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**—Original Article—**

# Involvement of calcium channels and intracellular calcium in bull sperm thermotaxis

**Md Anisuzzaman MONDAL[1)], Yuji TAKAGI[1)], Shoji A BABA[2)] and Koh-ichi HAMANO[1)]**

[1)]*Faculty of Agriculture, Shinshu University, Nagano 399-4598, Japan*
[2)]*Faculty of Science, Ochanomizu University, Tokyo 112-8610, Japan*

**Abstract.** Thermotaxis that sperm migrate to higher temperature area has been confirmed in rabbit and human. In this study, we examined the migration ability of bull sperm in a temperature gradient to confirm thermotaxis and elucidate the involvement of calcium in such thermotaxis, as well as the relation between sperm capacitation and bull fertility. Thermotaxis was evaluated in a temperature gradient of 34–42ºC using a cross-type column 22-mm long, 40-mm wide, and 100-μm deep. Significantly more sperm migrated to the high-temperature area of 39ºC in a 2ºC temperature gradient, and to 40ºC in a 1ºC temperature gradient. In calcium-free, BAPTA containing medium, and EGTA containing medium, the migrated sperm ratio in the two temperature areas was almost the same. In media containing lanthanum, ruthenium red, and 2APB, we could not confirm thermotaxis. Pre- and post-capacitated sperm migrated to the high-temperature area, expressing thermotaxis. The sperm from high-fertility bulls showed clear thermotaxis. Based on these results, thermotaxis of bull sperm was confirmed and the involvement of both calcium channels and intracellular stored calcium in thermotaxis was suggested. Although the sample size of bulls was quite small, the difference in thermotaxis may have been associated with bull fertility. Sperm thermotaxis evaluation has potential as a predictor of bull fertility.
**Key words:** Bull, Calcium, Sperm, Thermotaxis

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Mammalian sperm must migrate in the female reproductive tract in order to penetrate and fertilize an ovulated egg in the ampulla. Recently, it was reported that the migration of sperm in the female reproductive tract associated with fertilization is at the very least regulated by chemotaxis and thermotaxis [1–4]. Furthermore, the involvement of rheotaxis has been confirmed [5], and the mechanisms of taxis in sperm migration associated with fertilization have been elucidated [6]. Before arriving at the ampulla, which is the fertilization site, sperm motility is important for passing the utero cervix and tubal junction, and is essential for fertilization. The involvement of chemotaxis, thermotaxis, and rheotaxis in the migration of sperm has been previously reported [6]. David *et al*. [7] and Hunter and Nichol [8] reported that the difference in temperature between the isthmus and ampulla of the oviduct at ovulation were approximately 2 and 0.7ºC in rabbit and boar, respectively.

These findings raise the possibility that difference of temperature in the reproductive tract induces a sperm to seek the fertilization site. Bahat *et al*. [9] reproduced temperature difference of 2ºC in the rabbit reproductive tract, and reported positive thermotaxis of capacitated rabbit sperm migration to the high-temperature area. These same researchers also confirmed positive thermotaxis in human sperm with a similar experiment [10]. Bahat *et al*. theorized that thermotaxis and chemotaxis were involved complementarily in the fallopian tubes site [4, 9]. Because the chemoattractants are spread by oviduct peristalsis at ovulation, sperm migration to the fertilization site by chemotaxis alone is difficult. Thermotaxis is effective for migration of sperm in the oviduct which temperature difference is created between the isthmus and ampulla; around the egg, the sperm is primarily induced by chemotaxis and is not regulated by thermotaxis [9].

Bahat *et al*. [10] reported that calcium ion is involved in the thermotaxis of human sperm. Also, they found that the involvement of $IP_3R$ and PLC in human sperm thermotaxis, but not the transient receptor potential (TRP), which is one of the calcium ion channels. Kawanishi *et al*. [11] demonstrated the positive thermotaxis of mouse sperm and suggested involvement of the calcium ion channel TRPV4 in mouse sperm thermotaxis. Hamano *et al*. [12] examined thermotaxis in *Trpv4* gene-deficient mouse sperm, and confirmed the involvement of the TRPV4 calcium channel. Thermotaxis is thought to be an extremely important physiological function in mammalian sperm fertilized in the female reproductive tract. However, the regulation and the mechanism of thermotaxis in bull sperm have not yet been established.

