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GENETIC DISEASE

Response to controlled ovarian stimulation and oocyte quality in women with myotonic dystrophy type I

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Abstract

Purpose In women the relationship between myotonic dystrophy type I and fertility remains controversial. The objective of this study was to evaluate the ovarian reserve, ovarian response to stimulation and oocyte quality in these patients.

Materials and methods We compared 15 myotonic dystrophy type I patients with 39 age matched controls with isolated male factor infertility necessitating ICSI.

Results All parameters of ovarian reserve (day 3 FSH and E2, antral follicle count and delta E2) were significantly better in the controls. Despite having significantly lower doses of gonadotrophin, the control group attained a higher number of retrieved oocyte–cumulus complexes ($p < 0.04$). Analysis of cytoplasmic and extracytoplasmic dysmorphism did not reveal any difference between the two groups. Fertilisation rate and top grade embryos on day 3 were similar in both groups.

Conclusion The present study suggests that though women with myotonic dystrophy type I have a reduced ovarian reserve and respond poorly to controlled ovarian stimulation, there is no impact on oocyte and embryo quality. Hence suggesting that successful ART is feasible with appropriate selection in women with mild myotonic dystrophy.

Capsule Women with myotonic dystrophy type I have reduced ovarian reserve and respond poorly to controlled ovarian stimulation, though there is no impact on oocyte quality.

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Introduction

Myotonic dystrophy type I (DM1) is the commonest neuromuscular disease affecting adults (1). It is an autosomal dominant disease characterized by structural and functional abnormalities of the muscle membrane protein, myotoninkinase, which is involved in protein phosphorylation (2). The mutation that causes DM1 is the expansion of the CGT triplets at the 3′ end of the gene, which, in turn, is located on chromosome 19 (19q12) (3, 4).

Clinical picture is characterized by progressive muscle weakness, cataract, endocrine abnormalities, mental retardation and cardiorespiratory and gastrointestinal dysfunction (5). Patients with DM1, especially males, are reported to have reduced fertility (6, 7). However, such an association between female fertility and DM1 remains controversial (8–10).

The objective of this retrospective case control study was to evaluate whether ovarian function is affected in women with DM1. In this regard, we compared DM1 patients with age matched controls with isolated male factor infertility necessitating ICSI.

Materials and methods

Patient selection

We analysed the computerised records of women with DM1 ($n = 36$), who were referred to our unit for PGD (preimplantation genetic diagnosis) from 2002 to 2006. All women went through a multidisciplinary consultation

with a geneticist, embryologist and a gynaecologist. Following initial consultation, among the 36 couples in which the female partner was suffering from DM1, 12 opted out of the PGD programme due to social reasons and five couples choose to have spontaneous conception with prenatal diagnosis. Nineteen women with DM1 underwent assessment of ovarian reserve using a scoring system of: female age, BMI, antral follicle count on day 3 of the menstrual cycle along with FSH and E2 levels. Following initial blood test on day 3, all women were administered 300 IU of recombinant FSH (Gonal-F; Serono) and a second blood sample was taken for E2 on day 4. The rise in E2 levels in response to stimulation (delta E2 = day 4 E2–day 3 E2) was calculated. The starting dose of gonadotrophin stimulation was determined based on ovarian reserve score (11).

Following assessment of ovarian reserve 4 DM1 couples did not proceed to PGD due to evidence of poor ovarian reserve (FSH ≥ 15, delta E2 ≤ 100 and AFC < 10). Fifteen couples went through 21 cycles of ICSI treatment followed by embryo biopsy on day 3 for preimplantation genetic diagnosis. In order to avoid bias only first cycles of each couple was analysed. Ovarian response and oocyte quality of 15 DM1 patients was compared with consecutive 39 age matched controls that had ICSI treatment during the same period for isolated severe male factor infertility. The inclusion criteria for the control group included women with proven ovarian function (day 3 serum FSH, LH, day 21 progesterone and ultrasonographic follicular monitoring confirming ovulation).

