

Correlation between Rab3A Expression and Sperm Kinematic Characteristics

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Abstract

Ras-related (Rab) proteins, integral members of the monomeric G-protein family, play a pivotal role in regulating intracellular vesicular transport. These proteins contribute to male reproductive processes, specifically in acrosome formation, exocytosis, and sperm motility. Although a prior study indicated a correlation between Rab3A and sperm motility, including motion kinematic parameters such as mean dance, this association has only been explored within a limited sample size. Therefore, further verification is required to confirm the correlation between Rab3A and sperm motility parameters. In the present study, Rab3A expression, sperm motility, and motion kinematic parameters were analyzed in 150 boar spermatozoa. Additionally, correlations between Rab3A expression and sperm kinematic characteristics were evaluated statistically. The results revealed significant associations between Rab3A protein expression levels and various motion kinematic parameters. Specifically, Rab3A levels exhibited positive correlations with average path velocity ($p < 0.05$), mean amplitude of lateral head displacement ($p < 0.05$), and curvilinear velocity ($p < 0.01$). Consequently, it is proposed that Rab3A protein plays a crucial role in male fertility through its correlation with sperm kinematic characteristics, making it a potential marker for sperm motility-related assessments.

Keywords: Rab proteins, Rab3A, Sperm motility, Correlation, Male fertility

INTRODUCTION

Ras-related (Rab) proteins, part of the monomeric G-protein family, play a crucial role in regulating intracellular vesicular transport. These proteins are predominately expressed in organelles and various transport vesicles (Pereira-Leal & Seabra, 2000; Wu et al., 2010; Goody et al., 2017). Functionally, Rab proteins are key players in membrane trafficking, including processes such as vesicle formation, vesicle movement, and membrane fusion (Alory & Balch, 2001; Stenmark, 2009; Hutagalung & Novick, 2011). Additionally, they contribute to the establishment and maintenance of the Golgi structure by controlling Golgi trafficking (Smith et al., 1990; Liu & Storrie, 2012; Goud et al., 2018).

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Conflict of interests

The authors declare no potential conflict of interest.

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Authors' contributions

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Ethics approval

All procedures were performed in accordance with the Guidelines for the Ethical Treatment of Animals and approved by the Institutional Animal Care and Use Committee of Kyungpook National University (KNU 2021-0207).

Numerous studies have highlighted the importance of Rab proteins in male reproduction. They are implicated in the regulation of male meiosis (Shan & Sun, 2021), with Rab 12 displaying high expression levels in Sertoli cells (Iida et al., 2005) and Rab8B being associated with dynamic junctions in the testes (Lau & Mruk, 2003). During spermatogenesis, primordial sperm cells undergo structural changes, where the Golgi apparatus transforms into the acrosome in the sperm head (Nakamura et al., 2015; Kumar et al., 2016; Lin et al., 2017). Membrane trafficking involving Rab proteins is reported to play an important role in acrosome biogenesis, and Rab proteins are essential factors for the acrosome reaction and exocytosis (Moreno et al., 2000; Ramalho-Santos et al., 2001). In recent years, studies have investigated the involvement of Rab proteins in sperm motility. For example, Bae et al. demonstrated that various Rab proteins are present in the sperm head and tail, with several Rab proteins correlating with motion parameters (Bae et al., 2019, 2022a).

Rab3A plays a vital role in plasma membrane repair (Vieira, 2018) and is crucial for lymphocyte chemotaxis, regulating directional leukocyte motility (Constantin & Laudanna, 2010). It has also been linked to acrosomal formation and exocytosis in spermatozoa (Yunes et al., 2000). Furthermore, Rab3A exhibits correlations with specific sperm motility and motion kinematics, such as medium sperm motility and mean dance (Bae et al., 2022b). Although prior studies suggest an association between Rab3A and sperm motility, these findings are based on small population samples and provide limited information. Therefore, there is a need to identify additional sperm motility-related markers associated with Rab3A through more objective evaluations using larger sample sizes. The present study aims to evaluate the correlation between Rab3A and sperm motility parameters by assessing Rab3A expression levels and sperm kinematic characteristics in 150 boar spermatozoa. Additionally, statistical analysis was performed to estimate the correlations between Rab3A protein expression and sperm kinematic characteristics.

