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Testicular Sertoli cell function in male systemic lupus erythematosus

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Objective. To assess the testicular Sertoli cell function in male SLE patients.
Methods. Thirty-four consecutive patients were prospectively selected to evaluate serum inhibin B. Clinical features, treatment, semen analysis, urological evaluation, testicular ultrasound, hormones and anti-sperm antibodies were determined.
Results. Patients were subdivided into two groups: low serum inhibin B (Group 1, $n=8$) and normal levels (Group 2, $n=26$). The median sperm concentration ($P=0.024$), total sperm count ($P=0.023$) and total motile sperm count ($P=0.025$) were lower in Group 1. Inhibin B levels were positively correlated with sperm concentration ($r=0.343$), total motile sperm count ($r=0.357$), and negatively correlated with follicule-stimulating hormone (FSH) ($r=0.699$) and luteinizing hormone ($r=0.397$). The median serum inhibin B was lower in SLE patients treated with intravenous cyclophosphamide **(**IVCYC) compared with those without this therapy ($P=0.031$). Further evaluation of the 26 SLE patients with normal inhibin B and FSH levels revealed that medians of inhibin B/FSH ratio were lower in SLE patients with oligozoospermia compared with normozoospermia ($P=0.004$). This ratio was also lower in SLE patients treated with IVCYC than those without this therapy ($P=0.04$). In contrast, inhibin B serum level alone did not discriminate the later group of patients ($P=0.12$).
Conclusions. This is the first study to identify a high frequency of testicular Sertoli cell dysfunction in male SLE associated with semen abnormalities. Further prospective studies are necessary to determine if inhibin levels and inhibin B/FSH ratio will be an earlier and useful marker of IVCYC toxicity in these patients.

Key words: Systemic lupus erythematosus, Male, Sperm, Hormone, Sertoli cell, Inhibin B.

Introduction

SLE is a multisystem autoimmune disease involving patients during their reproductive years with a remarkable low proportion of male gender [1, 2]. Novel therapeutic approaches have improved survival and emphasize the relevance of future quality of life, including testicular function and fertility.

Inhibin is a heterodimeric glycoprotein hormone produced almost exclusively by testes' Sertoli cells [3, 4]. It is composed of an α-subunit disulphide-linked with β-subunits; either with the β_A-subunits to form inhibin A or with the β_B-subunits to form inhibin B [5, 6]. Inhibin B is the male active physiological form of inhibin in circulation, and therefore is the most important endocrine marker for monitoring the gonadal function in men [3, 5–8]. The findings that castration resulted in undetectable inhibin B levels evidenced that circulating inhibin B was produced by the testes [3].

Furthermore, inhibin B regulates pituitary follicle-stimulating hormone (FSH) release by a negative feedback loop [9], which is reinforced by reports of a strong negative correlation not only between inhibin B and FSH [10–12], but also with luteinizing hormone (LH) [12] in subfertile men. Inhibin B concentrations may provide useful information about seminiferous tubules function [10] and seems to be a direct marker of spermatogenesis [12]. Moreover, inhibin B has been used as an important Sertoli cell function parameter in male Hodgkin's disease survivors treated with chemotherapy [5].

We have recently studied 35 male SLE patients and identified severe sperm abnormalities associated with intravenous cyclophosphamide (IVCYC) therapy and with elevated FSH levels [13]. There is however no systematic study in the literature assessing inhibin B levels in male SLE patients and its relevance to sperm abnormalities.

The aim of this study was to evaluate testicular Sertoli cell function in male SLE patients and its possible association with sperm abnormalities determined by urological evaluation, testicular Doppler ultrasound, hormone profile and anti-sperm antibodies. Inhibin B levels were also analysed in the context of SLE clinical features, disease activity and treatment.

Patients and methods

SLE patients

Thirty-four SLE male patients aged between 15 and 45 yrs regularly followed at the Pediatric Rheumatology Unit or the Lupus Clinics of the Rheumatology Division, University of São Paulo, were selected for this study from January 2003 to January 2006, as previously reported [13]. All patients fulfilled the ACR SLE classification criteria [14]. Exclusion criteria were hydrocoele, hypospadia, cryptorchidism, testicular infection (e.g. mumps), testicular cancer, orchitis, testicular vasculitis, ureteral impairment, previous history of any scrotal or inguinal surgery (e.g. varicocoelectomy, vasectomy, hernia repair), diabetes mellitus, previous or current history of alcohol or tobacco use and refusal to collect sperm sample or incomplete evaluation. The local ethical committee approved this study and an informed consent was obtained from all participants and when necessary from their respective parents.

