韓國家畜繁殖學會誌 23(1) : 29~36(1999)
Korean J. Animal Reprod.

Effects of Methylxanthine Derivatives on Induction and Enhancement of Hamster Epididymal Sperm Motility

Park, Y. S.*, S. J. Song**, H. J. Lee***, S. J. Lee****, N. H. Kim*, H. T. Lee* and K. S. Chung***

Animal Resource Research Center, Konkuk University*

Hamster 정소상체 정자의 운동성 유도와 증가에 영향을 미치는 Methylxanthine Derivatives의 효과

박용석*, · 송상진** · 이호준*** · 이상진**** · 김남형* · 이훈택* · 정길생***

건국대학교 동물자원연구센터*

요 약

본 연구는 Hamster 정소상체 정자의 운동성에 영향을 미치는 methylxanthine derivatives의 효과를 알아보고자 수행하였다. Pentoxifylline을 첨가하였을 때 운동 형태에 대한 첨가효과는 정소상체 체부에서 차이를 보였으며, 농도에 대한 변화는 1 mM 농도군에서 현저한 차이를 나타냈다. 2-Deoxyadenosine 첨가군에서도 pentoxifylline 첨가군과 같이 특히 VCL이 정소상체 체부 정자부터 증가하였으며 다른 농도군에 비해 1 mM과 2 mM에서 차이가 났다. Hypoxanthine 첨가군은 1 mM 농도에서 다른 첨가군에 비해 운동 형태가 증가하였다. 그러나 pentoxifylline과 adenosine 첨가군과는 달리 농도를 달리하여도 뚜렷한 운동형태의 변화는 관찰되지 않았다. VCL과 VAP는 pentoxifylline 1 mM과 2-Deoxyadenosine 1 mM 첨가군에서 정소상체 체부 정자의 운동성이 증가하고 hypoxanthine 1 mM 첨가군도 유의하게 증가하였다. 결론적으로 hamster 정소상체 정자의 운동성은 적정한 농도의 methylxanthine derivatives의 첨가로 증가됨을 알 수 있었다.

(Key words : Hamster, Pentoxifylline, 2-Deoxyadenosine, Hypoxanthine)

I. INTRODUCTION

Mammalian spermatozoa leaving the testis do not have the ability to fertilize eggs. In the study of maturation of spermatozoa in the epididymis, sperm motility, morphology, and fertilizing ability have been improved during epididymal transit in species such as the rat (Brandan and Rumery, 1964), rabbit (Bedford, 1966) and hamster (Horan and Bedford, 1972). Fertilizing ability and motility of spermatozoa are acquired during passage through the epididymis (Yanagimachi, 1970). Spermatozoa present in the proximal region of the caput epididymis are immotile and unable to fertilize eggs. Maturational changes in mammalian spermatozoa occuring along the length of the epididy-

** 삼성제일병원 불임연구실(Infertility Research Laboratory, Samsung Cheil Hospital)
*** 을지의과대학 생리학교실(Department of Physiology, Eulji Medical Center)
**** 삼육의명대학 동물자원과(Department of Animal Sciences, Sahmyook College)

mis are their pattern and extent of motility. In hamster, the caput epididymal spermatozoa are either immotile or extremely sluggish with very little progressive motility and from the distal corpus to the proximal cauda a drastic transition of motility occurred. The acquisition of forward or progressive motility in the corpus epididymal spermatozoa is more obvious and distinctly noticeable in the rat (Hinton et al., 1979). A well defined transition point is exhibited just above the flexure of the cauda epididymis. The quality of spermatozoa depends on the efficiency of storage in the distal cauda region and the rate at which spermatozoa pass from the proximal to the distal cauda region (Moore and Akhondi, 1996). Sperm concentrated in the cauda epididymis are capable of motility, but are maintained in a state of quiescence in most mammals.

