Indian Journal of Biochemistry & Biophysics
Vol. 59, June 2022, pp. 632-639

Immunocytochemical evidence of histamine 1 and histamine 2 receptors on mice sperm

Nor AzimahAhmad@Aziz[1], Noor Hashida Hashim[2]* & Yusmin Mohd Yusuf[2,3]

[1]Institute of Advanced Studies; [2]Centre for Foundation Studies in Science, & [3]Centre of Biotechnology and Agriculture Research, Universiti Malaya, Kuala Lumpur-50603, Malaysia

Received 29 November 2021; *revised 22 June 2022*

Histamine is a biogenic amine which is synthesised by L-histidine decarboxylase enzyme (HDC). The histamine 1 and 2 antagonist administrations have been highly reported to cause detrimental effect on sperm parameters, which arisen the speculation of histamine 1 (H1R) and histamine 2 (H2R) receptors might be present in sperm. The present study was aimed to provide evidence on the localisation of H1R and H2R on mice sperm through immunocytochemistry. The sperm was harvested from cauda epididymis. After one hour of incubation, sperm suspension was smeared onto a poly-lysine-coated slide and allowed to dry before fixation and permeabilisation processes. The primary antibody encoded for receptors was exposed to the fluorescently tagged antibody; fluorescein isothiocyanate (FITC) conjugate followed by nuclear staining with 4′, 6-diamino-2-phenylindole dihydrochloride (DAPI). The testis, stomach, and skin were used as the positive controls. Our data showed that both receptors have been expressed on the midpiece and acrosome of mice. The present result was the first discovery of the presence and immunolocalisation of H1R and H2R on mice sperm. Therefore, present study proposes that these receptors could be involved in calcium regulatory mechanism and protein phosphorylation which are responsible for fertilisation-related processes.

Keywords: Histamine receptors, Immunocytochemistry, Localisation, Sperm

Histamine is an important biogenic amine which mainly synthesised by L-histidine decarboxylase enzyme (HDC) through decarboxylation process in most mammalian tissues[1]. Mast cells, macrophage, basophils, platelets, and enterochromaffin-like cells (ECL) are the major contributing cells that produce, store, and release histamine content by secretory granules through degranulation process upon the trigger from external stimulus[2,3]. The histaminergic activity is mediated by histamine receptor subtypes namely H1R, H2R, H3R, and H4R. These receptors are specific to be incorporated with their corresponding G-proteins that transmit the extracellular signal to intracellular signal transduction cascades, to perform the bodily events[4]. The histamine 1 receptor (H1R) is ubiquitously expressed in the endothelium, smooth muscle cells, respiratory tract, cardiac tissues, and skin. This receptor is well characterised to exert contractile effects resulting an increase in vascular permeability as well as the contraction of non-vascular smooth muscle which is involved in inflammation and allergic response[5,6]. In contrast, the relaxation of smooth muscles, increase vascular permeability and bronchodilation are mediated by histamine 2 receptor (H2R). The regulation of gastric acid secretion in parietal cell of stomach is the best characterised response mediated by H2R for homeostatic control[7,8]. In modulating the immune system, H2R is complementing the pharmacological reaction of H1R by expressing themselves in immune cells like monocytes, macrophages, and lymphocytes. The histamine 3 receptor (H3R), histaminergic neurons which are found in restricted population of neurons mediates various functions of brain in the central nervous system, mainly to modulate parasympathetic and sympathetic nerves. This receptor is also important for sensory functions, such as pain and itch in the peripheral nervous tissues[9-12]. The H4R is best-known to involve in immunoregulatory system. Hence, its expression is relatively high in bone marrow and haematopoietic cells such as eosinophils and dendritic cells[13,14].

As one of important modulators in physiological processes, it is undeniable that histamine does exist in male gonad and plays pivotal roles in male reproductive system such as testosterone production,

*Correspondence:
E-mail: nhhpasum@um.edu.my

spermatogenesis, secondary male characteristics, and sexual behaviour[15]. The histaminergic activity in male was earlier demonstrated through the localisation of mast cells and the ability of histamine to stimulate testicular steroid production *in vitro* in the testis of golden hamster. The HDC enzyme expression was then found on mouse germ cell and its mRNA was localised predominantly in the cell inside the seminiferous tubules, adding in testicular histamine milieu despite the mast cells as the main source of histamine production. In fact, the same report has demonstrated that sperm could synthesise histamine *in vitro* upon the addition of calcium ionophore A23187. Intriguingly, the presence of H1R and H2R has not been investigated at the cellular level of sperm despite extensively reported of these receptors in various testicular cells like Leydig cells, testicular macrophages, and in distinct regions of testis. The H1R and H2R antagonist administrations have been extensively investigated in various species[15,16]. These histamine receptors antagonist caused detrimental effect on testicular morphometric and sperm parameters (sperm count, motility, viability, and morphology) which in the worst scenario led to male infertility[16]. These findings, involvement of histamine receptors in testicular cells and histamine receptors antagonist bring about a thought of how histamine antagonists could deteriorate the sperm parameters without histamine receptors on it. Therefore, the objective of present study is to identify the presence of histamine receptors in the cellular level on mice sperm for better understanding on histamine mechanism in sperm physiology and male reproductive system.