Testicular cell function

The testicular cell function was determined by serum inhibin B levels at study entry, blinded to the semen analysis by the specialist (T.S.O.). This hormone was measured in an enzymatically amplified two-site two-step sandwich-type immunoassay. In the assay, duplicated samples were incubated in microtitration wells, which have been coated with anti-inhibin β_B (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). Intra- and inter-assay coefficients of variation were limited to 3.5–5.6% and 6.2–7.6%, respectively. The normal ranges were 74–470 pg/ml (12- to 17-yrs old) and 60–300 pg/ml (18- to 50-yrs old).

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Hormonal status

Hormonal determinations were performed at study entry. Abnormal results were repeated for confirmation. FSH, LH, prolactin, total testosterone, triiodothyronine (T3), tetraiodothyronine (T4), free T4 and thyrotropin [thyroid-stimulating hormone (TSH)] levels were detected by fluoroimmunoassay using kits from DELPHIA^{R} time-resolved fluoroimmunoassay (WALLAC Ou, Turku, Finland). Intra- and inter-assay coefficients of variation were limited to 3.5 and 2.1%, respectively. The normal ranges were: FSH (1–10.5 IU/l), LH (1–8.4 IU/l), prolactin (2–10 ng/ml), total testosterone (271–965 ng/dl), T3 (70–204 ng/dl), T4 (4.3–12.5 μg/dl), free T4 (0.4–1.6 ng/dl) and TSH (0.5–6 μU/ml).

Urological evaluation

A systematic clinical examination of the genitalia was performed in patients by the same expert urologist (M.C.) and includes evaluation of testicles, epididymis, vas deferens, scrotum and penis. The secondary sexual characteristics were evaluated according to Marshall and Tanner's pattern criteria of pubertal changes [15]. Testicular volumes were measured using the Prader orchidometer, which consists of 12 ellipsoid models graded from 1 to 25 ml (from 1 to 6, 8, 10, 12, 15, 20 and 25) [16]. The normal testicular volume in Brazilian post-pubertal adolescents and adults ranges between 12 and 25 ml [17]. The patients were examined in a warm room with temperature not inferior to 22°C, in both the standing and supine positions, and with and without Valsalva manoeuver. Varicocoele was graded according to the following criteria: Grade I (small) palpable only with concurrent Valsalva manoeuver, Grade II (medium) palpable with patient standing and Grade III (large) visible through scrotal skin, palpable with patient standing [18].

Testicular Doppler ultrasound

Ultrasound was performed in all SLE patients by an expert sonographer using a 14-MHz sector scanner (Logic 9-GE-, Milwaukee, WI, USA) blinded to the semen analysis. Testes were scanned in axial and longitudinal planes, and at least two measurements of length, width and thickness were obtained. The largest measurement in each dimension was recorded and used to calculate the testicular volume according to the formula for an ellipsoid (length $\times$ width $\times$ thickness $\times$ 0.52). The normal value in male post-pubertal adolescents and adults is 15 ± 8 ml [19].

Semen analysis

Semen analysis was performed according to the guidelines of the World Health Organization (WHO) [20, 21] by two expert medical technologists (A.B. and K.A.) blinded to disease diagnosis. All SLE patients were asked to provide at least one semen sample collected by masturbation at the collection room in the laboratory and processed within 1 h of liquefaction, after 48–72 h of sexual abstinence, within 1 month after acceptance of the study. Semen specimens were liquefied at 37°C for 30 min and sperm concentration and motility were checked manually under a microscope with a 20 $\times$ positive phase-contrast objective, and an overall magnification of 200$\times$. Microscopic images were transferred through a video system to a computer-assisted semen analyser, attached to the optical microscope and digitized, according to a special software program. The specimens were analysed by manual hand count as well as by a Computer Assisted Semen Analysis Systems (CASA) under 400 $\times$ magnification, using an HTM-2030 (Hamilton Thorne Research, Beverly, MA, USA). Each slide was scanned to estimate the number of spermatozoa per field equivalent to 1 ml, to obtain an approximate sperm concentration in millions of spermatozoa per millilitre of semen. Sperm motility was determined by analysis of at least five microscopic fields in a systematic way to classify 200 spermatozoa. The motility of each spermatozoa was graded 'a' (rapid progressive motility), 'b' (slow or sluggish progressive motility), 'c' (non-progressive motility) and 'd' (non-motility). Sperm morphology included evaluation of sperm head, neck, mid-piece and tail by two medical technologists (A.B. and K.A.) blinded to disease diagnosis [20]. A patient was considered to have oligozoospermia when a sperm concentration <20 million/ml were found in the ejaculate. Asthenozoospermia was defined as normal sperm motility ('a' + 'b') in <50%. Teratozoospermia was defined as normal sperm morphology in <30% by WHO criteria [21]. Sperm morphology was additionally evaluated according to Kruger strict criteria, in which <14% normal morphology is associated with subfertility [22].

