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Impact of Experimental Diabetes and Insulin Replacement on Epididymal Secretory Products and Sperm Maturation in Albino Rats

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ABSTRACT

The present study is aimed to explore the impact of experimental diabetes and insulin replacement on epididymal secretory products, sperm count, motility, and fertilizing ability in albino rats. Prepubertal and adult male Wistar strain rats were made diabetic with a single intraperitoneal injection of streptozotocin (STZ), at 120 and 65 mg/kg body weight for prepubertal and adult rats, respectively. After 3 days of STZ administration, insulin was given to a group of diabetic rats at a dose of 3 U/100 g body weight, subcutaneously and killed after 20 days of treatment. STZ-diabetes significantly reduced the epididymal tissue concentrations of testosterone, androgen-binding protein, sialic acid, glycerylphosphoryl choline, and carnitine, suggesting its adverse effects on the secretory activity and concentrating capacity of epididymal epithelium. Impaired cauda epididymidal sperm motility and fertility (in vivo) of STZ-diabetic rats imply the defective sperm maturation. Insulin replacement prevented these changes either partially or completely. From the above findings, it is evident that STZ-diabetes has an adverse effect on sperm maturation, which may be due to the decrease in the bioavailability of testosterone and epididymal secretory products. J. Cell. Biochem. 108: 1094–1101, 2009.

KEY WORDS: CARNITINE; DIABETES; EPIDIDYMIS; GLYCERYLPHOSPHORYL CHOLINE; SIALIC ACID; STREPTOZOTOCIN

Both type-1 (insulin-dependent) and type-2 (non-insulin-dependent) diabetes mellitus have adverse effects on sexual and reproductive functions in adolescent boys and men, which include impairment of spermatogenesis, reduced serum testosterone and seminal fluid volume, impotency, loss of libido, ejaculation difficulties, and infertility [Dinulovic and Radonjic, 1990; Steger and Rabe, 1997; Betancourt-Albrecht and Cunningham, 2003]. Studies carried out using semen samples of diabetic patients revealed oligozoospermia, impaired motility of the sperm and increased number of abnormal spermatozoa [Steger and Rabe, 1997; Betancourt-Albrecht and Cunningham, 2003]. Alterations to the seminal characters encountered under diabetic state indicate impaired sperm maturation, which may be due to altered epididymal structure and function. The maintenance of a constant internal milieu in the epididymal lumen is regulated by androgens, which influence the capacity of epididymal epithelium for absorption and secretion [Ezer and Robaire, 2002; Wong et al., 2002]. Subnormal androgenic status prevailing in diabetic patients [Dinulovic and Radonjic, 1990; Steger and Rabe, 1997; Sudha et al., 2000; Betancourt-Albrecht and Cunningham, 2003] was found to be the most probable cause for the impairment of secretory activity of epididymal epithelium and impeding sperm maturation. Hence, it is hypothesized that the epididymal function may undergo modification under diabetic state owing to subnormal androgenic status [Dinulovic and Radonjic, 1990; Steger and Rabe, 1997; Sudha et al., 2000; Betancourt-Albrecht and Cunningham, 2003] and altered epididymidal histoarchitecture [Soudamani et al., 2005]. To test this hypothesis, the present study was designed to delineate the impact of a diabetogenic agent, Streptozotocin (STZ)-induced diabetes and insulin replacement on the secretory products such as sialic acid, glycerylphosphoryl choline (GPC), and carnitine.

Sialic acid binds to the sperm surface as a terminal sugar of sialoglycoprotein and increases the negative surface charge on sperm during their epididymal sojourn [Yanagimachi et al., 1972]. Sialic acid also plays a major role in stabilizing the plasma and acrosomal membranes of the sperm, in maintaining the spermatozoa in a decapitated state, regulating the ionic balance in the epididymal fluid, and in the antigenic interaction between the sperm and the

