Int. J. Morphol.,
42(3):805-813, 2024.

Immunohistochemical Localization of Glucose Transporter GLUT1 and GLUT3 in the Testes of some Mammals

Localización Inmunohistoquímica del Transportador de Glucosa GLUT1 y GLUT3 en los Testículos de Algunos Mamíferos

Bassam A. Alahmadi[1]; Amin A. Seleem[1,2]; Fakhr El-Din M. Lashein[2]; Amira H. Badr[2] & Abeer A. Ahmed[2]

ALAHMADI, B. A.; SELEEM, A. A.; LASHEIN, F. E. M.; BADR, A. H. & AHMED, A. A. Immunohistochemical localization of glucose transporter GLUT1 and GLUT3 in the testes of some mammals. *Int. J. Morphol., 42(3)*:805-813, 2024.

SUMMARY: Glucose has an essential role in the proliferation and survival of testicular tissue. Glucose transporters (GLUTs) are responsible for glucose uptake across cell membranes. In the present work, two main isoforms GLUT1 and GLUT3 were investigated in the testes of Laboratory mouse (BALB/c), Lesser Egyptian jerboa (*Jaculus jaculus*), Golden hamster (*Mesocricetus auratus*), and Desert Hedgehog (*Paraechinus aethiopicus*). Immunofluorescent localization of GLUT1 and GLUT3 showed considerable species differences. The lowest expression of GLUT1 and GLUT3 was localized in the testis of Laboratory mouse (BALB/c), the highest GLUT1 localization was detected in the testis of Lesser Egyptian jerboa *(Jaculus jaculus*), and the highest GLUT3 immunofluorescent localization was observed in the testis of Hedgehog (*Paraechinus aethiopicus*). The results imply that GLUT3 is the principal glucose transporter in the studied testes, which is related to species differences. The different immunolocalization of GLUT in examined testes suggests using various transport systems for energy gain in different species.

KEY WORDS: GLUT1; GLUT3; Glucose transporter; Hedgehog testis; Jerboa testis; Hamster testis.

INTRODUCTION

The majority of cells require glucose which serves as the fundamental fuel molecule, an essential metabolic substrate for energy demand, and vital for the proliferation and survival of mammalian cells including testicular cells (Alves *et al*., 2013). Glucose plays a supply of energy for testicular development and a crucial part in the continuous development and quality of sperm cells (Williams & Ford, 2001; Miki, 2007). Glucose is a hydrophilic molecule and cannot pass through the plasma membrane, thus GLUTs or glucose facilitative transporter proteins are responsible for moving glucose across cell membranes in a particular way of various tissues (Gorovits & Charron, 2003). Glucose is taken through GLUT and converted into intermediate metabolite (Alves *et al*., 2013). GLUTs contain thirteen members (GLUT1 to GLUT12 and HMIT), which display various kinetic characteristics and substrate specificities (Joost & Thorens, 2001). Nualart *et al*. (2009) noted the classification of GLUTs into three groups based on their dependence on cellular energy. Class I contains GLUT-1 and GLUT-4. Fructose transporters including GLUT-5, 7, 9, and 11 are known as Class II. GLUT-6, 8, 10, 12, and the proton (H+)-driven myo-inositol transporter (HMIT) are found in class III (Joost & Thorens, 2001). The highest rates of glucose uptake are found in the testes, where particular glucose transporters regulate continuous spermatogenesis (Alves *et al*., 2014). Various expressions in GLUTs have been documented in the testes of different animals (Galardo *et al*., 2008; Oliveira *et al*., 2012). GLUT expression varies between species and is species-dependent i.e., rat testes have been shown to express GLUT1, GLUT2 was localized in the testes of mice and rats, and GLUT3 in the testes of mice, rats, and humans (Kokk *et al*., 2004; Hahn *et al*., 2017). GLUT8 is expressed in spermatids and spermatozoa and is involved in capacitation and fertilization processes (Banerjee *et al*., 2014). GLUT3 is considered a key mediator, prevalent in the testis, and reported in the cells of the seminiferous tubules, thus it is essential for successful fertilization (Burant & Davidson, 1994; Kishimoto *et al*. 2015; Sarkar & Singh, 2017). The sperm tails express GLUT3 in large amounts to utilize glucose and/or fructose, whereas sperm need a lot of ATP, which is mostly provided by glucose in the female reproductive tract

[1] Biology Department, Faculty of Science, Taibah University, Saudia Arabia.
[2] Zoology Department, Faculty of Science, Sohag University, Egypt.

Received: 2024-01-03 Accepted: 2024-02-25

(Simpson *et al*., 2008). Kishimoto *et al*. (2015) reported abundant expression of GLUT3 in the spermatocytes and spermatids. To the best of our knowledge, there is no published work about the comparative expression of GLUT 1 and 3 in different species of mammals. Thus, the current study examines the cellular expression of GLUT-1 and GLUT-3 in the testis of Laboratory mouse (Albino) (BALB/c), Lesser Egyptian jerboa (*Jaculus jaculus*), Golden hamster (*Mesocricetus auratus*), and Desert Hedgehog (*Paraechinus aethiopicus*).

MATERIAL AND METHOD

Animals: Laboratory mouse (Albino) (BALB/c) was obtained from the Pharmacy Faculty, Taibah University, Al-Madinah Almunawwarah, KSA. Golden hamster (*Mesocricetus auratus*) was obtained from a local market shop in AlUla Government. Lesser Egyptian jerboa (*Jaculus jaculus*) and Desert Hedgehog (*Paraechinus aethiopicus*) were trapped from AlUla Government, KSA from 2015 to 2017 (Seleem, 2020). Animals were kept in standard lab conditions and pelleted food and water were available at all times. All experiments were done and approved according to the guidelines Animal Experiment Ethical Committee of Sohag University (Approval number: CSRE-22-23). The studied animals were anesthetized with ketamine/xylazine (Sotoudeh & Namavar, 2022) and then euthanized by decapitation and saved in 10 % formalin for future studies.

