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Expression of GLUT-1 and GLUT-3 on spermatogenesis and fertility in rat testis with diabetes mellitus: an experimental study

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ABSTRACT

Introduction: Diabetes mellitus is a major public health problem worldwide that may cause organ failure and dysfunction such as reproduction dysfunction, including infertility. Glucose metabolism is an important process in spermatogenesis. Glucose Transporters (GLUTs) play an important role in transferring glucose into cells to carry out the sperm capacitation process and maintain sperm motility. Although glucose is needed in spermatogenesis, hyperglycaemic conditions may cause detrimental effects on male fertility. This study was conducted to analyze the impact of hyperglycaemic status in rats on testicular function of spermatogenesis, specifically regarding GLUT-1 and GLUT-3.

Methods: This study was an experimental study done in the integrated research and testing laboratory of Universitas Gadjah Mada from June to August 2018. This study used male Wistar rats, randomly divided into three groups as two intervention groups given Streptozotizin (STZ) induction for 4 and 8 weeks and one control group. The minimum sample size in each group was 9. Furthermore, the mRNA expression of GLUT-1 and GLUT-3 genes were assayed by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).

Results: This study showed an increase in GLUT-1 and GLUT-3 expression in rats that experienced hyperglycemia for 4 weeks ($p = 0.008$ and $p = 0.002$, respectively). Increased expression of GLUT-1 and GLUT-3 also occurred in rats that experienced hyperglycemia for 8 weeks, but the increase was not statistically significant ($p=0.36$ and $p=0.079$).

Conclusion: Hyperglycaemia may cause an increase in GLUT-1 and GLUT-3 expression. This expression indicates that GLUT-1 and GLUT-3 are very sensitive to glucose levels changes and may play a role in spermatogenesis changes in patients with diabetes mellitus.

Keywords: diabetes mellitus, fertility, GLUT-1, GLUT-3, spermatogenesis.
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INTRODUCTION

Diabetes mellitus is one of the world's major health issues. In 1980, it was estimated that 108 million of the world's population had diabetes mellitus. This number increased 4-fold in 2014 to 422 million cases. Diabetes mellitus is known to be associated with infertility. About 90% of people with diabetes have problems with sexual function, including decreased libido, impotence, and infertility.[1-3] Several studies hypothesized that diabetes has the potential to reduce male fertility function through pre-testicular, testicular, and post-testicular mechanisms. On the pre-testicular axis, type 2 diabetes mellitus is closely related to obesity. This condition can lead to hyperleptinemia, decreased secretion of pulsatile gonadotropin-releasing hormone (GnRH), decreased Leydig cell function, and decreased serum levels gonadotropins and testosterone.[4] At the testicular level, glucose concentration dramatically affects the process of spermatogenesis. In the tubular, the glucose level is maintained within a low threshold by the Sertoli cells. Passive glucose transport is mediated by glucose transporters (GLUT). Glucose metabolism in spermatogenesis plays a role in maintaining basic cell activity and maintaining the motility ability of mature sperm.[4]

Insulin signaling is crucial in the maturation process of rat spermatozoa. GLUT distribution also plays a vital role in transferring glucose into cells and indirectly in the maturation and maintenance of sperm motility during the capacitation process.[4] Diabetes and experimentally induced diabetes have shown that type 1 or type 2 diabetes can cause adverse effects on male fertility, namely decreased sperm quality and sperm motility. According to the facts on

Table 1. Primers used in this study

	Gene	
	GLUT-1	GLUT-3
Sense primer	5'-AGCAGCAAGAAGCTGACGGGTC-3'	5'-TCAGGCTCCACCCTTTGCGGA-3'
Antisense primer	5'-CGCCGGCCAAAGCGGTTAAC-3'	5-TGGGGTGACCTTCTGTGTCCCC-3'
Amplification size	291	216

Table 2. Expression of GLUT-1 and GLUT-3 in this study

Group	Mean	P-value
GLUT-1		
Control	10.1 ± 1.3	0.008
Group 1	18.5 ± 4.3	
Group 2	23.1 ± 5.0	0.36
GLUT-3		
Control	9.5 ± 2.6	0.002
Group 1	17.4 ± 2.2	
Group 2	20.02 ± 3.9	0.079

previous studies, the authors suggest the urge for further studies. This study aims to analyze the effect of hyperglycaemic status in rats on testicular function of spermatogenesis, specifically regarding GLUT-1 and GLUT-3.