Anti-sperm antibodies

Anti-sperm antibodies were performed at the study entry for SLE patients and were determined by direct immunobead test using Immunobead® rabbit anti-human Ig (IgA, IgG and IgM) kits (Irvine Scientific, Santa Ana, CA, USA). The direct immunobead-binding test detects antibodies that bind to the sperm cell surface (sperm head, mid-piece and/or tail). At least 50% of the motile spermatozoa ('a' + 'b') must be coated with immunobeads before the test is considered to be clinically significant [24]. Quality control was defined as recommended by the manufacturer (Irvine Scientific, Santa Ana, CA, USA): negative control should have a score <10% bead attachment and positive control a score >20% bead attachment. If the results were not as anticipated, the assay was repeated with fresh reagents.

Clinical evaluation, laboratory evaluation and treatment

An extensive clinical evaluation was performed at entry by the two rheumatologists (R.M.S. and P.M.F.S.), followed by a careful chart review including previous clinical, laboratory and therapeutic data. SLE manifestations were defined as: cutaneous lesions (malar or discoid rash, oral ulcers, vasculitis or photosensitivity), articular involvement (arthralgia or non-erosive arthritis), neuropsychiatry disease (seizure, psychosis, depression or peripheral neuropathy), renal involvement (proteinuria $\geq$0.5 g/24h, presence of cellular casts, persistent haematuria $\geq$10 red blood cells per high-power field or renal failure), cardiopulmonary disease (serositis, myocarditis, restrictive lung disease and pulmonary hypertension) and haematological abnormalities (haemolytic anaemia, leukopenia with a white blood cell count <4000/mm^3, lymphopenia <1500/mm^3 on two or more occasions, and thrombocytopenia with platelet count <100 000/mm^3 in the absence of drugs or infection). SLE disease activity and cumulative damage at the time of study entry were measured in all patients, using the SLEDAI [23] and the SLICC/ACR Damage Index [24]. Anti-double-stranded DNA (anti-dsDNA) antibodies were detected by indirect IF using *Crithidia luciliae* as substrate.

Data concerning the period of therapy (pre-pubertal or post-pubertal period), the current dosage and the cumulative doses of drugs (prednisone, chloroquine diphosphate, MTX, AZA, IVCYC, cyclosporin and mycofenolate mofetil) were determined. The time and duration of IVCYC therapy were also evaluated. A regimen of 7-monthly pulses of IVCYC followed by 2–3 yrs every 3 months was indicated for diffuse or focal proliferative glomerulonephritis.

Statistical analysis

Results are presented as the median for continuous variables and as the number (%) for categorical variables. Data were compared by *t*-tests or by the Mann–Whitney U-test for continuous variables to evaluate differences between SLE patients according to low or normal serum inhibin B. For categorical variables, differences were assessed by Pearson's chi-square test or Fisher's exact test. Pearson's coefficient was used to evaluate correlations between serum inhibin B and FSH, serum inhibin B and LH,

and serum inhibin B and sperm analysis. *P*-values <0.05 were considered significant.

Results

Testicular cell function and hormonal status

Thirty-four SLE patients were subdivided into two groups: low serum inhibin B (Group 1) and normal levels (Group 2). Eight SLE patients (23.5%) had low serum inhibin B levels [Group 1, median 11.05 pg/ml (7–48.67)] and 26 (76.5%) had normal serum levels [Group 2, median 141.05 pg/ml (65.59–265.45)] ($P = 0.0001$). Elevated FSH levels were detected in 100% of the patients of Group 1 compared with none in the normal serum inhibin B Group ($P = 0.0001$), as shown in Table 1. The medians of FSH and LH were significantly higher in Group 1 compared with Group 2 (17.7 *vs* 4.2 IU/l, $P = 0.0001$; 8.1 *vs* 3.95 IU/l, $P = 0.001$; respectively). The frequencies of elevated LH levels were similar in both groups (25% *vs* 7.7%, $P = 0.229$). The inhibin B levels were negatively correlated with serum FSH levels ($r = 0.699$, $P = 0.0001$) and LH levels ($r = 0.397$, $P = 0.02$). No significant differences were found between median and frequency of abnormal levels of the other hormones in both studied groups: prolactin, total testosterone, T3, T4, free T4 and TSH (Table 1).

