RESEARCH ARTICLE

J Anim Sci Technol 2026;68(1):96-104
https://doi.org/10.5187/jast.2024.e108

JAST
Journal of Animal Science and Technology
pISSN 2672-0191 eISSN 2055-0391

Investigating the correlation of glucose-regulated protein 78 with sperm motility and kinematic parameters insights into male fertility

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Received: Jul 29, 2024
Revised: Oct 22, 2024
Accepted: Nov 9, 2024

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Abstract

Glucose-regulated protein 78 (GRP78), which belongs to the heat shock protein 70 family, is a chaperone essential for maintaining cellular balance in the endoplasmic reticulum. It acts as a receptor on the cell surface, affecting cell growth and survival. Recent research has emphasized the involvement of GRP78 in protein folding, endoplasmic reticulum stress signaling, and cancer cell proliferation and highlighted its presence on sperm cells, where it contributes to spermatogenesis and sperm capacitation. Despite these findings, the association of GRP78 with sperm motility and motion kinematic parameters remains insufficiently understood. This study assessed the correlation between GRP78 and various sperm motility and kinematic parameters, which are essential for the capacitation process. GRP78 expression was positively correlated with linearity and straightness and negatively correlated with total sperm motility and beat cross frequency. However, GRP78 expression did not exhibit a significant correlation with progressive sperm motility, average path velocity, curvilinear velocity, straight-line velocity, and amplitude of lateral head displacement. These results provide novel insights into the correlation between GRP78 and sperm motility, as well as its potential implications for male fertility.

Keywords: Glucose-regulated protein 78 (GRP78), Sperm motility and motion kinematics, Male fertility

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Competing interests
No potential conflict of interest relevant to this article was reported.

Funding sources
This study was conducted with the support of the Gyeongsangbuk-do agricultural and fishery R&D activation project. In addition, this research was supported by a grant from the Nakdonggang National Institute of Biological Resources (NNIBR), funded by the Ministry of Climate, Energy and Environment (MCEE) of the Republic of Korea (grant number: NNIBR20261107) and the Korea Environment Industry & Technology Institute (KEITI), through the project to make multi-ministerial national biological research resources a more advanced program funded by the MCEE (grant number: RS-2021-KE001786).

Acknowledgements
Not applicable.

Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

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Conceptualization: Uwamahoro C, Lee WJ, Bae JW, Kwon WS.
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INTRODUCTION

Glucose-regulated protein 78 (GRP78), which belongs to the heat shock protein 70 (HSP70) family, is a recognized chaperone present in the endoplasmic reticulum (ER). It is essential for maintaining cellular balance in different cell types [1]. GRP78, also known as immunoglobulin heavy chain-binding protein (BiP), is also found on the cell surface, where it functions similarly to a receptor and controls cell growth and viability [2–4]. In addition to aiding in accurate protein folding, preventing the aggregation of intermediates, and directing misfolded proteins for degradation by the proteasome, GRP78 can interact with calcium ions (Ca^{2+}) and regulate ER stress signaling [5–7]. Furthermore, GRP78 has been detected on the cancer cell surface. It is associated with the activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway and the enhancement of cancer cell proliferation [8,9]. GRP78 also regulates the proliferation and apoptosis of prostate cells through the AKT/mammalian target of rapamycin (mTOR) pathway and plays a pivotal role in epithelial–mesenchymal transition (EMT) and oxidative stress (OS) [6].

Furthermore, GRP78 has been detected on the sperm cell surface during spermatogenesis in humans. It is also present in the testes of both mice and humans [10,11]. Different researchers have demonstrated that the interaction between the sperm and zona pellucida is influenced by GRP78 through a calcium-dependent pathway, impacting sperm capacitation [12,13]. GRP78 also plays a crucial role in sperm maturation, suggesting its potential influence on sperm function [14]. The molecular mechanism by which GRP78 affects sperm capacitation by interacting with the PI3K/3-phosphoinositide-dependent protein kinase-1 (PDK1)/AKT pathway has been studied, and GRP78 has been found to potentially affect sperm capacitation [11]. However, despite extensive documentation, the association of GRP78 with sperm motility and motion kinematic parameters remains unclear. Sperm motility and motion kinematics serve as crucial indicators of sperm movement within the female genital tract, ultimately influencing successful sperm–oocyte interaction [15]. Therefore, this study assessed the association of GRP78 with sperm motility and motion kinematic parameters.