MATERIALS AND METHODS

1. Sperm sample preparation

Total 150 individual semen samples were collected from healthy mature Duroc boars (24–36 months old) using the gloved-hand technique. These samples were diluted with a broad extender [1:1 (v/v)] in Beltsville thawing solution (37 mg/mL glucose, 6 mg/mL sodium citrate, 1.25 mg/mL EDTA, 1.25 mg/mL sodium bicarbonate, and 0.75 mg/mL potassium chloride). The extended semen was stored in a low-temperature incubator (17°C) and processed within 2 h of collection (Jang et al., 2022).

2. Sperm motility and motion kinematic parameters

Sperm motility and motion kinematic parameters were analyzed using a computer-assisted sperm analysis (CASA) program (IVOS® II, Hamilton Thorne, Beverly, MA, USA). Postincubation, 3 µL of the sample was placed on a preheated Makler counting chamber at 37°C (Sefi-Medical Instruments, Haifa, Israel). Measurements included total sperm motility (MOT, %), progressive sperm motility (PRG, %), curvilinear velocity (VCL, µm/s), straight-line velocity (VSL, µm/s), average path velocity (VAP, µm/s), mean amplitude of lateral head displacement (ALH, µm), beat-cross frequency (BCF, Hz), linearity (LIN, %), and straightness (STR, %).

3. Enzyme-linked immunosorbent assay

Rab3A protein expression in spermatozoa was determined using an enzyme-linked immunosorbent assay. Spermatozoa were treated with rehydration buffer to extract total proteins [7 M

urea, 2 M thiourea, 4% (w/v) 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate, 1% (w/v) octyl β-D-glucopyranoside, 24 mM phenylmethylsulfonyl fluoride, 1% (w/v) dithiothreitol, 0.05% (v/v) Triton X-100, and 0.002% (w/v) bromophenol blue] at 4°C for 1 h. Protein concentration was measured following the Bradford protein-binding protocol (Bradford, 1976). Extracted protein (50 μg) was loaded into 96-well plates and incubated overnight at 4°C. The plates were then washed with PBS containing 0.05% Tween-20 (PBST) and blocked with a blocking solution [1% (w/v) bovine serum albumin in PBST] for 90 min at 37°C. Subsequently, the plates were incubated with anti-Rab3A antibody (1:5,000; Abcam, Cambridge, UK) for 90 min at 37°C, followed by incubation with goat anti-rabbit IgG H&L (HRP) antibody (1:5,000; Abcam) for 90 min at 37°C. Tetramethylbenzidine solution was used to activate peroxidase for 15 min at room temperature. Activation was terminated with 1 N sulfuric acid, and Rab3A protein signals were detected at 450 nm using a microplate reader (Gemini Em; Molecular Devices, Sunnyvale, CA, USA).

4. Statistical analysis

Data were analyzed using SPSS (ver. 26.0, IBM, Armonk, NY, USA). The correlation between Rab3A protein expression and sperm motility was analyzed using Pearson correlation coefficients. Student's two-tailed t-test was used to compare sperm motility values. Data are presented as means ± SEM, with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Rab proteins are integral to the intracellular vesicular transport system, playing a critical role in both preserving and establishing the Golgi apparatus through membrane trafficking regulation (Smith et al., 1990; Stenmark, 2009; Liu & Storrie, 2012). These proteins are also recognized for their involvement in male fertility, contributing to the origin of the acrosome from the Golgi apparatus during spermatogenesis (Moreno et al., 2000; Ramalho-Santos et al., 2001). Additionally, Rab proteins contribute to acrosome formation and reaction as well as sperm motility (Bae et al., 2022a). Specific Rab proteins, such as Rab8B, are associated with dynamic junctions in the testes (Lau & Mruk, 2003), and Rab2A serves as a biomarker for boar fertility evaluation (Kwon et al., 2015). Notably, Rab3A is a protein associated with acrosomal formation and exocytosis in spermatozoa (Yunes et al., 2000).

The fertility of mammalian spermatozoa is contingent of a specific duration within the female reproductive tract. During this period, sperm motility and motion kinematic parameters undergo changes with hyperactivation. Sperm motility is vital for navigating the tract and penetrating barriers, such as the zona pellucida (Jung et al., 2022). Furthermore, numerous studies have found a positive correlation between sperm motility and fertilization (Bongso et al., 1989; Donnelly et al., 1998), emphasizing the sperm motility's pivotal role in successful fertilization. Specifically, Rab3A protein is correlated with medium sperm motility and mean dance (Bae et al., 2022b). However, for a more precise understanding, additional verification is essential to establish the correlation between Rab3A and sperm motility; therefore, the present study was performed to investigate this potential correlation.

