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**EFFECT OF THEOPHYLLINE ON MOTILITY
 OF BULL SPERMATOZOA IN VITRO**
 (With 2 Tables)

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تأثير الثيوفيللين على حركة الحيوانات المنوية للثيران
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تم في هذا البحث إضافة عقار الثيوفيللين بجرعات مختلفة (0.08, 0.17, 0.25, 0.5 مجم/سم من الخفف) للسائل المنوي للثيران أثناء فترة تخزينه عند درجات مئوية 4 درجات مئوية. وقد ازدادت أيضا فترة تخزين السائل المنوي مع احتفاظ الحيوانات المنوية بحركتها النشطة كما لوحظ أيضا أن الحيوانات المنوية التي تفقد حركتها من صدمة البرد تعاود حركتها بعد إضافة هذه المادة. كذلك أدى إضافة عقار الثيوفيللين إلى زيادة فترة إختزال صبغة الميتلين الزرقاء وزيادة مقاومة الحيوانات المنوية لمحلول الملح بتركيز 1%.

SUMMARY

Addition of different levels of theophylline (0.08, 0.17, 0.25, 0.5 mg/ml) during preservation (4°C) of bull semen in egg yolk citrate medium had a stimulatory effect on sperm motility. It increased the preservation time of bull semen by delaying the loss of sperm motility. Immotile bull sperm due to cold shock could also be mobilized by activity of theophylline. Moreover, theophylline reduced the metabolic activity and increase the resistance of bull semen to 1% NaCl solution.

INTRODUCTION

Despite the fact that no steady correlation between sperm motility and fertilizing ability has been found, spermatozoal motility is still considered one of the most important parameters in the evaluation of semen quality. Little is known about the mechanisms involved in initiation and maintenance of sperm motility after ejaculation.

There are several physiological substances that play role in stimulating or inhibiting sperm motility. Acetylcarnitine and LH stimulated human sperm motility at 37°C (MIZUTANI and SCHILL, 1985). The fertilizing ability of epididymal rabbit spermatozoa was improved in the presence of carnitine (CASILLAS and CHAIPAYUNGPAN, 1979). Other substances stimulate the spermatozoal motility by different mechanisms. These

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FAYED & HATTAB

substances are called motility stimulating factors (MSF) and include theophylline, dibutyryl-cAMP and kailikrein (SCHILL & HABERLAND, 1974; SCHOENFELD *et al.*, 1975; THOMPSON *et al.*, 1980; SCHILL & LITTICH, 1981 and TURNER & GILES, 1982). Moreover, caffeine has been shown to stimulate buffalo spermatozoal motility, *in vitro* (SINGH *et al.*, 1986).

A number of enzymes associated with the structural and functional integrity of spermatozoa have been reported to be affected with certain antifertility agents (ADEYEMO *et al.*, 1981; TSO *et al.*, 1982 and KALLA, 1982). Motility and survival of sperm vary according to their storage temperature. With reference to body temperature, cooling and heating are inhibitory to sperm. Cold shock reduced sperm motility possibly due to a leakage of sperm enzymes or slowing down of the Na - K ion exchange pump (SINGH *et al.*, 1986).

The objective of the present study was to investigate the effect of theophylline on motility of bull spermatozoa preserved at 4°C. The effect of theophylline on cold shocked spermatozoa was also investigated.

MATERIAL and METHODS

Semen samples were collected biweekly from 4 Baladi bulls and those having an initial motility of 80% and above were utilized. Neat semen (0.5 ml) diluted with 0.5 ml sodium citrate (2.9%) was maintained at 4°C and 37°C for 3 hours to find out the effect of cold shock on the individual motility of spermatozoa. Then 0.5 mg theophylline (Sigma Chemical Company, St. Louis, USA) was added for both samples and kept at 37°C to see the effect of theophylline as motility stimulant in cold treated and control spermatozoa. The motility was evaluated after 60 minutes.

Semen samples were diluted at a ratio of 1 : 15 in egg yolk citrate extender (10% egg yolk and 2.9% sodium citrate). Theophylline was added to the diluted semen at different concentrations (0.08, 0.17, 0.25 and 0.5 mg/ml). Control semen samples contained no theophylline. The motility of spermatozoa was determined immediately after the addition of theophylline, then all samples were kept at 4°C and the motility was assessed daily for 8 days. All motility determinations were carried out at room temperature.

The methylene blue reduction test was carried out in control and theophylline treated semen samples to determine the metabolic activity of the spermatozoa. Also the resistance of the spermatozoa to 1% Na solution was determined in control and treated samples.