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epididymal epithelium [Yanagimachi et al., 1972; Rajalakshmi et al., 1976]. Sialic acid acts as a lubricant, immunoprotectant, and helps in facilitating the downward transport of spermatozoa from the caput epididymis [Yanagimachi et al., 1972; Rajalakshmi et al., 1976]. GPC, another secretory product of epididymis, maintains osmotic pressure balance in the lumen, and plays an important role in the maintenance of epididymal pH and sperm membrane stability by inhibiting phospholipase A_2 activity, which is involved in acrosomal reaction [Robaire and Hermo, 1988; Mitra and Chowdhury, 1994]. Fatty acids released during GPC synthesis have been proposed to be utilized by spermatozoa as the energy source [Mitra and Chowdhury, 1994]. Seminal GPC is considered as an index of epididymal secretory activity [Robaire and Hermo, 1988]. Among the secretory products, carnitine, an essential cofactor, was found to be involved in the mitochondrial transport and β-oxidation, thus producing acetyl-CoA [Robaire and Hermo, 1988], which acts as a substrate for the oxidative process that produces energy for the sperm forward motility [Pruneda et al., 2007]. A positive correlation among seminal carnitine with sperm motility, number of motile spermatozoa, and fertility rate has been reported previously [Jeulin and Lewin, 1996]. Quantification of these secretory products will pave way for a better understanding with regard to the reproductive disorders prevailing in diabetic men. In addition, sperm count, sperm forward motility, sperm abnormalities, and fertility test (in vivo) were undertaken to delineate the impact of diabetes on fertility.

MATERIALS AND METHODS

ANIMALS

Healthy prepubertal (40 days old; 70–80 g) and adult (100 days old; 180–200 g) male Wistar strain rats (*Rattus norvegicus*) were used in the present study. Rats were kept in clean cages in a temperature-controlled environment with 12 h light/dark cycle. This study was approved by the Institutional Animal Ethical Committee (IAEC). Both prepubertal (18) and adult (18) rats were divided into the following groups, each consisting of six rats. Control: Rats received vehicle (0.1 M citrate buffer, pH 4.5) alone. Diabetic: Rats were given a single intraperitoneal injection of STZ at a dose of 120 mg/kg body weight for prepubertal and 65 mg/kg body weight for adult rats, dissolved in 0.1 M citrate buffer, after overnight fasting. Insulin-treated diabetic: After 3 days of STZ administration, one set of diabetic rats was given insulin (Lente: Porcine origin) at a dose of 3 U/100 g body weight, subcutaneously, daily in two equally divided doses at 8.00 a.m. and 6.00 p.m. After 20 days of treatment, rats were killed by decapitation. Both the epididymes were gently dissected out, cleaned off from adhering fat and connective tissues, and weighed accurately on a torsion balance. After weighing, each epididymis was divided into caput, corpus, and caudal segments, in accordance with the guidelines of Hamilton [1975].

SEPARATION OF EPIDIDYMAL SPERM AND TISSUE

Epididymal spermatozoa were separated according to the method of Brooks [1981], and the sperm-free epididymidal tissues were used for assays of various biochemical parameters.

ASSAY OF EPIDIDYMIDAL TISSUE TESTOSTERONE

Steroids from epididymidal tissues were extracted using ethyl acetate/iso-octane (1.1) mixture according to the method of Jean-Faucher et al. [1985]. After evaporation, the residue was dissolved in steroid hormone assay buffer and used for the estimation of testosterone using a solid-phase RIA kit obtained from DiaSorin (Italy).

ASSAY OF EPIDIDYMIDAL ANDROGEN-BINDING PROTEIN

The concentration of androgen-binding protein (ABP) was estimated using the method of Danzo et al. [1990]. Specific binding was determined by subtracting the amount of radioactivity in the tubes containing unlabelled 5α-DHT from that in the tubes containing [^{3}H]5α-DHT alone.

ASSAY OF BIOCHEMICAL PARAMETERS

Sialic acid was estimated by the method of Warren [1959]. GPC concentration was determined following the method of Wirthensohn and Guder [1985]. Carnitine was estimated using the method of Pearson et al. [1974].

SPERM COUNT

Epididymidal spermatozoa were counted in accordance with the method of Zaneveld and Polakoski [1977].

