Evidence tables male fertility preservation

Who should be informed about potential infertility risk?			
Gupta et al. Testicular biopsy for fertility preservation in prepubertal boys with cancer: Identifying preferences for procedure and reactions to disclosure practices Journal of Urology 2016; 196 (1):219-224			
Study design & Main study objective	**Participants and relevant characteristics**	**Relevant results (per outcome)**	**Additional remarks**
1. Study design Cross sectional multi-center study with in-depth interviews 2. Main study objective To measure and compare parent, male cancer survivor and health professional willingness to accept the risk of TBx in pre-pubertal boys and to identify reactions to disclosure practices regarding biopsy 3. Additional study characteristics, if relevant Interviews conducted between July 2012 and September 2013 'Threshold technique' used is clearly described in appendices and measured willingness to accept the risks associated with TBx with	1. Type and number of participants 153 parents of pre-pubertal boys with cancer 77 male survivors of childhood cancer 30 pediatric oncology health professionals 2. Age (at diagnosis) of participants Parents (age of their child at diagnosis): ≤12 years, median 4 years Survivors: ≤12 years, median 5 years Health providers: NA 3. Number of participants per diagnosis • Parents (diagnosis of their child): 106(69.3%) leukaemia/lymphoma: 11(7.2%) sarcoma 17(11.1%) brain tumour 19(12.4%) other • Survivors: 53(69.7%) leukaemia/lymphoma 10(13.2%) sarcoma 4(5.3%) brain tumour	1. Outcome(s) definition Outcome 1: Measure of desire for information about TBx for FP and Reactions to practices related to disclosure of information Outcome 2: Measure of relative willingness of each group to accept risk associated with TBx and Predictors of relative willingness 2. Results outcome 1 *Desire for information about testicular biopsy for fertility preservation* 90% survivors and 94% parents would have wanted information about testicular biopsy prior to commencement of therapy regardless of whether or not testicular biopsy was available at treating institution Parents reported the preference of having information about testicular biopsy regardless the risk of infertility 3. Results outcome 2 *Barrier to testicular tissue cryopreservation* Parents and patients perceived a >30% risk of infertility, a >25% chance of complications of testicular biopsy, a >$500 per year storage cost, and a >14% chance that	1. Strengths - Use of a novel approach to assessing the acceptability of an as yet experimental procedure that may meet needs otherwise unmet - 3 relevant subgroups of participants with good number in each - 3 institutions in Canada included in the study (multicenter) 2. Limitations - Lack of ethnic/cultural diversity in participant groups (identified by authors) - Risk of selection bias: number of those approached who declined to participate is given, but not reason for non-participation - Study undertaken outside of the 'real life' situation in which decisions around fertility

reference to 4 relevant considerations: • Risk of infertility • Risk of complications from bx • Likelihood of technology developing sufficiently to allow successful future use of tissue • Requirement for family to cover costs of storage of tissue until used In-depth interviews were also conducted with a subset of each participant group to explore information disclosure practices. Threshold technique followed by indepth guided interview of subgroup	9(11.8%) other • Health providers: NA <u>4. Additional participants characteristics, if relevant</u> - Parents: 38(24.8%) Male 103(67.3%) White 26(17%) Asian 5(3.3%) Hispanic 19(12.4%) Other 1. Survivors: Boys received at least 2 months of cancer therapy and either still receiving therapy, or post-therapy 62(80.5%) White 5(6.5%) Asian 2(2.6%) Hispanic 8(10.4%) Other 1. Healthcare providers: 15(50%) Female 29(96.7%) White 25(83%) physician 2(6.7%) NP 2(6.7%) RM 1(3.3%) SW	technology will evolve as barriers for testicular tissue cryopreservation Health professionals perceived a >29% risk of infertility, a >13.5% chance of complications, a >14% chance that that technology will evolve, and >$391 storage cost per year as barriers for testicular tissue cryopreservation <u>4. If applicable, results per additional outcomes</u> Predictors: - Survivors more likely to accept TBx with lower risk of infertility or lower chance of technology evolving as they aged (p= 0.05) - Greater household income associated with a lower minimum infertility risk (p= 0.05), and higher yearly costs (p= 0.04) - No demographic variables were associated with TBx desirability scores for HP Choose TBx vs. no biopsy overall: 110(72%) parents 52(67%) survivors 22(73%) HP	preservation are made (identified by authors) - Risk of interviewer induced bias - Risk of reporting bias

TBx: testicular biopsy; NA: not applicable; NP: nurse practitioners; SW: social worker; HP: health provider

Who should be informed about potential infertility risk?			
Gupta et al. Assessing information and service needs of young adults with cancer at a single institution: the importance of information on cancer diagnosis, fertility preservation,diet and exercise**.** Supportive Care in Cancer 2013; 21:2477-2484			
Study design & Main study objective	**Participants and relevant characteristics**	**Relevant results (per outcome)**	**Additional remarks**
1. Study design Single center cross-sectional (survey) study 2. Main study objective To identify the information and service needs of young adults with cancer to inform a program development 3. Additional study characteristics, if relevant Survey conducted November 2010 Adapted existing survey to use Likert Scale of importance (1-10) Study participants were asked how important it was to them to get information on a certain resource as part of a program	1. Type and number of participants 243 cancer patients receiving treatment, or within 5 years of completion of treatment 61.3% male 40.1% currently receiving cancer treatment 2. Age (at diagnosis) of participants NR Age at study: median 28 years (17-35 years) 3. Number of participants per diagnosis 23(9.5%) brain tumour 19(7.8%) breast, ovarian, cervical cancers 46(18.9%) leukaemia 69(28.4%) lymphoma 21(8.6%) sarcoma 40(16.5%) testicular cancers 25(10.5%) colon, other cancers 4. Additional participants characteristics, if relevant 162 (66.7%) Single/never married 68 (28%) Married/common-law	1. Outcome(s) definition Outcome 1: Importance of information on fertility effects from treatment and fertility preservation Outcome 2: Importance of information on treatments for infertility and other options for having children 2. Results outcome 1 and outcome 2 *Desire for information in fertility preservation discussion* Survey question: *Information about effects of cancer treatment on your ability to have children in the future and how to preserve your fertility before starting treatment* Median 10 (range 1-10); mean (SD) 8.77 (2.23) males: mean 8.45 (2.34) females: mean 9.28 (1.94) Survey question: *Information on treatment for infertility and other options for having children (i.e. artificial insemination, in vitro fertilization, surrogacy, adoption etc)* Median: 9 (range 1-10); mean (SD) 7.81 (2.85) males: mean (SD) 7.50 (2.90) females: mean (SD) 8.30 (2.72) 3. If applicable, results per additional outcomes - Females rated information on FP methods (p=0.004) and risk of infertility (p=0.033) as more important than did males	1. Strengths - The questionnaire used was an existing published item, adapted to reflect the study site, and piloted - Participants were representative of a wide range of diagnoses relevant in this age group, and of both on and off treatment groups 2. Limitations - Single center study (results have low external validity) - Risk of selection bias: no information on how the 243 patients were selected - Convenience sample (survey administered to those attending ambulatory care centre of Canadian adult tertiary cancer centre) - No report of ethnicity of participants - Actual questions of survey not included in report

for young adult cancer survivors, or have it included in the program Fertility items were 2 out of 18 questions Item responses averaged for entire sample	49 (20.3%) Have existing children	- Presence of existing children did not significantly impact on importance of information regarding risk to fertility from cancer treatment (p=0.65) - Those who had completed active therapy showed a trend towards rating receiving information about fertility as more important than those on active treatment p=0.052	- Risk of reporting bias (as use of survey)

NR: not reported; SD: standard deviation; FP: fertility preservation; SD: standard deviation

Who should be informed about potential infertility risk?			
Wyns et al. Fertility preservation in the male pediatric population: factors influencing the decision of parents and children. Hum Reprod 2015;30:2022-30.			
Study design & Main study objective	**Participants and relevant characteristics**	**Relevant results (per outcome)**	**Additional remarks**
1. Study design Cross sectional study Single-center survey from Belgium 2.Main study objective To critically analyse the multidisciplinary collaborative care pathway (MCCP) in the pediatric population,	1. Type and Number of Participants Prepubertal boys and adolescents aged 0-18 years diagnosed with cancer between May 2005 and May 2013. Eligible patients: 348 of which 120 returned questionnaire; only 78 questionnaires included responses to Part 2.	1. Outcome definitions • Factors influencing the FP decision • Feelings of patients and their parents, with a view to better fulfilling their expectations 2. Results Response by patients, parents or both: - Parents considered their child (91.4% of adolescents and 26.2% of children aged <12 years, but >7yr) capable of understanding and participating in the decisional process - Reasons for not understanding and participating in the decision process were immaturity of the child (5.7%), poor general health (2.9%)	1. Strengths A large study. Closed-ended questionaire followed by response options to minimize random errors in the data collection process and allow quantitative interpretation. 2. Limitations Recall bias due to the to the time interval between the actual FP procedure and the survey. Single-center survey, thus does not allow generalizability of results to other places.

focusing on factors influencing the decision, and to elucidate and characterize the feelings of patients and their parents, with a view to better fulfilling their expectations. 3. Study years May 2005 to May 2013 4. Follow-up Mean ±SD 3.4 ± 2.3 years (ie) Time from diagnosis to the time of the survey was	Parents gave their answers for 22 patients under 12 years of age and 3 patients aged 12–18yrs 2. Age at diagnosis Mean ± SD: 6.05 ± 3.74 years (range 0.1–143 months) for boys aged <12 yr 14.41 ± 1.5 years (range 144–212 months) for boys aged 12–18 yr 3. Number of responded participants per diagnosis Acute lymphoblastic Leukemia 33(27.7%) Acute myeloid leukemia 2(1.68%) Non-Hodgkin's lymphoma 13 (10.9%%) Hodgkin's lymphoma 6(5.0%) Medulloblastoma 3 (2.5%) Nephroblastoma 4 (3.3%) Neuroblastoma 9 (7.6%) Osteosarcoma 9 (7.6%) Retinoblastoma 7 (5.9%) Ewing's sarcoma 6 (5.0%) Rhabdomyosarcoma 6 (5.0%)	- No discrepancy between patient and parent decisions was noted, indicating that decisions were essentially made jointly - Information was provided mainly by 64/78 (82%) oncologist, 7/78 (8.9%) GP, 5/78 (6.4%) specialist and 2/78 (2.5%) by nurses; Although nurse support was limited in this study, it appeared to be relevant for 16.6% of adolescents Emotional state of parents during discussion of FP (barrier) - 52% of adolescents and 23.5% of children felt anxious at the time of discussion - Reasons were concern about future fertility, rather than the method of FP, 46% of boys aged 12–18 years considered the FP method challenging because of poor general health, lack of experience with masturbation and its taboo or embarrassing nature - 76% of children and 48% of adolescents considered their health to be more important than the ability to have a family - Family support was considered important for 75% of adolescents and 58% of children, and medical support was considered important for 50% of adolescents and 42% of children; Nursing support was relevant for 16.6% of adolescents. Understanding information: (facilitator) - Majority of boys aged >12 years reported information to be clear (72%), complete (80%) and understandable (90.9%) - Only 33.3% of boys aged <12 years were able to comprehend the information, the youngest being 11 years old (although, respectively, 71.4 and 57.9% of subjects found it to be complete and clear) Satisfaction with information: - 19% was not satisfied with the fertility preservation information content (completeness)	There is no availability of preexisting validated questionnaires or gold standard for this type of study. 3. Risk of bias 1. Selection bias: high risk Reason: 120/348 (34.5%) eligible patients returned their questionnaires (44 patients died, 14 lost to fup, 8 declined to participate, some did not return their questionnaire.) 2. Attrition bias: High risk Reason: A total of 78/120 (65%) gave information on FP issues and have responded to questions on communication, emotional state and perceptions during discussion of FP, reasons for refusal etc. 3. Detection bias: Unclear Reason: Unclear if outcome assessors were blinded. 4. Confounding: High risk Reason: did only bivalent analysis. Thus did not adjust for confounders.