In this study, the CASA program was used to estimate sperm motility and motion kinematics parameters. The analysis included average sperm motility, motion kinematic parameters, and Rab3A expression, yielding the following results: MOT = 88.0980% ± 0.4104%; PRG = 65.0233% ± 0.9996%; VAP = 99.5033 ± 0.8861 μm/s; VCL = 186.0728 ± 2.1710 μm/s; VSL = 65.5099 ± 1.1006 μm/s; ALH = 7.3627 ± 0.0915 μm; BCF = 36.2617 ± 0.2233 Hz; LIN = 37.0036% ± 0.7310%; STR = 65.5908% ± 0.8952%; and Rab3A = 0.0771 ± 0.0007 (Table 1; Fig. 1). Interestingly, Rab3A

Table 1. Sperm kinematic characteristics and Rab3A expression levels

MOT	PRG	VAP	VCL	VSL	ALH	BCF	LIN	STR	Rab3A
88.0980±0.4104	65.0233±0.9996	99.5033±0.8861	186.0728±2.1710	65.5099±1.1006	7.3627±0.0915	36.2617±0.2233	37.0036±0.7310	65.5908±0.8952	0.0771±0.0007

Sperm motility and motion kinematic parameters are presented as means±SEM. n=3.

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 head lateral displacement (µm); BCF, beat-cross frequency (Hz); LIN, linearity [%; (VSL / VCL) × 100]; STR, straightness [%; (VSL / VAP) × 100].