Histology and Immunohistochemistry: The testes of Laboratory mouse (BALB/c), Lesser Egyptian jerboa (*Jaculus jaculus*), Golden hamster (*Mesocricetus auratus*), and Desert Hedgehog (*Paraechinus aethiopicus*) were dissected out, cleaned, and immersed in fixative (10 % formal alcohol) for 4 h. Dehydration process was done by graded concentrations of ethanol. The clearing process was done by xylene and toluene, then infiltration in paraffin at a 60 ºC oven. Tissues embedded in paraffin were cut to a thickness of 5 µm using a Leica Biosystems, Shanghai, China, RM 2125RTS microtome. Harris hematoxylin and eosin and periodic acid Schiff reaction (PAS) were used to stain the sections (Survarna *et al*., 2013). Immunofluorescence staining of GLUT1 and GLUT3 was done according to Sarkar & Singh (2017). Sections on positive slides were rinsed in xylene for deparaffinization and in descending series of ethyl alcohol to water for rehydration. Citrate buffer (pH=6) was applied to the sections for an hour at 100 °C to retrieve the antigen. To remove endogenous peroxidase activity, the slides were rinsed in 3 % hydrogen peroxide in methanol for 10 min at room temperature. 5 % bovine serum albumin (BSA) was applied to the sections for two hours to prevent non-specific antibody binding. Primary antibody diluted in blocking solution was incubated on sections overnight (Rb Anti-GLUT-1 PAb, dilution, 1:100, E2840, Spring Bioscience, USA) (Rb Anti-GLUT-3 PAb, dilution, 1:100, E3270, Spring Bioscience, USA). After each step, phosphate buffer (pH=7.4) was used for washing. Secondary antibody FITC-conjugated, goat anti-rabbit IgG (Sigma Aldrich, USA) (1:1000) was applied to the sections for 2 h in the dark at room temperature. Afterward, sections were coverslipped with vectashield mounting media (Sigma). The slides were photographed by a camera (AxioCam ERc5s) under a fluorescent microscope (Axio Scope A1, Zeiss, Germany). Negative control sections were prepared to evaluate the specificity of the immunoreactivity, where the primary antibody was omitted from the procedures. The quantifying immunofluorescent color of GLUT 1 and GLUT 3 was calculated by Fiji (https:// fiji.sc/).

RESULTS

The hematoxylin and eosin stain showed a normal appearance of seminiferous tubule cells in the studied testes, which consists of spermatogonia, spermatocytes, spermatids, and spermatozoa in Laboratory mouse (BALB/c) (Fig. 1A), Lesser Egyptian jerboa (*Jaculus jaculus*) (Fig. 1B), Golden hamster (*Mesocricetus auratus*) (Fig. 1C), and Desert Hedgehog (*Paraechinus aethiopicus*) (Fig. 1D). PAS staining showed a negative occurrence of carbohydrates in the testis structure of Laboratory mouse (BALB/c) (Fig. 2A). PAS reaction was positive in the tails of sperms in the testis of Lesser Egyptian jerboa (*Jaculus jaculus*) (Fig. 2B), heads of sperms in the testis of Golden hamster (*Mesocricetus auratus*) (Fig. 2C), and dark PAS staining in the tails and heads of desert Hedgehog (*Paraechinus aethiopicus*) (Fig. 2D).

Varying in the intensity of GLUT 1 and GLUT 3 immunofluorescent localization was observed mainly in the testes of studied animals (Figs. 3 and 4; Table I and II). Immunolocalization of GLUT 1 in the testis of Laboratory mouse (BALB/c) (A) was absent in all cells of seminiferous tubules (Fig. 3A; Table I). Expression of GLUT 1 in the testis of Lesser Egyptian jerboa (*Jaculus jaculus*) was faint in spermatogonia and spermatocytes and moderate in spermatids, but it was not found in the spermatozoa (Fig. 3B; Table I). Immunofluorescence of GLUT 1 in the testis of the Golden hamster (*Mesocricetus auratus*) was moderate in spermatogonia, spermatocytes, spermatids, and spermatozoa (Fig. 3C; Table I). Weak immunofluorescence of GLUT 1 was detected in spermatogonia, spermatocytes, and spermatozoa but the absence of signals were noted in spermatids in the testis of Desert Hedgehog (*Paraechinus aethiopicus*) (Fig. 3D; Table I).

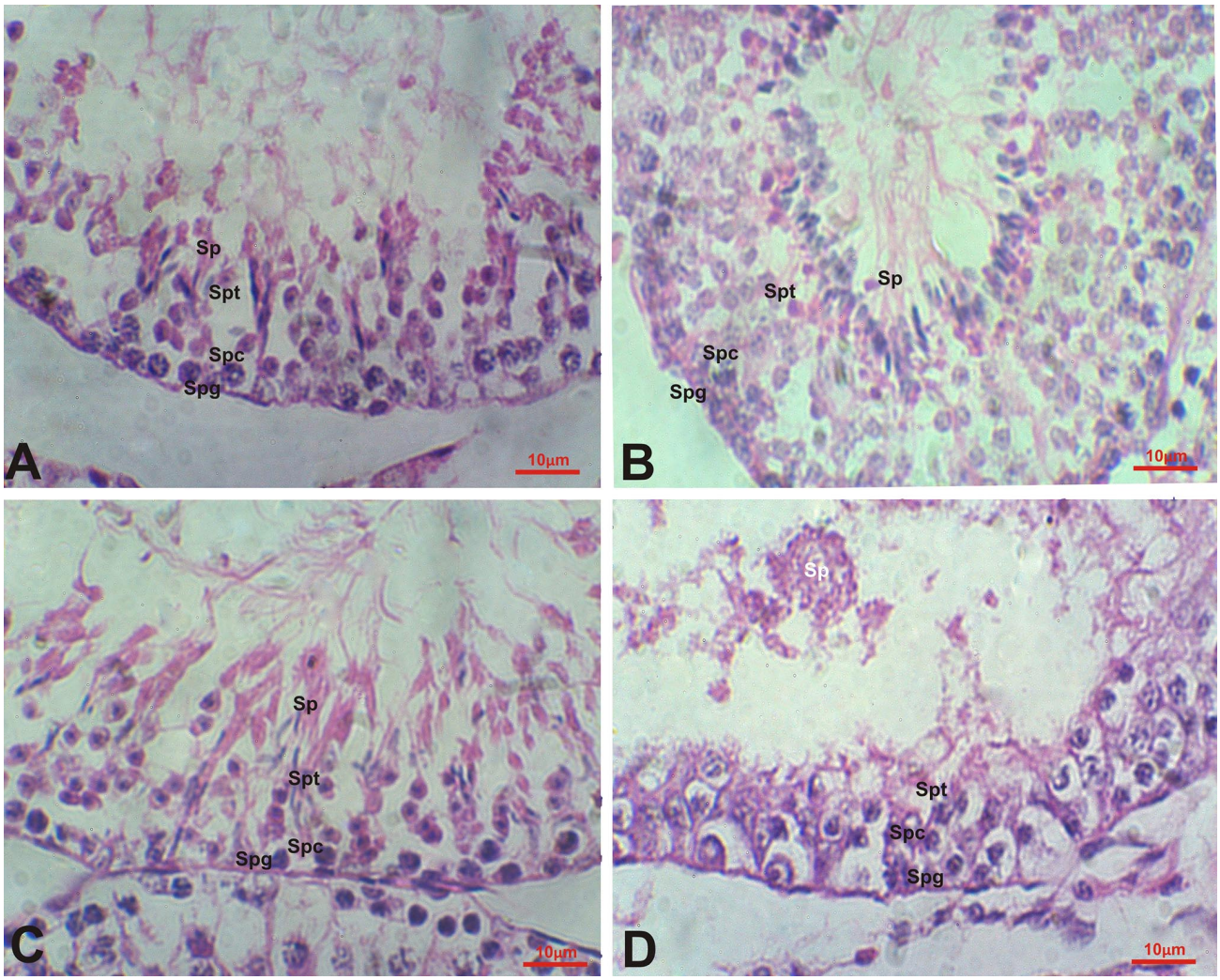

Fig. 1. Light photomicrograph of a transverse section of H&E through the testes of Laboratory mouse (BALB/c) (A), Lesser Egyptian jerboa (*Jaculus jaculus*) (B), Golden hamster (*Mesocricetus auratus*) (C), and Desert Hedgehog (*Paraechinus aethiopicus*) (D). Scale bar in A, B, C, and D is 10 µm.

