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Male diabetes mellitus and assisted reproduction treatment outcome

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James Mulholland was a talented and enthusiastic student in the final semester of his medical training. He conducted the majority of the review and collation of the results used in this study. Tragically, James passed away prior to graduation. The publication is dedicated to his memory.

Abstract The long-held view that diabetes has little effect on male reproductive function has been challenged by findings that the condition influences fertility in numerous previously undetected ways. This retrospective chart review of 3000 couples determined the incidence of couples with a male diabetic seeking assisted reproduction treatment and assessed any relationship between male diabetes and IVF/intracytoplasmic sperm injection (ICSI) outcome. Eight (2.7%) couples were found with a diabetic male partner, of which 18 couples underwent assisted reproduction treatment (five IVF, 12 ICSI, one both), with fertilization rates (IVF 68%, ICSI 62%) similar to non-diabetic patients (IVF 70%, ICSI 71%) and no difference in embryo quality. Two men had retrograde ejaculation and two were azoospermic. Other than reduced sperm motility, the remaining 14 had normal World Health Organization semen parameters. Embryo transfers produced one pregnancy (5% combined IVF/ICSI pregnancy rate/cycle) giving a lower-than-expected rate (28.8%). The pregnancy rate from seven FETs (29%) was comparable to the expected (21.3%). Compared with non-diabetics, approximately three times more couples with diabetic men sought treatment, with a larger percentage having 'unexplained' infertility. Fertilization rates and embryo quality did not differ but pregnancy rates were lower in couples with a diabetic male.

KEYWORDS: assisted reproduction techniques, infertility, male diabetes, pregnancy rates

Introduction

The incidence of diabetes mellitus (DM) is rising rapidly worldwide, with an estimated 366 million people living with the disease by 2030, (World Health Organization, 2002). With this expanding population of increasingly younger diabetics, health systems around the world face major challenges in understanding and, subsequently, dealing effectively with the chronic complications of the condition.

The increasing incidence of DM, particularly in affluent Western societies, is contemporaneous with falling fertility and birth rates (Hamilton and Ventura, 2006; Lutz, 2006).

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Undoubtedly, fertility rates are subject to many social factors, including availability of contraception, declining ideal family size and the delay of pregnancy for career and/or economic considerations (Carlsen et al., 1992; Skakkebaek et al., 2006). However, with the proportion of couples now seeking medical treatment for subfertility estimated to be as high as 17% (Gnoth et al., 2005), there are serious concerns that there may also be a progressive innate deterioration in human reproductive health. Within these concerns about fertility, a decline in male fertility, specifically decreasing sperm quality, has received significant attention (Jensen et al., 2002).

Other than erectile dysfunction (Sexton and Jarow, 1997) and retrograde ejaculation (Bettocchi et al., 2008), which are long acknowledged as sequelae of the condition, the impact of DM on male reproductive health remains controversial. Studies on DM's influence on both the hypothalamic—pituitary—gonadal axis (Baccetti et al., 2002; Ballester et al., 2004; Dinulovic and Radonjic, 1990; Garcia-Diez et al., 1991) and semen quality (Ali et al., 1993; Handelsman et al., 1985; Niven et al., 1995; Vignon et al., 1991) have yielded varying, often conflicting results. It is not surprising, therefore, that there is some scepticism amongst fertility specialists about its importance and, as a result, DM has been largely ignored when assessing male fertility. Due to this uncertainty, even the precise proportion of couples with a diabetic male who seek assistance for infertility remains unclear (Agbaje et al., 2007).

The association of DM with decreased male fertility is much clearer in the various animal models that are employed to study the condition: all show significantly decreased fecundity (Ballester et al., 2004; Scarano et al., 2006; Shrilatha and Muralidhara, 2007). Employing molecular approaches, a study has recently reported that DM is associated with: (i) subtle yet important changes in the metabolomic profile of the testis (Mallidis et al., 2009a); (ii) the increased presence in the male reproductive tract of a group of compounds that are implicated in numerous diabetic complications, i.e. advanced glycation end-products (Mallidis et al., 2008) and their receptor (Mallidis et al., 2007); and (iii) a significantly higher percentage of both sperm nuclear and mitochondrial DNA damage (Agbaje et al., 2007).