Exclusion criteria for the control group included women with polycystic ovarian syndrome, congenital or acquired uterine abnormality, tubal pathology, who had a follicle >10 mm or evidence of ovarian pathology on cycle day 3 of stimulation.

The objective of this study was to evaluate whether ovarian function is affected in women with DM1. In this regard, we assessed the ovarian reserve, ovarian response to stimulation and oocyte quality (maturity and morphology) in DM1 patients who underwent PGD in comparison to age matched controls with proven ovarian function who underwent ICSI for isolated male factor infertility.

Both groups underwent ovarian stimulation using midluteal down-regulation protocol with gonadotrophin releasing hormone (GnRH) analogue (Suprefact nasal spray, Hoechst UK, Hounslow, Middlesex, UK) and human menopausal gonadotrophin (Menopur, Ferring, Gentilly, France), as previously described (11). Transvaginal oocyte retrieval was performed 36 h after human chorionic gonadotrophin (HCG) administration.

Assessment of oocyte and embryo quality

Four hours after egg collection, oocytes were incubated for <30 s in culture medium containing 80 IU/ml hyaluronidase (Type VIII, H-3757 Sigma) and stripped off their cumulus and corona radiata cells by gentle pipetting. Oocytes obtained from both the DM1 group and the control group were inseminated by ICSI. This was to prevent contamination by sperm DNA in DM1 group and severe male factor infertility necessitating ICSI in the control group.

Maturation and morphological features of the oocytes were investigated immediately before ICSI. Analyzed anomalies included dark central granulation of the cytoplasm, refractile bodies, vacuoles, aggregation of smooth endoplasmic reticulum (sER) presence of abnormal zona pellucida and irregular first polar body (12).

ICSI procedure was performed on MII oocytes using conventional techniques (13). Oocytes with two pronuclei and two polar bodies 14–18 h after ICSI were considered as normally fertilized.

Day 3 embryo morphology was checked in all patients regardless of day of transfer. Each embryo was evaluated for the following: (1) cell number, (2) presence of equal sized cells, (3) good blastomere expansion, i.e. blastomeres touching the zona with minimal perivitelline space, (4) presence of cytoplasmic pitting, (5) signs of compaction and (6) the degree of fragmentation (14).

In patients with DM1, embryo biopsy for PGD analysis was carried out on cleavage stage on day 3. Embryo transfer was performed under ultrasound guidance (day 5 in the DM1 group and day 3 or 5 in the control group).

This study exclusively focused on oocyte quality and preimplantation embryo development. Implantation and pregnancy rates were not addressed because of heterogeneous study populations for this outcome (e.g. selection of embryos following biopsy among women with DM1 and different days of transfer).

To our knowledge this is the first published study assessing oocyte morphology in women with DM1.

The statistical analysis was performed by using SPSS software, version 10.0 (SPSS, Chicago, IL). Normally distributed metric variables were tested with the *t* test. Ordinal variables or not-normally distributed metric variables were analyzed with the Mann–Whitney *U* test. If more than two groups had to be analyzed, normally distributed metric variables with equal variances (Levene test) were examined by means of one-way ANOVA test. Multiple comparisons were made with Bonferroni test. For not-normally distributed metric variables or for variables with unequal variances, the Kruskal–Wallis one-way ANOVA test for ranks was employed. For correlation analysis, Spearman's rank correlation coefficient was used. All tests were two-tailed with a confidence level of 95% ($p < 0.05$).

Table 1 The baseline characteristics of myotonic dystrophy (DM1) and control groups

Variable	DM1(n=15)	CONTROL (n=39)	p value
Age (years)	33.35±4.23	33.55±2.70	NS
Body mass index (kg/m^2)	24.37±3.11	22.73±2.81	NS
Duration of infertility (years)	1.15±2.44	3.01±1.87	<0.001
Day 3 FSH (IU)	7.86±2.97	6.54±1.77	<0.03
Day 3 estradiol (pmol/mL)	166.64±96.84	182.45±62.86	<0.01
Delta estradiol (pmol/mL)	253.83±124.80	448.37±196.37	<0.002
Antral follicular count (n)	8.44±3.33	12.53±2.78	<0.001

Values are expressed as mean±SD.
NS Not significant

Results

There were no significant differences in female age and BMI between the two groups. Duration of infertility was significantly higher in the control group. All parameters of ovarian reserve (day 3 FSH and E2, antral follicle count and delta E2) were significantly better in the controls than in the DM1 group (Table 1).

