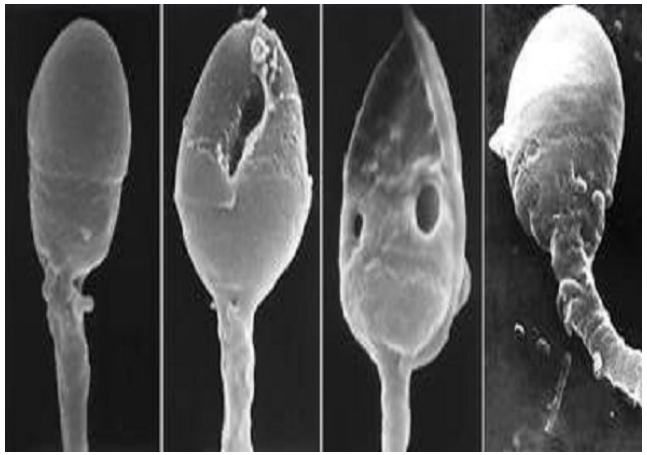
UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI MEDICINA
UOC Andrologia e Medicina della Riproduzione
Centro Regionale Specializzato di Crioconservazione dei Gameti Maschili
Centro Regionale Specializzato per la sindrome di Klinefelter
Direttore: Prof. Carlo Foresta

Preservare la fertilità maschile

Pierfrancesco Palego
U.O.C. Andrologia
e Medicina della Riproduzione



Regione del Veneto

giunta regionale

- ALLEGATO E) -

COMITATO CONSULTIVO REGIONALE DI BIOETICA

Allegato alla dgr

Parere del Comitato sul tema della n. 126 del 26 GEN. 2001

"CRIOCONSERVAZIONE DEI GAMETI MASCHILI"

Il Comitato riconosce l'importanza di curare l'infertilità maschile e/o di coppia, o di prevenirla nel caso di interventi che possano comprometterla, attraverso la pratica della crioconservazione del seme maschile. La sua attuazione può essere ottenuta con costi contenuti ed efficaci risultati. Ne consegue che la crioconservazione del seme maschile può essere opportunamente posta a carico del Servizio Sanitario Regionale.

1. **Criteri di accesso:** si ritiene eticamente riconoscibile il diritto all'accesso ai seguenti soggetti:

   a) soggetti in trattamento di Procreazione Assistita nell'ambito del Servizio Sanitario Regionale;

   b) soggetti che siano in procinto di sottoporsi a terapie potenzialmente dannose per la funzione riproduttiva.

4. **Accesso da parte di minori:** il Comitato rileva che non vi sono ostacoli di natura etica perché il minore possa richiedere ed ottenere il prelievo dei gameti in previsione di terapie potenzialmente dannose per la funzione riproduttiva. Al di sotto del sedicesimo anno di età va richiesto il consenso di chi esercita la potestà genitoriale.

abbiano compiuto, nel limite minimo, il quindicesimo anno di età e, nel limite

Associazione Italiana di Oncologia Medica

Società Italiana Endocrinologia

SIGO
SOCIETA' ITALIANA
DI GINECOLOGIA E OSTETRICIA

RACCOMANDAZIONI AIOM- SIE - SIGO SU ONCOFERTILITÀ

2. Ogni giorno in Italia vengono diagnosticati almeno 30 nuovi casi di tumore in pazienti di età inferiore ai 40 anni, pari al 3% della casistica generale.

4. I trattamenti antiblastici quali chemioterapia, radioterapia e terapie biologiche, sebbene abbiano, da una parte, migliorato significativamente la sopravvivenza dei pazienti affetti da tumore, dall'altra sono associati a un elevato rischio di infertilità temporanea o permanente. Tale tasso d'infertilità è variabile e dipende da molteplici fattori quali: tipo della neoplasia, classe, dose e posologia del farmaco impiegato, estensione e sede del campo di irradiazione, dose erogata e suo frazionamento, età e sesso del paziente, anamnesi di pregressi trattamenti per infertilità.

USA:

- > 1 milione di sopravvissuti da HL, NHL, Leucemie;
- 130.000 nuovi casi annui;
- sotto i 50y predominano HL (64%) e ALL (75%); con % curative rispettivamente di 80% e 40-90%;
- 17-20% di NHL e AML in paz sotto i 50y con % cura attorno al 60%;
- HSCT/BMT: nel 2009 109.000 sopravvissuti, proiezione per il 2020 di 242.000 (circa 2% di tutti i “cancer survivors”) e il 70% va incontro al trapianto (allogenico o autologo) sotto i 40y; fertilità post-trattamento tra 3 e 8%.

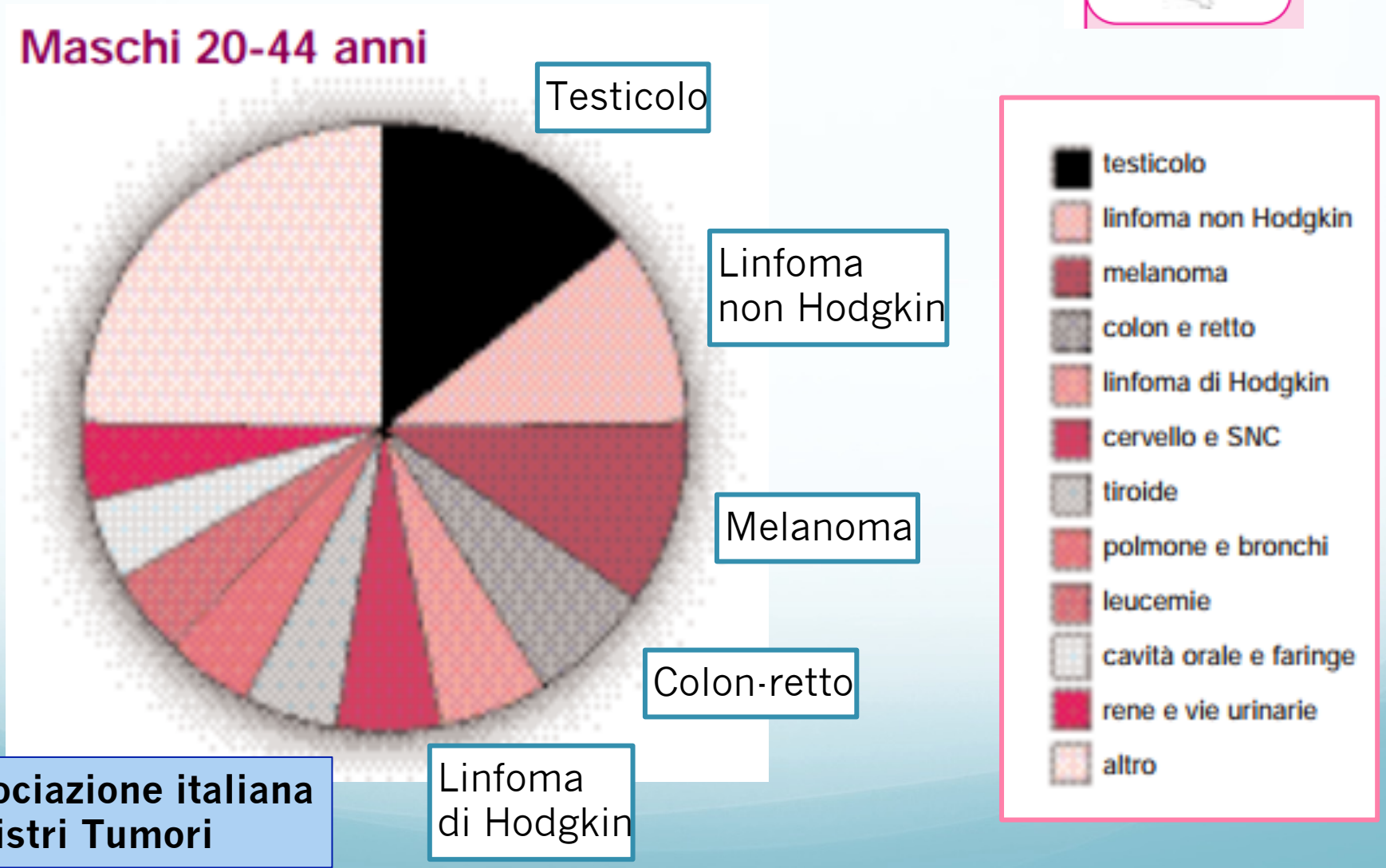
Carter et al, Bone Marrow transplantation 2006
Loren AW, Am Soc Haematology 2015
Leader A et al, BJH 2011

I tumori più frequenti in età 20-39 anni. Maschi.

Casi annui stimati in Veneto nel 2014 e % sul tot. tumori 20-39 anni.

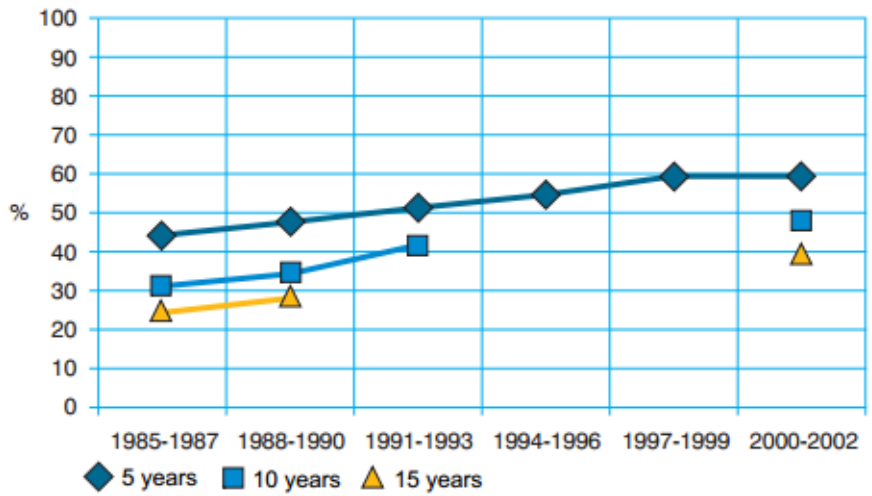
SEDE	Casi/anno	% sul tot tumori
Testicolo	88	22.3%
Cute melanomi	53	13.5%
Tiroide	35	8.9%
Linfoma di Hodgkin	34	8.5%
Linfomi non Hodgkin	28	7.2%
Totale eccetto pelle	395	100%

I tumori più frequenti nei giovani adul

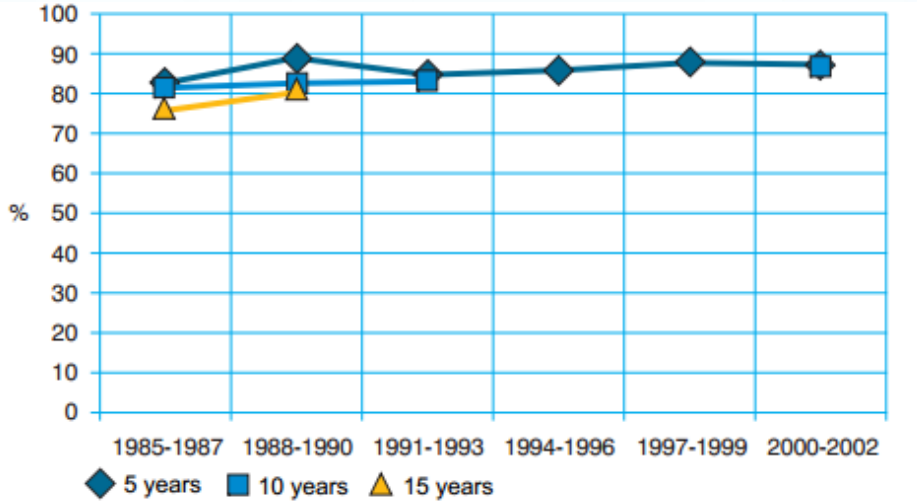


Associazione italiana Registri Tumori

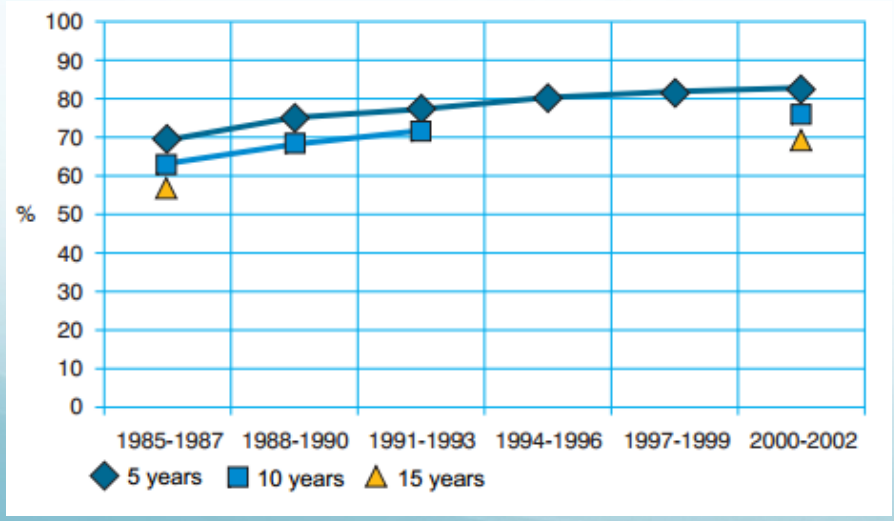
Sopravvivenza per tumore in Itali



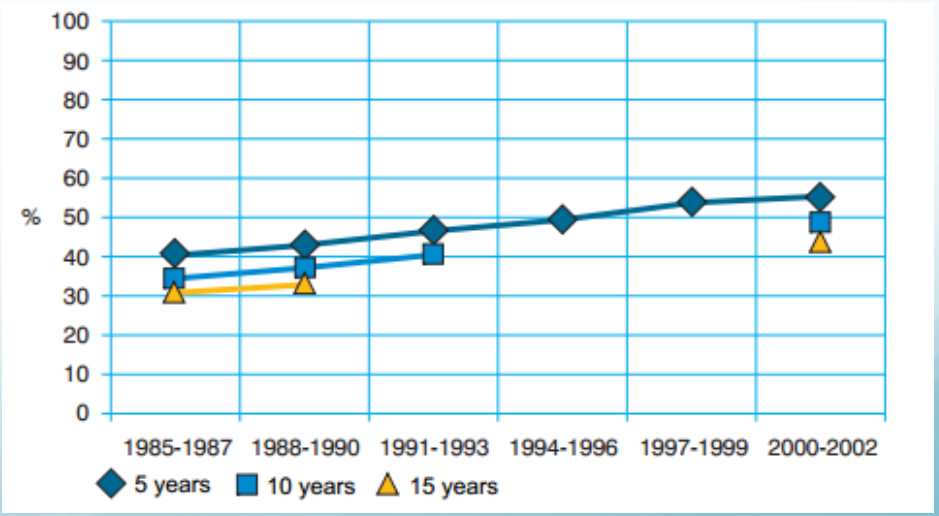
Linfomi non Hodgkin



Tumori del testicolo



Linfomi di Hodgkin

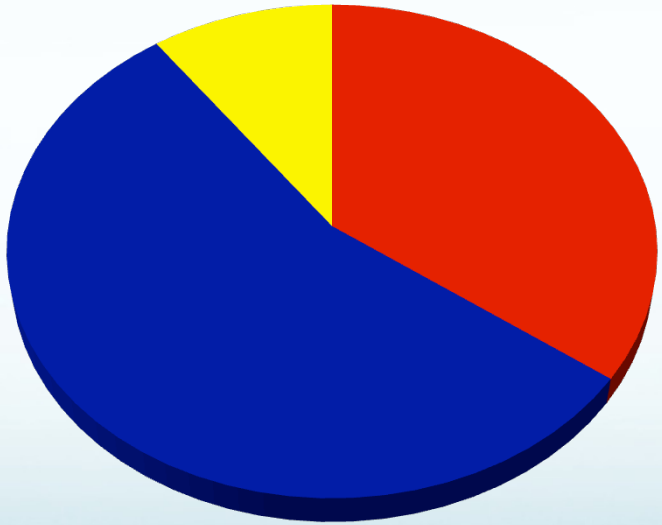


Tutti tumori

CRIOCONSERVAZIONI TOTALI
N. 5416

Altre patologie 10%

Infertilità 35%



Tumori 55%

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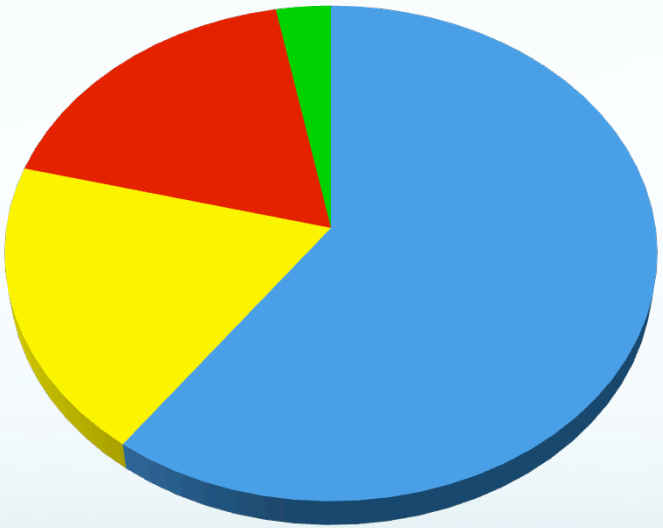
Distribuzione tumori alla crioconservazione

Linfoma non Hodgkin 3%

Altri Tumori 18%



Linfoma
di Hodgkin 19 %

Tumori del testicolo 60%

U.O.C. Andrologia e Medicina della Riproduzione

Preservare la fertilità: l'informazione negata - Dossier ProFert, 2012 (Milano-Roma-Bologna-Napoli)

roduttiva (…), ma è davvero questo che succede in Italia, nella realtà quotidiana? Una indagine condotta nei principa

acità futura di procreare, ci si accorge di come **i pazienti, sia uomini che donne, non abbiano a disposizione tutti**

della vita (dunque anche la riproduzione) dopo la guarigione. **Persino in quegli ospedali dove la preservazione d**

logia stessa, è un elemento di speranza per i pazienti e per i loro familiari, e rappresе

bjh review

Fertility considerations and preservation in haemato-oncology patients undergoing treatment

Avi Leader,[1] Michael Lishner,[1,2] Jennia Michaeli[3] and Ariel Revel[3]

[1]Department of Medicine A, Meir Medical Centre, Kfar Saba, [2]Sackler Faculty
Obstetrics and Gynaecology, Hadassah Hebrew University Hospital, Jerusalem,

"…One study showed that 51% of men diagnosed with cancer wished to preserve their reproductive potential, whereas this figure rose to 77% among childless men…" (Schover et al, 2002).

