

Effects of Magnetic Pole on Orientation of Bull Sperm Treated by Dithiothreitol or Heparin

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ABSTRACT : Effect of the north (N) or south pole (S) of a magnet and dithiothreitol (DTT) or heparin sodium (H) on the orientation of bull sperm were studied. Sperm were collected from four Japanese Black bulls of Okinawa Prefectural Livestock Experimental Station were treated with various concentrations of DTT or H during the first and sixth days and were then exposed them to N or S with a magnetic field of 3,000 Gauss for 24 hours. Experimental results showed that both N and S significantly increased the perpendicular oriented percentage of bull sperm in most treatments not treated or treated with DTT or H. Anisotropy of magnetic field of bull sperm was the main cause which induced them to orient perpendicular to the magnetic direction. The results of the experiment also showed that, in most cases, the two polarities had the same effect on the orientation of bull sperm. However, in some cases, N exerted a stronger effect on the perpendicular percentage of bull sperm than S, such as with the 20 mM DTT treatment at the first and sixth days and the 100 units H treatment at the sixth day. These cases showed that the perpendicular oriented percentage of bull sperm at N was significantly higher than that of S. It was only the 100 units heparin treatment at S both at the first and sixth days that upward perpendicular orientation was higher than that at N. This might be due to the fact that protamin-DNA structure of a small number of bull sperm nuclei slightly changed because of heparin action. (*Asian-Aust. J. Anim. Sci.* 2001. Vol. 14, No. 2 : 155-162)

Key Words : Magnetic Pole, Bull Sperm, Orientation, Dithiothreitol, Heparin

INTRODUCTION

Some experiments have been made on the effect of the magnetic field on components of the body such as sperm and erythrocyte. Those experiments examined the influence of magnetic field on two aspects. The first is the activity of those components as shown by their viability, motility or velocity. For this aspect, Suga et al. (1995) and Suga and Shinjo (1997) conducted experiments on the effect of weak magnetic fields (600 and 1000 Gauss) on frozen cattle sperm and reported that the viability and motility of bull sperm exposed to the magnetic field was higher than non exposed sperm. Similarly, Formiki et al. (1990) examined the motility of Danube salmon sperm (*Hucho hucho* L.) under a weaker magnetic field of about 70-100 mT (1T=10,000 Gauss) and demonstrated that magnetic field increased the percentage of activated spermatozoa and prolonged the time of different phases of their movement. The second aspect is the magnetic orientation of sperm and erythrocyte. For this aspect, Higashi et al. (1995) studied bull sperm, intact erythrocytes and glyceraldehyde fixed erythrocytes and showed that bull sperm oriented with their flat head and tail perpendicular to the magnetic direction. Suga et al. (2000) also observed the magnetic orientation of bull sperm treated by

dithiothreitol (DTT) or heparin (H) separately, under the exertion of a strong magnetic field (5,400 Gauss) and pointed out that by both treatments with DTT or H, a high percentage of perpendicular orientation occurred.

The results of the above authors showed clearly that magnetic field significantly increased the viability and motility of sperms or induced their orientation (sperm and erythrocyte) perpendicular or parallel to the magnetic direction. However, those experiments were carried out with the samples placed between the north (N) and south (S) poles of two magnets. Therefore an experiment was conducted on bull spermatozoa treated with DTT or H and exposed to N or S independently, to examine the effect of the polarity on the magnetic orientation of bull sperm.

MATERIALS AND METHODS

This experiment used sperm of bulls (*Japanese Black*), which were reared in Okinawa Prefectural Livestock Experimental Station. Semen samples were collected from four bulls and mixed in a plastic vial in amounts such that each bull was represented by the same number of sperm. The mixed semen sample was put in an incubator at 4°C for one day. This treated semen sample was used throughout the experiment. After settling of the semen sample, 100 µl of semen suspension were added to 2 ml of a salt solution containing 1% triton X-100 at room temperature (approximately 25°C). This sperm suspension was incubated with gentle rocking in a water bath at 38°C

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for one hour. After incubation, 100 μ l of demembrated sperm suspension were added to 2 ml of each of a salt solution containing 20 mM DTT, 2,000 mM DTT, 100 units H or 10,000 units H. These semen samples were placed in an incubator at 4°C for 6 days.