Studies of intrauterine insemination (IUI) and IVF outcomes have found a major difference in the percentage of DNA fragmentation in spermatozoa between those who successfully conceived and those who did not (Duran et al., 2002; Tomlinson et al., 2001). Sperm DNA integrity has also been associated with decreased embryo quality and an increase in miscarriage rates (Morris et al., 2002). Interestingly, an early study of the effects of male diabetes on reproductive outcome (Babbott et al., 1958) found the only difference in the partners of diabetic men compared with non-diabetics, was a significantly higher rate of miscarriage. The implication of each of these reports alone and combined is that DM confers a significant, yet elusive, negative male influence on reproductive success.

The aims of this study were to determine the incidence of couples with male diabetes attending this fertility centre for treatment and to assess whether there was a relationship between the presence of DM in the male and assisted reproduction treatment outcome.

Materials and methods

A retrospective chart review was performed which involved manually reviewing the available charts of patients who had attended the fertility clinic within the last 5 years. Patients were included in the study if male DM was documented at the time of first consultation for subfertility issues. Diabetic males were excluded from the study if they and their partner did not subsequently undergo any form of assisted reproduction (IVF, ICSI or frozen embryo transfer (FET)). Withdrawal from treatment and/or subsequent treatment was solely the decision of the couples and was not based on recommendations from their attending physicians.

Subjects were anonymized and the data divided into three information streams: (i) male; (ii) female; and (iii) assisted reproduction treatment.

For the males, information was recorded for date of birth, type and duration of diabetes, smoking status, nature of any treatment given. Light microscopic semen analysis information was recorded including volume of ejaculate, extent of liquefaction after 30 min at 37°C, sperm concentration, the percentage of spermatozoa judged rapidly motile, the percentage with normal morphology (early samples: World Health Organization (WHO) general criteria; later samples: strict criteria; World Health Organization, 1999) and the antibody status as determined by the mixed antiglobulin reaction test.

For the females, information was recorded for date of birth, parity and presence of ovulation, endometriosis or tubal disease, smoking status and use of any medication.

For the assisted reproduction treatment, information was recorded for number of cycles, numbers of eggs harvested, number normally fertilized, embryo grade at transfer, whether embryos were frozen and clinical outcomes (no pregnancy, biochemical pregnancy, miscarriage, singleton/multiple live birth, singleton/multiple stillbirth).

Means for the various semen parameters were compared with the clinic results as a whole for the period Jan—Dec 2007. The outcomes for IVF, ICSI and FET were considered individually and the success rates were compared with the Human Fertilisation and Embryology Authority (HFEA) figures for success rates for the Belfast Regional Fertility Centre (BRFC) in 2007.

Results

The trawl of 3000 charts revealed 80 couples where the male was known to be diabetic. Of these, 18 diabetic men and their partners were identified who had undergone assisted reproduction treatment between 2004 and 2007. Male age (median ± SD) ranged from 28 to 51 years (36.1 ± 6.8). Their partners' ages ranged from 24 to 41 years (33.9 ± 5.5).

In the HFEA mandatory returns the primary aetiology of infertility for the study group was attributed to: unexplained for 10 couples (56%); idiopathic male factor (by WHO criteria; World Health Organization, 1999) for four couples (22%); polycystic ovarian syndrome for two couples (11%); hyperprolactinaemia for one couple (6%) and endometriosis for one couple (6%). All female partners were nulliparous. One subject had had three previous miscarriages and another one miscarriage.

