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**Avaliação da função gonadal em pacientes do sexo
masculino com síndrome antifosfolípide**

Tese apresentada à Faculdade de
Medicina da Universidade de São
Paulo para obtenção do título de
Doutor em Ciências

Programa de Ciências Médicas
Área de concentração: Processos
Inflamatórios e Alérgicos

Orientador: Prof. Dr. Clovis Artur
Almeida da Silva

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realização de uma importante
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LISTA DE ABREVIATURAS E SIGLAS

Apl	Anticorpos antifosfolípidos
CFEV	Ciclofosfamida endovenosa
DP	Desvio padrão
FMUSP	Faculdade de Medicina da Universidade de São Paulo
FSH	Hormônio foliculoestimulante
LH	Hormônio luteinizante
Ig	Imunoglobulina
IMC	Índice de massa corpórea
LES	Lúpus eritematoso sistêmico
OMS	Organização Mundial de Saúde
SAF	Síndrome antifosfolípide
SAFP	Síndrome antifosfolípide primária
SAF-LES	Síndrome antifosfolípide associada ao lúpus eritematoso sistêmico
SLEDAI	Do inglês “ <i>Systemic Lupus Erythematosus Disease Activity Index</i> ”
SLICC/ACR-DI	Do inglês “ <i>Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index</i> ”
TSH	Hormônio tireoestimulante

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RESUMO

Rabelo-Júnior CN. Avaliação da função gonadal em pacientes do sexo masculino com síndrome antifosfolípide [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2011.

INTRODUÇÃO. A síndrome antifosfolípide (SAF) é uma condição trombofílica autoimune associada a títulos elevados e persistentes de anticorpos antifosfolípidos. Caracteriza-se por trombozes em diversos órgãos, incluindo os testículos. **OBJETIVO.** Realizar uma avaliação global da função gonadal em pacientes masculinos com SAF primária (SAFP) e SAF associada ao lúpus eritematoso sistêmico (SAF-LES). **MÉTODOS.** Estudo transversal realizado em 22 pacientes (12 com SAFP e 10 com SAF-LES) e 20 controles saudáveis pareados por sexo e idade. Os pacientes foram avaliados em relação a dados demográficos, exame urológico, ultrassonografia testicular, perfil hormonal, análise do sêmen, anticorpos antiespermatozóides e características clínicas e laboratoriais. **RESULTADOS.** A mediana da idade atual foi semelhante nos pacientes com SAFP e controles ($p=0,27$), assim como naqueles com SAF-LES e controles ($p=0,31$). Disfunção erétil foi significativamente maior nos pacientes com SAFP comparado aos controles (25% vs. 0%, $p=0,044$), e nos SAF-LES comparado aos controles (30% vs. 0%, $p=0,029$). Com relação à antropometria do pênis, a análise dos subgrupos de pacientes com ($n=7$) e sem ($n=5$) trombozes arteriais prévias demonstrou que a mediana da circunferência do pênis foi significativamente menor em SAFP com trombose arterial *versus* sem trombose arterial [8,1 (6-10) vs. 10,2 (10-11) cm,

p=0,007], bem como também observado em pacientes com SAF-LES com (n=2) e sem (n=8) eventos arteriais prévios [7,5 (7-8) vs. 9,18 (8-10,5) cm, p=0,039]. A mediana da circunferência do pênis foi significativamente menor nos pacientes com SAFP com disfunção erétil *versus* sem essa alteração [7,5 (6-9,5) vs. 9,5 (7,5-11) cm, p=0,039], assim como no grupo de SAF-LES [8,17 (8-8,5) vs. 9,14 (7-10,5) cm, p=0,0397]. Com relação à avaliação da função testicular, todos os parâmetros foram semelhantes nos pacientes com SAFP e controles (p>0,05). Por sua vez, as medianas de concentração e de mobilidade dos espermatozóides foram significativamente menores nos pacientes com SAF-LES comparado aos controles [41,1 (0-145) vs. 120,06 (34,5-329) x 10⁶/mL, p=0,003; 47,25 (0-87,5) vs. 65,42 (43-82)%, p=0,047; respectivamente], assim como a frequência de oligo/azoospermia (40% vs. 0%, p=0,007). A análise dos pacientes com SAF-LES mostrou que as medianas da concentração e da contagem total de espermatozóides foram significativamente menores nos que usaram ciclofosfamida endovenosa *versus* os que não usaram tal medicação [6,87 (0-23,5) vs. 63,9 (7,5-145) x 10⁶/mL, p=0,04; 16,12 (0-55,5) vs. 226,25 (8,5-471) x 10⁶, p=0,035; respectivamente]. **CONCLUSÕES.** Diminuição do tamanho peniano nos pacientes com SAFP e SAF-LES com disfunção erétil associada foi evidenciada, além de disfunção testicular secundária ao uso de agentes alquilantes nos pacientes com SAF-LES.

DESCRITORES: Síndrome antifosfolípide, sexo masculino, sêmen, lúpus eritematoso sistêmico, fertilidade, anticorpos antiespermatozóides.

ABSTRACT

Rabelo-Júnior CN. Evaluation of gonadal function in male patients with antiphospholipid syndrome [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2011.

INTRODUCTION. Antiphospholipid syndrome (APS) is an autoimmune thrombophilic condition associated with persistent high titers of antiphospholipid antibodies. It is characterized by thrombosis in various organs including the testes. **OBJECTIVE.** To perform a global testicular assessment in male primary antiphospholipid syndrome (PAPS) and secondary systemic lupus erythematosus-APS (SLE-APS) patients, and healthy controls. **METHODS.** A cross-sectional study was conducted in 22 APS (12 PAPS and 10 SLE-APS) male patients, and 20 healthy controls. They were assessed by demographic data, systematic urological examination, testicular ultrasound, hormone profile, sperm analysis, antisperm antibodies, clinical features and treatment. **RESULTS.** The median of current age was similar in PAPS patients and controls ($p=0.27$), likewise in SLE-APS and controls ($p=0.31$). Erectile dysfunction was significantly higher in PAPS patients compared than controls (25% vs. 0%, $p=0.044$), and in SLE-APS and controls (30% vs. 0%, $p=0.029$). Regarding the penile anthropometry, the analysis of subgroups of PAPS patients with ($n=7$) and without ($n=5$) previous arterial thrombosis demonstrated that the median circumference penis was significantly lower in PAPS with arterial thrombosis *versus* without [8.1 (6-10) vs. 10.2 (10-11) cm, $p=0.007$], as also observed in

SLE-APS patients with (n=2) and without (n=8) previous arterial events [7.5 (7-8) vs. 9.18 (8-10.5) cm, $p=0.039$]. In addition, the median penis circumference was significantly lower in PAPS patients with erectile dysfunction *versus* without this alteration [7.5 (6-9.5) vs. 9.5 (7.5-11) cm, $p=0.039$], likewise in SLE-APS patients [8.17 (8-8.5) vs. 9.14 (7-10.5) cm, $p=0.0397$]. Regarding gonadal evaluation, these parameters were uniformly normal in PAPS *versus* controls ($p>0.05$). In contrast, the median of sperm concentration and sperm motility were significantly lower in SLE-APS patients compared to controls [41.1 (0-145) vs. 120.06 (34.5-329) $\times 10^6/\text{mL}$, $p=0.003$; 47.25 (0-87.5) vs. 65.42 (43-82)%, $p=0.047$; respectively], likewise the frequency of oligo/azoospermia (40% vs. 0%, $p=0.007$). The analysis of SLE-APS patients showed that the median of sperm concentration and total sperm count were significantly lower in SLE-APS patients treated with intravenous cyclophosphamide *versus* untreated [6.87 (0-23.5) vs. 63.9 (7.5-145) $\times 10^6/\text{mL}$, $p=0.04$; 16.12 (0-55.5) vs. 226.25 (8.5-471) $\times 10^6$, $p=0.035$; respectively]. **CONCLUSIONS.** We have identified reduced penile size in PAPS and SLE-APS patients with deleterious erectile function, and testicular dysfunction due to alkylating agents in SLE-APS patients.

DESCRIPTORS: antiphospholipid syndrome, male, sperm, systemic lupus erythematosus, fertility, antisperm antibodies.

1 INTRODUÇÃO

A síndrome antifosfolípide (SAF) é uma condição trombofílica autoimune caracterizada por trombooses venosas e/ou arteriais associadas ou não à morbidade gestacional, na presença de títulos elevados e persistentes de anticorpos antifosfolípides (aPL).¹ Cerca de 8% a 18% dos pacientes afetados são do sexo masculino, podendo ser classificada em primária (SAFP) ou secundária a outras doenças, principalmente ao lúpus eritematoso sistêmico (SAF-LES),^{2,3} e pode acometer um grande número de órgãos e sistemas, incluindo os testículos.⁴

A avaliação testicular nas doenças reumatológicas autoimunes exige um exame urológico completo para detectar alterações no esperma devido à idade,^{5,6} anormalidades genitais,⁷ disfunção do eixo hipotálamo-hipofisário, anticorpos antiespermatozóides⁸ e efeitos do tratamento medicamentoso utilizado, especialmente os agentes imunossupressores.⁹⁻¹⁵

Já foram relatadas anormalidades graves no esperma associadas à atrofia testicular e níveis elevados de hormônio foliculoestimulante (FSH)¹¹ em pacientes com LES tratados com ciclofosfamida endovenosa (CFEV)^{12,13} e redução das dimensões do pênis em pré-púberes com LES sem SAF e/ou disfunção erétil.¹⁶ Trombose testicular secundário à SAFP ou SAF-LES foi descrita em poucos casos na literatura.^{3,17-21} Porém, não existe até o presente momento nenhum estudo sistemático avaliando a função gonadal global, incluindo avaliações testicular e peniana, em pacientes com estas condições.

2 OBJETIVOS

- 1 Avaliar função gonadal em pacientes com SAFP e SAF-LES e controles sadios.
- 2 Verificar possíveis associações entre disfunção gonadal em pacientes com SAFP e SAF-LES com: dados demográficos, índice de massa corpórea (IMC), exame urológico, disfunção erétil, análise do sêmen, perfil hormonal e achados clínicos e laboratoriais.

3 MÉTODOS

3.1 Pacientes com SAF e controles

Foram selecionados inicialmente 38 pacientes do sexo masculino com SAF. Dezesesseis pacientes foram excluídos devido a: vasectomia (três pacientes), recusa em coletar as amostras do sêmem (nove pacientes) e associação com outras doenças reumáticas em quatro (poliarterite nodosa em dois, artrite reumatóide em um e espondilite anquilosante em um).

Nenhum dos sujeitos do estudo tinha hidrocele, hipospádia, criptorquidia, infecção testicular, câncer testicular, orquite, vasculite testicular, disfunção ureteral, história prévia de qualquer cirurgia testicular ou inguinal, diabetes mellitus, história prévia ou atual de uso de álcool e tabaco.

3.2 Desenho e população do estudo

Foi conduzido um estudo transversal em 22 pacientes do sexo masculino portadores de SAF (12 com SAF-P e 10 com SAF-LES) acompanhados regularmente no Ambulatório de SAF da Divisão de Reumatologia do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (FMUSP) no período de maio de 2008 a fevereiro de 2011. Todos os pacientes preencheram os critérios de Sapporo para o diagnóstico de SAF.²² O grupo controle incluiu 20 indivíduos saudáveis seguidos no Grupo Pré-vasectomia da Divisão de Urologia do Hospital Universitário da FMUSP. Todos os pacientes do estudo e controles estavam em estágio puberal de Tanner 5.²³

3.3 Aprovação por comissão de ética

Este estudo foi aprovado pela Comissão de Ética para Análise de Projetos de Pesquisa do Hospital das Clínicas da FMUSP (CAPPesq). O termo de consentimento livre e esclarecido foi assinado por todos os participantes.

3.4 Dados demográficos e índice de massa corpórea

Foram registradas a idade atual, idade de espermarca (primeira ejaculação) e raça nos pacientes com SAF e controles, enquanto a idade no início da doença e duração da doença nos pacientes com SAF. O IMC foi definido pela fórmula: $IMC = \text{peso (em quilogramas)} / \text{altura (em metros)}^2$ ao quadrado.

