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Effect of Caffeine and Heparine on Acrosome Reaction and Protein Pattern of Korean Native Cattle Sperm *In Vitro*

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카페인과 헤파린 처리가 한우정자의 첨체반응과 단백질상에 미치는 영향

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요 약

본 연구는 수정능획득유기물질로 알려진 카페인과 헤파린을 병행처리하여 한우 정자의 첨체반응율과 생존율을 알아보고 수정능획득과정 중에 단백질의 변화상을 전기영동방법으로 조사하였다. 동결융해후 정자의 생존율은 90%이상이었으나 전배양처리후 0.5시간에 70%로 감소하고 2시간 이후에는 35%로 감소하였다. 정자의 첨체반응율은 동결융해후 정상정자가 85.7%였으나 전배양시간에 따라 53.4%에서 14.3%로 감소하였다. 동결융해후 첨체가 소실된 생존정자는 9.4%였으나 전배양시간에 따라 16.7%에서 21.5%로 증가하였고 첨체가 소실된 죽은 정자는 동결융해후 3.3%에서 전배양시간에 따라 17.2%에서 46.8%로 증가하였다. 원정액에서는 분자량 20,000정도에서 동결융해후 전자보다 특이적으로 많은 단백질량을 나타내었으나 동결융해 후 정자를 0.5에서 2.0시간으로 전배양하였을 때 전배양시간에 따른 단백질상의 변화는 대조구와 큰 차이가 없었다.

(Key words : caffeine, heparine, preincubation, acrosome reaction, electrophoresis, protein pattern, Korean Native cattle, *in vitro*)

INTRODUCTION

A traditional method of producing high potential animals has been through A.I. Animal producers have long been working on the efficient method of producing animals with large economic merit. Recent development of reproductive biotechnologies has brought a means of accelerating the efficacy of animal production. Among them, the techniques of *in vitro* fertilization(IVF) and embryo transfer today are widely practiced in livestock species to improve productivity and genetic potential. Especially, the application of IVF technique in cattle is of great potential. Calves have been produced by large through IVF. The capacitation of sperm is an important factor in the process of *in vitro* fertilization. For sperms and eggs to be fertilized, physiological and functional changes must be achieved. This process is called the capacitation(Austin, 1951; Chang, 1951). For a sperm to penetrate the zona pellucida and fertilize an egg, the acrosome reaction should occur(Barros et al, 1967). To date, the direct method for capacitation is the evaluation of hyperacti-

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vation, which is only possible in hamster. To detect the capacitation of bovine sperm, sperm were fertilized with zona-free hamster ova(Brackett et al., 1982, Graham and Foote, 1984), Zona-free bovine ova(Fulka, 1992), and *in vitro* matured bovine ova(Ball et al, 1983, Lenz et al, 1983), which is the direct method of detecting sperm capacitation. However, these direct methods are practically difficult because they were time-demanding and costly. The objective of this study was to examine the effects of heparin and caffeine on acrosome reaction of Hanwoo (Korean Native cattle) sperms and the pattern of protein change of sperms during the process of acrosome reaction.

MATERIALS AND METHODS

The frozen sperms from Hanwoo were thawed for 30 seconds at 35℃ in water bath, following the method of Niwa and Ohgoda(1988). Adding 5mM /ml caffein plus 10μg /ml heparine in Bo medium(Brackett and Oilphant, 1975), they were preincubated at 39℃ in 5% CO_2 incubator for 0, 0.5, 1.0, and 2.0 hrs, respectively. The rate of acrosome reaction(Didion et al., 1989) was measured for 200 sperms per slide. The preincubated sperms were centrifuged for 10 min at 200g. The supernatant was freeze-dried and stored at −20℃ before the electrophoresis and the sperm pellet was washed twice with saline. The electrophoresis(Laemmli, 1970) was done with SDS-PAGE.

RESULTS AND DISCUSSION

The survival rate of frozen-thawed bovine sperm was over 90%(Fig. 1). However, after treated with 5mM /ml of caffeine plus 10μg /ml of heparin for 0.5 hr and 2 hr, it was decreased to 70% and 35%, respectively. As shown in Table 1, after treating with 5mM /ml of caffeine plus 10μg /ml of heparin, the proportion of live intact-sperm was 85.7% after thawing but decreased to 53.4~14.3% by increasing the preincubation time from 0.5 hr to 2 hr. The percent-

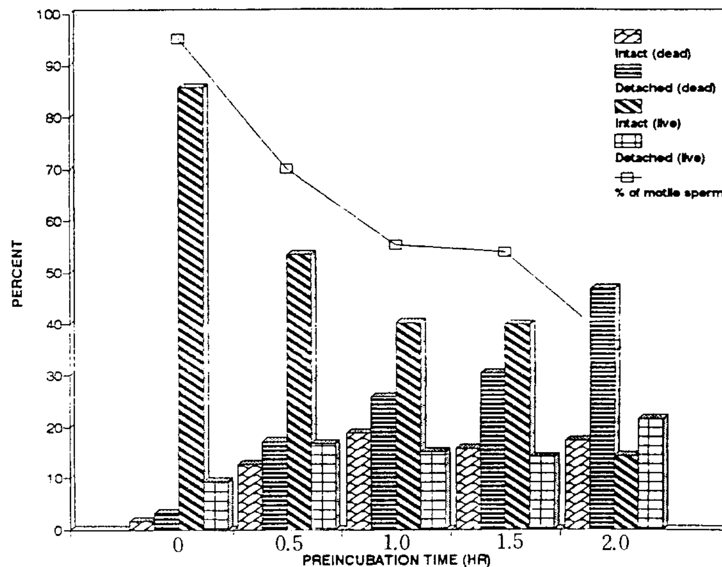

Fig. 1. Changes in percentage of motile and acrosome-reacted sperm at various in incubation times under caffeine and heparin treatment.