CAUDA SPERM FORWARD MOTILITY

After the rats were anesthetized, epididymis was exposed by scrotal incision, and the spermatozoa were expressed out by cutting the distal end of the cauda epididymidal tubule. Spermatozoa with epididymal fluid was diluted with physiological saline and placed on a thin glass slide, and the forward motility (rate) of 100 spermatozoa/rat was observed under microscope using precalibrated ocular micrometer [Ratnasooriya, 1984].

FERTILITY ASSESSMENT (IN VIVO)

Three batches of experiments (control/STZ-diabetic/insulin replaced) were conducted, and each batch consisted of six adult male rats. Two proven fertile proestrus female rats were cohabitated with one adult male rat and were observed for the presence of spermatozoa in the vaginal smear or vaginal plug to confirm mating. Rats that became pregnant were taken into consideration to assess the fertility of male rats.

STATISTICAL ANALYSIS

Data were analyzed using one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test to assess the significance between control and experimental groups by using a computer-based software, SPSS (version 7.5).

RESULTS

BIOAVAILABILITY OF TESTOSTERONE

In control rats of both age groups, caput had the maximal concentration of testosterone followed by corpus and caudal segments. Induction of diabetes resulted in a significant decrease in

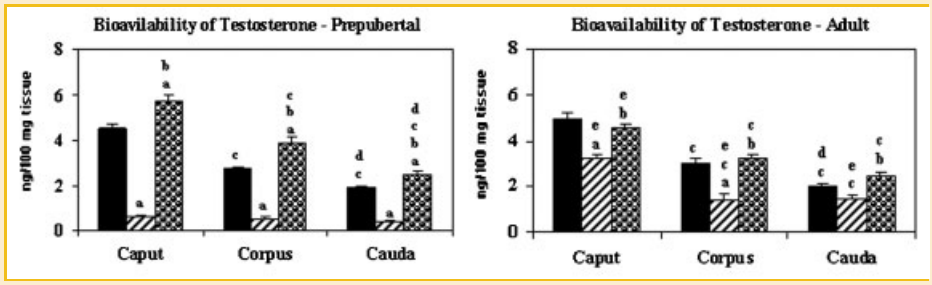

Fig. 1. Effect of STZ-diabetes and insulin replacement on the bioavailability of testosterone in the caput, corpus, and cauda epididymides of prepubertal and adult rats. (■) Control, (▨) STZ-diabetic, (▩) insulin replacement. Each bar represents mean ± SEM of six animals. Significance at $P < 0.05$. a: versus control; b, versus STZ-diabetic; c, versus caput; d, versus corpus; e, versus prepubertal.

the bioavailability of testosterone in all the three segments of prepubertal and adult rats, and the magnitude of diminution was comparatively greater in prepubertal rats. Insulin replacement on the other hand, elevated the bioavailability of testosterone in all the three segments in prepubertal rats while in adults, insulin replacement prevented the diminution in the epididymal tissue concentration of testosterone and maintained the same in par with control rats (Fig. 1).

ABP

In control rats, regional differences in ABP concentration occur irrespective of the age. At both ages, caput had the maximal concentration of ABP. In control rats, an age-dependent increase in ABP concentration was evident only in corpus region. After STZ treatment, there was a significant decrease in ABP concentration in all the three segments of prepubertal and adult rats. Insulin replacement prevented the decrease in ABP concentration in the corpus of prepubertal rats and in the caput and corpus segments of adult rats. However, in the caput of prepubertal rats and caudal segment of adult rats, it was able to do so partially. On the other hand, ABP concentration in the caudal segment of prepubertal rats remained unresponsive to insulin replacement (Fig. 2).

SIALIC ACID

In control rats, sialic acid exhibited an age-dependent increase in corpus and caudal segments alone. Among the three segments, caudal segment had the maximal concentration of sialic acid, followed by corpus and caput regions, regardless of the age. STZ-diabetes brought down the levels of sialic acid in all the three-epididymidal regions of prepubertal and adult rats. Insulin replacement prevented the diminution of sialic acid in the caput region of adults alone. In the corpus and caudal segments of adult rats and all the three segments of prepubertal rats, sialic acid concentration was only partially maintained (Fig. 3).