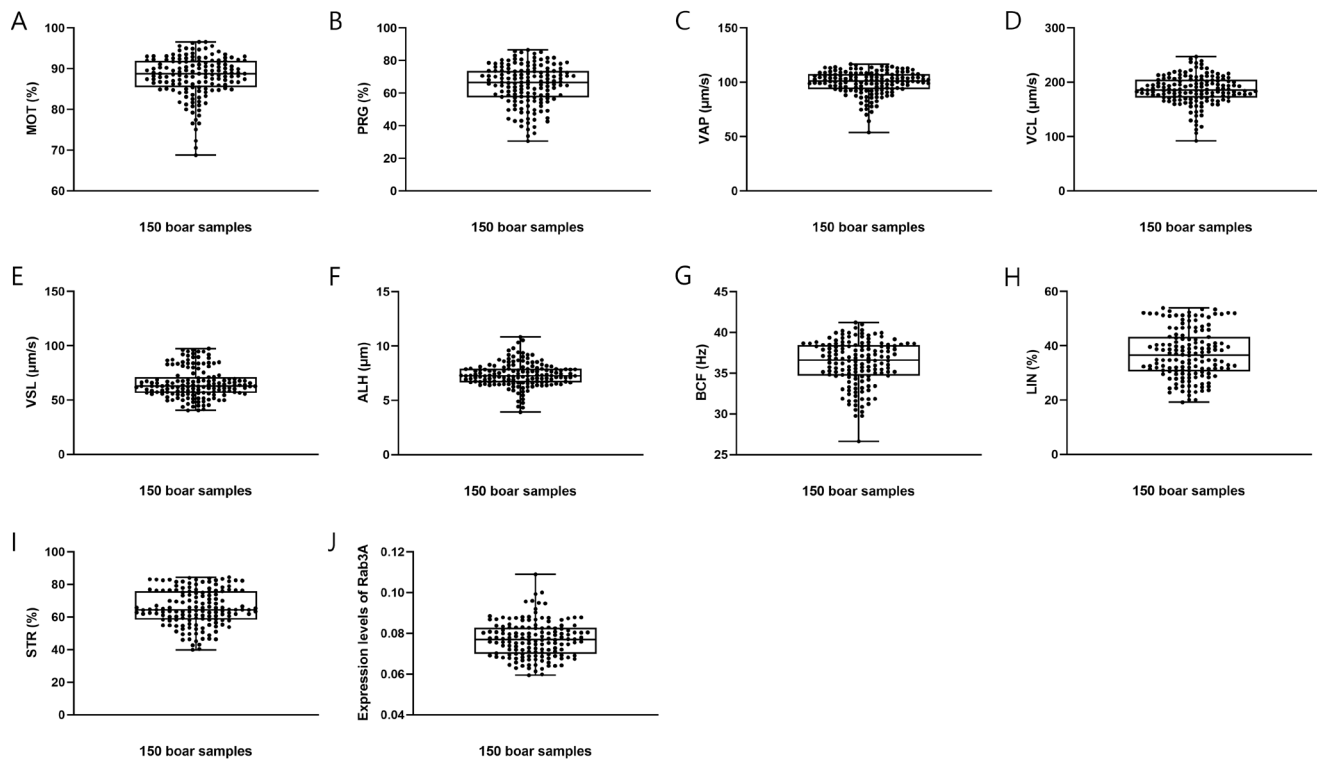


Fig. 1. Measurement of sperm motility and Rab3A expression levels in 150 boar spermatozoa samples. (A) MOT, total sperm motility (%). (B) PRG, progressive sperm motility (%). (C) VAP, average path velocity (µm/s). (D) VCL, curvilinear velocity (µm/s). (E) VSL, straight-line velocity (µm/s). (F) ALH, mean amplitude of head lateral displacement (µm). (G) BCF, beat-cross frequency (Hz). (H) LIN, linearity (%). (I) STR, straightness (%). (J) Rab3A expression levels. n=3.

protein expression levels exhibited a positive correlation with several motion kinematic parameters. Specifically, Rab3A was positively correlated with VAP, the velocity over the average trajectory of the sperm cell ($p < 0.05$; Table 2; Fig. 2A), ALH, the sperm head displacement along its curvilinear trajectory around the mean trajectory ($p < 0.05$; Table 2; Fig. 2C), and VCL, the instantaneous velocity along the total trajectory of the sperm cell ($p < 0.01$; Table 2; Fig. 2B) (Suarez & Dai, 1992; Rodríguez-Gil et al., 2007; Bae et al., 2022a). Several motion parameters, including VCL, ALH, linearity (LIN: VSL / VCL), and straightness (STR: $VSL / VAP \times 100$), are used to categorize hyperactivation (Hinrichs & Loux, 2012). Hyperactivation indicates alterations in sperm motility, enabling penetration of the zona pellucida and fertilization of the oocyte. Notably, VCL, ALH, and LIN are associated with hyperactivated motility. Furthermore, sperm motion parameters, such as VCL, VSL, VAP, and ALH, have been linked to the outcomes of *in vitro* fertilization and intrauterine insemination (Aghazarian et al., 2021).

In a prior study, Rab3A was associated with litter size; consequently, it can serve as a biomarker for predicting fertility (Bae et al., 2022b). In the present study, Rab3A was associated with various

Table 2. Correlations between sperm kinematic characteristics and Rab3A expression levels

	PRG	VAP	VCL	VSL	ALH	BCF	LIN	STR	Rab3A
MOT	.320**	.262**	.084	.011	-.106	.317**	-.032	-.140	.115
PRG		.192*	-.301**	.813**	-.434**	-.010	.794**	.811**	.002
VAP			.789**	.497**	.538**	-.064	-.114	-.103	.175*
VCL				-.002	.903**	-.086	-.632**	-.545**	.224**
VSL					-.141	-.281**	.758**	.805**	.005
ALH						-.339**	-.669**	-.541**	.187*
BCF							-.210**	-.267**	-.007
LIN								.964**	-.125
STR									-.107

* $p < 0.05$, ** $p < 0.01$. $n = 3$.

MOT, total sperm motility (%); PRG, progressive sperm motility (%); VAP, average path velocity ($\mu\text{m/s}$); VCL, curvilinear velocity ($\mu\text{m/s}$); VSL, straight-line velocity ($\mu\text{m/s}$); ALH, mean amplitude of head lateral displacement (μm); BCF, beat-cross frequency (Hz); LIN, linearity [%; $(\text{VSL} / \text{VCL}) \times 100$]; STR, straightness [%; $(\text{VSL} / \text{VAP}) \times 100$].

