm motility and vitality [6]. High levels of ROS have been shown to cause impaired sperm function in human sperm (Griveau, J. F., & Lannou, D. L. (1997). It should be remembered that the most favorable temperature for the migration and survival test was 36 °C because the highest fertility rate (mobility and vitality) was noted at this temperature.

5. Conclusion

This study highlights the influence of temperature on the survival or even the fertility of spermatozoa by measuring the mobility and vitality of the spermatozoa after improvement and incubation at 25 and 36 ° C. The results of the effect of incubating samples at 25 ° and 36 ° C on the mobility and vitality of human spermatozoa, which were followed in different incubation times (1,2, 3 and 4h) showed that 'after 1 hour of incubation the mobility and vitality at 25 ° C dropped considerably compared to that of the sample at 36 ° C. After 2 hours of incubation time, we note that the two samples drop drastically but still with the samples at 36 °C which have kept the best mobility and vitality. This same phenomenon was also noted at 2 and 3 hours of incubation at 25 and 36 °C. It should be remembered that the most favorable temperature was 36 ° C because the highest fertilization rate was noted at this temperature.

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