GLUT3 immunofluorescent in the testis of Laboratory mouse (BALB/c) showed weak expression in the spermatozoa with a complete absence of signals in spermatogonia, spermatocytes, and spermatids (Fig. 4A; Table II). Faint GLUT 3 expression was detected in the round spermatids of the testis of Lesser Egyptian jerboa (*Jaculus jaculus*) and a complete absence of signals in the spermatogonia, spermatocytes, and spermatozoa (Fig. 4B; Table II). The testis of the Golden hamster (*Mesocricetus auratus*) exhibited weak signals of GLUT 3 in spermatozoa and moderate signals in spermatogonia, spermatocytes, and spermatids (Fig. 4C; Table II). The

Table I. GLUT 1 immunofluorescent localization in the testes of studied animals.

Species	Testis cells			
	spermatogonia	spermatocytes	spermatides	spermatozoa
Laboratory mouse (BALB/c)	(-)	(-)	(-)	(-)
Lesser Egyptian jerboa (*Jaculus jaculus*)	(+)	(+)	(++)	(-)
Golden hamster (*Mesocricetus auratus*)	(++)	(++)	(++)	(++)
Desert Hedgehog (*Paraechinus aethiopicus*)	(+)	(+)	(-)	(+)

Strong expression (+++); moderate expression (++); faint expression (+); no expression (-)

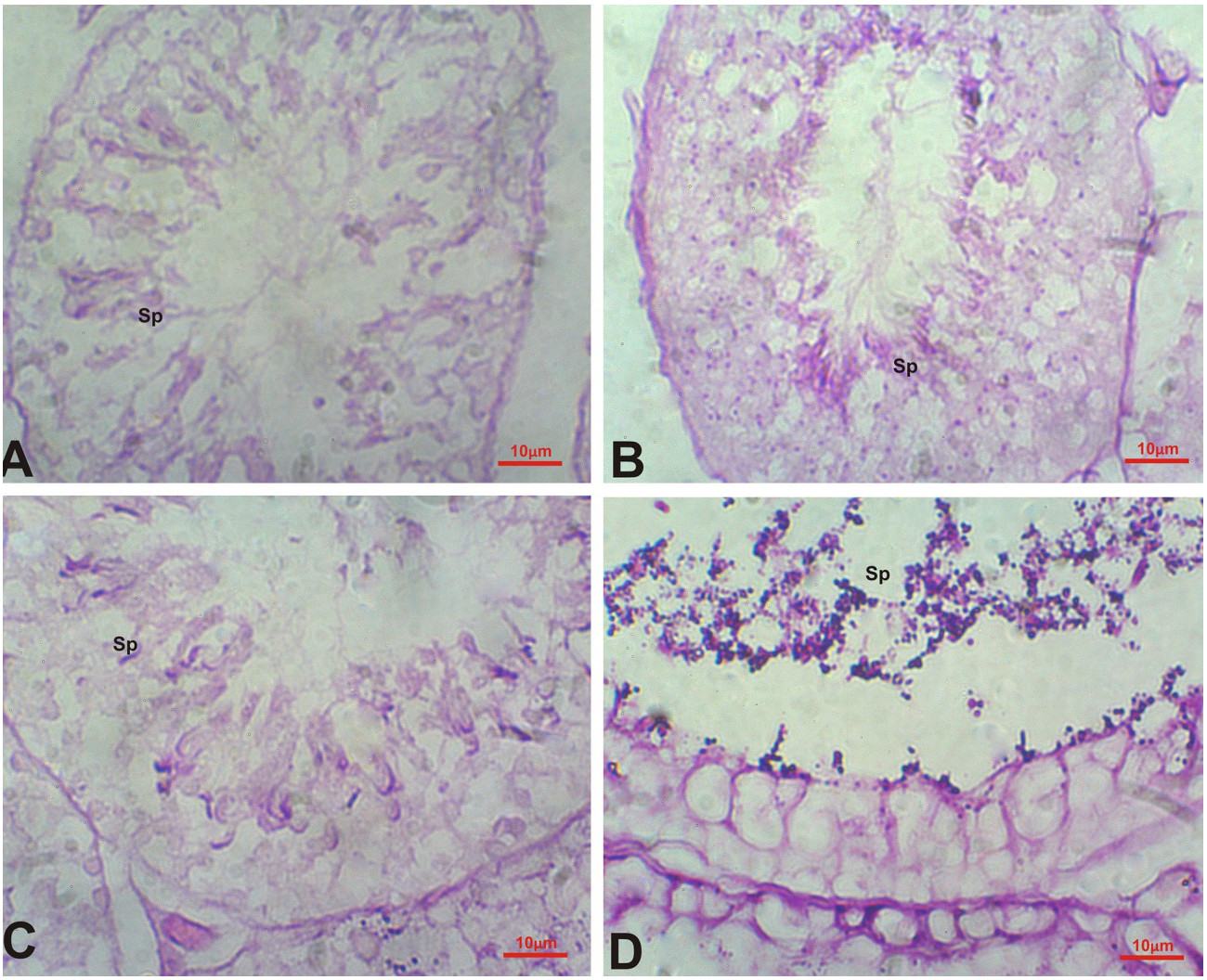

Fig. 2. Light photomicrograph of a transverse section of PAS staining through the testes of Laboratory mouse (BALB/c) (A), Lesser Egyptian jerboa (*Jaculus jaculus*) (B), Golden hamster (*Mesocricetus auratus*) (C), and desert Hedgehog (*Paraechinus aethiopicus*) (D). Scale bar in A, B, C, and D is 10 µm.

testis of Desert Hedgehog (*Paraechinus aethiopicus*) showed strong immunofluorescent localization of GLUT3 in spermatozoa and moderate expression in spermatogonia, spermatocytes, and spermatids (Fig. 4D; Table II). The study reported that the highest GLUT 1 expression was in the testis of Lesser Egyptian jerboa (*Jaculus jaculus*) and the lowest GLUT 1 expression was in the Laboratory mouse (BALB/c). The highest GLUT 3 immunofluorescent localization was observed in the testis of Hedgehog (*Paraechinus aethiopicus*) and the lowest GLUT 3 expression was noted in the testis of the Laboratory mouse (BALB/c).