MATERIALS AND METHODS

Preparation of spermatozoa

All chemicals were bought from Sigma, unless otherwise stated. In total, 57 individual semen samples were collected from healthy mature Duroc boars aged 24–36 mon at Gyeongsan Swine Gene using the gloved hand technique. The collected semen samples were diluted using a broad extender (1:1 [v/v] Beltsville thawing solution: 37 mg/mL glucose, 6 mg/ml sodium citrate, 1.25 mg/mL ethylenediaminetetraacetic acid (EDTA), 1.25 mg/ml sodium bicarbonate, and 0.75 mg/mL potassium chloride) to achieve a final concentration of 3×10^9 sperm cells/ml [16]. After dilution, the semen samples were cooled and kept at 17℃ until analysis. The semen samples were washed to eliminate seminal plasma and dead spermatozoa using a discontinuous Percoll gradient (70% [v/v] and 35% [v/v]) [17,18].

Sperm motility and motion kinematics

The computer-assisted sperm analysis (CASA) program (IVOS® II, Hamilton Thorne) was used to measure sperm motility and motion kinematics. In brief, 3 μL ($30–40 \times 10^6$ cells/ml) of the samples were added to a preheated Makler counting chamber (Sefi Medical Instrument) at 37℃ [19]. Images were analyzed using the FSA 2016 program. Subsequently, sperm motility and motion

Writing – original draft: Uwamahoro C, Kwon WS.
Writing – review & editing: Uwamahoro C, Jo JH, Jang SI, Jung EJ, Lee WJ, Bae JW, Kim DH, Yi JK, Oh DY, Nam SW, Chung EJ, Ha JJ, Kwon WS.

Ethics approval and consent to participate
All processes were performed in accordance with the guidelines and approved by Institutional Animal Care and Use Committee of Kyungpook National University (KNU 2021-207).

kinematics (MOT = total sperm motility [%], PRG = progressive sperm motility [%], VAP = average path velocity [μm/s], VCL = curvilinear velocity [μm/s], VSL = straight-line velocity [μm/s], ALH = mean amplitude of lateral head displacement [μm], BCF = beat cross frequency [Hz], LIN = linearity [%; VSL/VCL × 100], and STR = straightness [%; VSL/VAP × 100]) were assessed [16].

Enzyme-linked immunosorbent assay

The expression levels of GRP78 in individual spermatozoa samples from Duroc boars were assessed using enzyme-linked immunosorbent assay (ELISA) [16]. The sperm samples were incubated in rehydration buffer containing 7 M urea, 2 M thiourea, 4% (w/v) 3-([3-cholamidopropyl] dimethylammonio)-1-propanesulfonate (CHAPS), 1% (w/v) octyl β-D-glucopyranoside, 24 μM PMSF, 1% (w/v) dithiothreitol (DTT), 0.05% (v/v) Triton X-100, and 0.002% (w/v) bromophenol blue at 4℃ for 1 h [16,20,21]. Then, the samples were centrifuged at 10,000×g for 5 min to separate the suspension. The amount of protein in the sample was measured using the Bradford protein-binding method [22]. Solubilized proteins (50 μg/well) were coated onto 96-well immunoplates and incubated overnight at 4℃. Subsequently, the plates were washed in Dulbecco's phosphate-buffered saline (DPBS) with 0.05% Tween-20 (PBST) and blocked with blocking solution (1% [w/v] bovine serum albumin [BSA] in DPBS containing PBST) for 90 min at 37℃. The plates were then incubated with GRP78 polyclonal antibody (1:5,000; MyBioSource) for 90 min at 37℃. Subsequently, the plates were incubated for 90 min at 37℃ with anti-rabbit IgG horseradish peroxidase (HRP)-conjugated secondary antibody that had been diluted with blocking solution (1:5,000; Abcam). The plates were then incubated with 3,3',5,5'-tetramethylbenzidine (TMB) solution for 15 min at room temperature (RT) to activate peroxidase. The reaction was stopped by adding 1 N sulfuric acid. Finally, the signal was detected at 450 nm using a microplate reader (Gemini EM; Molecular Devices Corporation).