Methylxanthine derivatives act a stimulating factor on sperm motion by inhibiting cyclic adenosine 3',5'-monophosphate (cAMP) phosphodiesterase, leading to an increase in the intracellular cAMP concentration (Lewis et al., 1993). Pentoxifylline, a phosphodiesterase inihibitor in the methylxanthine group, and 2-Deoxyadenosine, an analogue of adenosine, are well known as stimulants of sperm motility. It has been reported that in severe male infertility, pentoxifylline is effective on sperm motion characteristics (Tesarik and Mendoza, 1993).

Therefore, this study was carried out to determine effect of methylxanthine derivatives on hamster epididymal sperm motility pattern.

Ⅱ. MATERIALS AND METHODS

1. Animals

Adult male golden hamsters (100~120g) were housed under a constant light:dark region of 14 : 10 hours and allowed water and feed *ad libitium*. Male hamsters in 9~12 months of age which have a large, firm and turgid epididymis were used.

2. Media and reagents

The media used for the incubation of hamster spermatozoa were modified Tyrode's solution supplemented with albumin, lactate and pyruvate (TALP). TALP [NaCl (110 mM), KCl (5 mM), $NaHCO_3$ (24.9 mM), $NaH_2PO_4 \cdot H_2O$ (0.36 mM), $MgCl_2 \cdot 6H_2O$ (0.49 mM), $CaCl_2 \cdot 2H_2O$ (2.4 mM), glucose (5 mM), sodium lactate (6.26 mM), and sodium pyruvate (0.125 mM)] was dissolved in deionized water and adjusted pH to 7.2 and then BSA (3 mg/ml) was added. The osmolarity of medium was 290~300 mOsmol. All inorganic salts were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO. USA).

3. Preparation of epididymal spermatozoa

To obtain epididymal spermatozoa, animals were killed by CO_2 asphyxiation and the testis and epididymis were exposed through a scrotal incision. Longitudinal incisions were made and spermatozoa were collected from three regions of epididymis. Spermatozoa were released by gently swirling the tissue in medium blotted free of blood and gently pierced with a 23 gauge needle so as to puncture. The sperm suspension was transferred to fresh TALP and incubated in CO_2 incubator (5% CO_2 and 95% in air maintained at 37℃). All media were overlaid light mineral oil and equilibrated with CO_2 prior to use at 37℃. About 5 μl of the sperm aliquot was collected and the concentration of spermatozoa and the number of motile spermatozoa were determined.

