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Effect of adding L-Carnitine as a sperm stimulant to the culture media for asthenozoospermic patients

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Abstract

Background: By increasing the percentage of morphologically normal sperm (MNS) and active sperm motility, pentoxifylline (PX) and L-carnitine (LC) have been demonstrated to play a significant effect in in vitro sperm activation. This, in turn, impacts the success of fertilization potential.

Objective: The current study aims to investigate how sperm motility stimulants (PX and LC) affect the medium used in asthenozoospermic patients' in vitro sperm activation.

Subjects, Material and methods: This study included 75 male patients with asthenozoospermia. Using seminal fluid analysis guidelines from the World Health Organization, those patients were divided into three categories. All the above groups underwent *in vitro* sperm activation with Ham's F-12 (control), PX and LC culture media for each semen sample which underwent macroscopical and microscopical examination. Certain sperm function parameter (sperm concentration, active sperm motility and MNS percentages) were recorded.

Results: After *in vitro* sperm activation, highly significant improvement in active sperm motility and MNS percentages in both PX and LC groups I comparison with control group. LC group shows a highly significant improvement on certain sperm function parameters (active sperm motility, morphologically normal sperm percentage) in comparison with other treated culture media.

Conclusion: Adding LC to the culture media is the best sperm stimulant, which shows a highly significant improvement on certain sperm function parameters (active sperm motility, morphologically normal sperm percentage) in comparison with other treated culture media.

Keys: Asthenozoospermia, Pentoxifylline and L-carnitine

Introduction

A drop in sperm motility to less than 40% or in progressive motility to less than 35% is indicative of asthenospermia. It is a common reason for infertility in men. Fine structural abnormalities of the sperm flagellum are suspected to be the cause of many cases of severe asthenozoospermia. These can occasionally be brought on by hereditary factors. It is obvious that these "primary" aberrations are permanent because they often affect all or most of the sperm in the ejaculate and are homogeneous.

The diagnostic criteria are [1, 2]:

1. Moderate asthenospermia, count >20 $\times 10^6$ /ml and motility 20-40%

2. Severe asthenospermia, count >20 $\times 10^6$ /ml and motility <20%

3. Oligoasthenospermia, count <20 $\times 10^6$ /ml and motility <20%.

4. One in 5000 males are reported to have complete asthenozoospermia, or 100% immotile spermatozoa in the ejaculate.

One of the main reasons for infertility or decreased fertility in men is asthenozoospermia, which lowers the quality of sperm. The percentage of viable spermatozoa in full asthenozoospermia varies between 0 and 100%. Even with ICSI, the diagnosis suggests a poor prognosis for fertility. It is

highly crucial to distinguish between two different categories of patients with complete asthenozoospermia, i.e. virtual or total asthenozoospermia. While absolute asthenozoospermia can be linked to metabolic deficits, sperm flagellum ultrastructural abnormalities, necrozoospermia, or idiopathic causes, the former group may have some motile spermatozoa after significant semen processing [2-4]. Normal motility is a sign of normal seminal plasma development, normal maturation in the epididymis, and normal spermatozoal axoneme formation during spermatogenesis in the testis.

The methylxanthine pentoxifylline (PX) functions as a phosphodisterase inhibitor, meaning that it prevents cyclic adenosine monophosphate (cAMP) phosphodiesterase from working, which raises the amount of cAMP inside cells. PX have gained popularity once more in assisted human reproduction because of the role that cAMP plays in the acrosome response second-messenger system and sperm kinematics [6] . The agonist-induced acrosome reaction, fertilization rates, sperm motility, and kinematics are all frequently enhanced by treatments that raise intracellular cAMP concentrations[7]. Furthermore, PX may be able to scavenge reactive oxygen radicals, according to certain theories [8, 9].

Carnitine is a quaternary ammonium compound biosynthesized in the liver and kidney from the amino acids lysine and methionine in the presence of vitamin C**.** Patients with asthenozoospermia exposed to LAC had a high percentage of motile ejaculated sperm cells [10.11]. Following testicular sperm exposure to LC in vitro, an improvement in sperm motility was also seen [12].

