

ORIGINAL ARTICLE

High concentration of dopamine treatment may induce acceleration of human sperm motility

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Abstract

Purpose: In humans, catecholamines (including dopamine) have been identified in semen and fallopian tubes, while dopamine D2 receptors (D2DR) are found in the sperm midpiece region. How dopamine dose affects human sperm function and whether dopamine treatment is useful in assisted reproductive technology is unclear.

Methods: Sperm samples were obtained from patients with normal semen parameters undergoing fertility treatment. We investigated the effects of dopamine treatment on tyrosine phosphorylation and sperm motility. Sperm motility was analyzed using the computer-assisted sperm analysis (CASA) system.

Results: This study revealed that various dopamine concentrations (0.1–100 μ M) did not increase sperm tyrosine phosphorylation. Progressive motility increased substantially when treated with high concentrations of dopamine (10 and 100 μ M) and was blocked by raclopride (a D2DR antagonist). After 24-h sperm culture, the addition of 10 μ M dopamine significantly increased curvilinear velocity and amplitude of lateral head displacement, which are indicators of hyperactivation.

Conclusion: Dopamine did not affect tyrosine phosphorylation, but increased sperm motility. High concentrations of dopamine were more effective to accelerate sperm motility in cases where sperm motile capacity was low.

KEYWORDS

dopamine, dopamine receptor, neurotransmitter agents, sperm motility, spermatozoa

1 | INTRODUCTION

Male infertility constitutes 50% of infertility cases,^{1–3} with defective sperm function being the most common cause. Male infertility is diagnosed using the semen analysis parameters defined by the World Health Organization.⁴ Sperm motility is a vital factor in achieving pregnancy. In vivo fertilization requires sperm to travel through the cervix, uterus, and oviduct to reach the ampulla of the

fallopian tubes where it fertilizes the oocyte. In case of asthenozoospermia, the sperm cannot reach the fertilization point,⁵ resulting in infertility. During conventional in vitro fertilization (cIVF), the sperm can easily reach the oocyte surrounded by the cumulus cells. Intracytoplasmic sperm injection (ICSI) is an artificial reproductive technology that is an effective method to overcome male infertility. It has been reported that in cases of asthenozoospermia, the fertilization rate is lower through cIVF than through ICSI.⁶ Therefore,

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the infertile sperm may have decreased fertilization functions such as capacitation, hyperactivation, and acrosome reaction. Moreover, ICSI has been reported to reduce embryo quality compared with cIVF.⁷⁻⁹ In male infertility treatment, cIVF is an important option for improved embryo quality. Therefore, activating sperm function is essential to increase the probability of pregnancy through either natural conception or infertility treatment.

Ejaculated mammalian sperm acquire capacitation ability through numerous factors when migrating through the female reproductive tract.¹⁰ When sperm reaches the ampulla of the fallopian tube, an acrosome reaction is induced, leading to fertilization. A previous study demonstrated that it was possible to induce capacitation *in vitro*.¹¹ These methods are currently being used worldwide in assisted reproductive technology for successful *in vitro* fertilization. Both mammalian ejaculated and injected sperm are exposed to various factors such as hormones, enzymes, and ions in the uterus,¹² some of which can induce capacitation by interacting with specific receptors. Interestingly, it has been reported that mammalian sperm carries neurotransmitter receptors generally found in the central nervous system. These receptors can modulate sperm function in many species.¹³⁻¹⁷

