ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

ORIGINAL ARTICLE

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Keywords:
male infertility, mitochondrial function, type 1 diabetes mellitus

Received: 7-Apr-2015
Revised: 1-Jul-2015
Accepted: 21-Jul-2015

doi: 10.1111/andr.12097

Reproductive function in male patients with type 1 diabetes mellitus

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SUMMARY

This study was undertaken to evaluate conventional and some of the main bio-functional spermatozoa parameters, serum gonadal hormones and didymo-epididymal ultrasound features in patients with type 1 diabetes mellitus (DM1). DM1 affects an increasing number of men of reproductive age. Diabetes may affect male reproduction by acting on the hypothalamic–pituitary–testicular axis, causing sexual dysfunction or disrupting male accessory gland function. However, data on spermatozoa parameters and other aspects of the reproductive function in these patients are scanty. Thirty-two patients with DM1 [27.0 (25.0–30.0 years)] and 20 age-matched fertile healthy men [28.0 (27.25–30.75 years)] were enrolled. Patients with diabetic neuropathy, other endocrine disorders or conditions known to alter spermatozoa parameters were excluded. Each subject underwent semen analysis, blood withdrawal for fasting and post-prandial glycaemia, hormonal analysis and didymo-epididymal ultrasound evaluation before and after ejaculation. Patients with DM1 had a lower percentage of spermatozoa with progressive motility [10.0 (7.0–12.75) vs. 45.0 (42.0–47.75) %; $p < 0.01$] and a higher percentage of spermatozoa with abnormal mitochondrial function than controls [47.0 (43.0–55.0) vs. 2.0 (1.0–5.0) %; $p < 0.01$]. Patients also had greater post-ejaculatory diameters of cephalic [11.5 (10.2–13.6) vs. 6.0 (4.0–7.0) mm; $p < 0.01$] and caudal epididymis [5.5 (4.00–7.55) vs. 3.0 (2.0–4.0) mm; $p < 0.01$] compared to controls, suggesting a lack of the physiological post-ejaculation epididymal shrinkage. Correlation analysis suggested that progressive motility was associated with fasting glucose ($r = -0.68$; $p < 0.01$). The other parameters did not show any significant difference. Patients with DM1 had a lower percentage of spermatozoa with progressive motility, impaired mitochondrial function and epididymal post-ejaculatory dysfunction. These findings may explain why patients with DM1 experience fertility disturbance. Larger multi-centric studies are necessary to confirm these results.

INTRODUCTION

Currently, diabetes mellitus (DM) is one of the most common diseases, which threatens the health of the world population. More than 346 million people worldwide are living with diabetes and this figure is expected to double by 2030 without global intervention. In Italy, in 2011, nearly 3 million people (4.9%) had diabetes (http://www.salute.gov.it/; Bruno *et al.*, 2005; Diaz-Valencia *et al.*, 2015).

The prevalence of DM increases with age: among the people older than 75 years, at least one in five people is affected; under 74 years, diabetes is more prevalent among men. However, in recent years, the average age at diagnosis is dropping more and more, especially for type 1 DM (DM1). Indeed, more than 90% of these patients are diagnosed before the age of 30. In Italy, about 300 000 people have DM1 and the incidence of this condition is increasing worldwide. Between 2001 and 2009, the prevalence of DM1 in people under 20 years increased by 23% – a growth rate of 3% each year. Therefore, a correct assessment of these patients, in addition to all the well-known complications related to DM, should include the evaluation of the reproductive function (http://www.salute.gov.it/; Bruno *et al.*, 2005; Diaz-Valencia *et al.*, 2015).

Recently, the number of live births in a population-based, retrospective cohort of men with childhood-onset DM1 (2819 men) was investigated. The results of this study showed that men with diabetes had a smaller number of live births than the controls; the hazards ratio of having a first child for diabetic men compared with controls was 0.77 (95% CI 0.72, 0.83). Later age at onset of diabetes was associated with a higher rate of having a first child among men ($p = 0.04$) (Sjöberg *et al.*, 2013).

Despite these premises, knowledge of spermatozoa parameters in patients with DM1 is very scanty and mostly limited to conventional spermatozoa parameters. Therefore, the aim of this study was to evaluate the effect of DM1 on both conventional and some of the main biofunctional spermatozoa parameters. In

particular, mitochondrial function, spermatozoa apoptosis and chromatin/DNA integrity were evaluated. Furthermore, didymo-epididymal morphometry (carried out before and after ejaculation) was performed by ultrasound scan before and after ejaculation.

