ORIGINAL ARTICLE

Impact of Ornithine Decarboxylase on Semen Parameters and Functional Status of Spermatozoa in Type 2 Diabetes Mellitus

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Abstract

Aim: Type 2 diabetes mellitus (T2DM) represents a spectrum of metabolic disorders and affects all the physiological system including reproduction. Even though enormous research is carried out to focus the etiologies of infertility in T2DM, till date, the role of polyamines which modulate three multiple biochemical activities including nucleic acid and protein synthesis as well as cell signaling that are important for spermatogenesis are ignored. Limited studies are available on ornithine decarboxylase (ODC); an enzyme involved in the biosynthesis of polyamine and maintains the homeostasis of polyamines. Further, these studies are confined to animals only. Hence, an attempt was made to uncover the role of ODC in fertility of T2DM individuals. **Materials and Methods:** A total of 230 T2DM males and 50 control male subjects with proven fertility and non-diabetic aged between 25 and 50 years were recruited from different diabetic centers and clinics in and around Mysore, South India. Semen analysis and sperm function tests were carried out according to WHO guidelines. The level of ODC was estimated using enzyme-linked immunosorbent assay kit. **Results:** Our results demonstrate a strong association of type 2 diabetic conditions with reduced vitality of sperm, nuclear chromatin decondensation, and hypo-osmotic swelling among sperm function test along with a significant drastic reduction in the ODC levels. Reduced levels of ODC might be inadequate for homeostasis of polyamine. **Conclusion:** Overexpression of antizyme could have resulted in reduced level of ODC resulting in perturbation of normal spermatogenesis in T2DM individuals.

Key words: Ornithine decarboxylase, sperm function test, spermiogram, type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetics mellitus (T2DM) is a global health issue estimated to affect 382 million people worldwide with the prevalence of 8.3%.[1] The term "DM" describes a metabolic disorder of carbohydrate, fat and protein resulting from defects in insulin secretion, insulin action or both.[2] The DM could induce long-term damages, dysfunctions and failures of various organs, including retinopathy, nephropathy, peripheral neuropathy, autonomic neuropathy, etc.[3] An important complication of diabetes is the inconsistency in the male reproductive potentiality. Many studies in both human and animals have confirmed the deleterious effect of diabetes on sexual functions, such as semen parameters, nuclear DNA fragment, and chromatin quality.[4-6] Mallidis *et al*.[7] demonstrated that T2DM induces subtle molecular changes that are important for sperm quality and function.

The polyamines spermidine and spermine and their diamine precursor putrescine are ubiquitous small basic molecules that are essential for cell proliferation, survival, and apoptosis. They modulate multiple biochemical activities including nucleic acid, protein synthesis as well as cell signaling.[8] Cellular and tissue polyamine contents are tightly regulated by different processes that include polyamine biosynthesis, catabolism, and transport.[9] The previous studies with mice overproducing ornithine decarboxylase (ODC) have demonstrated the importance of polyamine homeostasis for normal mammalian spermatogenesis.[10] The physiological function of polyamines in the male reproductive system is

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poorly understood, despite the observation that crystallization of spermine phosphate in human semen was already made by Leeuwenhoek in the 17th century. The biosynthesis of polyamines is characteristically high in mammalian prostate gland due to its secretory function. In the rodent testis, polyamines and their biosynthetic enzymes are unevenly distributed in different types of testicular cells. Although polyamines have been implicated in the induction of cell proliferation, growth and differentiation, their role in testicular physiology has remained elusive.

