

10.

Conclusiones

10 CONCLUSIONES

10.1 Estudio clínico del hombre seropositivo al VIH-1 que acude a un Centro de Reproducción Asistida

El estudio clínico andrológico del hombre seropositivo al VIH-1 está plenamente justificado. La presencia de anticuerpos anti-VIH-1 en sangre en hombres que desean tener hijos ha dejado de tener como única solución la utilización de semen de banco.

Es necesario hacer una importante labor informativa para que estos hombres acudan a centros de reproducción especializados y eviten realizar coitos no protegidos.

10.2 Calidad seminal del hombre seropositivo al VIH-1

Los pacientes seropositivos al VIH-1 presentan una disminución significativa del volumen seminal ($P = 0,04161$), del porcentaje de movilidad traslativa ($P = 0,00002$) y del porcentaje de espermatozoides normales ($P = 0,00001$) respecto a un grupo de candidatos a donantes de semen.

Se observa una disminución significativa del volumen seminal ($P = 0,0369$) y del porcentaje de espermatozoides normales ($P = 0,0291$) en el grupo de pacientes que han recibido terapia antirretroviral respecto a los que no.

A pesar de las alteraciones encontradas, la mayoría de los pacientes mantienen una calidad seminal suficiente para beneficiarse de técnicas de IA.

10.3 Lavado de semen de hombre seropositivo al VIH-1 para su utilización en técnicas de reproducción asistida

La técnica de doble lavado empleada en este estudio presenta una baja tasa de recuperabilidad espermática (6,8%). Es necesario partir de una muestra original de buena calidad para recuperar suficientes espermatozoides móviles que permitan realizar una IA con posibilidades reales de gestación.

10.4 Determinación del RNA y DNA del VIH-1 en semen lavado con la técnica de PCR

Los datos presentados en este trabajo demuestran que el 96,4% de las muestras son útiles para el uso en técnicas de reproducción asistida. El hecho de que se detecte un 3,6% de positivos hace insistir más si cabe en que tan solo deben utilizarse para reproducción asistida las muestras una vez analizadas por PCR, recomendación que también realizan otros autores (Chrystie *et al.*, 1998).

10.5 Aplicación de técnicas de reproducción asistida en parejas con hombre seropositivo al VIH-1

La IA en ciclo estimulado con semen lavado y testado para el VIH-1 es una técnica eficaz para aplicarla a parejas serodiscordantes (hombre seropositivo) que desean tener hijos genéticamente propios. Se consiguen altas tasas de gestación por ciclo (26,3%) y se reduce la probabilidad de contagio de la pareja respecto a la práctica de coito no protegido.

Aplicando la técnica de IA, los hombres en un estadio más avanzado de la enfermedad (grupos A3, B3, C1, C2 y C3 del CDC) presentan una

probabilidad menor de gestación estadísticamente significativa respecto a los pacientes de los grupos A1, A2, B1 y B2 (30,2% vs 18,5, $P < 0,01$).

La técnica de FIV-ICSI con semen lavado, congelado y testado para la presencia de RNA y DNA viral del VIH-1 es una técnica útil que permite solucionar los problemas de esterilidad masculina a los pacientes seropositivos al VIH-1. La técnica de FIV-ICSI puede aplicarse como recurso en los pacientes en los que la IA haya fallado y a mujeres que presenten obstrucción tubárica.

Las tasa de embarazo aplicando FIV-ICSI en estos pacientes es alta (49,2%/ciclo). La mayoría de los ciclos de FIV-ICSI son por causa masculina y las mujeres no presentan problemas de esterilidad. Aunque el número de gestaciones es aun pequeño, es necesario ajustar más el número de embriones a transferir en estas parejas para reducir la tasa excesivamente alta de partos gemelares (45,4%).

11.