MATERIALS AND METHODS

The study was conducted in 32 patients with DM1 and 20 healthy fertile (pregnancy in the previous 6 months) men (controls), aged 18–35 years. Patients with one of the following conditions were excluded from the study:

- Chronic complications of diabetes: neuropathy, nephropathy, retinopathy.
- Dyslipidemia.
- Hypertension.
- Body mass index ≥30.0 kg/m^2.
- Presence of mono- and/or bilateral varicocoele.
- Diagnosis of male accessory gland infection/inflammation.
- Congenital and/or acquired obstruction of proximal and/or distal spermatozoa ducts.
- Testicular volume <12 mL (according to Prader's orchidometer).
- Hypogonadism = total testosterone <3 ng/mL or 10.4 nM (Dandona & Rosenberg, 2010), hyperprolactinemia = a level above the upper limit of normal after a single measurement of serum levels (Melmed *et al.*, 2011), hyperestrogenism = >42 pg/mL (Reinsberg *et al.*, 2009), thyroid disorders (Völzke *et al.*, 2007).
- Cryptorchidism.
- Cigarette smoking.
- Recent drugs and/or alcohol abuse.

Two semen samples were obtained from each patient and control. A blood sample was collected from the antecubital vein, at 8.30 AM, to measure serum concentrations of luteinizing hormone, follicle stimulating hormone, testosterone, 17ß-estradiol, prolactin and glycated hemoglobin (HbA1c). Fasting glycemia and post-prandial plasma glucose levels (2 h after lunch) were detected for each patient.

The following biofunctional spermatozoa parameters were assessed by flow cytometry analysis: (i) mitochondrial membrane potential (MMP); (ii) early signs of apoptosis by evaluating phosphatidylserine (PS) externalization; (iii) degree of chromatin compaction; and (iv) DNA fragmentation. Ultrasound evaluation of the testicular volume and epididymal diameters was performed, before and after ejaculation, by the same clinician (SLV).

All subjects signed a written informed consent before enrolment and they were aware that their data would be used for clinical research purposes. The protocol was approved by the internal review board (approval no. 01/2013).

Spermatozoa analysis

Semen samples were collected by masturbation into a sterile container after 3–5 days of sexual abstinence. After liquefaction, semen was analyzed according to the World Health Organization criteria (WHO, 2010).

Flow cytometric spermatozoa analysis

The analysis was conducted with an EPICS XL Flow Cytometer (Coulter Electronics, IL, Milan, Italy), equipped with an argon laser at 48 nm and three fluorescence detectors: green (FL-1 at 525 nm), orange (FL-2 to 575 nm), and red (FL-3 at 620 nm), as reported previously (Perdichizzi *et al.*, 2007). For each sample, 100 000 events were measured at a low flow velocity and analyzed using SYSTEM II, version 3.0. Spermatozoa MMP was evaluated after staining with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine chloride (JC-1), PS externalization by double staining with annexin V and propidium iodide (PI), chromatin compactness after PI staining and DNA fragmentation using TUNEL assay.

JC-1 staining

Mitochondrial membrane potential was evaluated by staining with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzimidazolylcarbocyanine chloride (JC-1), a lipophilic probe, as reported previously (Perdichizzi *et al.*, 2007). Briefly, 0.5–1 × 10^6 spermatozoa were incubated for 10–15 min at 37 °C in the dark with JC-1 (Codisan S.p.A. Catania, Italy) and analyzed by flow cytometry.

Annexin V-PI staining

Staining with annexin V/PI was performed using a commercial kit (Annexin-FITC kit, Beckman Coulter S.r.l., Milan, Italy), as previously reported (Perdichizzi *et al.*, 2007). Briefly, 0.5 × 10^6 spermatozoa were re-suspended in 0.5 mL of binding buffer, incubated with 5 μL of annexin V-FITC and 20 μL of PI, for 10–15 min in the dark, and immediately analyzed. Signals were detected through FL-1 (FITC) and FL-3 (PE) detectors. The annexin V/PI analysis identified the different cell populations. In particular, annexin and PI negative spermatozoa were considered viable cells, annexin positive and PI negative were regarded as PS externalized spermatozoa (early apoptotic cells) and annexin and PI positive as late apoptotic cells.