Table II. GLUT 3 immunofluorescent localization in the testes of studied animals.

Species	Testis cells			
	spermatogonia	spermatocytes	spermatides	spermatozoa
Laboratory mouse (BALB/c)	(-)	(-)	(-)	(+)
Lesser Egyptian jerboa (*Jaculus jaculus*)	(-)	(-)	(+)	(-)
Golden hamster (*Mesocricetus auratus*)	(++)	(++)	(++)	(+)
Desert Hedgehog (*Paraechinus aethiopicus*)	(++)	(++)	(++)	(+++)

Strong expression (+++); moderate expression (++); faint expression (+); no expression (-).

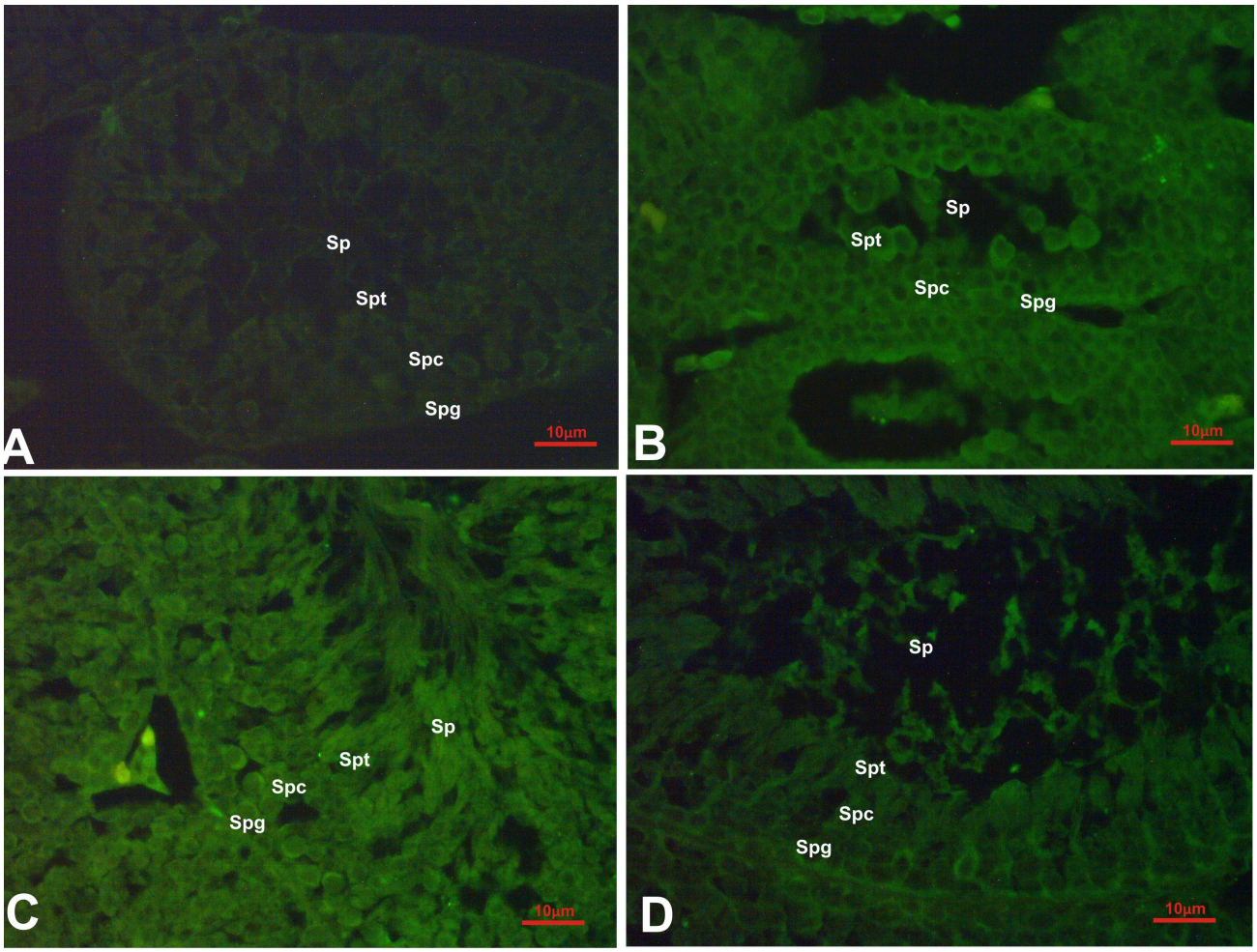

Fig. 3. Photomicrographs of GLUT1 immunofluorescent in the testes of Laboratory mouse (BALB/c) (A), Lesser Egyptian jerboa (*Jaculus jaculus*) (B), Golden hamster (*Mesocricetus auratus*) (C), and Desert Hedgehog (*Paraechinus aethiopicus*) (D). spermatogonia (Spg), spermatocytes (Spc) and spermatides (Spt), and spermatozoa (Sp). Scale bar in A, B, C, and D is 10 µm.

DISCUSSION

The current study showed different immunoreactivity of GLUT1 and GLUT3 in the testicular tissue of Laboratory mouse (BALB/c), Lesser Egyptian jerboa (*Jaculus jaculus*), Golden hamster (*Mesocricetus auratus*), and desert Hedgehog (*Paraechinus aethiopicus*). GLUT 1 was not observed in the sperms of studied animals and was observed with different localization in spermatogonia, spermatocytes, and spermatids with considerable species differences. Previous studies have shown that GLUT 1 was not present in the testes of either humans or mice (Kokk *et al*., 2004, 2005). GLUT1 immunoreactivities were not detected in the seminiferous tubules of the mouse and rat testis, but it was localized in vascular endothelial cells dispersed in the interstitial tissue (Kishimoto *et al*., 2015). GLUT1 immunoreactivity was not found in the mature spermatozoa and was expressed only in the spermatocytes of rat testes (Ibberson *et al*., 2002). Hahn *et al*. (2017) reported that GLUT1 has no significant effect on testicular function. The results of a Western blot analysis showed that rat testes had strong GLUT1 expression and there is a low level of GLUT1 expression in human testes (Burant & Davidson, 1994). Burant & Davidson (1994) and Kokk *et al*. (2007), stated that GLUT1 was expressed in vascular and stromal elements in the rat testis. According to other reports, GLUT 1 is present in the head sperm of rats, humans, and bulls (Angulo *et al*., 1998).