ODC, EC 4.1.1.17 converts the amino acid ornithine to the diamine putrescine, which is the obligatory precursor in the biosynthesis of the polyamines spermidine and spermine. Expression of ODC is quick possible because of its extremely short half-life of with few minutes to 1 h (the shortest half-life of any known enzyme). Its degradation is regulated by a unique regulatory protein named antizyme (AZ), which is induced by polyamine-mediated translational frameshifting.[11,12] In adult rodent testis, the levels of ODC mRNA are relatively elevated although in some cases no correlation with ODC activity has been established.[13] A detailed analysis of the expression of ODC mRNA in rat and mouse seminiferous epithelia suggested that polyamines may play an important role during late meiosis and early spermiogenesis.[14] The importance of polyamine metabolism in the testis was reinforced by the generation of transgenic mice overexpressing ODC in this tissue that presented reduced fertility. A remarkable change in testicular morphology and impaired spermatogenesis was observed with ODC activity and putrescine content in the testis of the transgenic mice.[15] A study by Mallidis *et al*.[16] showed fourteen-fold decrease in the expression of ODC, which is responsible for the production of spermine and spermidine, compounds responsible for cell growth that help in stabilizing the structure of DNA in T2DM.

DM is associated with very subtle disorders, either directly or indirectly also with physiology and functional status of the reproductive system and sexual dysfunction in all its forms.[17] Despite extensive research, the precise role of the ODC/polyamine system in cell physiology remains to be solved. Diabetes has a direct influence on the health of semen. It is widely believed that spermine was uniquely present in semen.[18] In view of this hypothesis, in this study, we made an attempt to analyze the impact of the levels of ODC on semen parameters and functional status of spermatozoa in T2DM individuals.

MATERIALS AND METHODS

The present investigation was conducted in 230 T2DM males aged between 25 and 50 years and was recruited from different diabetic centers and clinics in and around Mysore, India. A total of 50 control male subjects with proven fertility without Artificial reproductive techniques and non-diabetic were considered as control group. The study was approved by the Institutional ethical clearance committee, University of Mysore and concerned hospitals in and around Mysore. The study was approved by the institutional ethical clearance committee of University of Mysore and concerned hospitals (IHEC-UOM No.12/Res/2009-10). Informed written consent letters were taken from the participants before including them in this study. Subjects were interviewed to collect information about family, medical, reproductive history and lifestyle factors which includes age; the duration of married life, premature ejaculation and psychological status of the subjects. Semen analyses were carried out in both cases and control subjects according to the WHO guidelines.[19]

Semen collection and preservation

The semen samples were collected after 3-5 days of ejaculatory abstinence according to WHO guidelines.[19] In case of oligospermia or azoospermic patients, semen samples were collected thrice on alternate 3-5 days of ejaculatory abstinence. The semen samples were centrifuged; pellets were examined and confirmed for the azoospermic and oligospermic conditions.

Semen analysis

Physical examination such as liquefaction time, color, pH, and viscosity was recorded after liquefaction. Microscopic examinations were carried out to record the count, density, motility, and morphology of the sperm according to WHO guidelines.[19] Single-blinded semen analysis was performed for control and diabetic individuals.

Sperm function tests

Nuclear chromatin decondensation (NCD) test: This test was carried out to check the ability of decondensation of nuclear chromatin *in vitro* in spermatozoa. Semen sample was centrifuged to separate plasma. The pellets were washed with 0.05 M borate buffer. One volume of sample was mixed with nine volumes of EDTA-SDS mixture and incubated at 37°C for 60 min. An equal volume of glutaraldehyde borate buffer was added. A drop of this mixture was transferred onto clean glass slide and covered with cover slip and observed under microscope in ×400 magnification. The number of condensed and decondensed heads was counted. If more than 70% of spermatozoa showed decondensed nuclear chromatin, then it was considered as normal.

Hypo-osmotic swelling test (HOS)

Integrity of plasma membrane was performed using this test. HOS was prepared using fructose and sodium citrate in equal proportion. 1 ml of this solution was incubated at 37°C for 10 min and 100 µl of semen sample was mixed. It was incubated at 37°C for 30 min. This mixture was dropped on pre-cleaned

glass slide, covered with cover slip and observed under microscope in ×400 magnification. The percentage of coiled (curled) tail was recorded. If more than 60% of spermatozoa showed coiled tail, then it was considered as normal.