4. Effect of methylxanthine derivatives on epididymal sperm motility

Sperm were obtained described as above. Spe-

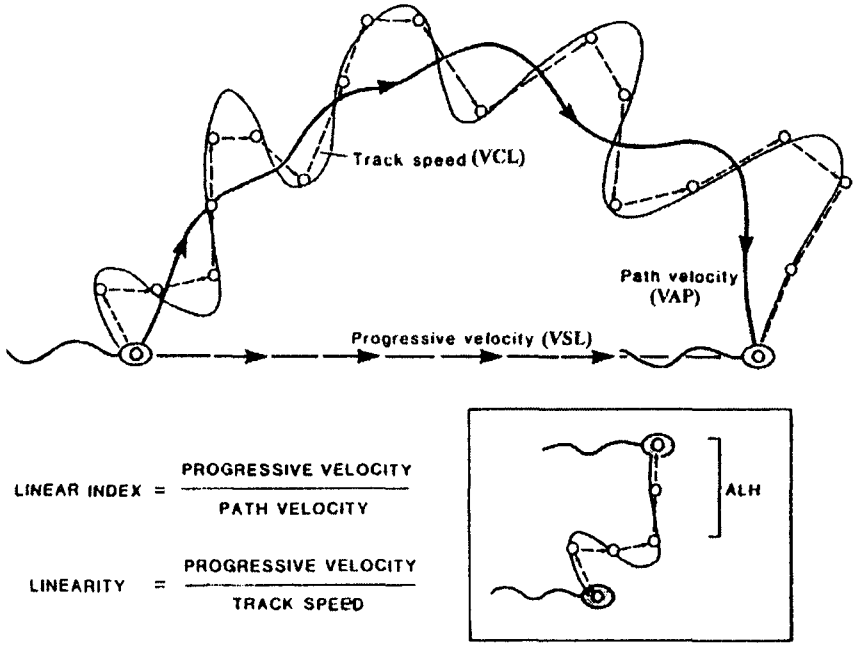

Fig. 1. The movement characteritics of spermatozoa. The track speed is known as the curvilinear velocity (VCL) and represents the total distance travelled by the sperm head in unit time; path velocity is also known as average path velocity (VAP) and represents the average path followed by the sperm head in unit time and is generally plotted as the 5 point running average; progressive velocity is also known as straight line velocity (VSL) and represents the straight line distance between the beginning and the end of the tract divided by the time elapsed; ALH represents the amplitude of lateral sperm head displacement and corresponds to the mean width of the lateral sperm head oscillation. Two measurement of the linearity of sperm movement are : straightness (STR) calculated as STR = VSL/VAP and linearity (LIN) calculated as LIN = VSL/VCL. (Taken from Aitken, J., 1995).

rm suspensions were divided, one part remains as control and the other treated with pentoxifylline (1, 2 and 3 mM), 2-deoxyadenosine (1, 2 and 3 mM) and hypoxanthine (1, 2 and 3 mM). Sperm motility parameters were assessed after a 30 min incubation (5% CO_2 and 95% in air maintained at 37℃) or various incubation times, i.e. 1, 2, 3 and 4 hr.

5. Analysis of sperm motility using Computer-Assisted Sperm Analysis (CASA) system

For motility analysis using Computer-Assisted Sperm Analysis (CASA) System, sperm suspension was transferred to the Makler chamber and analysis were performed using the HTM-Master C Motility Analyzer, Version 10.6 (Hamilton Thorn Research Inc., Danvers, MA, USA). The HTM-Master C Analyzer consists of a stereoscopic optical system, 486 kb memory computer, monitor and printer. A part from determining the sperm count and the number of motile spermatozoa, the analyzer provides data with re-

spect to seven other characteristics and always tracking the head of the spermatozoa which is the center of brightness. The other characteristics of sperm motility which are determined include VCL (which is the track speed of the sperm obtained by dividing thre total distance traveled by the sperm during an acquisition by the time elapsed), VSL (which is the straight line distance between the beginning and end of a sperm track divided by the time elapsed), VAP (which is the track speed along the average path of each sperm), STR or straightness (VSL/VAP), LIN or linearity (VSL/VCL), ALH (which refers to the mean width of the sperm head oscillation along the sperm track as it swims) and BCF (which is the frequency with which the track crosses the path in either direction). The definition of sperm kinematic parameter was summarized in Fig. 1.

6. Statistical Analysis

The data were analyzed by χ^2 test. A value of $P<0.05$ was considered to be statistically significant.

Ⅲ. RESULTS AND DISCUSSION

Changes of motility patterns of hamster epididymal spematozoa were observed. Kinematic parameters using a Computer Assisted Sperm Analysis (CASA) system has been validated for rat epididymal spermatozoa. (Yeung et al., 1992). Spermatozoa were prepared in media and mixed with each concentrations of pentoxifylline as described in materials and methods. After 30 min, the continuous presence of pentoxifylline, sperm motility was observed (Table 1). These spermatozoa showed a significant increased in motion parameters when exposed to concentrations of 1 mM pentoxifylline. An increase in the mean VCL value was one of the most conspicuous effects of pentoxifylline. The VCL showed a

Table 1. Kinematic parameters of a variety of epididymal spermatozoa to various concentrations of pentoxifylline

