



## Effect of *Origanum vulgare* essential oil supplementation on advanced parameters of mobility and vitality in human normozoospermia samples

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### ABSTRACT

Male infertility, which corresponds to half of the cases in a couple infertility, is assessed primarily by sperm parameters such as sperm motility and vitality. These, according to WHO standards, provide information on the fertility potential of a man. Thus, patients with abnormal sperm parameters are more likely to fail in medically assisted procreation because a lack of sperm mobility and vitality reveals aberrations in the paternal genome. Phytotherapy, which highlights numerous molecules known for their therapeutic and anti-infectious, antioxidant, antibacterial, antifungal and other properties, seems to be suitable for improving spermatoc parameters. Also, *Origanum vulgare*, an essential oil that has already proven itself in traditional medicine and known for various curative actions, would be an asset in spermiology? Thus, in our study, we evaluated the effect of a supplementation of this essential oil in fresh sperm ejaculates. The vitality and mobility of spermatozoa were the parameters studied after 24, 48 and 72 hours of suspension of a clean fraction of spermatozoa in *Origanum vulgare*.

### 1. INTRODUCTION

Today, phytotherapy has become the basis of treatments to alleviate the problem of health, due to the natural properties that its plants have and their interest that grows in various applications (FALCH et al., 2013). In fact, thanks to the virtues of its plants, it is possible to remedy the problems of infertility, which is a growing scourge that affects many couples in the 21st century (Mbaye et al., 2020). Even if most of the time, the blame is put on the woman, male infertility does exist and represents 50% of the cases shown by quantitative (sperm count) and

qualitative (decrease in mobility, vitality and morphology of spermatozoa) medical analysis (Mbaye et al., 2021). Thus, in our study, we will focus on qualitative abnormalities and specifically on motility, vitality and sperm count. Because of the importance of these two parameters on the chances of conceiving either by in vitro fertilization (IVF) or naturally and whose veracity is no longer to be demonstrated. In order to improve the low success rate of pregnancies and to give hope to these couples in need. With this in mind, we have tested the effect of essential oils of *Origanum vulgare* (oregano) on the mobility and vitality of the patients' sperm after 24, 48 and 72 hours.

### MATERIALS AND METHODS

#### Chemicals

All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored under standard physicochemical conditions.

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### Collection of human semen

This study was performed at the Laboratory of Medical Analysis and Reproductive Biology, “Labomac”, Casablanca, Morocco. We established a study group of 25 samples from male volunteers diagnosed as normozoospermic (progressive motility < 32%). Then, the samples were collected after 3 to 4 days of abstinence in sterile, labeled containers (Oukaci and Chikhoun, 2017). For liquefaction, samples were stored at room temperature until use. We checked at a time interval of 10 minutes until liquefaction was done. Microscopic analysis was performed according to World Health Organization (WHO) standards and guidelines.

### Semen preparation and processing

After one hour of semen production, sperm analysis (detection of count, motility and vitality) was performed using a 20 µm Makler counting chamber and sterile collection tips. Semen pretreatment was performed using the density gradient optimization technique. For this purpose, 1ml of PurSperm® 70%; 1ml of PurSperm® 40% and 1ml of semen sample were respectively added in a 10ml vial tube for each treatment and centrifuged at 500 rpm for 20 minutes. The 500 µl pellet was then divided into two equal aliquots (of 250 µl each), one of which was supplemented with 10 µl of ferticult and then each tube was divided into two. The first two tubes contained respectively 125µl of the pellet (control) and the other 125µl of the pellet plus 10µl of essential oil. Then the last two tubes contained respectively 130µl of the pellet (control) and the other 130µl of the pellet plus 10µl of essential oil, all incubated at room temperature for 24h, 48h and 72h (figure 1).