Classes of chemotherapy and their mechanisms of action Giwercman et al, 2000, Vakalopoulos I et al, 2015

Class of agent	Name of drugs	Mechanism	Cell cycle
Alkylating agents	Cyclophosphamide, nitrogen mustard, chloroethyl nitrosurea, busulfan, chlorambucil, melphalan, thiotepa	Cross-link DNA strand, interrupt RNA and protein synthesis	Non-specific
Cisplatin and analogues	Cisplatin, carboplatin	Interferes with DNA synthesis without affecting normal RNA and protein synthesis	Possibly specific (G2 arrest)
Vinca alkaloids (aneuploidy inducers)	Vincristine, vinblastine	Bind tubulin and cause dissociation of the microtubule apparatus	Specific: G1 and S phase
Antimetabolites	Methotrexate, aminopterin, 5-fluorouracil, cytarabine	Inhibit cellular metabolites by acting as false substrates for reactions required in DNA or RNA synthesis	Non-specific
Topoisomerase interactive agents (radiomimetics)	Bleomycin, actimomycin, doxorubicin, daunorubicin	Interact with enzyme-DNA complex. Prevents resealing of the top I-mediated DNA single strand breaks	Specific: G2 arrest/S-phase apoptosis

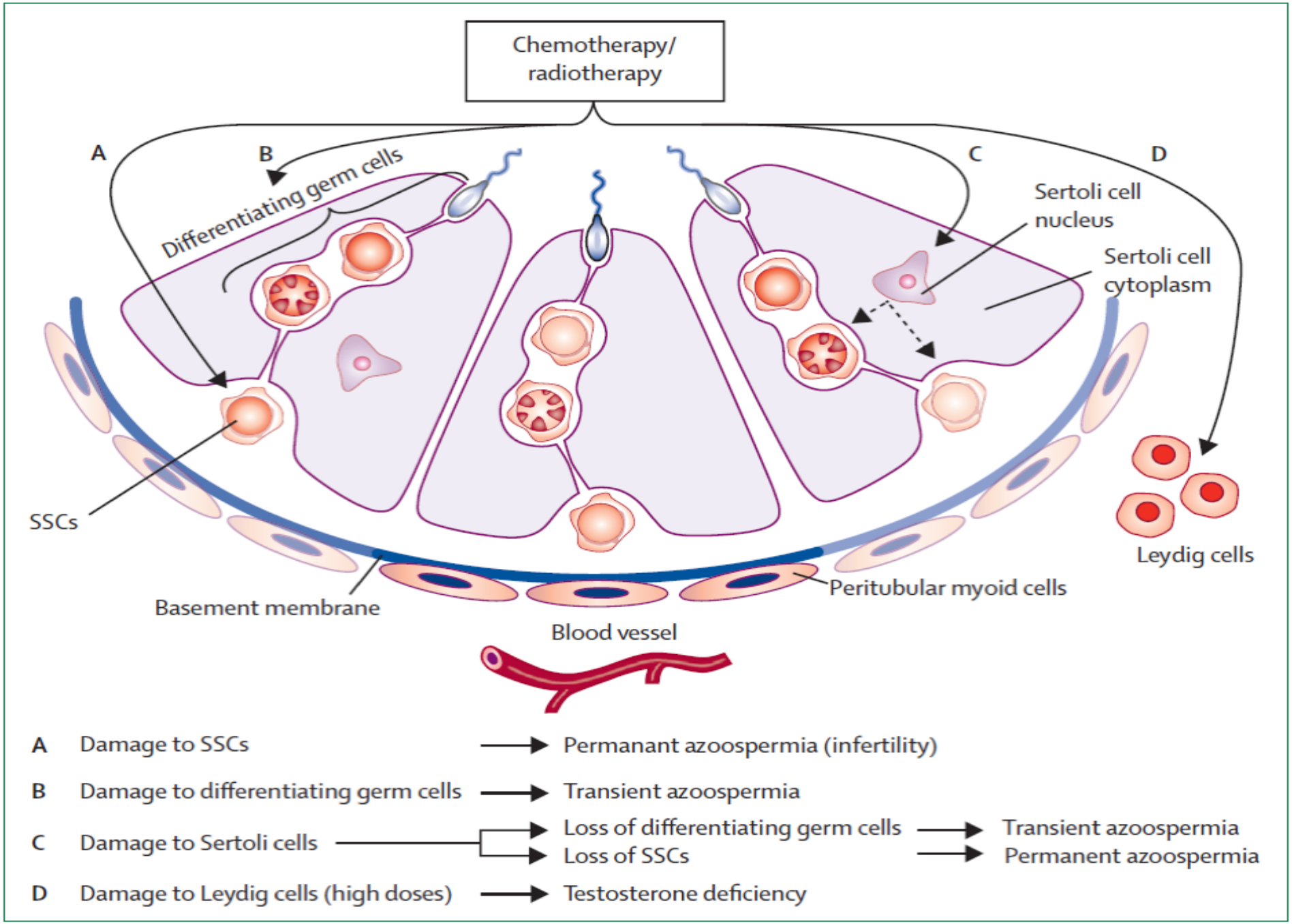
Classes of chemotherapy and their mechanisms of action Giwercman et al, 2000, Vakalopoulos I et al, 2015

Table 1. Examples of different cytotoxic agents

Group	Agent	Mode of Action	Degree of Gonadotoxicity
Alkylating agents		Addition of alkyl groups to DNA, altering DNA structure and function	
	Chlorambucil		Prolonged azoospermia
	Cyclophosphamide		
	Procarbazine		
	Melphalan		
	Carmustine		Azoospermia in adulthood after treatment before puberty
	Lomustine		
	Busulfan		Azoospermia likely, but always given with other highly sterilizing agents
	Ifosfamide		
Platinum analogs		Formation of DNA adducts, DNA interstrand cross-links	
	Cisplatin		Prolonged azoospermia
	Carboplatin		Prolonged azoospermia not often observed at indicated doses

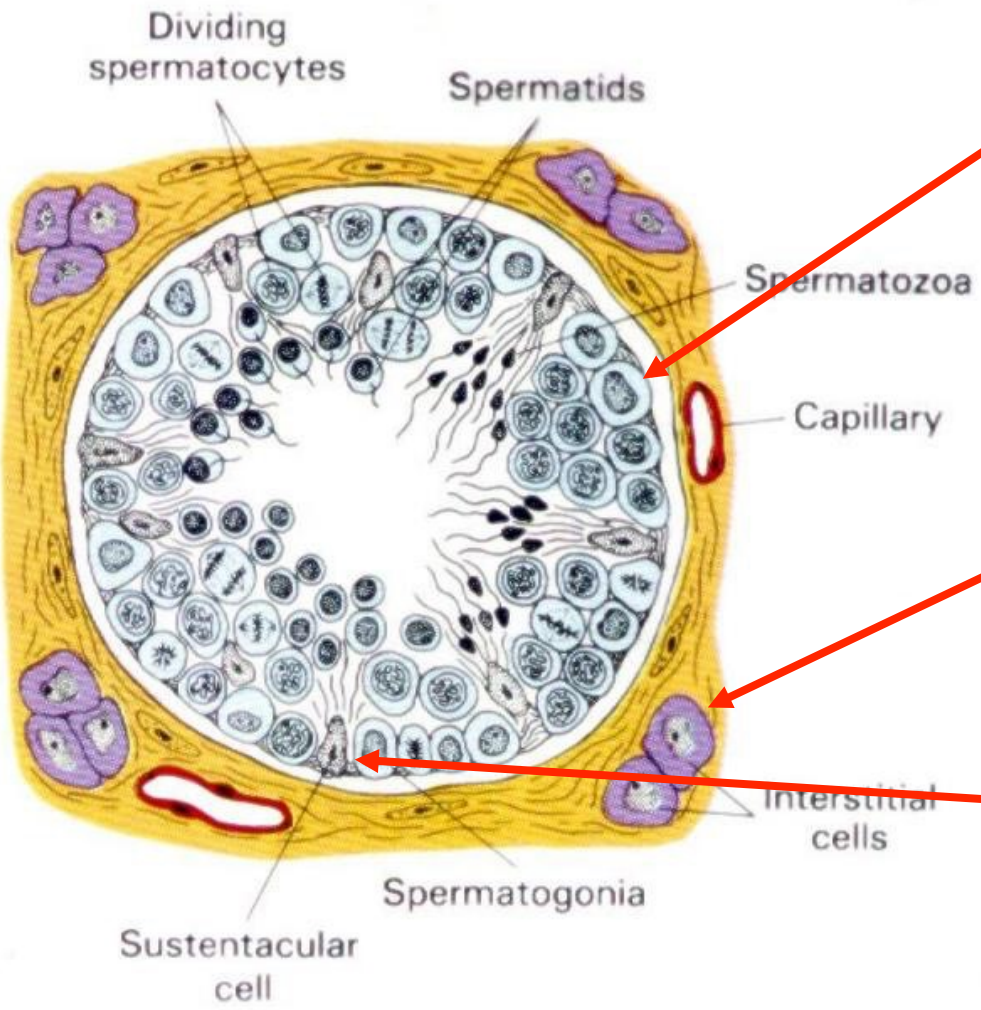
Table 2 Risk of treatment-related infertility with the main anticancer therapies (modified from the original [9])

Degree of risk	Type of anticancer treatment	
	Women	Men
High risk (>80 % risk of permanent amenorrhea in women; prolonged azoospermia in men)	-HSC transplantation with cyclophosphamide/TBI or cyclophosphamide/busulfan -External beam radiation to a field that includes the ovaries -CMF, CEF, CAF, TAC x 6 cycles in women ≥ 40 years	-Radiation > 2.5 Gy to testis -Chlorambucil (1.4 g/m2) -Cyclophosphamide (19 g/m2) -Procarbazine (4 g/m2) -Melphalan (140 mg/m2) -Cisplatin (500 mg/m2) -BCNU (1 g/m2) and CCNU (500 mg/m2)
Intermediate risk (40 % - 60 % risk of permanent amenorrhea in women; likelihood of azoospermia in men especially when given with other sterilizing agents)	-BEACOPP -CMF, CEF, CAF, TAC x 6 cycles in women age 30–39 -AC x 4 cycles in women ≥ 40 years -AC or EC x 4 → Taxanes	-Busulfan (600 mg/kg) -Ifosfamide (42 g/m2) -BCNU (300 mg/m2) -Nitrogen mustard -Actinomycin D
Low risk (<20 % risk of permanent amenorrhea in women; only temporary reductions in sperm counts in men especially when not given with other sterilizing agents)	-ABVD in women ≥ 32 years -CHOP x 4–6 cycles -CVP -AML therapy (anthracycline/cytarabine) -ALL therapy (multi-agent) -CMF, CEF, CAF, TAC x 6 cycles in women ≤ 30 years -AC x 4 cycles in women ≤ 40 years	-Carboplatin (2 g/m2) -Doxorubicin (770 mg/m2) -Thiotepa (400 mg/m2) -Cytosine arabinoside (1 g/m2) -Vinblastine (50 g/m2) -Vincristine (8 g/m2)
Very low or no risk (risk of permanent amenorrhea in women; temporary reductions in sperm count in men but additive effects are possible)	-ABVD in women < 32 years -Methotrexate -Fluorouracil -Vincristine -Tamoxifen	-Amsacrine -Bleomycin -Dacarbazine -Daunorubicin -Epirubicin -Etoposide -Fludarabine -Fluorouracil –6-mercaptopurine -Methotrexate -Mitoxantrone, -Thioguanine -Prednisone -Interferon-α
Unknown risk (risk of permanent amenorrhea in women; effect on sperm production in men)	-Monoclonal antibodies (trastuzumab, bevacizumab, cetuximab) -Tyrosine kinase inhibitors (erlotinib, imatinib)	-Oxaliplatin -Irinotecan -Monoclonal antibodies (trastuzumab, bevacizumab, cetuximab)



Anderson RA et al, Lancet Diabetes Endocrinol, 2015

Radiotherapy



4 Gy
interphase death
permanent damage to germ cells (spermatogonia)

Leydig cells
20-30 Gy: hypogonadism

spermatogenesi
0.1 – 1.2 Gy

Modified from Dohle GR, Int J Urol 2010

TABLE 1. Radiotherapy Protocols With High or Intermediate Impact on Ovarian and Testicular Function

High risk of prolonged azoospermia in men or amenorrhea in women
Total Body Irradiation (TBI) for bone marrow transplant/stem cell transplant[9,15,16]
Testicular radiation dose > 2.5 Gy in adult men[9,17]
Testicular radiation dose ≥ 6 Gy in prepubertal boys[18,19]
Pelvic or whole abdominal radiation dose ≥ 6 Gy in adult women[20–22]
Pelvic or whole abdominal radiation dose ≥ 10 Gy in postpubertal girls[21–24]
Pelvic radiation or whole abdominal dose ≥ 15 Gy in prepubertal girls[21–24]
Intermediate risk
Testicular radiation dose 1-6 Gy from scattered pelvic or abdominal radiation[13,16]
Pelvic or whole abdominal radiation dose 5-10 Gy in postpubertal girls[21,24]
Pelvic or whole abdominal radiation dose 10-15 Gy in prepubertal girls[21,22,24]
Craniospinal radiotherapy dose ≥ 25 Gy[14]

J Pediatr Hematol Oncol • Volume 32, Number 5, July 2010
Dohle GR, Int J Urol 2010

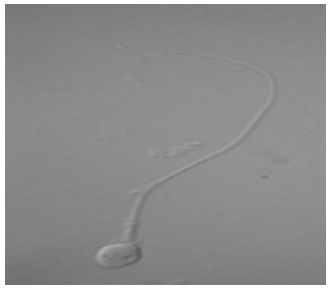
EFFETTI DELLE TERAPIE SULLA SPERMATOGENESI

Impact of lymphoma treatments on spermatogenesis and sperm deoxyribonucleic acid: a multicenter prospective study from the CECOS network

Louis Bujan, M.D., Ph.D.,[a,b] Marie Walschaerts, Ph.D.,[a] Florence Brugnon, M.D., Ph.D.,[b,c] Myriam Daudin, M.D.,[a,b] Isabelle Berthaut, Ph.D.,[b,d] Jacques Auger, M.D., Ph.D.,[b,e] Jacqueline Saias, M.D.,[b,f] Ethel Szerman, Ph.D.,[b,g] Nathalie Moinard, D.Pharm.,[a,b] Nathalie Rives, M.D., Ph.D.,[b,h] and Sylvianne Hennebicq, M.D., Ph.D.[b,i]

Treatment groups according to different drugs used.