	Hepatoblastoma 4(3.4%) Brain tumor 7 (5.9%) Astrocytosis 2(1.68%) Ependymoma 1(0.8%) Benign pathologies 7 (5.9%) <u>4. Additional patients characteristics, if relevant</u> 42 patients (35%) did not receive information on FP issues	<u>Acceptance and refusal rate: (barrier)</u> - One-third of the patients lack information about FP options when seen by the oncologist - FP acceptance rates were 74% for boys aged <12 and 78.6% for boys 12-18 years - 6/78 (7.7%) adolescents and 13/78 (16.7%) children under the age of 12 years refused to undergo FP procedures - Reasons for refusal were the urgency of cancer treatment, diminished general health, the FP procedure not being a priority or the experimental status of FP before puberty - Wishing to avoid an additional procedure was not an issue for FP acceptance - Satisfaction about completeness of information provided to patients and parents positively impact decision to preserve fertility ($p=0.04$) - Hope for future parenthood positively impact decision to preserve fertility ($p<0.01$) - Timing of FP information, healthcare provider who proved the FP information and anxiety were not significantly associated with decision to preserve fertility	

Who should be informed about potential infertility risk?			
Quinn et al. Fertility Preservation and Adolescent/Young Adult Cancer Patients: Physician Communication Challenges. J Adolesc Health 2009;44(4):394-400			
Study design & Main study objective	**Participants and relevant characteristics**	**Relevant results (per outcome)**	**Additional remarks**
1. Study design Cross sectional study with qualitative semistructured in-depth interviews 2. Main study objective To examine barriers experienced by physicians in discussing cancer-related fertility issues with patients aged 12-18yrears 3. Additional study characteristics, if relevant - Study used a subset of data from a larger study examining knowledge, attitudes, and behaviors of pediatric oncologists - All interviews were tape recorded and transcribed. The transcripts were read	1. Type and number of participants 24 Pediatric oncologists working in 15 clinics in Florida (US) *Response rate*: 41% participated (59 asked to participate) 2. Age (at diagnosis) of participants NA 3. Number of participants per diagnosis NA 4. Additional participants characteristics, if relevant NA	1. Outcome(s) definition Outcome 1: Healthcare system barriers Outcome 2: Perception of parent/patient desire for FP information Outcome 3: Awareness of FP resources Outcome 4: Patient characteristics that may impact FP discussions Outcome 5: Issues unique to adolescent patients 2. Results outcome 1: - Perceptions that the financial costs of FP were too high for most families (FP not covered by insurance) - Combination of lack of resources and lack of training or guidelines for having discussions 3. Results outcome 2: - About half of physicians said the cancer diagnosis is such a shock that an issue like fertility is often put on the ”back burner” - Other half thought that parents and teens do want this information but are either to embarrassed to discuss it or have no background on the topic and do not know how to begin a discussion 4. Results outcome 3: - One third of physicians were aware of sperm banking facilities	1. Strengths Provides information on barriers to discussing FP in pediatric oncology, implying that new methods of communication between all parties must be examined and utilized 2. Limitations - Results cannot be generalized to other pediatric hematology/oncology physicians or other populations - Authors state that interview may have limited the amount of in-depth discussion on any one topic - Risk of selection bias: responders more interested in the topic and more likely to engage in discussions about and/or encourage FP might have been participants - Risk of interviewer induced bias

through and the content analyzed through intuitive analysis. Key themes were identified - Author used theoretical saturation, in which each new participant we recruited refined new theoretical constructs. Midway in the data analysis we ascertained no new information was emerging; thus, we perceived we had reached theoretical saturation and made no further attempts to recruit additional physicians		- Remainder said their facility had no FP resources or they were unaware of resources for females (except oophoroypexy) - Physicians typically had low levels of knowledge about resources to refer patients to for FP procedures or consultations - Few pediatric oncologsist reported that the nationally distributed educational brochure they used was not always relevant to the local level and needed improvement <u>5. Results outcome 4:</u> - Most were comfortable in a general sense - However, many experienced barriers related to patient specific diagnosis or socioeconomic situation (ranged from perceived cultural or religious differences to knowing a family could not afford FP) <u>6. Results outcome 5:</u> - All found that it is an important issue to address for teens who have reached puberty - Most agreed that these conversations were awkward because resources were usually limited and there was a fine line between establishing a sense of trust with the patient, while not excluding parents - Conversations about fertility were related to issues of sexuality, and this was a source of embarrassment for both the patient and parents <u>4. If applicable, results per additional outcomes</u>	

NA: not applicable; FP: fertility preservation

Who should be counselled about fertility preservation?				
van Beek et al. Inhibin B is superior to FSH as a serum marker for spermatogenesis in men treated for Hodgkin lymphoma with chemotherapy during childhood. Human Reprod 2007;22:3215-3222				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Multi-centre cohort study 2. Treatment era 1974-1998 3. Follow-up Median 15.5 (5.6-30.2) years after treatment	1. Type and number of participants 56 male survivors of childhood Hodgkin lymphoma 2. Diagnoses 56 (100%) Hodgkin lymphoma 3. Age at diagnosis Median 11.4 (3.7-15.9) years 4. Age at follow-up Median 27.0 (17.7-42.6) years 5. Controls No controls	1. Chemotherapy - ABVD or EBVD: 56 (100%); Adriamycin 25mg/m^2 or epirubicin 30 mg/m^2, bleomycin 10 mg/m^2, vinblastine 6 mg/m^2, dacarbazine 250 mg/m^2 (days 1 and 8) - MOPP: 40 (71.4%) of whom 14 (25.0%) 3-4 cycles, 26 (46.4%) ≥6 cycles; Mechlorethamine 6mg/m^2(days 1 and 8), vincristine 2 mg/m^2 (days 1 and 8), prednisone 40 mg/m^2/day (days 1 – 14), procarbazine 100 mg/m^2/day (days 1 – 14) 2. Radiotherapy - Involved field irradiation: 7 (12.5%) - Abdominal irradiation: 0 (0.0%) - Pelvic irradiation: 0 (0.0%)	1. Outcome definitions - Oligozoospermia: sperm cell density <20 x 10^6/mL - Severe oligozoospermia: sperm cell density <5 x 10^6/mL - Decreased inhibin B: <150 ng/L - Increased FSH: >7.0 U/L 2. Results Sperm concentration (n=21 assessed): - MOPP$^-$: 49.1 (28-63) x 10^6/mL (n=4) - MOPP$^+$: 1.1 (0-72) x 10^6/mL (n=17) p<0.05 - MOPP$^-$: 0/4 (0.0%) azoospermia or oligozoospermia (all 4 normospermia) - MOPP$^+$: 9/17 (52.9%) azoospermia; 1/17 (5.9%) oligozoospermia; 3/17; (17.6%) severe oligozoospermia Inhibin B (n=38 assessed): - MOPP$^-$: 144.0 (93.0-274.0) ng/L (n=12) - MOPP$^+$: 16.5 (0.0-173.0) ng/L (n=26) p<0.01 FSH (n=56 assessed): - MOPP$^-$: 3.0 (1.7-6.0) U/L (n=16) - MOPP$^+$: 16.8 (1.3-51.0) U/L (n=40) p<0.01	1. Remarks 3 men reported 5 spontaneously conceived pregnancies. 2 men treated without MOPP each fathered 1 child. 1 man treated with 6 MOPP cycles fathered 1 child and reported 2 spontaneous abortions. 2. Risk of bias A. Selection bias High risk Reason: 56/100 (56%) eligible patients included in this study, however, there were no differences in age, disease characteristics and treatment between the included 56 male survivors and the 44 not included. B. Attrition bias High risk Reason: 21/56 (37.5%) of patients had sperm concentrations assessed. C. Detection bias Unclear

			Risk factors for decreased sperm concentration in multivariable analysis: - Number of MOPP cycles: beta -6.25 ($p<0.05$) - Age at diagnosis: beta -6.18 ($p<0.05$) - Number of EBVD/ABVD cycles, radiotherapy (mantle or mediastinal), puberty at diagnosis, presence of B-symptoms and follow-up duration ($p>0.05$) (no effect measures reported) Risk factors for decreased inhibin B in multivariable analysis: - Number of MOPP cycles: beta -21.59 ($p<0.05$) - Other factors not significant Risk factors for increased FSH in multivariable analysis: - Age at diagnosis: beta 1.4 ($p<0.05$) - Number of MOPP cycles: beta 2.57 ($p<0.01$) - Other variables not significant	Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome. D. Confounding Low risk Reason: Multiple linear regression analyses were performed, including number of MOPP cycles, age at diagnosis, number of EBVD/ABVD cycles, radiotherapy (mantle or mediastinal), puberty at diagnosis, presence of B-symptoms and follow-up duration.

Abbreviations: FSH, follicle-stimulating hormone.

Who should be counselled about fertility preservation?				
Brignardello et al. Gonadal status in long-term male survivors of childhood cancer. J Cancer Res Oncol 2016;142:1127-32.				
Study design Treatment era Years of follow-up	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Retrospective cohort study	1. Type and number of participants 199 male CCS aged <18 yr at cancer diagnosis	1. Chemotherapy - Any: 187 (94.0%) - Alkylating agents: 147 (73.9%)	1. Outcome definitions - Hypogonadism: testosterone <3.0 ng/dl; further subclassified as primary or central, depending on	1. Strengths Cohort size, long follow-up duration. 2. Limitations

2. Treatment era 1985-2007 3. Follow-up Median 14.01 (IQR 10.08-17.76) yr	with ≥1 clinic visit after age 18 yr 2. Diagnoses Hematological malignancies n=145 (ALL n=72, HL n=40, NHL n=21, AML n=12) brain tumours n=28, sarcomas n=15, other n=11 3. Age at diagnosis 0-4 yr: n=45; 5-10 yr: n=57; 10-18 yr: n=97 4. Age at follow-up >18 yr 5. Controls No controls	- Alkylating agents and platinum agents: 23 (11.6%) 2. Radiotherapy - Any: 125 (62.8%) - TBI: 33 (16.6%) - Cranial: 38 (19.1%) 3. Surgery Number patients treated with surgery not given, but in discussion mentioned that some were treated with surgical excision 4. Other treatments HSCT: 48 (24.1%)	gonadotropin levels (no further information reported) - Spermatogenesis damage: FSH >10.0 UI/l and inhibin B <100.0 pg/ml 2. Results Abnormal gonadal function: - Normal gonadal function: 102/194 (52.6%) - Spermatogenesis damage: 68/199 (34.2%) and confirmed in 41 patients in whom semen analysis was performed - Primary hypogonadism: 16/199 (8.0%) - Secondary hypogonadism: 13/199 (6.5%) - 33/33 (100%) treated with TBI had abnormal gonadal function: spermatogenesis damage n=17 primary hypogonadism n=13 central hypogonadism n=3 - 46/48 (95.8%) treated with HSCT had abnormal gonadal function Risk factors for spermatogenesis damage and primary hypogonadism in multivariable logistic regression analysis: - Alkylating + platinum agents vs. alkylating agents only: OR 9.22 (95% CI 2.17-39.23) - Other chemotherapy or none vs. alkylating agents only: OR 0.19 (95% CI 0.05-0.76) - Any radiation vs. none: OR 8.72 (95% CI 3.94-19.30)	- It is not stated how many participants were treated with both radiotherapy and chemotherapy. The result that ”the risk of gonadal dysfunction was higher in patients treated with radiotherapy” may be biased. - Definitions of primary or central hypogonadism are not given. - Testosterone assay not described, time of assessment not given (Testosterone fluctuates during the day and may give false negative results when assessed in afternoon and not twice). - Primary hypogonadism (n=16) will always result in impaired spermatogenesis (n=68). When considering spermatogenesis damage the results are displayed incorrectly. - Reference values for normal semen analysis not given. - Assumption bias: men with FSH >10 U/l and inh B <100 ng/l levels may have compensated spermatogenesis and may father children without assisted reproduction. Even so, the prognostic value for sperm concentration/progressive motility and morphology for fecundity is poor. Result without semen analysis parameters should be interpreted with caution. - Cohort unclear: total cohort 199, in text endocrine levels available at last visit n=194, Table 2 cohort n=186; gonadal dysfunction n=84 (elsewhere described as n=92): unclear.

			- Period of first cancer diagnosis 1990-1999 vs. 1985-1989: OR 1.48 (95% CI 0.43-5.10) - Period of first cancer diagnosis 2000-2007 vs. 1985-1989: OR 1.24 (95% CI 0.32-4.87) - Age at cancer diagnosis 5-9 vs. 0-4 yr: OR 1.08 (95% CI 0.40-2.93) - Age at cancer diagnosis ≥10 vs. 0-4 yr: OR 0.64 (95% CI 0.25-1.68) - Brain tumours vs. hematological malignancies: OR 0.98 (95% CI 0.36-2.63) - Sarcomas vs. hematological malignancies: OR 3.69 (95% CI 1.11-12.22) - Other tumours vs. hematological malignancies: OR 1.13 (95% CI 0.33-3.89)	3. Risk of bias A. Selection bias Unclear Reason: "all patients referred to the transition unit for CSS in Turin, Italy" the protocol for referral is not described: it is unclear if pre-selection for referral was made. B. Attrition bias Low risk Reason: of the referred eligible cohort only 11 males were lost to follow up and in at least 194 patients reproductive hormone levels were available (97.5%). C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome. D. Confounding Low risk Reason: analyses were adjusted for age at cancer diagnosis, period of cancer diagnosis and treatment.

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CCS, childhood cancer survivors; IQR, inter quartile range; HL, Hodgkin lymphoma; HSCT, hematopoietic stem cell transplantation; NHL, non-Hodgkin lymphoma; TBI, total body irradiation.