Testicular cell function and sperm abnormalities

The semen analysis in SLE patients according to low or normal inhibin B levels is shown in Table 2.

Macroscopic examination of the semen revealed no significant differences between the medians of sexual abstinence, sperm volume and sperm pH in Groups 1 and 2 (Table 2).

The microscopic investigation demonstrated that Group 1 had lower median sperm concentration (2×10^6/ml *vs* 56.5×10^6/ml, $P = 0.024$), total sperm count (6×10^6 *vs* 133×10^6, $P = 0.023$) and total motile sperm count (3×10^6 *vs* 69.5×10^6, $P = 0.025$) compared with Group 2. The percentages of oligozoospermia and decreased levels of total sperm count were higher in Group 1 *vs* Group 2 (75% *vs* 26.9%, $P = 0.033$; 87.5% *vs* 26.9%, $P = 0.004$). Further analysis of the grading system of spermatozoon's motility showed that only the median of non-motility spermatozoa was significantly higher in Group 1 compared with Group 2 (46.2% *vs* 33.2%, $P = 0.035$). No significant differences were observed between the medians of sperm morphology by the Kruger strict criteria and WHO in the two groups studied. All other evaluated parameters were similar in both groups (Table 2).

TABLE 1. Hormone profile in SLE patients according to low (Group 1) or normal (Group 2) inhibin B levels

Variables	Group 1 ($n = 8$)	Group 2 ($n = 26$)	*P*
Inhibin B, pg/ml	11.05 (7–48.67)	138.31 (65.59–265.45)	0.0001
Decreased levels, *n* (%)	8 (100)	0	0.0001
FSH, IU/l	17.7 (10.9–25)	4.2 (1.0–9.7)	0.0001
Elevated levels, *n* (%)	8 (100)	0	0.0001
LH, IU/l	8.1 (5.5–15.6)	3.95 (1.4–15.1)	0.001
Elevated levels, *n* (%)	2 (25)	2 (7.7)	0.229
Prolactin, ng/ml	7.9 (1.9–36.5)	9.8 (3.8–36.2)	0.34
Elevated levels, *n* (%)	2 (25)	7 (26.9)	1.0
Total testosterone, ng/dl	412 (187–728)	519 (80–1,259)	0.417
Decreased levels, *n* (%)	2 (25)	3 (11.5)	0.57
T3, ng/dl	130 (115–166)	139 (85–210)	0.64
Elevated levels, *n* (%)	0	1 (3.8)	1.0
T4, μg/dl	8.5 (6.67–11.2)	9.0 (6.2–13.2)	0.542
Elevated levels, *n* (%)	1 (12.5)	0	1.0
Free T4, ng/dl	1.0 (1.0–1.3)	1.0 (0.7–1.46)	0.392
TSH, μU/ml	1.6 (0.57–5.38)	2.0 (0.27–3.00)	0.598
Decreased levels, *n* (%)	1 (12.5)	0	0.235

Values of hormones are expressed in median (range). normal ranges: FSH (1–10.5 IU/l), LH (1–8.4 IU/l), Prolactin (2–10 ng/ml), total testosterone (271–965 ng/dl), T3 (70–204 ng/dl), T4 (4.3–12.5 μg/dl), free T4 (0.4–1.6 ng/dl), TSH (0.5–6 μU/ml).

Inhibin B levels were positively correlated with sperm concentration ($r = 0.343$, $P = 0.047$) and total motile sperm count ($r = 0.357$, $P = 0.038$). The medians of inhibin B values were lower in SLE patients with oligozoospermia (<20×10^6/ml), decreased levels of total sperm count (<40×10^6) and decreased levels of total motile sperm count (<10×10^6/ml) than in patients with normal sperm analysis [65.6 pg/ml (7–147.9) *vs* 151.1 pg/ml (7–265.5), $P = 0.001$; 57.1 pg/ml (7–147.9) *vs*159.1 pg/ml (7–265.5), $P = 0.001$; and 65.6 pg/ml (7–147.9) *vs* 138.3 pg/ml (7–265.5), $P = 0.01$; respectively]. FSH levels were negatively correlated with total sperm count ($r = -0.347$, $P = 0.044$) and total motile sperm count ($r = -0.370$, $P = 0.031$).