GPC

An age-dependent increase in GPC concentration was evident in all the three segments. In both prepubertal and adult rats, GPC concentration was greater in caput, followed by cauda and then corpus segment. STZ-diabetes decreased the levels of GPC, regardless of age and segment. After insulin replacement, GPC concentration was partially maintained in the caput and caudal segments of prepubertal and adult rats. In the corpus segment of adult rats, GPC concentration was maintained at the normal level, whereas in the same region of prepubertal rats, insulin replacement failed to prevent the decrease in GPC concentration (Fig. 4).

CARNITINE

Similar to sialic acid, carnitine also exhibited an age-dependent increase in all the three segments. In both prepubertal and adult control rats, caudal segment had the maximal concentration of carnitine. STZ-diabetic and insulin-replaced groups of adult rats

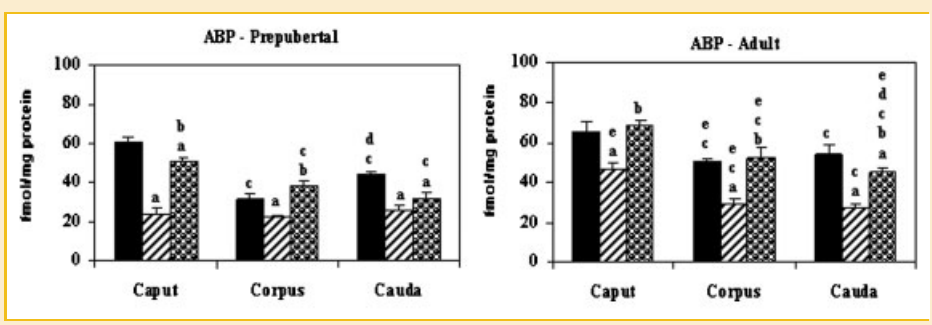

Fig. 2. Effect of STZ-diabetes and insulin replacement on androgen-binding protein (ABP) in the caput, corpus, and cauda epididymides of prepubertal and adult rats. (■) Control, (▨) STZ-diabetic, (▩) insulin replacement. Each bar represents mean ± SEM of six animals. Significance at $P < 0.05$. a, versus control; b, versus STZ-diabetic; c, versus caput; d, versus corpus; e, versus prepubertal.

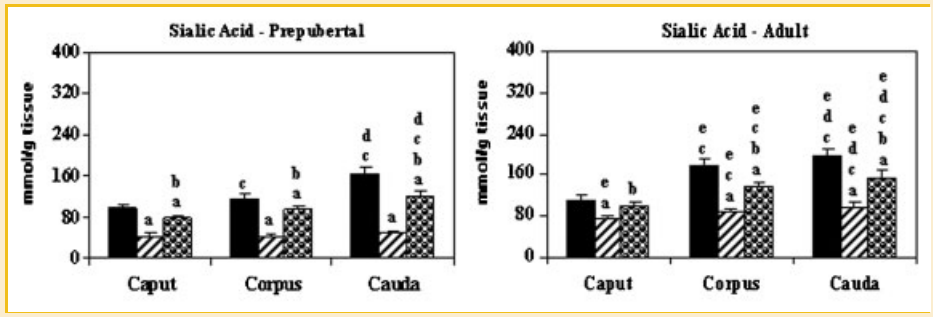

Fig. 3. Effect of STZ-diabetes and insulin replacement on sialic acid concentration in the caput, corpus, and cauda epididymides of prepubertal and adult rats. (■) Control, (▨) STZ-diabetic, (▩) insulin replacement. Each bar represents mean ± SEM of six animals. Significance at $P < 0.05$. a, versus control; b, versus STZ-diabetic; c, versus caput; d, versus corpus; e, versus prepubertal.

also exhibited a similar trend (i.e., increase in the caudal segment) compared with other segments. STZ-diabetes resulted in a significant decrease in carnitine concentration in all the segments of prepubertal rats and in the caudal segment of adult rats. Insulin replacement prevented the decrease in carnitine concentration in the corpus segment of prepubertal and in the caudal segment of adult rats. Whereas in the caudal segment of prepubertal rats, insulin replacement was able to prevent the diminution in carnitine concentration only partially (Fig. 5).