Statistical analysis

SPSS software (v.27, IBM SPSS) was used to analyze the data. The Pearson correlation coefficient was calculated to determine the association between GRP78 and sperm motion parameters. All data are expressed as the mean ± SEM. The statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

GRP78 is a well-known chaperone located in the ER. It plays a crucial role in maintaining cellular homeostasis across various cell types [1]. Moreover, studies have revealed that GRP78 is present on the cell surface, where it acts like a receptor to regulate cell growth and survival [1,14]. GRP78 has also been detected in sperm cells and has been reported to participate in both spermatogenesis and sperm capacitation [7,11,23]. Spermatogenesis is a thoroughly studied sequence of events that begins with prospermatogonia and culminates in the production of mature spermatozoa capable of fertilization [7]. However, mature sperms cannot fertilize eggs immediately after ejaculation [24]. For successful fertilization, the ejaculated sperm cells must undergo a unique process after spending a certain amount of time in the female reproductive tract to gain complete fertilizing ability; this process is known as capacitation [25].

During the capacitation process, changes occur in sperm motility and motion kinematic parameters, leading to hyperactivation [26]. Sperm motility is essential for navigating through the female reproductive tract and penetrating barriers, such as the zona pellucida, in which GRP78 plays a role [27,28]. Moreover, numerous studies have demonstrated a positive correlation between sperm motility and fertilization, underscoring its crucial role in successful sperm-oocyte interaction

 https://doi.org/10.5187/jast.2024.e108

[19,29]. In particular, GRP78 has been associated with sperm motility and motion kinematics [11]. However, further verification is required to precisely establish the correlation between GRP78 and sperm motility. Hence, this study assessed the association of GRP78 with sperm motility parameters (MOT and PRG) and kinematic parameters (VAP, VCL, VSL, ALH, BCF, LIN, and STR). VAP represents sperm velocity along its path [30,31]. VCL represents the instantaneous swimming speed of the sperm, determined by the frequency, wavelength, and amplitude of the flagella [30]. VSL represents the straight-line trajectory of the sperm cell [30], while ALH refers to the displacement of the sperm head along its curvilinear path relative to the average trajectory [30,32]. BCF refers to the number of lateral oscillatory movements of the sperm head around its average path, also known as head displacement frequency [30–32]. LIN is the ratio of linear velocity to VCL, calculated as VSL/VCL [30]. STR, or VSL/VAP, is the ratio of linear velocity to mean velocity [30].

In this study, sperm motility and motion kinematic parameters were evaluated using the CASA program (Table 1). The average sperm motility and motion kinematic parameters were as follows: MOT = 89.711 ± 0.498%, PRG = 62.819 ± 1.664%, VAP = 99.646 ± 1.418 μm/s, VCL = 189.410 ± 3.948 μm/s, VSL = 62.224 ± 1.757 μm/s, ALH = 7.531 ± 0.164 μm, BCF = 35.875 ± 0.408 Hz, LIN = 34.852 ± 1.269%, and STR = 62.456 ± 1.530% (Table 1). Interestingly, GRP78 exhibited a positive or negative correlation with sperm motility and various motion kinematic parameters. In particular, GRP78 was negatively correlated with MOT ($r = -0.4073$, $p < 0.01$) and BCF ($r = -0.2740$, $p < 0.05$; Table 2 and Fig. 1A and 1B). In contrast, GRP78 was positively correlated with LIN ($r = 0.2696$, $p < 0.05$) and STR ($r = 0.2928$, $p < 0.05$; Table 2 and Fig. 1C and 1D), which could help the sperm navigate through the female reproductive tract and reach the egg [33]. Interestingly, reduced LIN and STR in sperm movement have been linked to infertility issues in humans [23]. While high motility and frequent head movements are generally important for fertilization, a more focused and linear path may be advantageous in some cases [34,35]. Sperm motility parameters are crucial for successful fertilization, as they are closely linked with hyperactivation a necessary condition for effective sperm-oocyte interaction [34]. High-quality motility not only enhances the likelihood of sperm reaching and fertilizing the oocyte but also serve

Table 1. Sperm motility, motion kinematic parameters, and GRP78 levels in each sample