METHODS

An experimental study was conducted at the anatomical pathology laboratory, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, from June to August 2018. This study used male Wistar rats, with the total sample of 24 which were randomly divided into three groups, namely two intervention groups and one control group, after a week of adaptation. The control group consisted of 8 rats that did not receive any intervention. Group 1 consisted of 8 rats that were induced with Streptozotizin (STZ) at a dose of 40 mg/kgBW for 4 weeks, while Group 2 consisted of 8 rats that were induced with STZ at the same dose for 8 weeks. Healthy male Wistar rats, aged 12-16 weeks, weighing 250-300 grams, and blood sugar levels > 300 mg/dl after being induced were included as samples for this study. Wistar male rats that showed behavioral changes at the time of the study and died were excluded. Subsequently, RNA was extracted from testicular tissue samples and examined for mRNA expression of GLUT-1 and GLUT-3 genes using RT-PCR.

The data that has been collected is tabulated in tabular form to find the mean of GLUT-1 and GLUT 3 expressions in each group. The data will be analyzed using SPSS version 15.0. This study has obtained ethics approval from the Medical and Health Research Ethics Committee (MHREC) of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/DR. Sardjito General Hospital, Yogyakarta No. KE/FK/068?EC/2019, prior to the study conducted.

RESULTS

In this study, the testes of rats from the three groups were taken for further testing. The rat testes were put in paraffin, and Sertoli cells were isolated. The primers analyzed can be seen in Table 1.

In this study, it was found that there was an increase in the expression of GLUT-1 in the group induced by hyperglycemia for 4 weeks when compared to the control group ($p < 0.05$). Increased expression of GLUT-1 was also found after 8 weeks of hyperglycaemic exposure. However, this increase was not statistically significant ($p > 0.05$). Exposure to hyperglycemia for 4 weeks can induce a significant increase in GLUT-3 expression ($p<0.05$). This increase was also observed at week 8 of hyperglycemia exposure but was not statistically significant ($p > 0.05$). The mean of GLUT-1 and GLUT-3 expression can be seen in Table 2.

DISCUSSION

Sertoli metabolism has been linked to male fertility issues, particularly in cases of diabetes mellitus or hyperglycemia. Some of these patients' infertility cases have minor morphological and motility abnormalities. According to the previous study, the chances of a successful pregnancy are lower if the male partner has a history of diabetes mellitus.[3] GLUT-3 is the predominant GLUT subtype among class I GLUTs in mammalian testes. GLUT 3 and GLUT 1 are subtypes of the glucose pump in Sertoli cells located in the Blood Testes Barrier (BTB) in mammals, including humans. However, the exact cellular localization of these GLUTs, GLUT-1 and GLUT-3, has yet to be determined. An immunohistochemical study showed that GLUT-3 was expressed in all rat testicular seminiferous epithelial cells, including sperm. In another study, it was explained that testicular tissue and Sertoli cells in 8 and 20-day old rats were immunoreactive for GLUT-3 and GLUT-1.[4,5]

In this study, it was found that there was an increase in the expression of GLUT-1 and GLUT 3 in the first 4 weeks after the induction of hyperglycemia. The expression of these genes continued to increase until week 8. However, the increase was not statistically significant. This indicates that the glucose pump in spermatogenesis metabolism has a compensatory system for hyperglycaemic conditions to prevent excessive glucose from entering cells which can cause cell damage.[6,7] This increase in the glucose pump could be due to a rise in ATP-forming activity. This condition can result in a disproportionate amount of oxidant energy, which damages the sperm cell wall.[7] One of the limitations of this study was the small sample size. As a result, it was hoped that similar studies with a larger sample size could be conducted in the future.

CONCLUSION

Hyperglycaemia conditions can cause an increase in the expression of GLUT-1 and GLUT-3. This shows that GLUT-1 and GLUT-3 are very sensitive to changes in glucose levels and maybe one of the causes of changes in the process of spermatogenesis in patients with diabetes mellitus.

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CONFLICT OF INTEREST

We declare that there were no conflicts of interest in this study.

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AUTHOR CONTRIBUTION

All of the authors are equally contributed to the study.

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