Evaluating the decondensation of bull sperm nuclei

The decondensation of bull sperm nuclei in this experiment was evaluated by measuring the sperm head area. Measurement of the sperm head area was according to the method of Suga et al. (2000). Decondensation of bull sperm was examined at the first and sixth days before exposing them to the magnetic field. Fifty μ l of sperm suspension were removed from each tube and mixed with 100 μ l of eosin B solution (2% in the salt solution). The lengths and widths of 15 randomly selected sperm heads were examined in wet preparation under a differential interference contrast microscope at 1,000 \times magnification. The surface area of each sperm head nucleus was calculated from the formula: $\text{area} = \{ \text{the long axis of sperm head} \times \text{the short axis of sperm head} \times \pi \} / 4$.

Method of exposure of semen samples to the north or south pole of the magnet

After measuring the head area, semen samples were exposed to a static magnetic field of about 3,000 Gauss in N or S for 24 hours at room temperature (approximately 25°C). This magnetic field was generated by a Nd-Fe-B permanent magnet. Method of exposure of semen samples to the magnetic pole is

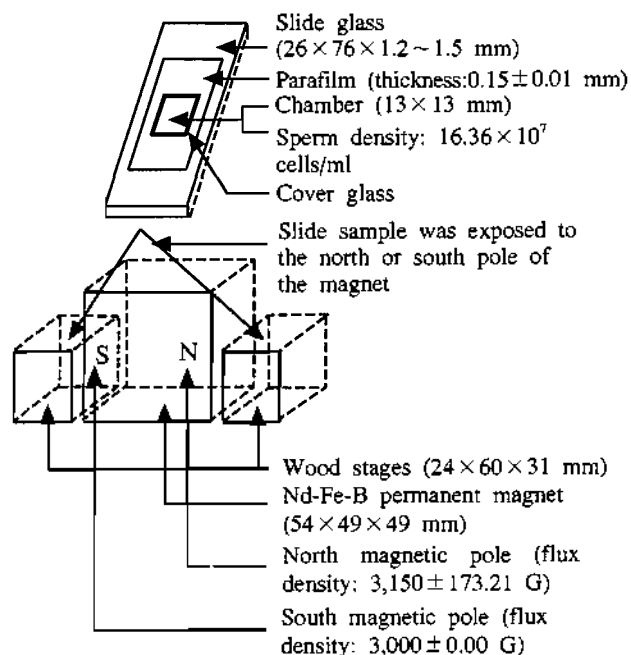


Figure 1. Method of exposure of bull sperm to the north or south pole of the magnet

described in figure 1. Fifty μ l sperm suspensions were removed from each tube and dropped into the clipping area on the slide (slide was prepared before). After dropping the semen sample into the slide chamber, a cover glass was used immediately. This manipulation needed to be done carefully to prevent air bubbles penetrating the slide chamber. The cover glass was moved in any direction to create a random direction for bull sperm, because non-motile sperm oriented themselves with flagellum towards the flow of the fluid. Then, the slide chamber was sealed with nail enamel around the border to keep the sample fluid from flowing out or evaporating. The slide chambers were put on wood stages placed at N or S (see figure 1). The control chamber of the sample was put in a part of the room not affected by artificial magnetic field.

Method to measure the magnetic oriented sperm

Methods of ranking and measuring of bull sperm orientation to the magnetic direction in this study were similar to those of Suga et al. (2000). The orientation of bull sperm to the magnetic direction was divided into eight categories, as described in figure 2. However, in this experiment, we reclassified from the eight bull sperm orientational categories of figure 2 into some main orientational categories; these were perpendicular and parallel (see table 2), upward head perpendicular, downward head perpendicular, leftward