The current results demonstrated the significance of GLUT-3 as a transporter, whereas it was mainly observed in spermatids and sperms depending on the species. GLUT3 has a seven-fold greater affinity than Glut1, Glut2, and Glut4 for glucose in tissues (Frolova & Moley, 2011; Zhang *et al*.,

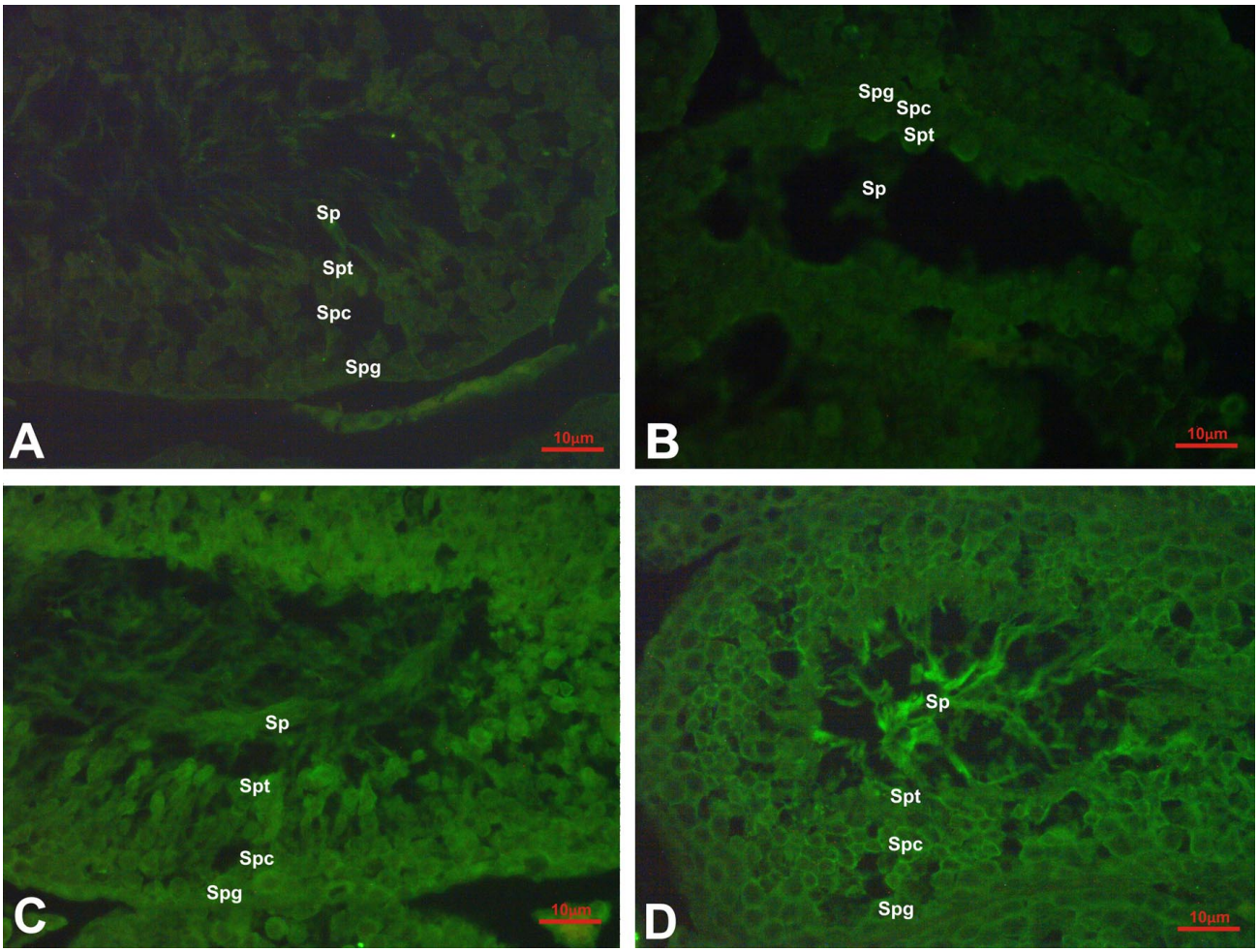

Fig. 4. Photomicrographs of GLUT3 immunofluorescent in the testes of Laboratory mouse (BALB/c) (A), Lesser Egyptian jerboa (*Jaculus jaculus*) (B), Golden hamster (*Mesocricetus auratus*) (C), and Desert Hedgehog (*Paraechinus aethiopicus*) (D). spermatogonia (Spg), spermatocytes (Spc), spermatides (Spt), and spermatozoa (Sp). Scale bar in A, B, C, and D is 10 µm.

2012; Martins *et al*., 2013). The actual GLUT3 cellular localization in the mammalian testis has not been determined yet, however, it is the predominant subtype among class I GLUTs (Kokk *et al*., 2004; Kishimoto *et al*., 2015). GLUT3 distribution pattern was different from one species to another (Burant & Davidson, 1994; Rauch *et al*., 2006; Kishimoto *et al*., 2015). Supporting these findings, GLUT3 was expressed in the spermatocytes and spermatids (Hahn *et al*., 2017). GLUT3 is expressed in mature sperms in the testes of humans, mice, and rats (Rauch *et al*., 2006; Kishimoto *et al*., 2015). GLUT 3 protein levels have been shown in human, mouse, bull, and rat testicular cells (Burant & Davidson, 1994; Banerjee *et al*., 2014). Mature sperms need GLUT3 expression to facilitate the uptake of glucose for successful fertilization (Urner & Sakkas, 1999; Simpson *et al*., 2008). Supporting these findings, GLUT 3 was found in the spermatids and spermatozoa tail of the testis of Desert Hedgehog (*Paraechinus aethiopicus*), which suggests that spermatozoa use glucose and fructose as a main energy substrate and GLUT3 plays an essential role in the trafficking of sugars during the processes of fertilization and capacitation, as well as in providing fuel to spermatozoa. According to Roy and Krishna, 2013a, b, GLUT 5 has a crucial role in the movement of the spermatozoa's fuel, which is needed for long-term storage in the female reproductive system of bats. Angulo *et al*. (1998) documented the localization of GLUT-3 in the sperm midpiece of bulls, rats, and humans. GLUT-3 was expressed in the canine sperm membrane, where sperm motility and glucose metabolism were found to be closely correlated (Rigau *et al*., 2002). GLUT-3 localization was reported by Medrano *et al*. (2006), in boar spermatozoa. GLUT 3 might have to maintain a role in the production of basic energy, which is required for testis survival (Mueckler, 1990). From birth until senescence, there was a minor difference in the expression of GLUT 3 in the testes of the mice (Banerjee *et al*., 2014). In contrast, a limited

localization of GLUT3 immunoreactivity was observed in the rat testis (Rauch *et al*., 2006). Kishimoto *et al.* (2015) found that GLUT3 was localized in the germ cell line of mouse and rat testis except in spermatogonia.