Treatment groups	Type of protocols
ABVD group	ABVD
	R-ACVBP
	ACVBP
ABVD + radiotherapy group	ABVD
	ABVDP
	ABVDP + VABEM
	EBVP
CHOP group	ACVBP
	CHOP
	R-ACVBP + MTX + VP16 + Cy
	R-CVP
	ACVBP R + MTX + IFM + VP16
	R-ACVBP +MTX
	R-CHOP
	R- mini CHVP + interferon
	R-ACVB + MTX+ VP16 + IFM + Cy
	R-CHOP
MOPP-ABV group	BEACOPP + ABV
	BEACOPP



Fertil Steril 2014

TABLE 1

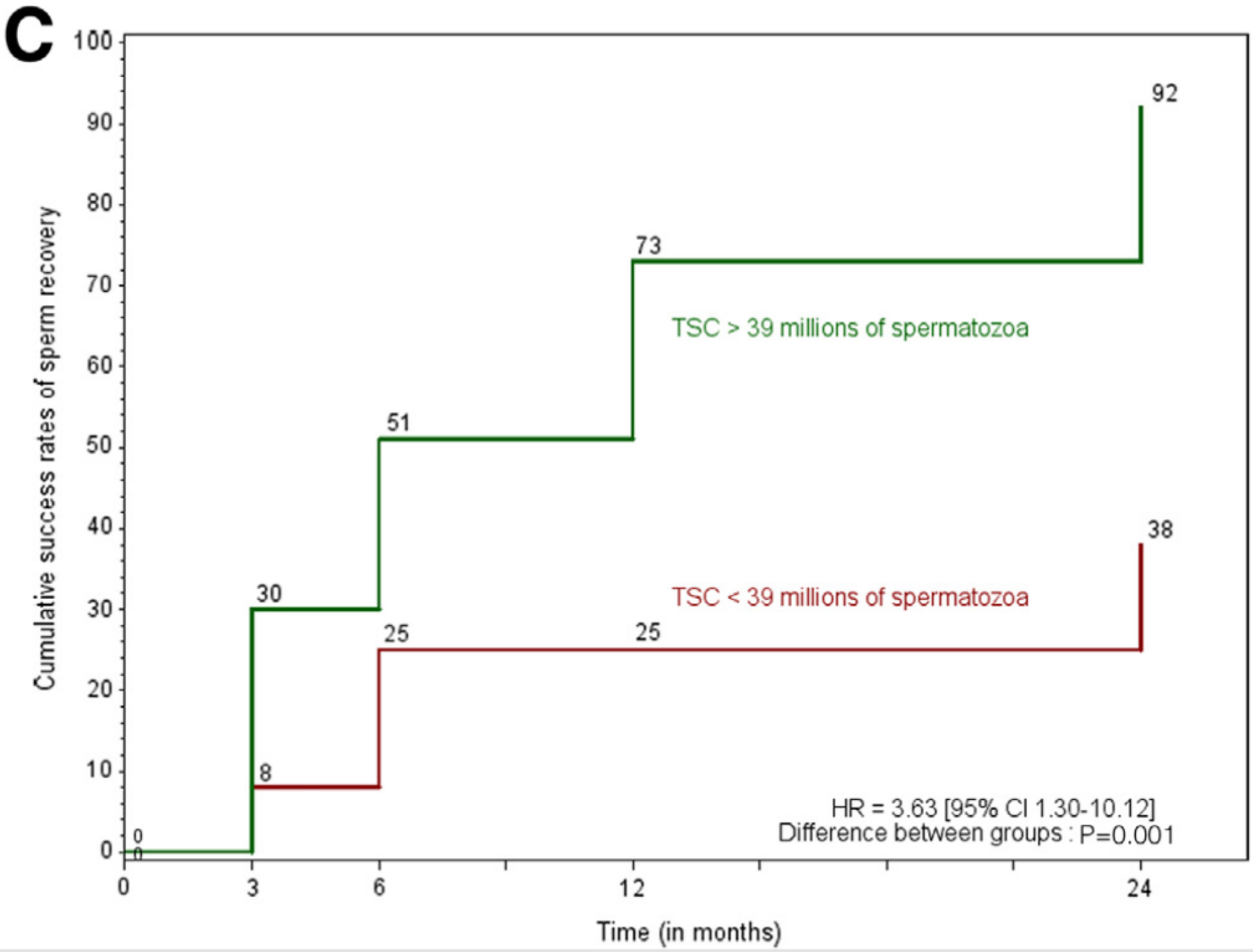
Sperm characteristics at follow-up time points according to treatment.

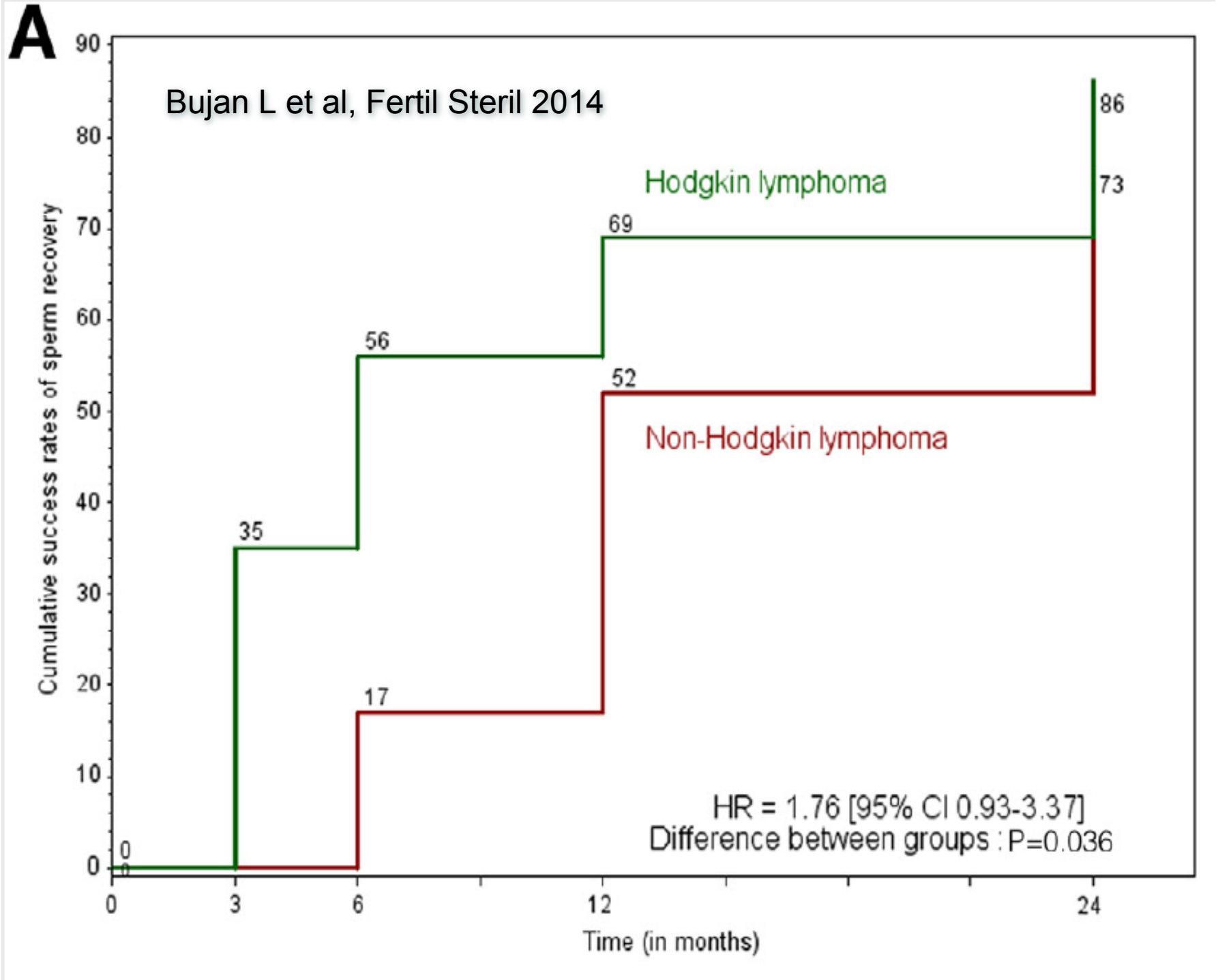
Charactrtistics	Before treatment	After treatment			
		3 mo	6 mo	12 mo	24 mo
ABVD, n	18	14	16	14	12
Sperm count, 10^6/mL	45.62 ± 47.08	7.88 ± 7.71[a]	15.56 ± 17.24[a]	28.49 ± 30.32	51.52 ± 52.23
Vitality, %	58.61 ± 13.26	58.09 ± 23.37	54.62 ± 25.45	66.83 ± 23.60	67.33 ± 6.96[a]
Motility, %	30.89 ± 12.64	30.45 ± 16.58	29.00 ± 15.72	35.21 ± 14.29	42.00 ± 13.74[a]
Total sperm count, 10^6/ejaculate	174.41 ± 336.96	33.45 ± 41.48[a]	60.61 ± 103.71[a]	143.91 ± 205.51	242.20 ± 208.85
Total motile sperm count, 10^6/ejaculate	47.48 ± 69.08	15.83 ± 17.11	23.75 ± 33.44	57.37 ± 97.50	104.86 ± 93.50
ABVD + Radiotherapy, n	39	34	28	30	19
Sperm count, 10^6/mL	57.89 ± 47.02	22.07 ± 29.04[a]	33.91 ± 32.33[a]	49.71 ± 52.53	59.08 ± 47.22
Vitality, %	61.10 ± 17.92	62.68 ± 17.68	64.08 ± 21.45[a]	60.48 ± 21.36	68.37 ± 15.13
Motility, %	42.00 ± 13.48	35.63 ± 15.86	37.89 ± 18.14	36.55 ± 18.09	44.58 ± 13.23
Total sperm count, 10^6/ejaculate	217.45 ± 222.05	74.89 ± 130.30[a]	126.25 ± 150.62	184.27 ± 214.18	241.24 ± 220.21
Total motile sperm count, 10^6/ejaculate	88.16 ± 82.12	31.20 ± 50.12[a]	55.78 ± 65.40[a]	82.54 ± 97.38	97.33 ± 74.17[a]
CHOP, n	13	11	13	11	9
Sperm count, 10^6/mL	78.09 ± 74.87	0.59 ± 1.24[a]	9.54 ± 21.73[a]	36.16 ± 78.26[a]	45.78 ± 57.92
Vitality, %	63.33 ± 20.60	19.75 ± 29.67[a]	26.14 ± 34.14	59.00 ± 33.97	62.00 ± 33.34
Motility, %	39.62 ± 15.87	13.75 ± 22.00[a]	18.18 ± 26.39[a]	28.00 ± 27.00	36.43 ± 28.09
Total sperm count, 10^6/ejaculate	215.16 ± 174.23	1.18 ± 2.37[a]	32.09 ± 90.66[a]	126.88 ± 281.71	165.76 ± 222.91
Total motile sperm count, 10^6/ejaculate	83.46 ± 69.00	0.69 ± 1.50[a]	13.79 ± 30.16[a]	75.47 ± 153.30	99.01 ± 109.24
MOPP-ABV, n	5	2	5	4	2
Sperm count, 10^6/mL	45.58 ± 41.24	0.00 ± 0.00	0.02 ± 0.04	7.00 ± 14.00	177.00 ± 250.32
Vitality, %	53.00 ± 9.38	Nm	0.00 ± 0.00	41.00 ± 57.98	86.00 ± -
Motility, %	40.00 ± 9.46	Nm	3.20 ± 7.16	16.67 ± 28.87	30.00 ± 42.43
Total sperm count, 10^6/ejaculate	129.63 ± 122.18	0.00 ± 0.00	0.07 ± 0.16	14.70 ± 29.40	460.20 ± 650.82
Total motile sperm count, 10^6/ejaculate	56.07 ± 60.20	Nm	0.01 ± 0.03	9.80 ± 16.97	276.12 ± 390.49

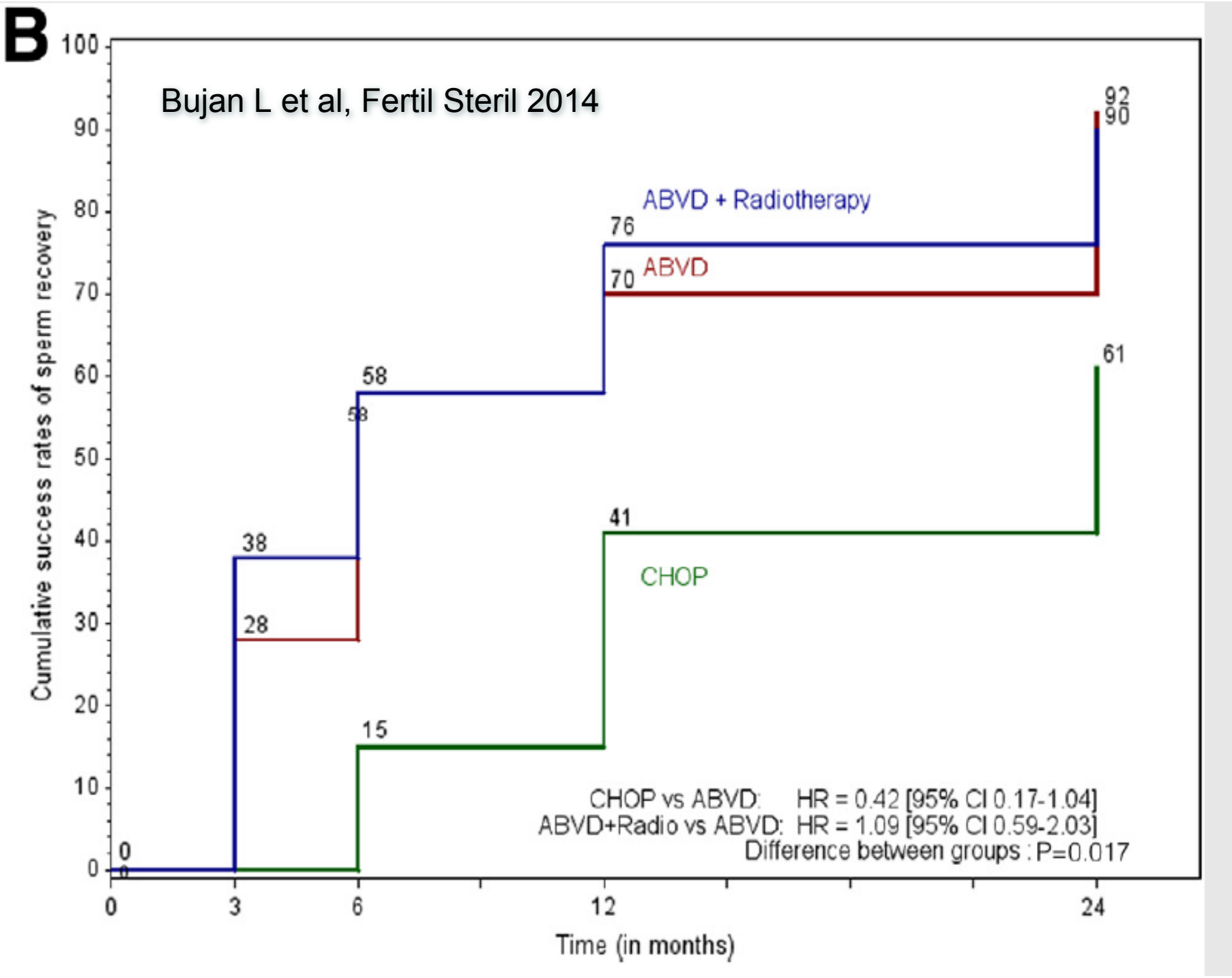
Note: Values are mean ± standard deviation. Nm = not measurable.