PI staining

Spermatozoa PI staining was performed as reported previously (Perdichizzi *et al.*, 2007). About 1 × 10^6 spermatozoa were incubated in LPR DNA-Prep Reagent containing 0.1% potassium cyanide, 0.1% NaN_3, non-ionic detergents, salts and stabilized (Beckman Coulter S.r.l.) in the dark at room temperature for 10 min. Subsequently, spermatozoa were incubated in Stein DNA-Prep Reagent containing 50 μg/mL of PI (<0.5%), RNAsi type A (4000 U/mL), <0.1% NaN_3, salts and stabilized (Beckman Coulter S.r.l.) in the dark at room temperature for 30 min.

TUNEL assay

TUNEL assay was carried out using the Mebstain Apoptosis TUNEL kit (DBA, Milan, Italy), as reported previously (Perdichizzi *et al.*, 2007). The negative control was obtained by not adding TdT at the reaction mixture; the positive control was obtained by pre-treating spermatozoa with 1 μg/mL of RNAse-free deoxyribonuclease I (Sigma Chemical, Sigma-Aldrich St. Louis, MO, USA) at 37 °C for 60 min before labeling.

Statistical analysis

All statistical analyses were completed using SPSS v. 19 software (SPSS Inc, IBM Corp, Somers, NY, USA). The continuous variables, presented as median (interquartile range), were tested by Mann–Whitney *U*-test or Kruskal–Wallis test according to their non-normal distribution (normality of variable distribution was tested by Kolmogorov–Smirnov test). Spearman's or Pearson's correlation coefficients were used to test the associations

between different variables. Multivariate linear regression models were performed for factors exhibiting significant correlation. A p value lower than 0.05 was considered statistically significant.

RESULTS

Patients with DM1 had a lower percentage of spermatozoa with progressive motility and a higher percentage of spermatozoa with abnormal mitochondrial function than controls. Patients also had greater post-ejaculatory diameters of cephalic and caudal epididymis compared to controls. The other parameters did not show any significant difference (Table 1).

The percentage of spermatozoa with progressive motility was significantly lower in DM1 patients with a disease duration >10 years compared to patients with shorter disease duration ($p < 0.05$) (Fig. 1). CEAE (caput epididymis after ejaculation) and TEAE (tail epididymis after ejaculation) were significantly higher in DM1 patients with a disease duration >10 years compared to patients with shorter disease duration ($p < 0.05$). Finally, patients with disease duration >5 years had a significantly higher percentage of spermatozoa with low MMP compared to patients with disease duration <5 years (Fig. 2).

On Spearman's correlation analysis, progressive motility was associated with fasting glucose ($r = -0.68$; $p < 0.01$), percentage of spermatozoa with low MMP ($r = -0.62$; $p < 0.01$), CEAE ($r = -0.65$; $p < 0.01$) and TEAE ($r = 0.49$; $p < 0.01$). Fasting glucose was further associated with percentage of spermatozoa with low MMP ($r = 0.70$; $p < 0.01$), CEAE ($r = 0.68$; $p < 0.01$) and TEAE ($r = 0.58$; $p < 0.01$).

Moreover, daily number of insulin units, HbA1C levels and duration of diabetes were not associated with progressive motility and percentage of spermatozoa with low MMP.

Multivariate linear regression analysis adjusted for clinical and endocrine variables showed that progressive motility was associated with fasting glucose ($r = -0.32$; $p < 0.01$) and CEAE ($r = -0.37$; $p < 0.01$) and low MMP was associated with CEAE ($r = 0.53$; $p < 0.01$) and TEAE ($r = 0.50$; $p < 0.01$).

DISCUSSION

We found that patients with DM1 had lower spermatozoa progressive motility compared to healthy, age-matched fertile men. This abnormality is related to fasting glycemia and the reduction becomes more important in patients with diabetes lasting longer than 10 years.