Agradecimientos

11 AGRADECIMIENTOS

Este trabajo no habría sido posible sin el esfuerzo personal, científico y humano de un amplio grupo de personas. Agradezco a todos los que han intervenido directa o indirectamente permitiéndome ser portavoz de los estudios aquí presentados. No quisiera desaprovechar este apartado sin hacer constancia explícita de cada persona que ha contribuido a la conclusión de estas páginas.

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Los infectólogos nos han ayudado mucho en la comprensión y el trato de esta enfermedad y quiero agradecer el asesoramiento recibido del Dr. Clotet y del Dr. Gatell en el desarrollo de este estudio.

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12.

Anexos

12 ANEXOS

12.1 ANEXO I. CONSENTIMIENTO INFORMADO

El Sr.....con DNI nº.....

y la Sra.....con DNI nº.....

por el presente escrito manifiestan:

- 11) Que el Sr. es seropositivo al VIH y
la Sra. es seronegativa al VIH.
- 21) Que desean tener hijos y solicitan se les efectue Inseminación Artificial con semen del marido.
- 31) Que conocen que el semen se procesará o lavará en el laboratorio para aislar los espermatozoides móviles y que será sólo esta fracción la que se utilizará para la Inseminación.
- 41) Que antes de efectuar la Inseminación se estudiará si contiene o no el VIH-1 (Virus del SIDA).
- 51) Que la mujer ha de hacerse un análisis de sangre para investigar la presencia del VIH-1 el mismo día de la inseminación.
- 61) Que sólo se efectuará la Inseminación si el estudio del VIH-1 da negativo, tanto en la sangre de la mujer como en la fracción de semen que se va a utilizar para la Inseminación.
- 71) Que la mujer se compromete a repetir el análisis de VIH-1 en sangre al mes de la Inseminación haya quedado o no gestante.
- 81) Que las técnicas utilizadas para lavado de semen y estudio del VIH-1 en el semen una vez lavado no son 100% seguras. La pareja por tanto asume el riesgo de que la mujer se contagie de VIH-1 mediante la Inseminación si bien dicho riesgo se considera mínimo, claramente inferior a que efectuasen coito sin preservativo.
- 91) Si la mujer no se hace el análisis de VIH-1 en sangre al mes de la Inseminación, haya quedado o no gestante un posible contagio de la mujer al VIH-1 no podrá ser atribuido a la Inseminación.
- 101) Que la práctica de la Inseminación no supone que la mujer vaya a quedar gestante. Pueden requerirse varios ciclos de inseminación y puede que no se consiga el embarazo. Ello va en función del grado de fertilidad de la pareja.
- 111) Que conocen las repercusiones legales, éticas y biológicas y económicas de la citada técnica.
- 121) Que han sido informados con toda claridad, y han entendido, todo el proceso. En prueba de ello libremente y con total conocimiento firman este documento.

Barcelona, a de de

Fdo.

Fdo.

12.2 ANEXO II. FORMULACIÓN DEL MEDIO HTF

Componente	(mM)
Cloruro Sódico	101,6
Cloruro Potásico	4,69
Sulfato Magnésico, Anhídrido	0,20
Fosfato Potásico	0,37
Cloruro Cálcico, Anhídrido	2,04
Bicarbonato Sódico	25,0
Glucosa	2,78
Piruvato Sódico	0,33
Lactato Sódico	21,4
Penicilina G	100 U/mL
Estreptomina Sulfato	50 mg/mL
Rojo Fenol	10 mg/L

12.3 ANEXO III. MATERIAL PARA LA CUANTIFICACIÓN DE RNA Y DETECCIÓN DE DNA PROVIRICO DEL VIH-1

Material Inventariable

Autoclave. Termociclador Perkin-Elmer 9600. Termociclador Minicicler MJ. Nevera. Microcentrífuga. Cámara de flujo. Estufa o incubador de placas. Lector de placas. Vórtex. Congelador. Lavador automático. Baño de hielo.