Because conventional sperm examination techniques (number, morphology and viability of sperm) cannot evaluate fertilizing ability directly, functional changes such as sperm migration and penetration ability have been studied. As examination of *in vitro* sperm migration might predict migration ability within the female reproductive tract, such migration has been examined to evaluate fertility [13]. No evaluation method has yet been established to predict the conception

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Correspondence: K Hamano (e-mail: khamano@shinshu-u.ac.jp)

rate of artificially inseminated bovine females, however.

In this study, we examined bull sperm migration and the expression of thermotaxis *in vitro* using a temperature gradient to elucidate the thermotaxis of sperm and its involvement in the fertilization mechanism. We also examined the involvement of calcium and related substances to clarify the expression mechanism of bull sperm thermotaxis. We examined thermotaxis in different bulls to evaluate bull fertility.

## Materials and Methods

### *Media and chemicals*

We used BO medium (BO) (Brackett & Oliphant [14]) containing 1% bovine serum albumin (BSA) (Wako, Tokyo, Japan) for washing, dilution of sperm, and column construction. The sperm capacitation-inducing medium (HC) was prepared by supplementation of 15 μg/ml heparin (Mochida, Tokyo, Japan) and 5 mM caffeine (SIGMA-Aldrich, St Louis, MO, USA) to BO medium. Sperm pre-incubation was conducted in a micro drop of BO or HC in a $CO_2$ incubator with 38.5ºC, 5% $CO_2$/95% air. To examine the effect of calcium on thermotaxis, we used BO without calcium (Ca-) and BO containing double concentration: 4.5 mM calcium (2Ca). We added 1 mM ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) and 0.5 μM 1,2-bis (o-aminophenoxy) ethane-N,N,N‘,N’-tetraacetic acid (BAPTA-AM) (SIGMA) of the calcium chelating agent into the BO. To inhibit a calcium channel, we added 0.1 mM lanthanum (general calcium channel inhibitor; SIGMA), 1 mM verapamil (voltage-dependent L-type calcium channel inhibitor; SIGMA), 40 μM mibefradil (voltage-dependent T-type calcium channel inhibitor; SIGMA), 10 μM ruthenium red (TRP calcium channel inhibitor; SIGMA), 10 μM SKF96365 (TRP calcium channel inhibitor; Wako), and 0.25 mM 2-Aminoethyl diphenylborinate (2APB) (intracellular calcium-releasing inhibitor; SIGMA) to the BO.

### *Semen*

Semen was obtained from three Holstein Friesian bulls that had different conception rates after artificial insemination (AI) of cows. Field fertility of bulls, classified by conception rates of inseminated cows, was examined 60–90 days non-return rates post AI. Bulls A, B and C had conception rates of 65% (324/495 heads), 57% (585/1029 heads), and 34% (95/277 heads), respectively. Evaluation of thermotaxis was conducted using the semen from bull B, and we evaluated sperm thermotaxis of the three bulls, A, B and C, with different fertility.

### *Chamber preparation*

Sperm motility and thermotaxis were estimated using manufactured analysis chambers (Fig. 1-A). We constructed each chamber with a slide glass (S2215: Matsunami, Tokyo, Japan) (Fig. 1-A: a) and a cover glass (22 × 40: Matsunami) (Fig. 1-A: b) adhered by double-stick tape (100-μm thick; No. 7046; Teraoka, Tokyo, Japan) (Fig. 1-A: c1–c4) to create a cross-type column (Fig. 1-A: d). The cross-type column was 10-mm long vertically (width: 5 mm), 40-mm broad (width: 3 mm) and connected along 13-mm vertically (width: 1 mm) to the slide glass with double-stick tape. The cover glass was adhered and the chamber was filled with BO (Fig. 1-A). Both ends of the wide column were covered with mineral oil once the sperm was introduced.