Dopamine, the predominant neurotransmitter in the mammalian central nervous system, regulates various functions, including cognition, emotion, and endocrine function. Dysfunctional dopamine systems are associated with neurological and psychiatric disorders, including Parkinson's disease and schizophrenia.¹⁸ Dopamine receptors are G-protein-coupled receptors and can be further divided into five subtypes (D1–D5) based on their pharmacology, biochemistry, and anatomic distribution.¹⁹ These dopamine receptor subtypes are of two categories: dopamine type 1 (D1)-like receptors and dopamine type 2 (D2)-like receptors. Stimulation of the D1-like receptors (D1DR and D5DR) activates adenylyl cyclase (AC) and increases cyclic adenosine 3', 5'-monophosphate (cAMP) accumulation,²⁰ whereas stimulation of the D2-like receptors (D2DR, D3DR, and D4DR) inhibits AC activation.¹⁸ D2-like receptors such as D2DR have been reported to modulate intracellular calcium levels by triggering the release of intracellular calcium stores or by acting on ion channels.²¹

It has been reported that D2DR is expressed in the sperm of many species, including humans, mice, rats, bulls, and boars.^{22,23} In boar sperm, stimulation of D2DR elevates tyrosine phosphorylation and accelerates sperm motility.²³ Recently, it has been reported that dopamine increases some human sperm functions, such as motility parameters and the acrosome reaction, and these effects are considered to be induced by tyrosine phosphorylation.²⁴ However, whether dopamine treatment has a positive effect on sperm tyrosine phosphorylation and how dopamine induces human sperm function are currently unknown. In addition, whether dopamine treatment is useful in assisted reproductive technology, particularly for male infertility, has not been tested. Therefore, in this study, we investigated the *in vitro* effect of dopamine on tyrosine phosphorylation and dopamine treatment to infertile patients useful for improve sperm motility.

2 | MATERIALS AND METHODS

2.1 | Human sperm preparation

Human sperm samples were obtained from 37 normozoospermic patients between the ages of 28 and 47 years old from an infertility clinic (Sendai ART Clinic, Miyagi, Japan). Samples constituted surplus sperm that had not been used for assisted reproductive technologies. All procedures in this study were approved by the Ethics Committee of Tohoku University (Miyagi, Japan) and the Sendai ART Clinic. Informed consent was acquired from all the sample donors. Human semen was collected with masturbation after 2–7 days of sexual abstinence. After 20 min of liquefaction treatment, semen analysis was performed, and normozoospermic patient semen samples were selected according to the World Health Organization criteria (sperm concentration > 15 million/ml, sperm motility > 40%, sperm morphology > 4%).⁴ Motile sperm samples were prepared using density gradient centrifugation methods. The semen was briefly applied to 5 ml Gradient™90 (CooperSurgical), a single-layer density gradient liquid, and was centrifuged at 292×g for 20 min at room temperature (RT, 20–25°C). After discarding the supernatant, the sediment was resuspended in Sperm Wash (CooperSurgical) and washed twice. The sperm suspension was used for further experiments.

2.2 | Western blot

The sperm samples from three patients were washed with saline and prepared at a concentration of 120–125×10⁶ cells/ml. Samples of mouse brain and mouse sperm were used as positive controls. C57BL/6N mice were purchased from Japan SLC (Shizuoka, Japan). Mouse brains were additionally processed for adequate protein extraction with the following treatments. Radioimmunoprecipitation buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 1% protease inhibitor; Nacalai Tesque) was added to the mouse brain samples followed by sonication to extract proteins. The mouse brain samples were then centrifuged (7938×g, 5 min, 4°C) and the supernatant was collected. Equal volumes of 2× sample buffer (Nacalai Tesque) was added to each of the human sperm, mouse brain, and sperm, and the extracted samples were subjected to heat shock at 100°C for 5 min. The proteins were separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 1% bovine serum albumin (BSA, Merck KGaA) in Tris-buffered saline containing 0.1% Tween 20 (TBS-T, pH 7.5–7.8) for 30 min at RT. After being washed three times with TBS-T, the membranes were incubated (overnight at 4°C) with mouse monoclonal anti-D2DR antibody (sc-5303, 1:200; Santa Cruz Biotechnology), and washed three times with TBS-T. The membranes were incubated for 2 h at RT with horseradish peroxidase (HRP)-conjugated anti-mouse IgG antibody (1:2000; Promega). After three washes with TBS-T, the membranes were treated with Chemilumi-One (Nacalai Tesque), and images were obtained using the LAS-3000-mini Lumino Image Analyzer (Fujifilm).