SPERM CONTENT AND CAUDA EPIDIDYMIDAL SPERM FORWARD MOTILITY

In adult control rats, sperm content was greater in the cauda segment followed by caput and corpus segments. Upon induction of diabetes, sperm content was significantly reduced, irrespective of the regions. Insulin replacement prevented the decrease in sperm content only in the caput segment, whereas in the corpus and caudal segments it was only partial. Cauda epididymidal sperm forward motility declined in STZ-diabetic rats, which was prevented by the immediate replacement of insulin (Fig. 6).

FERTILITY INDEX

STZ-diabetes decreased the fertility rate, which is reflected in the number of animals became pregnant. Insulin replacement prevented the decrease in the fertilizing ability of diabetic rats (Fig. 7).

DISCUSSION

Epididymis derives testosterone from the circulation and testicular fluid [Ezer and Robaire, 2002]. STZ-induced diabetes exerted an adverse effect on the tissue concentration of testosterone. The quantum of decrease in the bioavailability of testosterone varied with age and the impact being greater in prepubertal rats. In the adult rats, even though such an adverse effect of diabetes was evidenced, there was a segmental variation in the bioavailability of testosterone, suggesting specific uptake of testosterone by caput, corpus, and caudal segments. Local availability of testosterone at the level of epididymis and its conversion to DHT are indispensable for the maintenance of normal structure and function of epididymis [Ezer and Robaire, 2002; Wong et al., 2002]. Decreased bioavailability of testosterone in caput, corpus and cauda epididymides may be attributed to the decreased synthesis, secretion and transport of testosterone. Impaired steroidogenic function of Leydig cells in diabetic rats [Benitez and Perez-Diaz, 1985; Murray et al., 1985; Sudha et al., 2000], reduced secretion of testosterone into the testicular interstitial fluid and decreased concentration of intratesticular testosterone [Sudha et al., 2000] lend support to the above view. Reduced concentration of epididymidal ABP observed in the present study also strengthens the view of reduced transport of testosterone to the epididymis. Insulin replacement, surprisingly, enhanced the bioavailability of testosterone more than the control. This may be due to the reduced activity of 5α-reductase or

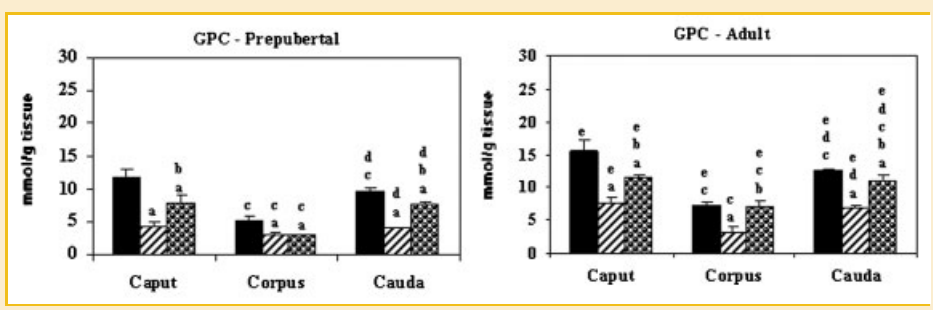

Fig. 4. Effect of STZ-diabetes and insulin replacement on GPC concentration in the caput, corpus, and cauda epididymides of prepubertal and adult rats. (■) Control, (▨) STZ-diabetic, (▩) insulin replacement. Each bar represents mean ± SEM of six animals. Significance at $P < 0.05$. a, versus control; b, versus STZ-diabetic; c, versus caput; d, versus corpus; e, versus prepubertal.