Materials and Methods

Male outbred ICR mice aged 9 to 12 weeks old were acclimatized for two weeks prior to experiment. The mice were maintained in a room temperature at 25-27°C with 12 h source of light and 12 h in dark per day. Pellet and drinking water were provided *ad libitum*. The sawdust bedding was changed once a week to ensure the hygienic condition. The experiment was approved by Universiti Malaya Animal Ethics Committee (Ethics number: FAR/3/03/2015/FSB (R)).

Sperm collection from cauda epididymis

Sperm were harvested from cauda epididymis and cut open in 500 µL Toyoda Yokohama Hosi (TYH) medium. The sperm suspension was incubated in CO_2 incubator (Thermo Scientific, USA) for an hour with 5.0% CO_2 at 37°C. Sperm suspension, 10 µL was smeared onto poly-lysine coated slide (Thermo Scientific, USA). Three smeared slides were prepared and one slide was assigned as a negative control. The sperm were allowed to dry and adhered on slide prior to fixation process.

Immunocytochemical study

The smeared sperm were first fixed in 2.0% of paraformaldehyde (Sigma Aldrich, USA) for 2 h. The sperm were washed with 1X phosphate buffered saline (PBS) (Oxoid, England) thrice prior to permeabilisation in 1% TritonX-100 (Sigma Aldrich, USA) for an hour. After a series of washing, the sperm smeared slide was incubated in 0.1 M PBS supplemented with 3 mg/mL bovine serum albumin (BSA) (Sigma Aldrich, USA) for an hour at room temperature, 27°C. The sperm were then incubated with specific primary antibody for overnight at 4°C. Primary antibody used was rabbit polyclonal antibody against H1R (1:100) and purified goat polyclonal antibody against H2R (1:100) (Santa Cruz Biotechnology, Inc). After three washes with PBS, sperm were incubated with secondary antibody, fluorescein isothiocyanate (FITC) conjugate goat anti-rabbit IgG and mouse anti-goat IgG (1:200) (Santa Cruz Biotechnology, Inc), for H1R and H2R, respectively for 2 h at 4°C in dark condition. These receptors detection was done for three replicates on each set of experiment. The primary antibody was omitted from sperm slide that assigned as negative control. After three washes with PBS, sperm were counter stained and mounted with 4′, 6-diamino-2-phenylindole dihydrochloride (DAPI) (Santa Cruz Biotechnology, Inc). The expression of histamine receptors was acquired by using fluorescence microscope (Nikon, USA) assisted with NIS – Elements Viewer 4.20 software under 40X magnification.

Immunohistochemical study

The testis, stomach and skin were harvested for positive control. Skin and stomach were positive controls for H1R and H2R, respectively while testis for both receptors. These positive control tissues were fixed in 10% formalin (Merck, USA) for 24 h prior to tissue processing and paraffin wax (Paraplast, USA) embedding. Each specimen was sectioned into 5 µM thick. Ribbon of sectioned sample was cut and adhered to poly-lysine coated slide (Thermo Scientific, USA)

for immunohistochemical study. All the positive control specimen was treated similar to the procedure as in immunocytochemical study. The expression was observed by using fluorescence microscope (Nikon, USA) assisted with NIS – Elements Viewer 4.20 software under 40X magnification.

Histological study

This study was conducted to identify and verify the morphology of specimen mainly at the region of expressed receptors. The consecutive ribbon of positive control specimen was adhered to normal glass slide for histological study. The specimen was first deparaffinised in two changes of xylene for 3 min each, followed by rehydration in a series of descending alcohol. After 3 min immersion in distilled water, the specimen was stained with hematoxylin solution for 20 min. The specimen was immersed in acid alcohol for a few seconds and placed under running tap water for 5 min prior to staining in eosin solution for 5 min. The specimen was then dehydrated in a series of ascending alcohol, 95% twice for 2 min each and 100% thrice for 3 min each prior to clearing with xylene. The DPX was dropped onto the specimen before mounting with cover slip and ready to be observed by using light microscope (Nikon, USA) assisted with NIS – Elements Viewer 4.20 software.

Results

Specific receptor was detected through the emission of green fluorescence of FITC dye for the expression of H1R (Fig. 1A) and H2R (Fig. 2A) on mice sperm. The counterstain agent, 4′, 6-diamino-2-phenylindole dihydrochloride (DAPI) emitted blue fluorescence that representing cell nuclei detection. The immunofluorescence microscopy showed that the expression of H1R and H2R were consistently expressed mainly on the midpiece and acrosome of sperm.