Parameter[1]	Concentration	Caput	Corpus	Cauda
VCL	Control	14.6 ± 4.3	37.9 ± 5.5	99.7 ± 10.0*
	0.5 mM	16.1 ± 2.0	40.3 ± 1.2	104.7 ± 4.0*
	1 mM	29.3 ± 4.9	132.9 ± 10.5	232.6 ± 25.4*
	2 mM	33.6 ± 2.8	70.2 ± 5.0	163.4 ± 16.8*
	3 mM	25.3 ± 0.9	63.9 ± 4.7	120.9 ± 14.3*
VAP	Control	13.0 ± 4.0	35.5 ± 5.4	96.7 ± 11.3*
	0.5 mM	13.6 ± 1.7	36.9 ± 1.2	99.1 ± 2.5*
	1 mM	24.5 ± 1.1	102.3 ± 4.5	216.8 ± 23.6*
	2 mM	27.3 ± 5.0	65.0 ± 3.2	144.1 ± 13.7*
	3 mM	22.4 ± 1.0	60.4 ± 5.5	111.1 ± 12.0*
VSL	Control	11.9 ± 4.1	32.4 ± 5.0	91.0 ± 8.8*
	0.5 mM	12.1 ± 1.5	32.7 ± 2.3	87.2 ± 5.9*
	1 mM	14.9 ± 1.6	61.3 ± 3.2	181.5 ± 22.3*
	2 mM	24.5 ± 4.7	59.2 ± 4.9	137.8 ± 16.1*
	3 mM	15.3 ± 0.6	55.8 ± 5.1	96.5 ± 3.3*

[1]VCL ; curvilinear velocity, VAP ; average path velocity, VSL ; straight line velocity

* $P<0.01$

Table 2. Kinematic parameters of a variety of epididymal spermatozoa to various concentrations of 2-deoxyadenosine

Parameter[1]	Concentration	Caput	Corpus	Cauda
VCL	Control	14.6 ± 4.3	37.9 ± 5.5	99.7 ± 10.0*
	0.5 mM	12.4 ± 1.8	33.7 ± 3.1	98.9 ± 5.8*
	1 mM	24.9 ± 3.3	110.1 ± 8.4	223.2 ± 11.1*
	2 mM	29.7 ± 1.3	89.3 ± 17.2	204.5 ± 14.4*
	3 mM	22.4 ± 1.9	47.9 ± 3.8	137.7 ± 14.5*
VAP	Control	13.0 ± 4.0	35.5 ± 5.4	96.7 ± 11.3*
	0.5 mM	11.5 ± 1.8	31.4 ± 3.2	94.7 ± 4.6*
	1 mM	21.7 ± 2.4	100.3 ± 5.6	206.9 ± 11.1*
	2 mM	18.7 ± 1.1	52.4 ± 4.4	212.5 ± 30.2*
	3 mM	20.3 ± 2.9	44.0 ± 5.7	106.7 ± 10.0*
VSL	Control	11.9 ± 4.1	32.4 ± 5.0	91.0 ± 8.8*
	0.5 mM	9.4 ± 1.0	29.3 ± 3.8	90.1 ± 4.5*
	1 mM	14.2 ± 1.3	39.7 ± 5.1	189.0 ± 14.8*
	2 mM	13.0 ± 2.3	33.6 ± 4.1	148.6 ± 36.7*
	3 mM	13.5 ± 2.4	40.3 ± 3.9	96.4 ± 7.1*

[1]VCL ; curvilinear velocity, VAP ; average path velocity, VSL ; straight line velocity

* $P<0.01$

high value in corpus and cauda spermatozoa exposed to 1 mM pf pentoxifylline. The relative VCL increase with pentoxifylline treatment was expressed as a percentage of the control value. VAP and VSL value also increased in response to 1 mM concentration.