No.	MOT	PRG	VAP	VCL	VSL	ALH	BCF	LIN	STR	GRP78
1	79.800	42.600	95.810	225.960	51.270	10.850	26.650	22.760	52.710	0.098
2	81.500	44.700	53.750	92.120	41.570	3.920	39.490	46.670	78.280	0.111
3	83.300	48.800	108.280	226.220	57.550	9.800	30.870	26.240	53.170	0.096
4	84.200	64.900	99.030	179.420	62.740	7.170	38.340	36.180	63.730	0.110
5	84.200	78.300	112.540	193.740	94.630	7.310	34.980	49.410	83.040	0.085
6	84.800	77.200	102.810	167.930	84.830	6.940	31.820	51.880	81.790	0.116
7	84.900	65.400	113.090	243.120	69.450	9.610	34.790	29.590	61.470	0.091
8	85.000	55.000	70.170	106.390	56.470	4.330	32.900	53.430	78.970	0.087
9	85.100	44.300	106.630	216.020	49.510	8.610	34.720	23.400	46.400	0.092
10	85.100	67.400	85.820	127.960	66.420	5.110	33.430	52.770	75.980	0.092
11	85.900	50.000	108.310	209.990	55.670	7.810	37.220	26.880	51.040	0.099
12	86.300	61.800	86.270	167.450	51.990	6.470	39.320	32.660	60.180	0.085
13	86.500	55.500	112.710	214.130	61.700	7.940	38.310	29.550	55.230	0.087
14	86.700	64.700	98.200	197.720	60.560	7.890	36.040	32.210	61.930	0.088
15	88.000	63.200	98.710	194.230	61.190	7.850	35.630	31.940	60.730	0.096

Table 1. Continued

No.	MOT	PRG	VAP	VCL	VSL	ALH	BCF	LIN	STR	GRP78
16	88.000	62.600	107.070	233.050	62.630	9.430	33.040	27.800	58.110	0.087
17	88.100	44.200	102.200	211.440	45.390	8.640	35.570	23.170	46.360	0.083
18	88.200	79.700	100.860	165.510	83.940	6.770	31.820	52.130	81.920	0.109
19	88.400	57.200	112.700	211.420	62.010	7.650	40.210	29.890	54.930	0.099
20	88.600	81.800	101.030	166.920	83.900	6.790	32.210	51.220	81.690	0.102
21	88.700	57.900	88.070	180.320	51.650	7.680	36.200	32.010	61.160	0.090
22	88.800	58.600	85.890	162.060	51.730	6.610	38.440	34.210	61.850	0.086
23	88.800	79.100	108.850	179.270	92.100	7.090	29.760	51.770	83.240	0.097
24	89.000	61.000	99.140	191.910	57.360	7.520	35.950	30.830	58.070	0.081
25	89.200	70.200	105.220	222.480	68.400	9.040	35.160	31.630	64.480	0.084
26	89.300	80.700	104.680	172.160	86.500	7.030	31.880	51.980	81.640	0.080
27	89.500	52.100	101.360	201.560	55.230	8.040	35.400	27.860	54.190	0.095
28	89.500	81.000	115.740	215.940	92.810	8.070	35.410	43.310	78.280	0.086
29	89.800	62.400	92.040	182.610	54.770	7.460	34.660	30.760	59.360	0.095
30	89.900	57.900	104.450	192.880	58.690	6.990	39.360	30.940	54.930	0.076
31	90.200	70.500	102.090	181.100	65.680	6.890	38.540	36.990	63.480	0.093
32	90.300	68.700	103.870	185.500	66.070	6.920	38.300	36.430	63.660	0.083
33	90.400	78.800	111.210	208.900	87.000	7.880	35.720	42.360	76.840	0.085
34	90.800	49.200	107.010	218.390	51.810	9.200	32.920	25.020	49.420	0.087
35	91.100	66.600	101.700	182.360	62.940	6.690	38.830	34.910	60.730	0.070
36	91.700	41.700	102.630	205.520	46.480	7.780	37.130	23.200	45.210	0.085
37	91.800	58.700	98.960	197.040	54.520	7.900	34.020	28.380	55.360	0.094
38	92.000	56.500	103.190	202.790	55.840	7.750	36.440	28.100	53.450	0.087
39	92.000	81.600	109.080	208.740	83.830	8.270	32.720	41.420	76.240	0.090
40	92.100	73.500	81.280	118.160	63.490	4.910	33.130	53.900	76.420	0.081
41	92.300	35.400	101.870	211.080	40.450	8.130	38.730	20.050	39.880	0.092
42	92.400	70.100	98.730	194.090	61.790	8.160	34.330	33.910	63.550	0.073
43	92.600	63.100	90.490	165.290	56.040	6.750	39.810	34.700	62.510	0.095
44	92.700	59.800	114.440	235.460	61.060	9.380	34.830	26.920	53.850	0.077
45	92.700	37.700	100.480	205.210	43.900	7.370	41.230	21.710	43.070	0.086
46	92.700	70.500	108.460	213.250	71.410	9.200	31.850	34.170	65.790	0.096
47	92.800	59.200	104.340	195.450	57.820	7.260	38.670	29.830	54.520	0.084
48	93.000	48.200	100.360	206.990	50.000	7.750	39.730	24.310	48.840	0.083
49	93.500	71.000	93.380	176.260	58.290	7.120	38.230	34.720	63.390	0.077
50	93.500	43.000	95.830	197.280	43.740	7.500	38.810	23.670	46.610	0.075
51	93.500	60.400	103.650	194.830	56.630	7.240	38.310	28.830	52.620	0.072
52	94.200	76.800	88.870	159.220	60.370	6.380	40.300	39.320	68.330	0.095
53	95.000	70.500	96.860	191.220	57.920	7.900	37.670	31.800	60.600	0.072
54	95.600	85.000	97.870	159.970	79.970	6.640	32.420	51.220	80.110	0.091
55	96.300	78.700	90.830	159.800	62.690	6.400	39.980	40.320	69.060	0.089
56	96.600	71.100	92.800	174.580	57.510	7.280	38.020	34.700	63.210	0.079
57	96.600	64.200	98.140	195.940	56.840	8.190	34.610	30.600	58.370	0.090
Average	89.711 ± 0.498	62.819 ± 1.664	99.646 ± 1.418	189.410 ± 3.948	62.224 ± 1.757	7.531 ± 0.164	35.875 ± 0.408	34.852 ± 1.269	62.456 ± 1.530	0.089 ± 0.001