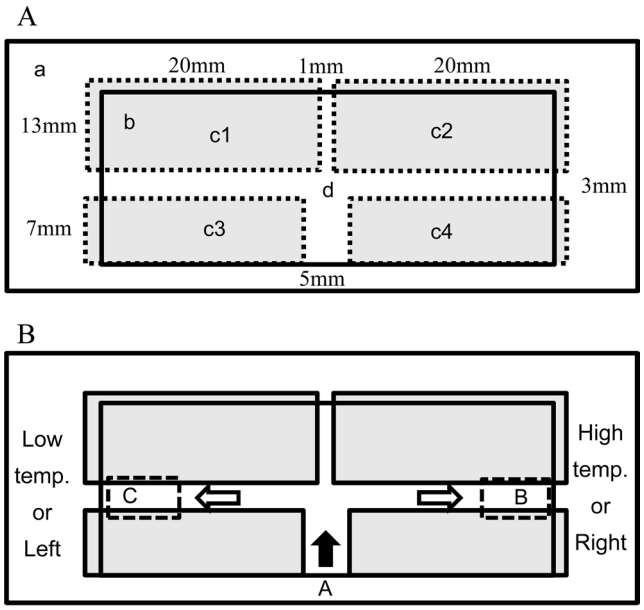


**Fig. 1.** (A) Sperm motility and thermotaxis were estimated using analysis chambers (Fig 1-A). The chamber was constructed with a slide glass (S2215: Matsunami, Tokyo, Japan) (a) and a cover glass (22 × 40: Matsunami) (b; a solid-line square) adhered by double-stick tape (100-μm thick; No. 7046, Teraoka, Tokyo, Japan) (c1–c4; dotted-line squares) to create a cross-type column (d). The cross-type column was 10-mm vertical long (width: 5 mm), 40-mm broad (width: 3 mm), and connected vertically for 13 mm (width: 1 mm) onto the slide glass with double-stick tape; the cover glass was then adhered, and the column was filled with BO. Both ends of the wide column were covered with mineral oil after the sperm was introduced. (B) A total of $10^4$ sperm were introduced at the end of the vertical column of the cross-type column (A), and allowed to incubate for 20 min. Sperm motility was then evaluated by examining the ratio of motile sperm that arrived at the 10-mm ends of both wide columns (B, C). After immobilizing the sperm by warming to 60ºC, thermotaxis was evaluated by measuring the number of sperm that had arrived at the ends of both wide columns (B, C).

### *Sperm preparation*

Frozen semen from the three bulls was thawed in a water bath at 38.5ºC and diluted with an equal volume of BO. Diluted semen was washed three times by centrifugation at 1500 rpm for 5 min. Sperm concentration was adjusted to $1 \times 10^7$/ml.

### *Evaluation of sperm motility and thermotaxis*

We set the temperature gradient using a device manufactured by special order: the Thermo Plate (Tokai Hit, Shizuoka, Japan). We evaluated sperm motility and thermotaxis by examining sperm migration ability in a temperature gradient. In the no-temperature-gradient condition, we set the three ends of the vertical column and wide column to 38ºC. In the temperature-gradient condition with

a 1 or 2ºC difference, the low-temperature end (Fig. 1-B: C) at the wide column was more than 34ºC, the high temperature end (Fig. 1-B: B) was less than 42ºC.

To begin the experiment, $10^4$ sperm was introduced at the end of the vertical column of the cross column (Fig. 1-B: A), and allowed to incubate for 20 min. We then evaluated the sperm's motility by examining the ratio of motile sperm that arrived at the 10-mm ends of both wide columns (Fig. 1-B: B, C). After immobilizing the sperm by warming to 60ºC, thermotaxis was evaluated by measuring the number of sperm that had arrived at both ends of the wide columns, and by the ratio of that number of sperm divided by the total number at both ends of the wide columns (Fig. 1-B: B, C).

*Statistical analysis*

Each experiment was replicated at least three times. The significance of the differences in sperm motility and migrated sperm numbers were determined with Student's *t*-test and the Welch's F test. For this part of the analysis, multiple range tests were adapted.

## Results

*Effect of temperature on sperm motility*

Table 1 presents the results for sperm motility under different temperatures, and migration to both wide column ends. Motility at 38ºC was 52.7%, significantly higher than at 3ºC (41.3%). There was no significant difference in number or ratio of migrated sperm to the right and left ends of the wide column.