### Motility test on CASA

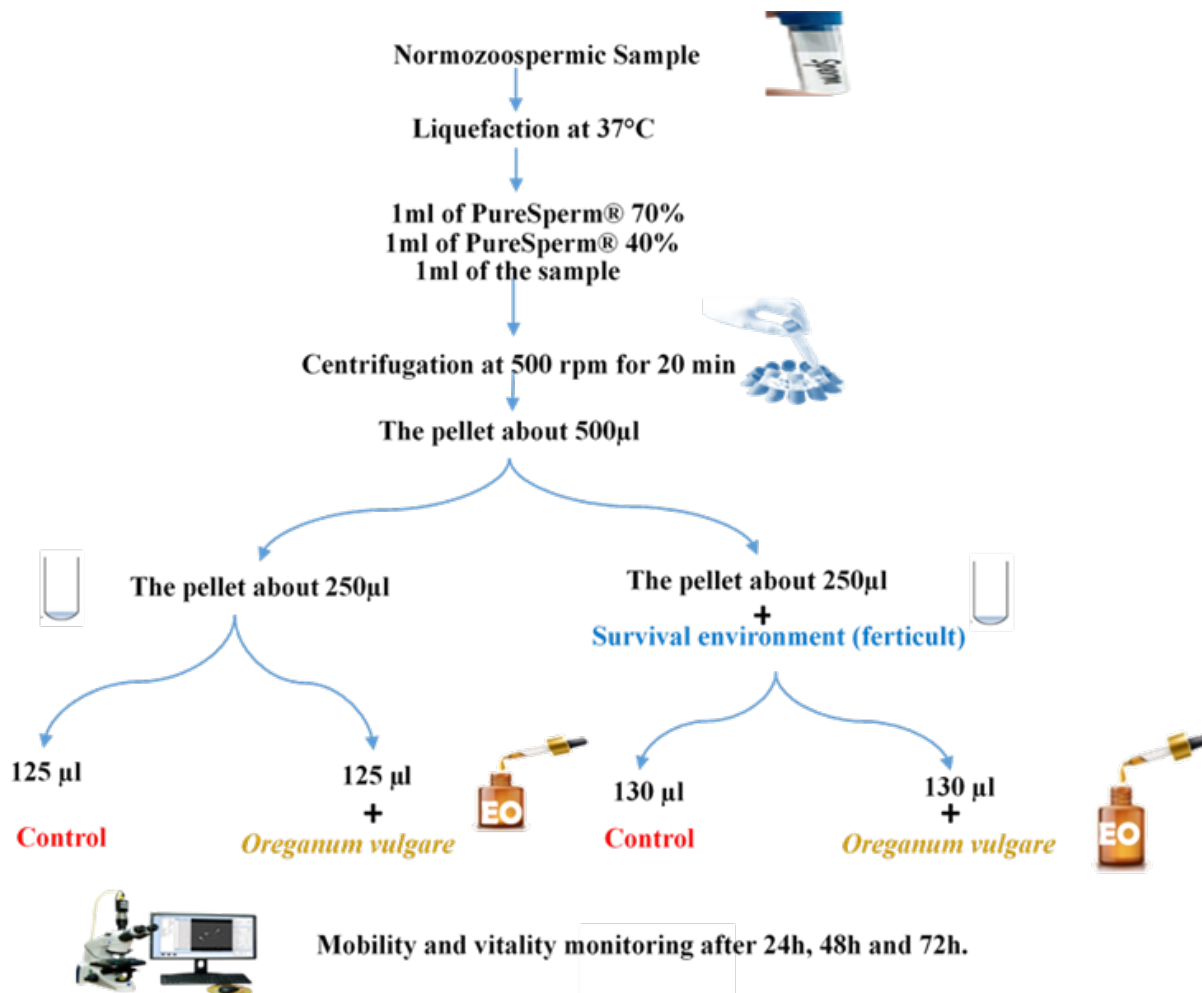
The effect of sage, oregano essential oils on human sperm motility was monitored at different contact times: 24h, 48h, and 72h at room temperature. The protocol consists of depositing a 20-µl aliquot of the mixture (sperm/oil, control) on a 20-µm Makler counting chamber. The latter is put under observation at CASA “Computer Assisted Sperm Analysis” just after the mixture is deposited (Schill et al., 2006) (Figure)

### Vitality test

The evaluation of sperm viability was performed with a 2% eosin stain (Hirano et al., 2001). From there, we proceeded with the following protocol: One drop of semen and one drop of 2% eosin solution were mixed. Subsequently, a smear is taken for each treatment and 100 spermatozoa are counted on different fields of the smear and the percentage of those that are dead “pink” or alive “white” is evaluated under a white light microscope with x40 magnification using a laboratory counter. This test is performed after contact between spermatozoa and the different essential oils studied, in a contact time range of 24h, 48h and 72h after incubation at room temperature (Brahiti et al., 2016).

### Statistical analysis

The data obtained in our experiment were statistically studied. The results of the essential oil supplementation studied on the physiological parameters (motility and vitality) of human spermatozoa were performed by Student’s t-test. All the graphs and histograms represented in this study were made using the software: Graph Pad Prism7.

