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The effect of in vitro exposure to certain metformin doses on the motility of human spermatozoa.

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ABSTRACT

Metformin is an anti-hyperglycemic medication used to treat type 2 diabetes mellitus. Regardless of its anti-diabetic effect, metformin has a non-genomic action and it could be a beneficial additive to treat the sperm which considered as transcriptionally dormant cell and also a molecule that could rapidly adjust the metabolism of spermatozoa to adapt it to the surrounding environment. Sixty-four normozoospermic semen sample included in the study. Each sample incubated for 30 minutes with metformin (2.5 and 0.5 µM) and SFA was conducted to each sample.
There was a significant decrease in total motility (progressive and non-progressive) in samples incubated with both concentrations compared with the neat samples. While the samples incubated with 0.5 µM shows a lower motility compared with 2.5 µM. Metformin significantly reduced total motility of fresh spermatozoa at both concentrations.

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KEYWORD

Metformin, Spermatozoa, Motility, AMPK

1. Introduction

Metformin (1,1-dimethylbiguanide hydrochloride) is an anti-diabetic and oral hypoglycemic agent it considered as a standard medication for the treatment of type 2 diabetes mellitus **(LaMoia and Shulman, 2021 [1])**.

Metformin is also used in patients with type 1 diabetes mellitus in combination with insulin **(Beysel et al., 2018 [2])**. It is considered also as a safe alternative drug for women with gestational diabetes **(Jorquera et al., 2020 [3])**.

Regardless of its anti-diabetic effect, metformin is also used to treat women with polycystic ovarian syndrome PCOS; it has the ability to decrease insulin resistance and significantly reduce blood androgen levels and body weight; metformin also considered as an effective ovulation induction agent in non-obese PCOS patients and associated with menstrual cycle regulation **(Gadalla et al., 2020 [4])**.

Metformin also has possessed anti-cancer properties **(Yu et al., 2019 [5]).** Recent meta-analysis showed that patients with T2DM receiving metformin had a lower risk of cancer occurrence compared to those taking other antidiabetic medications **(Hattum et al., 2022 [6]**; **Xin Jia et al., 2022 [7])**, especially pancreas, colon, and hepatocellular carcinoma (**Matar, S., 2021 [8]; Saraei et al., 2019 [9])**.

A number of chemicals have been added to the semen and have been found to improve sperm motility **(Nguyen et al., 2016 [10])**. One of these compounds is the biguanide metformin, which can modulate metabolism, reduce reactive oxygen species, activate the transcription factor Nrf2, and boost the expression of genes related to antioxidants **(Nguyen et al., 2019 [11])**.

Metformin can 1) decrease the activity of mitochondrial complex 1, which lowers the quantity of ROS, while also having an impact on the cytoplasm **(Bertoldo et al., 2014 [12])**. 2) AMP-activated protein kinase (AMPK), which is regarded as a critical regulator

of the energy balance within cells **(Martin-Hidalgo et al., 2018 [13])**. Is also stimulated by metformin. As a result, cells shift from an anabolic to a catabolic state **(Nowicka and Szymczak, 2021 [14])**. 3) Because of its non-genomic activity, metformin may be an effective supplement for treating sperm that are thought to be transcriptionally quiescent cells. It is also a chemical that can quickly change the metabolism of spermatozoa so that it can adapt to its environment **(Tartarin et al., 2012 [15]).**

2. Subject material and method

A sixty-four normozoospermic semen samples were used. For the removal of seminal plasma, the samples were centrifuged for 3 minutes at 3000 rpm; then, the supernatant were removed and resuspended with prewarmed metformin solutions in two concentrations (0.5 and 2.5 µM). Each sample were incubated with metformin for 30 minutes in 37℃, and then SFA was applied to record the differences that occurred in fresh sperm parameters.

1- Statistical analysis

Version 26 of the Statistical Package for Social Sciences (SPSS) was used to analyze the data. The data were provided as mean, SD, and ranges, expressed as frequencies and percentages for categorical data. In order to compare the continuous variables appropriately, an independent t-test and two-tailed Analysis of Variance (ANOVA) were utilized. A p-value of less than 0.05 was regarded as significant.