The medians of inhibin B/FSH ratio were lower in SLE patients with oligozoospermia, decreased levels of total sperm count and decreased levels of total motile sperm count than in patients with normal sperm analysis [8.68 (0.53-42.29) *vs* 38.39 (0.37–210.57), $P = 0.001$; 5.73 (0.39–42.29) *vs* 42.33 (0.37–210.57), $P = 0.001$; and 8.68 (0.53–42.29) *vs* 35.58 (0.37–210.57), $P = 0.01$; respectively].

Further evaluation of the 26 SLE patients with normal inhibin B levels (>60 pg/ml) and normal FSH levels revealed that the medians of inhibin B/FSH ratio were lower in SLE patients with oligozoospermia compared with normozoospermia [13.07 (8.69–42.29) *vs* 46.27 (13.7–210.57), $P = 0.004$], decreased or normal levels of total sperm count [15.25 (8.69–42.29) *vs* 46.27 (12.96–210.57), $P = 0.022$] and decreased or normal levels of total motile sperm count [21.58 (8.69–42.29) *vs* 42.33 (12.96–210.57), $P = 0.051$]. The medians of inhibin were lower in SLE patients with oligozoospermia compared with normozoospermia [81.14 pg/ml (65.59–147.91) *vs* 167.12 pg/ml (80.68–265.45), $P = 0.009$], whereas no difference was observed regarding decreased or normal levels of total sperm count [136.94 pg/ml (65.59–147.91) *vs* 167.12 pg/ml (75.19–265.45), $P = 0.069$] and decreased or normal levels of total motile sperm count [136.94 pg/ml (65.59–265.45) *vs* 151.1 pg/ml (75.19–260.88), $P = 0.059$]. The medians of FSH were higher in SLE patients with oligozoospermia compared with normozoospermia [5.8 IU/l (3.4–9.7) *vs* 3.4 IU/l (1.0–9.5), $P = 0.026$], whereas no difference was observed regarding decreased or normal levels of total sperm count

TABLE 2. Semen analysis in SLE patients according to low (Group 1) or normal (Group 2) inhibin B levels

Variables	Reference value	Group 1 ($n = 8$)	Group 2 ($n = 26$)	*P*
Macroscopic examination				
Sexual abstinence, days	≥2	3 (3–5)	4 (2–75)	0.169
Sperm volume, ml	≥2	1.75 (1–4)	2.37 (0.6–5)	0.426
Sperm pH	≥7.2	7.5 (7.0–8.0)	7.5 (7.0–8.0)	0.611
Concentration and count				
Sperm concentration, $\times 10^6$/ml	≥20	2 (1–84)	56.5 (0–892)	0.024
Oligozoospermia, *n* (%)		6 (75)	7 (26.9)	0.033
Total sperm count, $\times 10^6$	≥40	6 (1.4–221)	133 (0–1024)	0.023
Decreased levels, *n* (%)		7 (87.5)	7 (26.9)	0.004
Motility				
Total motile sperm count, $\times 10^6$	≥10	3 (0.7–112)	69.5 (0–577)	0.025
Decreased levels, *n* (%)		5 (62.5)	6 (23)	0.079
Sperm motility, %	≥50	57.2 (15–65)	60.2 (0–78.5)	0.465
Asthenozoospermia, *n* (%)		3 (37.5)	9 (34.6)	1.0
Rapid progressive motility (grade 'a'), %		0 (0–7.5)	1.5 (0–32)	0.070
Slow progressive motility (grade 'b'), %		25 (8–42.5)	30 (0–51)	0.730
Non-progressive motility (grade 'c'), %		20.5 (8–41)	22.5 (0–57.5)	0.839
Non-motility (grade 'd'), %		46.2 (34.5–84)	33.2 (0–89.5)	0.035
Morphology				
Kruger normal sperm forms, %	≥14	1.2 (0–5.5)	2.5 (0–27)	0.595
Teratozoospermia, *n* (%)		6 (75)	18 (69)	1.0
WHO normal sperm forms, %	≥15	7.2 (1–21.5)	13.2 (0–31)	0.309
Teratozoospermia, *n* (%)		6 (75)	14 (53.8)	0.422

Values are expressed in median (range). [a]Sperm motility reference value—≥50% motile (grades 'a' + 'b') or ≥25% with grade 'a' (WHO criteria) (24).