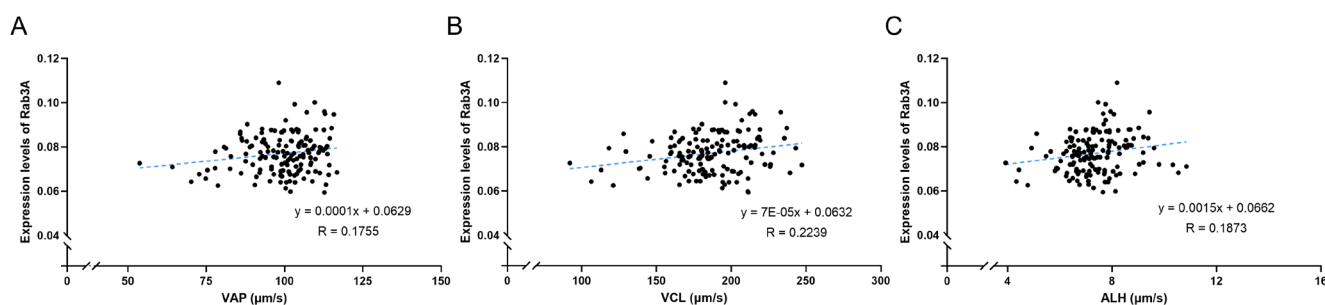


Fig. 2. Correlation between Rab3A expression levels and sperm kinematic characteristics. Correlations between Rab3A expression levels and (A) VAP, (B) VCL, and (C) ALH. $n = 3$. VAP, average path velocity ($\mu\text{m/s}$); VCL, curvilinear velocity ($\mu\text{m/s}$); ALH, mean amplitude of head lateral displacement (μm).

motion parameters, suggesting the protein's important role in motility-related fertility. Additionally, based on previous research (Bae et al., 2022b), Rab3A expression is correlated with medium sperm motility and mean dance (mean amplitude of head lateral displacement / linearity). Although prior studies on Rab3A primarily focused on acrosome formation and exocytosis, our results demonstrate its associated with various motion parameters. Thus, it is proposed that the Rab3A protein plays a crucial role in male fertility through its correlation with sperm kinematic characteristics. Overall, the data from this study can serve as foundational information for further exploration of the relationship between Rab proteins and sperm motility.

REFERENCES

Aghazarian A, Huf W, Pflüger H, Klätte T (2021) Standard semen parameters vs. sperm kinematics to predict sperm DNA damage. *World J Mens Health* 39:116-122.

Alory C, Balch WE (2001) Organization of the Rab-GDI/CHM superfamily: The functional basis for choroideremia disease. *Traffic* 2:532-543.

Bae JW, Hwang JM, Kwon WS (2022a) Prediction of male fertility using Ras-related proteins. *J Anim Sci Technol* 64:1024-1034.

Bae JW, Kim SH, Kim DH, Ha JJ, Yi JK, Hwang S, Ryu BY, Pang MG, Kwon WS (2019) Ras-related proteins (Rab) are key proteins related to male fertility following a unique activation

