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ORIGINAL ARTICLE *Puberty, Aging and HRT*

Anti-Müllerian hormone and inhibin B levels as markers of premature ovarian aging and transition to menopause in type 1 diabetes mellitus

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BACKGROUND: Serum anti-Müllerian hormone (AMH) levels decrease early during the transition to menopause and women with type 1 diabetes mellitus (DM1) experience menopause at a younger age. We hypothesized that older women with DM1 will have lower AMH levels than controls.

METHODS: We studied ovarian function in women with DM1 ($n = 66$) and healthy controls ($n = 58$), all <45 years old. Steroids, gonadotrophins, AMH and inhibin B levels were measured during the follicular phase.

RESULTS: Piece-wise regression analysis demonstrated that AMH levels begin to decrease at 33 years of age in both groups. This age limit was used to compare data in both groups. AMH levels were lower in DM1 women than in controls >33 years (4.1 ± 4.2 versus 9.5 ± 7.9 pmol/l, mean ± SD, $P = 0.006$). A higher proportion of women with DM1 showed AMH levels in the menopausal range compared with controls (16.7% versus 3.4%, respectively, $P = 0.02$). For all patients, those with DM1 exhibited lower inhibin B levels than controls (89.3 ± 51.7 versus 113.2 ± 76.0 ng/ml, $P < 0.05$). FSH and estradiol were similar in both groups. Regression analysis showed an earlier decline in AMH levels in women with DM1 than controls. Even after age adjustment, DM1 was a significant factor for the determination of inhibin B and AMH levels.

CONCLUSIONS: Lower AMH levels in women with DM1 during the fourth decade of life suggest the presence of an earlier decline in the ovarian follicle pool in these women. Further studies are needed to evaluate the mechanism of this complication.

Key words: menopause / type 1 diabetes mellitus / anti-Müllerian hormone / inhibin B / ovarian reserve

Introduction

In healthy women, menopause is an event that is associated with the exacerbation of several risk factors of cardiovascular disease and a decrease in bone mass (Martin and Manson, 2008). These abnormalities may be especially detrimental for women with type 1 diabetes mellitus (DM1) (Codner, 2008; Codner and Cassorla, 2009), who also experience menopause at a younger age (Snell-Bergeon *et al*., 2008). Dorman *et al*. (2001) observed that the age of the final menstrual period is 8 years earlier in women with DM1 compared with their sisters.

Several years before menopause, a period of irregular menses occurs that is termed 'menopausal transition' (Burger *et al*., 2007; Martin and Manson, 2008). Serum anti-Müllerian hormone (AMH), a glycoprotein produced by the granulosa cells of small ovarian follicles, decreases early during menopausal transition. Recently, Sowers *et al*. (2008a, b, c) followed a group of 50 women and observed that serum AMH levels began to decline 10 years before the final menstrual period, reaching a level below the detection limit 5 years before menopause. In contrast, the levels of estradiol (E_2) and FSH are normal until a couple of years before the cessation of menses (Sowers *et al*., 2008a, b, c).

Inhibin B is another hormone that is secreted by granulosa cells. Although it is not as indicative of ovarian aging as AMH (Sowers *et al*., 2008a, b, c), elevated levels of inhibin B suggest the presence

of autoimmune oophoritis (Tsigkou *et al*., 2008). This finding is useful in the evaluation of ovarian failure in DM1, since autoimmune oophoritis could be a cause of ovarian failure in these patients.

In order to assess the hypothesis that premature ovarian aging occurs in women with DM1, we performed a cross-sectional study to evaluate the levels of AMH, inhibin B, steroids and gonadotrophin in women with DM1. The primary hypothesis of this paper is that older women with DM1 have lower levels of AMH than an age-matched group of control women.

Study design and methods

Subjects

We studied the ovarian reserve in women with DM1 ($n = 66$) and healthy control women ($n = 58$). Both groups included only women who were younger than 45 years, and both were matched according to gynecological and chronological age and BMI. None of the women were pregnant or had received sex steroids or any drug known to affect ovarian function for at least 6 months. Gynecological age was defined as chronological age minus the age of menarche.