The other GLUT members participate in spermatogenesis, in which their distribution pattern is different from one species to another and their needs for the testis's glucose/fructose transport. The expression and regulation of Glut1, Glut2, Glut3, and Glut4 depend on a tissue and cell-specific manner. There is evidence of GLUT-1, 2, 3, 5, 6, 8, and 9 expression in the testes of mammals (Gómez *et al*., 2006). GLUT 8 was expressed with very high concentration in the rat testes (Doege *et al*., 2000; Chen *et al*., 2003). GLUT 8 has a role in mice testicular steroidogenesis, and expressed a significant variation during different stages (high during the reproductively active phase) and observed in interstitial cells (Leydig cells) in the interstitial tissue (Gómez *et al*., 2006; Kim & Moley, 2007; Banerjee *et al*., 2014). Also, the mouse testis's interstitial cells contained GLUT-8 and 9 (Kim & Moley, 2007). GLUT 2 and 5 were localized in the bull and rat testes (Angulo *et al*., 1998). The sperm heads of bulls, bats, boars, and rats express GLUT 5 (Kokk *et al*., 2004; Medrano *et al*., 2006; Roy & Krishna, 2013a,b) and in the dog sperm membrane (Rigau *et al*., 2002). GLUT-5 is recorded in the human testis (Burant *et al*., 1992). GLUT-5 is localized in the seminiferous tubule cells of the bat testis during various phases of the testicular cycle (Roy & Krishna, 2013a,b). The immunolocalization of GLUT1, GLUT2, GLUT3, GLUT5, and a low amount of GLUT4 in the spermatozoa of humans, rats, and bulls was verified by Angulo *et al*. (1998).

Gluts are membrane proteins that are expressed in particular tissues and are controlled by hormones and metabolism. The biological function of Gluts depends on tissue expression profiles, substrate specificity, and their kinetic properties (Wood & Trayhurn, 2003). GLUTs 1, 3, 4, and 5 are examples of facilitators of high-affinity transport because of their low Michaelis constant (Km), Glut3 has the highest affinity for glucose compared to the because of its low km (Korgun *et al*., 2001; Zhang *et al*., 2012). Glucose uptaking by GLUT is regulated by several factors i.e. such as hormones, growth factors, and cytokines (Oliveira *et al*., 2011; Rato *et al*., 2012; Sarkar & Singh, 2017). The different types of rat testis can be affected by proteins like insulin and some cytokines, which use GLUT in their signal transduction (Kokk *et al*., 2007). There is a correlation between androgenic hormones and the activity of GLUT members. A significant correlation was noted between the circulating testosterone level and steroidogenic protein in testicular tissue in aging-related changes in glucose levels and GLUT 8 expression (Banerjee *et al*., 2014). GLUT expression is essential in a variety of testicular tissues and for testosterone biosynthesis (Chen *et al*., 2003; Teixeira *et al*., 2012; Sarkar & Singh, 2017). Glucose entrance into the cell via GLUT1 and GLUT3 increases in response to hormones (Galardo *et al*., 2008). GLUT-5 was expressed in testicular cells correlated with the height of circulating testosterone during the period of mating and active spermatogenesis (Roy & Krishna, 2013a,b). Decreased testosterone synthesis or hypoandrogenism during mice aging leads to lower glucose availability through altered expression of GLUTs 1 and 3 (Rato *et al*., 2013). The ovary has a regulatory system to control the intake of glucose, whereas GLUT expression in the bovine ovary is controlled by insulin growth factor (IGF)-I, follicle-stimulating hormone, estradiol (E2), and interleukin-1 (Kodaman & Behrman, 1999; Nishimoto *et al*., 2006; Zhang *et al*., 2012). Gonadotropin-releasing hormone modifies glucose uptake and stimulates ovarian development by affecting Glut translocation or localization (Harris *et al*., 2012; Zhang *et al*., 2012). Previous studies documented expression of GLUT member in the testes of mice, rat, bat, dog, boar, golden hamster, bull, and human (Angulo *et al*., 1998; Kokk *et al*., 2004; Medrano *et al*., 2006; Roy & Krishna, 2013a,b; Banerjee *et al*., 2014; Kishimoto *et al*., 2015; Verma & Haldar, 2016; Hahn *et al*., 2017). There is no available data about the expression of GLUT in the Lesser Egyptian jerboa (*Jaculus jaculus*) and Desert Hedgehog (*Paraechinus aethiopicus*) testes. Also, a little data discussed the GLUT expression in the testis of a Golden hamster (*Mesocricetus auratus*). Therefore, the current work compared GLUT1 and GLUT3 expression levels in the examined testes.

CONCLUSION

In conclusion, the glucose uptake via GLUTs in testes remains to be elucidated with considerable species differences. Our findings corroborate earlier research showing that GLUT3 is the predominant in testicular cells across species. GLUT3 in the testis of Desert Hedgehog (*Paraechinus aethiopicus*) may be related to glucose uptake, which participates in the fertilization processes. Spermatogonia, spermatocytes, spermatids, and spermatozoa use different transport systems for energy substrates, which different from one species to another.

ALAHMADI, B. A.; SELEEM, A. A.; LASHEIN, F. E. M.; BADR, A. H. & AHMED, A. A. Localización inmunohistoquímica del transportador de glucosa GLUT1 y GLUT3. en los testículos de algunos mamíferos. *Int. J. Morphol., 42(3)*:805-813, 2024.

RESUMEN: La glucosa tiene un papel esencial en la proliferación y supervivencia del tejido testicular. Los transportadores de glucosa (GLUT) son responsables de la

absorción de glucosa a través de las membranas celulares. En el presente trabajo, se investigaron dos isoformas principales GLUT1 y GLUT3 en los testículos de un ratón de laboratorio (BALB/c), un jerbo egipcio menor (*Jaculus jaculus*), un hámster dorado (*Mesocricetus auratus*) y un erizo del desierto (*Paraechinus aethiopicus*). La localización inmunofluorescente de GLUT1 y GLUT3 mostró diferencias considerables entre especies. La expresión más baja de GLUT1 y GLUT3 se localizó en el testículo del ratón de laboratorio (BALB/c), la localización más alta de GLUT1 se detectó en el testículo del jerbo egipcio menor (*Jaculus jaculus*) y la localización inmunofluorescente de GLUT3 más alta se observó en el testículo de Erizo (*Paraechinus aethiopicus*). Los resultados implican que GLUT3 es el principal transportador de glucosa en los testículos estudiados, lo que está relacionado con diferencias entre especies. La diferente inmunolocalización de GLUT en los testículos examinados sugiere el uso de varios sistemas de transporte para ganar energía en diferentes especies.

PALABRAS CLAVE: GLUT1; GLUT3; Transportador de glucosa; Testículos de erizo del desierto; Testículos de Jerbo; Testículos de hámster.

REFERENCES

Alves, M. G.; Dias, T. R.; Silva, B. M. & Oliveira, P. F. Metabolic cooperation in testis as a pharmacological target: from disease to contraception. *Curr. Mol. Pharmacol., 7(2)*:83-95, 2014.