3.5 Avaliação urológica

A avaliação clínica da genitália incluiu a análise dos testículos, epidídimos, canal deferente, bolsa escrotal e pênis. O comprimento do pênis foi medido como a distância linear ao longo do lado dorsal que se estende desde a junção da pele pubopeniana até a ponta da glande no estado flácido, enquanto que a circunferência do pênis foi medida no meio do seu maior eixo.²⁴ A avaliação da disfunção erétil em pacientes e controles foi feita por meio de entrevista direta, durante a avaliação urológica, através de um questionário validado para a língua portuguesa.²⁵

3.6 Ultrassonografia testicular com doppler

O exame foi realizado cegamente em relação aos resultados da análise do sêmen e avaliação urológica nos pacientes e controles. A maior medida em cada dimensão foi gravada e usada para calcular o volume testicular de acordo com a fórmula (comprimento x largura x espessura x 0,52). A média normal do volume testicular $\pm$ desvio padrão (DP) em adolescentes do sexo masculino pós-púberes e adultos é de 15 ± 8 ml.^{11-13,26}

3.7 Avaliação hormonal

As dosagens hormonais foram realizadas no início do estudo nos pacientes com SAF e controles, incluindo: FSH, hormônio luteinizante (LH), testosterona total matinal, triiodotironina (T3), tetraiodotironina (T4), fração livre da tetraiodotironina (T4 livre), e hormônio tireoestimulante (TSH) detectados por fluoroimunoensaio usando kit Delfia de fluoroimunoensaio (Wallac, Turku, Finlândia). Os coeficientes de variação intra e interensaio foram de 3,5% e 2,1%, respectivamente. Os intervalos de variação para cada exame foram: FSH (1-10,5 UI/L), LH (1-8,4 UI/L), testosterona matinal total (271-965 ng/dL), T3 (7-20 ng/dL), T4 (4,5-12 mg/dL), T4 livre (0,7-1,5 ng/dL) e TSH (0,4-4,5 μ U/mL).

3.8 Avaliação do sêmen

A análise do sêmen foi realizada de acordo com as diretrizes da Organização Mundial de Saúde (OMS)⁷ por dois técnicos peritos. Todos os pacientes e controles coletaram duas amostras de sêmen com intervalo de

15-30 dias após 48-72 horas de abstinência sexual. Os espermatozóides foram analisados por contagem manual, bem como por um sistema de análise assistida por computador sob a ampliação de 400X, utilizando um aparelho HTM-2030. A motilidade dos espermatozóides foi classificada em "a" (motilidade progressiva rápida), "b" (motilidade progressiva lenta), "c" (motilidade não progressiva), e "d" (sem motilidade). A morfologia dos espermatozóides incluiu a avaliação das suas subdivisões (cabeça, pescoço, peça intermediária e cauda) seguindo critérios restritos da OMS e Kruger.²⁷ O paciente foi considerado como tendo azoospermia quando não foram encontrados espermatozóides na amostra e oligozoospermia quando a concentração de espermatozóides foi < 20 milhões/mL.⁹ A presença de anticorpos antiespermatozóides foi determinada pelo teste direto de Immunobead utilizando kits de Imunoglobulina (Ig) anti-humana de coelho (IgA, IgG e IgM) Immunobead R (Irvine Scientific, Santa Ana, CA, EUA) em todos os pacientes e controles. Pelo menos 50% dos espermatozóides móveis ("a"+"b") devem ter "Immunobeads" aderidos para o teste ser considerado clinicamente significativo. Um controle negativo deve ter menos que 10% de "beads" aderidos enquanto que o controle positivo deve ter mais que 20% de "beads" aderidos.⁷

3.9 Avaliação clínica e tratamento

Os seguintes parâmetros clínicos foram avaliados nos pacientes com SAF-P e SAF-LES: trombose venosa (documentada por trombose venosa profunda e/ou embolia pulmonar), trombose arterial (documentada

por acidente vascular cerebral, ataque isquêmico transitório ou infarto agudo do miocárdio), livedo reticular e trombocitopenia (contagem menor que 100.000 plaquetas, pelo menos em duas ocasiões distintas). Nos pacientes com SAF-LES, foram também mensurados a atividade da doença, bem como seus danos cumulativos no momento do início do estudo, usando o *Systemic Lupus Erythematosus Disease Activity Index* (SLEDAI)²⁸ e o *Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index* (SLICC/ACR-DI)²⁹, respectivamente. Foram avaliados dados relativos ao tratamento como o uso prévio de CFEV.

3.10 Análise imunológica

Anticorpos anticardiolipina IgM e IgG foram detectados por um kit comercial (Kit imunoenzimático, BINDAZYME™, Birmingham, UK) em todos os pacientes com SAF. Os valores de corte foram: < 11GPL/MPL U/mL (negativo), ≥11 e < 20 GPL/MPL U/mL (indeterminado) e ≥ 20 GPL/MPL U/mL (positivo).³⁰ Anticoagulante lúpico (LAC) foi detectado usando o tempo de tromboplastina parcial ativado (TTPA-Diagnostica Stago, França) e o tempo de veneno de víbora de Russel (dRVVT-Trinity Biotech, Wiclów, Irlanda, Reino Unido) de acordo com guidelines internacionais.³¹ O anti-beta-2-glicoproteína I IgM e IgG foi detectado pela técnica de ELISA (ORG 521 anti-beta-2-glicoproteína I IgG/IgM Mainz, Alemanha), com valores de corte de 8 U/mL para IgM e IgG. Os coeficientes de variação foram de 2,1-5,0% (intraensaio) e 2,6-7,95 (interensaio) para IgG e 2,1-3,8% (intraensaio) e 4,1-6,3 (interensaio) para IgM.

3.11 Análise estatística

Os dados foram comparados por testes t ou teste de Mann-Whitney (dados com distribuição normal e anormal, respectivamente) para variáveis contínuas para avaliar as diferenças entre pacientes com SAFP, SAF-LES e controles, e em SAFP e SAF-LES de acordo com trombose arterial anterior, disfunção erétil e uso de CFEV. Para as variáveis categóricas, as diferenças foram avaliadas pelo teste exato de Fisher. Valores de “p” inferiores a 0,05 foram considerados significativos.

4 RESULTADOS

4.1 Pacientes SAFP *versus* controles

Os dados demográficos, o IMC, a antropometria do pênis e o volume testicular pelo ultrassom em pacientes com SAFP e controles estão ilustrados na Tabela 1. A mediana da idade atual e idade da espermarca foi semelhante em ambos os grupos [37,5 (21-62) vs. 32,4 (18-54) anos, $p=0,270$; 13,1 (12-16) vs. 12,85 (11-15) anos, $p=0,224$; respectivamente]. O IMC foi significativamente maior nos pacientes com SAFP comparado aos controles [26,06 (15,7-34,6) vs. 22,2 (18-27) kg/m^2 , $p=0,006$]. As medianas do comprimento e da circunferência do pênis foram iguais na SAFP comparados aos controles [9,87 (7-13) vs. 10 (8-13) cm, $p=0,358$; 9 (6-11) vs. 9,4 (7-11) cm, $p=0,185$; respectivamente]. A disfunção erétil foi significativamente maior em pacientes com SAFP comparados aos controles (25% vs. 0%, $p=0,044$). Não foram encontradas diferenças no volume testicular pela ultrassonografia em ambos os grupos ($p>0,05$) (Tabela 1).

A análise do sêmen, anticorpos antiespermatozóides e avaliação hormonal em pacientes com SAFP e controles estão apresentados na Tabela 2. Os parâmetros de esperma, anticorpos e hormônios foram semelhantes nos dois grupos ($p>0,05$). Todos os pacientes SAFP e controles tinham dosagens de hormônios tireoidianos dentro da normalidade.

Tabela 1 – Dados demográficos, índice de massa corpórea (IMC), antropometria do pênis e volume testicular por ultrassonografia em pacientes com síndrome antifosfolípide primária (SAFP), síndrome antifosfolípide associada ao lúpus eritematoso sistêmico (SAF-LES) e controles

Variáveis	SAFP (n=12)	SAF-LES (n=10)	Controles (n=20)	P ¹	P ²	P ³
Dados demográficos e IMC						
Idade atual, anos	37,5 (21-62)	36,9 (21-57)	32,4 (18-54)	0,270	0,310	0,98
Idade do início da doença, anos	31,41 (18-61)	32,20 (14-57)				0,894
Duração da doença, anos	6,08 (1-18)	5,73 (0.3-13)				0,432
Idade da espermarca, anos	13,1 (12-16)	13,9 (12-16)	12,85 (11-15)	0,224	0,016	0,17
Raça caucasiana	6 (50)	4 (40)	7 (33)	0,473	1,0	0,691
IMC, Kg/m ²	26,06 (15,7-34,6)	26,9 (22-35,2)	22,2 (18-27)	0,006	0,003	0,649
Antropometria do pênis e disfunção erétil						
Comprimento, cm	9,87 (7-13)	10,35 (7,5-13)	10,02 (8-13)	0,358	0,575	0,561
Circunferência, cm	9 (6-11)	8,85 (7-10,5)	9,42 (7-11)	0,185	0,18	0,789
Disfunção erétil	3 (25)	3 (30)	0	0,044	0,029	1,0
Volume testicular						
Direito, mL	12,85 (7,2-18,4)	10,38 (3,9-16,7)	13,41 (8,5-20,6)	0,68	0,030	0,154
Esquerdo, mL	13,88 (7,7-20)	11,04 (7,2-17,2)	12,85 (6-21,2)	0,22	0,07	0,069

Valores expressos em mediana (intervalo) ou n (%), n – número de pacientes e controles, **P¹** - SAFP *versus* controles, **P²** - SAF-LES *versus* controles, **P³** - SAFP *versus* SAF-LES.

Tabela 2 – Análise do sêmen, anticorpos antiespermatozóide e avaliação hormonal em pacientes com síndrome antifosfolípide primária (SAFP), síndrome antifosfolípide associada ao lúpus eritematoso sistêmico (SAF-LES) e controles

Variáveis	Valores de referência	SAFP (n=12)	SAF-LES (n=10)	Controles (n=20)	P ¹	P ²	P ³
Análise do sêmen							
Volume do sêmen, ml	> 2	3,1±1,41	2,95±1,16	1,66 ±1,28	0,25	0,35	0,77
Concentração de espermatozoides, x 10 ⁶ /ml	> 20	141,5 (33-575)	41,1 (0-145)	120,06 (34,5-329)	0,65	0,003	0,046
Oligo/azoospermia		0	4 (40)	0	1,0	0,007	0,029
Contagem total de espermatozoides x 10 ⁶	> 40	388 (66-1131)	142,2 (0-471)	300,5 (41,3-1176)	0,4	0,06	0,036
Motilidade de espermatozoides %	> 50	61,29 (25-80)	47,25 (0-87,5)	65,42 (43-82)	0,4	0,047	0,139
Espermatozoides normais (OMS) %	> 15	21,12 (10-42,5)	11 (0-27)	23,95 (10-45)	0,45	0,002	0,026
Espermatozoides normais (Kruger) %	> 4	5,79 (1,5-16,5)	2,65 (0-8)	7,65 (3-18)	0,27	0,02	0,06
Anticorpos antiespermatozóide %		21,08 (8-60)	17,2(9-23)	16,9 (0-32)	0,35	0,92	0,403
Avaliação hormonal							
FSH, UI/litro	1-10,5	5,06 (1-26,8)	9,73 (2,4-25)	3,32 (1-5,9)	0,207	0,025	0,199
Níveis elevados		1(8)	4(40)	0	0,375	0,007	0,135
LH, UI/litro	1-8,4	4,48 (1-13,1)	6,87 (1,6-21,7)	3,49 (1,8-5,8)	0,171	0,074	0,327
Níveis elevados		1(8)	2(20)	0	0,375	0,103	0,571
Testosterona total, ng/dl	271-965	533 (309-803)	434,3 (91-891)	552,5 (230-1078)	0,381	0,09	0,242
Níveis reduzidos		0	0	1 (5)	1,0	1,0	1,0

Valores expressos em média ± desvio padrão, mediana (intervalo) ou n (%), n – número de pacientes e controles, OMS – Organização Mundial de Saúde, FSH – hormônio foliculoestimulante, LH – hormônio luteinizante, P¹ – SAFP versus controles, P² – SAF-LES versus controles, P³ – SAFP versus SAF-LES.

4.2 Pacientes SAFP

A análise de subgrupos de pacientes com SAFP com (n=7) e sem (n=5) trombose arterial prévia demonstrou que a mediana da circunferência do pênis foi significativamente menor nos pacientes com eventos arteriais [8,1 (6-10) vs. 10,2 (10-11) cm, $p=0,007$], enquanto a mediana do comprimento do pênis foi semelhante em ambos os grupos [9,1 (7-12) vs. 10,9 (9,5-13) cm, $p=0,08$]. Quarenta e dois por cento dos pacientes com SAFP e trombose arterial anterior tinham disfunção erétil em comparação com nenhum paciente do subgrupo sem evento arterial ($p=0,204$) (Tabela3).

