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Comparative study on the effect of platelet-activating factor, caffeine liquid and pentoxifylline on asthenospermia by CASA and Sperm Survival Test (SST)

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ABSTRACT

The inability to be pregnant after at least 12 months of unprotected sexual activity is a sign of infertility, which can affect either the male or female reproductive system. The aim of this work was to compare between Pentoxifylline (PX) versus Platelet-activating factor (PAF) and Caffeine liquid (CL) on sperm activation in Asthenozoospermia casas.In this clinical experiment with a prospective randomized double blind design, 82 male infertile couples who had been having regular sex for at least two years participated, all patients were Asthenospermia according to WHO criteria 2010 aged from 22 to 47 years old, normal ejaculates will serve as the study's sperm supplies. Each sample was divided into three tubes and control tube named Group (1): Control. Group (2): PX. Group (3): PAF. Group (4): CL. The results indicated that adding CL to the semen resulted in an increase in the sperm motility after one hour. However, that significance faded on the subsequent readings. Adding PAF led to a significant increase in sperm motility but less than CL. Adding PX led to a significant increase in sperm motility but less than PAF. Motility decreased after one hour. However, it was significantly better than the baseline. At the two and three hours readings, motility became less than the first hour. In conclusions CL has more pronounced and durable effect on sperm activation in Asthenospermia cases than of PX and PAF. It is better to use semen prepared by CL within one hour of preparation, as it has more pronounced and durable effect within this period.

***Keywords*:** Asthenospermia**,** Caffeine liquid, sperm activation, Pentoxifylline, Platelet-activating factor.

INTRODUCTION

A disorder of the male or female reproductive system known as infertility is defined as the inability to conceive following at least a year of unprotected sexual activity. Infertility affects millions of individuals of reproductive age worldwide and impacts their families and communities. Between 48 million couples and 186 million people worldwide are thought to be infertile (WHO, 2021). Asthenospermia is one of the key factors contributing to male infertility and improving sperm motility are linked to improved outcomes following either in vitro fertilization or intrauterine insemination (Tournaye and Cohlen, 2012). Tu *et al.* (2020) mentioned that one of the main reasons of male infertility is asthenozoospermia (AZS), which is characterized by low or nonexistent sperm motility. Multiple substances have been developed to improve sperm motility in males with sperm motility issues. The use of additives to increase sperm motility, involving but not restricted to cyclic

adenosine monophosphate (cAMP), human follicular fluid, xanthine, caffeine liquid (CL), Pentoxifylline (PX), and platelet activating factor (PAF), has been studied (Archer and Roudebush, 2013). Dadgar *et al.* (2023) found that PX causes the phosphodiesterase (PDE) enzyme to be inhibited, which raises intracellular cAMP levels and increases cellular glycolysis and adenosine triphosphate (ATP) energy synthesis in response. This will cause increasing in sperm motility. On the other hand, pellet activating factor is is being employed as a unique treatment alternative ad is characterized by an autologous concentration of human platelets that is three to five times greater than the normal level of thrombocytes in whole blood (Xu *et al.*, 2020). Megakaryocyte-derived platelets have granules that contain many secretory proteins. Through growth factors, they belong to the cytokine, chemokine, and chemo family and their thick granules have a role in regulating and speeding up the healing of wounds (Hamdan *et al.*, 2021).

Caffeine liquid (CL) (1, 3, 7-trimethylxanthine) is present in coffee, tea, soft drinks (especially cola and energy drinks), and chocolate. Among the many physiological advantages of CL are their ability to stimulate the central nervous system, increase the production of catecholamines, relax smooth muscles, and increase heart rate. It is acknowledged to have both favorable and harmful health consequences (Ricci *et al.*, 2017). CL's effects range from stimulating the central nervous system to elevating catecholamine release, relaxing the muscles, and stimulating vital signs (Kumar *et al.*, 2022). CL is a cyclic nucleotide PDE inhibitor that significantly enhanced and preserved the respiration and motility of ejaculated spermatozoa and it may operate directly via influencing cellular metabolism, and its action is dependent on calcium ion concentration (Ramirez-Perez *et al.*, 2021).