The duration of male diabetes ranged from 7 to 38 years (median 17.1 years). Type of diabetes was recorded for only four patients (three type 1, one type 2). Patients reported their diabetes as being well controlled (38%), poorly controlled (27%) and those unknown or unrecorded (35%). In terms of smoking status: 10 males (56%) were non-smokers, two (11%) were smokers whilst the smoking status was unrecorded for six (33%). No identifiable cause for the infertility of the subjects was discernable during examination.

Semen analysis

Of the subject group of 18 diabetic males, two (11%) had retrograde ejaculation and two were azoospermic. Light microscopic semen analysis information was available for the remaining 14 subjects and the results are given in **Table 1**. None of the subjects tested positive for the presence of antibodies. Extent of liquefaction was normal in 13 subjects. A reduction in motility was found in 80% of samples as was a decrease in the percentage of normal forms in 60% of samples. Median values for ejaculate volume, sperm concentration and viability fell within the WHO normal reference ranges (World Health Organization, 1999).

Assisted reproduction

Of the subject group 18 couples decided to continue with treatment: five underwent 10 cycles of IVF; 12 underwent 19 cycles of ISCI and one couple underwent two cycles of IVF and one cycle of ICSI (**Table 2**). Five couples who underwent ICSI subsequently opted to utilize FET (seven cycles). For IVF treatments a HFEA-reported fertilization rate of 68% was achieved for 66 harvested eggs. The average fertilization rate was 70% for IVF treatments in Belfast in 2007. Despite 12 embryo transfers (normally two embryos per cycle) there were no pregnancies. For ICSI a fertilization rate of 62% was achieved for 198 harvested eggs. The HFEA reported fertilization rate was 71%. Despite 18 embryo transfers, there was only one clinical pregnancy (6% per transfer), representing a combined IVF/ICSI clinical pregnancy rate per embryo transfer of 3.3% for fresh cycles which is significantly below the expected rate of 27.9% for the BRFC in 2007. The results for thawed cycles (FET) were more encouraging, with a 29% clinical pregnancy rate compared with an expected rate of 21.3% but there were only seven episodes of FET. No miscarriages or complications during delivery were noted.

Discussion

Sometimes, in reproductive medicine — as in other branches of medicine — the effect of a condition on a treatment outcome is obscured. A situation further complicated when the condition is inadequately recorded causing its influence to be buried deeper amongst the general treatment results. As far as is known, this is the first time that anyone has endeavoured to determine the influence of male DM on assisted reproduction treatment outcome. The preliminary nature of this study and the small number of couples identified is readily acknowledged. However, the finding that, whilst the percentage of eggs that fertilized and the embryo numbers were in keeping with those of the general population in the study unit, the combined clinical pregnancy rate/transfer for ICSI and IVF was 3.3% against a general

Table 1 Semen analysis results for the diabetic males in couples who underwent assisted reproduction treatment.

Parameter	*Study result (n = 14)*	*World Health Organization normal criteria (1999)*
Semen volume (ml)	2.3 ± 0.7	≥2
Sperm concentration (10^6/ml)	86 ± 28.0	≥20
Motility (% grades a + b)	19.0 ± 8.1	≥50

Values are median ± 95% confidence interval.

Table 2 Assisted reproductive outcomes by treatment.

Treatment	*Patients*	*Cycles*	*Eggs harvested*	*Eggs fertilized*	*Normally fertilized eggs*	*Embryo transfers*	*Clinical pregnancies/cycle*	*Overall clinical pregnancies/cycle BRFC (2007)*[a]
IVF	6	12	66	45 (68)	35 (78)	12	0	119/438 (27.2)
ICSI	13	20	198	123 (62)	110 (89)	18	1 (5)	91/316 (28.8)
FET	5	7	—	—	—	7	2 (29)	30/141 (21.3)
Total	18[b]	39	264	168	145	37	3	240/895 (26.8)

Values are *n* (%).
[a]Data obtained from http://guide.hfea.gov.uk/guide/SuccessRate.aspx?Code=77&s=l&&nav=2.
[b]One couple underwent two cycles of IVF and one cycle of ICSI. BRFC = Belfast Regional Fertility Centre; FET = frozen embryo transfer; ICSI = intracytoplasmic sperm injection.

background assisted reproduction pregnancy rate of 30% should act as a clarion call for more in depth studies.