Data were statistically analyzed by the unpaired Student's t-test (SNEDECOR and COCHRAN, 1980).

RESULTS

Initial motility (80%) of neat bull semen declined to 44% and 15% after 3 hours storage at 37°C and 4°C respectively. Addition of theophylline, however, stimulated

THEOPHYLLINE, BULL SPERMATOZOA

the motility of cold-shocked and non cold-shocked spermatozoa to 74% and 94%, respectively (Table 1).

As shown in table (2), there was approximately 20% decrease in the sperm motility after 24 hours of semen storage at 4°C. On 8th day of preservation, sperm motility was as low as 5% in the control samples, while it was 20% or higher in treated samples. On all days of preservation, the sperm motility were more motile in theophylline treated samples than those in the control. Moreover, the motility of spermatozoa was found to be directly proportional to the concentration of theophylline (Table 2).

The methylene blue reduction time of theophylline treated semen was delayed to 19 ± 3.2 minutes while the untreated samples had 9 ± 1.7 minutes. Moreover, the spermatozoal motility of theophylline treated semen was 50% after addition of 15 ml 1% NaCl while the motility reduced to 0% after the addition of the same amount of NaCl to the control samples.

DISCUSSION

In the present study, theophylline has been found to stimulate sperm motility and increase their storage time. Methylxanthines have been found to stimulate sperm motility by inhibiting the phosphodiesterase enzyme and increasing the intracellular C-AMP (TURNER and GILES, 1982). Moreover, some nucleotides, such as ATP, ADP and AMP have the capacity to stimulate sperm motility and are specially useful in causing flagellar rotation. These nucleotides provide energy for metabolic processes of the germ cell (respiration and movement) (NELSON, 1975). Moreover, compounds were found to be useful in the therapy of human oligozoospermia, since a reduction of sperm number and/or motility causes a decrease of male fertilizing capacity (BIANCHI *et al.*, 1978).

In the present investigation, the stimulatory effect of theophylline on spermatozoal motility was in accordance with previous investigations (SCHOENFELD *et al.*, 1975 and TURNER *et al.*, 1978). The mechanism by which theophylline stimulates the motility is unknown. Since theophylline has a phosphodiesterase inhibiting activity, it may activate the sperm C-AMP dependent protein kinase, which in turn activates a calcium-controlled motility regulating protein (HOSKINS and CASILAS, 1975). Whatever the mechanism may be, it is evidently clear that theophylline does increase the motility of spermatozoa.

Caffeine (methylxanthin group) had a stimulatory effect on buffalo sperm motility preserved in egg-yolk citrate at 5°C. In addition, immotile sperm due to cold shock could also be mobilized by addition of caffeine (SINGH *et al.*, 1986). These results were similar to our findings.

Motility and survival of sperm vary according to their storage temperature. Our results demonstrated that a significant reduction in motility of bull sperm occurred after cold shock. Leakage of sperm enzymes and slowing down of Na-K pump might be a possible reason of motility reduction after cold shock. Theophylline addition stimulated the motility of cold-shocked spermatozoa. The mechanism by which theophylline

FAYED & HATTAB

induced this effect is unknown.

Theophylline not only stimulated the sperm motility but also reduced its metabolic activity and increased its resistance to NaCl. The mechanism by which theophylline induced these effects is not fully clear.

The possibility of using theophylline treated bull spermatozoa in artificial insemination programme without inducing a deleterious effect on the offspring and pregnancy rate is unknown. However, more trials need to be undertaken before the addition of theophylline for routine use.