Acrosomal intactness test

Quality of the acrosomal enzymes were analyzed using this test. Gelatin coated slides were prepared by spreading warm aqueous solution of gelatin onto a clean glass slide and kept horizontally at 40°C for 24 h. These coated slides were immersed in PBS-glutaraldehyde solution for 2 min and washed using distilled water and stored at 40°C. Semen was mixed with PBS-D-glucose in the ratio of 1:5 and incubated at 37°C for 10 min. Gelatin coated slides were allowed to reach room temperature. A drop of diluted semen samples was smeared over gelatin slide. This slide was placed onto a Petri dish containing a moistened filter paper and incubated for 2 h at 37°C. The slides were examined under phase contrast microscope in ×400 magnification. The percentage spermatozoa with halos surrounding the head were recorded. Values more than 50% were considered as normal.

Estimation of ODC

Of 230 T2DM, only 60 cases with severe variation in semen parameters, and out of 50 controls, only 15 subjects were further included for the estimation of the levels of ODC using a double antibody sandwich enzyme-linked immunosorbent assay obtained from the Qayee-Bio for Life Science Company Shanghai, China. The estimation of ODC was done by measuring the absorbance at 450 nm using thermo fisher microtiter plate reader.

Statistical analysis

The statistical analysis was performed using SPSS software version 14.0. Independent *t*-test was used to compare the semen parameters along with sperm function test and ODC activity among T2DM and control group. Results were reported as mean ± standard error. Differences were considered significant when $P < 0.05$.

RESULTS

In this study, 250 T2DM males and 50 control subjects were analyzed for semen parameters. T2DM subjects were categorized into different conditions according to their semen profile as shown in Table 1. Among them, more than 50% T2DM cases were normozoospermic without any variation in semen parameters but remaining 50% cases had substantial variation resulting in astheno (11%), oligo (9.4%), and azoospermic (6.3%). Severe defects in semen parameters were observed in oligoasthenoteratozoospermia condition but in less number [Table 1]. Table 2 depicts the comparison of semen parameters between T2DM and control subjects. No significant differences exist for count, motility, morphology, pH, etc., but vitality was highly compromised. Sperm vitality plays a major role in fertility of individuals, and hence vitality showed statistically significant differences between controls and T2DM subjects. Table 3 showed the functional potential of the sperm through different function tests. Scores for NCD and HOS tests were significantly different in T2DM and control group indicating failure in the decondensation of nuclear chromatin, which seems to be vital factor for healthy sperm. Similarly, permeability of plasma membrane was also highly compromised, which could be due to hyperglycemic condition in T2DM. The estimation of acid phosphatase and ODC in T2DM and control samples was given in Table 4. The levels of ODC were significantly decreased in different infertile conditions of T2DM individuals compared to control subjects indicating the importance of this enzyme for the synthesis of spermine and spermidine that are important for spermatogenesis [Figure 1]. The level of ODC in different conditions of T2DM males, according to their semen profile shows constant readings, but the level of control group shows the higher value of 3.6 ng/ml as shown in Figure 2.

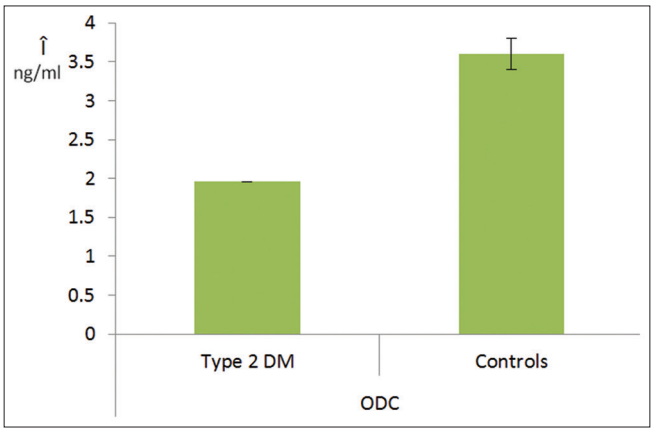

Figure 1: Estimation of the levels of ornithine decarboxylase in type 2 diabetes mellitus and control group