2.3 | Immunocytochemistry

Immunocytochemistry was performed to investigate D2DR localization in human sperm. The sperm samples from three patients were added to 2 ml phosphate-buffered saline (PBS, pH 7.4), and the suspension was collected after centrifugation ($1500\times g$ for 5 min at RT). The sperm were fixed with 10% formaldehyde (Nacalai Tesque) for 15 min at 4°C, washed with PBS, and stored at 4°C. The sperm samples were collected by centrifugation ($3890\times g$ for 5 min at 4°C) and permeabilized with 1% Triton X-100 in PBS for 15 min at 4°C. They were washed twice with PBS and blocked with 1% BSA in PBS for 60 min at 4°C. The sperm were incubated with mouse monoclonal anti-D2DR antibody (1:50) overnight at 4°C. After three washes with PBS, the sperm were incubated (1 h at RT) with Alexa Fluor 568-conjugated anti-mouse IgG (1:200; Thermo Fisher Scientific), MitoTracker® Red CMXRos (250 nM, Thermo Fisher Scientific), and Hoechst 33342 (1:2000; Thermo Fisher Scientific). Treated samples were then washed with PBS, mounted on glass slides, and covered with coverslips. Images were obtained using an FV3000 confocal laser scanning microscope (Olympus).

2.4 | Tyrosine phosphorylation

The sperm samples from eight patients were washed by centrifugation ($1168\times g$ for 5 min) with 2 ml saline and were prepared at a concentration of $10\text{--}15\times 10^6$ cells/ml in 5% Human Serum Albumin (HSA, Vitrolife) in Quinn's Fertilization medium (QF, CooperSurgical). The suspensions (200 μ l) were divided into 1.5-ml microtubes. Saline (control) or dopamine (Merck KGaA) dissolved in saline to final concentrations of 0.1, 1, 10, and 100 μ M was added to the suspensions from each patient, and the suspensions were cultured (3 h at 37°C) in 6% CO₂ and air. After incubation, the suspensions were collected by centrifugation ($1500\times g$ for 5 min) and were resuspended in 50 μ l saline. The same volume of 2 \times sample buffer was added to the suspensions, heated at 100°C for 5 min, and stored at -80°C. The acquired proteins were separated by 8% SDS-PAGE and transferred to polyvinylidene difluoride membranes. After 30 min of blocking with 1% BSA in TBS-T, the membrane was incubated overnight at 4°C with anti-phosphotyrosine 4G10 antibody (05-321, 1:2000; Merck KGaA) or anti- α -tubulin (sc-32293, 1:20000; Santa Cruz Biotechnology) as an internal control. After three washes with TBS-T, the membrane was treated with HRP-conjugated anti-mouse IgG (1:2000; Promega) for 2 h at RT. The membranes were reacted with Chemilumi-One, and images were obtained using the LAS-3000-mini Lumino Image Analyzer. Densitometric analyses were performed on the regions containing 81 to 105 kDa using the Image Gauge v4.22 analysis software (Fujifilm).

2.5 | Sperm motility assay

The sperm samples were washed by centrifugation ($1168\times g$ for 5 min) with saline. Suspensions from each patient were prepared at a

concentration of $10\text{--}15\times 10^6$ cells/ml using 5% HSA in QF and were divided into 200 μ l aliquots. The suspensions from eleven patients were treated with dopamine dissolved in saline, to a final concentration of 0.1, 1, 10, and 100 μ M. The other suspensions from nine patients were treated with dopamine (10 μ M) in the presence or absence of raclopride (Merck KGaA), a D2DR antagonist, dissolved in saline. The concentration of raclopride added was 32 nM, which is the 50% inhibition concentration (IC₅₀). In both examinations, saline was added to the suspensions as a control. After the samples were cultured for 1 h at 37°C in 6% CO₂ and air, 20 μ l of the samples was analyzed once using a computer-assisted sperm analysis (CASA) system (LensHooke).