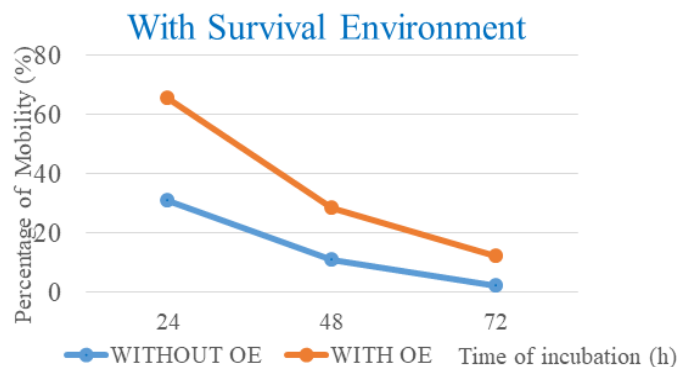


**RESULTS**

**Evaluation of the mobility and vitality of the samples supplemented with ferticult.**

**Mobility**

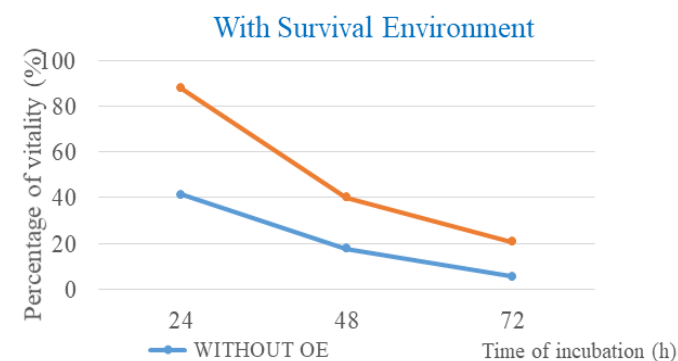
The results of the respective supplementation of survival medium (ferticult) and essential oil of oregano in normozoospermic samples after a contact time of 24h, 48h and 72h at room temperature shows an increase in mobility of about 65% in 24h, 30% in 48h and 15% in 72h compared to those of the control not supplemented with essential oil which gives respectively 30, 10 and 0% mobility in 24h, 48h and 72h (figure 2).



**Figure 2 :** The effect of essential oil supplementation with survival medium on sperm motility after enhancement

**Vitality**

The vitality of these same samples after 24h, 48h, and 72h under the same conditions gives the same variation profile as the mobility respectively 85%, 40% and 20% for the samples supplemented with oregano and 40%, 19%, and 5% for the samples not supplemented with oregano (control) after 24h, 48h and 72h of incubation at room temperature (figure 3).



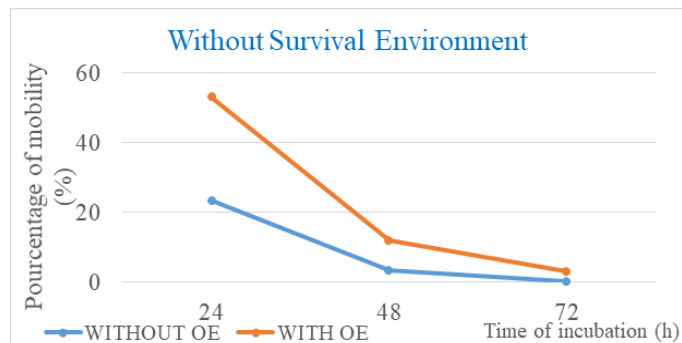
**Figure 3 :** The effect of essential oil supplementation with survival medium on sperm vitality after enhancement

**Evaluation of mobility and vitality of samples not supplemented with ferticult**

**Mobility**

The results of the supplementation of essential oil of oregano without survival medium (ferticult) in normozoospermic samples after a contact time of 24 hours, 48 hours and 72