Who should be counselled about fertility preservation?				
Chemaitilly et al. Leydig Cell Function in Male Survivors of Childhood Cancer: A Report From the St Jude Lifetime Cohort Study. J Clin Oncol 2019;37:3018-3031.				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Single-center cohort study 2. Treatment era 1970-2002 3. Follow-up Median 22.0 (range 7.5-49.8) years since diagnosis	1. Type and number of participants 1,516 adult male CCS aged ≥18 years who survived ≥5 years since diagnosis 2. Diagnoses Leukaemia 520 (34.3%), lymphoma 337 (22.2%), bone and soft tissue sarcomas 223 (14.7%), Wilms tumour 85 (5.6%), CNS tumour 148 (9.8%), neuroblastoma 68 (4.5%), retinoblastoma 45 (3.0%), carcinomas 23 (1.5%), germ cell tumour 20 (1.3%), other 47 (3.1%) 3. Age at diagnosis Range 0-≥15 years 4. Age at follow-up Mean 33.8 ± 9.2 years; Median 30.8 (range 18 1-63.8) years	1. Chemotherapy - Alkylating agents: 898 (59.2%) - CED >0-<4,000 mg/m^2: 133 (8.8%) - CED ≥4,000-<8,000 mg/m^2: 269 (17.7%) - CED ≥8,000-<12,000 mg/m^2: 245 (16.2%) - CED ≥12,000 mg/m^2: 251 (16.6%) 2. Radiotherapy - Testicular radiotherapy: 123 (8.1%) - 0-11.9 Gy: 65 (4.3%) - 12-19.9 Gy: 39 (2.6%) - ≥20 Gy: 19 (1.3%) 3. Surgery Unilateral orchiectomy: 35 (2.3%); Survivors with a bilateral orchiectomy were excluded from the study	1. Outcome definitions - Leydig cell failure: morning serum levels of total testosterone < 250 ng/dL (or 8.67 nmol/L) and LH > 9.85 IU/L - Leydig cell dysfunction: morning serum levels of total testosterone ≥ 250 ng/dL and LH > 9.85 IU/L 2. Results Leydig cell function at most recent SJLIFE visit vs. controls: - Point prevalence Leydig cell failure: 104 (6.9%; 95% CI 5.6%-8.2%) vs. 8 (4.8%; 95% CI 1.5%-8.0%); p=0.30 - Point prevalence Leydig cell dysfunction: 223 (14.7%; 95% CI 13.0%-16.5%) vs. 4 (2.4%; 95%CI 0.1%-4.7%); p>0.001 Risk factors for Leydig cell failure in multivariable logistic regression analysis: - Testicular radiation dose >0-11.9 Gy vs. none: OR 3.1; 95% CI 1.4-7.2 (p=0.007) - Testicular radiation dose 12-19.9 Gy vs. none: OR 97.3; 95% CI 29.2-323.6 (p<0.001) - Testicular radiation dose ≥20 Gy vs. none: OR 220.0; 95% CI 26.0-1,858.8 (p<0.001)	1. Remarks Participants with hormone levels indicative of Leydig cell failure before replacement and those without pretreatment laboratory data but whose medical records specifically documented Leydig cell failure as the reason for treatment were considered to have Leydig cell failure. These found associations of identified risk factors and Leydig cell failure remained significant after the exclusion of nine survivors who were receiving treatment for Leydig cell failure but lacked laboratory data supporting the diagnosis. 2. Risk of bias A. Selection bias High risk Reason: Out of 2,880 potentially eligible male survivors 1,701 (59.1%) agreed to participate in the study. B. Attrition bias Low risk

	5. Controls 168 age- and sex-matched community controls recruited among friends and non–first degree relatives of current and former patients		- CED >0->4,000 mg/m^2 vs. none: OR 0.5; 95% CI 0.2-1.7 (p=0.28) - CED 4,000-<8,000 mg/m^2 vs. none: OR 3.4; 95% CI 1.7-6.8 (p<0.001) - CED 8,000-<12,000 mg/m^2 vs. none: OR 2.9; 95% CI 1.4-6.0 (p=0.005) - CED ≥12,000 mg/m^2 vs. none: OR 5.6; 95% CI 2.8-10.9 (p<0.001) - Unilateral orchiectomy yes vs. no: OR 2.4; 95% CI 0.5-10.7 (p=0.25) - Age at diagnosis 5-9.9 years vs. 0-4.9 years: OR 1.8; 95% CI 1.0-3.3 (p=0.06) - Age at diagnosis 10-14.9 years vs. 0-4.9 years: OR 1.1; 95% CI 0.6-2.2 (p=0.73) - Age at diagnosis ≥15 years vs. 0-4.9 years: OR 0.8; 95% CI 0.4-1.8 (p=0.66) - Age at study 26-35.9 years vs. 18-25.9 years: OR 2.5; 95% CI 1.1-5.7 (p=0.026) - Age at study 36-45.9 years vs. 18-25.9 years: OR 3.7; 95% CI 1.6-8.6 (p=0.003) - Age at study ≥46 years vs. 18-25.9 years: OR 5.3; 95% CI 2.0-13.6 (p<0.001) - Non-Hispanic black ethnicity vs. non-Hispanic white ethnicity: OR 1.8; 95% CI 1.0-3.4 (p=0.06) - Other ethnicity vs. non-Hispanic white ethnicity: OR 1.3; 95% CI 0.4-4.5 (p=0.69) - Body mass index <18.5 kg/m^2 vs. ≥18.5-24.9 kg/m^2: OR 1.1; 95% CI 0.2-5.5 (p=0.90) - Body mass index 25-29.9 kg/m^2 vs. ≥18.5-24.9 kg/m^2: OR 1.1; 95% CI 0.6-2.0 (p=0.82) - Body mass index ≥30 kg/m^2 vs. ≥18.5-24.9 kg/m^2: OR 1.8; 95% CI 1.0-3.3 (p=0.06)	Reason: All patients had an outcome assessment. <u>C. Detection bias</u> Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome. <u>D. Confounding</u> Low risk Reason: important prognostic factors (i.e. age at diagnosis, age at assessment and gonadotoxic treatment) were taken adequately into account.

			<u>Risk factors for Leydig cell dysfunction in multivariable logistic regression analysis:</u> - Testicular radiation dose >0-11.9 Gy vs. none: OR 2..0; 95% CI 1.0-3.8 (p=0.05) - Testicular radiation dose 12-19.9 Gy vs. none: OR 17.7; 95% CI 5.8-50.1 (p<0.001) - Testicular radiation dose ≥20 Gy vs. none: OR 38.9; 95% CI 4.2-358.8 (p=0.001) - CED >0->4,000 mg/m^2 vs. none: OR 0.8; 95% CI 0.3-1.7 (p=0.52) - CED 4,000-<8,000 mg/m^2 vs. none: OR 3.3; 95% CI 2.0-5.3 (p<0.001) - CED 8,000-<12,000 mg/m^2 vs. none: OR 3.4; 95% CI 2.1-5.5 (p=0.005) - CED ≥12,000 mg/m^2 vs. none: OR 6.4; 95% CI 4.0-10.0 (p<0.001) - Unilateral orchiectomy yes vs. no: OR 4.1; 95% CI 1.7-9.5 (p=0.01) - Age at diagnosis 5-9.9 years vs. 0-4.9 years: OR 1.4; 95% CI 0.9-2.1 (p=0.15) - Age at diagnosis 10-14.9 years vs. 0-4.9 years: OR 1.3; 95% CI 0.8-2.0 (p=0.27) - Age at diagnosis ≥15 years vs. 0-4.9 years: OR 1.3; 95% CI 0.8-2.1 (p=0.23) - Age at study 26-35.9 years vs. 18-25.9 years: OR 1.7; 95% CI 1.1-2.7 (p=0.028) - Age at study 36-45.9 years vs. 18-25.9 years: OR 1.9; 95% CI 1.1-3.3 (p=0.014) - Age at study ≥46 years vs. 18-25.9 years: OR 3.0; 95% CI 1.6-5.3 (p<0.001) - Non-Hispanic black ethnicity vs. non-Hispanic white ethnicity: OR 1.5; 95% CI 1.0-2.3 (p=0.07)	

			- Other ethnicity vs. non-Hispanic white ethnicity: OR 1.2; 95% CI 0.5-2.7 (p=0.74) - Body mass index <18.5 kg/m^2 vs. ≥18.5-24.9 kg/m^2: OR 1.6; 95% CI 0.7-3.6 (p=0.26) - Body mass index 25-29.9 kg/m^2 vs. ≥18.5-24.9 kg/m^2: OR 0.6; 95% CI 0.4-0.9 (p=0.007) - Body mass index ≥30 kg/m^2 vs. ≥18.5-24.9 kg/m^2: OR 0.5; 95% CI 0.3-0.8 (p=0.002) Among 683 prospectively followed survivors, progression from normal function to Leydig cell dysfunction or Leydig cell failure (n=25) was significantly associated with higher CEDs (p=0 .025)	

Abbreviations: CCS, childhood cancer survivors; CED, cyclophosphamide equivalent dose.

Who should be counselled about fertility preservation?				
Green et al. Cumulative alkylating agent exposure and semen parameters in adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. Lancet Oncol 2014;15:1215-1223				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Single-center cohort study 2. Treatment era 1970-2002 3. Follow-up Median 21.0 years since diagnosis (10.5-41.6)	1. Type and number of participants 214 adult male CCS treated with alkylating agent therapy, but no radiotherapy, vasectomy or bilateral orchiectomy 2. Diagnoses ALL 70 (33%), AML 5 (2%), NHL 53 (25%), HL 2 (1%), NB 26 (12%), osteosarcoma 32 (15%), other 26 (12%) 3. Age at diagnosis Median 7.7 (0.01-20.3) yr 4. Age at follow-up Median 29.0 (18.4-56.1) yr 5. Controls No controls	1. Chemotherapy - Alkylating agents: 214 (100%) - Cyclophosphamide: 195 (91.1%) - Ifosfamide: 26 (12.1%) - Procarbazine: 2 (0.9%) - Chlormethine (mechlorethamine): 1 (0.5%) - Chlorambucil: 1 (<1%) - Busulfan: 3 (1.4%) - Cyclophosphamide equivalent dose (CED): Median 7,400 (1,000-41,311) mg/m^2 - Cisplatin/carboplatin: 44 (20.6%) - Dacarbazine: 3 (1.4%) 2. Radiotherapy 0 (0%) 3. Surgery Bilateral orchiectomy: 0 (0%)	1. Outcome definitions - Azoospermia: sperm concentration 0 - Oligospermia: sperm concentration >0 - <15 x 10^6/mL - Normospermia: sperm concentration ≥15 x 10^6/mL 2. Results Spermatogenesis and CED: - Azoospermia: 53 (24.7%); mean CED 10,830 mg/m^2 (SD 7,274) - Oligospermia: 59 (27.6%); mean CED 8,480 mg/m^2 (SD 4,264) - Normospermia: 102 (47.7%); mean CED 6,626 mg/m^2 (3,576) - Correlation CED and sperm concentration: r=-0.37 (p<0.0001) Risk factors for azoospermia as compared to normospermia in multinomial logistic regression analysis: - CED per 1,000 mg/m^2: OR 1.22; 95% CI 1.11-1.34 (p<0.0001) - Age at diagnosis per years: OR 0.97; 95% CI 0.91-1.05 (p=0.45) - Age at assessment per years: OR 0.99, 95% CI 0.94-1.05 (p=0.8)	1. Remarks Unable to identify threshold dose (substantial overlap). Impaired spermatogenesis unlikely when CED <4,000 mg/m^2 (88.6% normospermia in when CED <4,000 mg/m2). 2. Risk of bias A. Selection bias High risk Reason: Of the 549 men eligible, 226 (41%) agreed to participate; demographic and treatment characteristics were not equally distributed among the participants and non-participants. B. Attrition bias Low risk Reason: Of the 226 men who agreed to participate, 214 (94.7%) produced semen specimen. C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for

			Risk factors for oligospermia as compared to normospermia in multinomial logistic regression analysis: - CED per 1,000 mg/m^2: OR 1.14; 95% CI 1.04-1.25 (p=0.006) - Age at diagnosis per years: OR 0.95; 95% CI 0.89-1.02 (p=0.13) - Age at assessment per years: OR 0.97, 95% CI 0.92-1.03 (p=0.28)	important determinants related to the outcome. D. Confounding Low risk Reason: important prognostic factors (i.e. age at diagnosis and age at assessment) were taken adequately into account.

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CED, cyclophosphamide equivalent dose; HL, Hodgkin lymphoma; NB, neuroblastoma; NHL, non-Hodgkin lymphoma; OR, odds ratio; 95% CI, 95% confidence interval; SD, standard deviation.