The H1R was detected on the sperm acrosome and midpiece as shown in (Fig. 1B). The expression was compared to negative control of H1R (Fig. 1C & 1D).

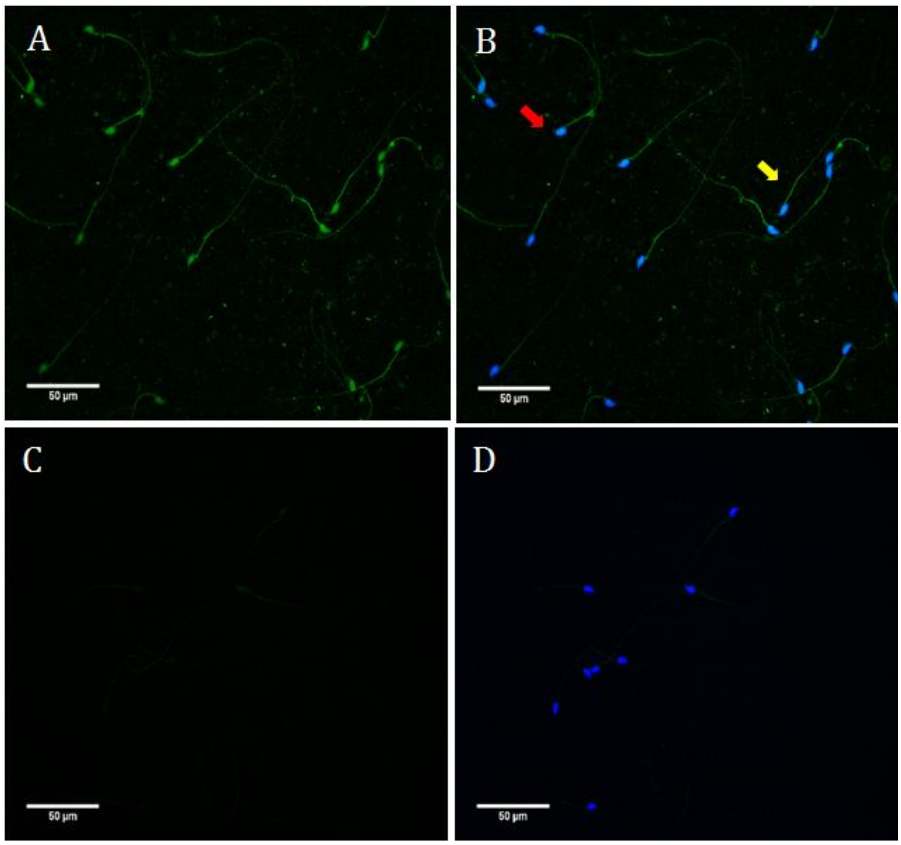

Fig. 1—Immunofluorescence localisation of H1R on mice epididymal sperm. (A) sperm stained with green fluorescence of FITC for H1R detection; (B) H1R was detected on the acrosome and midpiece; (C) negative control for H1R; and (D) negative control stained with DAPI. Green fluorescence indicated the expression of histamine receptors while blue emission was a counterstain of cell nuclei. The H1R was found on the acrosome (red arrow, stained in green) and midpiece (yellow arrow, stained in green) showed in Fig. 1B. Magnification: 40X (Scale bars at 50 µM)