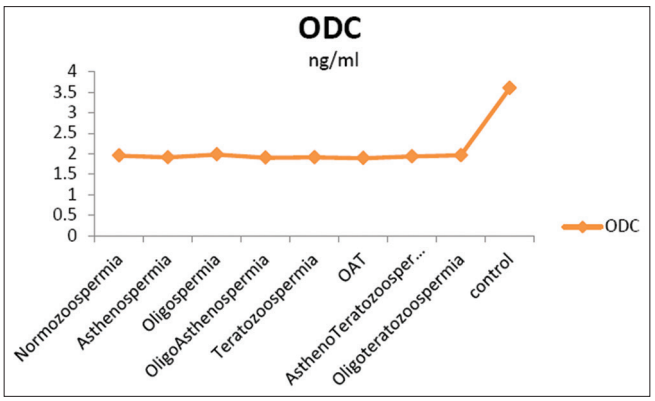

Figure 2: Quantity of ornithine decarboxylase in different infertile conditions in type 2 diabetes mellitus and control individuals

Table 1: Distribution of different infertile conditions in T2DM in comparison with controls

Condition	In percent	Coagulation		Liquefaction (30 min)		Colour (Greyish white)		pH (7.5-8)
		NR %	AB %	NR %	AB %	NR %	ABR %	
Normozoospermic	63.6	100	-	100	-	100	-	8.2±0.3
Oligospermia	9.4	75.6	24.4	69.2	30.7	63.2	36.8	8.2±0.1
Asthenospermia	11.05	84.4	15.6	100	-	84.3	15.7	8.2±0.2
Teratozoospermia	4.2	82.6	17.4	96.5	3.5	69.4	30.6	8.2±0.3
Oligoteratozoospermia	2.1	94.4	5.6	77.7	22.3	100	-	8.2±0.2
Asthenoteratoozoospermia	1.05	85.6	14.4	69.3	30.7	98.3	1.7	8.2±0.2
OAT	2.1	78.9	21.1	83.4	16.6	94.3	5.7	8.1±2.5
Azoospermia	6.3	74.4	25.6	78.9	21.1	84.9	15.1	8.2±0.3
Controls (*n*=50)	100	100	-	100	-	100	-	7.9±0.3

NR: Normal, AB: Absent, ABR: Abnormal, OAT: Oligoasthenoteratozoospermia, T2DM: Type 2 diabetes mellitus

Table 2: Comparison of semen parameters among control and T2DM cases

Semen Parameters	Condition	*n*	Mean±SE	*t*	Significant (two-tailed)	95% CI	
						Lower	Upper
pH	DM	60	7.8±0.04	0.687	0.494	0.133	0.274
	Control	16	7.7±0.06				
Count	DM	60	44.8±4.8	0.619	0.538	25.954	13.644
	Control	16	51±5.6				
Motility	DM	60	53.5±1.60	0.214	0.831	5.701	7.076
	Control	16	52.8±1.44				
Morphology	DM	60	16.1±1.47	0.674	0.503	8.128	4.020
	Control	16	18.18±2.05				
Vitality	DM	53	55.1±2.63	4.236	0.0001*	30.526	10.971
	Control	16	75.9±1.52				

*P<0.05. T2DM: Type 2 diabetes mellitus, DM: Diabetics mellitus

Table 3: Analysis of sperm function tests in among control and T2DM cases

Function tests	Condition	*n*	Mean±SE	*t*	Significant (two-tailed)	95% CI	
						Lower	Upper
NCD	DM	60	65±1.61	4.120	0.0001*	19.651	6.832
	Control	16	78.3±1.88				
HOS	DM	60	65±1.73	3.129	0.003*	16.593	3.667
	Control	16	75±1.20				
AIT	DM	60	55.2±1.57	0.928	0.357	4.002	10.930
	Control	16	51.7±4.36				