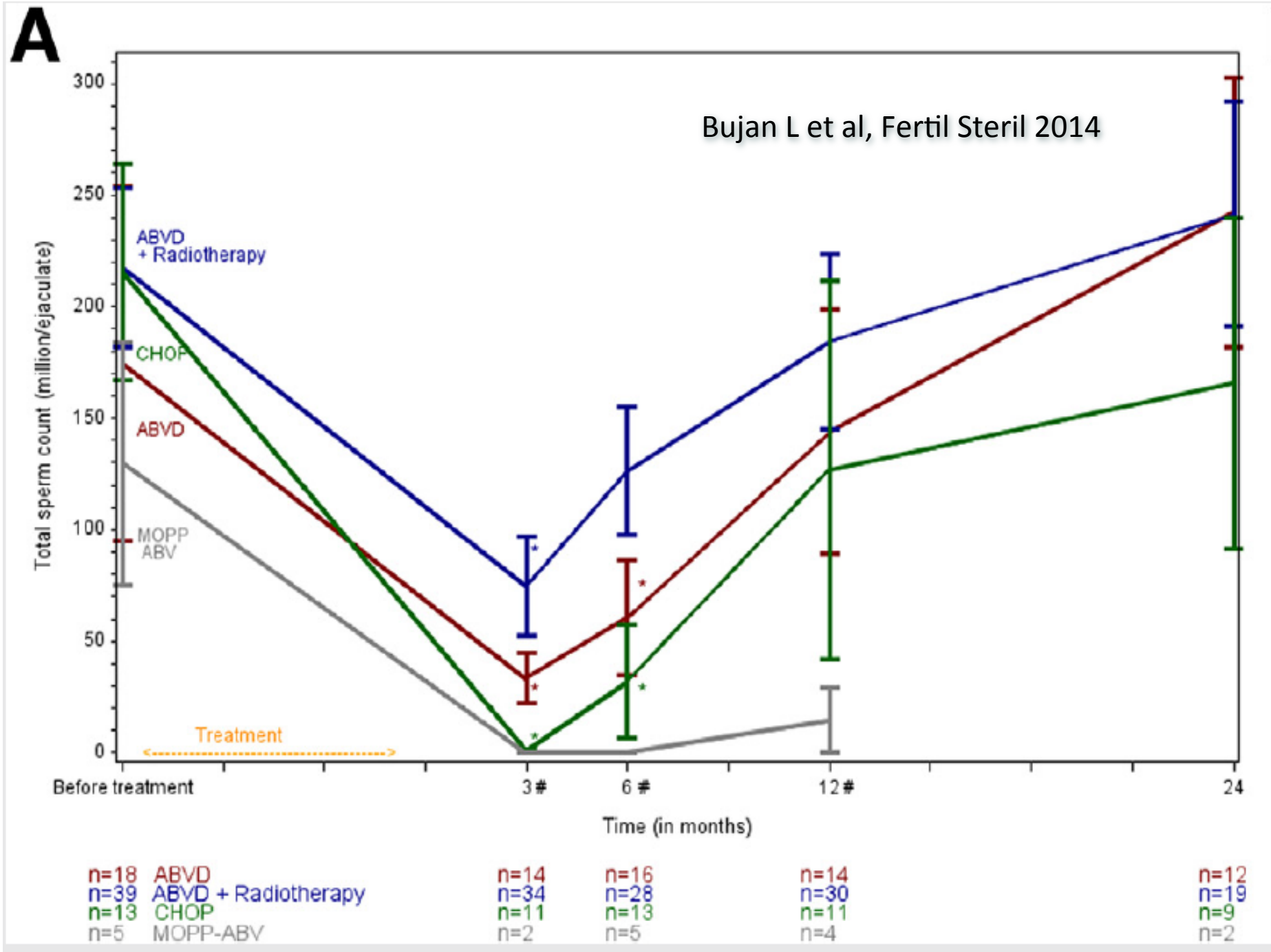
[a] $P < .05$, difference between before treatment and after treatment values (3, 6, 12, and 24 months).

Bujan. Lymphoma treatment and spermatogenesis. Fertil Steril 2014.


C
Cumulative success rates of sperm recovery
100
90
80
70
60
50
40
30
20
10
0
0
3
6
12
24
Time (in months)
92
73
51
38
30
25
25
8
0
0
TSC > 39 millions of spermatozoa
TSC < 39 millions of spermatozoa
HR = 3.63 [95% CI 1.30-10.12]
Difference between groups : P=0.001


A
Bujan L et al, Fertil Steril 2014
Hodgkin lymphoma
Non-Hodgkin lymphoma
86
73
69
56
52
35
17
0
0
HR = 1.76 [95% CI 0.93-3.37]
Difference between groups : P=0.036
Cumulative success rates of sperm recovery
Time (in months)
0
10
20
30
40
50
60
70
80
90
0
3
6
12
24


B
Bujan L et al, Fertil Steril 2014
Cumulative success rates of sperm recovery
Time (in months)
ABVD + Radiotherapy
ABVD
CHOP
92
90
76
70
61
58
58
41
38
28
15
0
0
CHOP vs ABVD: HR = 0.42 [95% CI 0.17-1.04]
ABVD+Radio vs ABVD: HR = 1.09 [95% CI 0.59-2.03]
Difference between groups : P=0.017

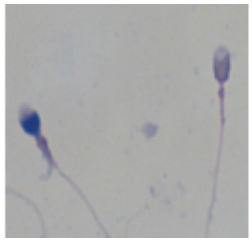

A
Bujan L et al, Fertil Steril 2014
Total sperm count (million/ejaculate)
300
250
200
150
100
50
0
ABVD
+ Radiotherapy
CHOP
ABVD
MOPP
ABV
Treatment
Before treatment
3#
6 #
12 #
24
Time (in months)
n=18 ABVD
n=39 ABVD + Radiotherapy
n=13 CHOP
n=5 MOPP-ABV
n=14
n=34
n=11
n=2
n=16
n=28
n=13
n=5
n=14
n=30
n=11
n=4
n=12
n=19
n=9
n=2

Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios

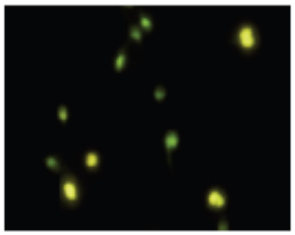
Transl Adv Urol Androl,2016

Ashok Agarwal[1], Ahmad Majzoub[2], Sandro C. Esteves[3], Edmund Ko[4], Ranjith Ramasamy[5], Armand Zini[6]

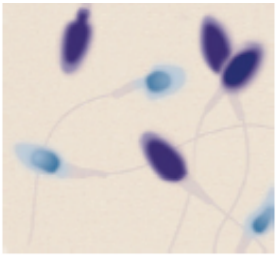
Arancio di acridina



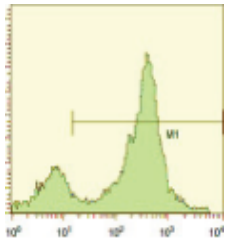
Blu di anilina



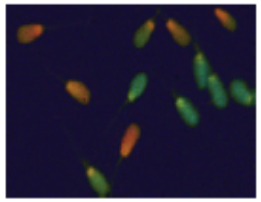
Cromomicina A3



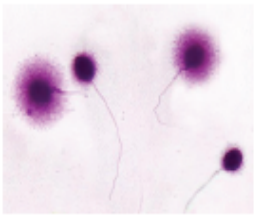
Blu di toluidina



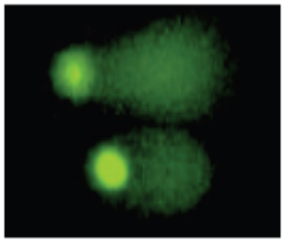
TUNEL test



Sperm chromatin structure assay (SCSA)

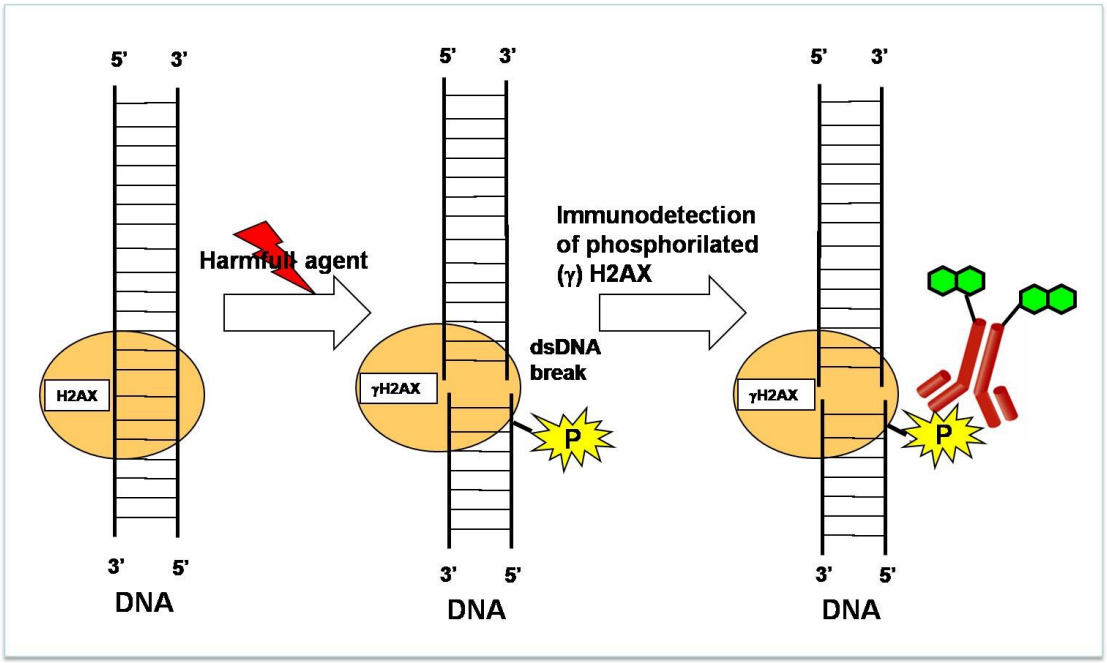


Sperm chromatin Dispersion test (SCD) Halo-test



COMET assay

γH2AX



Pro: Consente di ottenere un valore delle rotture sul doppio filamento. Il citofluorimetro consente l'analisi di numerose cellule in un breve tempo.

Cont.: nei soggetti gravemente oligozoospermici è necessario utilizzare un microscopio a fluorescenza.

Impact of lymphoma treatments on spermatogenesis and sperm deoxyribonucleic acid: a multicenter prospective study from the CECOS network

Louis Bujan, M.D., Ph.D.,[a,b] Marie Walschaerts, Ph.D.,[a] Florence Brugnon, M.D., Ph.D.,[b,c] Myriam Daudin, M.D.,[a,b] Isabelle Berthaut, Ph.D.,[b,d] Jacques Auger, M.D., Ph.D.,[b,e] Jacqueline Saias, M.D.,[b,f] Ethel Szerman, Ph.D.,[b,g] Nathalie Moinard, D.Pharm.,[a,b] Nathalie Rives, M.D., Ph.D.,[b,h]

TABLE 2

DNA fragmentation and abnormal chromatin (%).

		Lymphoma (HL + NHL) group				
			After treatment			
	Control group fertile men	Before treatment	3 mo	6 mo	12 mo	24 mo
N	51	71	38	38	43	34
DFI	8.6 [6.6–14.3]	15.9 [9.8–27.1][a,b]	17.4 [10.2–25.0][a,b]	15.0 [9.6–23.2][a,b,c]	16.7 [8.6–23.4][a,b,c]	14.4 [8.9–24.8][a,b,c]
HDS	5.3 [4.5–6.2]	6.1 [4.9–7.0][a]	6.2 [4.9–8.6][a]	5.4 [4.3–7.5]	5.8 [4.4–6.8]	5.7 [5.0–7.0]
DFI+HDS	14.3 [11.3–20.7]	22.5 [16.4–34.4][a,b]	24.8 [17.1–33.4][a,b]	20.8 [16.3–29.2][a,b,c]	22.2 [14.8–27.9][a,b,c]	21.9 [15.0–31.4][a,b]
TUNEL	7.9 [6.6–13.2]	12.7 [7.1–15.7][a]	7.6 [5.8–12.4][a,c]	9.8 [7.1–13.9][c]	9.3 [6.6–14.7][c]	10.5 [7.3–14.3]

Note: Values are medians [interquartile range: Q1–Q3]. DFI = DNA fragmentation index; HDS = highly DNA stainable cells were assessed by sperm chromatin structure assay; TUNEL = terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labelling assay.

[a] $P < .05$, difference between control group and lymphoma group.

[b] $P < .05$, difference between control group and lymphoma group, adjusted on abstinence duration and age.

[c] $P < .05$, difference between before-treatment and after-treatment values (3, 6, 12, and 24 months).

Bujan. Lymphoma treatment and spermatogenesis. Fertil Steril 2014.

Human Reproduction, Vol.25, No.8 pp. 1877–1883, 2010
Advanced Access publication on June 14, 2010 doi:10.1093/humrep/deq104

human reproduction

ORIGINAL ARTICLE *Andrology*

Sperm DNA integrity in cancer patients before and after cytotoxic treatment

SCSA

Sperm Chromatin Structure Assay

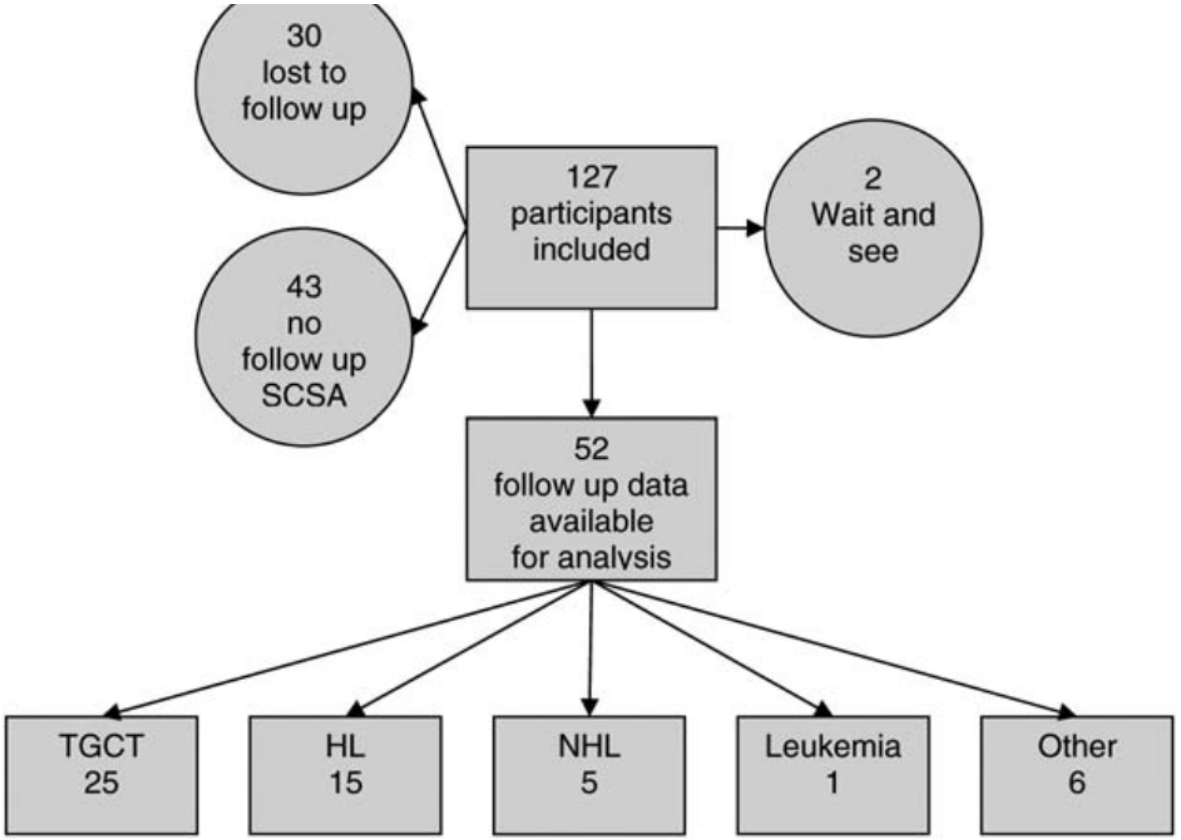


Table I Semen parar

	n		Other 19
Sperm count ($\times 10^6$)		9.7)	83.2 (8.6–1282.6)
Sperm concentration ($\times 10^6$		.2)	42.0 (4.3–283.0)
Progressive motility (%)		50.0)	42.0 (12.0–84.0)
Normal morphology (%)		.0)	6.0 (1.0–12.0)
DFI (%)		2.3)	11.1 (3.8–58.7)

All values are median (range). DFI i
compared with other diagnosis gr
patients ($P = 0.028$). TGCT, testic

entration was significantly lower
h NHL ($P = 0.041$) and TGCT
astrocytoma, rectal cancer etc.