The VSL in the presence of 1 mM pentoxifylline was higher than other concentrations. Incubation with pentoxifylline, a significant increase in VCL for all three groups compared with control. VSL and VAP both tended to decrease with pentoxifylline incubation. The majority of studies using pentoxifylline has been demonstrated the action on the sperm motion characteristics in 3.6 mM concentration. This concnetration has been show to increase the VCL and ALH of spermatozoa in suspension (Tesarik et al., 1993; Kay et al., 1993; Lewis et al., 1993), while Fuse et al. (1993) used 5 mM pentoxifylline to demonstrate silmilar effects. The VSL in the presence of 1 mM pentoxifylline was higher than other concentrations. Incubation with pentoxifylline, a significant increase in VCL for all three groups compared with control. VSL and VAP both tended to decrease with pentoxifylline incubation. The majority of studies using pentoxifylline has been demonstrated the action on the sperm motion characteristics in 3.6 mM concentration. According to Tesarik et al. (1993), in 3.6 mM concentration of pentoxifylline, the sperm motion characteristics were increased, the maximum motion was obtained within 10 min of incubation and the activity persisted for at least 2 hr after drug removal. However, pentoxifylline does not improve the percentage of motile spermatozoa. This concentration has been show to increase the VCL and ALH of spermatozoa in suspension (Tesarik et al., 1993; Kay et al., 1993; Lewis et al., 1993), while Fuse et al. (1993) used 5 mM pentoxifylline to demonstrate similar effects. Therefore, these data showed that the effectiveness of pentoxifylline

Table 3. Kinematic parameters of a variety of epididymal spermatozoa to various concentrations of hypoxanthine

Parameter[1]	Concentration	Caput	Corpus	Cauda
VCL	Control	14.6 ± 4.3	37.9 ± 5.5	99.7 ± 10.0*
	0.5 mM	13.3 ± 0.9	40.3 ± 2.6	114.8 ± 2.7*
	1 mM	23.1 ± 6.2	132.9 ± 30.4	167.7 ± 3.4*
	2 mM	16.7 ± 1.1	70.2 ± 2.1	139.2 ± 2.4*
	3 mM	14.7 ± 0.8	63.9 ± 2.2	118.1 ± 2.3*
VAP	Control	13.0 ± 4.0	35.5 ± 5.4	96.7 ± 11.3*
	0.5 mM	12.4 ± 0.8	36.9 ± 6.1	98.8 ± 1.2*
	1 mM	17.7 ± 1.4	102.3 ± 3.5	155.7 ± 3.4*
	2 mM	14.7 ± 1.3	65.0 ± 2.7	124.5 ± 2.5*
	3 mM	15.4 ± 2.6	60.4 ± 9.4	109.9 ± 1.6*
VSL	Control	11.9 ± 4.1	32.4 ± 5.0	91.0 ± 8.8*
	0.5 mM	12.4 ± 2.1	32.7 ± 5.7	95.3 ± 9.6*
	1 mM	15.5 ± 1.8	61.3 ± 3.1	126.0 ± 1.7*
	2 mM	13.5 ± 0.8	59.2 ± 2.4	108.5 ± 1.8*
	3 mM	14.1 ± 1.9	55.8 ± 2.9	107.0 ± 1.8*

[1]VCL ; curvilinear velocity, VAP ; average path velocity, VSL ; straight line velocity

* $P<0.01$

result in response to 1 mM concentration of spermatozoa recovered from the corpus and cauda regions.

Table 2 showed the effect and differences among motility parameters in various concentrations of 2-deoxyadenosine on epididymal sperm motility. Till now, 2-deoxyadenosine is known to elevate cAMP through direct action on adenyl cyclase (Aitken et al., 1986) and to enhance sperm motility and hyperactivation (Mbizvo et

Table 4. Kinematic parameters of maximum rise after treatment with methylxanthine derivatives