Patients with DM1 were included in this study if they fulfilled the following inclusion criteria: persistent severe insulinopenia, diagnosed with DM1 from the onset of the disease, diagnosed with DM1 before 30 years of age and duration of disease of at least 1 year. Most of the patients had DM1 for at least 3 years; only two patients had DM1 for a shorter period. The exclusion criteria were the following: type 2 or other types of diabetes; honeymoon period; abnormal thyroid function; elevated creatinine level, the presence of micro- or macrovascular complications; and the presence of other concomitant chronic conditions such as genetic syndromes, celiac disease, renal disease, liver disease, cardiac disease or undernourishment.

The control group included 58 healthy women who had normal fasting glucose levels without a history of hyperandrogenism, regular menstrual cycles and no history of any chronic disease. These women were friends of staff of the hospital and of the DM1 patients. Control women older than 35 years with mild menstrual irregularities were included in the study. Women with previous history of gestational diabetes or hypertension were not included in the study.

Study protocol

We first performed a complete physical examination. Weight was measured using a conventional Seca scale with a precision of 100 g, and height was measured with a Harpenden Stadiometer. Menstrual cycles were considered as irregular if they were longer than 35 days or shorter than 25 days or when the length difference between two successive cycles was greater than 7 days (Burger *et al*., 2005).

This protocol was approved by the institutional review board of the San Borja Arriarán Hospital. All the patients gave written informed consent. Patients younger than 18 years gave their assent before entering the study, and their parents gave informed consent.

Hormone assays

An early morning sample of blood was obtained from both groups of women during the follicular phase (days 1–7) for the measurement of AMH, inhibin B, gonadotrophins, testosterone, androstenedione, 17OH progesterone, dehydroepiandrosterone sulfate and sex hormone-binding globulin (SHBG), as described previously (Codner *et al*., 2005, 2006, 2007). The free androgen index was calculated as described previously (Codner *et al*., 2005). Steroids and gonadotrophins were measured as described previously (Codner *et al*., 2005, 2006, 2007). Serum AMH was assayed using the AMH/MIS enzyme-linked immunosorbent assay (ELISA) kit (Immunotech-Beckman, Marseilles, France) as described previously (Rey *et al*., 2005). The assay sensitivity was 0.7 pmol/l, and the intra- and inter-assay coefficients of variation were 5.3% and 8.7%, respectively. A serum AMH level equal to or lower than 1.23 pmol/l was considered to be in the menopausal range (van Disseldorp *et al*., 2008).

Serum inhibin B was measured using specific two-site ELISAs (Diagnostic Systems Labs, Webster, TX, USA). The assay sensitivity was 7 pg/ml, and the intra- and inter-assay coefficients of variation were 4.8% and 7.1%, respectively (Rey *et al*., 2005).

Statistical analysis

Continuous data are shown as mean ± SD. The variables had a normal distribution as assessed by a Kolmogorov–Smirnov test. Differences between the DM1 and control groups for continuous variables were assessed with a Student's *t*-test. The difference in the proportion of women having an AMH level in the menopausal range was evaluated using an independent χ^2 test. Correlations between AMH or inhibin B levels and other clinical or laboratory findings were evaluated with a Pearson correlation coefficient. The effect of DM1 adjusted by age on AMH or inhibin B levels was assessed using multiple linear regression. In addition, the effect of HbA1c (glycosylated hemoglobin), daily insulin dose and age of onset of DM1 on AMH and inhibin B levels were also evaluated using multiple linear regression in the DM1 group. The residues of the regression analysis did not have a normal distribution, which was solved by transforming AMH and inhibin B by the square root.

In order to determine the deflection point of AMH in both groups of women, piece-wise linear regression was utilized. This regression analysis allows for the determination of the effect of time on the dependent variable. Piece-wise analysis was performed using 5-year periods from the age of 18 years. The deflection point was used to determine the shift in the curve of AMH levels. Piece-wise regression showed that the deflection of AMH levels occurred at the age of 33 years in both DM1 ($P = 0.008$) and control women ($P = 0.05$) in our study sample. This age limit was used to compare clinical and hormonal data in both groups (Supplementary data 1). We are aware that performing the same study in larger samples might identify additional deflection points.

All statistical calculations were run on Stata 10.5 (College Station, Texas, USA) and GraphPad Prism, version 5.0, for Windows (Graph-Pad Software, San Diego, CA). A significance level of 5% was employed.