Subjects, Materials and Methods

75 male patients with asthenozoospermia were involved in the work.Those patients were classified into three subgroups according to WHO criteria of seminal fluid analysis [(1)]:

All patients underwent *in vitro* sperm activation with the following sperm motility stimulants for each semen sample:

1. Ham's F-12 medium (control medium).

2. Pentoxifylline (PX) supplemented culture media.

3. L-Carnitine (LC) supplemented culture media.

After three to five days of abstinence, semen samples were obtained by masturbating in a room close to the laboratory and placing them into sterile, dry, and clean disposable Perti-dishes [(1,13)] .The sample was sent straight to the lab, where it was incubated at 37°C until it completely liquefied. Next, semen samples were examined under a microscope and under a macroscopic microscope using WHO standards [(1, 13).]

Regaeding PX treated sample, liquefied semen sample was washed with suitable volume of culture medium (Ham's F-12) plus 0.1 ml of the prepared PX . The mixture was centrifuged at 1600–2000 revolutions per minute (rpm) for ten minutes. After discarding the supernatant, the pellet was again suspended in 0.5–0.75 ml of Ham's F-12 culture medium, and it was cultured for 30–45 minutes at 37°C.

In the LC treated sample, liquefied semen sample was washed with suitable volume of culture medium (Ham's F-12), 0.1 ml of the prepared HFF, 0.1 ml of the prepared LC solution. Similar steps of preparation of PX treated samples had been done.

One drop of the processed semen sample were taken from it's upper layer by a micropipette and examined microscopically.

Results

Table 1 showed that the activation of human sperms *in vitro* by a medium containing PX caused a highly significant ($P < 0.001$) decrease in sperm concentration (m/ml) compared to before activation (30.72 ±2.51 versus 57.44 ± 3.94).The percentages of active sperm motility (%) grade A, B and A+B were highly significantly increased($P < 0.001$) than that of before activation (41.20±2.73 versus 9.36 ± 1.27 for grade A, 44.16 ±1.51 versus 29.32± 2.00 for grade B and 85.64 ± 2.97 versus 38.68 ± 2.77 for grade A+B) as shown in table 1.

In vitro activation of human sperm with PX caused a highly significant increase ($P<0.001$) in the percentage of MNS compared to the results of before activation (71.08± 1.78 versus 53.16± 1.82) (table1). The number of round cells (cell/HPF) was highly significantly ($P<0.001$) decreased following the activation by a medium containing PX compared to before activation as shown in table 1

Table 1: Effect of adding Pentoxifylline (PX) to the culture medium on certain sperm function parameters of asthenozoospermic group

Certain Sperm function parameters		*In vitro* activation				*P* value
		Before activation		After activation		
		Mean	SE	Mean	SE	
Sperm concentration ($\times 10^6$/ml)		57.44	3.94	30.72	2.51	<0.001
Active sperm motility (%)	Grade A	9.36	1.27	41.20	2.73	<0.001
	Grade B	29.32	2.00	44.16	1.51	<0.001
	Grade A+B	38.68	2.77	85.64	2.97	<0.001

Morphologically normal sperm (%)	**53.16**	**1.82**	**71.08**	**1.78**	**<0.001**
Round cells (cells/HPF)	**2.58**	**0.51**	**0.0**	**0.0**	**<0.001**

Values are expressed as mean ± SE. Student's t-Test. No. samples=25

In table 2, the activation of human sperms *in vitro* by a medium containing L-carnitine (LC) caused a highly significant (P<0.001) decrease in sperm concentration (27.84 ±2.82) compared to before activation (57.44 ± 3.94). The percentages of active sperm motility (%) grade A, B and A+B were significantly (P<0.001) highly improved compared to before activation. *In vitro* activation of human sperm with LC caused a highly significant (P<0.001) improvement in the percentage of MNS compared to before activation (78.00± 2.10 versus 53.16± 1.82).The number of round cells (cell/HPF) was highly significantly (P<0.001) decreased following the activation by a medium containing LC compared to before activation as shown in table 2.