Parameter[1]	Methylxanthine	Caput	Corpus	Cauda
VCL	CON	14.6 ± 4.3	37.9 ± 5.5	99.7 ± 10.0*
	PTX	29.3 ± 8.1	132.9 ± 103.8	232.6 ± 6.3*
	ADE	24.9 ± 0.8	110.1 ± 87.2	223.2 ± 5.7*
	HX	23.1 ± 1.1	69.9 ± 30.4	167.7 ± 30.4*
VAP	CON	13.0 ± 4.0	35.5 ± 5.4	96.7 ± 11.3*
	PTX	24.5 ± 6.2	102.3 ± 6.1	98.8 ± 1.2*
	ADE	21.7 ± 2.9	102.3 ± 3.5	155.7 ± 3.4*
	HX	17.7 ± 1.4	65.0 ± 2.7	124.5 ± 2.5*
VSL	CON	11.9 ± 4.1	32.4 ± 5.0	91.0 ± 8.8*
	PTX	14.9 ± 3.6	32.7 ± 5.7	95.3 ± 9.6*
	ADE	14.7 ± 3.3	61.3 ± 3.1	126.0 ± 1.7*
	HX	15.5 ± 1.9	55.8 ± 2.9	107.0 ± 1.8*

[1]VCL ; curvilinear velocity, VAP ; average path velocity, VSL ; straight line velocity, CON ; control, PTX ; pentoxifylline, ADE ; 2-deoxyadenosine, HX ; hypoxanthine.

* $P<0.01$

al., 1993). Thus, addition of 2.5 mM of 2-deoxyadenosine, the proportion of motile sperm were increased to 21~39%, especially linear velocity and in the frequency of sperm head rotation (Aitken et al., 1986). Yovich et al. (1990) reported that pentoxifylline alone or 2-deoxyadenosine alone significantly improved the incidence of *in vitro* fertilization. The combination of both pentoxifylline and 2-deoxyadenosine is known to increase intracellular cAMP levels by stimulation of adenylate cyclase (Fraser and Monks, 1990). Incubation with 2-deoxyadenosine, compared with control, a significant increase in motility parameters were observed on 1 mM 2-deoxyadenosine. The concentration of 1 mM and 2 mM 2-deoxyadenosine showed significantly increased motility. However, in the presence of 2-deoxyadenosine, 0.5 mM concentration was decreased compared with control group.

The effect of hypoxanthine on sperm motility has been not reported. Therefore, this study tried to examine the effect of hypoxanthine on sperm motility. Addition of 1 and 2 mM hypoxanthine increased motility parameters of epididymal spermatozoa. As shown in Table 3, an increase in the VCL value was one of the most conspicuous effects of hypoxanthine.

When this drug was added to sperm suspensions, VCL was increased significantly and VAP and VSL also showed increased. However, motility parameter of 0.5 mM and 3 mM concentration was not different. Conclusively, supplement of hypoxanthine is effective to the epididymal spermatozoa. Table 4 showed the effects of proper concentration of methylxanthine derivatives. The effect of incubation with methylxanthine derivatives at concentrations of 1 mM was then evaluated. As the incubation time passed, the maximum stimulation effect was occurred after 1 hr incubation. The possibility of achieving the maximum effect after a short exposure of spermatozoa to methylxanthine derivatives and the relatively long persistence of the effect after subsequent drug removal are advantages for using in reproductive medicine (Tesarik et al., 1993). This result showed methylxanthine derivatives addition was to significantly increase sperm motility parameter, and pentoxifylline is the most effective agent compared with 2-deoxyadenosine and hypoxanthine. All motility patterns were similar and did not show any significant changes in early incubation (i.e., within 1 hr).

Ⅳ. SUMMARY

This study were performed the effect of methylxanthine derivatives on hamster epididymal sperm motility. To assess the effect of methylxanthine derivatives on motility kinematics of epididymal sperm, pentoxifylline, 2-deoxyadenosine and hypoxanthine were added to TALP medium. 1 mM of pentoxifylline, significantly increased VCL, VAP, VSL of spermatozoa obtained from corpus and cauda epididymis. With 1 mM of 2-deoxyadenosine, VCL, VAP, VSL of spermatozoa obtained from corpus and cauda epididymis were significantly increased, but 2 mM ADE for cauda spermatozoa was effective than 1 mM. In the case of hypoxanthine, various concentrations were not significantly effective, but 2 mM HX showed higher effect than other concentrations. pentoxifylline 1 mM and 2-deoxyadenosine 1 mM significantly increased VCL, VAP of corpus and cauda epididymal spermatozoa and VSL of cauda epididymal spermatozoa. From these results it could be concluded that the addition of methylxanthine increase motility kinematics of hamster spermatozoa collected from epididymis.