Alves, M. G.; Martins, A. D.; Cavaco, J. E.; Socorro, S. & Oliveira, P. F. Diabetes, insulin-mediated glucose metabolism and Sertoli/blood-testis barrier function. *Tissue Barriers 1(2)*:e23992, 2013.

Angulo, C.; Rauch, M. C.; Droppelmann, A.; Reyes, A. M.; Slebe, J. C.; Delgado-López, F.; Guaiquil, V. H.; Vera, J. C. & Concha, I. I. Hexose transporter expression and function in mammalian spermatozoa: cellular localization and transport of hexoses and vitamin C. *J. Cell Biochem., 71(2)*:189-203, 1998.

Banerjee, A.; Anuradha; Mukherjee, K. & Krishna, A. Testicular glucose and its transporter GLUT 8 as a marker of age-dependent variation and its role in steroidogenesis in mice. *J. Exp. Zool. A Ecol. Genet. Physiol., 321(9)*:490-502, 2014.

Burant, C. F. & Davidson, N. O. GLUT3 glucose transporter isoform in rat testis: localization, effect of diabetes mellitus, and comparison to human testis. *Am. J. Physiol., 267(6 Pt. 2)*:R1488-95, 1994.

Burant, C. F.; Takeda, J.; Brot-Laroche, E.; Bell, G. I. & Davidson, N. O. Fructose transporter in human spermatozoa and small intestine is GLUT5. *J. Biol. Chem., 267(21)*:14523-6, 1992.

Chen, Y.; Nagpal, M. L. & Lin, T. Expression and regulation of glucose transporter 8 in rat Leydig cells. *J. Endocrinol., 179(1)*:63-72, 2003.

Doege, H.; Schurmann, A.; Bahrenberg, G.; Brauers, A. & Joost, H. G. GLUT8, a novel member of the sugar transport facilitator family with glucose transport activity. *J. Biol. Educ., 275(21)*:16275-80, 2000.

Frolova, A. I. & Moley, K. H. Glucose transporters in the uterus: an analysis of tissue distribution and proposed physiological roles. *Reproduction, 142(2)*:211-20, 2011.

Galardo, M. N.; Riera, M. F.; Pellizzari, E. H.; Chemes, H. E.; Venara, M. C.; Cigorraga, S. B. & Meroni, S. B. Regulation of expression of Sertoli cell glucose transporters 1 and 3 by FSH, IL1 beta, and bFGF at two different time-points in pubertal development. *Cell Tissue Res., 334(2)*:295-304, 2008.

Gómez, O.; Romero, A.; Terrado, J. & Mesonero, J. E. Differential expression of glucose transporter SLC2A8 during mouse spermatogenesis. *Reproduction, 131(1)*:63-70, 2006.

Gorovits, N. & Charron, M. What we know about facilitative glucose transporters. *Biochem. Mol. Biol. Educ., 31(3)*:163-72, 2003.

Hahn, K. R.; Jung, H. Y.; Yoo, D. Y.; Kim, J. W.; Kim, Y. H.; Jo, Y. K.; Kim, G. A.; Chung, J. Y.; Choi, J. H.; Hwang, I. K.; *et al*. Immunohistochemical localization of glucose transporter 1 and 3 in the scrotal and abdominal testes of a dog. *Lab. Anim. Res., 33(2)*:114-8, 2017.

Harris, V. M.; Bendre, S. V.; Gonzalez De Los Santos, F.; Fite, A.; El-Yaman El-Dandachli, A.; Kurenbekova, L.; Abou-Samra, A. B. & Buggs-Saxton, C. GnRH increases glucose transporter-1 expression and stimulates glucose uptake in the gonadotroph. *J. Endocrinol., 212(2)*:139-47, 2012.

Ibberson, M.; Riederer, B. M.; Uldry, M.; Guhl, B.; Roth, J. R. & Thorens, B. Immunolocalization of GLUTX1 in the testis and to specific brain areas and vasopressin-containing neurons. *Endocrinology, 143(1)*:276-84, 2002.

Joost, H. G. & Thorens, B. The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). *Mol. Membr. Biol., 18(4)*:247-56, 2001.

Kim, S. T. & Moley K. H. The expression of GLUT8, GLUT9a, and GLUT9b in the mouse testis and sperm. *Reprod. Sci., 14(5)*:445-55, 2007.

Kishimoto, A.; Ishiguro-Oonuma, T.; Takahashi, R.; Maekawa, M.; Toshimori, K.; Twatanabe, M. & Iwanaga, T. Immunohistochemical localization of GLUT3, MCT1, and MCT2 in the testes of mice and rats: the use of different energy sources in spermatogenesis. *Biomed. Res., 36(4)*:225-34, 2015.

Kodaman, P. H. & Behrman, H. R. Hormone-regulated and glucose-sensitive transport of dehydroascorbic acid in immature rat granulosa cells. *Endocrinology, 140(8)*:3659-65,1999.

Kokk, K.; Veräjänkorva, E.; Laato, M.; Wu, XK.; Tapfer, H. & Pöllänen, P. Expression of insulin receptor substrates 1-3, glucose transporters GLUT-1-4, signal regulatory protein 1alpha, phosphatidylinositol 3-kinase and protein kinase B at the protein level in the human testis. *Anat. Sci. Int., 80(2)*:91-6, 2005.

Kokk, K.; Veräjänkorva, E.; Wu, X. K.; Tapfer, H.; Põldoja, E. & Pöllänen, P. Immunohistochemical detection of glucose transporters class I subfamily in the mouse, rat and human testis. M*edicina (Kaunas), 40(2)*:156-60, 2004.

Kokk, K.; Veräjänkorva, E.; Wu, X. K.; Tapfer, H.; Põldoja, E.; Simovart, H. E. & Pöllänen, P. Expression of insulin signaling transmitters and glucose transporters at the protein level in the rat testis. *Ann. N. Y. Acad. Sci., 1095*:262-73, 2007.

Korgun, E. T.; Demir, R.; Hammer, A.; Dohr, G.; Desoye, G.; Skofitsch, G. & Hahn, T. Glucose transporter expression in rat embryo and uterus during decidualization, implantation, and early postimplantation. *Biol. Reprod., 65(5)*:1364-70, 2001.

Martins, A. D.; Alves, M. G.; Simões, V. L.; Dias, T. R.; Rato, L.; Moreira, P. I.; Socorro, S.; Cavaco, J. E. & Oliveira, P. F. Control of Sertoli cell metabolism by sex steroid hormones is mediated through modulation in glycolysisrelated transporters and enzymes. *Cell Tissue Res., 354(3)*:861-8, 2013.