The aim of this work was to compare between Pentoxifylline versus platelet activating factor and Caffeine liquid on sperm activation in asthenozoospermia cases.

PATIENTS AND METHODS

This prospective randomized double blind clinical trial involved 82 guys who presented with infertility two years or more in present of regular intercourse, all patients were Asthenospermia according to WHO criteria 2010 aged from 22 to 47 years old, with no history of medical and testicular surgical interference, and normal prolactin hormone level, normal ejaculates will serve as the study's sperm supplies.

The study was done from march 2021 till October 2022, after approval from the Ethical Committee Sadat City University, Egypt. An informed written consent was obtained from the patients.

Exclusion criteria were diabetic, azoospermia, testicular biopsies, addiction to drugs.

All patients were subjected to: history taking, pprevious operations, medical disorders, medical treatment, surgical history. The study population was drawn into random samples using computers. An experienced technician used block randomization to complete all of the randomizations.

Semen collection and analysis:

The semen samples were obtained by masturbation after two to five days of abstention from sexual activity in sterile container without use of lubricant. At 37 °C, all patient semen samples underwent total liquefaction and examined under the CASA microscope (sperm classic analysis SCA® system, microptic A hamilition thorne company) and samples were analyzed in accordance with WHO guidelines from 2010.

Sperm washing procedure:

Equal volume of Earl's Balanced salt solution 1X media (HIMEDIA) was

added to the liquefied semen sample **i**n sterile conical tube, then centrifuge the tube after mix well for 10 min at 1500 rpm by IEC Centra CL2 centrifuge, discard the supernatant then put 1 ml of global® total® media (Cooper Surgical®) then re-suspend the pellet, and transfer it to 4 sterile tubes (0.2 ml to each) as follows:
Group (1): Control group without any activation.
Group (2): treated with PX.
Group (3): treated with PAF
Group (4): treated with CL

Materials Preparation:

Sperm separation medium: e.g., Earl's Balanced salt solution. Global® total® media (CooperSurgical®). 5 mL syringe. BD Precisionglide® syringe needles 1.0 ml needle, centrifuge, SPL 5 mL polystyrene tubes. Media under test: PX (C13H18N4O3, Sigma), PAF β-Acetyl-γ-O-hexadecyl-L-α-phosphatidylcholine hydrate, Sigma) and CL (sigma).

Control group (1): 0.2 ml of resuspended washed sample was taken; then 0.2 ml global total media were added.
Group (2): A stock solution of PX (1 mg/mL) was prepared in global total medium, a final PX concentration of 1.0 mg/mL, was obtained then 0.2 ml of resuspended washed sample, was taken then 0.2 ml of stock PX, and was added.
Group (3): A stock solution of PAF (1 mg/mL) in global total medium was prepared, 0.2 ml of resuspended washed sample was taken, and then 0.2 ml of PAF was added.
Group (4): A stock solution of CL (1 mg/mL) in global total medium was prepared, 0.2 ml of resuspended washed sample was taken then 0.2 ml of PAF was added.

All samples were incubated at HERACELLTM150L incubator (Thermo scientific) and incubated to 37°C, 5% CO2 and 95% N2, 10 microliters were taken and analyzed by casa microscope at 1,2,3hours. In each analysis test, the number of motile sperms and total motility will recorded according of WHO 2010 of semen parameters.

Sample Size Calculation:

A sequential sample size calculation was used:

$$n = 2\left[\frac{(Z_{\alpha/2}+Z_{\beta})*\sigma}{\mu_1-\mu_2}\right]^2$$

Where: n = required sample size,
Zα/2 = 1.96 (The critical value that divides the central 95% of the Z distribution from the 5% in the tail),
Zβ = 0.84 (The critical value that separates the lower 20% of the Z distribution from the upper 80%),
σ is the estimate of the standard deviation in the study group,
μ1 = mean in the group 1,
μ2 = mean in the group 2,
10% drop out was calculated.