Who should be counselled about fertility preservation?				
Green et al. Effect of cranial radiation on sperm concentration of adult survivors of childhood acute lymphoblastic leukaemia: a report from the St. Jude Lifetime Cohort Study. Human Reproduction 2017;32:1192-1201.				
Study design Treatment era Years of follow-up	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Single-center cohort study 2. Treatment era 1970-2002 3. Follow-up CRT: Mean 26.3 ± 6.3 yr No CRT: Mean 18.7 ± 6.0 yr	1. Type and number of participants 241 adult male childhood ALL survivors treated with alkylating agent therapy, but no vasectomy, patients receiving androgen replacement therapy, testicular radiation or CRT >26 Gy 2. Diagnoses 241 (100%) ALL 3. Age at diagnosis CRT: Mean 6.6 ± 4.4 yr	1. Chemotherapy - Cyclophosphamide: 241 (100%) - Mechlorethamine: 1 (0.4%) - Cyclophosphamide equivalent dose (CED) (mg/m^2) CRT patients: >0-<4000: 7 (7.7%) ≥4000-8000: 17 (18.7%) ≥8000 -12000: 50 (54.9%) ≥12000: 17 (18.7%) - CED (mg/m^2) no CRT patients: >0-<4000: 10 (12.2%) ≥4000-8000: 18 (22.0%) ≥8000 -12000: 50 (61.0%) ≥12000: 4 (4.9%) 2. Radiotherapy	1. Outcome definitions - Azoospermia: sperm concentration 0 - Oligospermia: sperm concentration >0 - <15 x 10^6/mL - Normospermia: sperm concentration ≥15 x 10^6/mL 2. Results Spermatogenesis (n=173): - Normospermia: 62 (35.8%) CRT: 32 (35.2%) No CRT: 30 (36.6%) - Azoospermia: 65 (37.6%) - Oligospermia: 46 (26.6%)	1. Remarks Large cohort study. 2. Risk of bias A. Selection bias High risk Reason: Of the 380 men eligible, 241 (63.4%) participated; treatment characteristics were not equally distributed among the participants and non-participants. B. Attrition bias High risk Reason: Of the 241 men who agreed to participate, 173

	No CRT: Mean 7.5 ± 5.0 yr 4. Age at follow-up CRT: Mean 32.9 ± 7.8 yr No CRT: Mean 26.2 ± 5.6 yr 5. Controls No controls	Hypothalamic-pituitary radiation >0-20 Gy: 81 (33.6%) >20-26 Gy: 53 (22.0%) 3. Surgery Bilateral orchiectomy: 0 (0%)	Risk factors for azoospermia or oligospermia in univariable log-binominal regression analysis: - CED (mg/m^2) ≥4000-8000 vs. >0-<4000: RR 1.46 (95% CI 0.71-2.99) - CED (mg/m^2) ≥8000-1200 vs. >0-<4000: RR 1.98 (95% CI 1.03-3.82) - CED (mg/m^2) ≥12000 vs. >0-<4000: RR 2.29 (95% CI 1.17-4.51) - CED per 1000 mg/m^2: RR 1.01 (95% CI 1.00-1.02) - CRT >0-20 Gy vs. 0: RR 0.99 (95% CI 0.70-1.28) - CRT ≥20-26 Gy vs. 0: RR 1.09 (95% CI 0.81-1.46) - Age at diagnosis (yr) 5-9 vs. <4: RR 1.3 (95% CI 1.05-1.61) - Age at diagnosis (yr) ≥10 vs. <4: RR 0.92 (0.69-1.23) - Age at semen analysis (yr) 26-35 vs. 18-25: RR 0.82 (95% CI 0.60-1.04) - Age at semen analysis (yr) ≥35 vs. 18-25: RR 0.94 (95% CI 0.69-1.26) - Time from diagnosis to semen analysis per yr: RR 1.00 (95% CI 0.98-1.01) Risk factors for azoospermia or oligospermia in multivariable log-binominal regression analysis: - CED (mg/m^2) ≥4000-8000 vs. >0-<4000: RR 1.42 (95% CI 0.70-2.89) - CED (mg/m^2) ≥8000-1200 vs. >0-<4000: RR 2.06 (95% CI 1.08-3.94) - CED (mg/m^2) ≥12000 vs. >0-<4000: RR 2.12 (95% CI 1.09-4.12) - Age at diagnosis (yr) 5-9 vs. <4: RR 1.3 (95% CI 1.05-1.61)	(71.8%) underwent semen analysis. C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome. D. Confounding Low risk Reason: important prognostic factors (i.e. age at diagnosis and age at assessment) were taken adequately into account (only the significant factors in univariable analysis were included in the multivariable analysis).

			- Age at diagnosis (yr) ≥10 vs. <4: RR 0.92 (0.69-1.23)	

Abbreviations: ALL, acute lymphoblastic leukaemia; CED, cyclophosphamide equivalence dose; CRT, cranial radiotherapy.

Who should be counselled about fertility preservation?				
Isaksson et al. High risk of hypogonadism in young male cancer survivors. Clinical Endocrinology 2018;88:432-441				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Multi-centre cohort study 2. Treatment era 1970-2002 3. Follow-up Mean 24.3 (±7.1) years after treatment	1. Type and number of participants 125 male childhood cancer survivors 2. Diagnoses 27(22%) leukaemia, 28 (22%) intracranial tumour, 21 (17%) lymphoma, 6 (4.8%) testicular cancer, 8 (6.4%) Wilms tumour, 6 (4.8%) bone tumour, 29 (23%) other 3. Age at diagnosis Median 9.6 (5.4-15.0) years 4. Age at follow-up Median 33.7 (30.2-40.1) years 5. Controls	1. Treatment subgroups - Chemotherapy: 29 (23%) of whom 16 (13%) alkylating agents - Cyclophosphamide equivalent dose (CED) >4000 mg/m^2: 10 (8.0%) - Radiotherapy to testes: 5 (4.0%) - Cranial radiotherapy: 12 (9.6%) - Cranial radiotherapy and chemotherapy: 16 (13%) - Other radiotherapy: 5 (4.0%) - Other radiotherapy and chemotherapy: 23 (18%) - Brain surgery: 15 (12%) - Surgery other than brain surgery: 15 (12%)	1. Outcome definitions Biochemical hypogonadism: - Primary hypogonadism: total testosterone <10 nmol/L, LH and FSH both >10 IU/L with FSH > LH or total testosterone <10 nmol/L, LH ≤10 IU/L and FSH >10 IU/L - Secondary hypogonadism: total testosterone <10 nmol/L, LH and FSH both ≤10 IU/L - Compensated hypogonadism: total testosterone ≥10 nmol/L, LH >10 IU/L - Or ongoing androgen replacement therapy 2. Results Mean (SD) testosterone levels childhood cancer survivors vs. controls: - Total testosterone: 15.4 (6.22) vs. 15.5 (6.01), p=0.87 - Free testosterone: 0.313 (0.104) vs. 0.311 (0.099), p=0.86	1. Remarks Treatment subgroup other radiotherapy and chemotherapy: 12/21 (57%) received alkylating agents with a median CED of 9593 mg/m^2. One case received high-dose chemotherapy followed by autologous BMT. 2. Risk of bias A. Selection bias High risk Reason: 125/427 (29%) eligible survivors included in this study. B. Attrition bias Low risk Reason: 121/125 (97%) of included survivors had an outcome measure. C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for

	125 age-matched controls from the Swedish Population Register		<u>Hypogonadism childhood cancer survivors (n=121 assessed) vs. controls (n=122 assessed):</u> - 31 (26%) vs. 17 (14%) - OR 2.1 (95% CI 1.1-4.1) - Survivors: primary hypogonadism n=7, secondary hypogonadism n=9, compensated hypogonadism n=2, ongoing testosterone replacement therapy n=13 - Controls: primary hypogonadism n=1, secondary hypogonadism n=16 <u>Risk factors for hypogonadism in bivariate logistic regression analysis (childhood cancer survivors vs. controls):</u> - Chemotherapy: OR 1.1 (95% CI 0.34-3.8) - CED >4000 mg/m^2: OR 2.0 (95% CI 0.36-11.0) - Radiotherapy to testes: OR 28.0 (95% CI 2.9-279.0) - Cranial radiotherapy: OR 4.4 (95% CI 1.1-18.0) - Cranial radiotherapy and chemotherapy: OR 1.7 (95% CI 0.42-6.7) - Other radiotherapy: OR 0.92 (95% CI 0.09-9.5) - Other radiotherapy and chemotherapy: OR 3.7 (95% CI 1.3-10.0) - Brain surgery: OR 0.94 (95% CI 0.19-4.7) - Surgery other than brain surgery: OR 1.0 (95% CI 0.26-3.9)	important determinants related to the outcome. <u>D. Confounding</u> Low risk Reason: survivors matched to controls.

Who should be counselled about fertility preservation?				
Jahnukainen et al. Semen quality and fertility in adult long-term survivors of childhood acute lymphoblastic leukemia. Fertility and Sterility 2011; 96(4): 837-42.				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Single-centre cohort study 2. Treatment era 1970-1995 3. Follow-up: Median 20 (11-30) years after treatment	1. Type and number of participants 51 male ALL survivors 2. Diagnoses 51 (100%) ALL 3. Age at diagnosis Median 5 (1-15) yr 4. Age at follow-up Median 29 (26-38) yr 5. Controls 56 age-matched males median 30 (25-36) yr	1. Chemotherapy Cyclophosphamide: 26 (51.0%) 1-6 g/m2: 13 (25.5%) 7-10 g/m2: 8 (15.7%) >20 g/m2: 5 (9.8%) 2. Radiotherapy Cranial radiation: 38 (74.5%) 18 Gy: 5 (9.8%) 24 Gy: 31 (60.8%) >24 Gy: 2 (3.9%) Testicular radiation: 18 (35.3%) 10 Gy: 2 (3.9%) 24 Gy: 16 (31.4%) Spinal radiation (6 Gy): 1 (2.0%) 3. Surgery 0 (0%)	1. Outcome definitions - Semen analysis - Hormone levels: testosterone, LH, FSH 2. Results Median (IQR) sperm concentration (10^6/mL) CCS vs. controls: - Controls (n=56): 50 (27-66) - No cyclophosphamide, no testicular irradiation (n=16): 41 (29-74); p>0.05 - ≤10 g/m^2 cyclophosphamide, no testicular irradiation (n=12): 35 (24-42); p>0.05 - >20 g/m^2 cyclophosphamide, no testicular irradiation (n=5): 1 (0-17); p<0.05 - Testicular irradiation (n=10) ± cyclophosphamide (n=8): 0; p<0.05 - Same results for total sperm count - No significant differences in percentage of motile sperm and morphologic normal sperm, semen volume Median (IQR) testosterone levels (pmol/L) CCS vs. controls: - Controls (n=56): 18.4 (14.7-24.0) - No cyclophosphamide, no testicular irradiation (n=16): 18.3 (13.6-20.1); p>0.05	1. Strengths - Semen analysis - Control group 2. Limitations - Small sample size especially when separating by treatment. - Multiple treatments in a given group make it difficult to link a given predictor to outcomes. 3. Risk of bias A. Selection bias: High risk Reason: Of 164 treated for ALL, only 77 were alive and 2 moved. 51/77 enrolled (66.2%). B. Attrition bias Low risk Reason: 47/51 (92.2%) survivors provided semen. C. Detection bias: Unclear Reason: unclear if the outcome assessors were blinded for determinants related to outcomes

			- ≤10 g/m^2 cyclophosphamide, no testicular irradiation (n=12): 12.7 (12.2-16.6); p<0.05 - >20 g/m^2 cyclophosphamide, no testicular irradiation (n=5): 13.4 (7.7-17.5); p<0.05 - Testicular irradiation (n=10) ± cyclophosphamide (n=8): 1.4 (0.9-8.9); p<0.05 <u>Median (IQR) LH levels (IU/L) CCS vs. controls:</u> - Controls (n=56): 3.3 (2.8-4.2) - No cyclophosphamide, no testicular irradiation (n=16): 3.5(2.7-4.6); p>0.05 - ≤10 g/m^2 cyclophosphamide, no testicular irradiation (n=12): 3.8 (3.0-4.5); p>0.05 - >20 g/m^2 cyclophosphamide, no testicular irradiation (n=5): 5.3 (2.6-7.0); p>0.05 - Testicular irradiation (n=10) ± cyclophosphamide (n=8): 6.4 (5.6-14.6); p<0.05 <u>Median (IQR) FSH levels (IU/L) CCS vs. controls:</u> - Controls (n=56): 3.2 (1.9-4.1) - No cyclophosphamide, no testicular irradiation (n=16): 2.5 (2.1-4.2); p>0.05 - ≤10 g/m^2 cyclophosphamide, no testicular irradiation (n=12): 4.7 (2.6-7.4); p<0.05 - >20 g/m^2 cyclophosphamide, no testicular irradiation (n=5): 11.1 (5.5-20.8); p<0.05	<u>D. Confounding:</u> Low risk Reason: survivors matched to controls

			- Testicular irradiation (n=10) ± cyclophosphamide (n=8): 10.8 (5.0-25.0); p<0.05	

Abbreviations: ALL, acute lymphoblastic leukaemia; CCS, childhood cancer survivors; FSH, follicle stimulating hormone; IQR, inter quartile range; LH, luteinizing hormone.