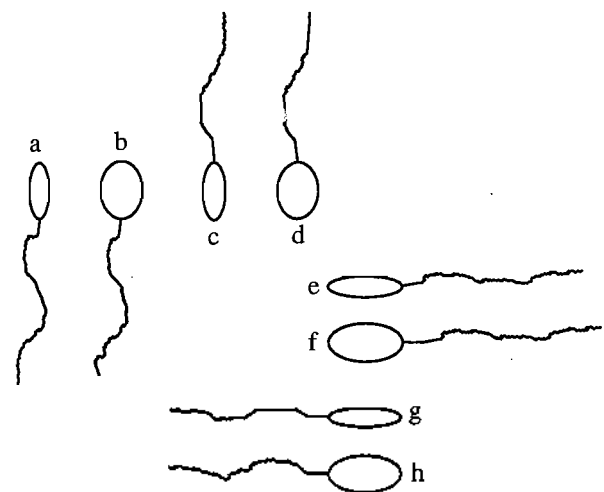


Figure 2. Classification of oriented bull sperm. a: Upward, with sperm flat head perpendicular to the magnetic direction, b: Upward, with sperm plane head perpendicular, c: Downward, with sperm flat head perpendicular, d: Downward, with sperm plan head perpendicular, e: Leftward, with sperm flat head parallel to the magnetic direction, f: Leftward, with sperm plane head parallel, g: Rightward, with sperm flat head parallel, h: Rightward, with sperm plane head parallel

head parallel and rightward head parallel (see table 3). The kind of perpendicular, consisting of upward head perpendicular (a plus b) plus downward head perpendicular (c plus d), was the orientation of bull sperm to the magnetic field direction with its long axis perpendicular. The kind of parallel, consisting of leftward head parallel (e plus f) plus rightward head parallel (g plus h), was the orientation of bull sperm to the magnetic direction with its long axis parallel.

Statistical analysis

Data in this study were analyzed by SAS (1990). For the data on head area of bull sperm, Duncan's multiple range test of GLM procedure was used to examine the difference between non-treated or treated with DTT or H. The t-test procedure was used to analyze the data on bull sperm head area between the first and sixth days. The magnetic orientation data were analyzed by using the Chi-square of frequency tests procedure for equal population to compare the difference of orientation between N and S, and between the first and sixth days.

RESULTS

Bull sperm head area

Mean and standard error of bull sperm head area treated with DTT or H are shown in table 1. This table showed that bull sperm head area had the highest values in experiments treated with 100 units and 10,000 units H at the first day, and were $34.05 \mu\text{m}^2$ and $33.77 \mu\text{m}^2$, respectively. For experiments treated with 20 mM and 2,000 mM DTT, bull sperm head areas were slightly smaller than those of the H treatments: $33.46 \mu\text{m}^2$ and $33.62 \mu\text{m}^2$, respectively. Table 1 also shows that bull sperm head area slightly

increased in most of the experiments treated with DTT or H at the sixth day compared to those at the first day except for the 2,000 mM DTT treatment. However, the differences in bull sperm head area in all of experiments were not significant when compared to each other and to the control treatment (table 1 and figure 3). Although bull sperm head area was not significantly different between treatments, the value of bull sperm head area in the 20 mM DTT, 100 units H treatments at both the first and sixth days and the 10,000 units H treatment at the sixth day was slightly bigger than the control treatment (table 1).

Magnetic orientation of bull sperm

Table 2 shows that perpendicular oriented percentages of bull sperm in most of treatments at both N and S were significantly higher than those of the control treatment at the first and sixth days (figure 4). In addition, perpendicular oriented percentages were significantly higher than the parallel oriented percentages. A difference between N and S for perpendicular oriented percentages was not found, except for the 20 mM DTT treatment at the first and sixth days and the 100 units H treatment at the sixth day. In these treatments perpendicular oriented percentages in N were significantly higher than those in S, as shown by 63.41% in N vs 56.21% in S of the 20 mM DTT treatment at the first day and 61.30% in N vs 56.45% in S of the 100 units H treatment at the sixth day. Table 2 also shows that perpendicular oriented percentages in most of treatments tended to be stable during treatment time except for the 100 units H treatment in N: perpendicular oriented percentages at the first day were significantly lower than those of the sixth day, 56.57% vs 61.30%, respectively.