Statistical analysis

Statistical analysis was done by computer through SAS (2003). SAS User's Guide: Version 9.1. (SAS Institute Inc., Cary, NC) The central tendency was calculated using the arithmetic mean. As a gauge of dispersion, standard error of deviation was employed. One-sided Chi-square test was used to compare frequencies between the study groups. The probability level P value 0.05 (5%) was used to determine the significance level to compare the means of the researched groups as, P value < 0.05 was considered significant.

RESULTS

Demographic data and Semen parameter

Patients suffered from infertility for a period ranged from 1 – 22 years with a mean 12.54 years. Their age had a mean of 31.11 years, weights ranged from 102 –

76.0 kg with a mean 83.84 kg, and heights ranged from 1.02 - 0.67 m with a mean 1.64 m and BMI ranged from 19.67 – 35.04 kg/m^2 with a mean 27.1 kg/m^2.

In Semen parameter before preparation, motile sperm percentage was 18 ± 27 % with a mean 30.20 %, million/ml, normal morphology percentage was 1- 2% with a mean 1.22 % and Number of sperm rang 34 - 38million/ml with a mean 23.86 (Table 1).

Table 1. Demographic data and semen parameter before preparation of study population.

Parameter	Rang	Mean
Age (years)	22 - 48	31.11
Weight (kg)	102 – 76.0	83.84
Height (meter)	1.02 - 0.67	1.64
Body mass index BMI (kg/m2)	19.67 – 35.04	27.1
Period of infertility (years)	1 - 22	12.54
Motile sperm (a+b) (%) active	18 - 27	30.20
Morphology (%)	1- 2	1.22
Number of sperm (million / ml)	34 - 38	23.86

Motile sperm percentage showed a significant difference between treatment by PX, CL and PAF at three hours compared with control. The highest motile sperm percentage was at the first hour after treatment with PX, followed by second hour. There were no significant differences between before and after treatments by PX at the third hours. Morphology percentage and the number of living sperm showed no significant differences between before and after treatment with PX, PAF and CL at three hours, but there were significant differences in the number of living sperm between patients treated by PX at third hour and before treatment in favour of treated with it. The morphology percentage and number of sperm showed no significant differences between before treated with CL, PAF and PX at the first, second and third hour (Table 2).

Table 2. Compered semen parameters between group (1) with groups (2), (3) and (4).

Group	Time	Motile sperm (a+b) (%) active	Morphology (%)	No. living sperms (million/ml)
G1: Without co-factor		30.20c ± 1.84	1.22a ± 0.11	23.86b ±2.77
G2: After add Pentoxifylline (PX)	1 h	46.24a ±1.84	1.20a ± 0.11	39.94a ±2.77
	2 h	39.56b ±1.84	1.16a ± 0.11	39.06a ±2.77
	3 h	32.68c ±1.84	1.15a ± 0.11	37.01a ±2.77
LSD		5.11	0.30	7.71
G1: Without co-factor		30.20c ± 1.84	1.22a ± 0.11	23.86b ±2.77
G3: After add platelet activating factor (PAF)	1 h	51.46a ±1.70	1.18a ±0.11	36.91c ±1.70
	2 h	45.01b ±1.73	1.22a±0.11	33.92ab ± 2.69
	3 h	36.91b ±1.71	1.20a ±0.11	32.66a±2.52
LSD		4.73	0.31	7.02
G1: Without co-factor		30.20c ± 1.84	1.22a ± 0.11	23.86b ±2.77
G4:After adding caffeine liquid (CL)	1 h	60.05a ±1.64	1.22a ±0.11	41.88c ±1.64
	2 h	50.78a ±1.72	1.23a ±0.11	31.33b ± 2.69
	3 h	41.88a ±1.73	1.29a ±0.11	34.11a ± 2.36
LSD		4.56	0.31	6.56

Means with the same letter are not significantly differences **LSD :Low stander difference.**

The motility sperm percentage showed a significant difference between after treatment with PX, platelet activating and CL at the first, second and third hours. The highest significant difference is between values of motility sperm

percentage in patients treated with CL then those treated with platelet activating factor, the last one was treated with PX (Table 3).