Who should be counselled about fertility preservation?				
Lopez Andreu et al. Persistent altered spermatogenesis in long-term childhood cancer survivors. Pediatr Hematol Oncol 2000;17:21-30				
Study design Treatment era Years of follow-up	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Single-centre cohort study 2. Treatment era Treatment era not mentioned 3. Follow-up Mean 13.6 (3.9-25.2) yr after treatment	1. Type and number of participants 43 male CCS >16 years of age at follow-up 2. Diagnoses ALL (n=21), AML (n=1), NB (n=8), GNB (n=1), GN (n=2), Wilms' tumour (n=9), mesoblastic nephroma (n=1) 3. Age at diagnosis Range 0.0-9.4 years 4. Age at follow-up Mean 20.2 (16.1-30.6) years 5. Controls 15 healthy volunteers aged ≤30 years; 373 patients aged ≤30 years consulting for infertility	1. Chemotherapy Cyclophosphamide: 9 (20.9%); 1200-27,200 mg/m^2 2. Radiotherapy - Cranial irradiation: 14 (32.6%); 18-46 Gy - Spinal irradiation: 3 (7.0%); 20-24 Gy (also treated with cranial radiation) - Testicular irradiation: 1 (2.3%); 30 Gy (also treated with cranial and spinal radiation) - Abdominal irradiation: 2 (4.7%); 30 Gy	1. Outcome definitions - Moderate oligozoospermia: sperm cell density 10-19 x 10^6/mL - Severe oligozoospermia: sperm cell density <10 x 10^6/mL - Moderate asthenozoospermia: 20-39% progressive motility - Severe asthenozoospermia: <20% progressive motility - Infertile: azoospermia or severe oligo-asthenozoospermia 2. Results Spermatogenesis: - Azoospermia: 8 (18.6%) - Moderate oligozoospermia: 1 (2.3%) - Severe oligozoospermia: 0 (0.0%) - Moderate asthenozoospermia: 8 (18.6%) - Severe asthenozoospermia: 3 (7.0%) - Moderate oligo-asthenozoospermia: 2 (4.6%) - Severe oligo-asthenozoospermia: 2 (4.6%) - Moderate oligozoospermia + severe asthenozoospermia: 3 (7.0%)	1. Remarks A forward-stepping logistic regression analysis was performed. In addition to cumulative cyclophosphamide dose, FSH level was included as an independent factor. Testicular volume was excluded from the model. Radiotherapy and follow-up duration were not included in the model. 1 survivor was on replacement therapy after 30 Gy testicular radiation and high-dose cyclophosphamide. 2. Risk of bias A. Selection bias: Unclear Reason: Unclear how many patients were included in the original cohort of survivors.

			- Infertile: 10 (23.2 %) <u>Risk factors for infertility in multivariable analyses:</u> - Cumulative cyclophosphamide dose significant (no effect measure reported) - FSH level significant (no effect measure and p-value reported) <u>Univariable results cyclophosphamide:</u> - 5/9 (55.6%) treated with cyclophosphamide azoospermic and 1/9 (11.1%) severe oligozoospermic - Cumulative cyclophosphamide dose negatively correlated with sperm count: r=-0.43 (p=0.004) - Cumulative cyclophosphamide dose negatively correlated with sperm motility: r=-0.45 (p=0.002) <u>Univariable results cranial radiotherapy:</u> - No correlation with sperm count, sperm motility, testicular volume, FSH level	<u>B. Attrition bias:</u> Low risk Reason: 90% of the eligible survivors were evaluated. <u>C. Detection bias:</u> Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome. <u>D. Confounding</u> High risk: Reason: Models did not include age, radiation therapy, other chemo agents, therapy duration/follow-up time.

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; FSH, follicle-stimulating hormone; GNB, ganglioneuroblastoma; GN, ganglioneuroma; NB, neuroblastoma.

Who should be counselled about fertility preservation?				
Mackie et al. Gonadal function following chemotherapy for childhood Hodgkin's disease. Med Pediatr Oncol 1996;27:74-78				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design National multi-centre trial cohort UK CCSG study 2. Treatment era Not reported 3. Follow-up Median 6 (2.5-11.1) years from diagnosis	1. Type and number of participants 58 postpubertal male survivors of childhood Hodgkin disease treated according to UKCCSG HD Trial 8201 2. Diagnoses 58 (100%) Hodgkin disease 3. Age at diagnosis Mean 12.2 (8.2-15.3) years 4. Age at follow-up Not reported 5. Controls No controls	1. Chemotherapy ChlVPP: 58 (100%); 6-8 cycles; Chlorambucil 504-672 mg/m^2, vinblastine, prednisolone, procarbazine 8,400-11,200 mg/m^2 2. Radiotherapy below diaphragm 0 (0.0%) 3. Surgery 0 (0.0%)	1. Outcome definitions: - Leydig cell dysfunction: LH >10 IU/L Testosterone <10 nmol/L - Abnormal gonadotropin levels: FSH >10 IU/L 2. Results Leydig cell dysfunction (↑ LH) (n=41 assessed): 10 increased (24.4%); range of these increased LHs, 10.3-18 IU/L ↓ testosterone levels (n=37 assessed): 5 decreased (13.5%); range of all testosterones, 4.3-37.8 nmol/L ↑ FSH levels (n=46 assessed): 41 increased (89.1%); range of increased FSHs, 10.8-40.7 IU/L Risk factors for Leydig cell dysfunction in multivariable regression analyses: - Amount of chemotherapy, NS - Age at treatment, NS - Follow-up duration, NS (no effect measures reported) Risk factors for ↑ FSH in multiple regression analyses:	1. Remarks - Relatively narrow range of cumulative doses - LH and testosterone only described clearly in survivors with increased FSH levels. - Note concerns about outcome definitions: Leydig cell dysfunction based on high LH rather than low testosterone; o Cutoff of 10 may be too high o High LH definition not appropriate if patient received cranial RT (not specified in this paper albeit unlikely) - Azoospermia present in 7 survivors in whom semen analyses were performed. All progressed spontaneously through puberty. - Unclear how "amount of chemotherapy" is defined. - Multiple regression performed for gonadotropins and Leydig cell dysfunction. However, methodology of testosterone analysis not clear. 2. Risk of bias A. Selection bias High risk

			- Age or pubertal status at time of treatment, NS - Follow-up duration, NS (no effect measures reported)	Reason: 101 out of 168 (60.1%) eligible male and female CCS included in this study. <u>B. Attrition bias</u> High risk Reason: 41 out of 58 (70.7%) CCS had LH measured and 37 out of 58 (63.8%) had testosterone measured <u>C. Detection bias</u> Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome. <u>D. Confounding</u> Low risk Reason: analyses were adjusted for age at treatment and follow-up duration.

Abbreviations: FSH, follicle-stimulation hormone; LH, luteinizing hormone; NS, not significant.

Who should be counselled about fertility preservation?				
Romerius et al. Hypogonadism risk in men treated for childhood cancer. Clin Endocrinol Metab 2009;94:4180-4186				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Single-centre cohort study 2. Treatment era 1970-2002 3. Follow-up Mean 20 years since diagnosis (± 7.3)	1. Type and Number of Participants 144 male childhood cancer survivors who were >18-45 years of age 2. Diagnoses Leukaemia: 26 (18.1%) Brain tumours: 31 (21.5%) Lymphoma: 32 (22.2%) Testicular cancer: 9 (6.3%) Wilms' tumour: 11 (7.6%) Others: 35 (24.3%) 3. Age at diagnosis Median 10 (0.1-17) years 4. Age at follow-up Median 30 (20-46) years 5. Controls 141 healthy fertile men (none on any fertility treatment)	1. Chemotherapy Chemotherapy alone, n=38 (26.4%) Of whom n=21 (14.6%) alkylating agents 2. Radiotherapy - Radiotherapy to testes, n=6 (4.2%) (mean dose 21 Gy) - Radiotherapy alone (nontesticular), n=17 (11.8%) - Mean cranial irradiation dose 38 Gy and mean dose directly on testis 21Gy 3. Chemotherapy and radiotherapy n=49 (34.0%) 4. Surgery - Brain surgery: n=17 (11.8%) - Surgery alone (except brain surgery): n=17 (11.8%)	1. Outcome definition Hypogonadism: - testosterone levels <10 nmol/L and/or LH >10 IU/L - or receiving androgen replacement therapy 2. Results Hypogonadism, survivors vs. controls: - n=33 (22.9%) vs. n=6 (4.3%) - OR 6.7 (95% CI 2.7-17.0) Risk factors for hypogonadism in binary logistic regression analysis; survivors vs. controls: • Leukaemias (31% hypogonadal): OR 10.0 (95% CI 3.1-32.0) • Brain tumours (19% hypogonadal): OR 5.4 (95% CI 1.6-18.0) • Lymphomas (31% hypogonadal): OR 10.0 (95% CI 3.4-31.0) • Testicular cancer (22% hypogonadal): OR 6.4 (95% CI 1.1-38.0) • Wilms' tumour (9.1% hypogonadal): OR 2.3 (95% CI 0.25-21.0) • Others (17% hypogonadal): OR 4.7 (95% CI 1.4-15.0) - Chemotherapy alone (26% hypogonadal):	1. Remarks - Blood sampling was done between 0800 and 1600 h; the proportion of truly hypogonadal men maybe lower than the reported 23%. - The definition of hypogonadism does not include free testosterone; BMI (mean 25 in the study group) can influence SHBG resulting in false low total testosterone. - The clinical relevance of testosterone deficiency is difficult to interpret because of the lack of information concerning complaints of hypogonadism. 2. Risk of bias A. Selection bias High risk Reason: 151 out of 397 (38.0%) eligible survivors included in the study. B. Attrition bias Low risk Reason: hormonal analyses were assessed in all 144 survivors.

			OR 8.0 (95% CI 2.7-24.0) - Radiotherapy to testes (83% hypogonadal): OR 110.0 (95% CI 11.0-1100.0) - Radiotherapy alone (nontesticular) (12% hypogonadal): OR 3.0 (95% CI 0.56-16.0) - Combination chemotherapy and radiotherapy (22% hypogonadal): OR 6.5 (95% CI 2.3-19.0) - Brain surgery (29% hypogonadal): OR 9.4 (95% CI 2.5-35.0 - Surgery alone (except brain surgery) (0% hypogonadal): OR 1.0	C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome. D. Confounding High risk Reason: controls were not matched to survivors and no correction for confounding factors in analyses.

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

Who should be counselled about fertility preservation?				
Siimes et al. Prophylactic cranial irradiation increases the risk of testicular damage in adult males surviving ALL in childhood. Med Pediatr Oncol 1993;21:117-121				
Study design Treatment era Years of follow-up	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Multi-centre (n=4)cohort study 2. Treatment era 1966-2003 3. Follow-up Mean 15.2 (4.0-25.0) yr from diagnosis	1. Type and number of participants 41 male childhood ALL survivors at least 1 yr off chemotherapy 2. Diagnoses 41 (100%) ALL 3. Age at diagnosis Mean 8.5 (range 1-16) yr	1. Chemotherapy - Cyclophosphamide: 23 (56.1%) - Cytosine arabinoside: 9 (22.0%) - Vincristine, prednisone, 6-mercaptopurine, methotrexate: 41 (100%) - Adriamycin: 21 (51.2%) - Asparaginase: 33 (80.5%) 2. Radiotherapy	1. Outcome definitions - ↓ testosterone levels: threshold level not reported - ↑ LH levels: threshold level not reported - Semen analysis, oligospermia: sperm concentration <20 million/mL 2. Results Testosterone levels: - Cranial radiotherapy: Mean 17.0 (±7.5) - No cranial radiotherapy: Mean 20.2 (±6.7) p=0.242	1. Remarks 3 patients treated with CRT had been started on testosterone supplementation from 4 to 9 years earlier. Their mean testosterone concentration was lower (9.8 U/L at 2 weeks after the preceding testosterone injection) than that of the other patients at time of the study.

	4. Age at follow-up Median 21.0 (18.0-27.0) yr 5. Controls No controls	- CRT 20-24 Gy: 17 (41.5%) - Radiation to fields including testes: 0 (0%) - Both cyclophosphamide and cranial radiotherapy: 12 (29%)	LH levels: - Cranial radiotherapy: Mean 8.2 (±8.1) - No cranial radiotherapy: Mean 6.0 (±3.4) p=0.456 Risk factors for lower (but not necessarily abnormal) testosterone in multivariable analysis: - Chemotherapy NS - Cranial radiotherapy NS - Age at diagnosis NS Semen analysis: Available in 18 patients: 3 (16.7%) azoospermia, 7 (38.9%) oligospermia	A forward-stepping linear regression analysis was used to identify factors independently associated with testosterone deficiency. 3 patients with testosterone supplementation excluded from analysis. Note limitations in interpreting LH level in patients who have received cranial radiotherapy (LH response may be blunted by central hypogonadism. 2. Risk of bias A. Selection bias Unclear Reason: Unclear how many patients were included in the original cohort of survivors. B. Attrition bias Low risk Reason: All patients had testosterone and LH samples taken. C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome. D. Confounding Low risk

				Reason: analysis was adjusted for chemotherapy, cranial radiotherapy and age at diagnosis.

Abbreviations: CRT, cranial radiotherapy; LH, luteinising hormone; NS, not significant.

Who should be counselled about fertility preservation?				
Tauer et al. Long-term imatinib treatment does not cause testicular toxicity in male adolescents with chronic myeloid leukemia and in a juvenile rat model. Klin Pediatr 2014;226:169-174.				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Prospective multicenter study 2. Treatment era 1978-2000 3. Follow-up Patients monitored over a mean period 39 (range 0-89) weeks and were on imatinib prior to entering the study with a median of 105 (range 12-296) weeks	1. Type and number of participants 13 boys with CML enrolled into the CML-PEAD II trial 2. Diagnoses CML n=13 3. Age at diagnosis Median 13.8 (range 7.9-18.7) yr 4. Age at follow-up Not applicable 5. Controls No controls	1. Chemotherapy Imatinib: 13 (100%) 260-300 mg/m^2 imatinib orally once daily for a minimum period of at least 3 months	1. Outcome definitions Testosterone and inhibin B levels compared to age-related reference ranges 2. Results Testosterone levels: - Compared to age-related reference ranges serum testosterone showed no rising or falling pattern during the course of treatment - Patients aged 11-15 yr showed highest variability of testosterone levels during therapy but within the age-related reference ranges Inhibin B levels: - Compared to age-related reference ranges inhibin B showed no rising or falling pattern during the course of treatment	1. Remarks Small study sample. 2. Risk of bias A. Selection bias Unclear Reason: unclear how many patients were included in the original cohort. B. Attrition bias Low risk Reason: 10/13 (76.9%) patients could be investigated at least 3 time points while the remaining 3 could be investigated once. C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome.