*Evaluation of sperm thermotaxis*

Table 2 shows sperm migration in 1ºC or 2ºC temperature-difference gradients. In the 2ºC difference gradient, the migrated sperm ratio in the high-temperature area of 40ºC was 62.5%, significantly higher than that in the low-temperature area (38ºC). In the 1ºC difference gradient, the migrated sperm ratio in the high-temperature area (39ºC) was 59.7%, significantly higher than that in the low-temperature area (38ºC).

*Calcium involvement in sperm thermotaxis*

We present the effects of calcium on sperm motility and thermotaxis in Table 3. The sperm migration ratio to the high-temperature area of 39ºC in both BO (58.9%) and 2Ca (59.4%) were significantly higher than that to the low-temperature area. Sperm migration ratio toward the high-temperature area of 39ºC in Ca- (56.7%), BO containing EGTA (56.6%) and BO containing BAPTA-AM (57.9%) were also higher than that to the low-temperature area, but the difference was not significant.

Table 4 lists the effects of the calcium channel inhibitors on sperm thermotaxis. In the BO with verapamil and 2APB, sperm motility was significantly lower than others, 43.6 and 41.8%, respectively. Similar to the control, in BO with mibefradil, sperm migration to the high-temperature area of 39ºC was significantly higher, at 59.0%. In the BO with verapamil and SKF, there was a tendency toward more sperm migration to the high-temperature area, but it was not significant. In the BO with lanthanum, ruthenium red, and 2APB, the ratio of migrated sperm to the high temperature area was almost same, 53.7, 55.9 and 53.5%, respectively.

**Table 1.** Bull sperm motility and migration at various temperatures (34–42ºC)

| Temp. (ºC) | Motility (%) | Left | | Right | |
|---|---|---|---|---|---|
| | | No. sperm | % | No. sperm | % |
| 34 | 41.3 ± 2.1 [a] | 179.4 ± 14.9 | 51.1 | 171.9 ± 22.4 | 48.9 |
| 36 | 46.6 ± 3.4 [ab] | 189.6 ± 25.2 | 47.6 | 208.5 ± 25.9 | 52.4 |
| 38 | 52.7 ± 5.2 [b] | 280.3 ± 28.1 | 46.5 | 322.4 ± 29.5 | 53.5 |
| 40 | 49.8 ± 3.2 [ab] | 288.7 ± 24.9 | 52.9 | 257.2 ± 33.5 | 47.1 |
| 42 | 44.0 ± 2.7 [ab] | 203.1 ± 25.5 | 47.7 | 222.6 ± 22.6 | 52.3 |

Each value represents the mean ± SEM of sperm from 3–5 experiments. [a, b] Indicates a significant difference ($P < 0.05$).

**Table 2.** Bull sperm migration in columns with temperature gradients (34–42ºC)

| Temp. (ºC) | Low temp | | High temp | |
|---|---|---|---|---|
| | No. sperm | % | No. sperm | % |
| 38 | 276.9 ± 36.4 | 45.2 | 335.7 ± 27.7 | 54.8 |
| 34–36 | 318.2 ± 39.5 | 46.9 | 360.3 ± 33.2 | 53.1 |
| 36–38 | 286.5 ± 35.7 | 44.5 | 357.2 ± 30.4 | 55.5 |
| 38–40 | 276.5 ± 26.3 | 37.5 | 461.7 ± 31.8 | 62.5 * |
| 40–42 | 284.2 ± 34.2 | 44.3 | 357.1± 35.6 | 55.7 |
| 36–37 | 256.1 ± 30.7 | 45.9 | 301.4 ± 33.2 | 54.1 |
| 37–38 | 286.1 ± 37.3 | 45.6 | 341.7 ± 31.5 | 54.4 |
| 38–39 | 294.1 ± 25.3 | 40.3 | 435.3 ± 31.2 | 59.7 * |
| 39–40 | 297.3 ± 28.5 | 46.1 | 347.1 ± 30.1 | 53.9 |
| 40–41 | 268.41 ± 33.5 | 43.9 | 342.6 ± 29.5 | 56.1 |

Each value represents the mean ± SEM of sperm from 3–5 experiments. * The number of sperm was significantly different between low and high temperature of the same temperature gradient ($P < 0.05$).