Next, to examine whether reduced sperm motility could be improved with dopamine, we added dopamine to 24-h cultures of the sperm samples. The sperm samples from each of 10 patients were similarly washed and were prepared at a concentration of $10\text{--}15\times 10^6$ cells/ml. The suspensions were divided into 200 μ l aliquots and were cultured at 37°C in 6% CO₂ and air for 24 h. Dopamine solution was added to a final concentration of 10 μ M, and the suspensions were cultured for 1 h at 37°C in 6% CO₂ and air. The suspensions were then analyzed once using the CASA system (LensHooke). Also, after 24-h culture, we recorded videos before and after dopamine treatment by using RI Viewer (CooperSurgical).

The parameters for sperm motility evaluation were as follows: total motility (%); progressive motility (%); average path velocity (VAP, μ m/s); curvilinear velocity (VCL, μ m/s); straight line velocity (VSL, μ m/s); linearity (LIN = [VSL/VCL] \times 100, %); straightness (STR = [VSL/VAP] \times 100, %); amplitude of lateral head displacement (ALH, μ m); wobble (WOB = [VAP/VCL] \times 100, %); and beat cross frequency (Hz).

2.6 | Statistical analysis

All evaluations were performed on at least three independent samples. Data are presented as mean (\pm SD) or medians (interquartile range). Statistical analyses were performed using the Kruskal-Wallis test, Steel test, and Wilcoxon signed-rank test. A *p*-value <0.05 was considered to indicate significant differences (**p* < 0.05).

3 | RESULTS

3.1 | Expression pattern of D2DR in sperm

Western blot and immunofluorescent staining were performed to determine the expression and localization of D2DR in human sperm. In Western blot analysis, a specific band at approximately 51 kDa (Figure 1A) was detected, similar to the mouse brain and sperm samples (positive control). This corresponds to the molecular size of D2DR at 48/51 kDa. Immunoreactivity of D2DR was observed in the midpiece of human sperm (Figure 1B).

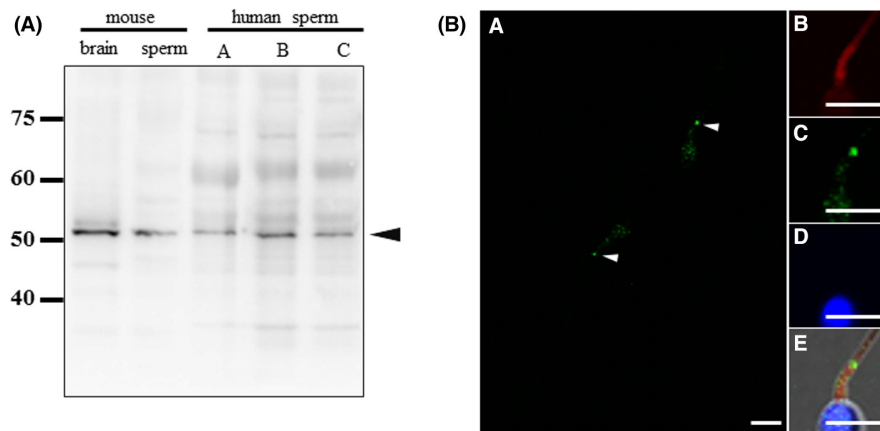


FIGURE 1 Detection of dopamine D2 receptor (D2DR) expression in human sperm. (A) D2DR expression was analyzed by Western blot analysis and immunocytochemistry. Mouse brain (lane 1) and sperm (lane 2) were prepared as positive controls. Note the human sperm samples from independent patients (lane 3–5) had specific bands corresponding to the positive control (arrowhead). The experiment was replicated twice. (B) Representative immunocytochemical images showing D2DR localization in human sperm. Immunoreactivity was detected as a spot in the midpiece region of sperm (arrowhead). Red: mitochondria (MitoTracker® Red CMXRos), Green: D2DR, Blue: nuclear (Hoechst 33342); scale bars = 5 μ m.