Table 3: Compered Semen parameters between groups (2), (3) and (4) after one, two and three hours of treatment.

Time	Group	Motile sperm (a+b) (%) active	Morphology (%)	No. sperms (million / ml)
after one hour:	G2: Pentoxifylline	46.24c ±1.72	1.20a ±0.11	39.94a ±2.88
	G3: platelet activating factor	51.46b ±1.72	1.18a ±0.11	35.13a ±2.88
	G4: caffeine liquid	60.05a ±1.72	1.22a ±0.11	32.56a ±2.88
LSD		4.79	0.30	8.03
after two hours	G2:Pentoxifylline	39.56c ±1.72	1.16a ±0.11	39.06a ± 2.69
	G3: platelet activating factor	45.01b ±1.72	1.22a ±0.11	33.92ab ± 2.69
	G4: caffeine liquid	50.78a ±1.72	1.23a ±0.11	31.33b ± 2.69
LSD		4.78	0.31	7.49
after three hours	G2: Pentoxifylline	32.68b ±1.71	1.15a ±0.11	37.01a ±2.60
	G3: platelet activating factor	36.91b ±1.71	1.20a ±0.11	32.66a ±2.60
	G4: caffeine liquid	41.88a ±1.71	1.29a ±0.11	34.11a ±2.60
LSD		4.77	0.30	7.25

Means with the same letter are not significantly differences LSD :Low stander difference.

The motility percentage showed a significant difference in the first, second and third hour between PX, platelet activating and CL with sample before treatment. The highest value was for those treated with CL and the lowest one was sample with no treatment. The morphology percentage and number of sperms showed no significant differences between before treatment with CL, PAF and PX at the first, second and third hours and after treatment and there was a significant differences between samples before and after treated with CL in favour of those treated with CL in the first hour. There were significant differences between samples after treatment with PX and CL and before treatment in favour of treated with PX in the second hour, also there were significant differences between samples after treat with PAF and before treatment in favour of those treated with PAF in the third hour (Table 4).

Table 4: Compered Semen parameters between groups (1), (2), (3) and (4) after one, two and three hours.

Time	Group	Motile sperm (a+b) (%) active	Morphology (%)	Number of sperms (million / ml)
	G1:Without co-factor	30.20d ± 1.73	1.22a ±0.11	23.86b ± 2.68
after one hour	G2: Pentoxifylline	46.24c ±1.73	1.20a ±0.11	39.94a ±2.68
	G3: platelet activating factor	51.46b ±1.73	1.18a ±0.11	35.13a ±2.68
	G4: Gcaffeine liquid	60.05a ±1.73	1.22a ±0.11	32.56a ±2.68
LSD		4.81	0.31	7.46
	G1:Without co-factor	30.20d ± 1.73	1.22a ±0.11	23.86c ± 2.53
after two hours	G2: Pentoxifylline	39.56c ±1.73	1.16a ±0.11	39.06a ± 2.53
	G3: platelet activating factor	45.01b ±1.73	1.22a ±0.11	33.92ab ± 2.53
	G4: Gcaffeine liquid	50.78a ±1.73	1.23a ±0.11	31.33b ± 2.53
LSD		4.81	0.31	7.03
	G1:Without co-factor	30.20c ± 1.73	1.22a ±0.11	23.86b ± 2.46
after three hours	G2: Pentoxifylline	32.68b ±1.73	1.15a ±0.11	37.01a ±2.46
	G3: platelet activating factor	36.91b ±1.73	1.20a ±0.11	32.66a ±2.46
	G4: Gcaffeine liquid	41.88a ±1.73	1.29a ±0.11	34.11a ±2.46
LSD		4.80	0.30	6.83