Table 3. Evaluation of testicular volume (by Prader and ultrasound) and varicocoele in SLE patients according to low (Group 1) or normal (Group 2) inhibin B levels

Variables	Group 1 ($n=8$)	Group 2 ($n=26$)	P
Testicular volume by Prader (ml)			
Right			
Decreased (<12), *n* (%)	15 (10–20)	15 (10–25)	0.475
Left	1 (12.5)	1 (3.9)	0.420
Decreased (<12), *n* (%)	15 (10–20)	15 (10–25)	0.344
	2 (25)	2 (7.7)	0.299
Testicular volume by US, ml			
Right			
Decreased (<7), *n* (%)	10.7 (3.8–20)	11.8 (4.8–21.8)	0.310
Left	3 (37.5)	3 (11.5)	0.126
Decreased (<7), *n* (%)	9.8 (3.8–22)	10.5 (3.28–22)	0.556
	2 (25)	5 (19.2)	0.147
Varicocoele			
Clinical (Grades I or II), *n* (%)	3 (37.5)	5 (19.2)	0.355
US, *n* (%)	5 (62.5)	8 (30.7)	0.211

Values are expressed in median (range). US: ultrasound.

[5.3 IU/l (3.4-9.5) *vs* 3.4 IU/l (1.0–9.7), $P=0.06$] and decreased or normal levels of total motile sperm count [4.3 IU/l (3.4–8.92) *vs* 3.3 IU/l (1.0–9.7), $P=0.201$].

Anti-sperm antibodies

The overall frequency of anti-sperm antibodies in SLE patients was 41%. The apparent lower frequency of positive anti-sperm antibodies in Group 1 compared with Group 2 did not reach significance (12.5% *vs* 50%, $P=0.102$).

Urological evaluation and testicular Doppler ultrasound

All SLE patients were P5 and G5 according to Marshall and Tanner's [15] pattern criteria of pubertal changes. The evaluation of testicular volume by both the Prader's [16] method and ultrasound, as well as the frequency of varicocoele in SLE patients according to the semen abnormalities is shown in Table 3. In the Prader's testicular volume evaluation, no significant differences were found between SLE patients with low or normal inhibin B levels for the median right and left testicle volume (15 ml *vs* 15 ml, $P=0.475$; 15 ml *vs* 15 ml, $P=0.344$; respectively). Testicular volume assessed by ultrasound revealed no differences between Groups 1 and 2 for the median right and left testicle volume (10.7 ml *vs* 11.8 ml, $P=0.310$; 9.8 ml *vs* 10.5 ml, $P=0.556$; respectively). The frequencies of varicocoele Grade I or II by clinical examination (37.5% *vs* 19.2%, $P=0.355$) and by testicular Doppler ultrasound (62.5% *vs* 30.7%, $P=0.211$) were alike comparing Groups 1 and 2.

Demographic, clinical features, SLE activity and damage

The distribution of demographic features showed that Groups 1 and 2 were similar regarding median current age [32.5 yrs (23–42) *vs* 27.5 yrs (15–45), $P=0.231$], age at disease onset [22 yrs (13–32) *vs* 20.5 yrs (1.6–40), $P=0.440$], disease duration [9.5 yrs (1–20) *vs* 6 yrs (1–21.5), $P=0.440$] and age of first ejaculation [13 (12–15) *vs* 12.5 (12–15) yrs, $P=0.141$].

The clinical manifestations, laboratorial alteration, scores of activity and damage in SLE patients were evenly distributed in Groups 1 and 2 (Table 4).

Previous and current therapy evaluation

All patients received drugs after puberty with the exception of two that were under steroid and chloroquine diphosphate therapies in the pre-pubertal period. The analysis of SLE therapy according to low or normal inhibin B is shown in Table 5.

Table 4. Clinical and laboratorial findings, scores of activity and damage in SLE patients according to low (Group 1) or normal (Group 2) inhibin B levels.