A mediana da circunferência do pênis foi significativamente menor nos pacientes com SAFP com disfunção erétil (n=3) comparado aos que não tiveram esta alteração (n=9) [7,5 (6-9,5) vs. 9,5 (7,5-11) cm, $p=0,039$]. Nenhuma diferença foi evidenciada na mediana do comprimento do pênis nos dois grupos [8,33 (7-11,5) vs. 10,22 (8-13) cm, $p=0,280$]. Os dados demográficos, IMC, volume testicular pelo ultrassom, análise do sêmen, anticorpos antiespermatozóides, perfil hormonal e características clínicas e laboratoriais foram semelhantes em pacientes com SAFP com e sem eventos arteriais ($p>0,05$; Tabela 3).

Tabela 3 – Dados demográficos, índice de massa corpórea (IMC), antropometria do pênis, volume testicular por ultrassonografia, análise do sêmen, anticorpos antiespermatozóide, avaliação hormonal e características clínicas e laboratoriais no início do estudo em pacientes com síndrome antifosfolípide primária (SAFP) com e sem eventos arteriais anteriores.

Variáveis	Valores de referência	SAFP Com evento arterial (n=7)	SAFP Sem evento arterial (n=5)	P
Dados demográficos e IMC				
Idade atual, anos		40,7 (24-62)	33 (21-53)	0,334
Duração da doença, anos		6,1 (1-16)	6 (2-18)	0,969
Idade da espermarca, anos		13,5 (12-16)	12,6 (12-13)	0,1
Raça caucasiana		3(42)	1(20)	0,417
IMC, Kg/m ²		27,2 (24-34,6)	24,4 (15,7-27,7)	0,27
Antropometria do pênis e disfunção erétil				
Comprimento, cm		9,1 (7-12)	10,9 (9,5-13)	0,08
Circunferência, cm		8,1 (6-10)	10,2 (10-11)	0,007
Disfunção erétil		3 (42)	0	0,204
Volume testicular	15 ± 8 ml			
Direito, mL		12,5 (7,2-17)	13,3 (8,2-18,4)	0,738
Esquerdo, mL		13,7 (7,7-20)	14 (12-17)	0,902
Análise do sêmen				
Volume do sêmen, ml	> 2	3,1 (1,5-6,8)	3,1 (2,5-3,5)	0,985
Concentração de espermatozóides x 10 ⁶ /ml	> 20	156 (33-575)	121,1 (55-255)	0,674
Contagem total de espermatozóides x 10 ⁶	> 40	416 (66-1131)	348,8 (165-637)	0,74
Motilidade de espermatozóides, %	> 50	58,2 (25-65)	65,6 (40-80)	0,419
Morfologia normal dos espermatozóides (OMS), %	> 15	21,2 (10-42,5)	20,9 (13,5-33)	0,95
Morfologia normal dos espermatozóides (Kruger), %	> 4	6,28 (1,5-16,5)	5,1 (1,5-10)	0,655
Anticorpos antiespermatozóide, %	>50%	25,7 (8-60)	14,6 (11-17)	0,164
Avaliação hormonal				
FSH, UI/litro	1-10,5	6,24 (0,8-26,8)	3,38 (1,3-5,3)	0,451
Níveis elevados		1 (14)	3 (60)	0,222
LH, UI/litro	1-8,4	4,65 (1-13,1)	4,24 (2,6-8)	0,82
Níveis elevados		1 (14)	0	1,0
Testosterona total, ng/dl	271-965	522,4 (417-622)	547,8 (309-803)	0,78
Níveis diminuídos		0	0	1,0
Características clínicas e laboratoriais, %				
Plaquetopenia		0	0	1,0
Livedo reticular		0	0	1,0
Anticardiolipina IgM, MPL U/mL	≥ 20	2 (28)	1 (20)	1,0
Anticardiolipina IgG, GPL U/mL	≥ 20	1 (14)	1 (20)	1,0
Anticoagulante lúpico		3 (42)	5 (100)	0,08
Anti-beta2-glicoproteína I IgM, U/mL	> 8	2 (28)	1 (20)	1,0
Anti-beta2-glicoproteína I IgG, U/mL	> 8	3 (42)	1 (20)	0,575

Valores expressos em mediana (intervalo) ou n (%), n – número de pacientes e controles, OMS – Organização Mundial de Saúde, FSH – hormônio foliculoestimulante, LH – hormônio luteinizante.

4.3 Pacientes SAF-LES *versus* controles

A mediana da idade da espermarca foi significativamente maior no grupo de SAF-LES comparado aos controles [13,9 (12-16) vs. 12,85 (11-15) anos, $p=0,016$]. Por sua vez, a mediana do volume testicular direito por ultrassom foi menor no SAF-LES [10,38 (3,9-16,7) vs. 13,4 (8,5-20,6) ml, $p=0,03$]. A frequência de disfunção erétil foi significativamente maior nos pacientes com SAF-LES comparado aos controles (30% vs. 0%, $p=0,029$). Não foram observadas diferenças em relação à antropometria do pênis e ao volume testicular esquerdo em ambos os grupos (Tabela 1). As medianas da concentração e da motilidade dos espermatozóides foram significativamente menores nos pacientes com SAF-LES comparados aos controles [41,1 (0-145) vs. 120,06 (34,5-329) $\times 10^6/\text{mL}$, $p=0,003$; 47,25 (0-87,5) vs. 65,42 (43-82) %, $p=0,047$; respectivamente]. As formas de espermatozóides normais avaliados pela OMS e Kruger foram significativamente menores no SAF-LES que nos controles [11 (0-27) vs. 23,95 (10-45)%, $p=0,002$; 2,65 (0-8) vs. 7,65 (3-18)%, $p=0,02$; respectivamente]. A frequência de oligo/azoospermia foi significativamente maior no grupo SAF-LES (40% vs. 0%, $p=0,007$). Da mesma forma, a mediana dos níveis de FSH e as frequências de FSH elevados foram significativamente maiores nesse grupo [9,73 (2,4-25) vs. 3,32 (1-5,9) UI/L, $p=0,025$; 40% vs. 0%, $p=0,007$; respectivamente] (Tabela 2). Todos os pacientes com SAF-LES e controles tinham dosagens de hormônios tireoidianos normais.

4.4 Pacientes SAF-LES

A análise de subgrupos de SAF-LES com (n=2) e sem (n=8) eventos arteriais prévios demonstrou que a mediana da circunferência peniana foi significativamente menor nos que tiveram trombose arterial [7,5 (7-8) vs. 9,18 (8-10,5) cm, $p=0,039$], com mediana de comprimento semelhante em ambos os grupos [8,25 (7,5-9) vs. 10,87 (8,5-13) cm, $p=0,08$] (Tabela 4).

A mediana da circunferência do pênis foi significativamente menor no subgrupo de SAF-LES com disfunção erétil (n=3) *versus* sem essa alteração (n=7) [8,17 (8-8,5) vs. 9,14 (7-10,5) cm, $p=0,0397$]. Nenhuma diferença foi evidenciada na mediana do comprimento do pênis em ambos os grupos [10,83 (9-12,5) vs. 10,14 (7,5-13) cm, $p=0,615$].

Os dados demográficos, IMC, volume testicular pelo ultrassom, análise do sêmen, perfil hormonal e características clínicas e laboratoriais foram semelhantes nos pacientes SAF-LES com e sem eventos arteriais prévios ($p > 0,05$) (Tabela 4).

A CFEV foi usada para o tratamento de nefrite lúpica em quatro pacientes. A mediana de tempo de duração entre a última dose de CFEV e o início do estudo foi de seis anos (variação de 2 a 9 anos). A mediana de duração da doença foi significativamente maior nos doentes tratados com CFEV (n=4) em comparação aos que não receberam essa medicação (n=6) [9,92 (5-13) vs. 3,38 (0,3-7) anos, $p=0,028$]. As medianas da concentração e da contagem de total de espermatozóides foram significativamente menores

no SAF-LES tratados com CFEV comparado aos que não usaram essa medicação [6,87 (0-23,5) vs. 63,9 (7,5-145) $\times 10^6$ /mL, $p=0,04$; 16,12 (0-55,5) vs. 226,25 (8,5-471) $\times 10^6$, $p=0,035$; respectivamente]. Não foram observadas diferenças na antropometria do pênis, volume testicular por ultrassom, anticorpos antiespermatozóide, perfil hormonal, atividade e dano cumulativo da doença em ambos os grupos ($p>0,05$) (Tabela5).

Tabela 4 - Dados demográficos, índice de massa corpórea (IMC), antropometria do pênis, volume testicular por ultrassonografia, análise do sêmen, anticorpos antiespermatozóide, avaliação hormonal, características clínicas e laboratoriais no início do estudo, atividade e dano cumulativo da doença em pacientes com síndrome antifosfolípide associada ao lúpus eritematoso sistêmico (SAF-LES) com e sem eventos arteriais anteriores.

Variáveis	Valores de referência	SAF-LES Com evento arterial (n=2)	SAF-LES Sem evento arterial (n=8)	P
Dados demográficos e IMC				
Idade atual, anos		39,5 (37-42)	36,25 (21-57)	0,538
Duração da doença, anos		5,5 (1-10)	5,78 (2-13)	0,935
Idade da espermarca, anos		14 (13-15)	13,87 (12-16)	0,904
Raça caucasiana		1 (50)	2 (25)	1,0
IMC, Kg/m ²		31,1 (27-35,2)	25,87 (22-31,3)	0,132
Antropometria do pênis e disfunção erétil				
Comprimento, cm		8,25 (7,5-9)	10,87 (8,5-13)	0,08
Circunferência, cm		7,5 (7-8)	9,18 (8-10,5)	0,039
Disfunção erétil		1 (50)	2 (25)	1,0
Volume testicular	15 ± 8 ml			
Direito, mL		7,2 (4,8-9,6)	11,17 (3,9-16,7)	0,213
Esquerdo, mL		9,15 (8,9-9,4)	11,51 (7,2-17,2)	0,082
Análise do semen				
Volume do sêmen, ml	> 2	3,5 (3,5-3,5)	2,81 (1,5-4,5)	0,086
Concentração de espermatozoides, x10 ⁶ /ml	> 20	53 (2-104)	38,12 (0-145)	0,721
Oligo/azoospermia		1 (50)	3 (37)	1,0
Contagem total de espermatozoides, x 10 ⁶	> 40	211 (6-416)	125 (0-471)	0,562
Motilidade de espermatozoides, %	> 50	26 (16-36)	52,6 (0-87,5)	0,143
Espermatozoides normais (OMS), %	> 15	9,5 (5-14)	11,37 (0-27)	0,769
Espermatozoides normais (Kruger), %	> 4	2,75 (1,5-4)	2,62 (0-8)	0,943
Anticorpos antiespermatozóide, %	>50%	17,5 (12-23)	17,12 (9-21)	0,918
Avaliação hormonal				
FSH, UI/litro	1-10,5	8,7 (4,2-13,2)	9,98 (2,4-25)	0,839
Níveis elevados		1 (50)	3 (37)	1,0
LH, UI/litro	1-8,4	5,95 (3,1-8,8)	7,1 (1,6-21,7)	0,786
Níveis elevados		1 (50)	2 (25)	1,0
Testosterona total, ng/dl	271-965	427,5 (321-534)	436 (91-891)	0,955
Níveis diminuídos		0	2 (25)	1,0
Características clínicas e laboratoriais, %				
Plaquetopenia		0	0	1,0
Livedo reticular		0	0	1,0
Anticardiolipina IgM, MPL U/mL	≥ 20	1 (50)	2 (25)	1,0
Anticardiolipina IgG, GPL U/mL	≥ 20	2 (100)	4 (50)	0,466
Anticoagulante lúpico		1 (50)	5 (62)	1,0
Anti-beta2-glicoproteína I IgM, U/mL	> 8	0	2 (25)	1,0
Anti-beta2-glicoproteína I IgG, U/mL	> 8	1 (50)	2 (25)	1,0
Atividade e dano cumulativo da doença				
SLEDAI		0 (0-0)	2,65 (0-12)	0,054
SLICC/ACR-DI		1 (1-1)	0,62 (0-4)	0,237

Valores expressos em mediana (intervalo) ou n (%), n – número de pacientes e controles, OMS – Organização Mundial de Saúde, FSH – hormônio foliculoestimulante, LH – hormônio luteinizante, SLEDAI – Systemic Lupus Erythematosus Disease Activity Index, SLICC/ACR-DI – Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

Tabela 5 - Dados demográficos, índice de massa corpórea (IMC), antropometria do pênis, volume testicular por ultrassonografia, análise do sêmen, anticorpos antiespermatozóide, avaliação hormonal, atividade e dano cumulativo da doença em pacientes com síndrome antifosfolípide associada ao lúpus eritematoso sistêmico (SAF-LES), tratados ou não com ciclofosfamida endovenosa (CFEV).