There are inherent difficulties with retrospective chart reviews including the possible absence of relevant information and ambiguity in recording patient data. The BRFC does not use a standard proforma for clinical histories and, therefore, this study was only able to include diabetes where it was noted to be present. Thus, it cannot absolutely be excluded that the incidence of DM is an under-representation. Whilst this study endeavoured to determine the type of diabetes mellitus for each of the subjects, this also was not always possible. Further, the duration of diabetes and an assessment of glycaemic control were sometimes also missing or simply recorded as 'well' or 'poorly' controlled. This information was taken from the patients and largely not substantiated by formal clinical assessment. This kind of verbal patient report is more open to bias and the usefulness of the information is, therefore, undermined. It would have been useful in this regard to have had HbA1C concentrations for each male. It is well recognized that poor glycaemic control is a good predictor of chronic complications for diabetic patients. A future prospective study would be designed to take these issues into account. Despite these inadequacies, the results remain striking.

On screening the female population, they were a representative cross-section of the female population undergoing assisted reproduction treatment in the unit: their mean age was slightly lower than average for the unit but they had a broad spectrum of infertility problems, with an incidence of polycystic ovarian syndrome of 11% and endometriosis 6%. However, most importantly, the incidence of unexplained infertility was over 50%. Given the outcome for these couples, it could be postulated that male DM is a relevant aetiological factor.

It is manifestly obvious that not all males with DM are infertile. However, the incidence of DM in males attending infertility units has been variously reported to be around 1%: in a large study of over 500 infertile couples in Italy, diabetes was found to be present in 1.18% of the male partners (Delfino et al., 2007). Other large studies of infertile men have reported diabetes to be present in 0.7% (Greenberg et al., 1978) and in 1.1% (Sexton and Jarow, 1997). In combination these three studies of 1401 infertile men identified 14 diagnosed diabetics (1%). This is significantly greater than the 0.2—0.3% prevalence of DM in the male reproductive age range. In the UK there are an estimated 46,000 diabetics in the age range 20—44 (King et al., 1998). In conjunction with the 2001 UK census information this gives an estimated incidence of diabetes of 0.22% in this age group. This is similar to the estimated expected incidence of 0.3% for the male reproductive population proposed by Sexton and Jarow (1997). Whilst this discrepancy between 0.3% and 1% may seem relatively small, it in fact equates to at least three times more diabetics presenting to fertility clinics than would otherwise be expected.

By contrast with IVF and ICSI fresh embryo transfers, the success rate for FET was more in keeping with that of the general population in this fertility unit. This may represent the fact that because these embryos were often frozen at the 8-cell stage and were therefore biased towards those of a higher innate quality, and because they had survived the freeze thaw processes, the embryos transferred in a frozen embryo cycle were self selected for those that were inherently stronger or of better quality. However, the number of FET cycles was small and these figures are, therefore, particularly open to bias.

In the study population presented in this paper, the incidence of retrograde ejaculation was 11%, which was higher than the 6% observed by Dinulovic and Radonjic (1990). However, again, this may reflect problems due to a bias in the population studied or the small population size in this study. Either way, retrograde ejaculation is a well-recognized complication of DM. The differences found in the semen profiles of this cohort of patients once again highlight the limitations of routine semen analyses. This study found reductions in sperm motility and morphology, results that are similar to some previous studies (Ali et al., 1993; Garcia-Diez et al., 1991) but contrasting to others (Handelsman et al., 1985; Vignon et al., 1991).

