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The Effects of Caffeine on Sperm Motility and In vitro Embryo Development for Different Bulls

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Abstract: *The effects of caffeine on the motility of frozen-thawed bull spermatozoa from 4 different bulls and their ability to fertilize in vitro matured oocytes was assessed. Frozen-thawed semen was divided into two groups. One group was incubated for 6 h in BO solution either with or without 2.5 mM caffeine. During the incubation period, the motility for each bull was observed at 0.5 h intervals. The other group was used to inseminate in vitro matured bovine oocytes. The addition of caffeine to the BO solution did not stimulate the motility of all bulls. The percentage of embryos that cleaved by the third day after insemination varied between bulls. In one bull, the percentage of cleaved embryos was significantly lower (P<0.05) than for the other bulls in the presence of caffeine. This percentage was also significantly lower (P<0.05) than that in the absence of caffeine in the same bull. These results indicated that caffeine do not stimulate the motility of frozen-thawed bovine spermatozoa in the BO medium, and that caffeine is not necessarily useful for the induction of in vitro sperm capacitation.*

Key words: *Bovine sperm motility, Caffeine, IVF, Embryo development.*

In mammals, highly motile spermatozoa are needed to penetrate to the zona pellucida of oocytes [1]. To stimulate sperm motility, some chemical agents including caffeine have been used for *in vitro* fertilization. Caffeine, a methylxanthin, is a cyclic nucleotide phosphodiesterase inhibitor that stimulates the sperm motility in man, pig and cattle *in vitro* [2–5]. The fertilization rates for mouse, bovine and porcine oocytes can be increased by caffeine treated spermatozoa [6–9]. Whether caffeine stimulates bovine spermatozoa is less clear [10, 11] and the relationship between bovine sperm motility and oocyte development after *in vitro* fertilization has not been well elucidated.

The present study was undertaken to evaluate the effects of caffeine on the motility and fertilizing capacity of bovine spermatozoa from 4 different bulls.

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Materials and Methods

Oocyte maturation

Bovine ovaries were obtained from a local slaughterhouse and brought to our laboratory in physiological saline (0.9%, NaCl) within 4 h after collection, and oocytes were aspirated from superficial follicles (1–6 mm in diameter) with a 20 G needle (Terumo, Tokyo, Japan) attached to 6 ml disposable syringe (Terumo). Oocytes attached with cumulus cells were selected, washed twice with TCM-199 (Gibco, MD., U.S.A.) supplemented with 2% calf serum (CS, Gibco). The oocytes were then transferred to 2.5 ml of 25 mM Hepes buffered TCM-199 supplemented with 5% CS and antibiotics (100 u/ml penicillin and 100 μg/ml streptomycin, Meiji Seika Co., Tokyo, Japan) and incubated for 24 h at 38.5°C in 5% CO_2 and 95% air under paraffin oil (Katayama Chemical Co., Osaka, Japan).

Semen preparation

Frozen semen from four Holstein bulls were thawed in a 37–39°C water bath for 1 min. After thawing, the semen was diluted stepwise (2, 4, 8 and 16 times, 2 min/step) with BO solution [12] with or without 5 mM caffeine (Katayama Chemical Co.). After the final dilution, the semen was centrifuged for 8 min (600 xg) at room temperature, and the sperm concentration adjusted to 1,800–1,900 × 10^4 sperm/ml with BO solution supplemented with 15 mg/ml bovine serum albumin (Fraction V, Katayama Chemical Co.). At this dilution, the final

concentration of caffeine was 0 or 2.5 mM.

To assess the sperm motility from each bull, 100 μl of semen was covered with paraffin oil and motility was assessed with an inverted microscope (x100 magnification) at 0.5 h intervals throughout a 6 h incubation period.

Matured oocytes were placed in 100 μl drops of semen (1,800–1,900 × 10^4 sperm/ml, 15–20 oocytes/drop) under paraffin oil and incubated for 6 h. The oocytes were then transferred to 2.5 ml of TCM-199 supplemented with 1% CS and antibiotics. On the 3rd day after insemination, these oocytes were removed from their cumulus cell layer and their development was assessed with an inverted microscope (× 100 magnification).