Table 1. Bull sperm head area (μm^2) after treatment with dithiothreitol (DTT) or heparin (H) during the first and sixth days

Items	Concentration of DTT or heparin (units/ml)	N ³	The first day		The sixth day	
			Mean \pm SE	Range	Mean \pm SE	Range
Control ¹		75	33.70 \pm 0.36 ^{a,A}	25.13-39.27 ^{a,A}	35.55 \pm 0.36 ^{a,A}	25.13-39.27 ^{a,A}
Nontreated and exposed ²		75	33.66 \pm 0.35 ^{a,A}	25.13-39.27 ^{a,A}	33.64 \pm 0.38 ^{a,A}	25.13-39.27 ^{a,A}
Treated with DTT						
	20 mM	75	33.46 \pm 0.44 ^{a,A}	25.13-47.12 ^{a,A}	33.78 \pm 0.35 ^{a,A}	25.13-43.20 ^{a,A}
	2,000 mM	75	33.62 \pm 0.41 ^{a,A}	25.13-39.27 ^{a,A}	33.35 \pm 0.34 ^{a,A}	25.13-39.27 ^{a,A}
Treated with heparin						
	100 units	75	34.05 \pm 0.42 ^{a,A}	25.13-43.20 ^{a,A}	34.17 \pm 0.36 ^{a,A}	28.27-47.20 ^{a,A}
	10,000 units	75	33.77 \pm 0.33 ^{a,A}	25.13-41.04 ^{a,A}	33.99 \pm 0.38 ^{a,A}	28.27-43.20 ^{a,A}

^{1,2} Not treated with dithiothreitol (DTT) or heparin (H).

³ Number of observations

^a Means within a column with like superscript are not different ($p > 0.05$).

^A Means within a row with like superscript are not different ($p > 0.05$).

Table 2. Comparison of two kinds of main orientations to the magnetic direction of bull sperm not treated or treated with dithiothreitol (DTT) or heparin (H) and then exposed to the north or south pole of a magnet (for 24 hours) with the magnetic line of force of 3,000 Gauss during the first and sixth days

Concentration of DTT or heparin (units/ml)	Polarity	First day			Sixth day		
		N ²	Perpendicular ³	Parallel ⁴	N	Perpendicular	Parallel
Control ¹		1148	50.31 ^a	49.69	1053	50.41 ^a	49.57
Not treated and exposed	North	1175	56.09 ^b	43.91*	1164	59.71 ^b	40.29*
	South	1173	55.75 ^b	44.25*	1143	57.48 ^b	42.52*
Treated with DTT and exposed							
20 mM	North	1137	63.41 ^b	36.59*	1155	61.90 ^b	38.10*
20 mM	South	1124	56.21 ^c	43.79*	1157	56.27 ^c	43.73*
2,000 mM	North	1122	58.91 ^b	41.09*	1076	57.53 ^b	42.47*
2,000 mM	South	1116	57.62 ^b	42.38*	1078	58.35 ^b	41.65*
Treated with heparin and exposed							
100 units	North	1165	56.57 ^{b,A}	43.43*	1150	61.30 ^{b,B}	38.70*
100 units	South	1108	59.12 ^b	40.88*	1178	56.45 ^c	43.55*
10,000 units	North	1131	57.12 ^b	42.88*	1124	59.70 ^b	40.30*
10,000 units	South	1086	58.01 ^b	41.99*	1080	59.35 ^b	40.65*

¹ Not treated with dithiothreitol (DTT) or heparin (H) and not exposed to the magnetic field.

² Weighted means of the number of sperms.

³ Sperm oriented each with its long axis perpendicular to the magnetic direction.

⁴ Sperm oriented each with its long axis parallel.

^{a,b,c} Oriented percentages in the same column with different superscripts differ ($p < 0.05 \sim 0.01$) when compared between treatments at the north and south poles of the magnet with control treatment.

^{A,B} Within the same row, oriented percentages of bull sperm with the different superscripts are significant ($p < 0.05 \sim 0.01$).