**Table 3.** Effect of Ca on bull sperm motility and migration in the column with a temperature gradient (38–39ºC)

| Medium | Motility (%) | Low temp | | High temp | |
|---|---|---|---|---|---|
| | | No. sperm | % | No. sperm | % |
| BO | 52.1 ± 6.1 | 293.1 ± 22.5 | 41.1 | 419.5 ± 32.4 | 58.9 * |
| Ca- | 48.4 ± 3.0 | 265.7 ± 27.8 | 43.3 | 347.5 ± 25.9 | 56.7 |
| 2Ca | 52.8 ± 6.3 | 269.3 ± 30.4 | 40.6 | 434.2 ± 33.9 | 59.4 * |
| ET | 46.5 ± 4.2 | 253.8 ± 39.6 | 43.4 | 331.2 ± 41.3 | 56.6 |
| BT | 43.9 ± 5.8 | 221.7 ± 33.4 | 42.1 | 304.9 ± 41.3 | 57.9 |

Each value represents the mean ± SEM of sperm from 3–5 experiments. Ca-; Ca free, 2Ca; double concentration of Ca, ET; BO containing EGTA, BT; BO containing BAPTA-AM. Sperm motility was evaluated by the ratio of migrating sperm. * The number of sperm was significantly different between low and high temperature of the same temperature gradient ($P < 0.05$).

*Thermotaxis and capacitation of sperm and bull fertility*

Table 5 shows the thermotaxis of capacitated sperm. The motility and total number of migrated sperm to both high- and low-temperature areas was lower than for pre-capacitation. Capacitated sperm migration to the high-temperature area of 40 and 39ºC were 60.4 and 59.1%,

**Table 4.** Effect of Ca channel inhibitor on bull sperm motility and migration in columns with a temperature gradient (38–39ºC)

| Medium | Motility (%) | Low temp | | High temp | |
|---|---|---|---|---|---|
| | | No. sperm | % | No. sperm | % |
| BO | 51.7 ± 3.3 [a] | 261.4 ± 25.1 | 39.6 | 398.5 ± 31.4 | 60.4 * |
| La3 | 45.1 ± 3.5 [ab] | 261.5 ± 38.2 | 44.9 | 320.9 ± 40.9 | 55.1 |
| verapamil | 41.5 ± 5.8 [b] | 200.8 ± 41.5 | 42.5 | 271.3 ± 44.3 | 57.5 |
| mibefradil | 47.1 ± 6.4 [ab] | 262.3 ± 25.4 | 40.5 | 385.1 ± 31.5 | 59.5 * |
| SKF | 47.9 ± 5.1 [ab] | 275.3 ± 29.3 | 43.1 | 362.8 ± 44.1 | 56.9 |
| RR | 45.8 ± 3.9 [ab] | 261.5 ± 37.8 | 44.1 | 331.7 ± 47.3 | 55.9 |
| 2APB | 40.7 ± 4.8 [b] | 211.5 ± 41.9 | 46.6 | 242.3 ± 39.5 | 53.4 |

Each value represents the mean ± SEM of sperm from 3–5 experiments. La3: lanthanum; RR: rutenium red. Sperm motility was evaluated as the ratio of migrating sperm. [a, b] The sperm motility was significantly different between the medium of sperm treatment ($P < 0.05$). * The number of sperm was significantly different between low and high temperature of the same temperature gradient ($P < 0.05$).

respectively, significantly higher than for the low-temperature area.

Table 6 shows the thermotaxis of the sperm from the three bulls with their fertility rates. There was no difference in sperm motility between the three bulls. The migrated sperm ratio to the high-temperature area of 39ºC of the high-fertility bulls A and B were 60.6, 59.5%, significantly higher than for the low-temperature area. Sperm migration to the high-temperature area of low fertility bull C was 52.4%, similar to the migrated sperm to the low-temperature area.

## Discussion

Thermotaxis in mammals is a transport-supporting mechanism for sperm penetration and egg fertilization in the female reproductive tract. The motility function of migrating sperm in relation to female conception rate and male fertility have been studied, and physiological conditions such as components and pH of secreting fluid, temperature, and contraction of the reproductive tract are known to strongly affect sperm migration. However, there are few studies regarding thermotaxis and chemotaxis in bull sperm, and the relation between migration ability of sperm and fertility of bulls is uncertain. In this study, we confirmed thermotaxis in bull sperm and examined its mechanisms, particularly the involvement of calcium and the relation between thermotaxis and sperm capacitation and bull fertility.