Variáveis	Valores de referência	SAF-LES Com CFEV (n=4)	SAF-LES Sem CFEV (n=6)	P
Dados demográficos e IMC				
Idade atual, anos		35 (23-47)	38,16 (21-57)	0,683
Duração da doença, anos		9,92 (5-13)	3,38 (0,3-7)	0,028
Idade da espermarca, anos		13,20 (12-14)	14,33 (13-16)	0,156
Raça caucasiana		1(25)	3(50)	0,571
IMC, Kg/m ²		25 (22-27)	28,2 (23,5 - 35,2)	0,218
Antropometria do pênis e disfunção erétil				
Comprimento, cm		9,0 (7,5-11)	11,08 (8,5-13)	0,135
Circunferência, cm		8,5 (7,0-10)	9,08 (8-10,5)	0,147
Disfunção erétil		1 (25)	2 (33)	1,0
Volume testicular	15 ± 8 ml			
Direito, mL		7,77 (3,9-12,4)	12,11 (9,1- 16,7)	0,125
Esquerdo, mL		9,9 (7,2 - 13)	11,8 (7,8 - 17,2)	0,33
Análise do sêmen				
Volume do sêmen, ml	> 2	2,25 (1,5-3,5)	3,41 (1,5-4,5)	0,119
Concentração de espermatozóides x 10 ⁶ /ml	> 20	6,87 (0-23,5)	63,9 (7,5-145)	0,04
Oligo/azoospermia		3 (75)	1 (16)	0,19
Contagem total de espermatozóides x 10 ⁶	> 40	16,12 (0-55,5)	226,25 (8,5-471)	0,035
Motilidade de espermatozóides,%	> 50	29,87 (0-55)	59,5 (36-87,5)	0,11
Espermatozóides normais (OMS),%	> 15	6,75 (0-20)	13,83 (4-27)	0,264
Espermatozóides normais (Kruger),%	> 4	1,75 (0-5)	3,25 (1-8)	0,367
Anticorpos antiespermatozóide, %	> 50%	19 (17-23)	16 (9-21)	0,251
Avaliação hormonal				
FSH, UI/litro	1-10,5	13,92 (4,3-25)	6,93 (2,4-24,9)	0,251
Níveis elevados		3(75)	1(16)	0,195
LH, UI/litro	1-8,4	8,57 (3,3-15,6)	5,73 (1,6-21,7)	0,51
Níveis elevados		2 (50)	1 (16)	0,5
Testosterona total, ng/dl	271-965	387,2 (91-891)	465,66 (322-586)	0,689
Níveis diminuídos		2 (50)	0	0,133
Atividade e dano cumulativo da doença				
SLEDAI		2,5 (0-6)	2,33 (0-8)	0,467
SLICC/ACR-DI		1,25 (0-4)	0,33 (0-1)	0,207

Valores expressos em mediana (intervalo) ou n (%), n – número de pacientes e controles, OMS – Organização Mundial de Saúde, FSH – hormônio foliculoestimulante, LH – hormônio luteinizante, SLEDAI – Systemic Lupus Erythematosus Disease Activity Index, SLICC/ACR-DI – Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

4.5 Pacientes SAFP versus SAF-LES

As medianas de concentração, contagem total e formas normais de espermatozóides foram significativamente maiores nos pacientes SAFP comparados ao SAF-LES [141,5 (33-575) vs. 41,1 (0-145) x 10⁶/mL, p=0,046; 388 (66-1131) vs. 142,2 (0-471) x 10⁶, p=0,036; 21,12 (10-42,5) vs. 11 (0-27)%, p=0,026; respectivamente]. A frequência de oligo/azoospermia foi significativamente menor no grupo SAFP versus SAF-LES (0% vs. 40%, p=0,029). Não foram observadas diferenças em dados demográficos, antropometria do pênis, volume testicular e perfil hormonal em ambos os grupos (p>0,05) (Tabelas 1 e 2).

5 DISCUSSÃO

Este foi o primeiro estudo que abordou especificamente a função gonadal em pacientes com SAF-P e SAF-LES em relação a controles saudáveis, mostrando claramente que estes pacientes têm diminuição das dimensões penianas relacionados à trombose arterial anterior. A antropometria do pênis anormal com disfunção erétil foi evidenciada nesses pacientes. Também foram verificadas importantes anormalidades nos espermatozóides em pacientes com SAF-LES que receberam CFEV.

A grande vantagem do presente estudo foi a avaliação sistemática peniana e testicular através de critérios estabelecidos para a antropometria do pênis,^{16,24} avaliações dos espermatozóides e anticorpos antiespermatozóides,^{5,6} e a inclusão apenas de pacientes pós-púberes e controles saudáveis. Além disso, a seleção dos pacientes com SAF e controles sem anormalidades testiculares e comorbidades é de extrema importância uma vez que estas alterações podem causar alterações gonadais.⁶

O desenvolvimento das dimensões do pênis ocorre apenas durante a puberdade e pode ser influenciado pela herança genética, disfunção do eixo hipotálamo-hipofisário e doenças crônicas, como descrito em nossos pacientes com LES.^{5,16} A produção de andrógenos pelos testículos e glândulas suprarrenais durante a puberdade é provavelmente o fator mais importante para o desenvolvimento dos órgãos genitais, como o pênis.³² Todos os nossos pacientes SAF-P e SAF-LES com redução da antropometria peniana tiveram início da doença após a espermarca, indicando que esta alteração não foi atribuída ao desenvolvimento puberal

durante a adolescência. Em contrapartida, nós observamos medianas reduzidas de circunferência peniana em pacientes lúpicos sem SAF associada pré-púberes em relação aos pacientes pós-púberes.¹⁶ Curiosamente os pacientes com SAFP e SAF-LES que tiveram trombooses arteriais prévias apresentaram diminuição das dimensões penianas, sugerindo que, além da trombose arterial dominante, microtrombos também podem ser instalados em outros órgãos, como o pênis e o corpo cavernoso.³³ Vale a pena ressaltar que todos os pacientes eram assintomáticos com relação ao pênis, sem edema e/ou dor. Não existem relatos de casos de trombose peniana em pacientes com SAF.

Notadamente, a disfunção erétil ocorreu nos pacientes com SAFP e SAF-LES com redução da circunferência do pênis. Essa disfunção sexual é caracterizada pela incapacidade do homem para conseguir uma ereção e mantê-la suficientemente razoável para o ato sexual.³⁴ A impotência sexual também foi relatada recentemente por nosso grupo em uma pequena população de SAF ligados à eventos arteriais,³⁵ no entanto as avaliações do pênis e dos testículos não foram realizados neste estudo. Anormalidades morfofuncionais do pênis em nossos pacientes com SAF, sem dúvida, podem provocar preocupações psicológicas e alteração na sua qualidade de vida relacionada à saúde.³⁴ Entretanto, apenas os homens com um comprimento flácido do pênis menor que quatro centímetros devem ser considerados candidatos para o aumento do pênis e nenhum dos nossos pacientes tinha micropênis.³⁶

Além disso, no grupo de SAF-LES, confirmamos nossa observação anterior de anormalidades no esperma, redução do volume testicular^{11-13,16} e alterações hormonais em homens com LES sem SAF associada.¹¹⁻¹³ O uso de ciclofosfamida parece contribuir para alterações importantes do sêmen em nossos pacientes SAF-LES ocasionando um dano persistente ou de longa duração para as células gonadais, como também observado em homens com LES sem SAF.¹²

Não há explicação clara para o atraso da espermarca nos pacientes SAF-LES, não sendo influenciado pela atividade da doença e/ou drogas, uma vez que todos eles tiveram o início da doença após a primeira ejaculação. Ao contrário, nós demonstramos anteriormente ocorrência tardia da menarca em paciente com LES juvenil³⁷⁻³⁹ e dermatomiosite juvenil⁴⁰ nas quais a doença ocorreu antes da primeira menstruação, em comparação com adolescentes brasileiros saudáveis.

Este estudo enfatiza a importância da criopreservação de esperma para técnicas futuras de reprodução assistida antes de usar CFEV.⁶ Além disso, nossos pacientes com SAFP tinha função gonadal normal, sendo que trombose testicular foi raramente relatado na literatura nestes pacientes.⁴

O seguimento destes pacientes está sendo realizado no Centro de Reprodução Humana do HC-FMUSP. A abordagem multidisciplinar e multiprofissional da infertilidade e disfunção erétil é fundamental para estes pacientes, melhorando a sua qualidade de vida relacionada à saúde e de suas respectivas parceiras. Outros estudos avaliando a função sexual,

envolvimento da vasculatura peniana e eficácia/segurança das drogas para disfunção erétil (inibidores de fosfodiesterase tipo 5) serão realizados futuramente nesta população de pacientes com trombose autoimune.

6 CONCLUSÕES

- 1 Pacientes com SAFP e SAF-LES apresentaram redução do tamanho do pênis associada à trombose arterial anterior e à disfunção erétil.
- 2 Pacientes com SAF-LES tiveram disfunção testicular devido ao uso de agentes alquilantes.

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7 APÊNDICES

APÊNDICE 1

Artigo “Primary antiphospholipid syndrome: morphofunctional penile abnormalities with normal sperm analysis”

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What is This?

PAPER

Primary antiphospholipid syndrome: morphofunctional penile abnormalities with normal sperm analysis

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Objective: To perform a global gonadal and sexual functions assessment in primary antiphospholipid syndrome (PAPS) patients. **Methods:** A cross-sectional study was conducted in 12 male PAPS patients and 20 healthy controls. They were assessed by demographic data, clinical features, systematic urological examination, sexual function, testicular ultrasound, seminal parameters according to the World Health Organization (WHO), seminal sperm antibodies, and hormone profile, including follicle stimulating hormone (FSH), luteinizing hormone (LH), morning total testosterone, and thyroid hormones. **Results:** The median of current age and age of spermatarche were similar in PAPS patients and controls (37.5 vs. 32.4 years, $p = 0.270$, and 13.1 vs. 12.85 years, $p = 0.224$, respectively), with a higher frequency of erectile dysfunction in the former group (25% vs. 0%, $p = 0.044$). Further analysis of PAPS patients with and without previous arterial thrombosis demonstrated that the median penis circumference was significantly lower in PAPS with arterial thrombosis than in PAPS without this complication (8.1 [6–10] vs. 10.2 [10–11] cm, $p = 0.007$). In addition, the median penis circumference was significantly lower in PAPS patients with erectile dysfunction than in patients without this complication (7.5 [6–9.5] vs. 9.5 [7.5–11] cm, $p = 0.039$). Regarding seminal analysis, the median sperm concentration, sperm motility, and normal sperm forms by WHO guidelines were comparable in PAPS patients and controls (141.5 [33–575] vs. 120.06 [34.5–329] $\times 10^6$ /ml, $p = 0.65$; 61.29 [25–80] vs. 65.42 [43–82]%, $p = 0.4$; 21.12 [10–42.5] vs. 23.95 [10–45]%, $p = 0.45$, respectively), and none of them had oligo/azoospermia. No differences were observed between PAPS patients and controls regarding the frequency of antisperm antibodies, testicular volume by ultrasound, or hormone profile (FSH, LH, morning total testosterone, and thyroid hormone) ($p > 0.05$). **Conclusions:** Normal testicular function has been identified in PAPS patients, in spite of morphofunctional penile abnormalities. Previous arterial thrombosis may underlie penile anthropometry alteration. *Lupus* (2011) 0, 1–6.

Key words: antiphospholipid syndrome; antisperm antibodies; fertility; penile; rheumatic disease; sperm

Introduction

Antiphospholipid syndrome (APS) is an autoimmune thrombophilic condition¹ that can occur with another autoimmune disease, most often systemic lupus erythematosus (SLE–APS),^{2,3} or in the isolated form – primary APS (PAPS). About 8% to 18% of affected patients are male and most organ

systems in the human body, including testicles,⁴ may be affected.

Testicular evaluation in autoimmune rheumatic disease requires a complete urological examination in order to assess sperm abnormalities due to aging,^{5,6} genital abnormalities,⁷ hypothalamic–pituitary-axis dysfunction, antisperm antibodies⁸ and effects of drug treatment, particularly immunosuppressive agents.^{9–15}

We have previously reported severe sperm abnormalities associated with cyclophosphamide treatment in male SLE patients^{12,13} and reduced penile dimensions not associated with erectile dysfunction in pre-pubertal SLE without APS.¹⁶ Of note, testicular thrombosis due to PAPS and SLE–APS has been described in a few case reports

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in the literature.^{3,17–21} There is, however, no systematic study assessing the overall gonadal function in PAPS patients with these conditions.