* Significant differences between perpendicular and parallel oriented percentages ($p < 0.05 \sim 0.01$).

A further analysis was carried out to examine the differences in the other orientational categories of bull sperm between N and S and its results are shown in table 3. This table showed that upward percentages of the non treated and H treatments in both N and S were significantly lower than those of the control treatment and downward percentages at the first and sixth days, except for the 10,000 units H treatment at the sixth day, in which no difference was found. For the group treated with DTT, upward percentages indicated significantly higher than those of the control treatment and the downward percentages at both the first and sixth days. Upward percentages at the first day were similar to the sixth day in most treatments. A difference between N and S for upward percentages was not found at either the first or sixth days, except for the 100 units H treatment at the sixth day, in which upward percentages of S were significantly higher than those of N, 43.31% vs 36.79%, respectively.

Table 3 also shows that leftward percentages of the non treated and exposed experiment in both N and S were significantly higher than those of the control treatment and the rightward percentages. For groups treated with DTT or H both at N and S, leftward percentages were significantly lower than those of the

control treatment and the rightward percentages at the first and sixth days except for the 100 units H experiment at the sixth day, in which leftward percentages were similar to the control treatment. The leftward percentages in most of the experiments tended to be stable during treatment time, and the difference between N and S for leftward percentages was not found, except for the 100 units H experiment at the sixth day in which leftward percentages in S were significantly higher than those in N, 43.66% vs 38.29%, respectively.

DISCUSSION

This experimental results showed that the average head area of bull sperm treated with DTT or H did not change compared to those of the control treatment (table 1). This is in good agreement with Motoishi et al. (1996). And is due to the fact that bull sperm nuclei contain only protamin I, which is maximally crosslinked by disulfide bridge (Balhorn, 1982). Thus, bull sperm nuclei were very stable. However, for the range in bull sperm head area, it might be said that a small number of bull sperm nuclei were decondensed by the 20 mM DTT, 100 units H treatments at the first and sixth days and 10,000 units H treatment at