*P<0.05. T2DM: Type 2 diabetes mellitus, NCD: Nuclear chromatin decondensation, DM: Diabetics mellitus

DISCUSSION

Spermatogenesis is the process that takes place in the seminiferous tubule of the testes, in which spermatogonia, the more primitive germ cells, proliferate and are first converted into spermatocytes that enter meiosis to yield spermatids that are finally transformed in spermatozoa. This event requires a sophisticated program of cell differentiation, further complicated by the requirements of germ cells to traverse the seminiferous epithelium, in an orderly manner, from locations adjacent to the basement membrane into the lumen of the tubules.[20] Polyamines are multifunctional organic bases that play an essential role in cell growth, differentiation, and malignant development.[21] They have many direct actions on DNA and RNA polymerase, methylase, hydrolases and actions involved in tRNA, rRNA

Table 4: Analysis of acid phosphatase and ornithine decarboxylase among control andT2DM cases

Biochemical analysis	Condition	*n*	Mean±SE	*t*	Significant (two-tailed)	95% CI	
						Lower	Upper
Acid phosphatase	DM	60	462±32.34	1.435	0.156	37.02837	227.34933
	Control	16	366±43.89				
Ornithine decarboxylase	DM	60	1.9±0.10	6.997	0.001*	2.11128	1.17534
	Control	16	3.6±0.24				

**P*<0.05. T2DM: Type 2 diabetes mellitus, DM: Diabetics mellitus

and mRNA in almost all somatic cells. Hence, these are ubiquitous cell components essential for normal growth of both eukaryotic and prokaryotic cells.[22] The three commonly occurring polyamines (putrescine, spermidine, and spermine) are synthesized from ornithine and/or arginine, putrescine being the first polyamine in these biosynthetic pathways.[23] Spermidine and spermine are generated from putrescine by the addition of aminopropyl groups derived from decarboxylated S-adenosyl Met.[23] The rate-limiting step in the formation of putrescine in animals and most fungi is the decarboxylation of ornithine by ODC.[22] The control of ODC degradation by the intracellular polyamine level and the AZ level[11,24] is proposed to assure homeostatic regulation of both the ODC activity and the polyamine concentrations in mammalian cells.

Spermiogram in this study completely deviates with global data; our findings significantly varies with previous studies.[4,25-29,30] Except significant decrease in sperm vitality all physical and microscopic parameters holds good with WHO[19] reference values in our study. This is the first report wherein except vitality; nil variation was observed in all other semen parameters in T2DM considering large sample size, unbiased sampling and confirmation done through 3 rounds of analysis.

Sperm function tests reveal functional status of sperm with reference to acrosomal enzymes, sperm NCD stability and membrane intactness. In this study, T2DM males exhibited a normal response to acrosome test consequently confirms a negative association between acrosome enzyme activity and fertility potential of spermatozoa in T2DM cases. Hyperglycemia would not have affected acrosome formation, and acrosomal proteins which are believed to be prerequisite modifications for eventually mature sperm to function properly as the fertilization process proceeds. This study shows reduced sperm chromatin decondensation in DM group which indicates abnormal protamine package henceforth DNA is prone to damage. Consequently, NCD of spermatozoa and subsequent male pronucleus formation are essential for fertilization and normal embryonic development, but these processes were not in healthy status in T2DM individuals. In this study, T2DM cases showed very poor response for HOS with lesser value from the normal range. This indicates the abnormality in the membrane intactness and the damages caused maybe due to different factors like environmental toxicants, ROS, etc., but the major factor could be hyperglycemic condition. The abnormality of plasma membrane of sperm was further evidenced by reduced vitality, the only variation reported in this study.