Results

The clinical characteristics of the patients are shown in Table I. Steroid, AMH, inhibin B and gonadotrophin levels are shown in Table II. For the groups as a whole, compared with controls the DM1 group had longer menstrual cycles, a higher prevalence of menstrual irregularities, higher levels of testosterone and androstenedione

Table I Clinical and anthropometric characteristics of women with DM1 and control women, including diabetes duration and HbA1c levels in patients with DM1

	Whole group		≤33 years		>33 years	
	Control	DM1	Control	DM1	Control	DM1
n	58	66	34	46	24	20
Age (years)	30.3 ± 8.7	27.7 ± 9.0	24.0 ± 5.1	22.9 ± 6.0	39.2 ± 3.3	38.7 ± 3.2
Gynecological age (years)	17.2 ± 8.8	15.1 ± 8.8	11.0 ± 4.9	10.3 ± 5.7	26.6 ± 3.3	25.7 ± 3.6
BMI (kg/m^2)	25.0 ± 3.5	24.8 ± 2.9	24.2 ± 3.4	24.5 ± 2.9	26.0 ± 3.4	25.6 ± 2.9
Duration of diabetes (years)		13.8 ± 7.9		10.8 ± 6.3		20.5 ± 7.4
HbA1c (%)		8.3 ± 2.0		8.5 ± 1.8		8.0 ± 1.6
Age at menarche (years)	12.7 ± 1.3	12.7 ± 1.3	12.8 ± 1.1	12.4 ± 1.6	12.6 ± 1.5	13.1 ± 1.6
Menstrual cycle length (days)	28.6 ± 2.0	32.3 ± 10.2**	28.6 ± 1.7	33.1 ± 11.7*	28.7 ± 2.5	30.4 ± 5.2
Menstrual irregularities (%)	1.7	21.2***	0	19.6**	4.2	25.0*
Pregnancies (*n*)	1.1 ± 1.4	0.8 ± 1.2	0.7 ± 1.4	0.6 ± 1.0	1.6 ± 1.5	1.4 ± 1.6
Number of live births	1.0 ± 1.4	0.6 ± 0.8	0.6 ± 1.3	0.5 ± 0.7	1.5 ± 1.4	1.0 ± 1.0

The women are also separated according to age, either younger or older than 33 years. Data are shown as mean ± SD.
*$P < 0.05$, **$P<0.01$ and ***$P < 0.001$ versus respective control group.

Table II Levels of steroids, gonadotrophins, AMH and inhibin B in patients with DM1 and control women (data are mean ± SD)

	Whole group		≤33 years		>33 years	
	Control	DM1	Control	DM1	Control	DM1
n	58	66	34	46	24	20
LH (IU/l)	6.7 ± 15.8	5.4 ± 5.5	4.8 ± 1.7	5.9 ± 6.3	9.5 ± 24.4	4.1 ± 2.3
FSH (IU/l)	7.4 ± 7.2	6.2 ± 3.4	5.4 ± 1.6	5.8 ± 2.9	10.1 ± 10.6	7.2 ± 4.1
Estradiol (pmol/l)	255.8 ± 122.5	271.2 ± 189.0	249.6 ± 123.7	237.8 ± 118.5	264.6 ± 122.9	344.6 ± 282.6
Testosterone (nmol/l)	1.5 ± 0.6	1.8 ± 0.8*	1.7 ± 0.5	2.1 ± 0.8*	1.3 ± 0.8	1.3 ± 0.0
FAI	3.9 ± 3.6	4.4 ± 3.9	4.7 ± 4.1	5.5 ± 4.2	2.9 ± 2.3	2.0 ± 1.4
SHBG (nmol/l)	53.2 ± 23.0	59.5 ± 29.3	51.7 ± 26.7	51.9 ± 27.6	55.3 ± 16.8	77.0 ± 26.0**
17OH progesterone (nmol/l)	3.6 ± 2.7	4.1 ± 2.5	4.2 ± 1.8	4.8 ± 2.3	2.9 ± 3.6	2.6 ± 2.2
DHEAS (μmol/l)	3.6 ± 1.57	3.9 ± 1.9	3.8 ± 1.2	3.9 ± 1.7	3.3 ± 1.9	3.9 ± 2.5
Androstenedione (nmol/l)	5.1 ± 2.0	6.2 ± 2.4*	5.5 ± 1.7	6.6 ± 2.4*	4.6 ± 2.5	5.3 ± 2.4
AMH (pmol/l)	14.2 ± 10.8	12.1 ± 9.2	17.6 ± 11.4	15.5 ± 8.7	9.5 ± 7.9	4.1 ± 4.2**
Inhibin B (pg/ml)	113.2 ± 76.0	89.3 ± 51.7*	127.5 ± 81.5	96.0 ± 55.6*	93.4 ± 64.0	74.0 ± 38.3

SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index. Conversion to metric units: testosterone: nmol/l × 0.2882 = ng/ml; androstenedione: nmol/l × 0.2865 = ng/ml; DHEAS: nmol/l × 370.37 = ng/ml; estradiol: pmol/l × 0.2725 = pg/ml; 17OH progesterone: nmol/l × 0.3300 = ng/ml; SHBG: nmol/l/34.67 = μg/dl; AMH: pmol/l × 0.1400 = μg/l.
*$P < 0.05$ and **$P < 0.01$ versus respective control group.

and lower levels of inhibin B. Similar abnormalities were also observed in women with DM1 ≤33 years of age when compared with their matched controls. The levels of AMH were similar in the two groups of women when all DM1 and control women, and those ≤33 years of age, were compared.

Although the prevalence of menstrual irregularities was increased in women with DM1 >33 years old, the length of the menstrual cycle was not affected. Lower levels of AMH and higher levels of SHBG were observed in DM1 patients in this age group. A higher proportion of women with DM1 had AMH levels in the menopausal range (16.7% versus 3.4%, respectively, $P = 0.02$).

To avoid the bias that would be introduced by the fact that cycle irregularities were higher in the DM1 group, we compared serum AMH levels exclusively in control ($n = 23$) and DM1 ($n = 15$) women of >33 years who were having regular menses. The clinical characteristics of the patients with normal cycles are shown in Supplementary data 2. In this subgroup analysis (not shown in Table II), AMH was lower in the DM1 patients (3.3 ± 3.9 pmol/l) than in the

controls (9.9 ± 7.8 pmol/l, $P = 0.002$). Androgen, estradiol and gonadotrophin levels were similar in both DM1 and control women >33 years old.

AMH levels were significantly affected by DM1 and age (Fig. 1A, Table III). This effect was observed mainly in DM1 women >33 years old (Fig. 1D). The levels of AMH were not affected by age or DM1 in women ≤33 years old (Fig. 1C). In DM1 and control women >33 years, the slope of the regression line of AMH levels according to age was similar, but it had a lower intercept in the DM1 group than in the controls ($F = 12.1$; DFn = 2; DFd = 41; $P < 0.0001$), which suggested an earlier decrease in the levels of AMH in women with DM1.

The levels of AMH were inversely correlated with the age at diagnosis of DM1 ($r = -0.3$, $P = 0.01$) and showed a positive correlation with daily insulin dose ($r = 0.4$, $P = 0.003$). When these factors were adjusted by age, however, neither age at diagnosis of DM1 nor daily insulin dose was significantly correlated with AMH levels. The levels of HbA1c and a pre-menarcheal onset of DM1 were not significantly correlated with AMH levels.

Inhibin B levels were lower in DM1 women, even adjusted for age (Tables II and III, Fig. 1B), and the differences in the elevation of the regression lines of the DM1 and control groups were significant ($F = 4.97$; DFn = 1; DFd = 120; $P = 0.05$). Patients with DM1 exhibited lower inhibin B levels in the entire group comparison and in women ≤33 years old, but not in those >33 years old. Regression analysis showed that DM1 affected inhibin B levels only in the younger group (Table III). Inhibin B levels were not correlated with metabolic control, daily insulin dose or age of onset of DM1.