				D. Confounding High risk Reason: no adjustment for important confounding factors.

Abbreviations: CML, chronic myeloid leukaemia.

Who should be counselled about fertility preservation?				
Tromp et al. Reproductive status in adult male long-term survivors of childhood cancer. Hum Reprod 2011;26:1775-1783				
Study design Treatment era Years of follow-up	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Single-centre cohort study 2. Treatment era 1966-2003 3. Follow-up Median 15 (range 5.0-39.0) yr since diagnosis	1. Type and number of participants 565 male childhood cancer survivors who survived ≥5 years from diagnosis and were ≥18 years at follow-up 2. Diagnoses Lymphoma 154 (27.3%) Leukaemia 125 (22.1%) Soft tissue sarcoma 70 (12.3%) Kidney tumour 64 (11.3%) Bone tumour 53 (9.4%) Brain/CNS tumour 47 (8.3%) Neuroblastoma 19 (3.3%) Testicular tumour 9 (1.9%)	1. Chemotherapy Alkylating agents: 336 (59.5%) 2. Radiotherapy - Pelvic/abdominal irradiation only: 51 (9.0%) - Cranial irradiation only: 120 (21.2%) - Cranial + pelvic/abdominal irradiation: 4 (0.7%) - TBI: 11 (1.9%) - Radiation to fields including testes: Not reported 3. Surgery testicular region: 38 (6.7%) Treatment; Combination chemotherapy and surgery n=172 (30.4%) 9 survivors(2.4%) chemotherapy not containing	1. Outcome definitions - ↓ testosterone levels: <11 nmol/L - ↑ LH levels: >15 U/L - ↑FSH levels: >10 U/L 2. Results ↓ testosterone levels (n=460 assessed): - 57 CCS (12.4%) - Mean testosterone level 17.2 nmol/L, SD 5.5 ↑ LH levels (n=489 assessed): - 14 CCS (2.9%) - Median LH level 6.0 U/L (1.0-40.0) ↑FSH levels (n=488 assessed): 161 (33%) - Median FSH level 6.0 U/L (0,1-72.7) Risk factors for lower (but not necessarily abnormal) testosterone in linear regression analysis:	1. Remarks - Higher incidence of decreased testosterone levels compared to elevated LH levels. Note higher LH threshold in this study, but note that one third of cancer survivors have elevated FSH levels. - Normal LH levels do not exclude testosterone deficiency in this group – clinically, testosterone and LH need be assessed together, especially in patients who received cranial irradiation (in whom LH response may be blunted by central hypogonadism), but data not reported - Difficult to interpret clinical relevance of testosterone risk factor analyses since most of the

	Endocrine tumour 5 (0.9%) Other 19 (4.3%) <u>3. Age at diagnosis</u> Median 7.8 years (0.0-17.8) <u>4. Age at follow-up</u> Median 21.0 years (18.0-46.0) <u>5. Controls</u> No controls	alkylating agents vinca alkaloid or antmetabolite	Model 1 – adjusted for age at diagnosis and follow-up duration: - TBI yes vs. no: beta -3.53 (p=0.036) Model 2 – adjusted for age at diagnosis, follow-up duration and all other variables as stated below: - All treatment variables not significant: procarbazine, cyclophosphamide, other alkylating agents (busulfan, carmustine, mechlorethamine, ifosfamide, lomustine, melphalan, temozolamide – not evaluated separately), cisplatin/carboplatin, antimetabolites, vinca alkaloids, anthracyclines, other chemotherapeutic agents, cranial irradiation, pelvic/abdominal radiation, other irradiation, TBI, surgery testicular region <u>Risk factors for elevated FSH in multivariable logistic regression analysis:</u> - Cyclophosphamide yes vs. no: OR 4.23 (95% CI 2.24-8.0) - Procarbazine yes vs. no: OR 3.79 (95% CI 1.76-8.17) - Other alkylating yes vs. no: OR 2.14 (95% CI 1.14-4.00) - Cisplatin/carboplatin yes vs. no: OR 2.29 (95% CI 0.89-5.89) - Antimetabolites yes vs. no: OR 1.15 (95% CI 0.63-2.07) - Anthracyclines yes vs. no: OR 1.06 (95% CI 0.56-2.00) - Vinca alkaloids yes vs. no: OR 2.80 (95% CI 1.07-7.30) - Other chemo yes vs. no: OR 0.90 (95% CI 0.50-1.59)	testosterones were in the normal range - The clinical relevance of testosterone deficiency is also difficult to interpret because of the lack of age-specific reference values of hormone levels and the lack of information concerning complaints of hypogonadism - This study does not distinguish between hypogonadotropic hypogonadism and hypergonadotropic hypogonadism 73 men reported that their partner had become pregnant: 120 conceptions resulted in 103 live births and 14 miscarriages. 56 (77%) natural conception. No data presented to prove paternity in these pregnancies. <u>2. Risk of bias</u> <u>A. Selection bias</u> High risk Reason: 565 out of 796 (71.0%) eligible survivors were included in this study. <u>B. Attrition bias</u> Low risk Reason: testosterone levels and LH levels were available in 81.4% and 86.5%, respectively. <u>C. Detection bias</u>

			- Pelvic/abdominal radiotherapy yes vs. no: OR 2.35 (95% CI 1.03-5.37) - Cranial radiotherapy yes vs. no: OR 0.55 (95% CI 0.28-1.07) - TBI not included in the model; All 11 survivors treated with TBI had ↑ FSH - Other radiotherapy yes vs. no: OR 1.78 (95% CI 0.95-3.34) - Surgery testicular region: OR 2.61 (95% CI 1.08-6.29) - Age at diagnosis, per yr: OR 1.08 (95% CI 1.02-1.16) - Follow-up duration, per yr: OR 1.06 (95% CI 1.01-1.12)	Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome (no information is available concerning time of the day of collection of blood samples). D. Confounding Low risk Reason: the analyses are adjusted for important factors (adjusted for age at diagnosis, follow-up duration and treatment).

Abbreviations: LH, luteinizing hormone; NS, not significant; SD, standard deviation; TBI, total body irradiation; CCS, childhood cancer survivors.

Who should be counselled about fertility preservation?				
Wilhelmsson et al. Adult testicular volume predicts spermatogenetic recovery after allogeneic HSCT in childhood and adolescence. Pediatr Blood Cancer 2014;61:1094-1100				
Study design Treatment era Years of follow-up	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
1. Study design Two-centre cohort study 2. Treatment era 1978-2000 3. Follow-up Mean 13 (4-28) years since HSCT	1. Type and number of participants 106 male CCS who received allogeneic HSCT >5 years after treatment and reached puberty at follow-up 2. Diagnoses ALL (n=44), AML (n=20), SAA (n=17), other (n=25); 68 malignant	1. Chemotherapy - Alkylating agents: 106 (100%) - Cyclophosphamide: 84 (79.2%); 120 mg/kg - Busulfan: 18 (17.0%) - Cytarabine: 16 (15.1%) - Melphalan: 3 (2.8%) - Etoposide: 2 (1.9%) 2. Radiotherapy - TBI: 71 (67.0%); 10-12 Gy - CRT for leukaemia: 14 (22%)	1. Outcome definitions Azoospermia: no spermatozoa detected in any field of a double-chambered haemacytometer before or after centrifugation 2. Results Spermatogenesis (n=31 assessed): - Azoospermia: 21 (67.7%) - TBI: 20/24 (83.3%) azoospermia, 4/24 (16.7%) non-azoospermia	1. Remarks Testicular volume measured with orchidometer or ruler & formula. If both testes measured, mean of both used. Adult testicular volume (n=74) documented by Tanner stage 5, reached final height, or age >18 years. In the multivariable analysis the effect of TBI was evaluated.

	HSCT (4 CML, 20 AML, 44 ALL), 38 non-malignant HSCT 3. Age at HSCT Mean 8.0 (1-17) years 4. Age at follow-up Mean 22 (12-42) years 5. Controls No controls	- Testicular irradiation for leukaemia: 8 (12%) - TNI: 5 (4.7%); 6 Gy 3. Therapy subgroups - sTBI + cyclophosphamide: 30 (28%) - fTBI + cyclophosphamide: 20 (19%) - fTBI + cyclophosphamide + etoposide: 2 (2%) - fTBI + cytarabine: 16 (15%) - fTBI + melphalan: 3 (3%) - Busulfan: 3 (3%) - Busulfan + cyclophosphamide: 15 (14%) - Cyclophosphamide only: 12 (11%) - Cyclophosphamide + TNI: 5 (5%)	- Cyclophosphamide-only: 0/3 (0%) azoospermia, 3/3 (100%) non-azoospermia - Busulfan-based: 1/4 (25%) azoospermia, 3/4 (75%) non-azoospermia - Leukaemia: 17/19 (89.5%) azoospermia, 2/19 non-azoospermia (10.5%) Predictors for active sperm production in multivariable logistic regression analysis: - No leukaemia diagnosis vs. leukaemia diagnosis: OR 19.8; 95% CI 1.9-210.3 (p<0.01) - Testicular volume ≥15 ml vs. <15 ml: OR 17.1; 95% CI 1.4-215.8 (p<0.03) - No TBI vs. TBI: p>0.05 (OR not mentioned) - FSH <10 IU vs. ≥10 IU: p>0.05 (OR not mentioned) Predictors for active sperm production in bivariate logistic regression analysis: - No leukaemia diagnosis vs. leukaemia diagnosis: OR 17.0; 95% CI 2.6-113.0 (p<0.003) - Testicular volume ≥15 ml vs. <15 ml: OR 14.2; 95% CI 2.1-98.1 (p<0.007) - No TBI vs. TBI: OR 30.0; 95% CI 2.8-322.1 (p<0.005) - FSH <10 IU/L vs. ≥10 IU/L: OR 0.8; 95% CI 0.7-1.0 (p<0.047)	However, 5 patients treated in the no TBI group had received TNI, hence potentially exposing the testes to radiotherapy (no details were provided to indicate if testicular shielding was used). In addition, the no TBI group was also treated with cyclophosphamide, busulfan, or both. Leukaemia was associated with azoospermia. This might be due to CRT and/or testicular irradiation. It is, however, unclear how many patients with sperm samples were treated with CRT and/or testicular irradiation. 2 out of 106 fathered a child. 2. Risk of bias A. Selection bias Low risk Reason: 106/123 (86.2%) eligible survivors participated in this study. B. Attrition bias High risk Reason: 31/106 (29.2%) semen analysis. C. Detection bias Unclear

				Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome. D. Confounding High risk Reason: Although multivariable analyses were performed, treatment with alkylating agents was not included in the models.

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CRT, cranial radiotherapy; FSH, follicle stimulating hormone; fTBI, fractionated TBI; HSCT, haematopoietic stem cell transplantation; OR, odds ratio; SAA, severe aplastic anaemia; sTBI, single fraction TBI; TBI, total body irradiation; TNI, total nodal irradiation; 95% CI, 95% confidence interval.

What male reproductive preservation methods should be offered?				
Chan et al. Testicular Sperm Extraction Combined with Intracytoplasmic Sperm Injection in the Treatment of Men with Persistent Azoospermia Postchemotherapy. Cancer 2001;92:1632-37				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Retrospective study 2. Treatment era NM Fertility preservation method: June 1995 to August 2000 3. Follow-up:	1. Type and Number of Participants 17 male patients previously treated with CT *Original cohort*: 198 patients who underwent TESE-ICSI for non-obstructive azoospermia 2. Diagnoses 16/17 (94%) Malignant diseases:	1. Fertility Preservation method 17 patients produced semen sample *Method of semen collection* Testicular sperm extraction (TESE) (After 1997, microdissection TESE.	1. Outcome definitions - Sperm retrieval rate: percentage of patients that underwent testicular biopsy and had live sperm obtained - Fertilization rate: percentage of embryos obtained by ICSI with two distinct pronuclei and two polar bodies - Clinical pregnancy rate: percentage of pregnancies with the presence of fetal heartbeats determined by transvaginal ultrasonography approximately 32 days after embryo transfer	1. Strengths This is one of two papers published that describes TESE-ICSI outcomes in patients that received chemotherapy and are azoospermic 2. Limitations - Very small number of patients - No controls - Retrospective - Two different biopsy techniques used - Varying chemotherapy/XRT

Mean 16.3 years after CT completion	6 (35%) HL 4 (24%) NSGCT 2 (12%) NHL 2 (12%) Leukaemia 1 (6%) Wilm's 1 (6%) Mediastinal GCT Other: 1 (6%) Nephrotic syndrome <u>3. Age at diagnosis</u> NM Age at 37.4 yrs at study <u>4. Age at follow-up</u> Mean 37.4 years (range 28-54) <u>5. Controls (if applicable)</u> No controls included <u>6. Additional study characteristics, if relevant</u> All pts azoospermic based on 2 semen analyses <u>7. Chemotherapy</u> - Varying CT regimens - Total cumulative dose of gonadotoxic agents not provided <u>8. Radiotherapy</u> 4 patients received XRT in addition to CT <u>9. Surgery</u>	Prior to 1997, random biopsy) Also combined with intracytoplasmic sperm injection (ICSI) 9 patients who underwent TESE-ICSI had sperm retrieval *Timing of intervention* After CT	- Live birth rate: not defined - Complications intervention <u>2. Results</u> *Sperm retrieval rate* 9/20 (45%) patients (mean 1.2 attempts per patient) *Clinical pregnancy rate* 3/9 (33%) patients *Live birth rate* 2/9 (22%) patients who had TESE-ICSI fathered 3 live births 1/9 (11.1%) pregnancies who had TESE-ICSI did not result in live birth *Complication of intervention (TESE)* All patients discharged home on same day No postoperative complication No correlation noted between TESE-ICSI outcome and the underlying conditions treated with CT	- One non-cancer patient <u>3. Risk of bias</u> <u>A. Selection bias</u> Unclear Reason: 17/198 (8.6%) patients included (inclusion those patients that received CT), unclear reasons for exclusion <u>B. Attrition bias</u> Low risk Reason: Outcomes assessed in all patients <u>C. Detection bias</u> Unclear unclear if outcome assessors were blinded for important determinants related to the outcome <u>D. Confounding</u> High risk / not applicable Reason: Although the authors noted that no correlation was detected with the chemotherapy received, the sample size was too small for this determination to be significant. No multivariate analysis performed.