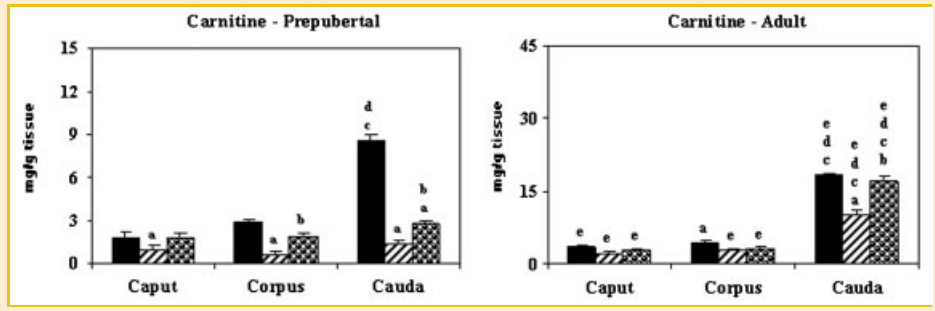

Fig. 5. Effect of STZ-diabetes and insulin replacement on carnitine concentration in the caput, corpus, and cauda epididymides of prepubertal and adult rats. (■) Control, (▨) STZ-diabetic, (▩) insulin replacement. Each bar represents mean ± SEM of six animals. Significance at $P < 0.05$. a, versus control; b, versus STZ-diabetic; c, versus caput; d, versus corpus; e, versus prepubertal.

aromatase that caused the accumulation of testosterone. Enhanced bioavailability also underscores the existence of local regulatory mechanisms in the epididymis. Prevention of adverse effects on testosterone bioavailability by insulin replacement ascertains the role of insulin on the synthesis, secretion, and transport of testosterone.

The data on ABP concentration revealed an age-dependent, segment-specific variation in control, STZ-diabetic and insulin-replaced groups of rats. The reduction in ABP concentration in caput, corpus, and caudal segments of the prepubertal and adult rats under diabetic state may be due to the decreased synthesis and secretion of ABP. Sertoli cells are the only source of ABP [Gunsalus and Bardin, 1991], and they secrete 20% of the available ABP across the basal membranes into the interstitial compartment and 80% into the lumen of the seminiferous tubules [Gunsalus and Bardin, 1991; Munell et al., 2002]. ABP is then transported via the rete testis and efferent ductules into the caput and cauda epididymides [Jeyaraj et al., 2005]. Both in vivo and in vitro studies have shown that the synthesis and secretion of ABP is regulated by androgens and follicle stimulating hormone (FSH) [Gunsalus and Bardin, 1991; Munell et al., 2002; Jeyaraj et al., 2005]. A previous study from this laboratory has shown decreased serum testosterone and FSH titers in diabetic rats [Sudha et al., 2000]. Therefore, the reduced concentration of ABP in epididymidal segments may be due to the cumulative effect of reduced androgenic and FSH stimulation on the production of ABP.

It has already been reported that the tubules of the caput epididymidis accumulate ^{3}H-testosterone more efficiently from the luminal surface in the presence of ABP [Danzo and Eller, 1976]. The function of ABP is to establish and maintain the high androgen levels required for sperm maturation in the epididymis [Gunsalus and Bardin, 1991; Munell et al., 2002]. Therefore, in the present study, the reduction in ABP concentration observed under diabetic state may have curtailed the transport of testosterone along the epididymidal segments, enforced changes in the binding, internalization and the subsequent delivery of 5α-DHT to the epididymidal tissue and sperm, and impeded with sperm maturational events. In the present study, partial restoration of normal serum testosterone titers observed in the insulin-replaced group of rats as well as in earlier studies [Benitez and Perez-Diaz, 1985; Murray et al., 1985; Sudha et al., 2000; Betancourt-Albrecht and Cunningham, 2003] may be responsible for the partial recovery of ABP in caput, corpus, and caudal segments of the epididymis. Probably, a critical intratesticular concentration of testosterone is required to ensure the normal transport of ABP from the testis to the epididymis.

The data with regard to sialic acid revealed a gradual increase in its concentration along the length of the epididymis with a maximum level being observed in the cauda epididymidal segment of both prepubertal and adult control rats. Sialic acids, free or bound to proteins as sialomucoproteins, are secreted by the epididymis of the rat, hamster, rabbit, monkey, and human [Yanagimachi et al.,