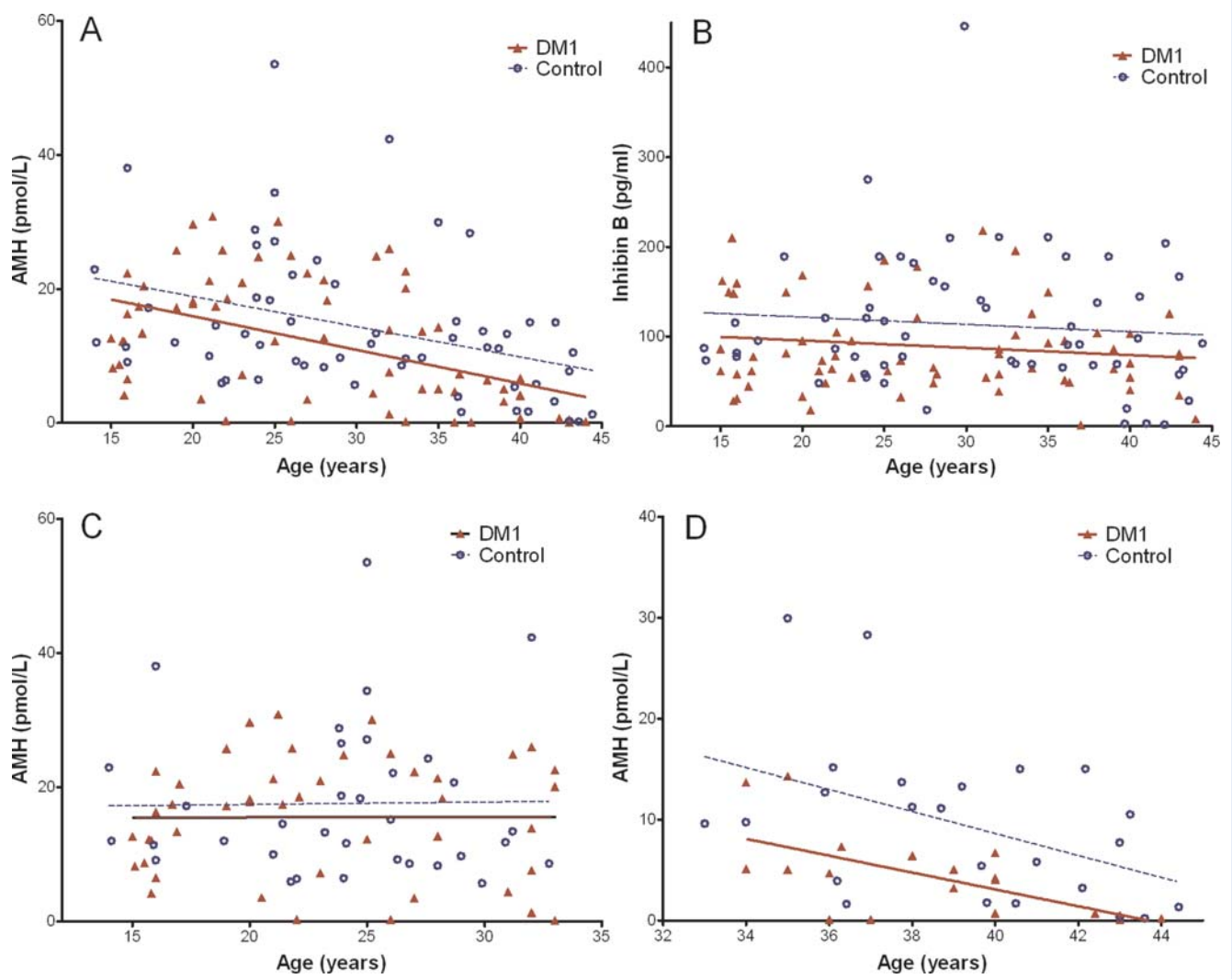

Figure 1 (**A**) Serum AMH levels in women with DM1 ($n = 66$) and controls ($n = 58$). The differences between the levels were significant ($P = 0.01$). (**B**) Serum inhibin B levels in women with DM1 and controls. $P = 0.05$, control versus DM1 levels. (**C**) Serum AMH levels in women with DM1 and controls, 33 years old or younger. Similar levels of AMH levels were observed in the two groups and age did not affect the AMH levels. (**D**) Serum AMH levels in women with DM1 and controls older than 33 years. $P < 0.0001$, control versus DM1 levels. Broken line and open blue circles, control women; black line and filled red triangles, women with DM1.

Table III Multivariate analysis of the effect of DM1 and age on the levels of AMH and inhibin B

Multivariate analysis for	B	SEM (B)	*P*-value
AMH levels: all subjects			
DM1	−0.62	0.23	0.01
Age	−0.85	0.01	<0.0001
AMH levels: women ≤33 years			
DM1	−0.32	0.30	0.28
Age	−0.01	0.03	0.66
AMH levels: women >33 years			
DM1	−1.15	0.32	0.0009
Age	−0.21	0.05	0.0002
Inhibin levels: all subjects			
DM1	−1.14	0.58	0.05
Age	−0.06	0.03	0.08
Inhibin B levels: women ≤33 years			
DM1	−1.4	0.7	0.04
Age	0.2	0.1	0.1
Inhibin B levels: women >33 years			
DM1	−0.3	0.2	0.1
Age	−0.8	1.0	0.4

'B' refers to the unstandardized coefficients of the estimated regression model. SEM, standard error of the mean of the coefficients.