Table 2: Effect of adding L-carnitine (LC) to the culture medium on certain sperm function parameters of asthenozoospermic group

Certain Sperm function parameters		***In vitro* activation**				**P value**
		Before activation		After activation		
		Mean	**SE**	**Mean**	**SE**	
Sperm concentration (×10^6/ml)		**57.44**	**3.94**	**27.84**	**2.82**	**<0.001**
Sperm motility (%)	**Grade A**	**9.36**	**1.27**	**47.60**	**2.73**	**<0.001**
	Grade B	**29.32**	**2.00**	**42.92**	**2.00**	**<0.001**
	Grade A+B	**38.68**	**2.77**	**90.12**	**3.33**	**<0.001**
Morphologically normal sperm (%)		**53.16**	**1.82**	**78.00**	**2.10**	**<0.001**
Round cells (cells/HPF)		**2.58**	**0.51**	**0.0**	**0.0**	**<0.001**

Values are expressed as mean ± SE. Student's t-Test. No. samples=25.

Table 3 showed that the preparation of human sperms in vitro by Ham's F-12 medium (control) caused a highly significant (P<0.001) decrease in sperm concentration (m/ml) compared to before activation (32.08 ±2.64 versus 57.44 ± 3.94).

The percentages of active sperm motility (%) grade A, B and A+B were significantly (P<0.001) highly improved than that of before activation (24.52 ±2.64 versus 9.36 ± 1.27 for grade A , 39.36 ±2.70 versus 29.32± 2.00 for grade B and 63.68 ± 4.68 versus 38.68 ± 2.77 for grade A+B). In vitro activation of human sperm with control Ham's-F12 caused a significant increase (P<0.05) in the percentage of MNS sperm compared to the results before activation (58.96± 2.08 versus 53.16± 1.82). The number of round cells (cell/HPF) was highly significantly (P<0.001) decreased following the activation by a medium containing Ham's F-12 compared to before activation as shown in table 3.

Table 3: Effects of Ham's-F12 medium (control) on certain sperm function parameters of asthenozoospermic group.

Certain Sperm function parameters		*In vitro* activation				P value
		Before activation		After activation		
		Mean	SE	Mean	SE	
Sperm concentration (×10^6/ml)		57.44	3.94	32.08	2.64	<0.001
Active sperm motility (%)	Grade A	9.36	1.27	24.52	2.64	<0.001
	Grade B	29.32	2.00	39.36	2.70	<0.001
	Grade A+B	38.68	2.77	63.68	4.68	<0.001
Morphologically normal sperm (%)		53.16	1.82	58.96	2.08	<0.001
Round cells(cells/HPF)		2.58	0.51	0.0	0.0	<0.001

Values are expressed as mean ± SE. Student's t-Test. No. samples=25.

Comparison of certain sperm parameters following *in vitro* activation among Ham's F-12 medium (control), adding Pentoxifylline and L-Carnitine media to the semen samples of asthenozoospermic group

In figure 1, there was a highly significant (P<0.001) increase in active sperm motility grade A in the three treated media used for *in vitro* activation compared to control. The best result (P<0.05) in the percentage of active sperm motility grade B was found in using a medium containing PX compared to other treated medium. Regarding sperm motility grade A and grade (A+B), there was a highly significant (P<0.001) increment by using a medium containing LC compared to other media (figure 1).

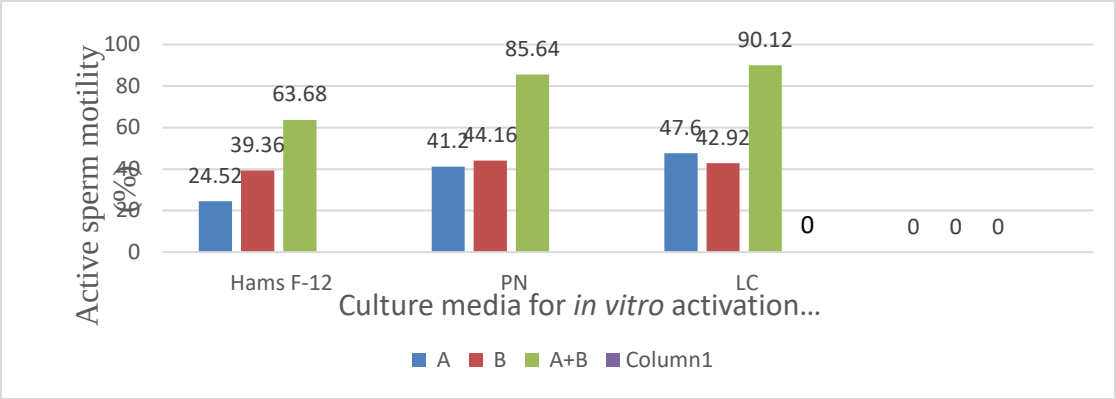

Figure 1: Comparison of active sperm motility grades following *in vitro* activation among Ham's F-12 medium (control), adding Pentoxifylline and L-Carnitine media to the semen samples of asthenozoospermic group. No. semen samples=25

There is a marked significance (P<0.001) increase in the percentage of MNS when LC medium (78.00± 2.10) used compared to other media (71.08± 1.78 PX and 58.96 ±2.08 in control medium) as shown in figure 2.