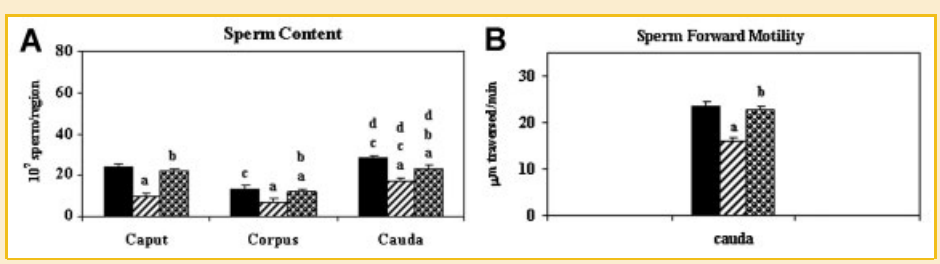

Fig. 6. Effect of STZ-diabetes and insulin replacement on sperm content (A) in the caput, corpus, and cauda epididymides and cauda epididymidal sperm forward motility (B) of adult rats. (■) Control, (▨) STZ-diabetic, (▩) insulin replacement. Each bar represents mean ± SEM of six animals. Significance at $P < 0.05$. a, versus control; b, versus STZ-diabetic; c, versus caput; d, versus corpus.

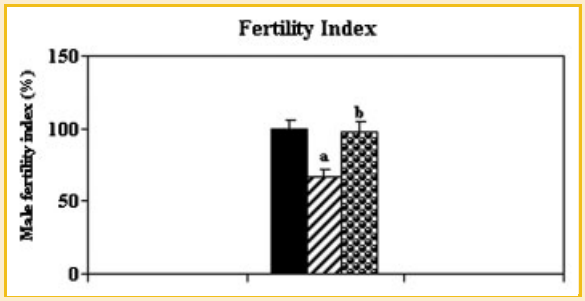

Fig. 7. Effect of STZ-diabetes and insulin replacement on the fertility index of adult male rats. (■) Control, (▨) STZ-diabetic, (▩) insulin replacement. Each bar represents mean ± SEM of six animals. Significance at $P < 0.05$. a, versus control; b, versus STZ-diabetic.

1972; Rajalakshmi et al., 1976]. In support of the present findings, earlier studies have shown that the level of sialic acid in the epididymal plasma was maximal in the cauda epididymidis [Yanagimachi et al., 1972; Rajalakshmi et al., 1976]. It is the region between the distal corpus and the proximal cauda of rat epididymis, when spermatozoa migrating along the duct sequentially encounter a sudden increase in the potential for motility and the development for fertilizing ability [Casillas, 1973]. Therefore, high levels of sialic acid in the caudal segment affirm maximal synthesis in this segment in view of a role for sialoproteins in the development and maintenance of the fertilizing ability of spermatozoa.

GPC levels are considered as an index of epididymal secretory activity [Robaire and Hermo, 1988]. The decrease in GPC concentration under diabetic state may be due to its impaired synthesis. The activity of phospholipase A2, which is responsible for the first hydrolytic step in the conversion of lecithin to GPC, is androgen-dependent [Wang et al., 1981]. As the synthesis and secretion of GPC are under androgenic control [Bjerve and Reitan, 1978; Robaire and Hermo, 1988], the decrease in the bioavailability of testosterone, observed in the current study, as well as serum testosterone and DHT levels, as reported earlier [Sudha et al., 2000], may be responsible for the decreased GPC concentration.

The normal flow of fluid and sperm concentration have been suggested to promote the synthesis of GPC [Robaire and Hermo, 1988]. Therefore, in the present study, the fall in sperm concentration observed after the induction of STZ-diabetes may also be correlated with the reduced concentration of GPC. As GPC also plays a role in maintaining the epididymal luminal fluid osmolarity [Robaire and Hermo, 1988] as well as in stabilizing the spermatozoal membrane [Mitra and Chowdhury, 1994], the low level of GPC observed after the induction of STZ-diabetes may have an adverse effect on the epididymal milieu making it not conducive for sperm maturation.

Accumulation of carnitine in the epididymis [Pruneda et al., 2007] and the secretion of acetyl carnitine in the epididymal fluid [Jeulin and Lewin, 1996] are shown to be associated with the acquisition of flagellar movement and sperm motility. The data on carnitine concentration revealed an age-dependent increase in the control, diabetic and insulin-replaced groups of rats. Among the regions, caudal segment exhibited the maximal concentration of carnitine. This is the area where there is a sudden increase in the potential for the motility of spermatozoa [Casillas, 1973]. This indicates that the accumulation of carnitine may directly be associated with the acquisition of flagellar movement [Jeulin and Lewin, 1996]. As carnitine is one of the factors responsible for sperm motility, any agent that decreases the level of carnitine may have definite impact on sperm motility. In diabetic rats, among the three regions, even though the caudal segment had maximal concentration of carnitine, the level was significantly lower than that of control rats. Therefore, in the present study, low levels of carnitine observed in STZ-induced diabetic rats could be one of the reasons for the impaired cauda epididymidal sperm motility in these animals.