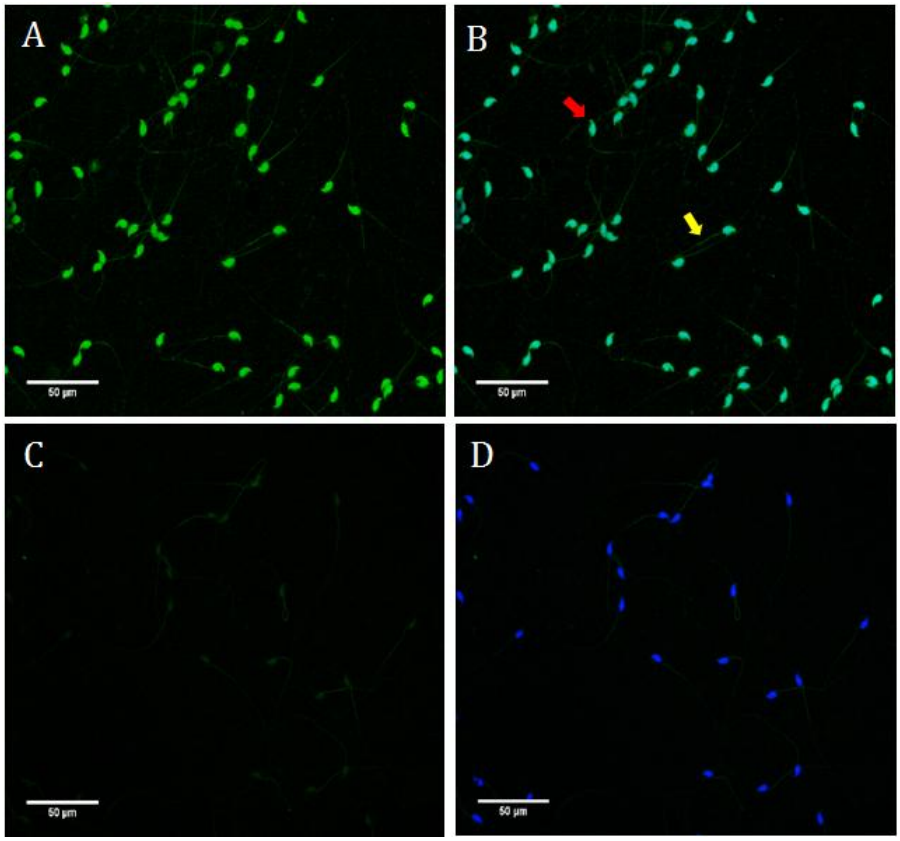

Fig. 2—Immunofluorescence localisation of H2R on mice epididymal sperm. (A) sperm stained with FITC for H2R detection; (B) H2R was detected on the acrosome and midpiece; (C) negative control; and (D) negative control stained with DAPI. The H2R was found on the acrosome (red arrow, stained in green) and midpiece (yellow arrow, stained in green) showed in Fig. 2B. Magnification: 40X (Scale bars at 50 µM)

The H2R expression was found on the similar locations as H1R, on the acrosome and the midpiece of sperm (Fig 2B) which comparable to its negative control (Fig. 2C & 2D).

The presence of H1R and H2R subtypes on mice sperm was comparable with the expression of positive control samples. Positive control was conducted using mice testis, skin, and stomach. The antibodies used for positive control samples were similar to that in immunocytochemical study.

Present result demonstrated the presence of H1R subtype on the Leydig cells of testis in (Fig. 3B). The expression of H2R subtype was also found on the same location of Leydig cells (Fig. 3D). The morphological feature of mice testis was identified through hematoxylin and eosin (H&E) staining method (Fig. 3A & 3C).

In the other positive controls, the H1R subtype was expressed on epidermis layer of skin (Fig. 4B) while the H2R subtype was well localised in parietal cell of stomach (Fig. 4D). The histology of skin (Fig. 4A) and stomach (Fig. 4C) were supplemented to study the tissue morphology.

Discussion

Extensive studies on the effect of sperm parameters upon histamine antagonist administration has strongly suggested that histamine receptor might be present on sperm cell. To the best of our knowledge, this is the first report on detection of histamine 1 (H1) and histamine 2 (H2) receptors presence on mice sperm through immunocytochemical study. Present study has discovered that H1R and H2R were exclusively present in the midpiece and acrosome of mice epididymal sperm through immunocytochemical study with immunofluorescence visualisation.

The localisation of these histamine receptors is largely correlated and supported by the mapping profile of guanine nucleotide-binding regulatory protein (G-protein) subunits on mature sperm considering histamine receptors are classified as G-protein coupled receptors (GPCRs)[4,17-19]. The extracellular stimuli require G-protein upon the receptor-ligand binding to convey the signals into intracellular. The signals are subsequently transduced by the activation signalling