Material Fungible y Reactivos

Kit de PCR-HIV Monitor de Roche. Kit de PCR de DNA provírico de Roche. Kit de extracción de DNA de Roche. Kit de visualización de resultados de DNA de Roche. Tubos eppendorf de 1,5; 0,5; y 0,2 mL. Puntas de pipeta de PCR de 100-1000 μ L; de 10-100 μ L; y de 1-20 μ L. Puntas de pipeta normales de 100-1000 μ L y de 10 -100 μ L. Guantes. Isopropanol frío. Etanol al 70%. Aceite Mineral.

12.4 ANEXO IV. CONDICIONES DE RECOGIDA DE SEMEN

- 1.- Guardar abstinencia sexual entre tres (3) y cinco (5) días. Esto incluye no efectuar coito ni masturbación ni haber tenido eyaculación en cualquier circunstancia, durante dichos días.
- 2.- No haber aplicado pomadas en el pene, al menos ocho (8) horas antes de recoger el semen.
- 3.- Recoger el semen por masturbación.
- 4.- Recoger TODO el semen. Si se pierden algunas gotas o se derrama, no traerlo al Laboratorio. Volver a recogerlo en OTRO frasco, guardando el periodo de abstinencia mencionado (entre 3 y 5 días).
- 5.- Recoger SOLO en el frasco que se da para ello. No es válido en preservativo.
- 6.- Traerlo al Laboratorio dentro de la primera hora después de recogerlo.
- 7.- Traer el frasco en posición vertical para no volcar su contenido.
- 8.- En días fríos, evitar que el semen recogido se enfríe demasiado: traerlo en bolsillo o pegado al cuerpo, pero no colocarlo cerca de fuentes de calor.

12.5 ANEXO V. MATERIAL INVENTARIABLE PARA FIV-ICSI

EQUIPO	MARCA	MODELO
BALANZA	SARTORIUS	BP210D
BAÑO	SELECTA	PRECIST-TERM
CALIENTATUBOS	MINITUBE	MULTIBLOK-HEATER LAB-LINE
CAMPANA FLUJO LAMINAR	TELSTAR	BH-100
CAMPANA FLUJO LAMINAR	TELSTAR	MICRO-H
CENTÍFUGA	JOUAN	C1000-55
CENTRÍFUGA	KUBOTA	5100
CRIOCONGELADOR BIOLÓGICO	PLANER	KRYO10 SERIES: AUTOSEEDER AS-1D CONTROLLER 10-20 PLANER LP4-C
CRIOCONGELADOR BIOLÓGICO	PLANER	KRYO10 SERIESIII PLANER LNP4-C
ESTIRADOR	NARISHIGE JAPAN	PB-7
ESTUFA	MERMMET	BM200
IMPRESORA	STAR	XB24-200 MULTIFONT
INCUBADOR	FORMA SCIENTIFIC	3194
INCUBADOR	FORMA SCIENTIFIC	STERI-CULT
MICROFORJA	NARISHIGE JAPAN	MF-9
MICROMANIPULADOR	NARISHIGE JAPAN	
MICROSCOPIO OPTICO	NIKON	ALPHAPHOT-2 YS2
MICROSCOPIO ESTEREOSCOPICO	NIKON	SMZ-2B
MICROSCOPIO ESTEREOSCOPICO	NIKON	SMZ-2T
MICROSCOPIO INVERTIDO	NIKON	DIAPHOT-300
MONITOR TV	SONY	KX-14CP1
OSMÓMETRO	FISKE ONE-TEN OSMOMETER	617-320-5656
PHMETRO	BECKMAN	PHL METER
PLACA CALENTADORA	MINITUB	GMBH
PLACA CALEFACTORA	SELECTA	PLACTRONIC CD6156100
PLETINA CALEFACTORA	LINKAM SCIENTIFIC	CO102
PLACA CALEFACTORA	MINITUB	MULTIBLOK-HEATER GT200
PURIFICADOR	MILLIPORE	MILLI-Q PF PLUS
PURIFICADOR	MILLIPORE	MILLI-RO PLUS 12
VIDEOIMPRESORA COLOR	SONY	CVP-M1E
VIDEO RECORDER	SONY	SL-HF-950 PAL/SECAM
VORTEX	HEIDOLPH	REAX 2000