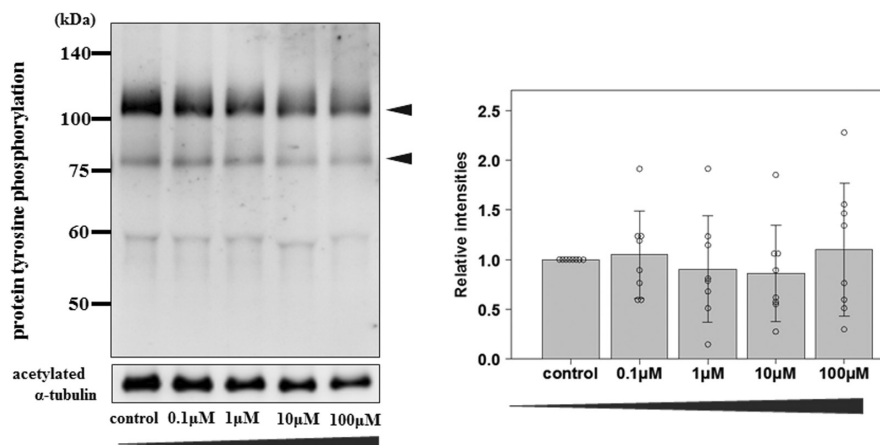


FIGURE 2 Effect of dopamine on sperm protein tyrosine phosphorylation. Human sperm were incubated with various dopamine concentrations for 3 h. The first panel shows the representative blotting image. Major human sperm tyrosine phosphorylation is 81 and 105 kDa (arrow heads). Second panel shows quantification of relative intensities of tyrosine phosphorylated proteins 81 to 105 kDa, and the comparison between control and dopamine treatments. The experiment was replicated eight times with independent sample preparations from patients indicated by plots. The relative intensities of the control protein were normalized to 1. Acetylated α -tubulin was used as the internal control. Data are shown as the mean \pm SD.

3.2 | Effect of dopamine on sperm protein tyrosine phosphorylation

Whether dopamine induces tyrosine phosphorylation of sperm proteins is an indicator of sperm capacitation. Human sperm were incubated with various dopamine concentrations (0–100 μ M) for 3 h and were analyzed using Western blot (Figure 2). Acetylated α -tubulin was used as the internal control and was detected in the same band region for all groups. The regions containing the major bands for human sperm tyrosine phosphorylation (81 to 105 kDa) were analyzed.²⁵ There was no significant difference between the intensity of the bands.

3.3 | Effect of dopamine on sperm motility

The effect of dopamine treatment on sperm motility, which is essential for fertilization, was evaluated. Progressive sperm motility increased significantly in samples incubated for 1 h with 10 and 100 μ M dopamine (Table 1). Moreover, when coculturing dopamine and raclopride (D2DR antagonist), progressive motility was not significantly different from that of the control (Table 2). Similarly, after the suspension (24-h culture) was treated with 10 μ M of dopamine for 1 h, progressive sperm motility was observed, but this was not significantly different. Furthermore, after a 24-h incubation, increased VCL and ALH were observed, which was significantly

TABLE 1 Dopamine treatment for 1 h increased progressive motility sperm rate ($n = 11$)