MOT = total sperm motility (%); PRG = progressive sperm motility (%); VAP = average path velocity (μm/s); VCL = curvilinear velocity (μm/s); VSL = straight-line velocity (μm/s); ALH = mean amplitude of lateral head displacement (μm); BCF = beat cross frequency (Hz); LIN = linearity (%, [VSL/VCL] × 100); STR = straightness (%, [VSL/VAP] × 100); and GRP78 = glucose-regulated protein 78.

https://doi.org/10.5187/jast.2024.e108

Table 2. Correlation between sperm motion parameters and GRP78 expression levels (n = 3)

	PRG	VAP	VCL	VSL	ALH	BCF	LIN	STR	GRP78
MOT	0.221	0.086	0.035	−0.069	−0.043	0.348^{**}	−0.123	−0.148	$−0.497^{**}$
PRG		0.134	−0.254	0.852^{**}	−0.229	−0.253	0.748^{**}	0.823^{**}	0.040
VAP			0.836^{**}	0.415^{**}	0.702^{**}	−0.172	$−0.287^{*}$	−0.205	−0.139
VCL				−0.024	0.935^{**}	−0.097	$−0.698^{**}$	$−0.570^{**}$	−0.197
VSL					−0.039	$−0.456^{**}$	0.718^{**}	0.801^{**}	0.173
ALH						$−0.327^{*}$	$−0.644^{**}$	$−0.494^{**}$	−0.093
BCF							$−0.334^{*}$	$−0.385^{**}$	$−0.270^{*}$
LIN								0.964^{**}	0.274^{*}
STR									0.293^{*}

MOT = total sperm motility (%); PRG = progressive sperm motility (%); VAP = average path velocity (μm/s); VCL = curvilinear velocity (μm/s); VSL = straight-line velocity (μm/s); ALH = mean amplitude of lateral head displacement (μm); BCF = beat cross frequency (Hz); LIN = linearity (%, [VSL/VCL] × 100); STR = straightness (%, [VSL/VAP] × 100); and GRP78 = glucose-regulated protein 78 (%).