Means with the same letter are not significantly difference LSD :Low stander difference

DISCUSSION

In the current study adding 0.2 mm PX to the semen led to elevation in the sperm motility at the first hour, however, that significance faded on the subsequent readings. In agreement with our findings, Satish *et al.* (2021) reported that PX, a PDE inhibitor, is used in assisted reproductive technology (ART) to enhance sperm motility, primarily to help select viable sperm in asthenozoospermic (AZS) ejaculates and testicular spermatozoa prior to intra cytoplasmic sperm injection (ICSI). In addition, others confirmed that PX has been utilized to treat sperm samples in assisted reproduction (Villani *et al.*, 2022). Also, in agreement with our findings, Nabi *et al.* (2017) reported that PX application led to a significant increase in progressive sperm motility. PX. was found to enhance human spermatozoa in vivo Okada *et al.* (1997) and in vitro (Kovačič *et al.*, 2006).

Several studies have reported no impact in normozoospermic cases, while others demonstrated a considerable enhancement in motility and increasingly the frequency of motile spermatozoa in AZS cases (Henkel and Schill, 2003). Banihani and Abu-Alhayjaa (2016) reported that adding PF at 5 mm to sperm samples increased significantly the progressive sperm motility by about 25.4%. Also, adding 5 mm PTX to semen samples significantly increased the number of progressive spermatozoa by about 36% compared to control (without PTX) (Banihani *et al.*, 2018). Moreover, in asthenozoospermic cases, researchers observed a beneficial impact of PTX on intracytoplasmic sperm injection (ICSI) results, involving fertilization, embryo quality, and pregnancy rates (Amer *et al.*, 2013).

In the same context, Hassanpour *et al.* (2010) on rats confirmed the current findings regarding PTX, however the impact of PX at a low dose (0.01 mM) on progressive motility and other kinematic parameters in rats was increased with progressive motility had a mean value of 62.5% compared to 51.1% in controls. However, at higher dosages, the motion parameters were unchanged (0.1 and 1 mM PTX) or decreased (10 mM PTX). In contrast to our findings, Mirshokraei *et al.* (2011) reported that the increasing motility of spermatozoa exposed to varied concentrations of PF did not vary significantly. This difference can be attributed to the variation of PTX concentration used.

In the current study adding 0.02 mm PAF to the semen led to a first hour enhancement in the sperm motility, while it was decreased after two and three hours. Ricker *et al.* (1989) observed that when human spermatozoa were treated with PAF at varying doses, they displayed a statistically significant increase in motility compared to control samples that had not been treated with PAF, with the rise being statistically significant in the group with the lowest starting motility.

In contrast with the present trsults Mirshokraei *et al.* (2011) reported that the progressive motility of spermatozoa exposed to varied concentrations of PAF did not vary significantly. In the current study PAF resulted in a significant increase in sperm motility from 28.6% at baseline to 50.28% immediately after installation. Motility was decreased after one hour (35%). However, it was significantly better than the baseline. At the two-hour reading, motility became less than that of group 1. Naz and Minhas (1995) reported adding PAF to human sperm increased sperm motility, promotes sperm penetration of cervical mucus, and improves sperm penetration. Odeh, *et al.* (2003) concluded that a lower quantity of PAF increases the motility of stallion spermatozoa, whereas a larger concentration promotes the acrosome response. Studies of Reinhardt *et al.* and Roudebush *et al.* (1999, 2000) demonstrated the presence of PAF

receptors on the surface of the plasma membranes of human sperm. However, Wu *et al.* (2020) negated any significant positive impact of in vitro application of PAF on sperm motility parameters.