Variables	Group 1 ($n=8$)	Group 2 ($n=26$)	P
SLEDAI	0 (0–12)	0 (0–16)	0.982
SLEDAI $\geq$4, *n* (%)	4 (50)	6 (22)	0.194
SLEDAI $\geq$8, *n* (%)	1 (12.5)	3 (11.5)	1.0
SLICC/ACR-DI	0.5 (0–3)	0 (0–2)	0.258
SLICC/ACR-DI $\geq$ 1, *n* (%)	3 (37.5)	1 (30.7)	1.0
Clinical manifestations			
Cutaneous, *n* (%)	6 (75)	22 (84.6)	0.61
Articular, *n* (%)	7 (87.5)	25 (96.2)	0.42
Renal, *n* (%)	6 (75)	18 (69)	1.0
Neuropsychiatry, *n* (%)	2 (25)	7 (26.9)	1.0
Haematological abnormality, *n* (%)	8 (100)	24 (92.3)	1.0
Cardiopulmonary, *n* (%)	5 (62.5)	11 (42.3)	0.43
Laboratorial finding			
Anti-dsDNA, *n* (%)	1 (12.5)	4 (15.3)	1.0

Values are expressed in median (range).

Table 5. Drug therapy in SLE patients according to low (Group 1) or normal (Group 2) inhibin B levels

Variables	Group 1 ($n=8$)	Group 2 ($n=26$)	P
IVCYC			
Current use, *n* (%)	0	0	–
Use after first ejaculation, *n* (%)	5 (62.5)	8 (30.8)	0.211
Cumulative dose, g	26.5 (12–39)	14.1 (11–31.5)	0.057
Number of pulse therapy, *n*	20 (12–31)	12 (6–19)	0.064
Duration of treatment, yrs	2.6 (1–4)	1.7 (0.6–3)	0.161
AZA			
Current use, *n* (%)	2 (25)	8 (30.8)	1.0
Use after first ejaculation, *n* (%)	4 (50)	18 (69.2)	0.41
Current dosage, mg	225 (150–300)	150 (125–250)	0.199
Cumulative dose, g	95.45 (30–138.3)	73.6 (3.5–150.5)	0.37
Prednisone			
Current use, *n* (%)	3 (37.5)	16 (61.5)	0.417
Current dosage, mg	20 (10–20)	15 (2.5–60)	0.571
Cumulative dose, g	23.8 (4.5–50)	26 (3.3–76)	0.887
Chloroquine diphosphate			
Current use, *n* (%)	5 (62.5)	20 (76.9)	0.648
Use after first ejaculation, *n* (%)	7 (88.8)	25 (96)	0.421
Current dosage, mg	250	250 (200–250)	0.742
Cumulative dose, g	494 (64–966)	260 (22.5–1478)	0.327
MTX			
Current use, *n* (%)	0	2 (7.7)	1.0
Use after first ejaculation, *n* (%)	1 (12.5)	8 (30.8)	0.403
Cumulative dose, g	0.84	1.75 (0.08–5.6)	0.245
Mycophenolate mofetil			
Current use, *n* (%)	0	4 (15.4)	551
Use after first ejaculation, *n* (%)	1 (12.5)	5 (19.2)	1.0
Current dosage, mg	0	2500 (2000–3000)	–
Cumulative dose, g	92	1098 (180–2447)	0.143

Values are expressed in median (range).

The median serum inhibin B was lower in SLE patients treated with IVCYC compared with those never under this therapy [75.19 pg/ml (7–265.45) *vs* 138.31 pg/ml (7–260.88), $P=0.031$] and the apparent higher frequency of IVCYC use after the first ejaculation in Group 1 did not reach statistical significance (62.5% *vs* 30.8%, $P=0.211$). A trend towards a higher median cumulative dose and number of pulse therapy was observed in Group 1 compared with Group 2 (26.5 g *vs* 14.1 g, $P=0.057$; and 20 *vs* 12 pulsed administration, $P=0.064$; respectively) (Table 5). The median of inhibin B/FSH ratio was lower in SLE patients treated with IVCYC compared with those never under this therapy [12.96 (0.39–68.06) *vs* 35.58 (0.37–210.57), $P=0.014$]. All 13 patients treated by IVCYC received this drug after the first ejaculation and none were under this therapy at study entry. The time between the last dose of IVCYC and evaluation of Sertoli cell function was 5.11 ± 3.72 yrs (0.4–12).