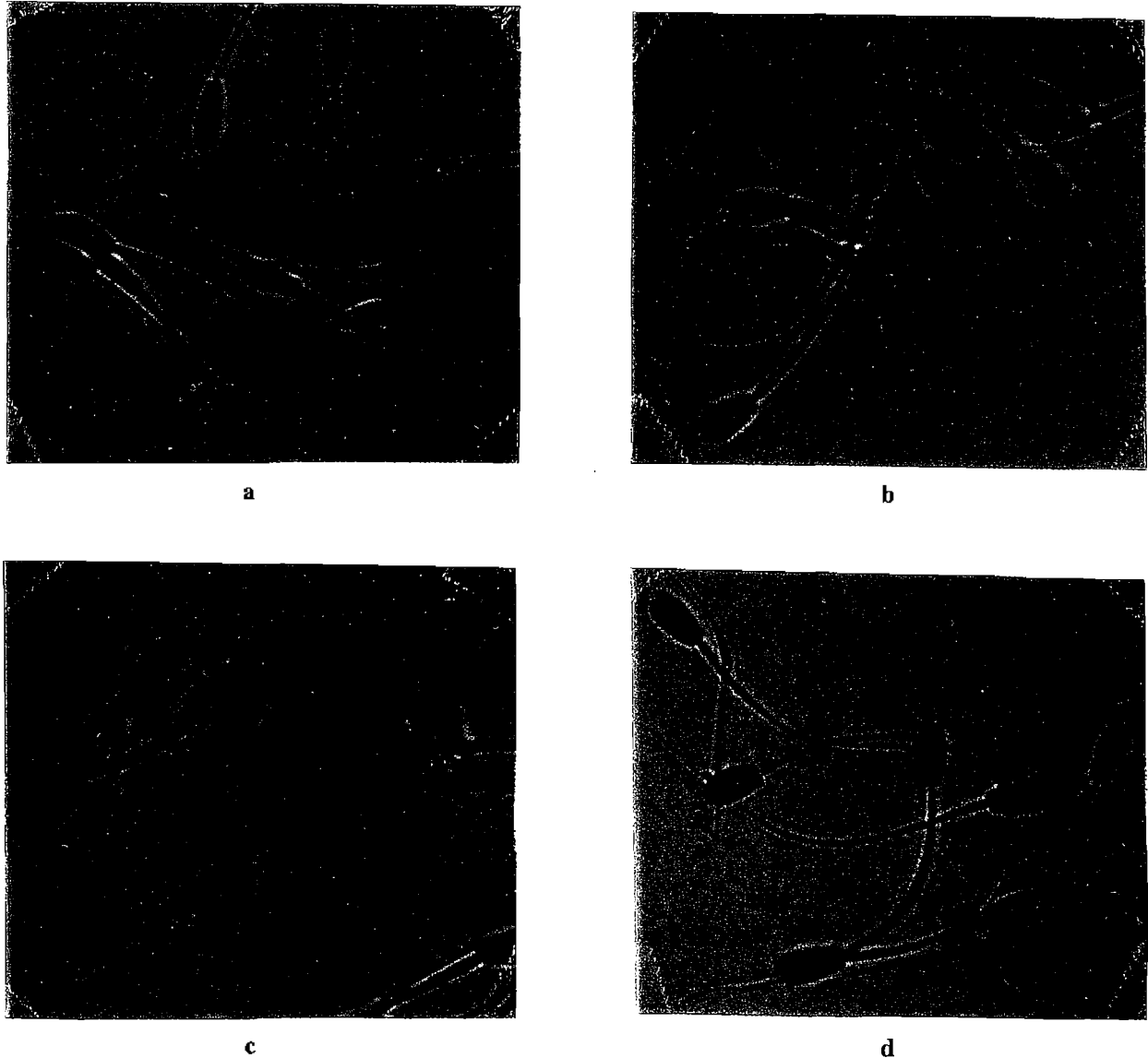


Figure 3. Shape of bull sperm in control treatment or treatment with dithiothreitol (DTT) or heparin (original magnification $400\times$). a: the control treatment, b: the 20 mM DTT treatment at the first day, c: the 100 units heparin treatment at the first day, d: the 10,000 units heparin treatment at the sixth day

the sixth day (table 1). This result is in agreement with Suga et al. (2000). For those experiments, the decondensation of bull sperm is due to the reduction of disulfide bond by DTT treatment or the change of the structure of chromatin by heparin treatment.

The perpendicular magnetic orientation in this experiment, after bull sperm were treated with DTT or H, both at N and S, was higher than in the control group. From this result, it could be said that the magnetic anisotropy of bull sperm induced them to orient perpendicular to the magnetic direction. No difference was found for the perpendicular oriented percentages of bull sperm between N and S in most of experiments at the first and sixth days. Thus, it can

be said that the magnetic pole had the same effect on the orientation of bull sperm. However, for the 20 mM DTT treatment at the first day and the 100 units H treatment at the sixth day, perpendicular oriented percentages in N were significantly higher than those in S. This might be due to a small number of bull sperm in two experiments decondensed, which pre-disposed the inside structure of bull sperm nuclei to changes.

As for the upward perpendicular and downward perpendicular, the DTT treatment showed that the upward percentages were significantly higher than those of the control treatment. This might be due to the fact that DTT was the agent which reduced the