Numerous studies now indicate that DM's influence on male fertility is subtle, causing adverse metabolic (Mallidis et al., 2009a), proteomic (Kriegel et al., 2010) and genetic and transcriptional changes (Mallidis et al., 2009b). DM has also been associated with increased numbers of spermatozoa with fragmented DNA (Agbaje et al., 2007), a factor whose corollary is decreased fecundity, and thus consistent with the current study's findings. The precise mechanism responsible for this destructive state remains unclear but the most plausible explanation put forward is that sperm DNA damage, in men with DM (Agbaje et al., 2008) and without DM (Aitken and De Iuliis, 2010) alike, results from oxidative stress. The identification of high concentrations of advanced glycation end-products in the male reproductive tract (Mallidis et al., 2008) raises the possibility that this group of compounds known to cause cell dysfunction, oxidative stress and DNA damage in various organs may also be involved in male infertility. A proposition supported by the mRNA profile of spermatozoa from DM men which shows significant differences in the expression of genes involved in oxidative stress, DNA metabolism, repair and replication indirectly regulated by advanced glycation end-products and their receptors or antiglycation (Mallidis et al., 2009b). As the influence of glycation is not restricted to the diabetic state but is present in every fundamental process of cellular metabolism and is a normal consequence of life, the further study of the fertility of male diabetics may have implications beyond the elucidation of processes unique to DM. It may also provide insights into hitherto unsuspected mechanisms which undermine male reproductive function in general.

References

Agbaje, I.M., Rogers, D.A., McVicar, C.M., et al., 2007. Insulin dependant diabetes mellitus: implications for male reproductive function. Hum. Reprod. 7, 1871—1877.

Agbaje, I.M., Rogers, D.A., McVicar, C.M., et al., 2008. Increased concentrations of the oxidative DNA adduct 7,8-dihydro-8-oxo-2-deoxyguanosine in the germ-line of men with type 1 diabetes. Reprod. Biomed. Online 16, 401—409.

Aitken, R.J., De Iuliis, G.N., 2010. On the possible origins of DNA damage in human spermatozoa. Mol. Hum. Reprod. 16, 3—13.

Ali, S.T., Shaikh, R.N., Ashfaqsiddiqi, N., et al., 1993. Serum and urinary levels of pituitary—gonadal hormones in insulin-dependent and non-insulin-dependent diabetic males with and without neuropathy. Arch. Androl. 30, 117—123.

Babbott, D., Rubin, A., Ginsburg, S.J., 1958. The reproductive characteristics of diabetic men. Diabetes 7, 33–35.

Baccetti, B., La Marca, A., Piomboni, P., et al., 2002. Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. Hum. Reprod. 17, 2673–2677.

Ballester, J., Munoz, M.C., Dominguez, J., et al., 2004. Insulin-dependent diabetes affects testicular function by FSH-and LH-linked mechanisms. J. Androl. 25, 706–719.

Bettocchi, C., Verze, P., Palumbo, F., et al., 2008. Ejaculatory disorders: pathophysiology and management. Nat. Clin. Pract. Urol. 5, 93–103.

Carlsen, E., Giwercman, A., Keiding, N., et al., 1992. Evidence for decreasing quality of semen during past 50 years. Br. Med. J. 305, 609–613.

Delfino, M., Imbrogno, N., Elia, J., et al., 2007. Prevalence of diabetes mellitus in male partners of infertile couples. Minerva Urol. Nefrol. 59, 131–135.

Dinulovic, D., Radonjic, G., 1990. Diabetes mellitus/male infertility. Arch. Androl. 25, 277–293.

Duran, E.H., Morshedi, M., Taylor, S., et al., 2002. Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. Hum. Reprod. 17, 3122–3128.

Garcia-Diez, L.C., Corrales Hernandez, J.J., Hernandez-Diaz, J., et al., 1991. Semen characteristics and diabetes mellitus: significance of insulin in male infertility. Arch. Androl. 26, 119–128.

Gnoth, C., Godehardt, E., Frank-Herrmann, P., et al., 2005. Definition and prevalence of subfertility and infertility. Hum. Reprod. 20, 1144–1147.