Statistical analysis

Embryo cleavage was analyzed by chi-square test [13].

Results

The sperm motility for each bull is shown in Fig. 1. Caffeine had no effect on the initial sperm motility of any of the bulls. By the end of the 6 h incubation period, the sperm were almost immotile in all caffeine treated groups and 3/4 of the non-treated group.

From 0.5 h in the incubation period, motilities of caffeine treated spermatozoa from 3 of 4 bulls (P-220, 222, 225) were slightly lower than those from each control group. In P-194, the motilities in both the presence or absence of caffeine were almost the same throughout the whole incubation period.

The number of embryos that had reached the 2 to 16 cell stage by the 3rd day after insemination is shown in Table 1. In 2 of 4 bulls (P-222 and 225), the percentage of cleaved embryos fertilized was higher for caffeine treated spermatozoa, but there was no correlation with

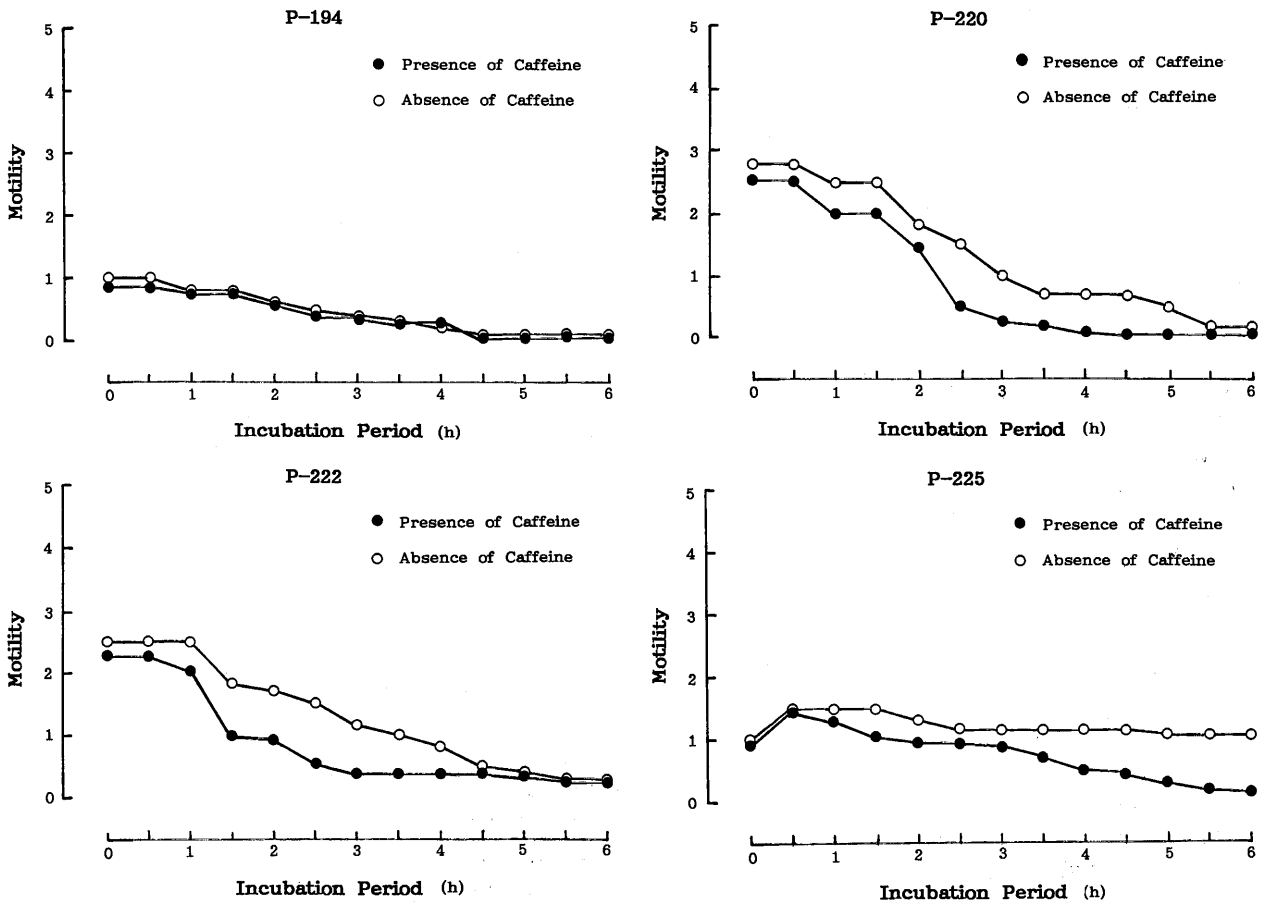

Fig. 1. The motility of sperm from four bulls (P-194, P-220, P-222 and P-225) in the presence (2.5 mM) or absence (0 mM) of caffeine during incubation for 6 h.

Table 1. The percentage of bovine embryos from the 2 to the 16 cell stage in the presence or absence of caffeine in each bull

Bull No.	Percentage of embryos %*			
	N	Presence of caffeine	N	Absence of caffeine
P-194	50	14.0	43	16.3
P-220	47	0.0**	40	12.5
P-222	16	31.3	15	13.3
P-225	46	19.6	51	7.8

*: Determined on the 3rd day after fertilization.
**: Significantly different ($P<0.05$) within a column and between presence and absence of caffeine.

sperm motility in the presence or absence of caffeine. For bull P-194 showed the same sperm motilities and fertilizing capacity in both the presence and absence of caffeine, but for bull P-220 no cleavage was observed in oocytes inseminated with caffeine treated sperm.

Discussion

This study shows that caffeine failed to stimulate sperm motility in any bull. Caffeine should enhance the concentration of cyclic AMP (cAMP) in bovine ejaculated sperm [3]. An increase in cAMP should increase the motility and oxygen consumption of sperm. Fraser and Monks [14] indicated that the increase in cAMP was related to the induction of acrosome reaction, although the mechanisms involved in the processes have not yet been clarified. Bird *et al.