12.6 ANEXO VI. MATERIAL FUNGIBLE PARA FIV-ICSI

TIPO	DESCRIPCIÓN	MARCA	REFERENCIA
CÁNULAS	MANDRIN	WALLACE	1816NST
CÁNULAS	TRANSFERENCIA	WALLACE	1816N
CÁNULAS	TRANSFERENCIA	LABOTECT	320200
CÁNULAS	TRANSFERENCIA	ROCKET	R57570
CÁNULAS	TRANSFERENCIA	FRYDMAN	
CAPILARES	BOROSILICATO 100 MM	CLARK EI	GC100T-10
CAPILARES	VIDRIO	DRUMMOND	1-000-0300
CONGELACIÓN	Propilenglicol (PROH)	SIGMA	P1009
CONGELACIÓN	PAJUELAS 0,5 ML	IMV	
CONGELACIÓN	Glicerol 99%	SIGMA	G2025
DESTILADOR	Pastillas de cloro	MILLIPORE	ZWCLO1F50
DESTILADOR	MILLIPAK-40	MILLIPORE	MPGL04SK2
DESTILADOR	PREFILTRO	MILLIPORE	CDPRM1206
DESTILADOR	KIT RO-PAK SQ1	MILLIPORE	CPROPO4S1
DESTILADOR	KIT Q-PAK	MILLIPORE	CPMQOO4R1
DESTILADOR	SAL / SACO 25 Kg	REGENERANTE	REGENIA
FILTROS	STERIVEX GS / 0,22 µM	MILLIPORE	SVGS01015
FILTROS	MILLEX AA / 0,8 µM	MILLIPORE	SLAA025BS
FILTROS	MILLEX GV / 0,22 µM	MILLIPORE	SLGV025BS
FILTROS	STERIVEX GS / 0,22 µM CAMPANA	MILLIPORE	SVGSB1010
FILTROS	MILLEX HV / 0,45 µM	MILLIPORE	SLHV025LS
FRASCOS	260 ML	NUNC	153732
FRASCOS	1900 ML	FALCON	353099
FRASCOS	2000 ML ROLLER	FALCON	353007
FRASCOS	800 ML	NUNC	156502
JERINGAS	50 ML TRES CUERPOS	BECTON-DIKINSON	300865
JERINGAS	10 ML TRES CUERPOS	BECTON-DIKINSON	
JERINGAS	1 ML TRES CUERPOS	ICO	
JERINGAS	1 ML TRES CUERPOS / AGUJA 25 G	ICO	
LIMPIEZA	JABÓN 7X	CHEMETRON	
MICROPIPETA	DENUDACIÓN	COOK	KFPIP-1140-5
MICROPIPETAS	ICSI 30º	COOK	KMPIP-1035
MICROSCOPIO	PORTAS 76 X 26 MM	MENZEL-GLÄSER	01.1101
MICROSCOPIO	PORTAS ESMERILADOS 76 X 26 MM	MENZEL-GLÄSER	
MICROSCOPIO	CUBRES 24 X 24 MM	MENZEL-GLÄSER	
MISCELANIA	PAPEL BALANZA 100 X 100	SCHLEICHER & SCUEL	347893
MISCELANIA	ESPARADRAPO HIPOALÉRGICO 2,5 CM	MICROPORE (3M)	1530-1
MISCELANIA	NITRÓGENO LÍQUIDO		
MISCELANIA	PARAFILM	AMERICAN NTL	
MISCELANIA	PAPEL FOTO COLOR	SONY	UPC-1010
MISCELANIA	BOMBONA CO2 PURO 4.0		
MISCELANIA	BOMBILLA LUPA 20V 150W	PHILLIPS	14501
MISCELANIA	BOMBILLA MICROSCOPIO		
MISCELANIA	PAPEL INDICADOR PH	MERCK	1.09557.001
OSMÓMETRO	ESTÁNDAR 850 MOSM	FISKE	00A085
OSMÓMETRO	ESTÁNDAR 50 MOSM	FISKE	00A005
OSMÓMETRO	ESTÁNDAR 290 MOSM	FISKE	00A030