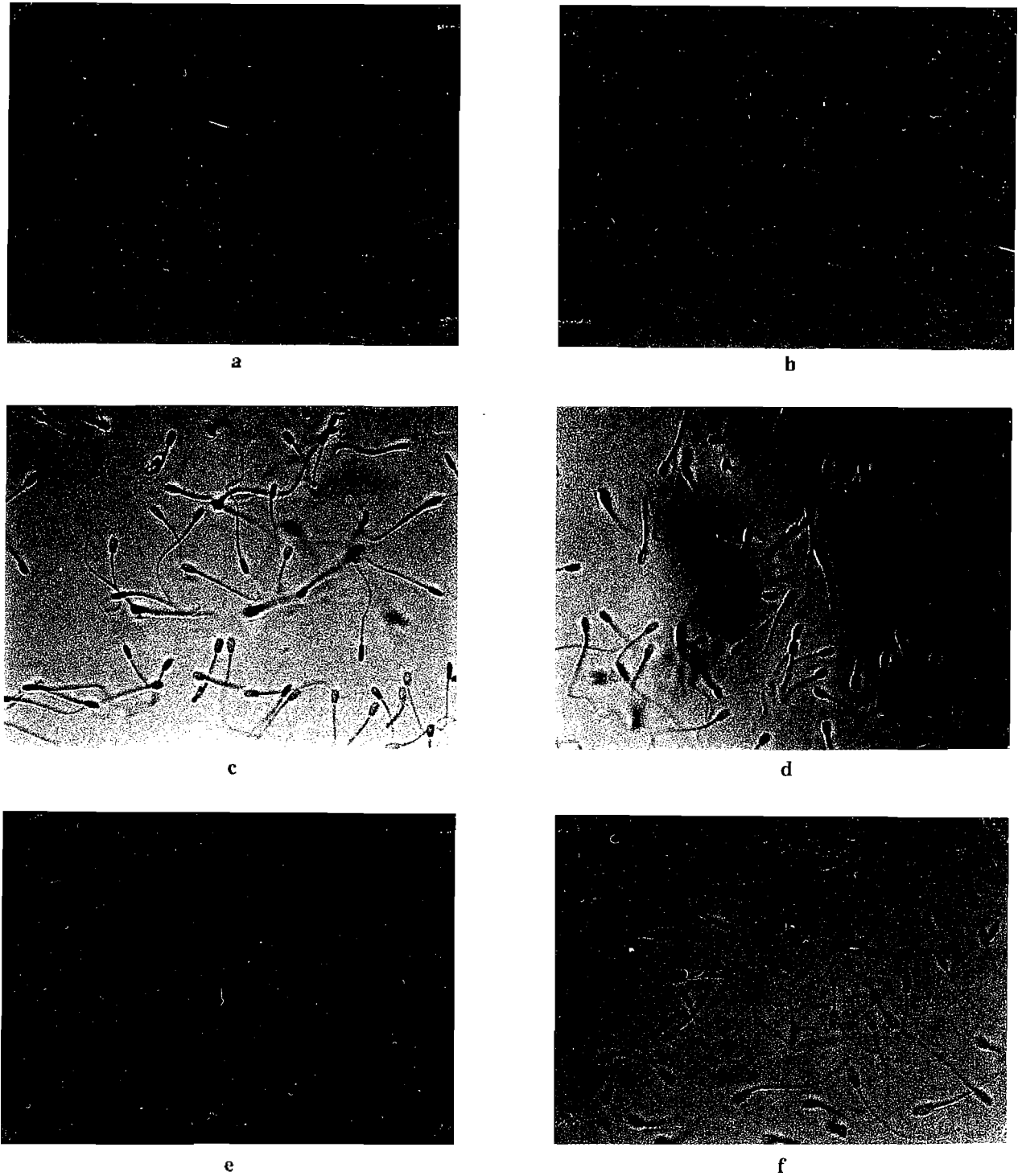


Figure 4. Magnetic orientation of bull sperm not treated or treated with DTT or heparin and then exposed to the north or south pole of a magnet (original magnification 200 \times). a: the control treatment, b: the non treated and exposed treatment in the north pole at the sixth day, c: the 20 mM DTT treatment in the north pole at the first day, d: the 20 mM DTT treatment in the south pole at the first day, e: the 100 units heparin treatment in the north pole at the sixth day, f: the 100 units heparin treatment in the south pole at the sixth day

disulfide bond of bull sperm, and this enabled bull sperm treated with DTT to orient upward perpendicular to the magnetic direction.