Sperm motility parameters	Control	Dopamine			
		0.1 μ M	1 μ M	10 μ M	100 μ M
Total motility (%)	90.0 (85.0–95.5)	90.0 (86.5–95.0)	88.0 (84.0–96.5)	92.0 (89.0–96.0)	92.0 (88.0–97.0)
Progressive motility (%)	83.0 (79.5–85.5)	83.0 (80.0–89.5)	82.0 (80.0–84.0)	90.0 (85.0–93.5)*	87.0 (85.0–91.0)*
VAP (μ m/s)	29.3 (25.0–34.6)	32.9 (28.2–35.0)	30.8 (28.2–34.4)	31.8 (26.9–39.2)	31.0 (27.7–39.6)
VCL (μ m/s)	55.0 (53.0–65.1)	58.0 (53.9–64.8)	59.0 (51.8–65.2)	55.0 (50.5–64.3)	62.0 (52.7–67.0)
VSL (μ m/s)	20.9 (16.6–26.5)	22.0 (18.4–29.4)	23.0 (15.7–26.5)	24.4 (18.7–31.7)	25.0 (18.5–36.5)
LIN (%)	42.0 (35.0–46.5)	38.0 (34.0–45.5)	38.0 (32.0–47.5)	40.0 (36.5–52.0)	38.0 (33.5–59.5)
STR (%)	72.0 (66.5–74.0)	69.0 (62.5–74.5)	73.0 (61.5–75.5)	76.0 (68.5–77.5)	71.0 (63.5–84.0)
ALH (μ m)	4.1 (3.7–4.8)	4.8 (4.4–5.2)	4.4 (4.1–5.4)	4.8 (4.0–5.2)	4.8 (4.0–5.6)
WOB (%)	55.0 (50.5–61.0)	52.0 (50.5–58.0)	56.0 (47.0–60.0)	53.0 (50.5–66.0)	56.0 (49.0–65.0)
BCF (Hz)	6.2 (5.9–7.1)	6.2 (5.6–6.9)	6.0 (6.0–6.4)	5.8 (5.5–6.9)	6.2 (5.7–7.0)

Abbreviations: ALH, amplitude of lateral head displacement; BCF, beat cross frequency; LIN, linearity; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight line velocity; WOB, wobble.

Data are shown as the median (IQR: interquartile range).

Asterisks denote a statistically significant difference vs. control group ($p < 0.05$, Kruskal-Wallis test and Steel test).

TABLE 2 Effect of treatment with 10 μ M dopamine and the dopamine D2 receptor (D2DR) antagonist, raclopride (32 nM), on several parameters of human sperm motility ($n = 9$)

Sperm motility parameters	Control	Dopamine 10 μ M	Dopamine 10 μ M + Raclopride 32 nM
Total motility (%)	89.0 (82.0–93.0)	95.0 (94.0–98.0)	91.0 (88.0–95.0)
Progressive motility (%)	86.0 (81.0–88.0)	95.0 (89.0–98.0)*	91.0 (83.0–94.0)
VAP (μ m/s)	35.4 (29.6–37.6)	33.5 (28.4–36.1)	31.2 (28.3–32.9)
VCL (μ m/s)	58.5 (57.5–61.8)	58.4 (54.4–69.5)	59.2 (53.0–66.9)
VSL (μ m/s)	29.7 (23.3–32.6)	26.3 (18.2–30.3)	26.0 (19.3–28.2)
LIN (%)	49.0 (47.0–52.0)	37.0 (34.0–46.0)	40.0 (40.0–48.0)
STR (%)	78.0 (76.0–79.0)	74.0 (64.0–78.0)	73.0 (72.0–80.0)
ALH (μ m)	4.1 (3.6–5.6)	5.0 (4.0–5.6)	4.8 (4.0–5.1)
WOB (%)	60.0 (55.0–64.0)	50.0 (48.0–59.0)	51.0 (49.0–60.0)
BCF (Hz)	6.6 (6.2–7.4)	6.4 (5.9–7.2)	6.8 (6.5–7.2)

Data are shown as the median (IQR). Asterisks denote a statistically significant difference vs. control group ($p < 0.05$, Kruskal-Wallis test and Steel test).

different to the control and treatment with 10 μ M dopamine for 1 h (Table 3). In some cases, dopamine treatment after 24-h incubation substantially increased motility (Movies [S1 and S2]).