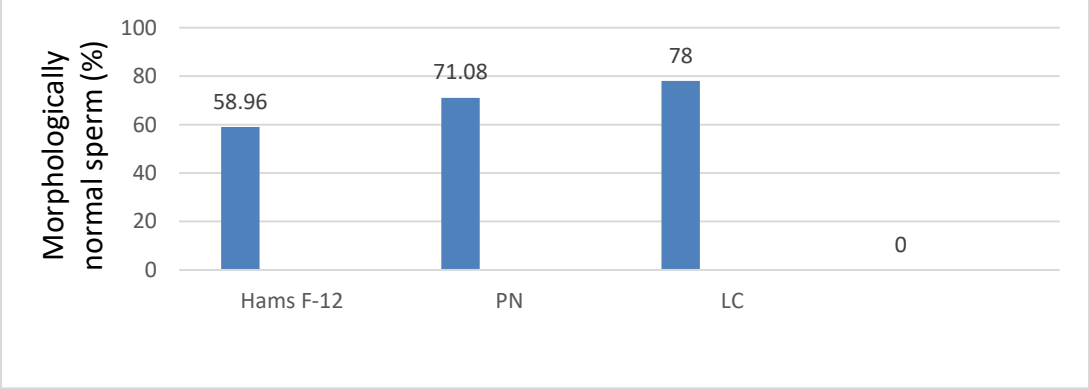

Figure 2 : Comparison of morphologically normal sperm following *in vitro* activation among Ham's F12 medium (control), or adding Pentoxifylline and L-Carnitine media to the semen samples of asthenozoospermic group .No. semen samples=25

Discussion

Pentoxifylline (PX) was shown to lower the concentration of sperm when added to asthenozoospermic semen in the current study.This resulted from the aberrant and dead sperms' low motility, which prevented them from

swimming up and migrating from the sperm pellet to the top layer of the culture media.

The results of this study are consistent with those of earlier studies that examine PX's function in in vitro sperm activation methods. Al-Dujaily (14) discovered that adding PX to mildly asthenospermic semen significantly increased sperm motility, most likely due to the drug's ability to shield the sperm plasma membrane. This finding is extremely helpful for the process of preparing semen for assisted reproductive procedures. . Rashidi [15] and Sato [16] demonstrated that PX increased sperm motility when it added to culture media and they suggested that treating sperm with PX is effective for *in vitro* fertilization. The mechanism of action of PX depends on inhibition of phosphodiesterase enzyme which will increase intracellular cyclic AMP levels and decrease its break down [15,16].

The data of this study indicated that a lower sperm concentration, a higher percentage of active motile and morphologically normal sperms were obtained by a semen samples treated by LC-containing culture media than other treated media in asthenozoospermic patient . This finding may be attributed to the effect of LC which improves sperm motility and chromatin quality *in vitro*[17].While PX have no effect on sperm chromatin quality [18]. Another hypothesis is that PX has a time-dependent influence on sperm motility. On the other hand, sperm motility increased with LC in comparison to the time-limited control sample [19] .It could be because PX is hazardous and exposure for longer than 90 minutes is not advised [19] ; on the other hand, LC are amino acids that are normally present in the testis's microenvironment [20] . Compared to PX, LC took longer to enhance motility. The different energy sources that spermatozoa employ after being exposed to LC could be

the cause. Sperm genetic material contributes to the effectiveness of ART regimens and provides a significant explanation for this impact [21]. Spermatozoa should carry out the final phases of protamination as part of their maturation process during the late spermiogenesis stages.

Conclusion

Adding LC to the culture media is the best sperm stimulant, which shows a highly significant improvement on certain sperm function parameters (active sperm motility, morphologically normal sperm percentage) in comparison with other treated culture media.

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