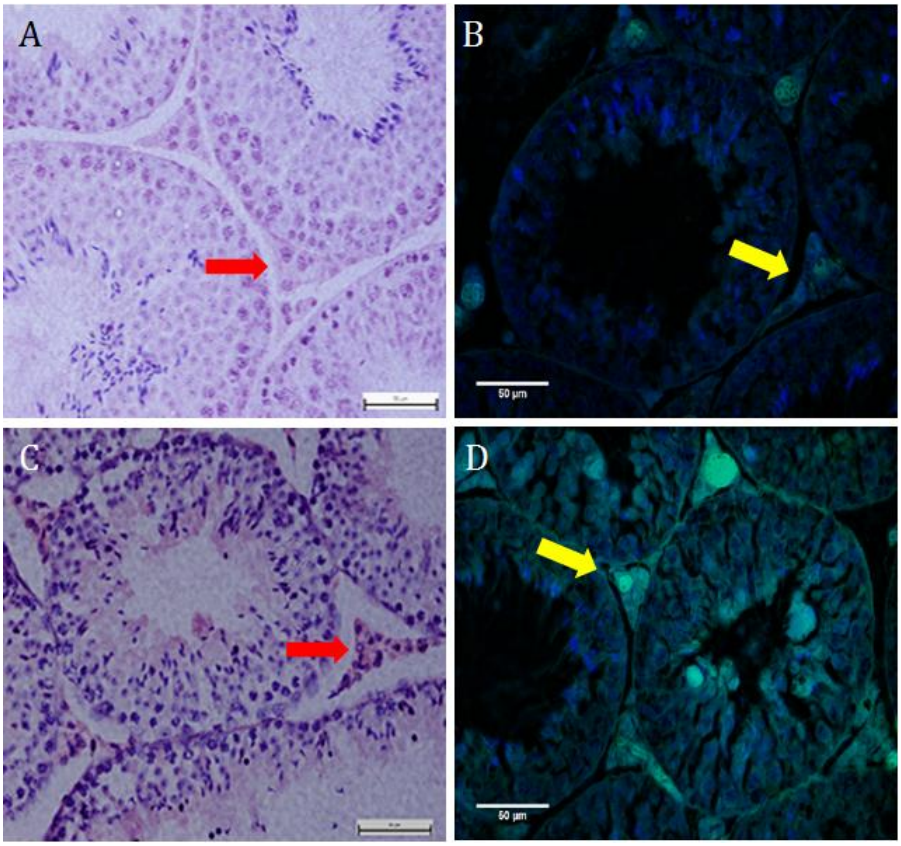

Fig. 3—Histological and immunofluorescence features of mice testis. (A and C) The histology of Leydig cells (red arrow); (B) Positive expression of H1R on Leydig cells (yellow arrow, stained in green); and (D) H2R was observed on Leydig cells (yellow arrow, stained in green). Magnification: 40X (Scale bars at 50 µM)

pathways to exert biological reactions[20]. Immunoblotting of membrane sperm and consecutive indirect immunofluorescence have been conducted to localise distinct subcellular G-protein subunits on sperm[21]. As a result, the finding has revealed that H1R corresponding G-protein, $G\alpha_{q/11}$ was detected in the acrosome, with highly located in the equatorial segment. However, this G-protein was not observed in the midpiece of sperm through this experimental approach until Baxendale and Fraser[22] have confirmed that $G\alpha_{q/11}$-protein subunit has been remarkably observed in the midpiece as well as the equatorial segment of mice acrosome. Therefore, the detection of H1R on the midpiece and acrosome from present study is highly relevant as $G\alpha_{q/11}$-protein subunit is present in the similar locations. The discovery of $G\alpha_s$ protein subunit on the midpiece and acrosome for both human and mice models has supported the localisation of H1R and H2R in these regions[22,23].

Given that, H1R signalling through $G\alpha_{q/11}$-protein stimulates the activation of phospholipase C (PLC) pathway which hydrolysed phosphatidylinositol-4, 5-biphosphate (PIP2) into diacyl glycerol (DAG) and inositol triphosphate (IP_3)[20]. The IP_3 binds to IP_3 receptor and releases the intracellular calcium into cytoplasm to be utilised. The DAG can further potentiate protein kinase C (PKC) to activate protein phosphorylation. An elevation of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) through H2R-$G\alpha_s$ activation also caused calcium release which can lead to acrosome reaction. On this basis, the H1R and H2R on the midpiece and acrosome would be suggested to facilitate the calcium mobilisation. This is in parallel as both locations are well-known to play role as calcium storage. In addition, the cAMP-dependant PKA pathway is acknowledged to play role in sperm motility regulation in concert with extracellular calcium. Meanwhile, the activation of PKA is being a crucial occurrence in protein phosphorylation. The involvement of protein phosphorylation in the motility regulation is in accordance with the increase in phosphotyrosine content of fibrous sheath proteins. On the contrary, the reduction of cAMP levels can cause poor sperm motility[24].