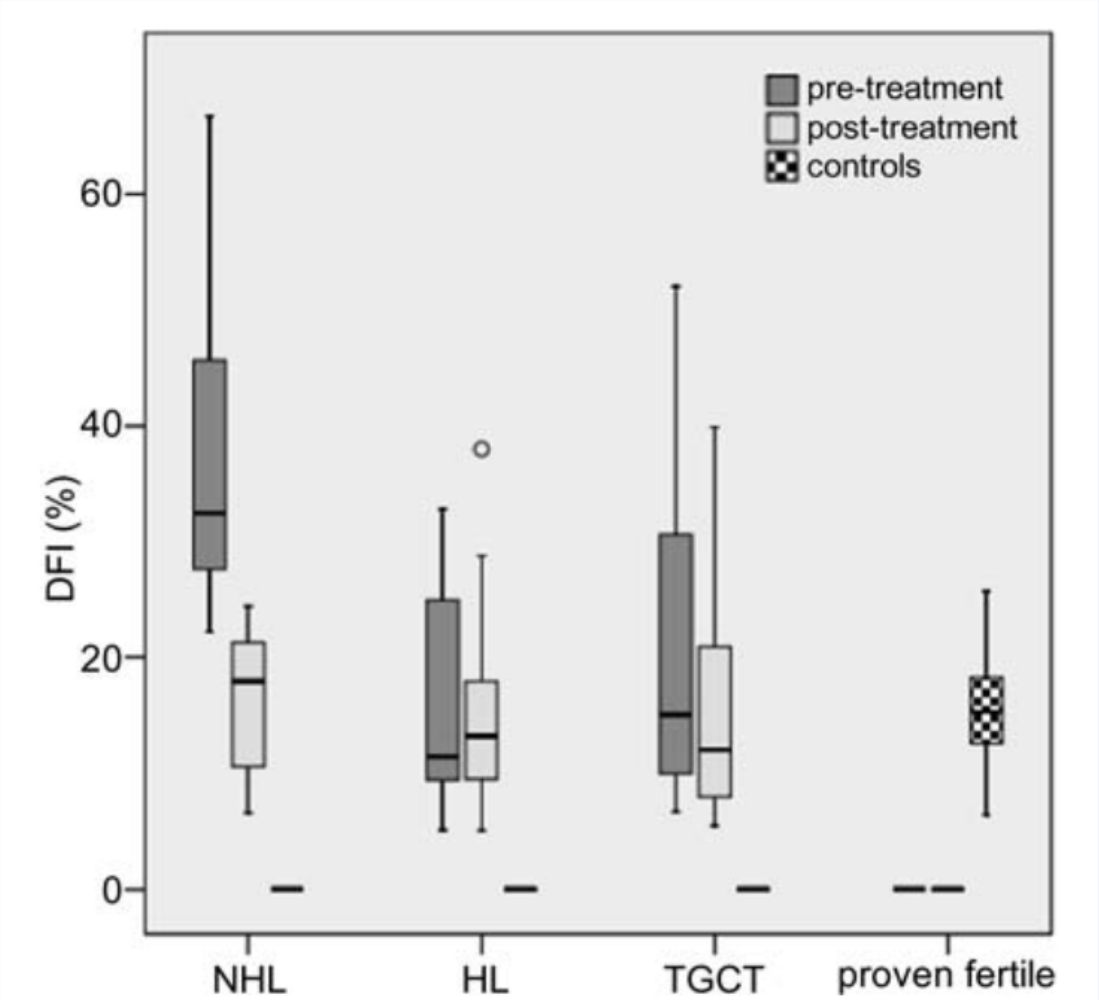


Figure 2 Pre- and post-treatment DFI values in the three most prevalent cancer diagnosis groups ($n = 5$ NHL, $n = 15$ HL, $n = 25$ TGCT) and 22 fertile controls. Boxes show the mean DFI with standard deviation, whiskers show the range of non-outlier DFI values, open circle represents an outlier.

Impact of chemotherapeutics and advanced testicular cancer or Hodgkin lymphoma on sperm deoxyribonucleic acid integrity

Fertil Steril 2010

Cristian O'Flaherty, Ph.D.,[a] Barbara F. Hales, Ph.D.,[a] Peter Chan, M.D.,[b,c] and Bernard Robaire, Ph.D.[a,b,c,d]

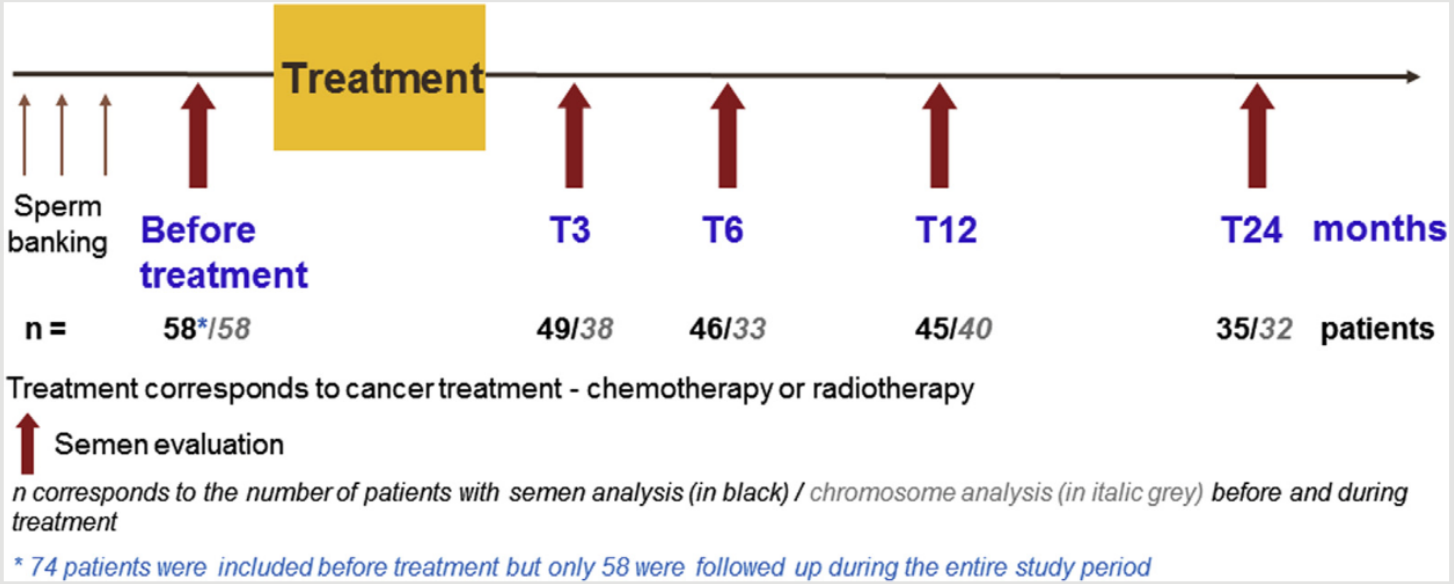
Livelli significativamente più alti di danno del DNA spermatico
casi vs controlli che persistono anche 2 anni dopo CT (vari schemi)


DANNI AI
CROMOSOMI
SPERMATICI

Impact of Hodgkin or non-Hodgkin lymphoma and their treatments on sperm aneuploidy: a prospective study by the French CECOS network

VOL. 107 NO. 2 / FEBRUARY 2017

SUPPLEMENTAL FIGURE 1

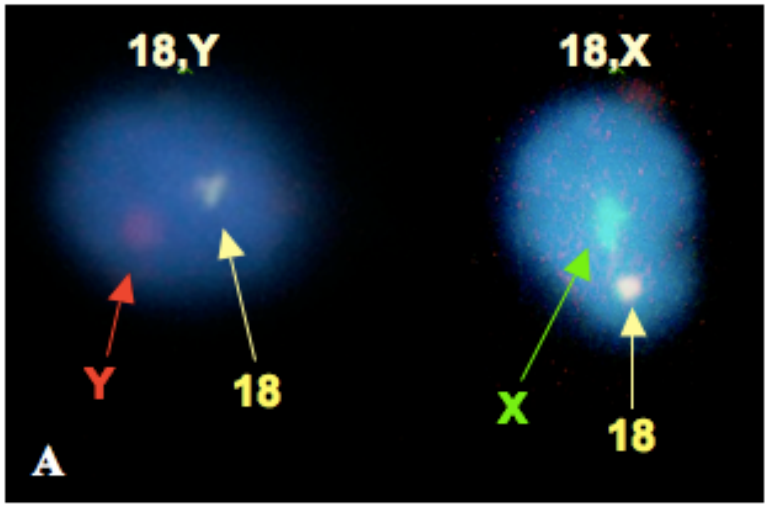


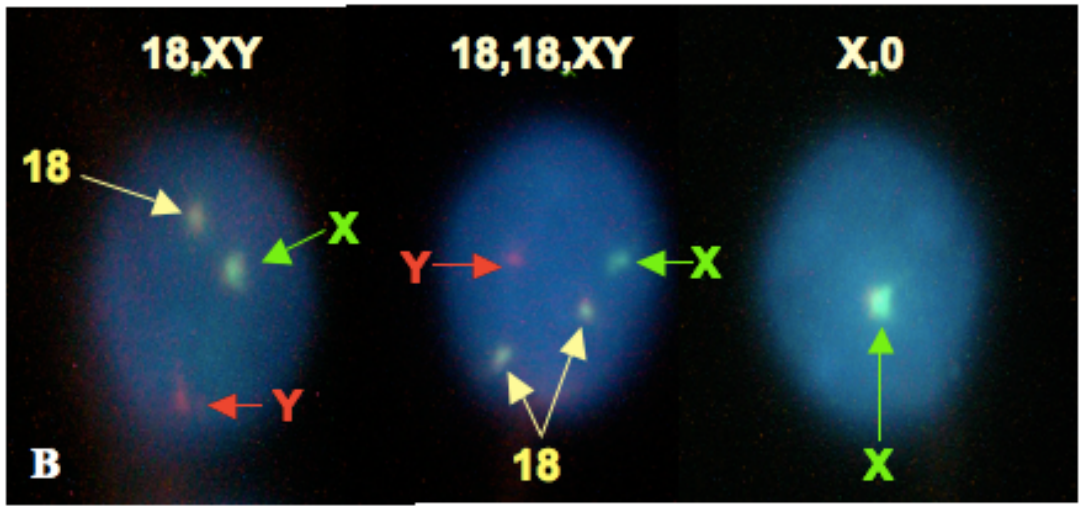
Design of the prospective study.

Martinez. Sperm aneuploidy and lymphoma. Fertil Steril 2016.

FISH Test

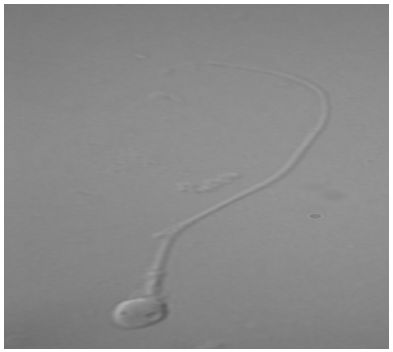
(Valutazione dei cromosomi spermatici 13,18, 21, X, Y)

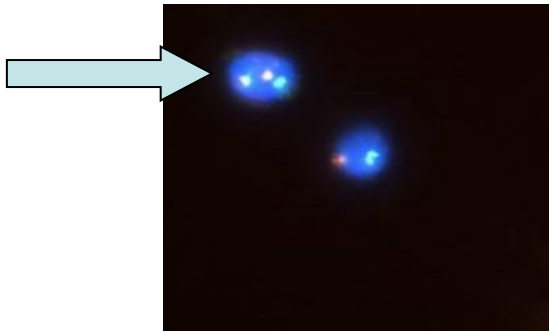


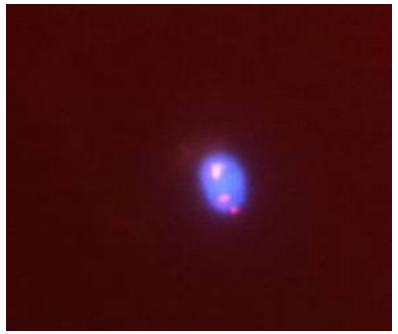


Spermatozoi euploidi

Spermatozoi aneuploidi







REVIEW Reproductive BioMedicine Online (2015) 31, 481–485

Implication of sperm chromosomal abnormalities in recurrent abortion and multiple implantation failure

Ana Lara Caseiro [a,*], Ana Regalo [a], Elisa Pereira [a], Telma Esteves [a], Fernando Fernandes [b], Joaquim Carvalho [a]

- La maggior parte degli studi rivela che il fallimento dell'impianto embrionario in corso di IVF potrebbe essere di origine maschile ed essere attribuibile ad un aumento delle aneuploidie spermatiche.
- L'etiologia dell'aborto ripetuto è determinata solo nel 30% dei casi. L'eventuale contributo paterno è poco investigato.

TABLE 1

Comparative analysis of sperm characteristics and chromosome content (in %) in the control group and the lymphoma group (HL and NHL) before treatment.

Parameter	Control group (n = 29)		Lymphoma group (n = 74)		HL group (n = 56)		NHL group (n = 18)	
Sperm characteristics								
Volume (mL)	3.50	3.09–5.09	3	2.09–4.09[a]	3.05	2.05–4.55[a]	2.90	2.50–3.70[a]
Sperm count (×10^6/mL)	86.25	19–126	46.39	22–75	45.89	22.50–64.35	48	19–102
Vitality (%)	71	64–82	61	53–73[a]	60	54–72[a]	65	51–80
Motility (%)	45	40–50	40	30–50	40	32–47	35	30–50
Total sperm count (×10^6/ ejaculate)	270	157–379	120	60–272[a]	120	59–234[a]	126	76–328

TABLE 2

Sperm characteristics according to treatment and time points in the 58 lymphoma patients.

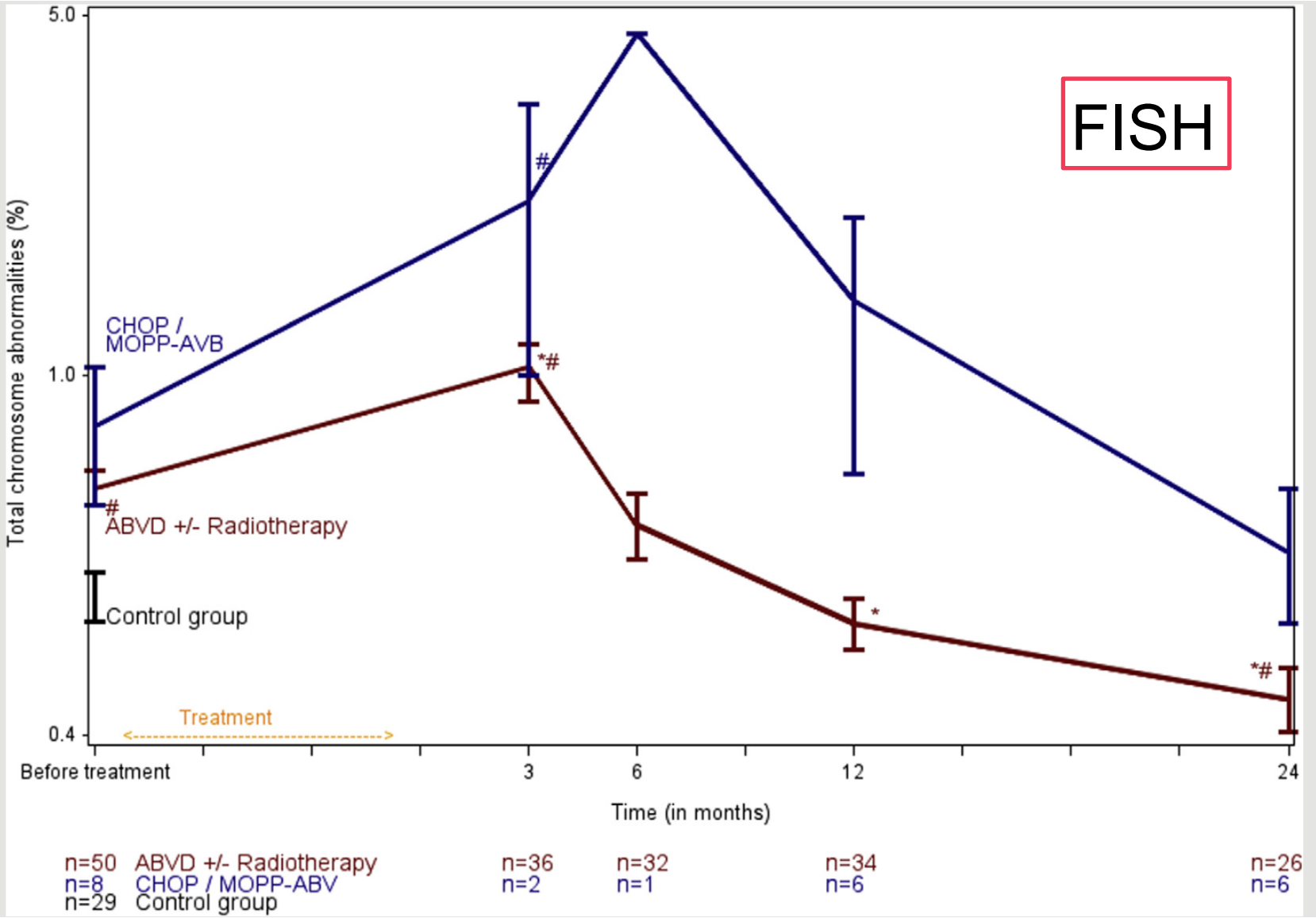
Treatment regimen	Before treatment		After treatment: 3 mo		6 mo		12 mo		24 mo	
ABVD ± radiotherapy										
N (%)[a]	50		41 (82)		38 (76)		37 (74)		28 (56)	
Volume (mL)	3.15	2.50–4.59	3.20	2.80–4.40	3.55	2.30–4.80	3.70	2.40–4.90	4.25	3–6.40[b]
Sperm count (10^6/mL)	40.50	22–66	10.80	6.59–20[b]	20.30	4.80–52[b]	40	16–65	42	28.30–75.50
Vitality (%)	60	52–74	64	52–78	66	50–75	66	60–76[b]	70	62–75[b]
Motility (%)	40	30–48	35	30–45	35	30–48	40	30–45	41	35–50[b]
Total sperm count (×10^6/ ejaculate)	130.83	59.78–272.59	36	16.79–89.25[b]	56.86	20.99–146.78[b]	128.95	55–271.82	168.40	106.54–329.79[b]
Total motile sperm count (×10^6/ejaculate)	53.61	18.34–112.01	15.92	3.99–35.70[b]	17.63	11.34–82.36[b]	51.58	19.73–124.02	97.60	39.24–157.66[b]
CHOP/MOPP-ABV										
N (%)[a]	8		8 (100)		8 (100)		8 (100)		7 (88)	
Volume (mL)	2.85	2.24–3.30	2.60	1.94–3.90	2.09	2.09–4.05	2.50	2.15–3.55	2.59	2.09–3.80
Sperm count (10^6/mL)	83	47.39–114.59	0	0–0.27[b]	0.01	0–7.55[b]	23.10	0.20–61.50	57.59	23–160
Vitality (%)	76	53–80	0	0–40	22	0–61	76	68–82	81	78–85
Motility (%)	48	35–57	0	0–30	16	0–50	50	30–50	55	30–60
Total sperm count (×10^6/ ejaculate)	277.92	115.74–376.15	0	0–0.57[b]	0.04	0–17.42[b]	53.59	0.50–212.23	269.50	48.29–607.99
Total motile sperm count (×10^6/ejaculate)	115.95	59.49–143.25	0	0–0.34[b]	0.04	0–20.69[b]	26.79	0.07–134.76	107.80	28.97–297.85

Note: Values are expressed as median and interquartile range (Q1–Q3) unless otherwise noted.