In a previous study, the evaluation of spermatozoa motility parameters, by a computerized image analysis system in 17 patients with DM1, showed no significant alterations compared

Table 1 Main parameters examined of patients with type 1 diabetes mellitus (DM1) and healthy fertile controls

	Controls ($n = 20$)	DM1 ($n = 32$)	p-value
Anthropometric parameters			
Age (years), median (IQR)	28.0 (27.25–30.75)	27.0 (25.0–30.0)	0.30
Weight (kg), median (IQR)	67.0 (64.5–73.5)	70.0 (60.5–76.0)	0.69
Height (m), median (IQR)	174.0 (170.0–177.0)	172.5 (168.5–176.0)	0.30
Body mass index (kg/m^2), median (IQR)	23.0 (21.25–24.75)	25.0 (22.25–27.75)	0.02
Waist circumference (cm), median (IQR)	88.0 (81.25–90.0)	88.0 (86.0–90.0)	0.25
Spermatozoa parameters			
Volume (mL), median (IQR)	2.5 (2.0–16.5)	3.0 (2.0–15.0)	0.97
Concentration (mil/mL), median (IQR)	50.0 (41.25–55.0)	45.0 (40.0–50.0)	0.26
Progressive motility (%), median (IQR)	45.0 (42.0–47.75)	10.0 (7.0–12.75)	<0.01
Normal forms (%), median (IQR)	8.0 (6.0–12.0)	7.0 (6.0–10.0)	0.72
Leucocytes (mil/mL), median (IQR)	0.55 (0.35–0.70)	0.60 (0.50–0.70)	0.96
Hormonal parameters			
LH (UI/L), median (IQR)	2.2 (1.72–2.57)	2.0 (0.8–2.55)	0.22
FSH (UI/L), median (IQR)	2.75 (2.50–3.50)	1.75 (0.32–3.3)	0.30
TT (ng/mL), median (IQR)	6.5 (5.0–8.0)	6.2 (4.8–7.8)	0.30
E2 (pg/mL), median (IQR)	8.0 (6.25–11.5)	8.3 (5.0–13.75)	0.24
PRL (ng/mL), median (IQR)	9.5 (7.25–12.75)	9.0 (6.0–12.0)	0.26
Flow cytometric parameters			
Low MMP (%), median (IQR)	2.0 (1.0–5.0)	47.0 (43.0–55.0)	<0.01
PS.Ext (%), median (IQR)	3.0 (2.0–3.0)	3.0 (2.0–4.0)	0.59
Abnormal chromatin (%), median (IQR)	2.5 (2.0–3.75)	3.0 (2.0–4.0)	0.39
DNAF (%), median (IQR)	2.5 (2.0–3.75)	3.0 (2.0–4.0)	0.46
Ultrasound parameters			
TV (mL), median (IQR)	20.5 (16.25–23.75)	20.0 (16.0–22.0)	0.65
CEBE (mm), median (IQR)	6.0 (4.0–7.0)	12.0 (10.0–13.75)	0.07
CEAE (mm), median (IQR)	3.0 (2.0–4.0)	11.5 (10.2–13.6)	<0.01
TEBE (mm), median (IQR)	6.5 (4.25–8.0)	6.0 (4.25–7.75)	0.92
TEAE (mm), median (IQR)	3.0 (2.0–4.0)	5.5 (4.0–7.55)	<0.01
Metabolic parameters			
Fasting glucose (mg/dL), median (IQR)	82.5 (74.75–89.0)	150.0 (133.0–166.0)	<0.01
Post-prandial glycaemia (mg/dL), median (IQR)	89.5 (80.75–93.5)	155.5 (134.0–200.0)	<0.01
Cholesterol (mg/dL), median (IQR)	147.5 (122.2–185.2)	154.0 (136.2–166.7)	0.72
Triglycerides (mg/dL), median (IQR)	122.5 (112.0–143.0)	131.0 (114.0–143.75)	0.50
Daily number of insulin units (UI)	–	38.0 (34.25–40.0)	–
HbA1c (nmol/mol)	–	45.0 (31.0–53.0)	–
Duration of diabetes	–	7.0 (3.0–12.75)	–

MMP: mitochondrial membrane potential; PS Ext.: phosphatidylserine externalization; DNAF: spermatozoa DNA fragmentation; TV: testicular volume; CEBE: caput epididymis before ejaculation; CEAE: caput epididymis after ejaculation; TEBE: tail epididymis before ejaculation; TEAE: tail epididymis after ejaculation.