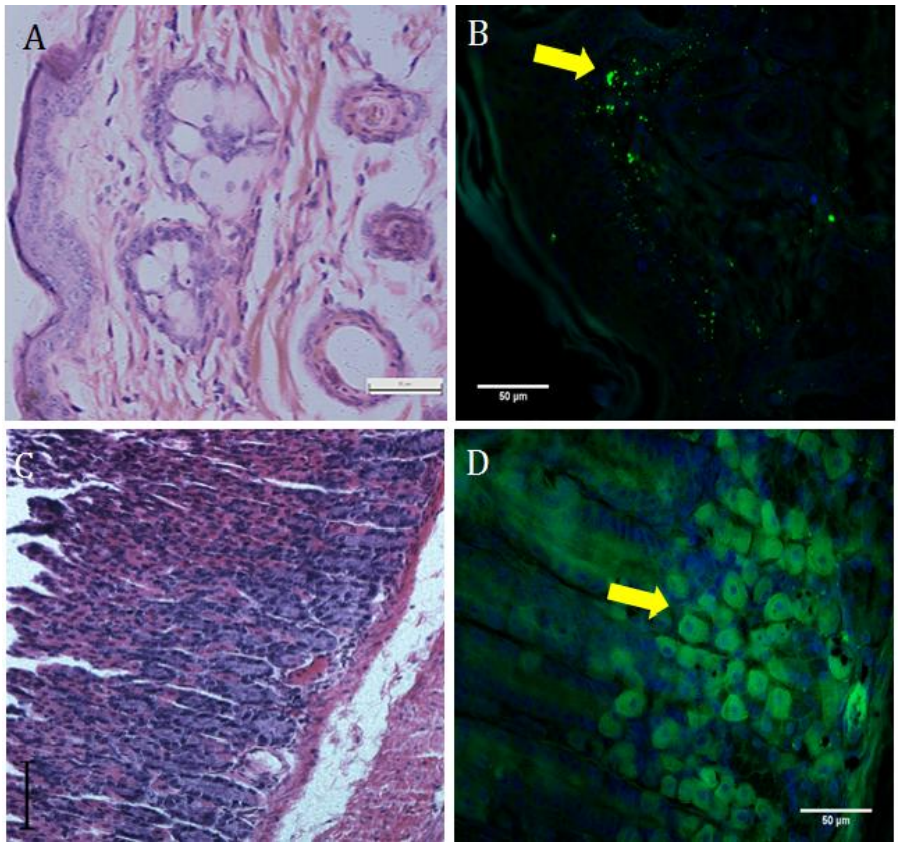

Fig. 4—Immunofluorescence visualisation on positive control samples. (A) Histological features of skin; (B) H1R was expressed in epidermis layer (yellow arrow, stained in green); (C) Histology of mice stomach; and (D) H2R was found in parietal cell of stomach (yellow arrow, stained in green). The blue stain represented cell nuclei. Magnification: 40X (Scale bars at 50 µM)

The calcium regulatory mechanism is an absolute requirement for sperm to regulate almost all sperm activities. Furthermore, there are many calcium channels in germ cells of spermatogonia and spermatozoa to exhibit the regulation of calcium signalling. Thus, the factor or activation that affect this intracellular messenger, would influence the sperm physiology and functions.

In accordance with previous reports on H1R and H2R antagonist administrations, the impaired sperm parameters are caused by an evoke of intracellular calcium in sperm[25,26]. The modulation of intracellular calcium by histamine is presumably mediated by H1R on the midpiece and acrosome *via* PLC signalling pathway. The inclined of intracellular calcium in sperm exhibits spermicidal activity indicated by complete loss of sperm viability, which in turn affects sperm count. Declined intracellular calcium is also associated with defective sperm motility[27,28]. Therefore, this indicates that calcium homeostasis regulation is required to ensure the competency of sperm is maintained prior to fertilisation and also to prevent male infertility.

With the discovery of both histamine receptors, H1R and H2R in the midpiece and acrosome would suggest that these receptors take part in modulation of calcium homeostasis in sperm which has significant contribution to sequential events of fertilisation-associated processes. The H1R and H2R presence on the midpiece may play role in sperm motility and capacitation. Calcium regulation in the midpiece regulates mitochondrial enzymatic activity to generate energy supply through adenosine triphosphate (ATP) supplementation to drive the flagella for sperm motility and hyperactivation. These types of sperm movement would lead to capacitation[29-31] which requires remarkable calcium for sperm to achieve fertilising ability. Both receptors, H1R and H2R on acrosome could possibly facilitate calcium regulation during acrosome reaction. The calcium influx can initiate acrosome reaction through calcium channel on the sperm plasma membrane[32]. Over past decades, the histamine release has been characterised upon *in vitro* acrosome reaction induced by calcium ionophore, A23187[33]. The protein phosphorylation that activated

by PKC and PKA from H1R and H2R signalling, respectively also carries out vital roles in capacitation and acrosome reaction[34].

In the light of this finding, it is reasonable to speculate that sperm has exhibited sperm-specific histaminergic pathway as individual cell. The H1R and H2R presence are consistently found in testicular tissues like various region of testis, Leydig cells, and testicular macrophages[15]. Taken together with H1R and H2R immunocytochemical detection on the midpiece and acrosome is the immunohistochemical evidence of Leydig cells upon the same receptors. As a result, these receptors are obviously found in the Leydig cells, making the present study is the first reported on the localisation of these receptors in the Leydig cells to support the molecular evidence from previous study[34]. This slight additional information is remarkable to emphasise these receptors contribution on testicular steroidogenesis. The previous study demonstrated that prolonged histamine deficiency in *HDC* gene knockout mice influenced the Leydig cell function. The absence of functional *HDC* gene showed the inability of Leydig cells to produce endogenous histamine. No basal and human chorionic gonadotropin-induced testosterone production response were identified in HDC-KO mice compared to the same parameters in wild type (WT) mice which endowed with *HDC* gene[35]. The expression of H1R and H2R genes in both groups have been evaluated after determining the basal and hCG treatments. As results, the stimulatory response of histamine concentrations is mediated by H2R. The augmentation of cAMP production through the coupling of H2R to adenylate cyclase system in Leydig cells which leads to stimulatory effect on the synthesis of steroid. This pathway enhances the increase of cAMP concentration and will direct cAMP sensitive calcium channel to be activated causing slightly higher calcium concentration. The H1R through $G\alpha_q$ protein activation results in IP_3 production which is significant in modulating steroid production in Leydig cells by either directly or by regulating calcium release to activate some calcium or calmodulin-dependent protein. The H1R stimulation will increase intracellular calcium directly proportional to cyclic adenylyl monophosphate (cAMP) level[15]. The H1R and H2R signalling within sperm may complement the same pattern as it is in the tissue level, Leydig cells.