- mechanism. *Reprod Biol* 19:356-362.
- Bae JW, Yi JK, Jeong EJ, Lee WJ, Hwang JM, Kim DH, Ha JJ, Kwon WS (2022b) Ras-related proteins (Rab) play significant roles in sperm motility and capacitation status. *Reprod Biol* 22:100617.
- Bongso TA, Ng SC, Mok H, Lim MN, Teo HL, Wong PC, Ratnam SS (1989) Effect of sperm motility on human *in vitro* fertilization. *Arch Androl* 22:185-190.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248-254.
- Constantin G, Laudanna C (2010) Leukocyte chemotaxis: From lysosomes to motility. *Nat Immunol* 11:463-464.
- Donnelly ET, Lewis SEM, McNally JA, Thompson W (1998) *In vitro* fertilization and pregnancy rates: The influence of sperm motility and morphology on IVF outcome. *Fertil Steril* 70:305-314.
- Goody RS, Müller MP, Wu YW (2017) Mechanisms of action of Rab proteins, key regulators of intracellular vesicular transport. *Biol Chem* 398:565-575.
- Goud B, Liu S, Storrie B (2018) Rab proteins as major determinants of the Golgi complex structure. *Small GTPases* 9:66-75.
- Hinrichs K, Loux SC (2012) Hyperactivated sperm motility: Are equine sperm different? *J Equine Vet Sci* 32:441-444.
- Hutagalung AH, Novick PJ (2011) Role of Rab GTPases in membrane traffic and cell physiology. *Physiol Rev* 91:119-149.
- Iida H, Noda M, Kaneko T, Doiguchi M, Mōri T (2005) Identification of rab12 as a vesicle-associated small GTPase highly expressed in Sertoli cells of rat testis. *Mol Reprod Dev* 71:178-185.
- Jang SI, Jo JH, Jung EJ, Lee WJ, Hwang JM, Bae JW, Kwon WS (2022) Development of an optimal protocol to induce capacitation of boar spermatozoa *in vitro*. *J Anim Reprod Biotechnol* 37:285-291.
- Jung EJ, Lee WJ, Hwang JM, Bae JW, Kwon WS (2022) Reproductive toxicity of ritonavir in male: Insight into mouse sperm capacitation. *Reprod Toxicol* 114:1-6.
- Kumar S, Lee HJ, Park HS, Lee K (2016) Testis-specific GTPase (TSG): An oligomeric protein. *BMC Genomics* 17:792.
- Kwon WS, Rahman MS, Ryu DY, Park YJ, Pang MG (2015) Increased male fertility using fertility-related biomarkers. *Sci Rep* 5:15654.
- Lau ASN, Mruk DD (2003) Rab8B GTPase and junction dynamics in the testis. *Endocrinology* 144:1549-1563.
- Lin YH, Ke CC, Wang YY, Chen MF, Chen TM, Ku WC, Chiang HS, Yeh CH (2017) RAB10 interacts with the male germ cell-specific GTPase-activating protein during mammalian spermiogenesis. *Int J Mol Sci* 18:97.
- Liu S, Storrie B (2012) Are Rab proteins the link between Golgi organization and membrane trafficking? *Cell Mol Life Sci* 69:4093-4106.
- Moreno RD, Ramalho-Santos J, Sutovsky P, Chan EKL, Schatten G (2000) Vesicular traffic and Golgi apparatus dynamics during mammalian spermatogenesis: Implications for acrosome architecture. *Biol Reprod* 63:89-98.
- Nakamura Y, Asano A, Hosaka Y, Takeuchi T, Iwanaga T, Yamano Y (2015) Expression and intracellular localization of TBC1D9, a Rab GTPase-accelerating protein, in mouse testes. *Exp Anim* 64:415-424.
- Pereira-Leal JB, Seabra MC (2000) The mammalian Rab family of small GTPases: Definition of

- family and subfamily sequence motifs suggests a mechanism for functional specificity in the Ras superfamily. *J Mol Biol* 301:1077-1087.
- Ramalho-Santos J, Moreno RD, Wessel GM, Chan EKL, Schatten G (2001) Membrane trafficking machinery components associated with the mammalian acrosome during spermiogenesis. *Exp Cell Res* 267:45-60.
- Rodríguez-Gil JE, Silvers G, Flores E, Jesús Palomo M, Ramírez A, Montserrat Rivera M, Castro M, Brito M, Bücher D, Correa J, Concha II (2007) Expression of the GM-CSF receptor in ovine spermatozoa: GM-CSF effect on sperm viability and motility of sperm subpopulations after the freezing–thawing process. *Theriogenology* 67:1359-1370.
- Shan MM, Sun SC (2021) The multiple roles of RAB GTPases in female and male meiosis. *Hum Reprod Update* 27:1013-1029.
- Smith CE, Hermo L, Fazel A, Lalli MF, Bergeron JJM (1990) Ultrastructural distribution of NADPase within the Golgi apparatus and lysosomes of mammalian cells. *Prog Histochem Cytochem* 21:1-120.
- Stenmark H (2009) Rab GTPases as coordinators of vesicle traffic. *Nat Rev Mol Cell Biol* 10:513-525.
- Suarez SS, Dai X (1992) Hyperactivation enhances mouse sperm capacity for penetrating viscoelastic media. *Biol Reprod* 46:686-691.
- Vieira OV (2018) Rab3a and Rab10 are regulators of lysosome exocytosis and plasma membrane repair. *Small GTPases* 9:349-351.
- Wu YW, Oesterlin LK, Tan KT, Waldmann H, Alexandrov K, Goody RS (2010) Membrane targeting mechanism of Rab GTPases elucidated by semisynthetic protein probes. *Nat Chem Biol* 6:534-540.
- Yunes R, Michaut M, Tomes C, Mayorga LS (2000) Rab3A triggers the acrosome reaction in permeabilized human spermatozoa. *Biol Reprod* 62:1084-1089.