Therefore, the aim of this study was to perform global testicular and sexual function assessments in male patients with PAPS, evaluating its possible association with clinical and laboratory parameters.

Patients and methods

PAPS patients and controls

We initially screened 38 male patients with APS without a medical history of hydrocele, hypospadias, cryptorchidism, testicular infection, testicular cancer, orchitis, testicular vasculitis, urethral impairment, previous history of any scrotal or inguinal surgery, diabetes mellitus, or tobacco use. None of the participants had a history of alcohol consumption or heavy occasional drinking before or at study entry. Of the 38 patients, 26 patients were excluded due to previous vasectomy ($n=3$), refusal to collect sperm sample ($n=9$), or association with other rheumatic diseases ($n=14$) (systemic lupus erythematosus, $n=10$; polyarteritis nodosa, $n=2$; rheumatoid arthritis, $n=1$; and ankylosing spondylitis, $n=1$). Therefore, a cross-sectional study was conducted in 12 PAPS patients regularly followed at the Antiphospholipid Outpatient Clinic of the Rheumatology Division of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo from May 2008 to February 2011. All patients fulfilled the Sapporo criteria for APS diagnosis.²² The control group included 20 healthy subjects followed at the Pre-vasectomy Group of the Urology Division of our University Hospital. All PAPS patients and controls were at Tanner stage 5.²³ The Local Ethics Committee approved this study and informed consent was obtained from all participants.

Global reproductive health evaluation

Demographic data and body mass index

Current age, age at spermatarche (first ejaculation), and race were recorded in PAPS patients and controls, and age at disease onset and disease duration in PAPS patients. Body mass index (BMI) was defined by the formula $\text{BMI} (\text{kg}/\text{m}^2)$.

Urologic evaluation and sexual function

A clinical assessment of the genitalia included evaluation of testicles, epididymis, vas deferens,

scrotum and penis. Penis length was measured as linear distance along the dorsal side of the penis extending from the pubopenile skin junction to the tip of the glans in the flaccid state, while penis circumference was measured at the middle of the shaft.²⁴ Sexual function in patients and controls was evaluated by the male sexual quotient, an self-administered questionnaire validated in Brazilian Portuguese.²⁵

Testicular Doppler ultrasound

Ultrasound was performed blinded to the results of the semen analysis in PAPS patients and controls. The largest measurement in each dimension was recorded and used to calculate the testicular volume according to the formula for an ellipsoid ($\text{length} \times \text{width} \times \text{thickness} \times 0.52$). The normal mean $\pm$ SD value in male post-pubertal adolescents and adults is 7–23 ml.^{11–13,26}

Hormone evaluation

Hormone determinations performed at study entry in PAPS patients and controls included: follicle-stimulating hormone (FSH), luteinizing hormone (LH), morning total testosterone, triiodothyronine (T3), tetraiodothyronine (T4), free tetraiodothyronine (free T4), and thyrotropin (TSH) detected by fluoroimmunoassay using DELFIA time-resolved fluoroimmunoassay kits (Wallac, 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), total morning testosterone (271–965 ng/dl), T3 (70–200 ng/dl), T4 (4.5–12 $\mu\text{g}/\text{dl}$), free T4 (0.7–1.5 ng/dl), and TSH (0.4–4.5 $\mu\text{U}/\text{ml}$).

Sperm evaluation

Sperm analysis was performed according to the guidelines of the World Health Organization (WHO)⁷ by two expert medical technologists. All PAPS patients and controls collected two semen samples at an interval of 15–30 days after 48–72 h of sexual abstinence. The spermatozoa were analyzed by manual hand count as well as by a computer-assisted semen analysis system under 400 $\times$ magnification, using an HTM-2030 (Hamilton Thorne Research, Beverly, MA, USA). Sperm morphology included evaluation of sperm head, neck, midpiece, and tail by WHO guidelines and Kruger strict criteria.²⁷ Azoospermia was defined as no spermatozoa in the ejaculate and oligozoospermia as sperm concentration <20 million/ml.⁹ The presence of antisperm antibodies was determined by direct Immunobead test using Immunobead^R rabbit anti-human Ig (IgA, IgG, and IgM) kits (Irvine Scientific,

Santa Ana, CA, USA) in all patients and controls. At least 50% of the motile spermatozoa ('a' + 'b') must be coated with Immunobeads before the test results are considered to be clinically significant. A negative control should have a score of <10% bead attachment, and a positive control should have a score of >20% bead attachment.⁷

Clinical evaluation

The following clinical parameters were evaluated in PAPS patients: venous thrombosis (documented deep vein thrombosis and/or pulmonary embolism), arterial thrombosis (clinically documented stroke, transient ischemic attacks or acute myocardial infarction), livedo reticularis and thrombocytopenia (<100,000/mm³ platelets, on at least two distinct occasions).

Serum immunologic analysis

Anticardiolipin antibodies were detected by a commercial kit (Enzyme Immunoassay Kit, BINDAZYME™, Birmingham, UK) in all APS patients. The cutoff values were: <11 GPL/MPL (units) U/ml negative, ≥11 and <20 GPL/MPL U/ml indeterminate, and ≥20 positive.²⁸ Lupus anticoagulant (LAC) was detected according to international guidelines.²⁹ Serum IgG and IgM anti-beta-2-glycoprotein I were detected by enzyme-linked immunoabsorbent assay (ELISA) (ORG 521 Anti-beta-2-Glycoprotein I IgG/IgM Mainz, Germany) with cutoff values of 8 U/ml for IgM and IgG, and with intra-assay variation 2.1–5.0% and inter-assay variation 2.6–7.95% for IgG and intra-assay variation 2.1–3.8% and inter-assay variation 4.1–6.3% for IgM.

Statistical analysis

Data were compared by *t*-test or Mann-Whitney test if numerical data presented a normal or non-normal distribution, respectively, to evaluate differences between PAPS patients and controls according to previous arterial thrombosis and erectile dysfunction. For categorical variables, differences were assessed by Fisher's exact test. *p* values less than 0.05 were considered significant.

Results

PAPS patients versus controls

The demographic data, BMI, penile anthropometry, testicular volume by ultrasound, sperm analysis, antisperm antibodies, and hormone evaluation in PAPS patients and controls are illustrated in

Table 1. The median of current age and age of spermatarche were similar in both groups (37.5 [21–62] vs. 32.4 [18–54] years, *p* = 0.270, and 13.1 [12–16] vs. 12.85 [11–15] years, *p* = 0.224, respectively), whereas the BMI was significantly higher in PAPS patients (26.06 [15.7–34.6] vs. 22.2 [18–27] kg/m², *p* = 0.006).

Erectile dysfunction was significantly higher in PAPS patients than in controls (25% vs. 0%, *p* = 0.044) (Table 1). The penis anthropometry, sperm parameters, antisperm antibodies and hormones were similar in PAPS patients and controls (*p* > 0.05, Table 1). All PAPS patients and controls had normal thyroid hormones.

PAPS patients

The subgroup analysis of PAPS patients with (*n* = 7) and without (*n* = 5) previous arterial thrombosis demonstrated that the median penis circumference was significantly lower in PAPS patients with arterial events (8.1 [6–10] vs. 10.2 [10–11] cm, *p* = 0.007), whereas a similar median penis length was found in both groups (9.1 [7–12] vs. 10.9 [9.5–13] cm, *p* = 0.08). Forty-two percent of PAPS patients with previous arterial thrombosis had erectile dysfunction, compared with no patients without arterial events (*p* = 0.204) (Table 2). Further analysis revealed that the median penis circumference was significantly lower in PAPS patients with (*n* = 3) erectile dysfunction vs. without (*n* = 9) this complication (7.5 [6–9.5] vs. 9.5 [7.5–11] cm, *p* = 0.039). No differences were evidenced in the median penis length between groups (8.33 [7–11.5] vs. 10.22 [8–13] cm, *p* = 0.280).

The demographic data, BMI, testicular volume by ultrasound, hormonal profile, sperm analysis, and clinic and laboratory features were similar in PAPS patients with and without previous arterial events (*p* > 0.05; Table 2).

Discussion

To our knowledge, this was the first study that specifically addressed gonadal and sexual function in PAPS patients, identifying a reduced penile size associated with previous arterial thrombosis.

The limited number of patients and the cross-sectional design are the drawbacks of this study. However, strengths of the study are that the data were collected prospectively and all patients received a close and pre-established protocol. Systematic penile and testicular evaluations were performed using established criteria for penis

Table 1 Demographic data and body mass index (BMI), penis anthropometry, testicular volume by ultrasound, sperm analysis, antisperm antibodies, and hormone evaluation in primary antiphospholipid syndrome (PAPS) patients and healthy controls

Variables	PAPS (n = 12)	Controls (n = 20)	p
Demographic data and BMI			
Current age, years	37.5 (21–62)	32.4 (18–54)	0.270
Age at disease onset, years	31.41 (18–61)		
Disease duration, years	6.08 (1–18)		
Age at spermarche, years	13.1 (12–16)	12.85 (11–15)	0.224
Caucasian race	6 (50)	7 (33)	0.473
BMI, kg/m ²	26.06 (15.7–34.6)	22.2 (18–27)	0.006
Penis anthropometry and erectile dysfunction			
Length, cm	9.87 (7–13)	10.02 (8–13)	0.358
Circumference, cm	9 (6–11)	9.42 (7–11)	0.185
Erectile dysfunction	3 (25)	0	0.044
Testicular volume			
Right, ml	12.85 (7.2–18.4)	13.41 (8.5–20.6)	0.68
Left, ml	13.88 (7.7–20)	12.85 (6–21.2)	0.22
Sperm analysis			
Sperm volume, ml	3.1 ± 1.41	1.66 ± 1.28	0.25
Sperm concentration, × 10 ⁶ /ml	141.5 (33–575)	120.06 (34.5–329)	0.65
Oligo/azoospermia	0	0	1.0
Total sperm count, × 10 ⁶	388 (66–1131)	300.5 (41.3–1176)	0.4
Sperm motility, %	61.29 (25–80)	65.42 (43–82)	0.4
Normal sperm forms by WHO guidelines, %	21.12 (10–42.5)	23.95 (10–45)	0.45
Normal sperm forms by Kruger, %	5.79 (1.5–16.5)	7.65 (3–18)	0.27
Antisperm antibodies, %	21.08 (8–60)	16.9 (0–32)	0.35
Hormone evaluation			
FSH, IU/liter	5.06 (1–26.8)	3.32 (1–5.9)	0.207
Elevated levels	1 (8)	0	0.375
LH, IU/liter	4.48 (1–13.1)	3.49 (1.8–5.8)	0.171
Elevated levels	1 (8)	0	0.375
Morning total testosterone, ng/dl	533 (309–803)	552.5 (230–1078)	0.381
Lower levels	0	1 (5)	1.0

Values expressed in mean ± SD, median (range) or n (%).

FSH: follicle-stimulating hormone, LH: luteinizing hormone, WHO: World Health Organization.

Reference values: FSH 1–10.5 IU/liter, LH 1–8.4 IU/liter, Morning total testosterone 271–965 ng/dl.

The values in bold: p < 0.05 (statistically significant).

anthropometry,^{16,24} sperm and antisperm antibody assessments,^{5,6} and the inclusion of only post-pubertal subjects. In addition, the restricted selection criteria of PAPS patients and controls without a medical history of testicular abnormalities and comorbidities is relevant, since these conditions may affect gonadal evaluation.⁶

Development of penis dimensions occurs only during puberty and may be influenced by genetic background, hypothalamic–pituitary-axis dysfunction and chronic diseases, as described in our SLE patients.^{5,16} Androgens produced by testis and adrenal glands at pubertal age are probably the most important factor in the development of the genital organs, including the penis.³⁰ All of our PAPS patients with reduction of penile anthropometry had disease onset after spermarche, indicating that their reduced penis dimension was not attributed to pubertal development during adolescence.

Interestingly, PAPS patients with previous arterial events had reduced penile circumference,

raising the possibility that chronic subclinical endothelial dysfunction involving corpus cavernosum may result in atrophy.³¹ We are currently performing Doppler evaluation to test this hypothesis. Of note, the entire group of patients was asymptomatic, without a history of penis edema or pain. To our knowledge there are no cases of clinical penis thrombosis in PAPS patients reported in the literature.