Estimation of ODC in the present study showed statistically significant variations between control group and T2DM individuals. Mallidis[7] showed 14 folds decrease in the ODC levels in T2DM subjects and our finding accord with the study. Reduced ODC levels were insufficient in the conversion of ornithine to polyamine such as putrescine, spermidine, and spermine. The importance of polyamine metabolism in the testis was reinforced by the generation of transgenic mice overexpressing ODC in this tissue that presented reduced fertility. Further analysis on the influence of polyamine levels on DNA synthesis, morphology and the number of cells at different stages of the cycle of seminiferous epithelium of ODC transgenic mice, revealed that putrescine stimulated DNA synthesis in spermatogonia, but reduced the number of meiotic and postmeiotic cells.[31] Taken together, these results suggested that polyamines may have a dual stimulatory or inhibitory action during spermatogenesis. This balance is maintained by AZ, a new paralogue of mammalian AZ, found to be expressed specifically in the testis.[10,32]

In this study, drastic reduction in ODC level could be due to overexpression of AZ might reduce the level of ODC resulting in perturbation of normal spermatogenesis in T2DM condition. AZ binds reversibly to monomers of ODC and promotes its proteolytic degradation by the 26S proteasome, AZ also downregulates polyamine uptake by cells. Activity of AZ is regulated by another unique protein. This AZ inhibitor (AZIN) is highly homologous to ODC but lacks its activity completely and is believed to prevent ODC degradation by trapping the AZ.[33] ODC AZ1 is the most common AZ family member and is believed to be the predominant factor in the regulation of ODC.[34] AZ2 appears to overlap functionally with AZ1 but is less abundant and has reduced ability to induce degradation of ODC.[35-37] AZ3 maintains polyamine homeostasis during spermatogenesis but does not mediate ODC degradation.[32,38] It was found that AZ3 transcription was restricted to testis germ cells, starting early in spermiogenesis and finishing in the late spermatid phase.[10] Since the AZ3 wave of expression follows the wave of high ODC expression that takes place during the early phases of spermatogenesis, it was postulated that the physiological role

of AZ3 was to abolish ODC activity to avoid the detrimental effects of putrescine during spermiogenesis.[10,12] Each of the AZ also binds to a second protein, designated ODC AZIN that is highly conserved with ODC at the sequence level but lacks decarboxylase activity.[39] In addition to regulating ODC protein levels in response to changing intracellular polyamine concentrations, the balance between AZ1 and AZIN also modulates the polyamine transport system. Elevated polyamine levels lead to repression of polyamine uptake and induction of excretion that is dependent on AZ1, but independent of interaction between AZI and ODC.[40,41] The identification of the expression of AZIN2 in the testis added new insights on the requirements of a more stringent control of polyamine levels in testicular cells. The expression of AZIN2 in the mouse testis presents a very well defined pattern, both at the spatial and temporal levels. *In situ* RNA hybridization experiments have demonstrated that AZIN2 mRNA is present in the inner part of the seminiferous tubules, where spermatids and spermatozoa are mainly located, suggesting that the expression of AZIN2 takes place in the haploid germinal cells.[42] One possibility is that AZIN2 may participate in the regulation of polyamine fluxes during the differentiation of the haploid cells that takes place during spermiogenesis. In turn, changes in polyamine pools have been reported during spermiogenesis,[43] concomitantly with processes of chromatin remodeling in which histones are replaced by protamines. Hence, reduced levels of ODC may be inadequate for homeostasis of polyamine in T2DM which in turn is essential for normal spermatogenesis.

CONCLUSION

The effects of diabetes on human reproductive function have been largely neglected beyond concerns. Even though research focused on the etiologies of infertility in T2DM, till date biosynthesis of polyamines mediated by ODC, which has prime importance for spermatogenesis is greatly ignored. A significant decrease in the ODC in this study in T2DM highlights its importance not only in spermatogenesis but also for functional potentiality of spermatozoa. For this reason, it is necessary to evaluate the ODC levels along with another laboratory diagnosis for the proper management of T2DM.