3. Results

Comparison in sperm parameters between fresh semen samples and semen samples incubated for half an hour with (2.5 and 0.5 µM) metformin **(Table 1)** shows the comparison in semen parameters between three groups of the study,

including fresh semen samples and semen samples incubated for a half hour with 2.5 µM and 0.5 µM metformin. The mean of sperm concentration and motility (progressive and non-progressive) were significantly higher in fresh samples than in other groups. In terms of immotile sperm, the results showed that there was a significant decrease in fresh samples than that in samples incubated for half an hour with (2.5 µM, 0.5 µM) metformin **(Figure1).** Lower significant differences (LSD) or post hoc tests were run to confirm the differences that occurred in the mean of sperm parameters between fresh semen samples and semen samples that were incubated for half an hour with 2.5 µM and 0.5 µM metformin **(Table 2).**

The mean concentration of sperm between semen samples incubated for 30 minutes with (2.5 µM and 0.5 µM) metformin showed that there were no differences between them, while sperm concentration was significantly higher ($p < 0.005$) in fresh samples than that in half-hour incubated with 2.5 µM metformin and half hour incubated with 0.5 µM metformin samples **(Table 2)** . Although, the mean of progressive motility was significantly higher ($p < 0.001$) in half-hour incubated with 2.5 µM metformin samples than that in half-hour incubated with 0.5 µM metformin samples, the fresh semen samples still showed a higher significant difference from both groups (2.5 µM, and 0.5 µM metformin incubated samples) as shown in **(Table 2)** No statistically significant differences in non-progressive motility parameters ($p \geq 0.05$) between half hour incubated with 2.5 µM metformin and half hour incubated with 0.5 µM metformin samples as shown in **(Table2)**. Even though there was a difference in fresh samples higher than those two groups (2.5 µM and 0.5 µM metformin incubated samples) with a p-value of 0.001 .The mean of immotile spermatozoa in half-hour incubated with 2.5 µM metformin samples was

lower than that in half-hour incubated with 0.5 µM metformin samples with a significant p-value ($p < 0.001$). Nevertheless, the fresh samples were lower than the two groups (2.5 µM and 0.5 µM incubated with metformin) with a high significant value ($p<0.001$) **(Table 2)**. The percentage of change in sperm parameters in half-hour incubated with 2.5 µM metformin samples is illustrated in **(Figure 2)**. It was obvious that sperm concentration, sperm progressive motility, and sperm non-progressive motility were decreased in half-hour incubated with 2.5 µM metformin samples when compared to fresh samples .While the percentage of immotile sperm was increased in half an hour and incubated with 2.5 µM metformin samples as compared to fresh samples **(Figure 2)**. The percentage of change in sperm parameters regarding samples incubated with 0.5 µM metformin for a half hour is shown in **(Figure 3)** Sperm parameters regarding concentration, progressive motility, and non-progressive motility showed a decrease in half-hour incubated with 0.5 µM metformin samples compared to fresh samples **(Figure 3)**.On the other hand, the percentage of immotile sperm was increased in half-hour 0.5 µM metformin incubated samples as it compared with the fresh samples **(Figure 3)**.

Table (1): Comparison in sperm parameters between fresh semen samples and semen samples incubated for half an hour with 2.5 and 0.5 µM metformin

sperm parameters	**Sample**			**F - value**	***p* - Value**
	Fresh Mean ± SD	30 min 2.5 µM Mean ± SD	30 min 0.5 µM Mean ± SD		
Concentration (10^6/ml)	30.51 ± 10.0	25.98 ± 8.5	25.98 ± 8.5	5.367	0.005
Progressive (%)	46.18 ± 12.0	37.79 ± 12.1	31.65 ± 9.2	27.155	0.001
Non-Progressive (%)	19.12 ± 7.3	12.01 ± 5.8	12.01 ± 5.8	26.706	0.001
Immotile sperm (%)	34.68 ± 10.9	50.18 ± 11.6	56.32 ± 9.1	71.412	0.001

Table (2): Lower Significant differences test (LSD) to confirm the difference in sperm parameters between fresh semen samples and semen samples incubated for half an hour with 2.5 and 0.5 µM metformin

Sperm parameter	Sample			
	Fresh Mean ± SD	**30 min 2.5 µM Mean ± SD**	**30 min 0.5 µM Mean ± SD**	***p* - Value**
Concentration (10^6/ml)	30.51 ± 10.0	25.98 ± 8.5	-	0.005
	30.51 ± 10.0		25.98 ± 8.5	0.005
	-	25.98 ± 8.5	25.98 ± 8.5	1.0
Progressive motility (%)	46.18 ± 12.0	37.79 ± 12.1	-	0.001
	46.18 ± 12.0	-	31.65 ± 9.2	0.001
	-	37.79 ± 12.1	31.65 ± 9.2	0.002
Non-progressive motility (%)	19.12 ± 7.3	12.01 ± 5.8	-	0.001
	19.12 ± 7.3	-	12.01 ± 5.8	0.001
	-	12.01 ± 5.8	12.01 ± 5.8	1.0
Immotile sperm (%)	34.68 ± 10.9	50.18 ± 11.6	-	0.001
	34.68 ± 10.9	-	56.32 ± 9.1	0.001
	-	50.18 ± 11.6	56.32 ± 9.1	0.001