The analysis of the 26 SLE patients with normal inhibin levels (>60 pg/ml), the median inhibin B/FSH ratio was lower in SLE patients treated with IVCYC than those never under this therapy [14.93 (11.12–68.06) *vs* 42.33 (8.69–210.57), $P = 0.04$]. In contrast, the median inhibin B was similar in SLE patients treated with IVCYC compared with those never under this therapy [109.46 (65.59–265.45) *vs* 159.11 pg/ml (77.48–260.88), $P = 0.12$]. Only a trend towards a increased FSH levels in SLE patients treated with IVCYC compared with those never under this therapy was observed [5.5 IU/l (3.4–9.7) *vs* 3.35 (1.0–9.5), $P = 0.052$].

No significant differences were observed comparing the two groups with regard to prednisone, chloroquine diphosphate, MTX, AZA and mycophenolate mofetil treatment (Table 5). One patient of each group used cyclosporin ($P = 0.421$).

Discussion

This is the first study that particularly focused testicular Sertoli cell dysfunction in SLE and revealed that decreased serum inhibin/FSH ratio may be more sensitive to identify sperm abnormalities, particularly in patients treated with IVCYC.

For years, FSH has been the most important indicator of seminiferous epithelia function and elevated levels indicate testicular damage [16]. In lupus patients, high levels of gonadotrophins (FSH and LH) [25–29] and hypoandrogenism (low testosterone and elevated FSH and LH) have been observed and primary gonad injury is perhaps the main causal factor [13].

However, inhibin B may be a more valuable and direct marker of testicular dysfunction [12] since this hormone is a direct product of the seminiferous tubules. Inhibin B levels are higher in normozoospermic men than in men with impaired gonadal function [7, 10, 11]. Moreover, its secretion is particularly induced during advanced stages of spermatogenesis [30]. In contrast, gonadotropin-releasing hormone (GNRH), oestradiol and testosterone levels may directly affect FSH. An additional benefit of inhibin B measurement is allowing a global evaluation of testicular tissue function, while needle biopsy may not represent the entire testis [10].

Our data confirm and extend previous observation that this hormone is related to sperm abnormalities in male infertility [12]. In this regard, sperm count, concentration and motility in lupus patients were also associated with Sertoli cell dysfunction. As expected, in SLE, this correlation was also true for FSH since an inverse relationship exists between circulating inhibin B and FSH in men with normal and abnormal spermatogenesis [10–12]. Likewise, this negative feedback has been reported for inhibin B and LH in infertile men [12] and further demonstrated herein for lupus.

Remarkably, the inhibin B/FSH ratio seems to be a more sensitive marker than either serum FSH or inhibin B alone for impaired spermatogenesis in SLE, since it identified a subpopulation of patients with sperm abnormalities and normal hormone levels. In contrast, testicular volumes by ultrasound and by Prader orchidometer were not as sensitive to detect subtle Sertoli cell dysfunction, as evidenced by other authors [5, 6, 12].

The near absence of Grade III varicocoele and the similar low frequency of Grade I/II observed in both studied groups suggest that this complication is not the major cause of sperm abnormalities. In fact, semen and hypothalamic–pituitary–testicular axis alterations are more commonly associated with severe varicocoele [31].

Anti-sperm antibodies (ASA) were observed bound to spermatozoan surface in ~10% of infertile male partners [32]. In SLE, almost half of the patients have these antibodies without any evidence of a deleterious effect in testicular dysfunction [13, 33]. Reinforcing this finding, the presence of anti-sperm antibody did not discriminate the low inhibin B group.

On the other hand, we identified chemotherapy as an important cause of persistent or long-lasting injury to Sertoli cells leading to significant low inhibin B levels. Our findings are in accordance with previous studies in cancer that showed a low inhibin B levels following multiple chemotherapy including IVCYC treatment [34, 35] and testicular irradiation [36]. Moreover, the present data confirm that inhibin B/FSH ratio may antedate isolated serum hormonal alterations [5].

Sperm cryopreservation prior to IVCYC was not obtained in any of our patients in reproductive age. Our study, however, emphasizes that cryopreservation of semen and subsequent *in vitro* fertilization is the best currently available option for post-pubertal males in order to guarantee the possibility of reproduction after immunosuppressive therapy [37].

In conclusion, the integrity of Sertoli cell function is altered in male SLE affecting inhibin B secretion. The index inhibin B/FSH may offer a better possibility of an earlier and useful marker of IVCYC toxicity in these patients, which needs to be confirmed by prospective studies.

Rheumatology key messages

- Testicular Sertoli cell function was evaluated by serum inhibin B levels.
- High frequency of testicular Sertoli cell dysfunction in male SLE was associated with semen abnormalities.

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