Discussion

To our knowledge, this is the first study to evaluate ovarian reserve in DM1 women. Our results show that middle-aged women with DM1 have a higher frequency of menstrual irregularities, lower AMH levels and an increased prevalence of menopausal AMH values compared with age-matched controls. We further demonstrate that AMH levels decrease earlier in DM1 patients and that Inhibin B levels are also lower in these women. Together, these findings suggest an earlier decline in the ovarian follicle pool in women with DM1.

In normal women, there is a decline in the quantity and quality of oocytes during mid-life that predicts menopause (Faddy *et al*., 1992). The serum levels of AMH, which is secreted by small follicles, have been shown to reflect the ovarian follicle pool (Visser *et al*., 2006). Furthermore, AMH levels are a good index of reproductive age (van Disseldorp *et al*., 2008), and the levels of this hormone can be used to predict the age of menopause (van Rooij *et al*., 2005). Women with higher levels of this hormone reach menopause later (van Disseldorp *et al*., 2008). The lower levels of AMH in DM1 women older than 33 years suggest the presence of a lower number of small growing follicles and a smaller ovarian reserve. Future follow-up of the patients in our study will address whether AMH levels can also predict the age of menopause in DM1 women.

Lower inhibin B levels were observed in DM1, even after adjusting for age. These data suggest a lower number of antral follicles in these patients (Groome *et al*., 1996; Knight and Glister, 2001). Early menopause in women with DM1 has been postulated to be associated with autoimmune oophoritis. Recently, Tsigkou *et al*. (2008) showed that the measurement of inhibin B levels may help with differentiating autoimmune premature ovarian failure from natural menopause, since the levels of this hormone are elevated in the former and diminished in the latter. The low inhibin B levels observed in our study are more compatible with non-immune-mediated follicular loss than with the presence of autoimmune oophoritis.

The mechanism of the advancement in the age of menopause in DM1 is unclear. In healthy non-diabetic women, apoptosis is the primary mechanism that leads to a decrease in the ovarian follicular pool (Tilly, 2001). This mechanism has been shown to be exaggerated in studies performed in diabetic animals (Chang *et al*., 2005). Several of the mechanisms involved in ovarian aging that have been described in healthy women may be exacerbated in women with DM1 (reviewed in Tatone *et al*., 2008). These mechanisms include protein damage by non-enzymatic protein glycosylation, altered vascular support, a reduced oxygen supply and elevated levels of vascular endothelial growth factor.

Women are born with a pool of ovarian follicles that will accompany them during their reproductive lives as 'resting' follicles. These follicles are subject to damage by environmental conditions (Tatone *et al*., 2008). Granulosa cells interact with each other, which is fundamental for the survival of the oocyte. These cells are altered when exposed to hyperglycemia, which was recently demonstrated in diabetic animals (Colton *et al*., 2003; Chang *et al*., 2005). In addition, the presence of elevated blood glucose levels in animals exacerbates several known mechanisms in the process of ovarian aging (Tatone *et al*., 2008). These studies have shown the presence of altered metabolic pathways (Downs and Utecht, 1999; Colton *et al*., 2003) and maturation of the follicles, in addition to increased apoptosis (Chang *et al*., 2005) in diabetic mice.

Glycosylation of proteins, which is a process enhanced by hyperglycemia, may have a role in deteriorating ovarian function in women with DM1. The presence of advanced glycosylation end products and the activation of their receptor are involved in physiological aging (Baynes, 2001; Tatone *et al*., 2008). These end-products are present in the ovarian tissue of healthy young women during their third decade of life (Diamanti-Kandarakis *et al*., 2007). It is possible that the ovary, similar to other tissues, also experiences the deleterious effects of the activation of these pathways.

The fact that AMH was diminished in the face of normal E_2 and FSH levels may be explained by the fact that steroids and gonadotrophin reach abnormal levels only when the final menstrual period is imminent and that they are less sensitive markers of the ovarian reserve (Sowers *et al*., 2008a, b, c).

We did not find any clinical factor related to diabetes that could explain the decline in AMH levels in these patients. Our study, however, excluded women who had micro- or macrovascular complications and abnormal creatinine levels. It is possible that including a larger group of women would identify the modifying factors that are involved in the early decay of ovarian function.