Table 2 The controlled ovarian hyperstimulation response of the myotonic dystrophy and control groups

Variable	Myotonic dystrophy (n=15)	Control (n=39)	p value
Starting dose of HMG (no. of ampules)	4.26±1.38	3.26±0.75	<0.04
Total dose of HMG (no. of ampules)	53.69±16.01	35.51±10.83	<0.001
Total duration of stimulation (days)	11.46±1.65	10.67±0.66	<0.01
Endometrial thickness on day of hCG administration (mm)	10.25±1.37	9.68±1.81	NS
Estradiol on the day of hCG administration (pmol/mL)	12103±4497	15897±4750	<0.007
No. of follicles >15 mm in diameter at hCG administration	10.38±4.71	13.43±4.17	<0.02
No. of oocytes retrieved	11.61±3.25	14.18±4.62	<0.04

Values are expressed as mean±SD.
NS Not significant

Despite having significantly lower doses of gonadotrophin stimulation ($p < 0.001$) for a shorter duration ($p < 0.01$), the control group attained a higher serum E2 level on the day of hCG injection ($p < 0.007$). The numbers of follicles >15 mm in diameter on the day of HCG administration and the number of retrieved oocyte–cumulus complexes was also significantly higher in the control group in comparison with the DM1 group (Table 2). Three cycles were cancelled in the DM1 group for poor ovarian response (less than eight follicles or E2 less than 1,000 pg/ml after 5 days of ovarian stimulation).

The number of metaphase II oocytes and the number of good-quality oocytes with no dysmorphism were similar in both groups (Table 3). Detail analysis of the cytoplasm revealed that percentage of oocytes with central granulation of the cytoplasm and evenly granular cytoplasm were comparable among the groups (Table 3). The incidence of vacuoles, refractile bodies and sER clusters were also similar among women myotonic dystrophy and control group. Extracytoplasmic dysmorphism (frequency of polar body fragmentation and abnormal zona pellucida) did not differ among the groups (Table 3).

Both in the DM1 and the control group, the fertilisation rate (60 vs 64%, respectively), percentage of top grade embryos (52 vs 57%) and average grade embryos (29 vs 30%) on day 3 were similar.

Table 3 Oocyte quality in myotonic dystrophy and control groups

Variable	Myotonic dystrophy (n=15)	Control (n=39)	p value
No. of metaphase II oocytes	10.50±3.23	11.26±4.41	NS
Percent of metaphase II oocytes	91	80	NS
No. of oocyte with normal cytoplasmic morphology	4.15±3.30	5.69±2.74	NS
Central granular cytoplasm (%)	2.94±2.04	2.78±2.04	NS
Evenly granular cytoplasm (%)	2.00±1.75	2.30±2.05	NS
Refractile body (%)	0.39±0.85	0.63±1.19	NS
Aggregation sER (%)	0.02±0.01	0.03±0.01	NS
Vacuolization (%)	0.33±0.69	0.28±0.56	NS
Fragmented polar body (%)	4.50±2.36	5.38±2.72	NS
Abnormal zona pellucida (%)	0.17±0.51	0.50±1.54	NS
No. of two pronucleated oocytes	8.9±4.0	7.3±3.8	NS

Values are expressed as mean±SD.
NS Not significant

Discussion

Our findings revealed a reduced ovarian reserve and a lower ovarian response to stimulation in women with DM1. Women with DM1 required a significantly greater amount of hMG ($p<0.001$), with delay in the day of HCG administration ($p<0.01$). The number of retrieved oocyte–cumulus complexes was also significantly higher in the control group in comparison with the DM1 group ($p<0.04$) The use of higher quantities of FSH could well be biased by a higher starting dose based on the earlier impression of reduced ovarian reserve in some MD cases.No woman with evident poor ovarian reserve enters our PGD programme on account of the inherently reduced IVF outcome. It represents a limitation of our methodology, as we could not evaluate the response to COS of women who were previously excluded.