Remarkably, erectile dysfunction occurred particularly in PAPS patients with reduced penile circumference. This sexual dysfunction is characterized by the inability of a man to achieve an erection and maintain it sufficiently for sexual performance.³² Sexual impotence was also recently reported by our group in a small population with APS linked to arterial events,³³ suggesting that erectile dysfunction may be linked to local subclinical endothelial cell dysfunction in PAPS patients.

The penis morphofunctional abnormalities in PAPS patients described herein are of major

Table 2 Demographic data, body mass index (BMI), penis anthropometry, testicular volume by ultrasound, sperm analysis, antisperm antibodies, hormone evaluation, and clinical and laboratory features at study entry in primary antiphospholipid syndrome (PAPS) patients with and without previous arterial events

Variables	PAPS with arterial events (n = 7)	PAPS without arterial events (n = 5)	p
Demographic data and BMI			
Current age, years	40.7 (24–62)	33 (21–53)	0.334
Disease duration, years	6.1 (1–16)	6 (2–18)	0.969
Age of spermarche, years	13.5 (12–16)	12.6 (12–13)	0.1
Caucasian race	3 (42)	1 (20)	0.417
BMI, kg/m ²	27.2 (24–34.6)	24.4 (15.7–27.7)	0.27
Penis anthropometry and erectile dysfunction			
Length, cm	9.1 (7–12)	10.9 (9.5–13)	0.08
Circumference, cm	8.1 (6–10)	10.2 (10–11)	0.007
Erectile dysfunction	3 (42)	0	0.204
Testicular volume			
Right, ml	12.5 (7.2–17)	13.3 (8.2–18.4)	0.738
Left, ml	13.7 (7.7–20)	14 (12–17)	0.902
Sperm analysis			
Sperm volume, ml	3.1 (1.5–6.8)	3.1 (2.5–3.5)	0.985
Sperm concentration, × 10 ⁶ /ml	156 (33–575)	121.1 (55–255)	0.674
Total sperm count, × 10 ⁶	416 (66–1131)	348.8 (165–637)	0.74
Sperm motility, %	58.2 (25–65)	65.6 (40–80)	0.419
Normal sperm forms by WHO guidelines, %	21.2 (10–42.5)	20.9 (13.5–33)	0.95
Normal sperm forms by Kruger, %	6.28 (1.5–16.5)	5.1 (1.5–10)	0.655
Antisperm antibodies, %	25.7 (8–60)	14.6 (11–17)	0.164
Hormone evaluation			
FSH, IU/l	6.24 (0.8–26.8)	3.38 (1.3–5.3)	0.451
Elevated levels	1 (14)	3 (60)	0.222
LH, IU/l	4.65 (1–13.1)	4.24 (2.6–8)	0.82
Elevated levels	1 (14)	0	1.0
Morning total testosterone, ng/dl	522.4 (417–622)	547.8 (309–803)	0.78
Lower levels	0	0	1.0
Clinical and laboratory features, %			
Thrombocytopenia	0	0	1.0
Livedo reticularis	0	0	1.0
IgM anticardiolipin, >20 MPL	2 (28)	1 (20)	1.0
IgG anticardiolipin, >20 GPL	1 (14)	1 (20)	1.0
Lupus anticoagulant	3 (42)	5 (100)	0.08
IgM anti-beta2-glycoprotein I, >8 U/ml	2 (28)	1 (20)	1.0
IgG anti-beta2-glycoprotein I, >8 U/ml	3 (42)	1 (20)	0.575

Values expressed as median (range) or *n* (%).

BMI: body mass index, FSH: follicle-stimulating hormone, LH: luteinizing hormone, PAPS: primary antiphospholipid syndrome, WHO: World Health Organization.

Reference values: FSH 1–10.5 IU/liter, LH 1–8.4 IU/liter, Morning total testosterone 271–965-ng/dl.

The values in bold: *p* < 0.05 (statistically significant).

concern, since they contribute to psychological distress, with a consequent reduction of health-related quality of life.³² In spite of this, our extensive evaluation revealed that PAPS patients have normal testicular function, a reassuring fertility preservation. In fact, testicular thrombosis was rarely reported in these patients.⁴ Moreover, only men with a flaccid length of less than 4 cm should be considered candidates for penile enlargement, and none of our PAPS patients had micropenis.³⁴

In conclusion, we have identified normal testicular function, in spite of morphofunctional penile abnormalities. Previous arterial thrombosis may underlie penile anthropometry alteration.

Conflict of interest

None declared.

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APÊNDICE 2

Artigo “Moderate/severe erectile dysfunction in patients with antiphospholipid syndrome”

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What is This?

CONCISE REPORT

Moderate/severe erectile dysfunction in patients with antiphospholipid syndrome

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Purpose: To evaluate sexual function of antiphospholipid syndrome (APS) patients using the Brazilian version of the validated International Index of Erectile Function (IIEF).

Materials and methods: Eleven APS male patients (Sapporo criteria) were age and race-matched with 22 healthy controls. Demographic and clinical data, drug use and antiphospholipid antibodies were evaluated. The IIEF was also self-applied.

Results: Mean age ($p=0.114$), frequency of Caucasian race ($p=1.00$) and married status ($p=0.438$) were similar in APS and controls. Mean disease duration was 8.8 ± 4.6 years. Erectile dysfunction (ED) was frequently observed in APS versus controls (45.5 vs. 4.5%, $p=0.0096$), especially moderate/severe ED ($p=0.0081$). The total IIEF score (49.6 vs. 67.1, $p=0.019$), erectile function (19.6 vs. 28.1, $p=0.005$) and intercourse satisfaction (7.8 vs. 11.9, $p=0.009$) were lower in patients than in controls. No differences were seen in orgasmic function ($p=0.114$), sexual desire ($p=0.123$) or overall satisfaction ($p=0.097$) between the groups. The comparison between APS patients with ED ($n=5$) and without ED ($n=6$) revealed more arterial events in APS with ED (100 vs. 16.7%, $p=0.0152$), and also longer disease duration (12 [7–16] vs. 5.5 [2–13] years, $p=0.031$). A trend towards lower venous events (20 vs. 83.3%, $p=0.0801$) and higher renal thrombotic microangiopathy (60% vs. 0, $p=0.0606$) was observed in APS patients with ED. Demographics, clinical manifestations, smoking and antiphospholipid antibodies positivity were similar in both groups.

Conclusion: To our knowledge, this was the first study that demonstrated moderate/severe ED in almost 50% of cases of a rare autoimmune disease. This alteration was linked to arterial events and longer disease duration. *Lupus* (2011) 0, 1–5.

Key words: antiphospholipid syndrome; erectile dysfunction; impotence; *International Index of Erectile Function (IIEF) Brazilian version*

Introduction

Sexual dysfunction in men with rheumatic diseases has been reported.¹ In respect of antiphospholipid syndrome (APS), a rare rheumatic disease that is characterized by vascular events, it is logical to speculate that the impairment of the penile vascular system could result in symptoms of erectile dysfunction (ED). However, no studies have evaluated erectile function in subjects with this thrombophilia.

The objectives of this study were, therefore, firstly to investigate the presence of ED using a specific questionnaire in patients with APS and compare it with a control population matched for age and race, and secondly to evaluate its possible association with clinical aspects of the disease.

Materials and methods

We screened 320 patients; 300 were excluded because they were female. Nine out of the remaining 20 were excluded due to not living in a partnership situation ($n=2$) or use of drugs that could influence sexual function ($n=7$; sildenafil [$n=1$], tadalafil [$n=1$], amlodipine [$n=2$], captopril [$n=1$] and losartan [$n=2$]). For this study, 11

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APS patients aged over 18 were included (Figure 1). All patients fulfilled the international criteria for APS according to the Sapporo criteria² and were followed up at our Outpatient Clinic of the Rheumatology Division, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP). The control group was composed of 22 healthy men, matched for age and race.

The inclusion criteria for APS patients and controls were having a sexual partner and being more than 18 years old. Exclusion criteria were penile impairment, diabetes mellitus, thyroid diseases, previous or current history of alcohol, tobacco use or illicit drugs, and use of any drugs that could induce erectile dysfunction, such as antidepressants, beta-blockers, alpha-blockers, central adrenergic blockers, testosterone, sildenafil, tadalafil and vardenafil.

We collected demographic data and reviewed medical records, characterizing APS and previous events of arterial thrombosis, venous thrombosis, the presence of thrombocytopenia, duration of disease, and presence of cardiovascular and cerebrovascular events (angina, acute infarction and stroke). Prior comorbidities and medication use were also included.

The International Index of Erectile Function (IIEF) is a 15-item questionnaire that was developed in English and validated as an instrument to assess erectile function in the Brazilian Portuguese version that was used in this study.³ This instrument examines five domains or dimensions: erectile function (questions 1–5 and question 15), orgasmic function (questions 9 and 10), sexual desire (questions 11 and 12), intercourse satisfaction (questions 6, 7 and 8) and overall satisfaction (questions 13 and 14). Each scale ranges from 0 to 5, so that the maximum score that can be obtained is 75. Lower scores indicate poor sexual function.

Figure 1 Flow chart of the selection process for identification of male APS patients in our Outpatient Clinic.

An IIEF score greater than 25 is defined as normal erectile function, 21–4 as mild ED, 16–20 as moderate ED and lower than 15 as severe ED.³

The Local Ethics Committee approved this study and informed consent was obtained from all participants.

Statistical analysis

Results are presented as mean $\pm$ standard deviation or median (range) for quantitative variables and frequencies (percentages) for qualitative variables. Statistical analysis was performed using GraphPad software. The Student *t* or Mann–Whitney test was used to compare means and χ^2 or Fisher's exact test for frequencies. *p* values less than 0.05 were considered significant.

Results

APS patients versus controls

Demographic evaluation of APS patients and controls revealed comparable mean age (46.2 ± 9.4 vs. 42.3 ± 6.0 years, $p = 0.114$), frequency of Caucasian race (81.8% vs. 81.8%, $p = 1.00$) and married status (63.6% vs. 77.3%, $p = 0.438$). Nine (82%) had primary APS and two had secondary APS. Patients had a mean disease duration of 8.8 ± 4.6 years. A lower frequency of occupational activities was observed in APS patients versus controls (54.5% vs. 100%, $p = 0.002$) and also a higher frequency of physical inactivity (81.8% vs. 27.3%, $p = 0.003$).

ED was observed in APS more frequently than in the control group (45.5% vs. 4.5%, $p = 0.0096$). Moderate/severe ED was also more evidenced in APS than in controls (36.4% vs. 0, $p = 0.0081$). The total IIEF score was lower in patients than in controls (49.6 vs. 67.1, $p = 0.019$), as well as its components, such as erectile function (19.6 vs. 28.1, $p = 0.005$) and intercourse satisfaction (7.8 vs. 11.9, $p = 0.009$). No difference was demonstrated in orgasmic function (7.7 vs. 9.6, $p = 0.114$), sexual desire (7.1 vs. 8.3; $p = 0.123$) or overall satisfaction (7.4 vs. 9.1, $p = 0.097$) between the two groups (Table 1).

APS patients with ED versus those without ED

Arterial events were significantly higher in APS patients with ED ($n = 5$) than in those without ED ($n = 6$) (100% vs. 16.7%, $p = 0.0152$). Likewise, the median disease duration was significantly higher in APS patients with ED than in those without this sexual dysfunction (12 [7–16] vs. 5.5

[2–13] years, $p=0.031$). A trend towards lower venous events (20% vs. 83.3%, $p=0.0801$) and higher renal thrombotic microangiopathy (60% vs. 0, $p=0.0606$) was observed in APS patients versus controls. The other demographics, clinical manifestations, smoking and antiphospholipid

antibodies positivity were similar in both groups (Table 2).

Discussion

To the best of our knowledge, this is the first study to have identified the presence of moderate/severe ED in almost 50% of APS patients. This alteration was linked to arterial events and longer disease duration.

A great advantage of the present study was the exclusion of secondary causes for sexual dysfunction, such as metabolic conditions. In fact, it is well known that patients with diabetes mellitus often have impotence.⁴ Additionally, it was relevant to exclude subjects who were on drugs that may interfere with sexual function, such as anti-hypertensives,⁵ or medications that improve this function, such as tadalafil and sildenafil.^{6,7} Another benefit was the sole inclusion of patients who fulfilled the international criteria for APS,² forming a homogeneous group for analysis.