[a] N = % of compliance with the survey.

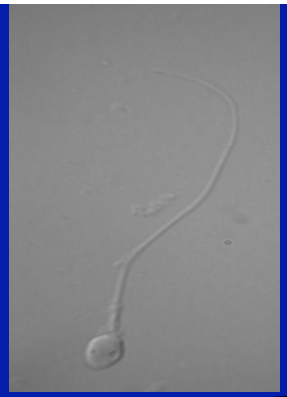
[b] $P < .05$, difference between before-treatment and after-treatment values (3, 6, 12, and 24 months).

Martinez. Sperm aneuploidy and lymphoma. Fertil Steril 2016.



Mean percentages of total chromosomal abnormalities and SEMs before and during posttreatment follow-up according to type of treatment (ABVD ± radiotherapy, or CHOP/MOPP-ABV) and in the control group. *$P<.05$, pre- and posttreatment difference. #$P<.05$, difference between controls and lymphoma patients.

Martinez. Sperm aneuploidy and lymphoma. Fertil Steril 2016.



DATI AMBIGUI E CONTRADDITTORI

- Differenti approcci metodologici usati
- Differenti popolazioni studiate in termini di neoplasia
- Variabilità dei trattamenti considerati
- bassa numerosità dei campioni

The determination of reproductive safety in men during and after cancer treatment

Fertil Steril 2013

Jeremy T. Choy, M.D. and Robert E. Brannigan M.D.
Department of Urology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois

- Sono necessari ulteriori studi clinici prospettici, con popolazioni ed end-points omogenei, per chiarire le conseguenze sullo stato gonadico, in particolare sull'integrità genomica dei gameti maschili, delle terapie anti-neoplastiche.

- Stilare linee-guida cliniche condivise al fine di fornire ai pazienti un counseling chiaro ed esaustivo sulle possibilità, il "timing" ed i rischi di concepimento a seguito dei trattamenti anti-neoplastici.

Risk of Birth Abnormalities in the Offspring of Men With a History of Cancer: A Cohort Study Using Danish and Swedish National Registries

Olof Ståhl, Heather A. Boyd, Aleksander Giwercman, Morten Lindholm, Allan Jensen, Susanne Krüger Kjær, Harald Anderson, Eva Cavallin-Ståhl, Lars Rylander

Manuscript received May 4, 2010; revised December 1, 2010; accepted December 7, 2010.

	Yes (n = 8670)		No (n = 1769095)			
Type of congenital abnormality	**No. of patients**	**Prevalence, %**	**No. of patients**	**Prevalence, %**	**RR (95% CI)†**	***P*‡**
Any	420	4.84	77844	4.40	1.12 (1.02 to 1.24)	.018
Any major	322	3.71	57067	3.23	1.17 (1.05 to 1.31)	.004
Selected groups of congenital abnormalities§						
Abdominal wall	3	0.03	511	0.03	1.2 (0.4 to 3.7)	.76
Alimentary tract atresia	14	0.16	1704	0.10	1.7 (1.0 to 2.8)	.054
Cardiovascular	88	1.01	17772	1.00	1.0 (0.9 to 1.3)	.92
Central nervous system	8	0.09	1915	0.11	0.9 (0.4 to 1.7)	.65
Chromosomal, non-Down	2	0.02	1179	0.07	0.3 (0.1 to 1.4)	.13
Cleft lip	17	0.20	2731	0.15	1.3 (0.8 to 2.0)	.33
Cleft palate	8	0.09	1410	0.08	1.2 (0.6 to 2.3)	.68
Club foot	15	0.17	2657	0.15	1.2 (0.7 to 1.9)	.58
Craniosynostosis	5	0.06	967	0.05	1.1 (0.4 to 2.5)	.90
Cystic kidney	7	0.08	547	0.03	2.6 (1.2 to 5.5)	.012
Diaphragmatic hernia	1	0.01	447	0.03	0.5 (0.1 to 3.2)	.43
Down syndrome	5	0.06	1941	0.11	0.5 (0.2 to 1.3)	.15
Hypospadias	27	0.31	4506	0.25	1.2 (0.8 to 1.8)	.30
Kidney dysgenesis, agenesis or hypoplasia	5	0.06	440	0.02	2.3 (1.0 to 5.6)	.061
Limb reduction	10	0.12	912	0.05	2.2 (1.2 to 4.2)	.011
Neural tube	5	0.06	777	0.04	1.3 (0.5 to 3.2)	.54
Phacomatosis	4	0.05	336	0.02	2.4 (0.9 to 6.5)	.078
Polydactyly	7	0.08	1659	0.09	0.8 (0.4 to 1.8)	.69
Pyloric stenosis	13	0.15	1493	0.08	1.8 (1.0 to 3.1)	.04
Skeletal	2	0.02	375	0.02	1.1 (0.3 to 4.4)	.91
Syndactyly	11	0.13	1510	0.08	1.5 (0.8 to 2.7)	.19

PROBLEMATICHE NEL MASCHIO PREPUBERE

- Crioconservazione da biopsia testicolare prima dell'inizio delle terapie oncologiche che possono impedire l'inizio della spermatogenesi
- Possibilità di crioconservare tessuto testicolare (spesso in piccola quantità, circa 0.1 cm^3) contenente cellule staminali spermatogoniali (SSCs) prima dei trattamenti oncologici e autotrapiantarle nel/i testicolo/i in futuro per ripristinare la spermatogenesi
- Essenziale eliminare il rischio di reintrodurre cellule maligne

- Più di 14 maschi di età inferiore a 15 anni ricevono una diagnosi di tumore ogni giorno (Howlader H et al 2011)
- Più dell[illegible] di tal[illegible] dal tumore (Howlader H[illegible]
- [illegible] no agli spe[illegible]zio del [illegible]enta una [illegible]plastici ne[illegible]guente sterilità

- ALL: 40% dei tumori sotto i 14 anni di età
-In più del 30% dei casi localizzazione testicolare confermata all'esame istologico

[illegible]mphoblastic Leukemia Therapy [illegible]ogonia Populations and Future Fertility

Mirja Nurmio, Victoria Keros, Päivi Lähteenmäki, Toivo Salmi, Markku Kallajoki, and Kirsi Jahnukainen

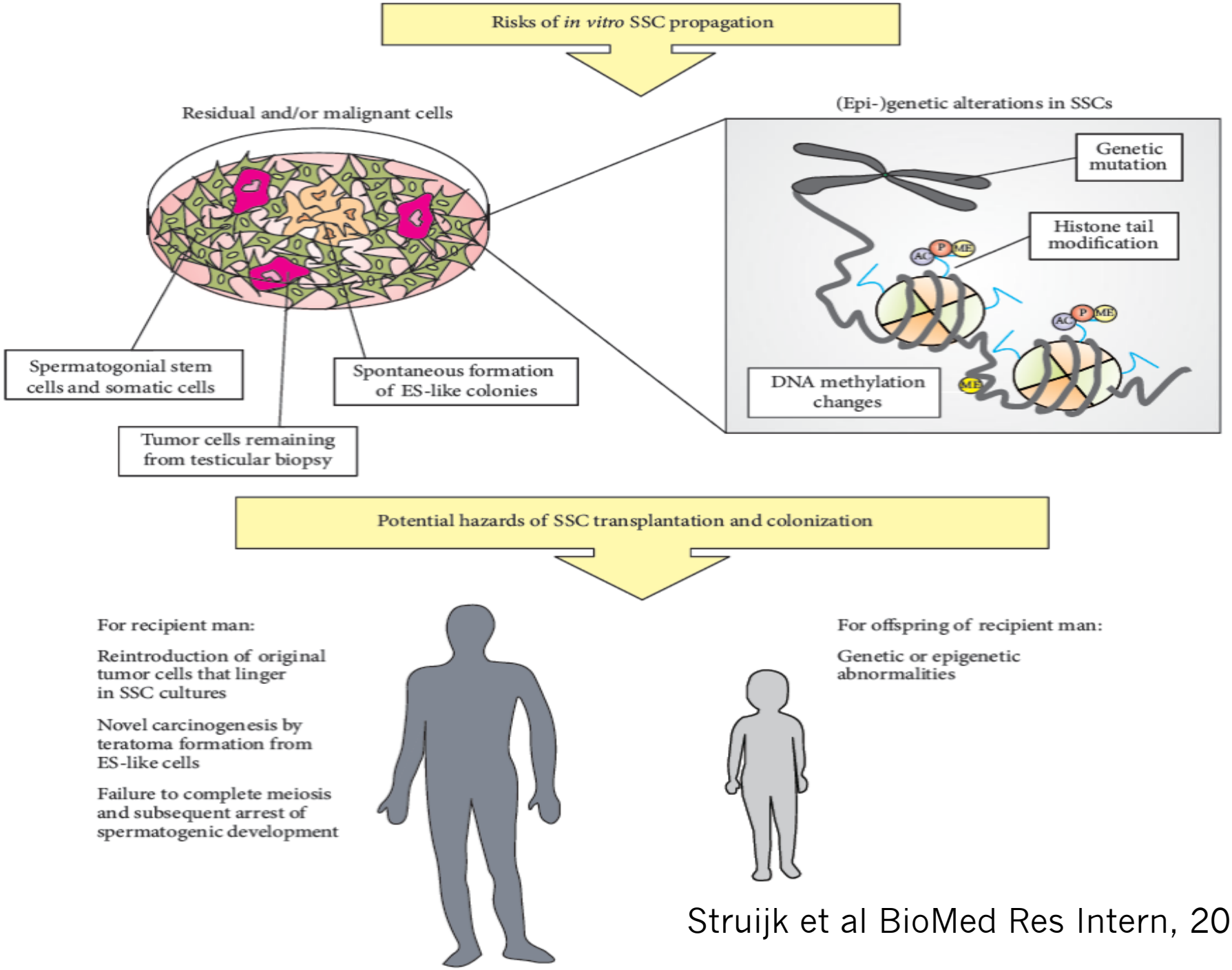
J Clin Endocrinol Metab, June 2009, 94(6):2119–2122

Eliminating acute lymphoblastic leukemia cells from human testicular cell cultures: a pilot study

Fertil Steril 2014

Hooman Sadri-Ardekani, M.D., Ph.D.,[a,b] Christa H. Homburg, M.Sc.,[c] Toni M. M. van Capel, B.Sc.,[d] Henk van den Berg, M.D., Ph.D.,[e] Fulco van der Veen, M.D., Ph.D.,[a] C. Ellen van der Schoot, M.D., Ph.D.,[c] Ans M. M. van Pelt, Ph.D.,[a] and Sjoerd Repping, Ph.D.[a]

- Le cellule di leucemia linfoblastica acuta (ALL) poste in coltura assieme alle cellule spermatogenetiche, dopo 26 giorni non sono più evidenziabili mediante tecnica "minimal residual disease" (MDR-PCR), anche quando presentavano concentrazioni iniziali elevate (40%).
- Dopo 52 giorni di coltura la positività di ZBTB16, UCHL1 e GPR125 (markers specifici degli spermatogoni) mediante RT-PCR confermava la presenza degli spermatogoni.



Struijk et al BioMed Res Intern, 2013

Review Article

Restoring Fertility in Sterile Childhood Cancer Survivors by Autotransplanting Spermatogonial Stem Cells: Are We There Yet?

2013

Robert B. Struijk, Callista L. Mulder, Fulco van der Veen, Ans M. M. van Pelt, and Sjoerd Repping

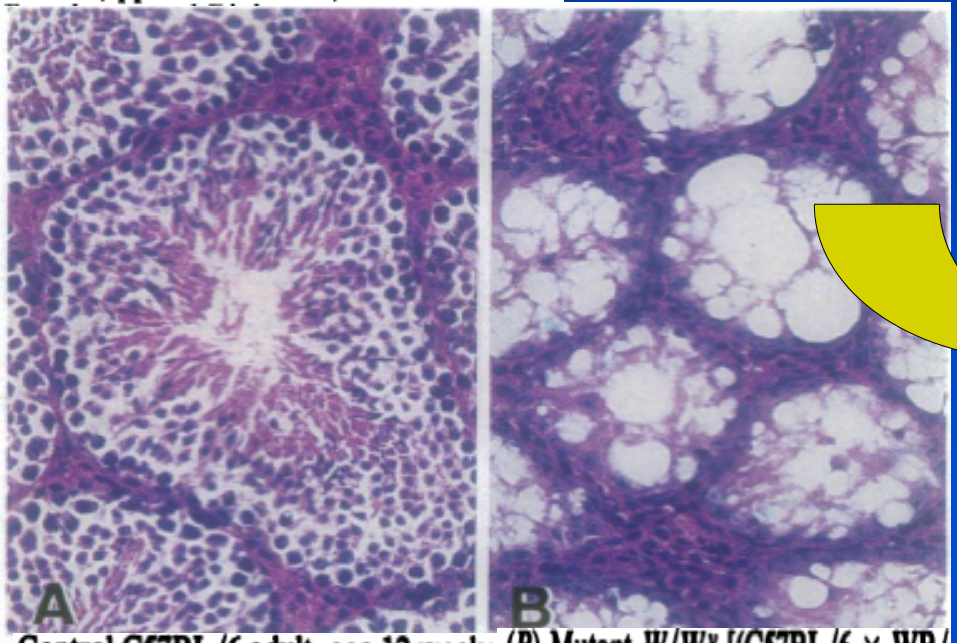
TABLE 1: Selected milestones in the history of spermatogonial stem cell research.