It is noteworthy that all our patients had only mild DM1 and usually the only clinical sign of the disease was weakness in facial muscles and in grip. No woman with severe DM1 was enrolled on account of the higher risks during pregnancy.

However even in the mild form of the disease, ovarian reserve and quantitative ovarian response was significantly diminished.

For the first time in the published medical literature, we also report the incidence of oocyte dysmorphism in women with DM1. A prognostic role of oocyte morphology in fertilisation has already been recognised. De Sutter et al. (15) found a severely reduced fertilization rate in vacuolized oocytes (40%) compared with gametes without vacuolization (69.6%). Cytoplasmic dysmorphism such as degree of central granulation and presence of sER clusters negatively correlates with ongoing pregnancy rate (16, 17). In another study presence of extracytoplasmic dysmorphism such as fragmented polar body was associated with poor fertilization rates and inferior embryo quality (18).

In our study the percentage of oocytes with cytoplasmic and extracytoplasmic dysmorphisms was similar in both groups, indicating that presence of DM1 is unlikely to affect oocyte quality.

It is of note that despite lower ovarian reserve and ovarian response to stimulation, women with DM1 had a normal fertilisation rate. Oocyte and embryo quality were also similar between women with DM1 and the control group. These findings suggest that successful ART is feasible in selected women with mild myotonic dystrophy.

There appears to be a consensus in the medical literature about the risk of decreased fertility in men with myotonic dystrophy. DM1 may cause testicular atrophy and oligozoospermia due to tubular lesions (6). Hortas et al. (7) reported that capacitation and acrosome reaction of spermatozoa of men with DM1 was deficient in comparison to controls.

However in women, the correlation between fertility and DM1 remains controversial. Dao et al. did a case–control study, in the Saguenay-Lac-St-Jean region of Canada (known to have a high prevalence of the disease), of 373 myotonic dystrophy patients to determine whether their fertility was affected by the disorder. They analysed several demographic parameters, such as number of children, the ages at the time of birth of the first and the last child, and the interval between consecutive births. This study found no difference in parameters of fertility between patients with DM1 and controls (8). Harper (5) failed to find a relationship between this multisystemic disease and fertility. Whereas, Ulloa-Aguirre et al. (9) reported a decreased fertility in women with DM1. They suggested a hypothalamic basis for ovarian dysfunction in these women. Feyereisen et al. compared the ovarian response of women with DM1 with women with X-linked disorder carriers. They concluded that women with DM1 have a moderate decrease in fertility (10).

The results of our study suggest a relatively poor response to COS among women with DM1 in comparison to age matched controls, though there was no difference in oocyte dysmorphism, fertilisation rate and embryo quality.

The mechanisms by which DM1 damages testicular functions while being relatively sparing of ovarian function remains to be elucidated. Though the number of CTG repeats broadly correlates with the overall severity of the disease but it has a predictive value only in the case of some clinical symptoms, and pathogenic mechanisms of DM1 may differ depending on the tissue. Marchini et al. (19) reported that sterility or hypogonadism were not related to the number of CTG repeats. Feyereisen et al. (10) found no statistically significant correlation between the ovarian status, response to COS, or cancellation rate for insufficient response and (CTG) triplet repeats.

In conclusion, despite the fact that the present study is retrospective with a limited number of cases, the findings suggest that women with DM1 have a relatively reduced ovarian reserve and respond poorly to COS, but there is no impact on oocyte and embryo quality. Reproductive potential remains relatively intact in comparison to men. Further studies with sufficient number of subjects are required to evaluate the mechanism of impact of DM1 on ovarian function.

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