Further, the accumulating mechanism of carnitine is also influenced by circulating androgens [Jeulin and Lewin, 1996; Pruneda et al., 2007] and androgen bound to the intraluminal ABP [Böhmer and Hansson, 1975]. Therefore, in the present study, the reduced concentration of carnitine in the caput, corpus and cadual segments may be due to the impaired uptake of carnitine from circulation or reduced concentrating capacity of epididymal epithelial cells, which may be secondary to the consequence of STZ-induced deprivation in serum testosterone and DHT levels [Benitez and Perez-Diaz, 1985; Murray et al., 1985; Sudha et al., 2000]. Low levels of epididymidal testosterone and ABP could also be the contributing factors for the low levels of carnitine observed in diabetic rats.

Sperm content was significantly reduced in caput, corpus, and caudal segments of adult diabetic rats, which is consistent with the clinical and experimental evidences showing oligozoospermia [Murray et al., 1985; Dinulovic and Radonjic, 1990; Steger and Rabe, 1997; Betancourt-Albrecht and Cunningham, 2003]. The reduced sperm content in caput, corpus, and cauda epididymides implies an adverse effect of STZ-induced diabetes on spermatogenesis. In support of this, histological studies undertaken in the testis of diabetic men and animals illustrate the spermatogenic arrest at the level of primary spermatocytes, desquamation of immature germ cells, malformation of elongated spermatids and loss of germ cells [Murray et al., 1985; Dinulovic and Radonjic, 1990; Steger and Rabe, 1997; Betancourt-Albrecht and Cunningham, 2003].

After spermatozoa leave the testis in an immature state, they undergo maturational changes and acquire the potential for progressive forward motility during passage through the caput and corpus epididymides [Yeung and Cooper, 2002]. Epididymal secretory products furnish a microenvironment that is considered vital for the acquisition of motility and viability of sperm [Wong et al., 2002; Yeung and Cooper, 2002]. Immature spermatozoa swim poorly or not at all, and mature spermatozoa express a high degree of progressive forward motility [Robaire and Hermo, 1988; Wong et al., 2002; Yeung and Cooper, 2002]. In the present study, the diminished forward motility of spermatozoa obtained from caudal segment is in correlation with the clinical observation of asthenozoospermia in diabetic patients [Dinulovic and Radonjic, 1990; Steger and Rabe, 1997; Betancourt-Albrecht and Cunningham, 2003]. Gatti et al. [2004] reported that sperm forward motility is the result of a balance between maturation of the flagellum and

inhibition of the flagellar machinery that keeps spermatozoa in an immotile state.

In the present study, the decreased fertility of diabetic rats as assessed by in vivo fertility test is probably the result of not only reduced sperm production, but also the abnormal spermatozoa and their impaired forward motility, which might have rendered them incapable of fertilizing the ova. The reduced synthesis or uptake of a number of secretory products such as sialic acid, GPC, and carnitine as well as defective sperm motility are clear manifestations of altered epididymal milieu and impaired sperm maturation, which may be a plausible reason for the diminished fertility of STZ-treated rats. Immediate insulin replacement helps to maintain these secretory products partially or completely in an age- and region-specific manner. Sperm forward motility and fertility (in vivo) remained normal in insulin-replaced group of rats.

CONCLUSION

On the basis of the present study, it is concluded that STZ-induced diabetes has an adverse effect on the epididymal secretory products, which may be due to subnormal androgenic status. The modified composition of secretory products, lead to defective sperm maturation and this may be responsible for the reduced fertility of STZ-diabetic rats. Amelioration of these changes either partially or completely by insulin replacement suggests that insulin is one of the hormones required along with testosterone for sperm maturational events.

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