4 | DISCUSSION

In this study, we detected coincident protein bands in human sperm, mouse sperm, and brain tissue using Western blot, corresponding with a previous study.²² The expression of D2DR was detected in the midpiece region of human sperm (Figure 1B), which is consistent with previous reports on the sperm of humans and other species such as rats, mice, bulls, and boars.^{22,23} The sperm midpiece is an essential region for oxidative metabolism and for promoting sperm motility. Thus, we hypothesized that dopamine could act to promote capacitation through increasing sperm motility. Two

experiments were conducted to assess the effect of dopamine on human sperm function with bicarbonate and HSA, which are used to treat infertility.

First, we investigated the tyrosine phosphorylation of sperm proteins, which showed no significant difference when treated with various concentrations of dopamine (Figure 2). Tyrosine phosphorylation is one of the indicators of sperm capacitation.^{25,26} It has been reported that cAMP elevation and subsequent protein kinase A activation is necessary for tyrosine phosphorylation in the sperm.²⁵ D2DR (Gai/o family of G-proteins) and dopamine-binding of D2DR inhibit AC activity following cAMP synthesis.^{18,27} Therefore, dopamine in human sperm was predicted to decrease the level of tyrosine phosphorylation. However, tyrosine phosphorylation was unaffected by dopamine treatment. Mammalian sperm contains soluble adenylylase (sAC), not coupled to G-protein, which is a distinct form of membrane-cyclase and is a major component of cAMP

TABLE 3 The sperm suspensions were cultured for 24 h and treated with 10 μM dopamine for 1 h: Analysis of several parameters of human sperm motility ($n = 10$)

Sperm motility parameters	Control	Dopamine 10 μM
Total motility(%)	79.0 (77.0–86.0)	84.0 (80.8–92.0)
Progressive motility(%)	75.0 (69.5–81.3)	82.5 (78.5–91.3)
VAP ($\mu\text{m/s}$)	30.6 (23.6–36.5)	34.1 (32.0–36.4)
VCL ($\mu\text{m/s}$)	54.7 (47.8–58.1)	60.2 (55.0–63.8)*
VSL ($\mu\text{m/s}$)	27.3 (20.2–33.8)	30.0 (28.1–33.8)
LIN(%)	50.0 (44.8–56.0)	51.0 (44.8–56.3)
STR(%)	82.0 (77.8–87.8)	84.0 (82.3–88.8)
ALH (μm)	3.7 (2.9–4.2)	4.3 (3.9–4.6)*
WOB(%)	57.5 (53.5–64.8)	57.0 (52.5–61.8)
BCF (Hz)	7.4 (6.8–7.7)	7.4 (7.2–7.7)

Data are shown as the median (IQR). Asterisks denote a statistically significant difference vs. control group ($p < 0.05$, Wilcoxon signed-rank test).

production in mammalian sperm.^{28,29} Stimulation of D2DR also increases intracellular calcium ion (Ca^{2+}) levels. An increase in the sperm Ca^{2+} levels has been reported to induce sAC activation, leading to cAMP synthesis.³⁰ In the present study, it is possible that the effect of dopamine on tyrosine phosphorylation was not detected because we used a medium that contained adequate bicarbonate, which is most important for the induction of tyrosine phosphorylation. However, our results suggest that dopamine applied in infertility treatment will not inhibit tyrosine phosphorylation.

Next, under conditions of infertility treatment, we demonstrated that treatment with a high concentration of dopamine immediately after sperm sample preparation significantly increased the progressive motility of the sperm, while other parameters were not significantly affected (Table 1). Moreover, raclopride (D2DR antagonist) with a high concentration of dopamine was observed to slightly (but not significantly) increase sperm progressive motility (Table 2). In this study, the use of raclopride at IC_{50} concentrations may have inhibited the action of some dopamine on sperm motility. It is possible that dopamine induces accelerated sperm motility through D2DR. Although, in this study, we used normozoospermia and induced sperm function in a medium that contained bicarbonate, the treatment of sperm immediately after sperm preparation with dopamine slightly induced accelerated sperm motility. To assume a case of male infertility with reduced sperm function, we treated sperm samples that had been cultured for 24 h. After the 24-h culture, the progressive motility rate significantly decreased (data not shown). One hour after treatment with a high concentration of dopamine, progressive motility increased compared with that of the control, but not significantly. VCL and ALH were significantly increased by treatment with a high dopamine concentration (Table 3). When sperm movement is shifted to hyperactivated motility, the flagellar bend and beat patterns become asymmetric and acquire a higher amplitude. This is reflected in the motility parameters (increased VCL and ALH, and