We examined sperm motility from 34 to 42ºC, mainly at 38ºC of bovine body temperature. Bull sperm showed significantly higher motility at 38ºC, compared to 34ºC or 42ºC. This suggests that bovine body temperature supports migration, fertilization, and involvement of thermotaxis in fertilization.

Temperature at the fertilization site of rabbits at ovulation is higher than the sperm storage site and increases, depending on a time course, after ovulation [15]. In particular, it has been confirmed that the difference in temperature at the fertilization site significantly increases from 0.8ºC at pre-ovulation to 1.6ºC post-ovulation [9, 15]. In this study, a high number of bull sperm migrated from a low- to a high-temperature area in a 2ºC or 1ºC temperature gradient; that

**Table 5.** Sperm motility and migration of non-capacitated and capacitated bull sperm in various temperature gradients (37–40ºC)

| Sperm capacitation | Temp. (ºC) | Motility (%) | Low temp | | High temp | |
|---|---|---|---|---|---|---|
| | | | No. sperm | % | No. sperm | % |
| non-capacitated | 37–38 | 50.3 ± 5.9 [a] | 280.5 ± 38.1 | 45.9 | 331.2 ± 29.6 | 54.1 |
| | 38–39 | 52.4 ± 5.0 [a] | 284.3 ± 22.5 | 39.9 | 428.7 ± 35.3 | 60.1 * |
| | 39–40 | 49.2 ± 4.9 [a] | 273.6 ± 25.4 | 44.8 | 337.1 ± 28.7 | 55.2 |
| capacitated | 37–38 | 41.3 ± 3.9 [b] | 213.9 ± 34.5 | 43.2 | 291.7 ± 36.5 | 57.7 |
| | 38–39 | 42.1 ± 4.8 [ab] | 197.4 ± 38.5 | 41.2 | 281.9 ± 37.9 | 58.8 * |
| | 39–40 | 38.9 ± 4.5 [b] | 169.1 ± 41.2 | 39.2 | 262.2 ± 34.3 | 60.8 * |

Each value represents the mean ± SEM of sperm from 3–5 experiments. Sperm capacitation was induced with treatment of incubation of sperm with media containing with heparin, caffein. [a, b] The sperm motility was significantly different with/without sperm capacitation ($P < 0.05$). * The number of sperm was significantly different between low and high temperature of the same temperature gradient ($P < 0.05$).

**Table 6.** Sperm motility and migration of fertility confirmed with temperature gradient (38–39ºC)

| Bull | Fertility (%) | Motility (%) | Low temp | | High temp | |
|---|---|---|---|---|---|---|
| | | | No. sperm | % | No. sperm | % |
| A | 65 | 51.5 ± 3.5 | 215.1 ± 41.5 | 38.8 | 338.9 ± 48.5 | 61.2 * |
| B | 57 | 49.8 ± 4.9 | 264.3 ± 23.2 | 40.6 | 387.4 ± 31.9 | 59.4 * |
| C | 34 | 53.5 ± 5.7 | 331.5 ± 33.1 | 48.4 | 353.6 ± 40.9 | 51.6 |

Each value represents the mean ± SEM of sperm from 3–5 experiments. Fertility of bull A, B and C revealed with conception ratio after AI were 65, 57 and 34%, respectively. * The number of sperm was significantly different between low and high temperature of the same temperature gradient ($P < 0.05$).

is, from a low 38ºC to high 40ºC or from a low 39ºC to high 40ºC. In animals in which thermotaxis has been confirmed, it has been reported that at the time of ovulation, the temperature gradient from the isthmus to the ampulla of the oviduct is 0.5–2ºC higher than body temperature [8–10]. It has also been confirmed that the intravaginal temperature of bovines was elevated 0.4ºC at estrus [16, 17]. Therefore, a temperature gradient of 39–41ºC, higher than body temperature (38–39ºC) [17, 18], is formed in the reproductive tract of the estrus bovine, and thermotaxis may be involved in supporting the migration of fertilizing sperm. In this *in vitro* study, the thermotaxis of bull sperm toward the 1–2ºC higher temperature was confirmed. Our findings suggest the involvement of thermotaxis in the fertilization mechanism of bull sperm.