12. Anexos

OSMÓMETRO	TUBOS DE MUESTRA Y ALGODONES	FISKE	110825
PHMETRO	TAMPÓN pH 4,0	CRISON	23-110-02-1
PHMETRO	TAMPÓN pH 7,02	CRISON	23-111-02-1
PHMETRO	SOL. CARGA ELECTRODO 4M KCl	BEKMAN	566467
PHMETRO	SOL. LIMPIEZA ELECTRODO	BEKMAN	566576
PIPETAS	1 ML	FALCON	357521
PIPETAS	2 ML	FALCON	357507
PIPETAS	5 ML	FALCON	357543
PIPETAS	10 ML	FALCON	357551
PIPETAS	25 ML	FALCON	357525
PIPETAS PASTEUR	LARGAS 23 CM	BRAND	7477-20
PIPETAS PASTEUR	CORTAS 15 CM	BRAND	7477-17
PLACAS	60 X 15 MM GRANDES	NUNC	150288
PLACAS	50 X 9 MM MICROMANIPULACIÓN	FALCON	351006
PLACAS	60 X 15 MM GRANDES POZO CENTRAL	FALCON	353037
PLACAS	60 X 15 MM GRANDES	FALCON	353004
PLACAS	35 X 10 MM PEQUEÑAS	NUNC	153066
PLACAS	35 X 15 MM PEQUEÑAS	FALCON	353001
PUNTAS	100 µL FINNTIPS CON FILTRO	LABSYSTEMS	94052200
REACTIVOS	HEPES 1 M	GIBCO BRL	15630-056
REACTIVOS	Rojo fenol	SIGMA	P5530
REACTIVOS	Aceite Mineral	SIGMA	
REACTIVOS	Hidróxido sódico (NaOH) 1 N	SIGMA	1.09557.001
REACTIVOS	Estreptomina (sulfato)	SIGMA	S1277
REACTIVOS	Glucosa (D+)	SIGMA	G7021
REACTIVOS	Hialuronidasa type IV	SIGMA	H3884
REACTIVOS	Polivinilpirrolidona (PVP) 60.000	ICN	102787
REACTIVOS	Polivinilpirrolidona (PVP) 40.000	SIGMA	PVP-40
REACTIVOS	Sacarosa	SIGMA	S1888
REACTIVOS	Penicilina G (sódica)	SIGMA	P3032
REACTIVOS	Cloruro magnésico	SIGMA	M2393
REACTIVOS	Sulfato magnésico	SIGMA	M1810
REACTIVOS	Fosfato potásico	SIGMA	P5655
REACTIVOS	Cloruro potásico	SIGMA	P5405
REACTIVOS	Cloruro cálcico	SIGMA	C7902
REACTIVOS	Bicarbonato sódico	SIGMA	S5761
REACTIVOS	Albúmina bovina type V (BSA)	SIGMA	A3156
REACTIVOS	Albúmina humana 5% (HSA)	GRIFOLS	860684
REACTIVOS	Ácido pirúvico (Piruvato sódico)	SIGMA	P5280
REACTIVOS	Ácido láctico (Lactato sódico)	SIGMA	L4263
REACTIVOS	Metanol	HISTOKIMIA	105
REACTIVOS	Cloruro sódico	SIGMA	S5886
SEGURIDAD	MASCARILLAS	DISPOTEX	13.01.00-00
SEGURIDAD	CONTENEDORES RESIDUOS		
SEGURIDAD	GORROS		
SEGURIDAD	GUANTES LÁTEX 61/2 SIN TALCO	PROFEEL	
SEGURIDAD	MASCARILLAS AZULES	ASEPTEX (3M)	
SEGURIDAD	GUANTES VINILO 6-7		
TUBOS	10 ML Fondo cónico	NUNC	347856
TUBOS	5 ML Fondo redondo	FALCON	352003