$^{*}p < 0.05$, $^{**}p < 0.01$.

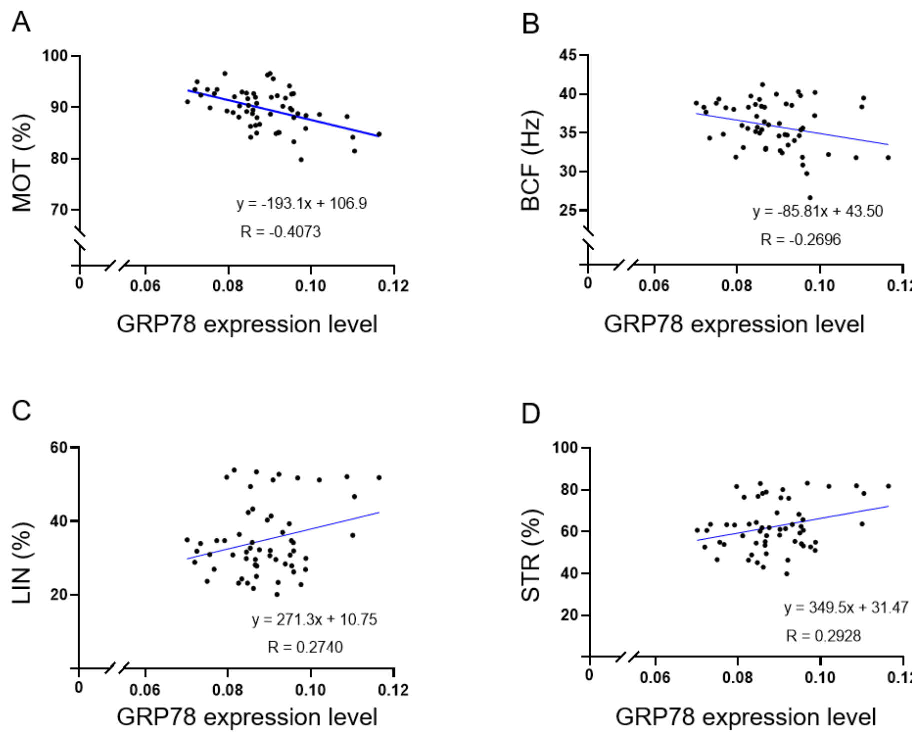

Fig. 1. Correction between glucose-regulated protein 78 (GRP78) expression level and various sperm motility and motion kinematic parameters. (A) Correlation between GRP78 expression level and sperm motility (%), (B) correlation between GRP78 expression level and beat cross frequency (Hz), (C) correlation between GRP78 expression level and linearity (%), (D) correlation between GRP78 expression level and straightness (%).

as a reliable indicator of reproductive potential [36]. Our results showed that GRP78 influences key motility parameters, thus it may affect sperm-oocyte interaction. Several motion parameters, such as VCL, ALH, LIN, and STR, are used to classify hyperactivation [26]. Hyperactivation involves changes in sperm motility that allow the sperm to penetrate the zona pellucida and fertilize the oocyte [26]. Various parameters, such as VCL, ALH, and LIN, are particularly associated with this enhanced motility [32]. In a previous study, GRP78 and Hsp60 exhibited no significant effect on PRG, VCL, and ALH. Moreover, none of the motility parameters, such as BCF, STR, and LIN, were modified by the presence of GRP78 or Hsp60 [13]. Similarly, in our study, GRP78 exhibited no significant correlation with PRG, VAP, VCL, VSL, and ALH. However, GRP78 exhibited a correlation with LIN, STR, MOT, and BCF. While GRP78 has been correlated with sperm motility and motion parameters, future research is needed to elucidate its effect on sperm motility and motion parameters in a perspective of reproductive technologies such as artificial insemination and in vitro fertilization.

In conclusion, this study elucidated the complex effects of GRP78 on sperm motility and kinematic parameters, which are crucial for successful sperm–oocyte interaction and fertilization. Although GRP78 shows a correlation with certain aspects of sperm motility and kinematics, its overall impact on sperm function may be multifaceted. Further research is warranted to investigate the precise mechanism by which GRP78 affects sperm function. Understanding this mechanism could have significant implications for improving male fertility.

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