REFERENCES

- Adeyemo, A.; Chang, G.Y.; Koide, S.S. and Segal, S.J. (1981): Effect of gossypol on the production and utilization of ATP by sea urchin spermatozoa. *Biol. Bull.* 161: 333.
- Bianchi, B. Mariani, M. and Menchini-Fabris, G.F. (1978): Modificazioni dei parametri seminologici in 140 pazienti dopo trattamento con TBM 78. Cited in: Treatment of male infertility. Bian. J. Schill, W. and Schwarzstein, L. (eds). 1st edition; Springer-Verlag Berlin Heidelberg New York (1982). PP. 13-152.
- Casillas, E.R. and Chaipayungpan, S. (1979): The distribution of carnitine and acetylcarnitine in the rabbit epididymis and the carnitine content of rabbit spermatozoa during maturation. *J. Reprod. Fert.* 56: 439-444.
- Hoskins, D.D. and Casillas, E.R. (1975): Function of cyclic nucleotides in mammalian spermatozoa. Cited in: Treatment of male infertility. Bian J., Schill, W. and Schwarzstein, L. (eds). 1st edition, Springer-Verlag Berlin Heidelberg, New York (1982), PP. 143-152.
- Kalla, N.R. (1982): Gossypol. The male antifertility agent. *IRCS Med. Science* 10: 766-769.
- Mizutani, T. and Schill, W.B. (1985): Motility of seminal plasma free spermatozoa in the presence of several physiological compounds. *Andrologia* 17: 150-156.
- Nelson, L. (1975): Spermatozoan motility. Cited in: Treatment of male infertility. Bian J., Schill, W. and Schwarzstein, L. (eds). 1st edition, Springer-Verlag Berlin Heidelberg, New York (1982), PP. 143-152.
- Schill, W.B. and Haberland, G.L. (1974): Kinin induced enhancement of sperm motility. *Hopp-Seylers Z. Physiol. Chem.* 355: 229-231.
- Schill, W.B. and Littich, M. (1981): Split- ejaculate insemination with and without the addition of kallikrein. *Andrologia*, 13: 121-126.
- Schoenfeld, C.Y.; Ameler, R.D. and Dubin, L. (1975): Stimulation of ejaculated human spermatozoa by caffeine. *Fert. Steril.* 26: 158-161.
- Singh, S.D.; Anari, M.R. and Benjamin, B.R. (1986): Effect of caffeine on motility of buffalo spermatozoa in vitro *Andrologia* 18: 33-36.
- Snedecor, G. and Cochran, W. (1980): *Statistical Methods*. 6th Ed. The Iowa State University Press: Ames, IA, USA.
- Thompson, W.A.J.; Traub, C. and Earshaw, J.C. (1980): Effect of a kallikrein on sperm motility. *Arch. Androl.* 5, Suppl. A. pp. 117.

THEOPHYLLINE, BULL SPERMATOZOA

- Tso, W.W., Fee, C.S. and Tso, M.W. (1982): Sensitivity of various spermatozoal enzymes to gossypol inhibition. Arch. Androl, 9: 31-32.
- Turner, T.T.; Addario, D. and Howards, S.S. (1978): Further observations on the initiation of sperm motility. Biol. Reprod. 19: 1095-1101.
- Turner, T.T. and Giles, R.D. (1982): The effect of cyclic adenine nucleotides, phosphodiesterase inhibitors, and cauda epididymal fluid on the motility of rat epididymal spermatozoa J. Androl. 3: 134-139.

Table (1): Effect of theophylline (0.5 mg/ml) on the motility of cold-shocked and non-cold shocked bull spermatozoa(%).

	cold-shocked Samples	non cold-shocked samples
	Motile sperm	Motile sperm
Initial motility	80.00 ± 5.0	85.00 ± 3.0
Prior to theophylline addition	15.00 ± 1.5	44.00 ± 3.5
After theophylline	74.00 ± 3.0	94.00 ± 2.0

Data are mean of 6 determinations ± S.E.

* Significantly higher ($p < 0.01$).

Table (2) Effect of theophylline on the motility of diluted bull semen

Preservation of semen (days)	control	Percentage of individual motility.			
		Theophylline 0.08 mg / ml	0.17	0.25	0.5
immediately	60.00± 3.0	75.00±3.0	85.00±3.0	93.00±3.0	95.00± 2.0
1	40.00± 3.0	65.00±3.0	80.00±2.0	80.00±3.0	95.00± 2.0
2	20.00± 2.0	60.00±3.0	70.00±2.0	75.00±3.0	80.00± 2.0
3	20.00± 1.0	60.00±2.0	65.00±2.0	70.00±2.0	70.00± 1.0
4	15.00± 1.0	55.00±2.0	60.00±2.0	65.00±2.0	65.00± 2.0
5	15.00± 1.0	45.00±2.0	50.00±3.0	60.00±2.0	60.00± 2.0
6	10.00± 1.0	40.00±2.0	50.00±2.0	50.00±2.0	60.00± 2.0
7	10.00± 1.5	20.00±1.0	40.00±2.0	50.00±2.0	60.00± 2.0
8	5.00± 1.0	20.00±0.2	40.00±2.0	50.00±2.0	60.00± 2.0
% to day	8.3%	26.7%	47.0%	53.8%	63.2%

Data are mean of 6 determinations ± S.E.

* The motility of theophylline treated spermatozoa at all concentration was significantly higher ($p < 0.01$) than the control during the 8 days storage.