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REFERENCES

1. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 2014;103:137-49.
2. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53.
3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33:S62-9.
4. Agbaje IM, Rogers DA, McVicar CM, McClure N, Atkinson AB, Mallidis C, *et al.* Insulin dependant diabetes mellitus: Implications for male reproductive function. Hum Reprod 2007;22:1871-7.
5. Kilarkaje N, Al-Hussaini H, Al-Bader MM. Diabetes-induced DNA damage and apoptosis are associated with poly (ADP ribose) polymerase 1 inhibition in the rat testis. Eur J Pharmacol 2014;737:29-40.
6. Cavallini G. Male idiopathic oligoasthenoteratozoospermia. Asian J Androl 2006;8:143-57.
7. Mallidis C, Agbaje I, McClure N, Kliesch S. The influence of diabetes mellitus on male reproductive function: A poorly investigated aspect of male infertility. Urologe A 2011;50:33-7.
8. Igarashi K, Kashiwagi K. Polyamines: Mysterious modulators of cellular functions. Biochem Biophys Res Commun 2000;271:559-64.
9. Pegg AE. Mammalian polyamine metabolism and function. IUBMB Life 2009;61:880-94.
10. Ivanov IP, Rohrwasser A, Terreros DA, Gesteland RF, Atkins JF. Discovery of a spermatogenesis stage-specific ornithine decarboxylase antizyme: Antizyme 3. Proc Natl Acad Sci U S A 2000;97:4808-13.
11. Hayashi S, Murakami Y, Matsufuji S. Ornithine decarboxylase antizyme: A novel type of regulatory protein. Trends Biochem Sci 1996;21:27-30.
12. Coffino P. Polyamines in spermiogenesis: Not now, darling. Proc Natl Acad Sci U S A 2000;97:4421-3.
13. Weiner KX, Dias JA. Regulation of ovarian ornithine decarboxylase activity and its mRNA by gonadotropins in the immature rat. Mol Cell Endocrinol 1993;92:195-9.
14. Kaipia A, Toppari J, Mali P, Kangasniemi M, Alcivar AA, Hecht NB, *et al.* Stage-and cell-specific expression of the ornithine decarboxylase gene during rat and mouse spermatogenesis. Mol Cell Endocrinol 1990;73:45-52.
15. Halmekytö M, Alhonen L, Wahlfors J, Sinervirta R, Eloranta T, Jänne J. Characterization of a transgenic mouse line over-expressing the human ornithine decarboxylase gene. Biochem J 1991;278:895-8.
16. Mallidis C. European society for human reproduction and embryology. Diabetes Linked to Male Infertility; Excess Sugars in the Body Have Direct Effect on Sperm Quality. Rockville, Maryland ,Washington: Science Daily; 2008.
17. Dinulovic D, Radonjic G. Diabetes mellitus/male infertility. Arch Androl 1990;25:277-93.