Figure 1 Upper panel: Conventional spermatozoa parameters in patients with type 1 diabetes mellitus (DM1) classified according to disease duration. Lower panel: Biofunctional spermatozoa parameters, evaluated through flow cytometry, in patients with DM classified according to the disease duration. MMP: mitochondrial membrane potential; PS: phosphatidylserine.

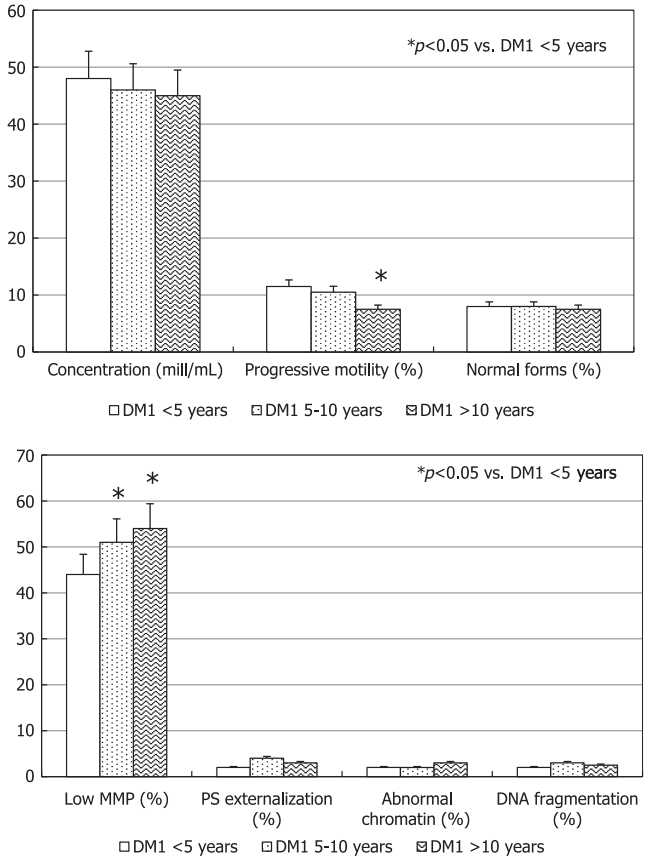
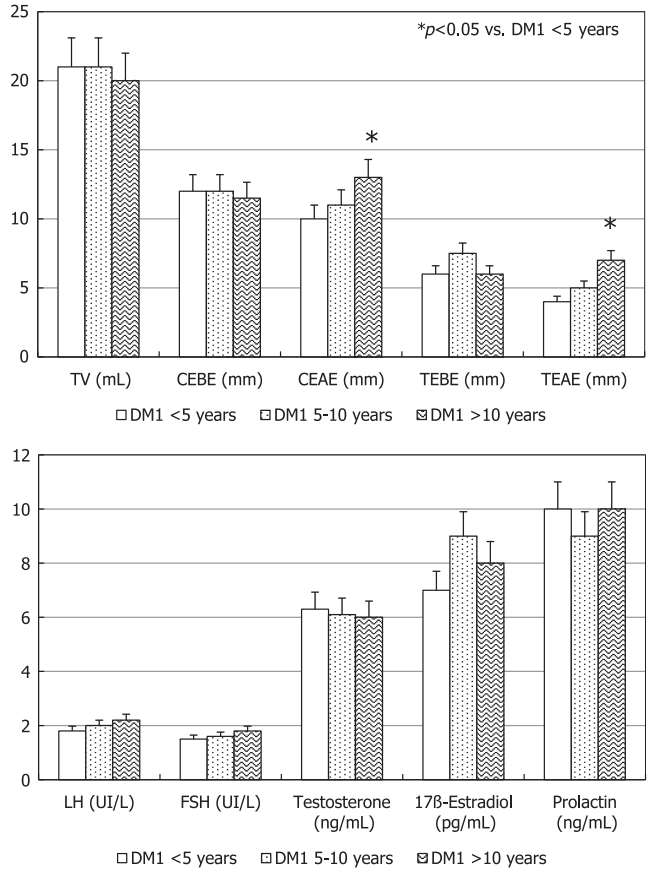