We previously measured AMH levels in DM1 patients with hyperandrogenism (Codner *et al*., 2007). We found that AMH levels are normal in DM1 women with polycystic ovary syndrome (PCOS), which is in contrast to the elevated levels that are observed in non-diabetic women with PCOS. This finding suggests that insulin acts as a co-gonadotrophin during the more advanced stages of

folliculogenesis, stimulating the growth of large follicles that do not secrete AMH. Whether insulin therapy has a role in the decline in ovarian function that is observed in our patients remains unclear.

Hyperandrogenism may be a frequent problem in women with DM1, especially in patients receiving intensive insulin treatment (Codner *et al*., 2006; Codner and Escobar-Morreale, 2007). Our data show that this problem is primarily observed in young women and that androgen levels are within the normal range in older women. One hypothesis explaining this observation could be that the decline in ovarian function that occurs with age in women with DM1 also affects the production of androgens.

A limitation of our study is that the menstrual cycle abnormalities observed in DM1 may have several causes. The irregularities of the menstrual cycle in DM1 patients have been associated with abnormalities in the hypothalamic-pituitary axis (Djursing *et al*., 1985; Codner and Cassorla, 2008) or PCOS (Codner *et al*., 2006; Codner and Escobar-Morreale, 2007). Our data suggest that ovarian aging may also represent an underlying mechanism.

In conclusion, our study shows that women with DM1 have prematurely aging ovaries, as demonstrated by an early decline in the levels of AMH and inhibin B. These findings suggest an earlier decline in the ovarian follicle pool compared with non-diabetic women. This phenomenon most likely occurs at the oocyte–cumulus cell complex and results from damage through several mechanisms. We speculate that a non-immune mechanism may play a role in the pathophysiology of this complication. Future studies should evaluate the precise mechanism that leads to this decline in AMH levels, as well as the relationship between this abnormality and reproductive function in women with DM1.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/

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References

Baynes JW. The role of AGEs in aging: causation or correlation. *Exp Gerontol* 2001;**36**:1527–1537.

Burger HG, Robertson DM, Baksheev L, Collins A, Csemiczky G, Landgren BM. The relationship between the endocrine characteristics and the regularity of menstrual cycles in the approach to menopause. *Menopause* 2005;**12**:267–274.

Burger HG, Hale GE, Robertson DM, Dennerstein L. A review of hormonal changes during the menopausal transition: focus on findings from the Melbourne Women's Midlife Health Project. *Hum Reprod Update* 2007;**13**:559–565.

Chang AS, Dale AN, Moley KH. Maternal diabetes adversely affects preovulatory oocyte maturation, development, and granulosa cell apoptosis. *Endocrinology* 2005;**146**:2445–2453.

Codner E. Estrogen and type 1 diabetes mellitus. *Pediatr Endocrinol Rev* 2008;**6**:228–234.

Codner E, Cassorla F. Puberty and ovarian function in girls with type 1 diabetes mellitus. *Horm Res* 2008;**71**:12–21.

Codner E, Cassorla F. Puberty and ovarian function in girls with type 1 diabetes mellitus. *Horm Res* 2009;**71**:12–21.

Codner E, Escobar-Morreale HF. Hyperandrogenism and polycystic ovary syndrome in women with type 1 diabetes mellitus. *J Clin Endocrinol Metab* 2007;**92**:1209–1216.

Codner E, Mook-Kanamori D, Bazaes RA, Unanue N, Sovino H, Ugarte F, Avila A, Iniguez G, Cassorla F. Ovarian Function during puberty in girls with type 1 diabetes mellitus: response to leuprolide. *J Clin Endocrinol Metab* 2005;**90**:3939–3945.

Codner E, Soto N, Lopez P, Trejo L, Avila A, Eyzaguirre FC, Iniguez G, Cassorla F. Diagnostic criteria for polycystic ovary syndrome and ovarian morphology in women with type 1 diabetes mellitus. *J Clin Endocrinol Metab* 2006;**91**:2250–2256.

Codner E, Iniguez G, Villarroel C, Lopez P, Soto N, Sir-Petermann T, Cassorla F, Rey RA. Hormonal profile in women with polycystic ovarian syndrome with or without type 1 diabetes mellitus. *J Clin Endocrinol Metab* 2007;**92**:4742–4746.