In contrast to other studies,⁸ we used a specific and standardized questionnaire to evaluate sexual function. The IIEF instrument is short and easy to

Table 1 Erectile dysfunction, severity and its components measured by IIEF of the 11 male APS patients and 22 healthy controls

	APS patients n = 11	Controls n = 22	p value
Erectile dysfunction, n (%)	5 (45.5)	1 (4.5)	0.0096
Mild	1 (9.1)	1 (4.5)	1.0
Moderate or severe	4 (36.4)	0	0.0081
Total score of IIEF	49.55 ± 22.41	67.14 ± 5.08	0.019
Erectile function	19.55 ± 10.36	28.14 ± 2.05	0.005
Orgasmic function	7.73 ± 3.50	9.59 ± 1.18	0.114
Sexual desire	7.09 ± 2.17	8.32 ± 1.49	0.123
Intercourse satisfaction	7.82 ± 4.96	11.95 ± 1.73	0.009
Overall satisfaction	7.36 ± 2.84	9.14 ± 1.17	0.097

Data expressed as mean (standard deviation) or percentage. Bold p values are significant. APS: antiphospholipid syndrome, IIEF: International Index of Erectile Function.

Table 2 Demographic data, clinical and laboratory features, and treatment of the five APS patients with and the six without erectile dysfunction

	APS with ED n = 5	APS without ED n = 6	p value
Demographic data			
Current age, years	52 (41–62)	43 (27–53)	0.073
Disease duration, years	12 (7–16)	5.5 (2–13)	0.0318
Caucasian race, n (%)	5 (100)	4 (66.6)	0.4545
Clinical and laboratory features, n (%)			
Arterial events	5 (100)	1 (16.66)	0.0152
Venous events	1 (20)	5 (83.33)	0.0801
Livedo reticularis	0	2 (33.33)	0.4545
Stroke	3 (60)	1 (16.66)	0.2424
Renal thrombotic microangiopathy	3 (60)	0	0.0606
Pulmonary thromboembolism	1 (20)	1 (16.66)	1.0
Deep venous thrombosis	1 (20)	4 (66.66)	0.2424
Dyslipidemia	1 (20)	0	0.4545
Previous history of smoking	1 (20)	1 (16.66)	1.0
Current smoking	0	3 (50)	0.1818
Systemic hypertension	2 (40)	1 (16.6)	0.5455
Angina	0	0	1.0
Laboratory features and treatment, n (%)			
Thrombocytopenia	0	3 (50)	0.1818
Lupus anticoagulant	4 (80)	3 (50)	0.5455
IgM anticardiolipin	1 (20)	4 (66.66)	0.2424
IgG anticardiolipin	1 (20)	2 (33.33)	1.0
Warfarin use	5 (100)	6 (100)	1.0

Data expressed as median (range) or percentage. Bold p values are significant. APS: antiphospholipid syndrome, ED: erectile dysfunction.

administer and understand. It is available in ten languages, including Portuguese,³ for use in clinical trials and has shown adequate sensitivity and specificity for detecting disorders related to erectile function in patients with ED. It has some limitations; for instance, it focuses on superficial erectile sexual response and evaluation of the limited partner relationship rather than investigating different aspects of sexual function or evaluating its components.³ It was developed with the aim of being a short, reproducible, culturally and psychometrically valid instrument, which can be used by physicians and researchers in clinical and therapeutic trials, as another parameter of evaluation of the efficacy/effectiveness of various interventions proposed today.³ Being a self-report, it may encourage imprecision in the results, but preserves intimacy and allows the individual to respond more comfortably to issues considered sensitive,⁹ for which self-reported questionnaires are preferred to interviews.³

Sexual dysfunction in men with rheumatic diseases has been reported.¹ In addition, ED in was found in 12% of ankylosing spondylitis (AS) patients¹⁰ and in none of our systemic lupus erythematosus patients without secondary APS.⁸ The IIEF was also applied to men with osteoporosis and 74% had ED, with scores lower than 22. The authors found ED in 41.4% of individuals aged 50 to 59 years, 30.3% of subjects aged 60 to 69 years and 16.3% in those aged 70 years or more. Patients with age greater than 70 years had severe ED, and those between 50 to 59 years had mild ED.¹¹ Moreover, a pilot study with AS (age between 29 and 48 years) showed significant improvement in four of the five domains of IIEF after 3 months of using anti-tumor necrosis factor (TNF)- α . The improvement in patients with ED was significant in three of five domains (erectile function, sexual satisfaction and sexual desire).¹²

ED in APS patients can be multifactorial, and the factors most associated with ED are age, disease activity and drug use.⁸ It is reasonable to speculate that a thrombotic mechanism may be implicated in the pathophysiology of this ED in APS. In accordance with this, there are some case reports showing thrombosis of penile vessels leading to ED.¹³ On the other hand, endothelial dysfunction represented by atherosclerosis plays a relevant role in ED. In fact, ED has been previously described in diabetic subjects with coronary artery disease.¹⁴

Interestingly, the ED observed in a large number of APS cases in this study was essentially moderate and severe. This relevant aspect was different in the

healthy population; indeed, mild, moderate and severe ED was demonstrated in 30.2%, 1.4% and 0.3% of the normal subjects, respectively.¹⁵

In summary, this study suggested that half of APS patients may have moderate/severe ED, evaluated by a specific questionnaire, and low intercourse satisfaction, and that this sexual dysfunction is associated with arterial events and long-term disease. One ongoing study from our group is currently evaluating penile vasculature, and possibly will add new knowledge in this field.

Funding

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Conflict of interest

None declared.

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APÊNDICE 3

Artigo "Morphofunctional penile alterations with severe sperm abnormalities in antiphospholipid syndrome associated with systemic lupus erythematosus"

Enviado em 28 de setembro de 2011 para Revista *Lupus*

28-Sep-2011

Dear Prof. Silva:

Your manuscript entitled "MORPHOFUNCTIONAL PENILE ALTERATIONS WITH SEVERE SPERM ABNORMALITIES IN ANTIPHOSPHOLIPID SYNDROME ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS" has been successfully submitted online and is being considered for processing.

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CONCISE ARTICLE

MORPHOFUNCTIONAL PENILE ALTERATIONS WITH SEVERE SPERM ABNORMALITIES IN ANTIPHOSPHOLIPID SYNDROME ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

Objective: To perform global gonadal and sexual function assessments in systemic lupus erythematosus related antiphospholipid syndrome (SLE-APS) patients.

Methods: A cross-sectional study was conducted in 10 SLE-APS male patients and 20 healthy controls. They were assessed by demographic data, clinical features, systematic urological examination, sexual function, testicular ultrasound, seminal parameters according to World Health Organization (WHO), seminal sperm antibodies and hormone profile including: follicle stimulating hormone (FSH), luteinizing hormone (LH), morning total testosterone and thyroid hormones.

Results: The median of current age was similar in SLE-APS patients and controls (36.9 vs. 32.4 years, $p=0.310$), with a higher frequency of erectile dysfunction in the former group (30% vs. 0%, $p=0.029$). Further analysis revealed that the median penis circumference was significantly reduced in SLE-APS patients with erectile dysfunction compared to patients without this complication [8.17 (8-8.5) vs. 9.14 (7-10.5) cm, $p=0.0397$]. In addition, SLE-APS patients with previous arterial thrombosis had a significantly reduced median penis circumference compared to those without this complication [7.5 (7-8) vs. 9.18 (8-10.5) cm, $p=0.039$]. Comparing SLE-APS patients and controls, the former had a significant lower median of sperm concentration (41.1 vs. $120.06 \times 10^6/\text{mL}$, $p=0.003$), percentages of sperm motility (47.25 vs. 65.42 %, $p=0.047$), normal sperm forms evaluated by WHO guidelines (11 vs. 23.95 %, $p=0.002$) and Kruger criteria (2.65 vs.

7.65 %, $p=0.02$). The median of FSH levels was significantly higher in SLE-APS group (9.73 vs. 3.32 IU/liter, $p=0.025$). Regarding seminal analysis, the median of sperm concentration and total sperm count were significantly lower in SLE-APS patients treated with intravenous cyclophosphamide *versus* untreated with this drug [6.87 (0-23.5) vs. 63.9 (7.5-145) $\times 10^6/\text{mL}$, $p=0.04$; 16.12 (0-55.5) vs. 226.25 (8.5-471) $\times 10^6/\text{mL}$, $p=0.035$, respectively].

Conclusions: We have identified that IVCYC underlies severe sperm alterations in SLE-APS and we also observed a novel association of reduced penile size with erectile dysfunction and previous arterial thrombosis in these patients.

Keywords: antiphospholipid syndrome, systemic lupus erythematosus, sperm, penile, fertility, antisperm antibodies.

INTRODUCTION

We identified severe sperm abnormalities associated with intravenous cyclophosphamide (IVCYC) use^{1,2} and reduced penile dimensions with no deleterious effect in erectile function in systemic lupus erythematosus (SLE) patients.³ In contrast, our group recently observed normal testicular function in primary antiphospholipid syndrome (PAPS) patients, in spite of morphofunctional penile abnormalities. Previous arterial thrombosis seemed to underlie this penile anthropometry alteration in PAPS.⁴ There is, however, no systematic study assessing the overall gonadal function in SLE-APS patients.

Therefore, the aim of this study was to perform global testicular and sexual function assessments in male SLE-APS evaluating its possible association with clinical and laboratorial parameters.

PATIENTS AND METHODS

SLE-APS patients and controls

We screened initially 38 male patients with APS without a medical history of hydrocele, hypospadias, cryptorchidism, testicular infection, testicular cancer, orchitis, testicular vasculitis, ureteral impairment, previous history of any scrotal or inguinal surgery, diabetes mellitus and tobacco use. None of the participants had history of alcohol consumption or heavy occasional drinking before or at study entry. Of the 38 patients, 28 patients were excluded due to previous vasectomy (n=3), refusal to collect sperm sample (n=9), PAPS (n=12) and APS associated with others rheumatic diseases (n=4) [polyarteritis nodosa (n=2), rheumatoid arthritis (n=1) and ankylosing spondylitis (n=1)]. Therefore, a cross-sectional study was conducted in 10 SLE-APS patients regularly followed at Antiphospholipid Outpatient Clinic of the Rheumatology Division of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo from May 2008 to February 2011. All patients fulfilled the Sapporo and American College of Rheumatology for APS⁵ and SLE diagnosis,⁶ respectively. The control group included 20 healthy subjects followed at the Pre-vasectomy Group of Urology Division of our University Hospital. All SLE-APS patients and controls were at

Tanner stage 5.⁷ The Local Ethics Committee approved this study and an informed consent was obtained from all participants

GLOBAL REPRODUCTIVE HEALTH EVALUATION

1. Demographic data and body mass index: Current age, age at spermatarche (first ejaculation) and race were recorded in SLE-APS patients and controls, while age at disease onset and disease duration in SLE-APS patients. Body mass index (BMI) was defined by the formula: BMI (Kg/m^2) weight in kilograms/height in meters.

2. Urologic evaluation and sexual function: A clinical assessment of the genitalia included evaluation of testicles, epididymis, *vas deferens*, scrotum and penis. These assessments were performed blinded to the results of the semen in SLE-APS patients and controls. Penis length was measured as linear distance along the dorsal side of penis extending from the pubopenile skin junction to the tip of the glands in the flaccid state, while penis circumference was measured at the middle of shaft.^{3,4} The evaluation of sexual function in patients and controls was acquired by the male sexual quotient, an self-administered questionnaire validated in Brazilian Portuguese.⁸

3. Testicular Doppler Ultrasound: Ultrasound was performed blinded to the results of the semen in SLE-APS patients and controls. The largest measurement in each dimension was recorded and used to calculate the testicular volume according to the formula for an ellipsoid (length X width X thickness X 0.52). The normal mean $\pm$ SD value in male post pubertal adolescents and adults is 7-23 ml.¹⁻⁴

4. Hormone evaluation: Hormone determinations performed at study entry in SLE-APS patients and controls, included: follicle-stimulating hormone (FSH), luteinizing hormone (LH), morning total testosterone, triiodothyronine (T3), tetraiodothyronine (T4), free tetraiodothyronine (free T4), and thyrotropin (TSH) detected by fluoroimmunoassay using DELFIA time-resolved fluoroimmunoassay kits (Wallac, 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), total morning testosterone (271–965 ng/dL), T3 (70–200 ng/dL), T4 (4.5–12 μ g/dL), free T4 (0.7–1.5 ng/dl) and TSH (0.4–4.5 μ U/mL).