We then examined the effect of calcium on bull-sperm thermotaxis to investigate the expression mechanism of thermotaxis. Calcium plays an important role in sperm hyperactivation [19], acrosome reaction [20] and chemotaxis [21]. In this study, we examined the effect of two kinds of chelating agents; EGTA acts outside of sperm, while BAPTA-AM acts specifically on the intracellular calcium. Although Bahat *et al*. [10] reported that internal $Ca^{2+}$ stores regulate thermotaxis in human sperm, we confirmed that both intracellular and extracellular calcium involved in bull sperm thermotaxis.

Influx and efflux of intracellular calcium are regulated by various mechanisms. Calcium influx is regulated by several types of calcium channels. The TRP channel is an important temperature-related calcium channel [22, 23], but it has been determined that it is not involved in human-sperm mechanisms [10]. Kawanishi *et al*. and Hamano *et al*. confirmed the involvement of TRPV4 in sperm thermotaxis using *Trpv4* gene-deficient mice [11, 12]. Recently Kumar *et al*. [24] showed that TRPV4 induced calcium influx into human sperm, and was strongly associated with fertilization. These findings suggest the different function of calcium channels by animal species.

In this study, investigation of the effects of intracellular and extracellular calcium ions on sperm cells, and the involvement of the calcium ion channel, revealed that calcium ions in the medium were necessary for induction of thermotaxis. In the calcium ion channel analysis, thermotaxis was not confirmed in either lanthanum (general calcium ion channel inhibitor) and SKF or RR (TRP calcium ion channel inhibitor). Involvement of these calcium channels in sperm thermotaxis has been suggested [10, 12]. Because verapamil (voltage-dependent L-type calcium ion channel inhibitor) and 2APB (intracellular calcium-releasing inhibitor) significantly inhibited motility as compared with other treatments, sperm migration ability by thermotaxis might be inhibited indirectly. The inhibitor of voltage-dependent T-type calcium ion channel (mibefradil), which also inhibits the CatSpar channel involved in sperm hyperactivation, did not affect sperm thermotaxis. From the above, we surmised that thermotaxis was regulated not only by single effects, but also by mechanisms such as the involvement of both intracellular and extracellular calcium, calcium channel and intracellular calcium release. To clarify the mechanism, more work needs to be done in the future.

We examined the thermotaxis of capacitated bull sperm induced by treatment with heparin and caffeine in this study. We demonstrated results that were different from those of humans [10, 15] in terms of expression of thermotaxis of bull sperm pre- and post-capacitation. The thermotaxis of bull sperm was confirmed as sperm migration to higher temperature areas at pre- and post-capacitation. Therefore, we considered bull sperm to show positive thermotaxis in the female reproductive tract at pre- and post-capacitation. Bull sperm injected into the reproductive tract at pre-capacitation expresses thermotaxis, by which sperm can recognize slight differences of temperature, and may support migration ability in the uterus and oviduct. In the temperature gradient of 38–39ºC, the sperm migration ability of bull C, which showed conception rates of 34% after AI, was lower than that of bulls A and B, which showed higher rates of 65 and 57%, respectively. Although individual differences between the three bulls and sperm migration at other temperature gradients are unknown, and the sample size of bulls here was quite small, the difference in thermotaxis of the three bulls may have been associated with their fertility. More definitive conclusions will require evaluation of a large number of bulls. Because thermotaxis of pre- and post-capacitation was involved in the migration of sperm in the female reproductive tract, it may be that bull fertility could be estimated by increasing the sample size to a large number of bulls.

In conclusion, bull sperm thermotaxis and the effect of calcium in the thermotaxis were confirmed. In particular, the involvement of both intracellular and extracellular calcium, the calcium channel and intracellular calcium release in the thermotaxis were suggested. Furthermore, although the sample size of bulls was quite small, the difference in thermotaxis may have been associated with bull fertility. Therefore thermotaxis may be a reliable tool for effectively analyzing sperm migration, and has potential as a predictor of bull fertility.

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