Year	Author	Highlighted findings	Species
1966	Clermont	Initial histological description of A_{pale} and A_{dark} spermatogonia	Human
1971	Huckins	Model for renewal and differentiation of spermatogonia and existence of "spermatogonial stem cells" (SSCs)	Rat
1994	Brinster and Avarbock	First successful transplantation of testis-derived cells from one mouse to another resulting in donor-derived F1 progeny	Mouse
1998	Nagano et al.	*In vitro* maintenance of SSCs for 4 months on a somatic feeder layer	Mouse
1999	Schlatt et al.	Xenotransplantation of primate testis cell suspensions from one primate into the testes of another	Macaque
2002	Nagano et al.	First report on successful colonization of mouse testes after xenotransplanting human SSCs	Human
2003	Kanatsu-Shinohara et al.	Prolonged *in vitro* propagation of SSCs using GDNF, without immortalization of the cells in culture	Mouse
2005	Keros et al.	Proof of successful cryopreservation of testicular biopsies without decreasing structural integrity	Human
2005	Kanatsu-Shinohara et al.	Long-term propagation of SSCs under serum free and feeder free conditions	Mouse
2009	Sadri-Ardekani et al.	Long-term propagation of adult SSCs *in vitro* with retainment of functionality	Human
2011	Sadri-Ardekani et al.	Long-term propagation of prepubertal SSCs with retainment of functionality	Human
2012	Hermann et al.	Production of functional sperm by infertile prepubertal macaques after autotransplantation, capable of fertilizing oocytes	Macaque

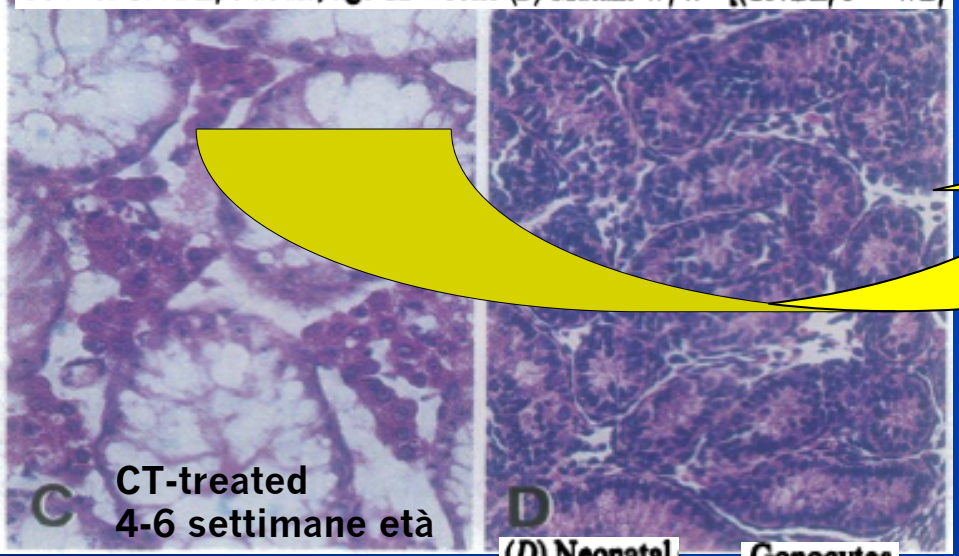
Spermatogenesis following male germ-cell transplantation

RALPH L. BRINSTER* AND JAMES W. ZIMMERMANN†

Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 11298–11302, November 1994



Control C57BL/6 adult, age 12 weeks (*B*) Mutant *W/W*^{v} [(C57BL/6 × WB/



CT-treated
4-6 settimane età

(*D*) Neonatal Gonocytes

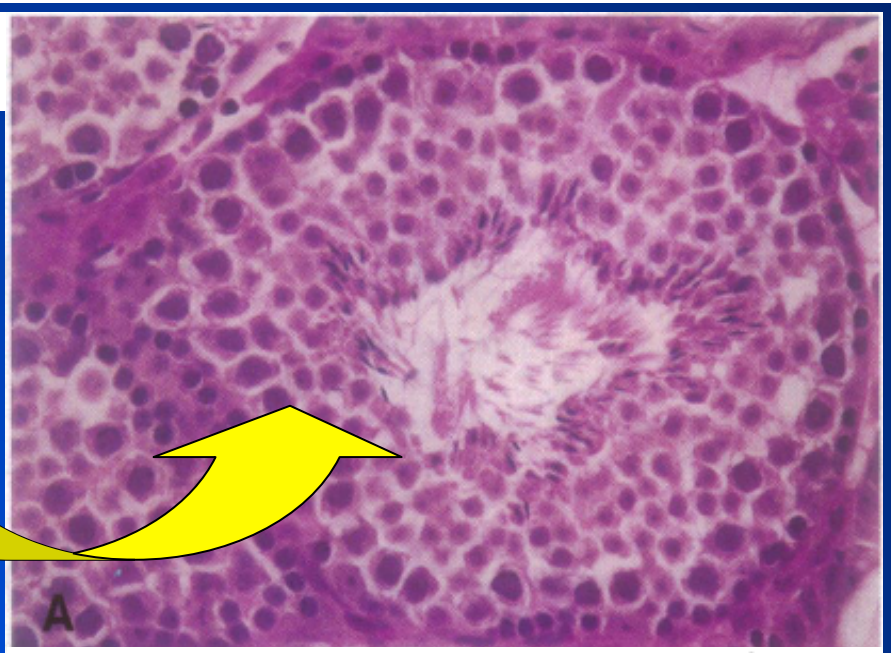
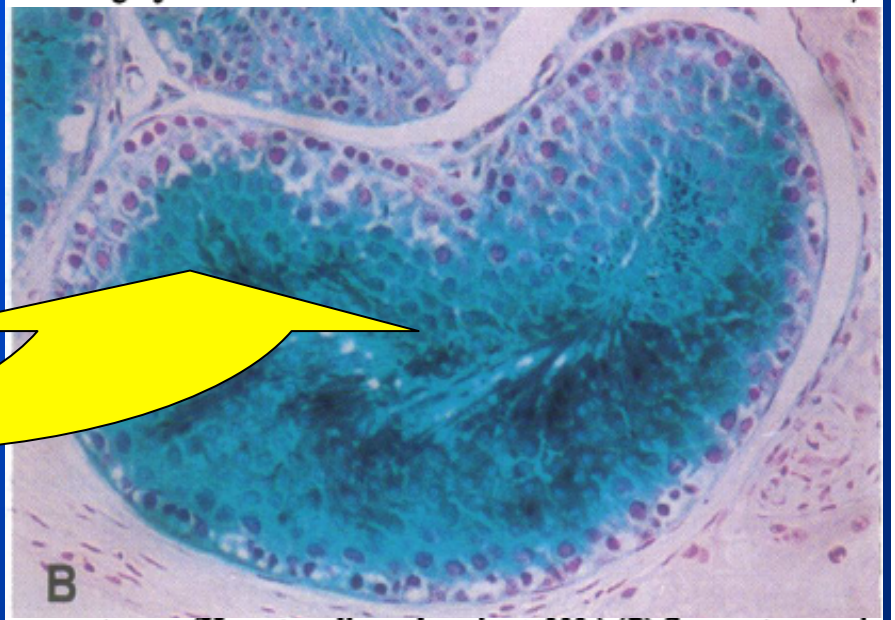


FIG. 5. (*A*) Spermatogenesis in the testes of a *W/W* mouse following injection of donor cells isolated from the testes of C57BL/6



spermatozoa. (Hematoxylin and eosin, ×300.) (*B*) Spermatogenesis in the testes of a busulfan-treated C57BL/6 × SJL male following injection of ZFlacZ donor cells. The testes of mice treated with

Review Article

Restoring Fertility in Sterile Childhood Cancer Survivors by Autotransplanting Spermatogonial Stem Cells: Are We There Yet?

2013

Robert B. Struijk, Callista L. Mulder, Fulco van der Veen, Ans M. M. van Pelt, and Sjoerd Repping

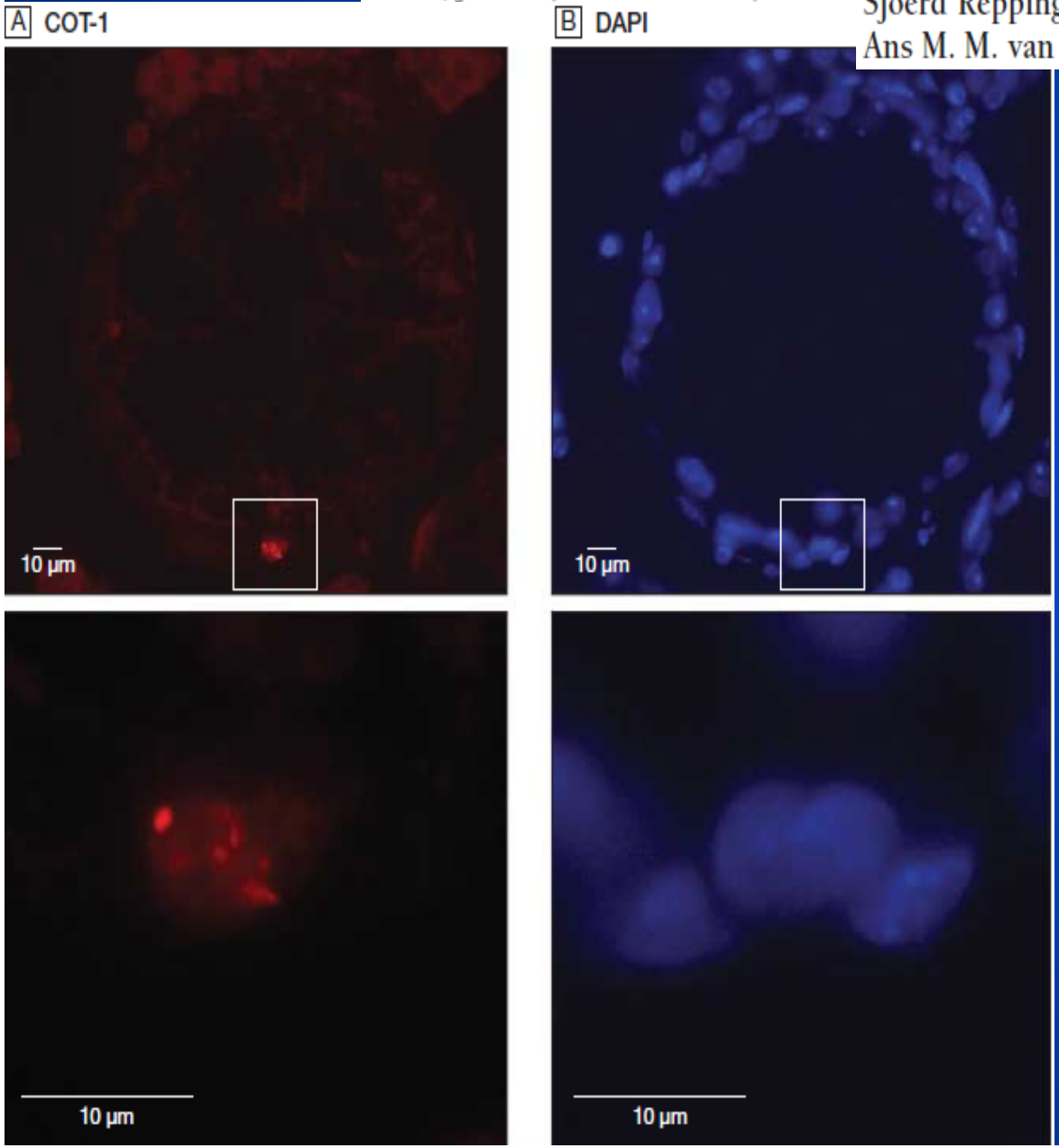
Table 1: Selected milestones in the history of spermatogonial stem cell research.

Year	Author	Highlighted findings	Species
1966	Clermont	Initial histological description of A_{pale} and A_{dark} spermatogonia	Human
1971	Huckins	Model for renewal and differentiation of spermatogonia and existence of "spermatogonial stem cells" (SSCs)	Rat
1994	Brinster and Avarbock	First successful transplantation of testis-derived cells from one mouse to another resulting in donor-derived F1 progeny	Mouse
1998	Nagano et al.	*In vitro* maintenance of SSCs for 4 months on a somatic feeder layer	Mouse
1999	Schlatt et al.	Xenotransplantation of primate testis cell suspensions from one primate into the testes of another	Macaque
2002	Nagano et al.	First report on successful colonization of mouse testes after xenotransplanting human SSCs	Human
2003	Kanatsu-Shinohara et al.	Prolonged *in vitro* propagation of SSCs using GDNF, without immortalization of the cells in culture	Mouse
2005	Keros et al.	Proof of successful cryopreservation of testicular biopsies without decreasing structural integrity	Human
2005	Kanatsu-Shinohara et al.	Long-term propagation of SSCs under serum free and feeder free conditions	Mouse
2009	Sadri-Ardekani et al.	Long-term propagation of adult SSCs *in vitro* with retainment of functionality	Human
2011	Sadri-Ardekani et al.	Long-term propagation of prepubertal SSCs with retainment of functionality	Human
2012	Hermann et al.	Production of functional sperm by infertile prepubertal macaques after autotransplantation, capable of fertilizing oocytes	Macaque

In Vitro Propagation of Human Prepubertal Spermatogonial Stem Cells

Hooman Sadri-Ardekani, MD
Mohammad A. Akhondi, PhD
Fulco van der Veen, MD, PhD
Sjoerd Repping, PhD
Ans M. M. van Pelt, PhD

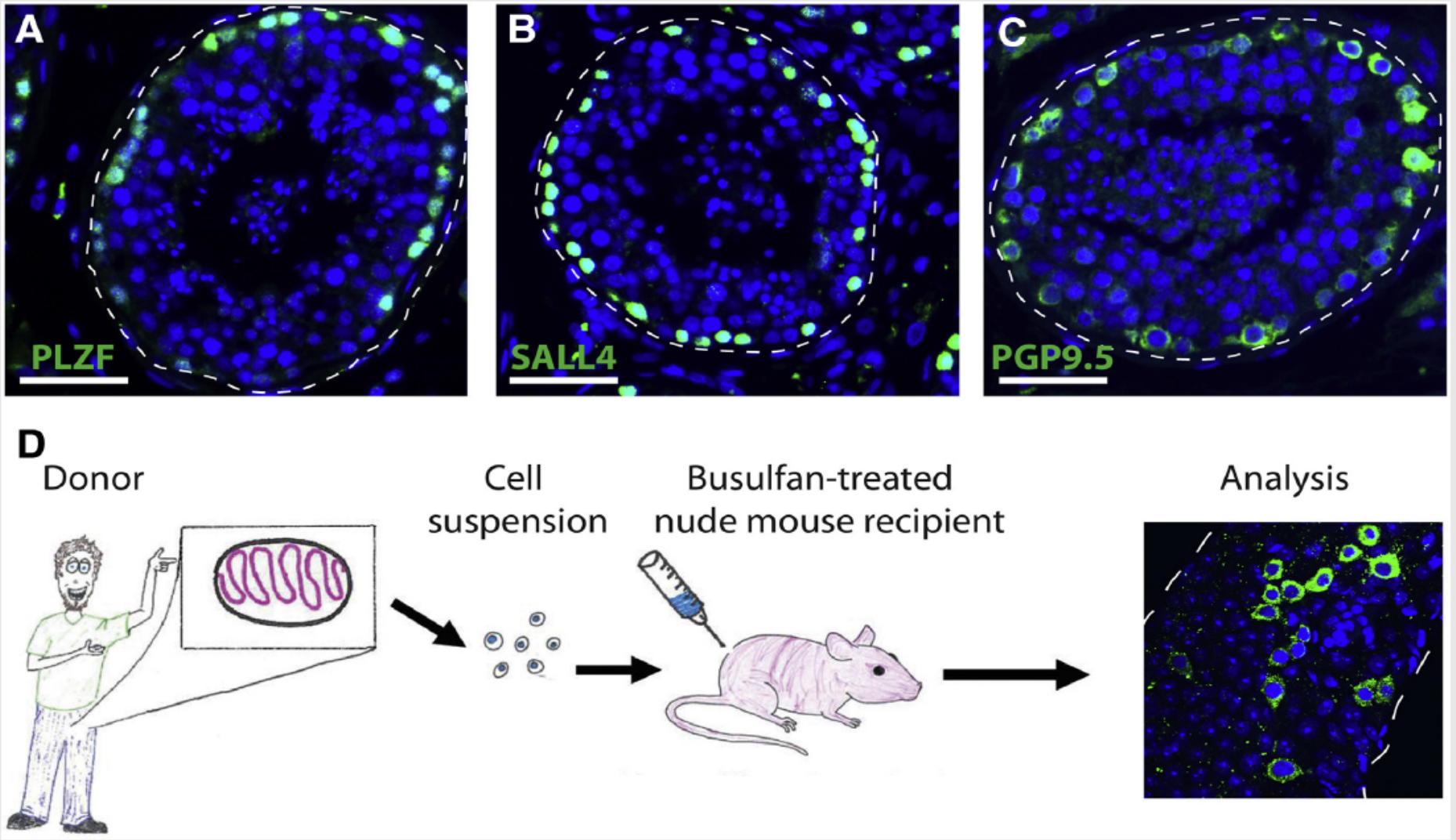
JAMA, June 15, 2011—Vol 305, No. 23



2 soggetti
6.5 anni
8 anni
Linfoma Hodgkin
Biopsia testicolare
Xenotrapianto
Topi trattati alchilant

- Le prime cellule staminali germinali (GSCs) compaiono dopo 2.5 settimane di coltura.
- SSCs isolate sono in grado di propagare in vitro fino a 20 settimane dopo lo xenotrapianto (soggetto di 6.5 anni) e dopo 15 settimane per il soggetto di 8 anni.
- Si stima che 35 giorni di coltura di SSCs o 58-83 giorni di coltura per GSCs siano necessari per raggiungere la crescita esponenziale di 1300 volte stimata come necessaria per ripopolare il testicolo umano adulto dopo l'autotrapianto

FIGURE 4



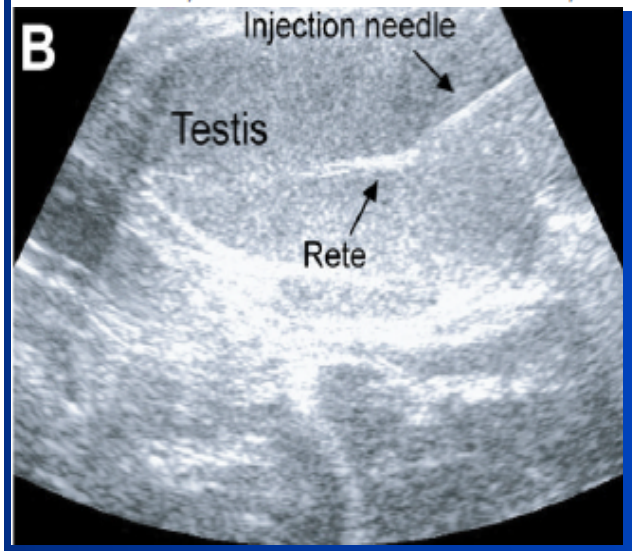
Experimental techniques to assay human spermatogonia. (A, B, and C) Expression of spermatogonia markers PLZF (A), SALL4 (B), and PGP9.5 (C) is limited to germ cells located on the basement membrane of human seminiferous tubules. Thus, they are reliable markers to screen test cell populations for human spermatogonia. DAPI (*blue*) stains all cell nuclei. Scale bar = 50 μm. (D) Human–to–nude mouse xenotransplantation assay. Human testicular tissue is made into a cell suspension and then transplanted into the testis of busulfan-treated infertile nude mice. Two months after the transplantation, the testes are recovered, the tunica is removed, and the seminiferous tubules are gently dispersed to make a whole mount. The tubules are then stained with antiprimate antibody (122) to recognize the colonies of human spermatogonia (*green*).