	17/17 testicular biopsy for sperm extraction 10. Other treatments -			

Abbreviations: NM: not mentioned; CT: chemotherapy; NSGCT: Mediastinal GCT; NHL: Non-Hodgkin lymphoma; HL: Hodgkin Lymphoma; XRT:chest radiation; ICSI: intracytoplasmic sperm injection; TESE: testicular sperm extraction; pts: patients ; XRT: chest radiation.

What male reproductive preservation methods should be offered?				
Corkum et al. Testicular wedge biopsy for fertility preservation in children at significant risk for azoospermia after gonadotoxic therapy. J Ped Surgery 2019;54:1901-1905				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Retrospective study 2. Treatment era TTC between 2015-2017 3. Follow-up: Median 1.4 years (interquartile range 0.9-2.2 years) since TTC	1. Type and Number of Participants 23 males who will be treated with high risk gonadotoxic therapy 2. Diagnoses Solid tumour (74%), hematological malignancy (17%), benign hematological disease (9%) 3. Age at diagnosis Median 10 (range 0.42-18) years 4. Age at follow-up NA 5. Controls (if applicable) No	1. Fertility Preservation method 23 (100%) TTC (unilateral wedge biopsy) *Timing of intervention* - 5 (21.7%) received one or two rounds of their planned chemotherapy prior to TTC - 6 (26%) underwent TTC at the time of disease relapse	1. Outcome definitions - Tissue dissection in pubertal patients - Complications 2. Results *Tissue dissection in pubertal patients before cryopreservation* 22 (96%) had normal testicular tissue with the presence of germ cells on histopathological analysis *Complications of intervention* - 0 (0%) intraoperative complications related to testicular wedge biopsy occurred - 1 (4.3%) developed a scrotal cellulitis three weeks after TTC after initiation of chemo- therapy; the superficial wound infection was successfully treated with intravenous antibiotics	1. Strengths 2. Limitations 3. Risk of bias A. Selection bias Unclear Reason: unclear if all patients that underwent TTC were included in the study group B. Attrition bias Low risk Reason: outcomes assessed for all 23 patients C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for

	6. Additional study characteristics, if relevant Tanner stage 1: 18 (78%) Tanner stage 2: 3 (13%) Tanner stage ≥3: 2 (9%) 7. Chemotherapy NM 8. Radiotherapy NM 9. Surgery NM 10. Other treatments 9 (39.1%) HSCT		- Median time from TTC to start of cancer therapy: 7 days with no unanticipated delays in treatment initiation	important determinants related to the outcome D. Confounding bias NA Reason: Only descriptive results, no analyses performed

Abbreviations: NA, not applicable; NM, not mentioned; TTC: testicular tissue cryopreservation.

What male reproductive preservation methods should be offered?				
Ming et al. Cryopreservation of testicular tissue in pre-pubertal and adolescent boys at risk for infertility: A low risk procedure. J Ped Urol 2018;14:274e1-e5.				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Retrospective study 2. Treatment era TTC between 2014-2016 3. Follow-up:	1. Type and Number of Participants 34 males who will be treated with high risk gonadotoxic therapy 2. Diagnoses Solid tumour (44%), leukaemia or lymphoma	1. Fertility Preservation method 34 (100%) TTC (unilateral open biopsy) *Timing of intervention* - 15 (44.1%) received chemotherapy prior to TTC	1. Outcome definitions Complications 2. Results *Complications of intervention* - 2 (5.9%) developed complications after biopsy: ipsilateral epididymo-orchitis (resolved with antibiotics) and an	1. Strengths 2. Limitations 3. Risk of bias A. Selection bias Low risk

NM	(35%), hematological disorders (21%) <u>3. Age at diagnosis</u> Mean 6.9 ± 4.4 years (range 0.7-15 years) <u>4. Age at follow-up</u> NA <u>5. Controls (if applicable)</u> No <u>6. Additional study characteristics, if relevant</u> Pre-pubertal: 32 (94%) Post-pubertal: 2 (6%) <u>7. Chemotherapy</u> NM <u>8. Radiotherapy</u> NM <u>9. Surgery</u> NM <u>10. Other treatments</u> 22 (64.7%) HSCT	- 1 (2.9%) medulloblastoma patient received prior cranial radiotherapy	ipsilateral torsed appendix testis (managed conservatively) - Both patients were preparing for stem cell transplant and there was no delay to transplant as a result of these complications - 0 (0%) had bleeding complications nor return visits to the operating room	Reason: all 34 consecutive patients were included in the study. <u>B. Attrition bias</u> Low risk Reason: outcomes assessed for all 34 patients <u>C. Detection bias</u> Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome <u>D. Confounding bias</u> NA Reason: Only descriptive results, no analyses performed

Abbreviations: NA, not applicable; NM, not mentioned; TTC: testicular tissue cryopreservation.

What male reproductive preservation methods should be offered?				
Stukenborg et al. Spermatogonial quantity in human prepubertal testicular tissue collected for fertility preservation prior to potentially sterilizing therapy Human Reprod 2018;33:1677-1683				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Retrospective study 2. Treatment era TTC between 2014-2017 3. Follow-up: NM	1. Type and Number of Participants 32 pre-pubertal males who will be treated with very high risk gonadotoxic therapy (HSCT or testicular radiotherapy) 2. Diagnoses Malignant hematological disease (40.6%), solid tumour (15.6%), benign hematological disorders (43.8%) 3. Age at diagnosis Range 0.7-13.1 years 4. Age at follow-up NA 5. Controls (if applicable) 14 testicular samples without testicular pathology from the biobank of the Department of Pathology, Karolinska University Hospital served as controls; Mean age: 5.6 ± 5.0 years	1. Fertility Preservation method 32 (100%) TTC (unilateral open biopsy; <20% of testicular volume of one testes sampled) *Timing of intervention* 20 (62.5%) testicular biopsy performed 1-45 days after a previous dose of chemotherapy	1. Outcome definitions Tissue dissection in pre-pubertal patients 2. Results *Tissue dissection in pre-pubertal patients before cryopreservation* Spermatogonia per transverse tubular cross-section: - Mean 1.7 ± 1.0 in patients treated with non-alkylating agents (no significant difference compared to controls) - Mean 0.2 ± 0.3 in patients treated with alkylating agents ($p<0.05$ compared to controls and non-alkylating agent group) - Mean 0.8 ± 0.9 in patients treated without chemotherapy ($p<0.05$ compared to controls) - Mean 4.1 ± 4.6 in controls - Among 5 boys exposed to CED ≥4000 mg/m^2 spermatogonia values were close to zero	1. Strengths 2. Limitations Detailed information regarding previous medical treatments and testicular volumes of patients included in the biobank were not available. 3. Risk of bias A. Selection bias Unclear Reason: unclear if all patients that underwent TTC were included in the study group B. Attrition bias Low risk Reason: outcomes assessed for all 32 patients C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome D. Confounding bias High

	6. Additional study characteristics, if relevant 7. Chemotherapy Alkylating agents: 6 (18.8%) Non-alkylating agents: 8 (25.%) 8. Radiotherapy NM 9. Surgery NM 10. Other treatments NM			Reason: Analyses were not adjusted for potential confounding factors

Abbreviations: NA, not applicable; NM, not mentioned; TTC: testicular tissue cryopreservation.

What male reproductive preservation methods should be offered?				
Uijldert et al. Development of the testis in pre-pubertal boys with cancer after biopsy for fertility preservation. Human Reprod 2017;32:2366-2372				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Prospective study 2. Treatment era TTC between 2011-2017 3. Follow-up: Range 0.08-1 year	1. Type and Number of Participants 64 pre-pubertal boys who will be treated with gonadotoxic therapy without the ability to ejaculate 78 boys underwent TTC, of whom 6 deceased during follow-up, 5 lost to follow-up, 2 orchiectomy of biopsied testes and 1 without a volume recorded 2. Diagnoses Solid tumour (87.5%), haematological cancer (7.8%), other (4.7%) 3. Age at diagnosis Mean 8.3 (range 0.5-15.5 years) 4. Age at follow-up NA 5. Controls (if applicable) No	1. Fertility Preservation method 64 (100%) TTC (unilateral biopsy never exceeding 50% of the testicular volume) *Timing of intervention* All patients underwent TTC prior to cancer treatment	1. Outcome definitions - Tissue dissection in pre-pubertal patients before cryopreservation - Testicular growth - Complications 2. Results *Tissue dissection in pre-pubertal patients before cryopreservation* - No spermatogonia: 1 (1.9%) - Spermatogonia only: 44 (68.8%) - Up to spermatocytes: 9 (14.1%) - Up to spermatids: 10 (14.1%) *Testicular growth* - After an initial decrease in testis volume in both the biopsied and non-biopsied testis during the first 6 months after surgery, an increase in mean volume was observed between 6 and 12 months after surgery in both the biopsied and non-biopsied testis - Overall, biopsy had no significant impact on testicular growth (p=0.519) - No significant differences between biopsied and non-biopsied testis when looking at subgroups defined by the stage of testicular development (spermatogonia only, development up	1. Strengths 2. Limitations 3. Risk of bias A. Selection bias Low risk Reason: 64 out 78 (82.1%) eligible patients were included in the study. B. Attrition bias Low risk Reason: outcomes assessed for all 64 patients. Regarding testicular growth, 64, 58 and 55 patients underwent the 1 month, 6 month and 12 month ultrasound, respectively. C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome. D. Confounding bias Low risk

	6. Additional study characteristics, if relevant NM 7. Chemotherapy NM 8. Radiotherapy NM 9. Surgery NM 10. Other treatments NM		until the spermatocyte stage or up until the spermatid stage) *Acute complications of intervention* - 1/78 (1.3%) post-operative bleeding - 2/78 (2.6%) wound infection one of which had a minor infection where no additional action had to be taken; the other boy was treated with antibiotics; complaints resolved within a few days without visible testicular damage; no second operation or orchiectomy was necessary in either case *Ultrasonographic abnormalities at 1 month in biopsied vs. contralateral testis (n=64)* - Calcifications: 2 (3.1%) vs. 2 (3.1%) - Epididymal cyst: 3 (4.7%) vs. 1 (1.6%) - Hydrocele: 4 (6.3%) vs. 1 (1.9%) - Extra-testicular haematoma: 5 (7.8%) vs. 0 - Intratesticular haematoma: 2 (3.1%) vs. 0 - Fibrotic lesion: 0 vs. 0 *Ultrasonographic abnormalities at 6 months in biopsied vs. contralateral testis (n=58)* - Calcifications: 2 (3.4%) vs. 2 (3.4%) - Epididymal cyst: 2 (3.4%) vs. 1 (1.7%) - Hydrocele: 2 (3.4%) vs. 0 - Extra-testicular haematoma: 0 vs. 0 - Intratesticular haematoma: 0 vs. 0 - Fibrotic lesion: 0 vs. 0	Reason: Biopsied testes compared to non-biopsied testes.

			Ultrasonographic abnormalities at 12 months in biopsied vs. contralateral testis (n=55) - Calcifications: 1 (1.6%) vs. 1 (1.6%) - Epididymal cyst: 0 vs. 1 (1.6%) - Hydrocele: 1 (1.6%) vs. 2 (3.1%) - Extra-testicular haematoma: 0 vs. 0 - Intratesticular haematoma: 0 vs. 0 - Fibrotic lesion: 4 (6.3%) vs. 0	

Abbreviations: NA, not applicable; NM, not mentioned; TTC: testicular tissue cryopreservation.