18. Cohen SS. A Guide to the Polyamines. Oxford: Oxford University Press; 1998.
19. World Health Organization. Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. New York: Cambridge University Press; 2010.
20. Lie PP, Cheng CY, Mruk DD. Coordinating cellular events during spermatogenesis: A biochemical model. Trends Biochem Sci 2009;34:366-73.
21. Pegg AE. Recent advances in the biochemistry of polyamines in eukaryotes. Biochem J 1986;234:249-62.
22. Marton LJ, Pegg AE. Polyamines as targets for therapeutic intervention. Annu Rev Pharmacol Toxicol 1995;35:55-91.
23. Slocum RD. Tissue and subcellular localization of polyamines and enzymes of polyamine metabolism. In: Slocum RD, Flores HE, editors. Biochemistry and Physiology of Polyamines in Plants. Boca Raton, FL: CRC Press; 1991. p. 23-40.
24. Hayashi S, Murakami Y. Rapid and regulated degradation of ornithine decarboxylase. Biochem J 1995;306:1-10.
25. Barták V. Sperm quality in adult diabetic men. Int J Fertil 1979;24:226-32.
26. Padrón RS, Dambay A, Suárez R, Más J. Semen analyses in adolescent diabetic patients. Acta Diabetol Lat 1984;21:115-21.
27. Ali ST, Shaikh RN, Siddiqi NA, Siddiqi PQ. Semen analysis in insulin-dependent/non-insulin-dependent diabetic men with/without neuropathy. Arch Androl 1993;30:47-54.
28. Niven MJ, Hitman GA, Badenoch DF. A study of spermatozoal motility in Type 1 diabetes mellitus. Diabet Med 1995;12:921-4.
29. Ranganathan P, Mahran AM, Hallak J, Agarwal A. Sperm cryopreservation for men with nonmalignant, systemic diseases: A descriptive study. J Androl 2002;23:71-5.
30. Delfino M, Imbrogno N, Elia J, Capogreco F, Mazzilli F. Prevalence of diabetes mellitus in male partners of infertile couples. Minerva Urol Nefrol 2007;59:131-5.
31. Hakovirta H, Keiski A, Toppari J, Halmekytö M, Alhonen L, Jänne J, *et al.* Polyamines and regulation of spermatogenesis: Selective stimulation of late spermatogonia in transgenic mice overexpressing the human ornithine decarboxylase gene. Mol Endocrinol 1993;7:1430-6.
32. Tosaka Y, Tanaka H, Yano Y, Masai K, Nozaki M, Yomogida K, *et al.* Identification and characterization of testis specific ornithine decarboxylase antizyme (OAZ-t) gene: Expression in haploid germ cells and polyamine-induced frameshifting. Genes Cells 2000;5:265-76.
33. Murakami Y, Ichiba T, Matsufuji S, Hayashi S. Cloning of antizyme inhibitor, a highly homologous protein to ornithine decarboxylase. J Biol Chem 1996;271:3340-2.
34. Matsufuji S, Miyazaki Y, Kanamoto R, Kameji T, Murakami Y, Baby TG, *et al.* Analyses of ornithine decarboxylase antizyme mRNA with a cDNA cloned from rat liver. J Biochem 1990;108:365-71.
35. Ivanov IP, Gesteland RF, Atkins JF. A second mammalian antizyme: Conservation of programmed ribosomal frameshifting. Genomics 1998;52:119-29.
36. Zhu C, Lang DW, Coffino P. Antizyme2 is a negative regulator of ornithine decarboxylase and polyamine transport. J Biol Chem 1999;274:26425-30.
37. Chen H, MacDonald A, Coffino P. Structural elements of antizymes 1 and 2 are required for proteasomal degradation of ornithine decarboxylase. J Biol Chem 2002;277:45957-61.
38. Snapir Z, Keren-Paz A, Bercovich Z, Kahana C. Antizyme 3 inhibits polyamine uptake and ornithine decarboxylase (ODC) activity, but does not stimulate ODC degradation. Biochem J 2009;419:99-103.
39. Albeck S, Dym O, Unger T, Snapir Z, Bercovich Z, Kahana C. Crystallographic and biochemical studies revealing the structural basis for antizyme inhibitor function. Protein Sci 2008;17:793-802.
40. He Y, Suzuki T, Kashiwagi K, Igarashi K. Antizyme delays the restoration by spermine of growth of polyamine-deficient cells through its negative regulation of polyamine transport. Biochem Biophys Res Commun 1994;203:608-14.
41. Mitchell JL, Judd GG. Antizyme modifications affecting polyamine homoeostasis. Biochem Soc Trans 1998;26:591-5.
42. López-Contreras AJ, Ramos-Molina B, Martínez-de-la-Torre M, Peñafiel-Verdú C, Puelles L, Cremades A, *et al.* Expression of antizyme inhibitor 2 in male haploid germinal cells suggests a role in spermiogenesis. Int J Biochem Cell Biol 2009;41:1070-8.
43. Quemener V, Blanchard Y, Lescoat D, Havouis R, Moulinoux JP. Depletion in nuclear spermine during human spermatogenesis, a natural process of cell differentiation. Am J Physiol 1992;263:C343-7.

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