In the current study, adding 0.02 mm CL to the semen led to increase in the sperm motility after one hour, which decreased after two and three hours. In agreements with current results the addition of 5 mM CL increased sperm motility in frozen equine sperm, hence enhancing the fertility rate of mares inseminated with equine semen treated with CL (Alves *et al.*, 2021). Campbell *et al.* (2020) indicated that yielded 48 percent post-thaw sperm motility after cryopreservation involved soaking the sperm in 10 mm of CL with PBS containing 10 percent v/v DMSO (Ca++, Mg++ free) and cooling the sperm at a rate of 32.1C/min for five minutes is important before submerging them in LN2.

In the current study, the effects of CL, PAF, and PTX on sperm activation in AZS cases during in vitro fertilization procedures were compared. PX was added to the semen in the current investigation, and this immediately increased the sperm's ability to move, but after three hours, CL increased this ability more effectively than PTX and PAF.

Conclusions:

Although PX, PAF and CL resulted in an immediate increase in motility, CL has more pronounced and durable effect on sperm activation in Asthenospermia cases than PX and PAF. It is better to use semen prepared by CL within one hour of preparation, CL has more pronounced and durable effect with the period.

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دراسة مقارنة لعامل تنشيط الصفائح الدموية وسائل الكافيين والبنتوكسيفيلين على وهن النطاف بواسطة CASA واختبار بقاء الحيوانات المنوية SST

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المستخلص

تعد عدم القدرة على الحمل بعد ١٢ شهرًا على الأقل من النشاط الجنسي غير المحمي علامة على العقم، والذي يمكن أن يؤثر على الجهاز التناسلي الذكري أو الأنثوي. كان الهدف من هذا العمل هو المقارنة بين البنتوكسيفيلين (PX) مقابل عامل تنشيط الصفائح الدموية (PAF) وسائل الكافيين (CL) على تنشيط الحيوانات المنوية في وهن النطاف. في هذه التجربة السريرية مع تصميم عشوائي محتمل مزدوج التعمية، شارك ٨٢ من الأزواج الذكور الذين يعانون من العقم والذين كانوا يمارسون الجنس بانتظام لمدة عامين على الأقل، وكان جميع المرضى يعانون من وهن النطاف وفقًا لـ وفقًا لمعايير منظمة الصحة العالمية لعام ٢٠١٠، الذين تتراوح أعمارهم بين ٢٢ إلى ٤٧ عامًا، سيكون القذف الطبيعي بمثابة إمدادات الحيوانات المنوية للدراسة. تم تقسيم كل عينة إلى ثلاثة أنابيب وأنبوب التحكم يسمى المجموعة (١): السيطرة. المجموعة (٢): (PX) ، المجموعة (٣): (PAF). المجموعة (٤): (CL). أشارت النتائج إلى أن إضافة CL إلى السائل المنوي أدى إلى زيادة في حركة الحيوانات المنوية بعد ساعة واحدة. لكن هذه الأهمية تلاشت في القراءات اللاحقة. أدت إضافة PAF إلى زيادة كبيرة في حركة الحيوانات المنوية ولكن أقل من CL. أدت إضافة PX إلى زيادة كبيرة في حركة الحيوانات المنوية ولكن أقل من PAF. انخفضت الحركة بعد ساعة واحدة. ومع ذلك، كان أفضل بكثير من خط الأساس. وفي قراءتي الساعتين والثلاث ساعات أصبحت الحركة أقل من الساعة الأولى. في الاستنتاجات اضافة CL إلى السائل المنوي كان له تأثير أكثر وضوحا ودائم على تنشيط الحيوانات المنوية في حالات وهن النطاف مقارنة بـ PX و PAF. من الأفضل استخدام السائل المنوي المحضر بواسطة CL خلال ساعة واحدة من التحضير، حيث أن تأثيره يكون أكثر وضوحًا ودوامًا خلال هذه الفترة.