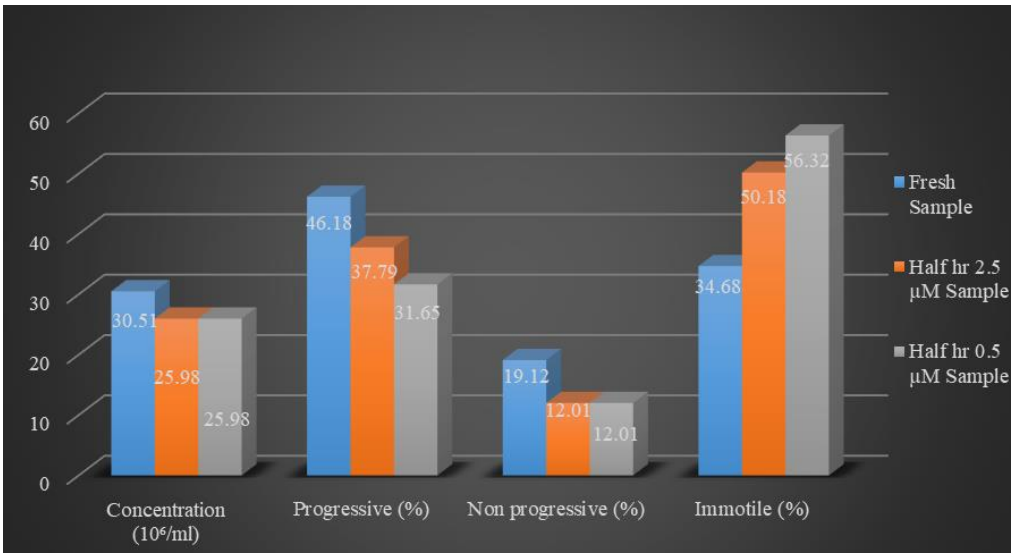


Figure (1): Sperm concentration and motility in fresh semen samples and semen samples incubated for half an hour with (2.5 and 0.5 µM) metformin

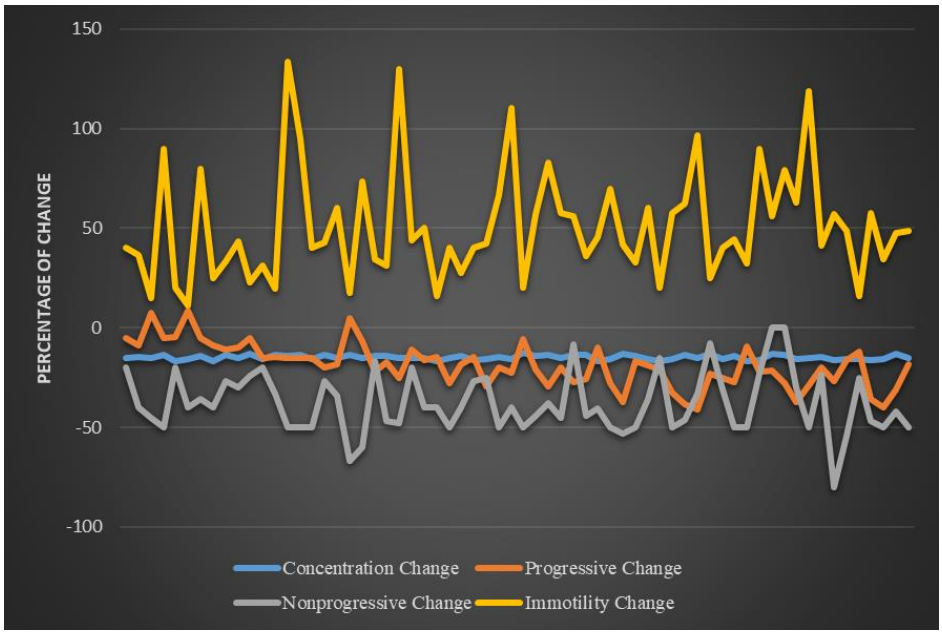
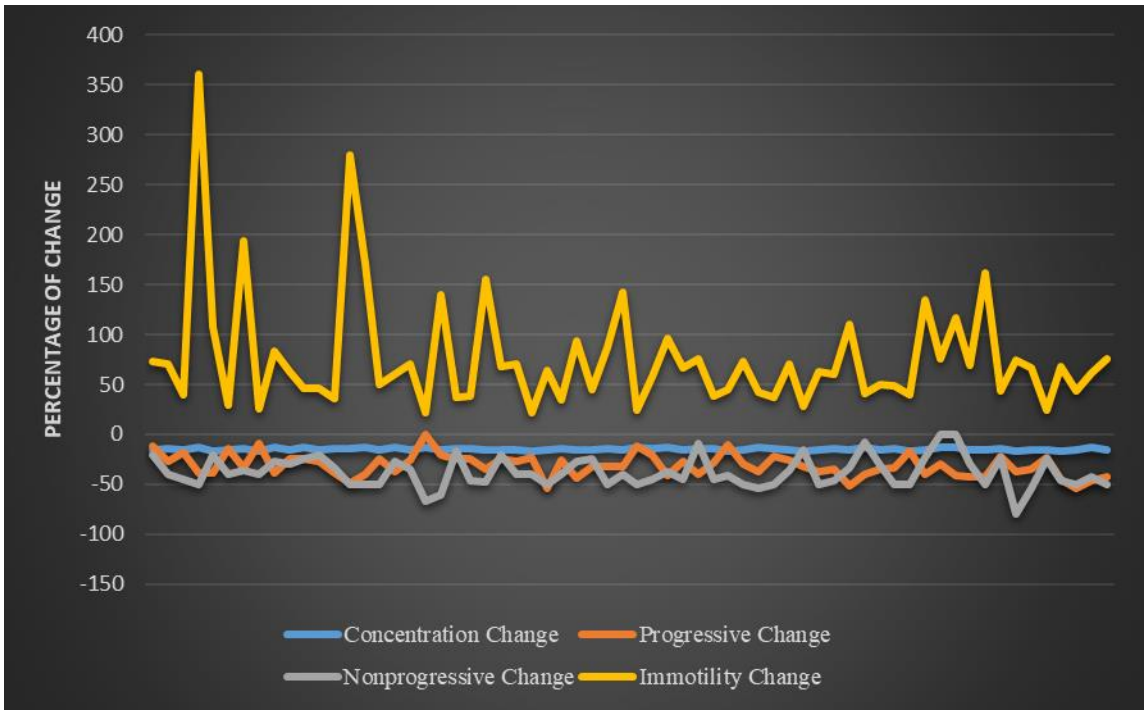