Figure 2 Upper panel: Ultrasound parameters in patients with type 1 diabetes mellitus (DM1) classified according to different disease duration. TV: testicular volume; CEBE: caput epididymis before ejaculation; CEAE: caput epididymis after ejaculation; TEBE: tail epididymis before ejaculation; TEAE: tail epididymis after ejaculation. Lower panel: Serum hormone concentrations in patients with DM1 classified according to disease duration.



to controls and no correlation with age, duration of diabetes or HbA1c levels (Niven *et al.*, 1995). Similarly, another study did not find any significant difference in the percentage of motile spermatozoa, evaluated by light microscopy, in 27 patients with DM1 compared to 29 non-diabetic controls (Agbaje *et al.*, 2007). On the other hand and in keeping with the data of the present study, spermatozoa motility decrease has been reported in a cohort of patients with DM1 and DM2 (Ali *et al.*, 1993) and in 32 adolescent patients with DM1 (Padrón *et al.*, 1984). When diabetic patients with or without neuropathy were compared, spermatozoa motility showed no significant difference suggesting, according to the authors, an endocrine basis for this abnormality (Ali *et al.*, 1993).

However, this hypothesis does not agree, at least in patients with DM1, with the results of the present study which showed no significant difference in the hormonal values between patients with DM1 and controls. This latter finding is further supported by a study that did not find any significant difference in serum testosterone levels in patients with DM1 (Padrón *et al.*, 1984). Therefore, other mechanisms should be hypothesized to explain spermatozoa motility lessening. These may include increased oxidative stress (Agbaje *et al.*, 2007), autoimmune disorder with the development of anti-spermatozoon antibodies and/or neuropathy which may alter seminal vesicle function (alteration evaluated by our group in patients with type 2 diabetes, there is no clinical experience in patients with DM1) (La Vignera *et al.*, 2012). In contrast to the reported lack of effect of neuropathy in a mixed cohort of DM1 and DM2 diabetic patients, significantly worse spermatozoa parameters, including motility, were found in DM1 patients with neuropathy and in those with poor metabolic control. Therefore, both neuropathy and poor metabolic control seem to be important factors for this deterioration (Padrón *et al.*, 1984). In contrast, we did not find any relationship between metabolic control (HbA1c) and conventional or biofunctional spermatozoa parameters and serum hormone concentration.

More recently, the reduced spermatozoa motility observed in patients with DM1 was also examined from the therapeutic point of view. In vitro, a previous study found an increment of spermatozoa motility in seminal samples obtained from patients with DM1 following incubation with L-arginine, polyamines putrescine, spermidine and spermine (Morales *et al.*, 2003). Similarly, Ali and Rakkah (2007) reported that the administration of sildenafil, a selective inhibitor of phosphodiesterase type 5 commonly used in the treatment of erectile dysfunction, to patients with DM1 and neuropathy is able to significantly improve spermatozoa motility with a mean increase of about 40%.

In addition to lower spermatozoa progressive motility, patients with DM1 also had a higher percentage of spermatozoa with lower MMP, associated with fasting glucose and the reduction of this parameter becomes more important in patients with diabetes lasting longer than 5 years. Indeed, we found a significant increase in low-quality spermatozoa (Amaral *et al.*, 2013) in patients with diabetes duration ranging from 5 to 10 years. Hence, the onset of a mitochondrial dysfunction was temporally more precocious than the declining spermatozoa progressive motility.

To the best of our knowledge, this is the first study showing an increased abnormal spermatozoa mitochondrial function in patients with DM. The only study exploring the mitochondrial status in patients with DM1 evaluated mitochondrial DNA in 27 patients with DM1. Diabetic patients had a higher median number of mtDNA deletions (4 vs. 3) compared with 29 control subjects. On this account the authors concluded that "diabetes is associated with increased spermatozoa nuclear and mtDNA damage that may impair the reproductive capability of these men" (Agbaje *et al.*, 2007). The results of the present study add further evidence to this hypothesis. Indeed, disruption of spermatozoa mitochondrial function may be responsible for the decline in spermatozoa motility observed in DM1 patients.

Mitochondrial membrane potential is an important indicator of the mitochondrial energetic status responsible for ATP production and therefore for the movement of the spermatozoa flagellum. This parameter is a good marker of mitochondrial function. Ultrastructural mitochondrial alterations appear to be associated with decreased spermatozoa motility in humans (Mundy *et al.*, 1995; Pelliccione *et al.*, 2011) and MMP alteration is associated with reduced spermatozoa motility (Amaral *et al.*, 2013). Recent findings suggest that this non-conventional parameter may be ameliorated by myoinositol (Condorelli *et al.*, 2012).