No difference was found for upward percentages between N and S in most of experiments; therefore the magnetic pole did not influence the upward

Table 3. Comparison of the main oriented directions of bull sperm (%) not treated or treated with dithiothreitol (DTT) or heparin (H) and then exposed to the magnetic line of force (for 24 hours) of about 3,000 Gauss in the north or south pole of the magnet during the first and sixth days

Concentration of DTT or heparin (units/ml)	Pole the magnet	Perpendicular				Parallel			
		First day		Sixth day		First day		Sixth day	
		Upward ²	Downward ³	Upward	Downward	Leftward ⁴	Rightward ⁵	Leftward	Rightward
Control ¹		50.35 ^a	49.65	50.19 ^a	49.81	50.79 ^a	49.21	49.62 ^a	50.38
Nontreated and exposed	North	43.94 ^b	56.06*	44.17 ^b	55.83*	58.53 ^b	41.47*	58.42 ^b	41.58*
	South	42.97 ^b	57.03*	42.16 ^b	57.84*	57.03 ^b	42.97*	57.20 ^b	42.80*
Treated with DTT and exposed									
20 mM	North	56.87 ^b	43.13*	56.22 ^b	43.78*	36.30 ^b	63.70*	38.72 ^b	61.28*
20 mM	South	56.25 ^b	43.75*	58.37 ^b	41.63*	37.31 ^b	62.69*	34.78 ^b	65.22*
2,000 mM	North	59.61 ^b	40.39*	56.54 ^b	43.46*	43.17 ^b	56.83*	42.67 ^b	57.33*
2,000 mM	South	61.84 ^b	38.16*	58.82 ^b	41.18*	44.40 ^{ab}	55.60*	40.76 ^b	59.24*
Treated with heparin and exposed									
100 units	North	37.18 ^b	62.82*	36.79 ^b	63.21*	39.33 ^b	60.67*	38.29 ^b	61.71*
100 units	South	40.46 ^b	59.54*	43.31 ^c	56.69*	41.94 ^b	58.06*	43.66 ^a	56.34*
10,000 units	North	44.27 ^b	55.73*	44.71 ^{ab}	55.29*	38.14 ^b	61.86*	41.81 ^b	58.19*
10,000 units	South	44.13 ^b	55.87*	42.43 ^b	57.57*	39.47 ^{ba}	60.53*	42.60 ^{ba}	57.40*

¹ Non treated with dithiothreitol (DTT) or heparin (H) and non exposed to the magnetic field.

² Sperm oriented each with its axis perpendicular to the magnetic direction and upward.

³ Sperm oriented each with its axis perpendicular to the magnetic direction and downward.

⁴ Sperm oriented each with its axis parallel to the magnetic direction and leftward.

⁵ Sperm oriented each with its axis parallel to the magnetic direction and rightward.

^{a,b,c} Oriented percentages in the same column with different superscripts differ ($p < 0.05 \sim 0.01$) when compared between experiments at the north and south poles of the magnet with the control treatment.

^{A,B} Oriented percentages in the same row between the first and sixth day with different superscripts differ ($p < 0.05 \sim 0.01$).

* Significant differences between upward and downward or between leftward and rightward oriented percentages ($p < 0.05 \sim 0.01$).

orientation of bull sperm. Nevertheless, for the 100 units H treatment, the upward percentages in N were lower than those in S at the sixth day. This may be due to the fact that H has an ability to combine protamin in the protamin-DNA structure of the sperm nuclei (Chargaff and Olson, 1938), leading to an increase in the area (table 1). As the protamin-DNA structure of bull sperm nuclei slightly changed, bull sperm were strongly magnetized to orient upward more at S than at N.

As shown in table 3, for the leftward percentages, the DTT and H effects were significantly lower than those of the control treatment. For DTT treatment, sperm orientation rightwards was similar to that of upwards. For the H treatment, direction of rightward tended to be same as downward (figure 4). This difference was explained earlier in the upward case.

No difference was observed for the leftward percentages in most of experiments between N and S. This indicated that the pole of the magnet did not affect bull sperm for their leftward orientation.

It is included that N and S have the same effect on the orientation of bull sperm. Moreover, both N

and S with a magnetic strength of about 3,000 Gauss induced bull sperm to orient perpendicular to the magnetic direction because of the magnetic anisotropy of the sperm.

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