Figure (2): Percentage of Change in sperm parameters in half-hour incubated with 2.5 µM metformin samples compared to fresh samples



Figure(3): Percentage of change in sperm parameters in half-hour incubated samples with 0.5 µM metformin compared to fresh samples

4. Discussion:

1- Characteristics of male patients in this study

According to the WHO 2010 classification, all of the male patients included in this study fell into the normozoospermia group. This criterion was adopted to ensure that any apparent changes on sperm parameters are only attributable to the use of metformin and not to any other cause by reducing sample variances in comparison to utilizing oligozoospermic or asthenozoospermic samples.

2- Effect of metformin on fresh semen parameters

In the present study, the effect of certain doses of metformin on fresh semen parameters was illustrated in **(Table 2)**. The results showed a significant decrease in total motility in fresh samples with both concentrations of metformin (2.5, 0.5) compared to fresh semen samples (p 0.001), while

the 0.5 µM showed a significant decline in motility compared to 2.5 µM .

This reduction in the number of motile sperm in the samples with metformin may be due to the ability of metformin to inhibit mitochondrial complex 1, thereby reducing mitochondrial function and cellular respiration, leading to reduce the ATP production, which is necessary for sperm motility characteristic.

Several studies conducted on human, board, and mouse sperm have shown similar results and found out that the exposure of sperm to metformin reduced motility at a different level and that depend on the concentration used, species of the animal, and the time of exposure and these studies indicated the reduction in motility is due to the inhibition of mitochondrial complex 1and, in turn, lowering the ATP production **(Bertoldo et al., 2014 [12]; Calle-Guisado et al., 2019 [16]; Hurtado de Llera et al., 2018 [17])** For instance, a study done on murine and bovine suggested that the decrease in motility is due to the continuous activation of AMPK which could lead to a dysregulation in ATP production **(Londoño-Vásquez et al., 2022 [18])**.

Recent study found out that the using of metformin in a therapeutically relevant concentration does not effect on the motility of fresh human spermatozoa at high doses; this study used a longer incubation period from (4 – 24) hours compared to the current study (30 minutes) **(Yang et al., 2020 [19]).**

Another study conflict with the current finding observed that incubation of sperm for 4 hrs. with metformin increases motility **(Li et al., 2020 [20]).** This observation may be due to the longer exposure time to metformin as well as the type of media used in that study, which contains high levels of glucose, pyruvate, and lactate, which may interfere with the motility inhibiting role of metformin, where Gravel et al. found that the effect of metformin depends on the metabolites in the culturing media. While in the present study, PBS was used, which has

no energy supplements in its content and, in turn, does not interfere with the effect of metformin **(Gravel et al., 2014 [21]).**

5. Conclusion

Metformin significantly reduced the total motility of fresh spermatozoa at both concentrations.

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Author Contribution Fatima Emad Al-nashme performed the study, and Wasan Adnan Al-jubory supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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