* [15] indicated that the addition of 2.5 mM caffeine, the same concentration as in our experiment, to the fertilization medium stimulated the motility of frozen-thawed bovine sperm. Caffeine also enhances sperm motility in man [4, 16]. Contrary to these reports, our results confirm those of Crister *et al.* [10], who observed no stimulating effect of caffeine on the motility of ejaculated bovine spermatozoa.

Sperm motility is important for sperm to fuse with the oolemma in mouse [1]. Pomeroy *et al.* [17] suggested that fertilization of mouse oocytes *in vitro* might be enhanced by a stimulating effect of caffeine on their sperm motility or acrosome reaction, but in the present study the sperm motilities in our bulls did not seem to be related to the development of *in vitro* fertilized oocytes. Bird *et al.* [15] observed that a high concentration of caffeine (5 mM) could have an adverse effect on bovine sperm motility as the penetration rate of hamster oocytes was significantly increased compared with those at a lower concentration of caffeine. Long *et al.* [18] reported that although bovine sperm motility was enhanced by the addition of a mixture of penicillamine, hypotaurine and adrenalin, this did not increase the number of oocytes that were penetrated *in vitro*. Further, Moore and Akhondi [19] suggested that sperm motility was a poor predictor of sperm fertilizing ability in rat *in vitro* fertilization. Considered with these reports, our result suggests that sperm motility is not a powerful predictor for *in vitro* fertilization of cattle.

In the present study, the number of oocytes which were cleaved after fertilization varied from bull to bull. Differences in males were one of the important factors influencing not only fertilization but also embryonic development [20], which suggests that further studies are needed.

We conclude that the sensitivity of bovine spermatozoa to caffeine in fertilization medium differs between bulls and that this influences their capacity for fertilization.

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