The purpose of pursuing the detection of histamine receptors presence, particularly H1R and H2R in murine sperm is to fill this important gap of the histaminergic mechanism at the cellular level, hence providing an additional information in sperm physiology and eventually in fertilisation. However, further investigation is required to quantitate the expression of histamine receptors on sperm which would give substantial information to this research frame.

Conclusion

This present study demonstrated the localisation of H1R and H2R were found on the midpiece and acrosome of mice sperm. The specific role of these receptors has not fully studied by far. Hence, further studies are required to elucidate the mechanism of action *via* receptors on various sperm function. The possible roles suggested have been drawn in regards with G-protein signalling pathways. Therefore, this finding could be a starting point to initiate extensive studies regarding the physiological effect of these receptors on sperm function.

Acknowledgement

High appreciation is given to lecturers and all staffs of Anatomy Department, Faculty of Medicine, Universiti Malaya for tremendous advice in histological works and immunofluorescence studies. This work was funded by University Research Grant (RG310-14AFR) Universiti Malaya.

Conflict of interest

All authors declare no conflict of interest.

References

1 Huang H, Li Y, Liang J & Finkelman FD, Molecular regulation of histamine synthesis. *Front Immunol*, 9 (2018) 1.
2 Fultz R, Engevik MA, Shi Z, Hall A, Herrmann B, Ganesh BP, Major A, Haag A, Mori-Akiyama Y & Versalovic J, Phagocytosis by macrophages depends on histamine H2 receptor signaling and scavenger receptor 1. *Microbiologyopen*, 8 (2019) 1.
3 Tiligada E& Ennis M. Histamine pharmacology: from Sir Henry Dale to the 21st century. *Br J Pharmacol*, 177 (2020) 469.
4 Seibel-Elhert U, Plank N, Inoue A, Bernhardt G & Strasser A, Label-free investigations on the G Protein dependent signaling pathways of histamine receptors. *Int J Mol Sci*, 22 (2021) 1.
5 Hirasawa N, Expression of histidine decarboxylase and its roles in inflammation. *Int J Mol Sci*, 20 (2019) 1.
6 Moriguchi T & Takai J, Histamine and histidine decarboxylase: Immunomodulatory functions and regulatory mechanisms. *Genes Cells*, 20 (2020) 443.
7 Engevik AC, Kaji I & Goldenring JR, The physiology of the gastric parietal cell. *Physiol Rev*, 100 (2020) 573.
8 Waldum H & Mjønes P, Towards understanding of gastric cancer based on physiological role of gastrin and ECL cells. *Cancers (Basel)*, 12 (2020) 3477.

9 PanulaP, Histamine, histamine H3 receptor, and alcohol use disorder. *Br J Pharm*, 177 (2020) 634.

10 ObaraI, Telezhkin V, Alrashdi I & Chazot PL, Histamine, histamine receptors, and neuropathic pain relief. *Br J Pharmacol*, 177 (2020) 580.

11 Fukasawa T, Yoshizaki-Ogawa A, Enomoto A, Miyagawa, Sato S & Yoshizaki A, Pharmacotherapy of itch- Antihistamines and histamine receptors as G protein-coupled receptors. *Int J Mol Sci*, 23 (2022) 6579.

12 Agrawal A, Kulkarni GT & Lakshmayya, Molecular docking study to elucidate the anti-pruritic mechanism of selected natural ligands by desensitizing TRPV3 ion channel in Psoriasis: An *in silico* approach. *Indian J Biochem Biophys*, 57 (2020) 578.

13 Mehta P, Miszta P, Rzodkiewicz P, Michalak O, Krzeczyński P & Filipek S, Enigmatic histamine receptor H4 for potential treatment of multiple inflammatory, autoimmune, and related diseases. *Life*, 10 (2020) 1.

14 Schirmer B & Neumann D, The function of the histamine H4 receptor in inflammatory and inflammation-associated diseases of the gut. *Int J Mol Sci*, 22 (2021) 6116.

15 Mondillo C, Varela ML, Abiuso AMB & Várquez R, Potential negative effects of anti-histamines on male reproductive function. *Reproduction*, 155 (2018) 211.