decreased LIN)³¹ estimated using the CASA system. Actually, some sperm movements were observed to be hyperactivation³² after cultured for 24 h and treated dopamine (S2). Therefore, dopamine may have induced sperm hyperactivation but may have had a low impact in normozoospermia under culture conditions of infertility treatment. Because treatment with a high concentration of dopamine in sperm with decreased motility was more effective in improving sperm motility, we believed that male infertility may benefit from a high concentration of dopamine.

Flagella are crucial in sperm motility, which is largely related to Ca^{2+} content in the midpiece.³³ Our analysis showed D2DR was present in the sperm midpiece region (Figure 1B). In in vitro experiments using LtK-mouse fibroblasts and mouse striatal slices, stimulation of D2DR was reported to result in increasing Ca^{2+} levels.^{21,34} It has been reported that high concentrations of Ca^{2+} are present in the human sperm midpiece region, and the sperm neck region expresses inositol 1,4,5-trisphosphate receptor (IP_3R).³⁵ Dopamine treatment induces the acrosome reaction, in which elevated Ca^{2+} in spermatozoa are the major induction factor.²⁴ These facts believed that dopamine may increase intracellular Ca^{2+} levels in human sperm through D2DR, with a contribution to accelerated motility and the induction of hyperactivation. However, the present study could not reveal that the stimulation of D2DR increases intracellular Ca^{2+} levels; whether it triggers an increase in intracellular Ca^{2+} in human sperm needs to be further investigated.

In the seminal plasma, concentrations of dihydroxyphenylalanine (DOPA), the precursor of dopamine, are higher than in the plasma.³⁶ Moreover, in patients with asthenozoospermia and oligozoospermia, the seminal plasma and blood dopamine concentrations are low compared with fertile men.³⁷ During the luteal period, dopamine is present in the female fallopian tube, with a higher concentration in the isthmus region (approximately 0.32 μM) than in the ampulla region (approximately 0.20 μM).³⁸ Our results show that dopamine-induced acceleration of sperm motility can be achieved by using higher dopamine concentrations (10 or 100 μM) than that in the semen or fallopian tube. It has been reported that human sperm motility is accelerated at dopamine concentrations close to those in the female reproductive tract.²⁴ Because the study²⁴ aimed to investigate the effect of dopamine on sperm function, HSA medium was not used, which is an important component for the acceleration of sperm motility. The current study was assumed to be used in assisted reproduction technology, and sperm function was adequately induced using culture media that contained bicarbonate and HSA, which are commonly used in infertility treatment. High concentrations of dopamine further improved sperm motility. Therefore, our results show that a high concentration of dopamine may be useful to improve sperm motility in infertility treatment.

Overall, our results demonstrate that dopamine can enhance human sperm motility without elevating tyrosine phosphorylation. Moreover, this study is the first to report that sperm motility reduced after 24-h culture can be accelerated by a neurotransmitter. This may be useful to improve sperm motility in patients with

infertility, and for successful conception either naturally or through artificial insemination.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

HUMAN RIGHTS

All procedures followed were in accordance with the ethical standards of the Responsible Committee on Human Experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all the men who participated in the study. This study was approved by the Institutional Review Board at Tohoku University and Sendai ART Clinic.

ANIMAL STUDIES

The animal studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by Tohoku University.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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