Colton SA, Humpherson PG, Leese HJ, Downs SM. Physiological changes in oocyte–cumulus cell complexes from diabetic mice that potentially influence meiotic regulation. *Biol Reprod* 2003;**69**:761–770.

Diamanti-Kandarakis E, Piperi C, Patsouris E, Korkolopoulou P, Panidis D, Pawelczyk L, Papavassiliou AG, Duleba AJ. Immunohistochemical localization of advanced glycation end-products (AGEs) and their receptor (RAGE) in polycystic and normal ovaries. *Histochem Cell Biol* 2007;**127**:581–589.

Djursing H, Hagen C, Nyboe Andersen A, Svenstrup B, Bennett P, Molsted Pedersen L. Serum sex hormone concentrations in insulin dependent diabetic women with and without amenorrhoea. *Clin Endocrinol (Oxf)* 1985;**23**:147–154.

Dorman JS, Steenkiste AR, Foley TP, Strotmeyer ES, Burke JP, Kuller LH, Kwoh CK. Menopause in type 1 diabetic women: is it premature? *Diabetes* 2001;**50**:1857–1862.

Downs SM, Utecht AM. Metabolism of radiolabeled glucose by mouse oocytes and oocyte–cumulus cell complexes. *Biol Reprod* 1999;**60**:1446–1452.

Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. *Hum Reprod* 1992;**7**:1342–1346.

Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP, McNeilly AS. Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* 1996;**81**:1401–1405.

Knight PG, Glister C. Potential local regulatory functions of inhibins, activins and follistatin in the ovary. *Reproduction* 2001;**121**:503–512.

Martin KA, Manson JE. Approach to the patient with menopausal symptoms. *J Clin Endocrinol Metab* 2008;**93**:4567–4575.

Rey RA, Codner E, Iniguez G, Bedecarras P, Trigo R, Okuma C, Gottlieb S, Bergada I, Campo SM, Cassorla FG. Low risk of impaired testicular

Sertoli and Leydig cell functions in boys with isolated hypospadias. *J Clin Endocrinol Metab* 2005;**90**:6035–6040.

Snell-Bergeon JK, Dabelea D, Ogden LG, Hokanson JE, Kinney GL, Ehrlich J, Rewers M. Reproductive history and hormonal birth control use are associated with coronary calcium progression in women with type 1 diabetes mellitus. *J Clin Endocrinol Metab* 2008;**93**:2142–2148.

Sowers MR, Eyvazzadeh AD, McConnell D, Yosef M, Jannausch ML, Zhang D, Harlow S, Randolph JF Jr. Anti-Mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. *J Clin Endocrinol Metab* 2008a;**93**:3478–3483.

Sowers MR, Zheng H, McConnell D, Nan B, Harlow S, Randolph JF Jr. Follicle stimulating hormone and its rate of change in defining menopause transition stages. *J Clin Endocrinol Metab* 2008b; **93**:3958–3964.

Sowers MR, Zheng H, McConnell D, Nan B, Harlow SD, Randolph JF Jr. Estradiol rates of change in relation to the final menstrual period in a population-based cohort of women. *J Clin Endocrinol Metab* 2008c; **93**:3847–3852.

Tatone C, Amicarelli F, Carbone M, Monteleone P, Caserta D, Marci R, Artini P, Piomboni P, Focarelli R. Cellular and molecular aspects of ovarian follicle ageing. *Hum Reprod Update* 2008;**14**:131–142.

Tilly JL. Commuting the death sentence: how oocytes strive to survive. *Nat Rev Mol Cell Biol* 2001;**2**:838–848.

Tsigkou A, Marzotti S, Borges L, Brozzetti A, Reis F, Candeloro P, Luisa Bacosi M, Bini V, Petraglia F, Falorni A. High serum inhibin concentration discriminates autoimmune oophoritis from other forms of primary ovarian insufficiency. *J Clin Endocrinol Metab* 2008; **93**:1263–1269.

van Disseldorp J, Faddy MJ, Themmen AP, de Jong FH, Peeters PH, van der Schouw YT, Broekmans FJ. Relationship of serum antimullerian hormone concentration to age at menopause. *J Clin Endocrinol Metab* 2008;**93**:2129–2134.

van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, Fauser BJ, Themmen AP, Te Velde ER. Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. *Fertil Steril* 2005;**83**:979–987.

Visser JA, de Jong FH, Laven JSE, Themmen APN. Anti-Mullerian hormone: a new marker for ovarian function. *Reproduction* 2006; **131**:1–9.

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