16 Ahmed EA & Abdel-Emam RA, The potential impact of 1st and 2nd generation antihistamines on male fertility. *Comp Clin Path*, 28 (2019) 1.

17 Conrad M, Söldner CA, Miao Y & Sticht H, Agonist binding and G protein coupling in histamine H2 receptor: A molecular dynamics study. *Int J Mol Sci*, 21 (2020) 6693.

18 Herrera-Zúñiga LD, Moreno-Vargas LM, Ballaud L, Correa-Basurto J, Prada-Gracia D, Pastré D, Curmi PA, Arrang JM & Maroun RC, Molecular dynamics of the histamine H3 membrane receptor reveals different mechanisms of GPCR signal transduction. *Sci Rep*, 10 (2020) 16889.

19 Xia R, Wang N, Xu Z, Lu Y, Song J, Zhang A, Guo C & He Y, Cryo-EM structure of the human histamine H1 receptor/G_q complex. *Nat Commun*, 12 (2021) 1.

20 Panula P, Chazot PL, Cowart M, Gutzmer R, Leurs R, Liu WLS, Stark H, Thurmond RL & Haas HL, International Union of Basic and Clinical Pharmacology. XCVIII. Histamine Receptors. *Pharmacol Rev*, 67 (2015) 601.

21 Merlet FS, Weinstein LK, Goldsmith P, Rarick TL, Hall J, Bisson JP & de Mazancourt P, Identification and localization of G protein subunits in human spermatozoa. *Mol Hum Reprod*, 5 (1999)38.

22 Baxendale RW&Fraser LR, Immunolocalization of multiple Gα subunits in mammalian spermatozoa and additional evidence for $G\alpha_s$. *Mol Reprod Dev*, 65 (2003) 104.

23 Fraser LR, Adeoya-Osiguwa S & Baxendale RW, First messenger regulation of capacitation *via* G protein-coupled mechanisms: a tale of serendipity and discovery. *Mol Hum Reprod*, 9 (2003) 739.

24 PereiraR, Sá R, Barros A & Sousa M, Major regulatory mechanisms involved in sperm motility. *Asian J Androl*, 19 (2017) 5.

25 Gupta A, Gupta S & Tiwary AK, Spermicidal efficacy of H_2-receptor antagonists and potentiation with 2', 4'-dichlorobenzamill hydrochloride: role of intrasperm Ca^{2+}. *Contraception*, 68 (2003) 64.

26 Gupta A, Khosla R, Gupta S & Tiwary AK, Influence of histamine and H_1-receptor antagonists on ejaculated human spermatozoa: role of intrasperm Ca^{2+}. *Indian J Exp Biol*, 42 (2004) 481.

27 Bejarano I, Espino J, Paredes SD, Ortiz A, Lozano G, Pariente JA & Rodríguez AB, Apoptosis, ROS and calcium signaling in human spermatozoa: relationship to infertility. *Male Infertility*, 20 (2012) 51.

28 Schmid TE, Grant PG, Marchetti F, Weldon RH, Eskenazi BM & Wyribek AJ, Elemental composition of human semen is associated with motility and genomic sperm defects among older men. *Hum Reprod*, 28 (2013) 274.

29 Alasmari W, Barratt CLR, Publicover SJ, Whalley KM, Foster E, Kay V, da Silva SM & Oxenham SK, The clinical significance of calcium-signalling pathways mediating human sperm hyperactivation. *Hum Reprod*, 28 (2013) 866.

30 Yoshida M & Yoshida K, Sperm chemotaxis and regulation of flagellar movement by Ca^{2+}. *Mol Hum Reprod*, 17 (2011) 457.

31 PiomboniP, Focarelli R, Stendardi A, Ferramosca A & Zara V, The role of mitochondria in energy production for sperm motility. *Int J Androl*, 35 (2012) 109.

32 Rahman MS, Kwon WS & Pang MG, Calcium influx and male infertility in the context of the sperm proteome: An update. *BioMed Res Int*, 2014 (2014) 1.

33 Safina F, Tanaka S, Inagaki M, Tsuboi K, Sugimoto Y & Ichikawa A, Expression of L-Histidine decarboxylase in mouse male germ cells. *J Biol Chem*, 277 (2002) 14211.

34 BarbonettiA, Vasallo MRC, Cordeschi G, Venetis D, Caboni A, Speranndio A & Francavilla F, Protein tyrosine phosphorylation of the human sperm head during capacitation: immunolocalization and relationship with acquisition of sperm-fertilizing ability. *Asian J Androl*, 12 (2010) 85.

35 Mondillo C, Falus A, Pignataro O & Pap E, Prolonged histamine deficiency in histidine decarboxylase gene knockout mice affects Leydig cell function. *J Androl*, 28 (2007) 86.