5. Sperm evaluation: Sperm analysis was performed according to the guidelines of the World Health Organization (WHO)⁹ by two expert medical technologists. All SLE-APS patients and controls collected two semen samples with interval of 15-30 days after 48-72 hours of sexual abstinence. The spermatozoa were analyzed by manual hand count as well as by a computer-assisted semen analysis system under 400X magnification, using

an HTM-2030. Sperm morphology included evaluation of sperm head, neck, midpiece, and tail by WHO guidelines⁹ and Kruger strict criteria.¹⁰ Azoospermia was defined as no spermatozoa in the ejaculate and oligozoospermia as sperm concentration < 20 million/mL.⁹ The presence of antisperm antibodies was determined by direct Immunobead test using Immunobead^R rabbit anti-human Ig (IgA, IgG, and IgM) kits (Irvine Scientific, Santa Ana, CA, USA) in all patients and controls. At least 50% of the motile spermatozoa ("a" + "b") must be coated with Immunobeads before the test results are considered to be clinically significant. A negative control should have a score of < 10% bead attachment, and a positive control should have a score of > 20% bead attachment.⁹

6. Clinical and treatment evaluations: The following clinical parameters were evaluated in SLE-APS patients: venous thrombosis (documented deep vein thrombosis and/or pulmonary embolism), arterial thrombosis (clinically documented stroke, transient ischemic attacks or acute myocardial infarction), livedo reticularis and thrombocytopenia (< 100,000/mm³ platelets, at least in two distinct occasions). SLE disease activity and cumulative damage at the time of study entry were measured in all patients, using the SLE Disease Activity Index (SLEDAI)¹¹ and the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SLICC/ACR-DI), respectively.¹² Data concerning the therapy were evaluated.

7. Serum immunologic analysis. Anticardiolipin antibodies were detected by a commercial kit (Enzyme Immunoassay Kit, BINDAZYME™, Birmingham, UK) in all APS patients. The cutoff values were: < 11 GPL/MPL U/mL negative, ≥ 11 and < 20 GPL/MPL U/mL indeterminate and ≥ 20 GPL/MPL U/mL positive.¹³ Lupus anticoagulant (LAC) was detected according to international guidelines.¹⁴ Serum IgG and IgM anti-beta-2-glycoprotein I were detected by Enzyme-Linked Immunoabsorbent Assay (ELISA) technique (ORG 521 Anti-beta-2-Glycoprotein I IgG/IgM Mainz, Germany) with cutoff values of 8 U/mL for IgM and for IgG with intra-assay variation: 2.1-5.0%; inter-assay variation: 2.6-7.95 to IgG and intra-assay variation: 2.1-3.8%; inter-assay variation: 4.1-6.3 to IgM .

8. Statistical analysis: Data were compared by *t*-test or Mann-Whitney's test if numerical data presented normal or abnormal distribution, respectively, to evaluate differences between SLE-APS patients and controls, and according to previous arterial thrombosis and erectile dysfunction in SLE-APS patients. For categorical variables, differences were assessed by Fisher's exact test. *P* values less than 0.05 were considered significant.

RESULTS

SLE-APS patients versus controls

The demographic data, BMI, penile anthropometry, testicular volume by ultrasound, sperm analysis, antisperm antibodies and hormone evaluation

in SLE-APS patients and controls are shown in Table 1. The median of current age was similar in both groups [36.9 (21-57) vs. 32.4 (18-54) years, $p=0.310$]. In contrast, the median age of spermarche and BMI were significantly higher in SLE-APS patients *versus* controls [13.9 (12-16) vs. 12.85 (11-15) years, $p=0.016$; 26.9 (22-35.2) vs. 22.2 (18-27), $p=0.003$; respectively] (Table 1).

The frequency of erectile dysfunction was significantly higher in SLE-APS patients compared to controls (30% vs. 0%, $p=0.029$). The median right testicular volume by ultrasound was significantly lower in SLE-APS patients [10.38 (3.9-16.7) vs. 13.4 (8.5-20.6) ml, $p=0.03$]. No differences were observed in penis anthropometry and left testicular volume in both groups (Table 1).

The median of sperm concentration and sperm motility were significantly lower in SLE-APS patients compared to controls [41.1 (0-145) vs. 120.06 (34.5-329) $\times 10^6/\text{mL}$, $p=0.003$; 47.25 (0-87.5) vs. 65.42 (43-82) %, $p=0.047$, respectively]. Normal sperm forms evaluated by WHO guidelines and Kruger strict criteria were significantly lower in SLE-APS patients [11 (0-27) vs. 23.95 (10-45) %, $p=0.002$; 2.65 (0-8) vs. 7.65 (3-18) %, $p=0.02$, respectively]. The frequency of oligo/azoospermia was significantly higher in SLE-APS patients (40 % vs. 0 %, $p=0.007$). The median of FSH levels and frequencies of elevated FSH were significantly higher in SLE-APS group [9.73 (2.4-25) vs. 3.32 (1-5.9) IU/liter, $p=0.025$; 40 % vs. 0 %, $p=0.007$; respectively] (Table 1). All SLE-APS patients and controls had normal thyroid hormones.

SLE-APS patients

The median penis circumference was significantly lower in SLE-APS patients with (n=3) erectile dysfunction *versus* without (n=7) this alteration [8.17 (8-8.5) vs. 9.14 (7-10.5) cm, p=0.0397]. No differences were evidenced in the median penis length in both groups [10.83 (9-12.5) vs. 10.14 (7.5-13) cm, p=0.615]. SLE-APS patients with previous arterial thrombosis (n=2) had a significantly reduced median penis circumference than those without arterial events (n=8) [7.5 (7-8) vs. 9.18 (8-10.5) cm, p=0.039], whereas the median of length in both groups was comparable [8.25 (7.5-9) vs. 10.87 (8.5-13) cm, p=0.08]. Testicular volumes, sperm analysis, antisperm antibodies, hormone profile, thrombocytopenia, SLEDAI, SLICC/ACR-DI, IgM anticardiolipin, IgG anticardiolipin, IgM anti-beta2-glycoprotein I, IgG anti-beta2-glycoprotein I and lupus anticoagulant were alike in patients treated with IVCYC and those never under this treatment (p>0.05). None of the SLE-APS patients reported history of penile edema or pain.

IVCYC was used for lupus nephritis treatment in four patients. The median duration of time from last dose of IVCYC to study entry was 6 years (2-9). The median of disease duration was significantly higher in those treated with (n=4) IVCYC compared never under this drug (n=6) [9.92 (5-13) vs. 3.38 (0.3-7) years, p=0.028]. The median of sperm concentration and total sperm count were significantly lower in SLE-APS patients treated with IVCYC *versus* untreated with this drug [6.87 (0-23.5) vs. 63.9 (7.5-145) x 10⁶/mL, p=0.04; 16.12 (0-55.5) vs. 226.25 (8.5-471) x 10⁶/mL, p=0.035, respectively] while no difference was observed for the other sperm

parameters ($p>0.05$). Penis anthropometry, testicular volumes, antisperm antibodies, hormone profile, SLEDAI, SLICC/ACR-DI were alike in patients treated with IVCYC and those never under this treatment ($p>0.05$).

DISCUSSION

Herein we have demonstrated that IVCYC underlies severe sperm alterations in SLE-APS patients and we also observed for the first time an association of reduced penile size with erectile dysfunction and previous arterial thrombosis in these patients.

The advantage of the present study was the use of a global protocol and the inclusion of only post-pubertal subjects, assuring a more homogeneous group regarding gonadal and sexual evaluation. The systematic penile, gonadal and sexual assessments were carried out using established criteria for penis anthropometry,^{3,4} sperm and antisperm antibodies,^{1,2,9} and sexual function.⁸ The rigorous exclusion criteria of SLE-APS patients and controls without a medical history of genital diseases and comorbidities is relevant since these alterations may affect gonadal evaluation.¹⁵ The low number of patients and the cross sectional design are limitations of this study.

The penile development is influenced by race, hypothalamic-pituitary-testicular axis dysfunction and chronic diseases, as described in our SLE without APS and in PAPS patients.^{3,4} Of note, SLE-APS patients evaluated herein with previous arterial events had reduced circumference penile size,

suggesting the possibility that chronic subclinical endothelial dysfunction involving corpora cavernosa or penis arteries may result in atrophy and sexual alteration.¹⁶ These findings were also observed in our PAPS patients⁴ and we are currently carrying out penis pharmacologic ultrasound in lupus patients with APS to evaluate the reduced penis blood flow and intimal proliferation of penile vessels. Interestingly, none of the patients reported history of penile edema or pain, as also observed in PAPS patients.⁴

Erectile dysfunction occurred particularly in SLE-APS patients with reduced penile circumference, as also evidenced in our previous study evaluating PAPS patients.⁴ This sexual dysfunction is characterized by the inability of man to achieve and maintain an erection sufficient for satisfactory sexual performance.⁸ Sexual impotence of our SLE-APS patients may be linked to local subclinical endothelial cells dysfunction, and may cause psychological problems with a consequent reduction of health-related quality of life.⁴ Further studies, including the evaluation of efficacy and safety of phosphodiesterase type 5 (PDE-5) inhibitors in this population of autoimmune thrombosis, will be necessary.

Additionally, in our lupus patients with APS, we have confirmed our previous observation of sperm abnormalities, reduced of testicular volume and gonadal hormone alterations in SLE without this thrombophilic disorder.¹⁻³ Importantly, the use of cyclophosphamide seemed to contribute to severe semen alterations in our SLE-APS patients leading a persistent or long-lasting damage to primordial sperm cells, as also observed in our male SLE without this condition.¹ This study emphasizes the importance of sperm

cryopreservation for future assisted reproductive techniques before alkylating agents use.^{15,17}

Of note, no clear etiology for SLE-APS delayed spermarche was identified herein. The possible influence of disease or drug was suggested in our previous study in female juvenile SLE¹⁸⁻²⁰ and juvenile dermatomyositis²¹ patients with late occurrence of menarche in which disease occurred before first menstruation compared with normal Brazilian adolescents. This was not, however, the case in male SLE-APS since all of our patients had disease onset after spermarche.

In conclusion, we have identified that IVCYC is the major factor for severe and potentially permanent damage to the tests in SLE-APS patients. We also observed for the first time an association of reduced penile size with erectile dysfunction and previous arterial thrombosis in these patients.

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Table 1 - Demographic data and body mass index (BMI), penis anthropometry, testicular volume by ultrasound, sperm analysis, anti-sperm antibodies and hormone evaluation in systemic lupus erythematosus related antiphospholipid syndrome (SLE-APS) patients and healthy controls.

Variables	SLE-APS (n=10)	Controls (n=20)	P
Demographic data and BMI			
Current age, years	36.9 (21-57)	32.4 (18-54)	0.310
Age at disease onset, years	32.20 (14-57)	-	-
Disease duration, years	5.73 (0.3-13)	-	-
Age at spermarche, years	13.9 (12-16)	12.85 (11-15)	0.016
Caucasian race	4 (40)	7 (33)	1.0
BMI, Kg/m ²	26.9 (22-35.2)	22.2 (18-27)	0.003
Penis anthropometry and erectile dysfunction			
Length, cm	10.35 (7.5-13)	10.02 (8-13)	0.575
Circumference, cm	8.85 (7-10.5)	9.42 (7-11)	0.18
Erectile dysfunction	3 (30)	0	0.029
Testicular volume			
Right, mL	10.38 (3.9-16.7)	13.41 (8.5-20.6)	0.03
Left, mL	11.04 (7.2-17.2)	12.85 (6-21.2)	0.07
Sperm analysis			
Sperm volume, ml	2.95 ±1.16	1.66 ±1.28	0.35
Sperm concentration, x10 ⁶ /ml	41.1 (0-145)	120.06 (34.5-329)	0.003
Oligo/azoospermia	4 (40)	0	0.007
Total sperm count, x 10 ⁶	142.2 (0-471)	300.5 (41.3-1176)	0.06
Sperm motility, %	47.25 (0-87.5)	65.42 (43-82)	0.047
Normal sperm forms byWHO guidelines,%	11 (0-27)	23.95 (10-45)	0.002
Normal sperm forms by Kruger,%	2.65 (0-8)	7.65 (3-18)	0.02
Antisperm antibodies, %	17.2 (9-23)	16.9 (0-32)	0.92
Hormone evaluation			
FSH, IU/liter	9.73 (2.4-25)	3.32 (1-5.9)	0.025
Elevated levels	4 (40)	0	0.007
LH, IU/liter	6.87 (1.6-21.7)	3.49 (1.8-5.8)	0.074
Elevated levels	2 (20)	0	0.103
Morning total testosterone, ng/dl	434.3 (91-891)	552.5 (230-1078)	0.09
Lower levels	0	1 (5)	1.0

Values expressed in mean±SD, median (range) or n (%), n – number of patients and controls, WHO - World Health Organization, FSH - follicle-stimulating hormone (reference values 1-10.5 IU/liter), LH - luteinizing hormone (reference values 1-8.4) IU/liter, morning total testosterone (271-965 ng/dl).