12. Anexos

TUBOS	10 ML Fondo redondo	FALCON	352001
TUBOS	15 ML Tapón azul, Fondo cónico	FALCON	352095
TUBOS	50 ML Tapón azul, Fondo cónico	FALCON	352074

13.

Abreviaturas

13 ABREVIATURAS

AIDS	<i>Acquired immune deficiency syndrome</i>
ARC	<i>AIDS Research Center</i>
ARV	<i>AIDS-associated retroviruses</i>
AZT	Zidovudina
BOE	Boletín Oficial del Estado
CDC	<i>Center for Disease Control</i>
CRSS	<i>Consortium for Retrovirus Serology Standardization</i>
CS	Calidad seminal
DNA	Ácido desoxiribonucleico
DGP	Diagnóstico genético preimplantacional
DTT	Dithiothreitol
EC	Estándar de cuantificación
ELISA	<i>Enzyme-linked immunoabsorbent assay</i>
EIA	<i>Enzyme immuno assay</i>
EOR	Especies oxígeno reactivas
ETS	Enfermedades de transmisión sexual
FDA	<i>US Food and Drug Administration</i>
FIV	Fecundación <i>in vitro</i>
FSH	Hormona folículo estimulante
HAART	<i>Highly active antiretroviral therapy</i>
HIS	Hibridación <i>in situ</i>
HSG	Histerosalpingografía
HLA	<i>Human leukocyte antigen</i>
HTF	<i>Human tubal fluid</i>
HTLV	<i>Human T-cell leukemia virus</i>
IA	Inseminación artificial
IAC	Inseminación artificial conyugal
IAD	Inseminación artificial con semen de donante
IA-IU	Inseminación artificial intrauterina

ICSI	<i>Intracytoplasmic Sperm Injection</i>
IFI	Inmunofluorescencia indirecta
IN	Integrasa
IP	Inhibidores de la proteasa
ITIn	Análogos de nucleósidos inhibidores de la transcriptasa inversa
ITInn	Inhibidores no nucleósidos de la transcriptasa inversa
LAV	<i>Lymphadenopathy-associated virus</i>
LH	Hormona luteinizante
LIA	<i>Lineal immuno-enzimatic assay</i>
MAR	<i>Mixed antiglobulin reaction</i>
MET	Microscopía electrónica de transmisión
NCS	No calidad seminal
OMS	Organización Mundial de la Salud
OR	<i>Odds ratio</i>
PCR	<i>Polymerase chain reaction</i>
PESA	<i>Percutaneous Epididymal Sperm Aspiration</i>
PNT	Protocolo normalizado de trabajo
PR	Proteasa
PVP	Polivinil pirrolidona
PZD	<i>Partial Zona Dissection</i>
RIPA	<i>Radio immuno-precipitation assay</i>
RNA	Ácido ribonucleico
RNase H	Ribonucleasa H
RPR	<i>Rapid plasma reagin</i>
RT	Retrotranscriptasa
SIDA	Síndrome de inmunodeficiencia adquirida
SUZI	<i>Subzonal Injection</i>
TRA	Técnicas de reproducción asistida
UDI	Uso de drogas inyectadas
UDVP	Uso de drogas por vía parenteral
VHC	Virus de la hepatitis C

13. Abreviaturas

VIH-1	Virus de la inmunodeficiencia humana tipo-1
VIH-2	Virus de la inmunodeficiencia humana tipo-2
WB	<i>Western blot</i>

14.

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