age of acrosome-detached viable sperm was 9.4% after thawing and slightly increased to 16.7~21.5% by increasing the preincubation time from 0.5 hr to 2 hr. The percentage of acrosome-detached dead sperm was 3.3% after thawing but increased to 17.2~46.8% by increasing the preincubation time from 0.5 hr to 2 hr. Among live sperm, there was no significant difference in percentage of acrosome-detached sperm with preincubation time. However, among dead sperm, there was significant difference in percentage of acrosome-detached sperm with preincubation time. Especially, in preincubation time of 2 hr, the percentage of acrosome detached sperm drastically increased to 63.8%. From this result, the percentage of acrosome reacted sperm in general was found to increase with preincubation time. The percentage of detached sperm among live sperm was not changed but the percentage of detached sperm among dead sperm was increased. As observed in this study, normal sperm changed to capacitated sperm, the capacitated sperm changed to acrosome reacted sperm, and then, they were dead, which is the general cell cycle of sperm. The same result was also found in the study of Didion et al.(1989).

From the result of electrophoresis(Fig. 2),

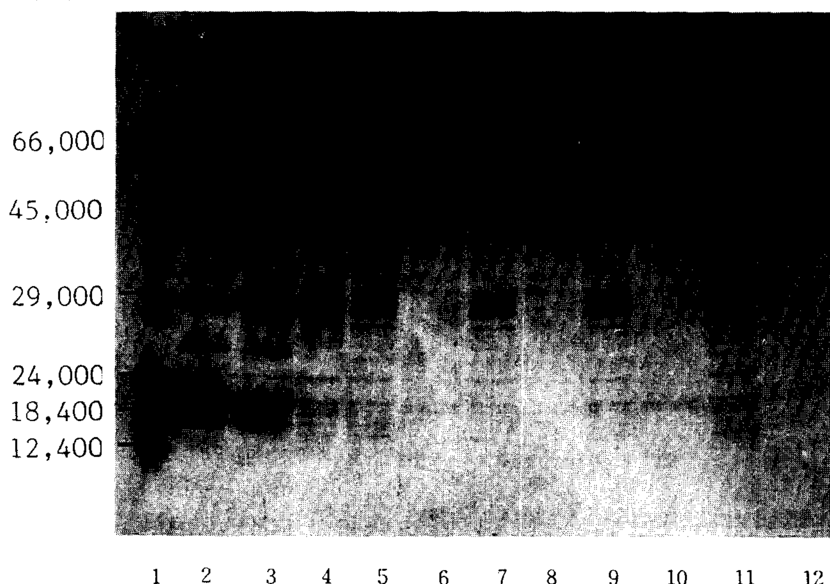

Fig. 2. Electrophoresis (SDS-PAGE) of bovine sperm treated with caffeine and heparin.

1. Standard protein
2. Seminal plasma of fresh semen
3. Sperm pellet from Eresh semen
4. Sperm pellet from Frozen-thawed semen
5. Sperm pellet from sperm preincubated for 0.5hr
6. Sperm suspension from sperm preincubated for 0.5hr
7. Sperm pellet from sperm preincubated for 1.0hr
8. Sperm suspension from sperm preincubated for 1.0hr
9. Sperm pellet from sperm preincubated for 1.5hr
10. Sperm suspension from sperm preincubated for 1.5hr
11. Sperm pellet from sperm preincubated for 2.0hr
12. Sperm suspension from sperm preincubated for 2.0hr

Table 1. Acrosome reaction(%) of frozen-thawed sperm preincubated in BO with caffeine and heparine

Sperm treatment	Incubation time(h)	Dead sperm		Live sperm		A+C	B+D
		Intact(A)	Detacted(B)*	Intact(C)	Detacted(D)*		
Caffeine	0	1.7	3.3	85.7	9.4	87.4	12.7
(5mM)	0.5	12.8	17.2	53.4	16.7	66.2	33.8
+	1.0	18.9	25.9	40.2	15.1	59.0	41.0
Heparin	1.5	15.8	30.3	39.8	14.2	55.6	44.5
(10μg/ml)	2.0	17.4	46.8	14.3	21.5	31.7	68.3
Heparin (10μg/ml)	0	1.5	4.0	89.5	5.0	91.0	9.0
	0.5	19.9	17.2	47.7	15.3	67.5	32.5
	1.0	17.7	25.1	37.4	19.9	55.0	45.0
	1.5	16.7	34.4	29.1	19.9	45.8	54.3
	2.0	12.8	39.5	26.6	23.2	37.4	62.7

* B: False acrosome-reacted(AR) sperm, D:true AR sperm.

fresh semen showed more protein content in 20, 000 dalton of molecular weight and also, no significant differences among the band patterns of sperm in different incubation time were observed. In 66,000 dalton of molecular weight, more protein content was found in the supernatant of thawed semen than that of fresh semen. No difference among protein band patterns was observed in capacitation of different incubation time. The larger amount of protein content in higher molecular weight was found, which was possibly due to the leakage of proteinase into the supernatant.

SUMMARY

After treated with 5mM/ml of caffeine plus 10μg/ml of heparin, the proportion of live intact-sperm was 85.7% after thawing. It was decreased to 53.4~14.3% by increasing the preincubation time from 0.5 hr to 2 hr. The percentage of acrosome-detached viable sperm was 9.4% after thawing and was slightly increased to 16.7~21.5% by increasing the preincubation time from 0.5 hr to 2 hr. There was no significant difference between acrosome reaction rate of Hanwoo sperm treated with different combination of caffein and heparine. More than 40% of acrosome reaction was found after 1 hr of preincubation. Protein band patterns were not changed in capacitation of different incubation time.

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