Greenberg, S.H., Lipshultz, L.I., Wein, A.J., 1978. Experience with 425 subfertile male patients. J. Urol. 119, 507–510.

Hamilton, B.E., Ventura, S.J., 2006. Fertility and abortion rates in the United States, 1960–2002. Int. J. Androl. 29, 34–45.

Handelsman, D.J., Conway, A.J., Boylan, L.M.P., et al., 1985. Testicular function and glycemic control in diabetic men. A controlled study. Andrologia 17, 488–496.

Jensen, T.K., Carlsen, E., Jorgensen, N.P., et al., 2002. Poor semen quality may contribute to recent decline in fertility rates. Hum. Reprod. 17, 1437–1440.

King, H., Aubert, R.E., Herman, W.H., 1998. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. Diabetes Care 21, 1414–1431.

Kriegel, T.M., Heidenreich, F., Kettner, K., et al., 2010. Identification of diabetes- and obesity-associated proteomic changes in human spermatozoa by difference gel electrophoresis. Reprod. Biomed. Online 19, 660–670.

Lutz, W., 2006. Fertility rates and future population trends: will Europe's birth rate recover or continue to decline? Int. J. Androl. 29, 25–33.

Mallidis, C., Agbaje, I., Rogers, D.P., et al., 2007. Distribution of the receptor for advanced glycation end products in the human male reproductive tract: prevalence in men with diabetes mellitus. Hum. Reprod. 22, 2169–2177.

Mallidis, C., Agbaje, I.M., Rogers, D.A.P., et al., 2008. Advanced glycation end products accumulate in the reproductive tract of men with diabetes. Int. J. Androl. 32, 295–305.

Mallidis, C., Green, B.D., Rogers, D., et al., 2009a. Metabolic profile changes in the testes of mice with streptozotocin-induced type 1 diabetes mellitus. Int. J. Androl. 32, 156–165.

Mallidis, C., Agbaje, I., O'Neill, J., et al., 2009b. The influence of type 1 diabetes mellitus on spermatogenic gene expression. Fertil. Steril. 92, 2085–2087.

Morris, I.D., Ilott, S., Dixon, L., Brison, D.R., 2002. The spectrum of DNA damage in human sperm assessed by single cell gel electrophoresis (Comet assay) and its relationship to fertilization and embryo development. Hum. Reprod., 990–998.

Niven, M.J., Hitman, G.A., Badenoch, D.F., 1995. A study of spermatozoal motility in type 1 diabetes mellitus. Diabet. Med. 12, 921–924.

Scarano, W.R., Messias, A.G., Oliva, S.U., et al., 2006. Sexual behaviour, sperm quantity and quality after short-term streptozotocin-induced hyperglycaemia in rats International. J. Androl. 29, 482–488.

Sexton, W.J., Jarow, J.P., 1997. Effect of diabetes mellitus upon male reproductive function. Urology 49, 508–513.

Shrilatha, B., Muralidhara, 2007. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: its progression and genotoxic consequences. Reprod. Toxicol. 23, 578–587.

Skakkebaek, N.E., Jorgensen, N., Main, K.M., et al., 2006. Is human fecundity declining? Int. J. Androl. 29, 211.

Tomlinson, M.J., Moffatt, O., Manicardi, G.C., et al., 2001. Interrelationships between seminal parameters and sperm nuclear DNA damage before and after density gradient centrifugation: implications for assisted conception. Hum. Reprod. 16, 2160–2165.

Vignon, F., Le Faou, A., Montagnon, D., et al., 1991. Comparative study of semen in diabetic and healthy men. Diabetes Metab. 17, 350–354.

World Health Organization, 1999. Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Interaction. University Press, Cambridge, United Kingdom.

World Health Organization. The Cost of Diabetes (Fact Sheet No. 236), 2002. Available from: <http://www.who.int/mediacentre/factsheets/fs236/en/>.

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