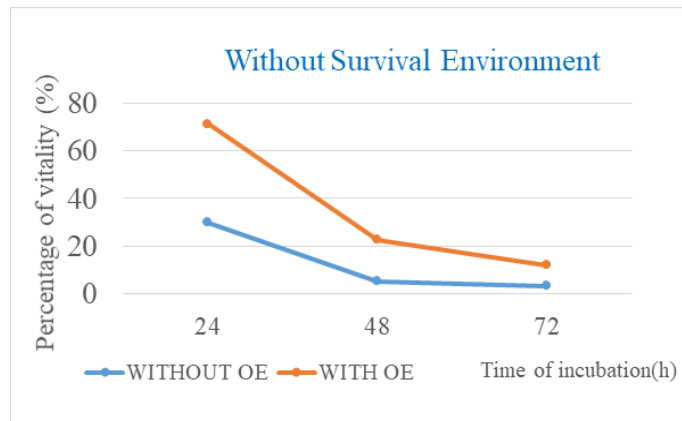
hours at room temperature shows an increase in mobility of about 52% in 24 hours, 10% in 48 hours and 0.3% in 72 hours compared to those of the control not supplemented with essential oil, which gives respectively 22-3 and 0% mobility in 24 hours, 48 hours and 72 hours (figure 4).



**Figure 4 :** The effect of essential oil supplementation without survival medium on sperm motility after enhancement

**Vitality**

The vitality of these same samples after 24, 48 and 72 hours under the same conditions gives the same variation profile as the mobility respectively 70%, 21% and 10% for samples supplemented with oregano and 30%, 5% and 2% for samples not supplemented with oregano (control) after 24, 48 and 72 hours of incubation at room temperature (Figure 5).



**Figure 5 :** L'effet de la supplémentation de l'huile essentielle sans milieu de survie sur la vitalité des spermatozoïdes après amélioration

**DISCUSSION**

The results of the respective supplementation of survival medium (ferticult) and essential oil of oregano in normozoospermic samples after a contact time of 24h, 48h and 72h at room temperature shows an increase in mobility of the order of 65% in 24h, 30% in 48h and 15% in 72h compared to those of the control not supplemented with essential oil which gives respectively 30, 10 and 0% of mobility in 24h, 48h and 72h (figure 2) The vitality of these same samples after 24h, 48h, and 72h under the same conditions gives the same variation profile as the mobility respectively 85%, 40% and 20% for the samples supplemented with oregano and 40%, 19%, and 5% for the samples not supplemented with oregano (control) after 24h, 48h and 72h of incubation at room temperature (figure 3).

The results of the supplementation of essential oil of oregano without survival medium (ferticult) in normozoospermic samples after a contact time of 24h, 48h and 72h at room temperature shows an increase in mobility of about 52% in 24h, 10% in 48h and 0.3% in 72h compared to those of the control not supplemented with essential oil which gives respectively 22-3 and 0% mobility in 24h, 48h and 72h. (Figure4)

The vitality of these same samples after 24h, 48h, and 72h under the same conditions gives the same variation profile as the mobility respectively 70%, 21% and 10% for the samples supplemented with oregano and 30%, 5%, and 2% for the samples not supplemented with oregano (control) after 24h, 48h and 72h of incubation at room temperature (figure5).

The results of the study of the supplementation of oregano essential oil on the advanced parameters of sperm motility and vitality show that oregano essential oil could be an alternative to improve the parameters of motility and vitality to help couples in need. After interpretation of the results of the effect of the essential oil supplementation on the advanced parameters of mobility and vitality, we can say that after incubation at room temperature, the samples with essential oil plus survival medium gave excellent results compared to the samples with survival medium and without essential oil at the level of mobility as well as vitality.

However, these results drop slightly after 48h of incubation. And totally after 72h of incubation at room temperature. However, these results drop according to the parameters (mobility and vitality) observed at the beginning, well before the addition of essential oil on the semen samples. On the other hand, in the control samples without survival medium and without essential oil, the results remain very basic (whether in mobility or vitality) compared to the samples containing essential oil and without survival medium, whether after 24h, 48h or even 72h, according to the experiments performed. From the results of the oreganum vulgare supplementation, it is clear that the results obtained with oreganum are better than those without the essential oil (Mousa *et al.*, 2020).

In general, the study carried out to show the improvement of oregano oil on the advanced parameters of sperm motility and vitality, so we can conclude that the essential oil of oregano could be a therapeutic alternative (Mbaye *et al.*, 2020). The exact mechanism by which essential oils improve the motility and vitality of human sperm, remains unknown and is therefore of great interest (De Iuliis *et al.*, 2009).

## CONCLUSION

The evaluation of the effect of oregano essential oil supplementation on the advanced parameters of sperm motility and vitality allowed us to evaluate the biological facades of this plant. The oil of oregano is an alternative to succumb to the infertility problems of couples. However, it would be very interesting to explore the mechanism by which oregano oil improves sperm parameters.

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