Finally, progressive motility and fasting glucose were associated with CEAE and TEAE (correlation analysis) and low MMP was associated with CEAE and TEAE (multivariate linear regression analysis).

Scrotal ultrasound scan revealed a peculiar aspect in patients with DM1 represented by the lack of physiological contraction of both the cranial and caudal portions of the epididymis after ejaculation. This alteration was significantly higher in patients with DM1 lasting longer than 10 years. This finding is an intriguing aspect of the present study suggestive of hypotony and/or atony of this anatomical structure, never explored in previous studies. Physiologically, after ejaculation, epididymal thickness, measured by ultrasound scan, decreases by approximately 3 mm as found in the control group. During ejaculation, the epididymis plays a fundamental role by collecting about 200 million spermatozoa, half of them in the cephalic and body stretch and the remaining in the caudal portion (Amann & Howards, 1980). The cephalic and caudal portions have poor innervation and are characterized by a spontaneous peristalsis capable of transferring immotile spermatozoa (El-Badawi & Schenk, 1967). On the contrary, the caudal epididymis has a rich adrenergic innervation involved in the emission phase of the ejaculatory process. However, an adrenergic denervation, obtained chemically or surgically, does not eliminate epididymal contractility (Kempinas *et al.*, 1998). This evidence suggests that epididymal contractility is mediated not only by a neuronal mechanism but also by non-neuronal factors (hormonal and non-hormonal). Oxytocin and endothelin-1 are considered the main hormones involved in the non-neuronal secretory dysfunction capable of altering the contractile function of the epididymis. Estrogens are involved in enhancing tissue sensitivity to the action of both oxytocin and endothelin-1, at least in the animal model (Vignozzi *et al.*, 2008). These aspects are worthy of further investigation in patients with DM1 with fertility problems. Recently, it has been reported that the epididymis is a potential target of the oxidative stress in patients with diabetes. In particular, immunohistochemical analysis allowed to highlight the presence of receptors for advanced glycation end products at the level of the epididymal region (Mallidis *et al.*, 2007). A possible involvement of oxidative response in the alterations of epididymal contractility is therefore plausible.

Diabetological interest, originality and limitations of the study

This study confirms previous data regarding a reduction in fertility potential in DM1 patients (Agbaje *et al.*, 2007; La Vignera *et al.*, 2012; Sjöberg *et al.*, 2013). On the basis of these data, as DM1 affects mostly males in fertile age, their diabetological counseling should provide the evaluation of this complication, through the examination of spermatozoa parameters. In particular, these alterations could be classified as another example of altered mitochondrial function in patients with DM1 (Blake & Trounce, 2014). To our knowledge, there are no studies that have evaluated simultaneously the spermatozoa parameters examined in this study (concentration, progressive motility, morphology, leukocytes, spermatozoa DNA fragmentation, chromatin compaction, spermatozoa apoptosis, mitochondrial function) in relation to the ultrasound characteristics in DM1 patients. The previous recent experience of our group, in particular the ultrasound characterization of the seminal vesicles associated with autonomic neuropathy, concerned DM2 but not DM1 patients. In our opinion, the limited number of patients and the lack of a multicenter study regarding these parameters represent the main limitations of this study, which must be validated by further studies. Finally, even the selection of controls among men who have recently induced pregnancy could be a potential bias of the study.

In conclusion, patients with DM1 have a decreased spermatozoa progressive motility, abnormal spermatozoa mitochondrial function and ultrasound features suggestive of post-ejaculatory epididymal hypotony/atony. All these parameters are significantly related to fasting glycemia and mitochondrial dysfunction seems to be the first to appear. These findings may explain the lower fecundity recently reported in men with DM1 (Sjöberg *et al.*, 2013).

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

S.L. and A.E.C. researched data, wrote, reviewed/edited the manuscript; R.A.C. did the spermatozoa parameter analysis and wrote the manuscript; M.D. and D.L. researched data; L.M. contributed to discussion and statistical analysis; G.R. performed the statistical analysis.

DISCLOSURE

The authors have nothing to disclose.

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