Medrano, A.; García-Gil, N.; Ramió, L.; Rivera, M. M.; Fernández-Novell, J. M.; Ramírez, A.; Pena, A.; Briz, M. D.; Pinart, E.; Concha, I. I.; *et al*. Hexose-specificity of hexokinase and ADP-dependence of pyruvate kinase play important roles in the control of monosaccharide utilization in freshly diluted boar spermatozoa. *Mol. Reprod. Dev., 73(9)*:1179-94, 2006.

Miki, K. Energy metabolism and sperm function. *Soc. Reprod. Fertil. Suppl., 65:*309-25, 2007.

Mueckler, M. Family of glucose-transporter genes: implications for glucose homeostasis and diabetes. *Diabetes, 39(1)*:6-11, 1990.

Nishimoto, H.; Matsutani, R.; Yamamoto, S.; Takahashi, T.; Hayashi, K G.; Miyamoto, A., Hamano, S. & Tetsuka, M. Gene expression of glucose transporter (GLUT) 1, 3 and 4 in bovine follicle and corpus luteum. *J. Endocrinol., 188(1)*:111-9, 2006.

Nualart, F.; Los Angeles García, M.; Medina, R. A. & Owen, G. I. Glucose transporters in sex steroid hormone related cancer. *Curr. Vas. Pharmacol., 7*:534-48, 2009.

Oliveira, P. F.; Alves, M. G.; Rato, L.; Laurentino, S.; Silva, J.; Sá, R.; Barros, A.; Sousa, M.; Carvalho, J. E. & Socorro, S. Effect of insulin deprivation on metabolism and metabolism-associated gene transcript levels of in vitro cultured human Sertoli cells. *Biochim. Biophys. Acta, 1820(2)*:84-9, 2012.

Oliveira, P. F.; Alves, M. G.; Rato, L.; Silva, J.; Sá, R.; Barros, A.; Sousa, M.; Carvalho, R. A.; Cavaco, J. E. & Socorro, S. Influence of 5a-dihydrotestosterone and 17b-estradiol on human Sertoli cells metabolism. *Int. J. Androl., 34*:e612-e620, 2011.

Rato, L.; Alves, M. G.; Dias, T. R.; Lopes, G.; Cavaco, J. E.; Socorro, S. & Oliveira, P. F. High-energy diets may induce a pre-diabetic state altering testicular glycolytic metabolic profile and male reproductive parameters. *Andrology, 1(3)*:495-504, 2013.

Rato, L.; Alves, M. G.; Socorro, S.; Carvalho, R. A.; Cavaco, J. E. & Oliveira, P. F. Metabolic modulation induced by oestradiol and DHT in immature rat Sertoli cells cultured in vitro. *Biosci. Rep., 32(1)*:61-9, 2012.

Rauch, M. C.; Ocampo, M. E.; Bohle, J.; Amthauer, R.; Yáñez, A. J.; Rodríguez-Gil, J. E.; Slebe, J. C.; Reyes, J. G. & Concha, I. I. Hexose transporters GLUT1 and GLUT3 are colocalized with hexokinase I in caveolae microdomains of rat spermatogenic cells. *J. Cell. Physiol., 207(2)*:397-406, 2006.

Rigau, T.; Rivera, M.; Palomo, M. J.; Fernandez-Novell, J. M.; Mogas, T.; Ballester, J.; Pena, A.; Otaegui, P. J.; Guinovart, J. J. & Rodriguez-Gil, J. E. Differential effects of glucose and fructose on hexose metabolism in dog spermatozoa. *Reproduction, 123(4)*:579-91, 2002.

Roy, V. K & Krishna, A. Changes in glucose and carnitine levels and their transporters in utero-tubal junction in relation to sperm storage in the vespertilionid bat, *Scotophilus heathi*. *J. Exp. Zool. A Ecol. Genet. Physiol., 319(9)*:517-26, 2013a.

Roy, V. K, & Krishna, A. The expression pattern of the glucose transporter GLUT-5 in the testis during the spermatogenic cycle of the vespertilionid bat *Scotophilus heathi. Gen. Comp. Endocrinol., 191*:59-64, 2013b.?

Sarkar, D. & Singh, S. K. Neonatal hypothyroidism affects testicular glucose homeostasis through increased oxidative stress in prepubertal mice: effects on GLUT 3, GLUT 8 and Cx43. *Andrology, 5(4)*:749-62, 2017.?

Seleem, A. A. Immunohistochemical localization of alpha-synuclein in the retina of some nocturnal and diurnal animals. *Biotech. Histochem., 95(5)*:360-72, 2020.

Simpson, I. A.; Dwyer, D.; Malide, D.; Moley, K. H.; Travis, A. & Vannucci, S. J. The facilitative glucose transporter GLUT3: 20 years of distinction. *Am. J. Physiol. Endocrinol. Metab., 295(2)*:E242-53, 2008.

Sotoudeh, N. & Namavar, M. R. Optimisation of ketamine-xylazine anaesthetic dose and its association with changes in the dendritic spine of CA1 hippocampus in the young and old male and female Wistar rats. Vet. Med. Sci., 8(6):2545-52, 2022.

Survarna, S. K.; Layton, C. & Bancroft, J. D. T*heory and practice of histochemical techniques.* 7th ed. New York, Churchill Livingstone, Elsevier, 2013. pp.435-54.

Teixeira, S. S.; Tamrakar, A. K.; Goulart-Silva, F.; Serrano-Nascimento, C.; Klip, A. & Nunes, M. T. Triiodothyronine acutely stimulates glucose transport into L6 muscle cells without increasing surface GLUT4, GLUT1, or GLUT3. *Thyroid, 22(7)*:747-54, 2012.

Urner, F. & Sakkas D. A. A possible role for the pentose phosphate pathway of spermatozoa in gamete fusion in the mouse. *Biol Reprod., 60(3)*:733-9, 1999.

Verma, R. & Haldar, C. Photoperiodic modulation of thyroid hormone receptor (TR-a), deiodinase-2 (Dio-2) and glucose transporters (GLUT 1 and GLUT 4) expression in testis of adult golden hamster, *Mesocricetus auratus. J. Photochem. Photobiol. B., 165*:351-8, 2016.

Williams, A. C. & Ford, W. C. The role of glucose in supporting motility and capacitation in human spermatozoa. *J. Androl., 22(4)*:680-95, 2001.

Wood, I. S. & Trayhurn, P. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br. J. Nutr., 89(1)*:3-9, 2003.

Zhang, C.; Niu, W.; Wang, Z.; Wang, X. & Xia, G. The effect of gonadotropin on glucose transport and apoptosis in rat ovary. *PLoS One, 7(8)*:e42406, 2012.

Corresponding author:
Amin A. Seleem
Zoology Department
Faculty of Science
Sohag University
EGYPT

E-mail: amin_seleem@science.sohag.edu.eg

ORCID: https://orcid.org/0000-0002-4633-7835