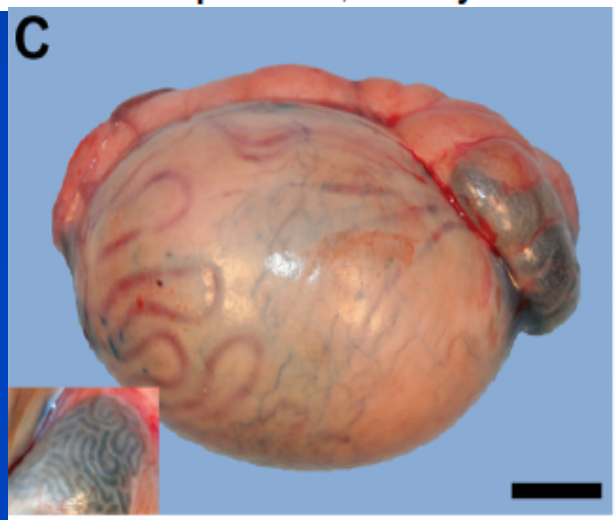
Valli. Stem cell therapies for male factor infertility. Fertil Steril 2014.

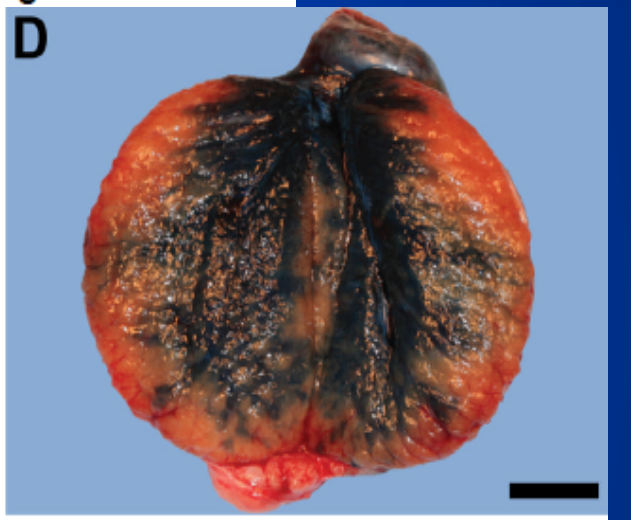
Spermatogonial stem cell transplantation into Rhesus testes regenerates spermatogenesis producing functional sperm

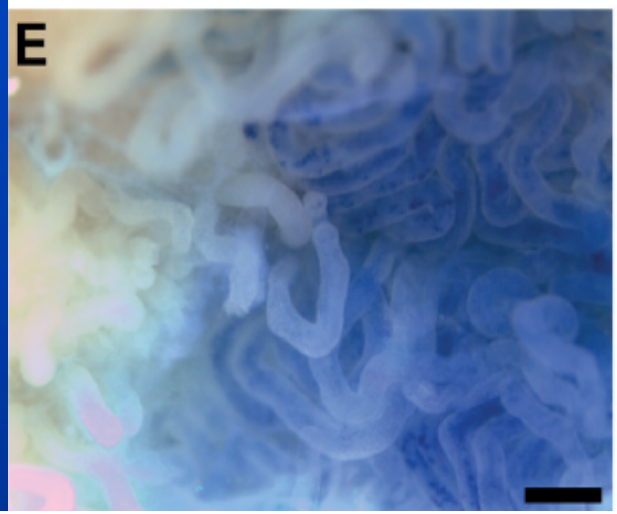
Brian P. Hermann[1,4,7,15], Meena Sukhwani[7], Felicity Winkler[7], Julia N. Pascarella[7], Karen A. Peters[7], Yi Sheng[1,7], Hanna Valli[6,7], Mario Rodriguez[7], Mohamed Ezzelarab[5], Gina Dargo[12], Kim Peterson[12], Keith Masterson[8], Cathy Ramsey[8], Thea Ward[11], Maura Lienesch[13], Angie Volk[13], David K. Cooper[5], Angus W. Thomson[5], Joseph E. Kiss[3,12], Maria Cecilia T. Penedo[11], Gerald P. Schatten[1,7], Shoukhrat Mitalipov[8,9,10], and Kyle E. Orwig[1,2,4,7,14]

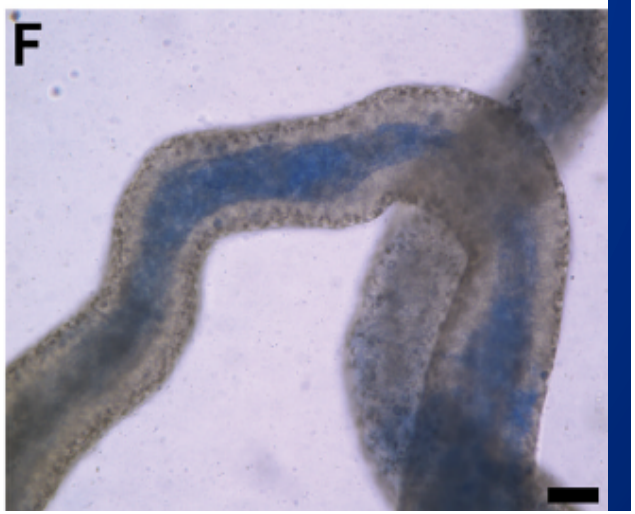
Cell Stem Cell 2012











SSCs del donatore sono reintrodotte nei tubuli seminiferi del testicolo residuo attraverso la rete testis. La loro presenza nel 60-80% dei tubuli seminiferi è confermata mediante blue dye

Spermatogonial stem cell transplantation into Rhesus testes regenerates spermatogenesis producing functional sperm

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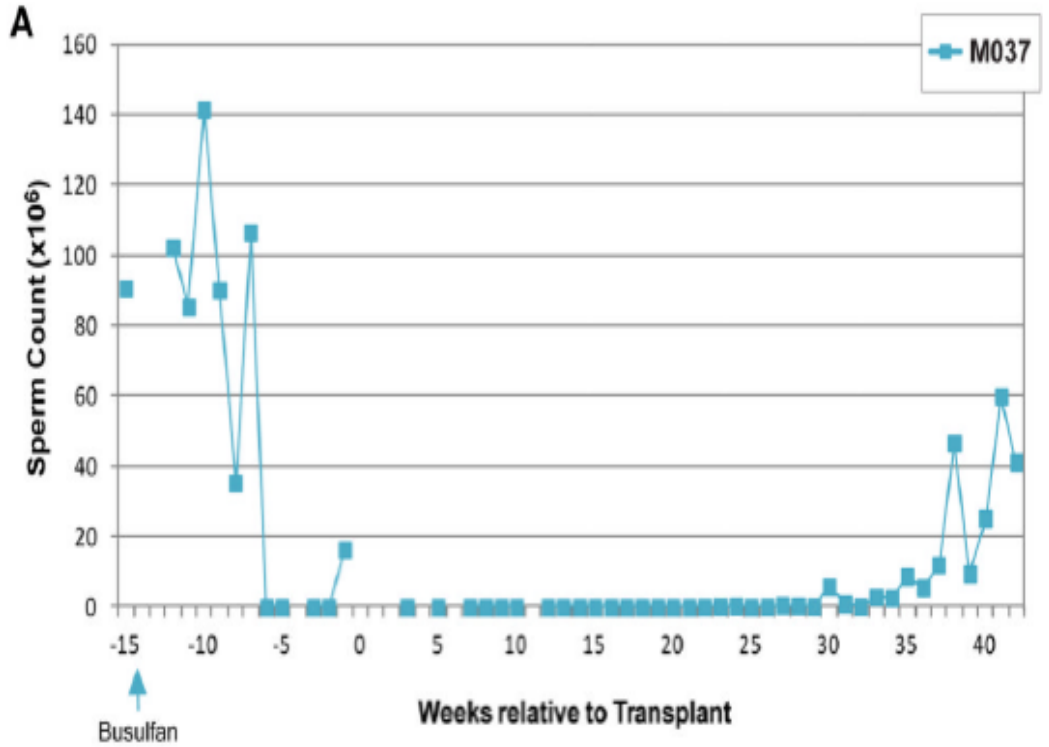
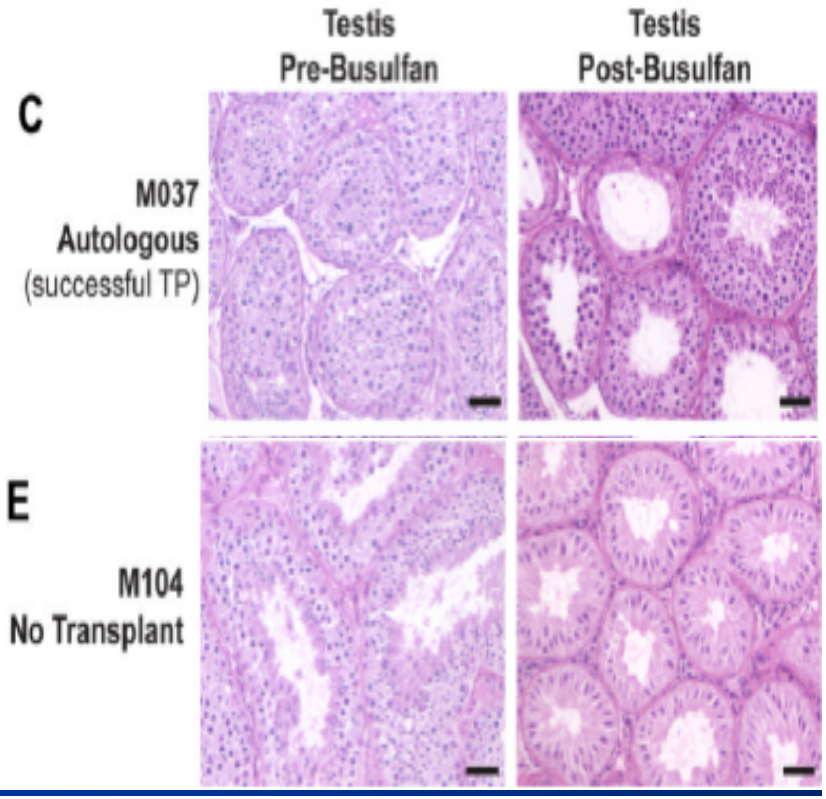
Cell Stem Cell 2012

TRAPIANTO AUTOLOGO
SSCs marcate con vettori lentivirus





Spermatozoi positivi al lentivirus si riscontrano
dalla 30° settimana fino a 63 settimane dopo autotrapianto in >75% (9/12) Rhesus adulti e

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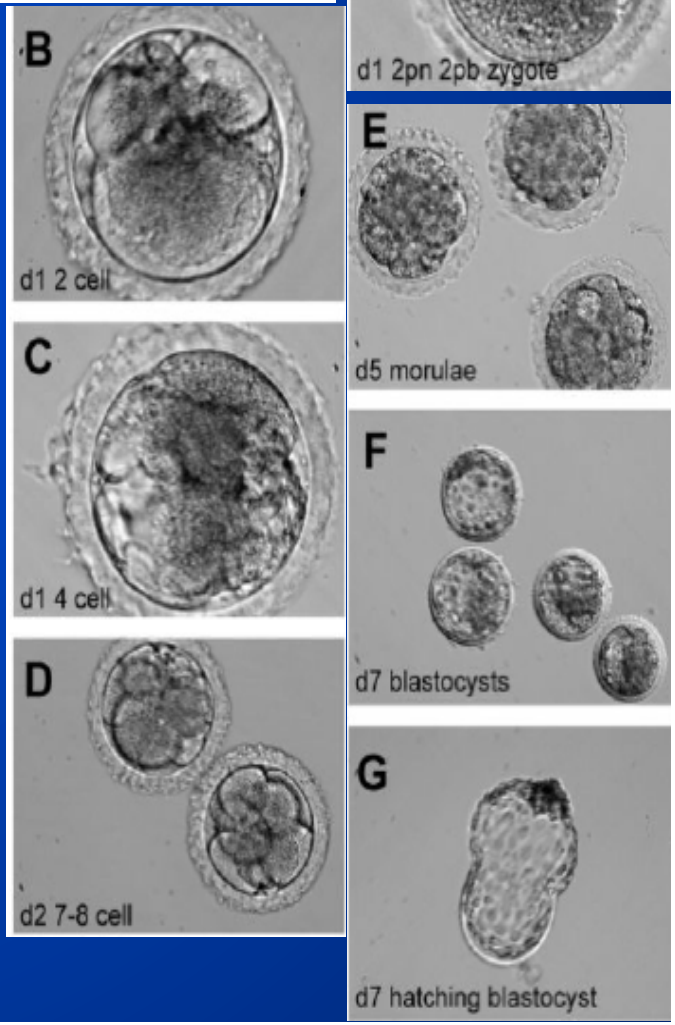
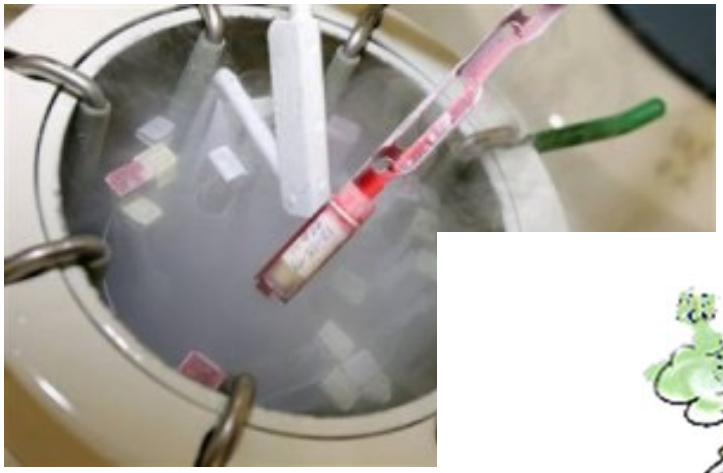
Cell Stem Cell 2012

- L'autotrapianto di SSCs ha avuto successo nel 70.5% dei casi mentre l'allotrapianto nel 33.3% dei casi in termini di recupero di spermatozoi nell'eiaculato
- La competenza e la funzione degli spermatozoi è stata dimostrata dalla fertilizzazione di ovociti Rhesus e dalla formazione di embrioni fino allo stadio di blastocisti





UOC Andrologia e Medicina della Riproduzione
Azienda Ospedaliera di Padova

GRAZIE PER L'ATTENZIONE!

"nothing of humanity was foreign to him"