V. REFERENCES

1. Aitken, R.J., A. Mattei, and S. Irvine. 1986. Paradoxical stimulation of human sperm motility by 2-deoxyadenosine. J. Reprod. Fert., 78:515-527.
2. Bedford, J.M. 1966. Development of fertilizing ability of spermatozoa in the epididymis of the rabbit. J. Exp. Zool., 163:319-324.
3. Brandan, R.J. and R.E. Rumery. 1964. The relationship of swimming movement of epididymal spermatozoa to their fertilizing capacity. Fertil. Steril., 15:571-577.
4. Fraser, L.R. and N.J. Monks. 1990. Cyclic nucleotides and mammalian sperm capacitation. J. Reprod. Fert. Suppl., 42:9-21.
5. Fuse, H., M. Sakamoto, S. Ohta, and T. Katayama. 1993. Effect of pentoxifylline on sperm motion. Arch. Androl., 31:9-15.
6. Aitken, J. 1995. Cell biology of human spermatozoa. In: Grudzinskas, J.G. and J.L. Yovich. eds. Gametes-The spermatozoon. Cambridge; Cambridge University Press, 206-237.
7. Hinton, B.T., H.M. Dott, and B.P. Setchell. 1979. Measurement of the motility of rat spermatozoa collected by micropuncture from the testis and from different regions along the epididymis. J. Reprod. Fert., 55:167-172.
8. Horan, A.M. and J.M. Bedford. 1972. Development of the fertilizing ability of spermatozoa in the epididymis of the Syrian hamster. J. Reprod. Fert., 30:417-423.
9. Kay, V.J., J.R.T. Coutts, and L. Robertson. 1993. Pentoxifylline stimulates hyperactivation in human spermatozoa. Hum. Reprod., 8:727-731.
10. Lewis, S.E.M., J.M. Moohan, and W. Thompson. 1993. Effects of pentoxifylline on human sperm motility in normospermic individuals using computer-assisted analysis. Fertil. Steril., 59:418-423.
11. Mbizvo, M.T., R.C. Johnson, and G.H.W. Baker. 1993. The effect of the motility stimulants caffeine, pentoxifylline and 2-deoxyadenosine, on hyperactivation of cryopreserved human sperm. Fertil. Steril., 59:1112-1117.
12. Moore, H.D.M. and M.A. Akhondi. 1996. Fertilizing capacity of rat spermatozoa is correlated with decline in straight-line velocity measured by continuous computer aided sperm analysis: Epididymal rat spermatozoa from the proximal cauda have a greater fertilizing capacity in vitro than those from the distal cauda or vas deferens. J. Androl., 17:50-60.
13. Tesarik, J. and C. Mendoza. 1993. Sperm treatment with pentoxifylline improves the fertilizing ability in patients with acrosome reaction insufficiency. Fertil. Steril., 60:141-148.
14. Yanagimachi, R. 1970. The movement of golden hamster spermatozoa before and after capacitation. J. Reprod. Fert., 23:193-196.
15. Yeung, C.H., G. Oberlander, and T.G. Cooper. 1992. Characterization of the motility of rat spermatozoa by computer-aided objective measurement. J. Reprod. Fert., 96:427-431.
16. Yovich, J.M., W.R. Edirisinghe, J.M. Cummins, and J.L. Yovich. 1990. Influence of pentoxifylline in severe male factor infertility. Fertil. Steril., 53:715-722.

(접수일자 : 1999. 1. 25. /채택일자 : 1999. 2. 20.)