What male reproductive preservation methods should be offered?				
Hagenäs et al. Clinical and biochemical correlates of successful semen collection for cryopreservation from 12 - 18-year -old patients: a single-center study of 86 adolescents. Human Reproduction 2010; 25(8):2031-2038				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Single-centre retrospective cohort 2. Treatment era December 1995 - May 2009 3. Follow-up: No long term follow-up	1. Type and Number of Participants 80 male patients with malignant disease (various) *Total cohort*: 86 male patients 2. Diagnoses 80/86 (93%) Malignant diagnosis: 19 (22%) HL 16 (20%) Testicular cancer 25 (31%) Leukaemia /NHL 20 (23%) Solid tumours 6/80 (7%) Other: 2 aplastic anemia	1. Fertility Preservation method 86 patients produced semen sampling for cryopreservation *Method of semen collection* - 74 (86%) patients masturbation - 11 (13%) patients electroejaculation - 1 (1.2%) Penile vibration	1. Outcome definitions Successful semen sampling via masturbation Successful semen sampling via electro-ejaculation Sperm concentration Motile spermatozoa Semen volume Determinants of successful semen collection 2. Results *Semen analysis before cryopreservation by masturbation* 65/74 (87.8%) patients with successful sample collected and cryopreserved 6/74 (8.1%) patients with azoospermia	1. Strengths Study based on standard clinical parameters (available in every-day practice) 2. Limitations - Retrospective cohort - Small cohort - No long-term follow-up data 3. Risk of bias A. Selection bias unclear Reason: unclear how the 86 patients were selected B. Attrition bias Low risk

	1 disseminated sclerosis 1 histiocytosis, 1 varicocele 1 Wegeners granulomatosis <u>3. Age at diagnosis</u> Median 16.2 years (12.2 - 17.9) <u>4. Age at follow-up</u> NA <u>5. Controls (if applicable)</u> NA <u>6. Additional study characteristics, if relevant</u> - FSH, LH, inhibin B, testosterone <u>7. Chemotherapy</u> Intervention prior to CT <u>8. Radiotherapy</u> NA <u>9. Surgery</u> NA <u>10. Other treatments</u> NA	*Timing of intervention* Cryopreservation before treatment *Timing of semen analysis* Before cryopreservation	3/74 (4%) patients with immotile sperm *Semen analysis before cryopreservation by electro-ejaculation* 6/12 (50%)patients with successful sample collected and cryopreserved 4/12 (33%) patients with azoospermia 2/12 (16.75) patients with immotile sperm *Successful semen sampling(total)* 71/86 (83%) patients with successful sample collected and cryopreserved 10/86 (11.6%) patients with azoospermia 5/86 (5.8%) patients with immotile sperm *Sperm concentration* - Median: 9.6 (range 0-284) million/ml - 33/86 (38.4%) patients had ≥20 million/ml sperm concentration - 43/86 (50%) patients had < 20 million/ml sperm concentration - 10/86 (11.6%) patients had azoospermia *Motile spermatozoa* Median: 45.5% (range 0-86%) 71/86 (83%) patients had motile spermatozoa; 5/86 (5.8%) patients with few immotile sperm *Semen volume (median)* HL 1.7 ml (0.1-5.9) Testicular cancer 3.0ml (0.5–6.9) Leukaemia /NHL 1.8 ml (0.1–6.2) Solid tumours 1.5 ml (0.03–6.1)	Reason: outcomes assessed in all patients <u>C. Detection bias</u> Unclear unclear if outcome assessors were blinded for important determinants related to the outcome <u>D. Confounding</u> High risk Reason: associations performed with Pearson's correlation but not with multivariate analysis

			Total sperm count (millions) HL 7 (0–243) Testicular cancer 8.7 (0.03–216) Leukaemia /NHL 46.8 (0.04–611) Solid tumours 34.1 (0.15–210) *Determinants of successful semen collection* - Testicular volume correlated with sperm concentration (R=0.283, p=0.046), and percentage of motile spermatozoa (R= 0.410, p=0.003) - Chronological age (but not reproductive hormones) correlated with sperm concentration (R=0.25, P=0.049)	

Abbreviations: NA: not applicable; NM: not mentioned; HL: Hodgkin Lymphoma; HL: Hodgkin Lymphoma; ALL: acute lymphoblastic leukaemia

What male reproductive preservation methods should be offered?				
Hovav et al. Electroejaculation before chemotherapy in adolescents and young men with cancer. Fertility and Sterility 2001; 75(4): 811-13.				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Retrospective clinical study 2. Treatment era 1998-1999 3. Follow-up:	1. Type and Number of Participants 6 male patients with cancer diagnoses prior to chemotherapy 2. Diagnoses PT1: Ewing sarcoma PT2: Osteosarcoma PT3: Osteogenic sarcoma	1. Fertility Preservation method 6 patients produced semen sample *Method of sample collection*	1. Outcome definitions Successful sperm retrieval as determined by semen analysis Complication of intervention 2. Results *Semen analysis before cryopreservation:* *Sperm count* Mean 16 x 10^6 (range 0-45 x 10^6) *Sperm motility*	1. Strengths study highlights the utility of electroejaculation for sperm retrieval prior to chemotherapy 2. Limitations - Small study - Effect of electrostimulation on long-term sperm viability and quality unknown - No long-term follow-up data

No long-term follow-up	PT4: Testicular germ-cell tumour PT5: Hodgkin lymphoma PT6: Testicular germ-cell tumour 3. Age at diagnosis Mean 18 years ±3 Range 15-22 years 4. Age at follow-up No long-term follow-up 5. Controls (if applicable) none 6. Additional study characteristics, if relevant - patients failed masturbation and refused vibratory stimulation to produce semen - 4 patients underwent more than one electroejaculation session 7. Chemotherapy - 8. Radiotherapy - 9. Surgery See intervention Before CT	Electroejaculation under general anesthesia to produce semen: - Antegrade semen collected directly - Retrograde semen collected in Ham-F10 medium which was extracted from the bladder, centrifuged, and concentrated to 1-2mL *Timing of intervention* Before anticancer therapy *Timing of semen analysis* Before cryopreservation	Mean 14% (range 0-53%) *Sperm count, sperm motility* PT1: 15 x 10^6 ; 6% PT2: 24 x 10^6; 53% PT3: 9 x 10^6; 0% PT4: 35 x 10^6; 33% PT5: 45 x 10^6; 10% PT6: 6.5 x 10^6; 20% *Complication of intervention* 0/6 patients with complications Electroejaculation can be performed 2-3 times every 48 hours without complications	3. Risk of bias A. Selection bias Unclear Reason: unclear how the 6 patients were selected B. Attrition bias Low risk Reason: outcomes assessed in all patients C. Detection bias Unclear Reason: unclear if outcome assessors were blinded for important determinants related to the outcome D. Confounding High risk Reason: descriptive study, no multivariate analysis performed

Abbreviations: NA: not applicable; NM: not mentioned; CT: chemotherapy; PT: patient

What male reproductive preservation methods should be offered?				
Kamischke et al. Cryopreservation of Sperm From Adolescents and Adults With Malignancies. Journal of Andrology 2004; 25: 586–592				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Single-center cohort study, retrospective 2. Treatment era April 1989 - March 2003 3. Follow-up NM	1. Type and Number of Participants 300 male patients with malignant disease <25 years of age *Original cohort:* 936 patients with malignant disease. Of these, 851 (91%) included: 111 (13%) adolescents <20 years; 740 (87%) adults <40 years Exclusion criteria: - Incomplete data, relapses, secondary cancers, known bilateral testicular cancer, unilateral testicular cancer in combination with contralateral intraepithelial neoplasia, or an unknown primary diagnosis 2. Diagnoses Testicular cancer 485 (57%) 28 (6%) adolescents 457 (94%) adults	1. Fertility Preservation method 851 patients produced semen sampling for cryopreservation *Method of sample collection* Unclear (?masturbation) *Timing of intervention* - Before initiation of anticancer treatment. - Except: 61% patients with testicular cancer had unilateral ablation of the testis before cryopreservation *Timing of semen analysis* Before cryopreservation	1. Outcome definitions Successful cryopreservation (defined as observation of at least a single motile sperm after the thawing procedure) Semen analysis Sperm count Total sperm motility Testes volume Live births 2. Results *Semen analysis before cryopreservation in patients <20 years* 110/111 (99.1%) patients with successful sample collected and cryopreserved 1/111 (0.9%) patient with azoospermia *Semen analysis after freezing and thawing in patients <25 years* 268/300 (89%) patients with at least a single motile sperm 32/300 (10.7%) patients without motile sperm *Total sperm motility (%)* <15 yrs: 38 ± 7 15- <16 yrs: 48 ± 5 16- <17 yrs: 48 ± 4 17- <18 yrs :57 ± 4	1. Strengths - Despite reasonable exclusion criteria 91% of possible participants included - Large cohort of adolescents: 111 (13% of total participants) 2. Limitations - No healthy controls - No follow-up 3. Risk of bias A. Selection bias Low risk Reason: 851/936 (91%) patients included, exclusion criteria reported B. Attrition bias Low risk Reason: outcomes assessed for all patients C. Detection bias Unclear Reason: unclear if outcome assessors were blinded for

	Lymphomas 187 (22%) 36 (19%) adolescents 151 (81%) adults Leukaemia's 68 (8%) 13 (19%) adolescents 55 (81%) adults Bone cancer 65 (8%) 20 (31%) adolescents 45 (69%) adults Other cancers 46 (5%) 14 (30%) adolescents 32 (70%) adults <u>3. Age at diagnosis (years)</u> NM <25 years 111/300 (37%) patients <20 years at cryopreservation <15: 11 (1.3%) 15- <16: 15 (1.8%) 16- <17:19 (2.2%) 17- <18:21 (2.5%) 18- <19: 19 (2.2%) 19- <20:26 (3%) 20- <25:189 (22.2%) 25- <30: 271 (31.8%) 30- <35: 191 (22.4%) 35- <40: 89 (10.5%) Split into diagnose groups: - Testicular cancer Adolescents 17.6 ± 0.3		18- <19 yrs: 56 ± 5 19- <20 yrs: 48 ± 5 20- <25 yrs: 49 ± 2 *Sperm concentration (million/mL)* <15 yrs: 38.9 ± 13.9 15- <16 yrs: 56.0 ± 24.4 16- <17 yrs: 46.6 ± 15.0 17- <18 yrs : 49.9 ± 20.1 18- <19 yrs: 36.6 ± 11.4 19- <20 yrs: 34.7 ± 6.5 20- <25 yrs: 34.8 ± 3.7 *Ejaculate volume (mL)* <15 yrs: 1.5 ± 0.3 15- <16 yrs:1.7 ± 0.3 16- <17 yrs: 3.4 ± 0.5 17- <18 yrs: 2.4 ± 0.2 18- <19 yrs: 3.0 ± 0.3 19- <20 yrs: 3.0 ± 0.4 20- <25 yrs: 3.6 ± 0.1 *Sperm count (million/ejaculate)* <15 yrs: 38 ± 10.2 15- <16 yrs:73 ± 23.6 16- <17 yrs: 174.4 ± 58.7 17- <18 yrs :113.2 ± 35.6 18- <19 yrs: 119.7 ± 38.4 19- <20 yrs: 106.9 ± 20.5 20- <25 yrs: 135 ± 18.9 - Testicular volume significantly correlated with age (r = 0.24, P = .0096), ejaculate volume (r = 0.26,P = .0058), testosterone (P = .0048), and sperm count (P = .0064)	important determinants related to the outcome <u>D. Confounding</u> High risk Reason: multivariate analysis not performed

	Adults 29.2 ± 0.2 - Lymphomas Adolescents 17.5 ± 0.3 Adults 27.5 ± 0.4 - Leukaemias Adolescents 17.5 ± 0.5 Adults 27.1 ± 0.7 - Bone cancer Adolescents 17.0 ± 0.4 Adults 27.0 ± 0.8 - Other cancers Adolescents 17.1 ± 0.4 Adults 30.1 ± 0.9 <u>4. Age at follow-up</u> NM <u>5. Controls (if applicable)</u> - <u>6. Additional study characteristics, if relevant</u> - <u>7. Chemotherapy</u> NM <u>8. Radiotherapy</u> NM <u>9. Surgery</u> NM <u>10. Other treatments</u> Not reported		- No significant correlation between testicular volume and FSH *Pregnancy in patients <20 years* 1/1 (100%) patient who had IVF-ICSI achieved pregnancy but resulted in early abortion *Pregnancy in adults >20 years* 9 clinical pregnancies resulted in 11 live births (including 3 sets of twins) and 1 abortion from 11 adult patients	

Abbreviations: NA: not applicable; NM: not mentioned; yrs: years; IVF/ICSI: in vitro fertilization/ intracytoplasmic sperm injection; ART: assisted reproduction

What male reproductive preservation methods should be offered?				
Kliesch et al. Cryopreservation of Semen From Adolescent Patients With Malignancies. Med Pediatr Oncol. 1996;26:20-7				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Retrospective cross- sectional study 2. Treatment era Unclear 3. Follow-up: NM	1. Type and Number of Participants 28/239 (11.7%) male patients with malignant diseases (various) aged < 20 years *Total cohort:* 239 male patients: 210 patients aged >20 years; 29 patients aged 14-20 years 2. Diagnoses Testicular cancer Hodgkin's disease Leukaemia Other tumours (osteosarcoma, melanoma, astrocytoma, rhabdomyosarcoma of epididymis, Ewing's sarcoma) Other diseases (non-cancer) 3. Age at diagnosis (years) NM	1. Fertility Preservation method 239 patients produced semen sampling for cryopreservation *Method of sample collection* Unclear (?masturbation) *Timing of intervention* Before cancer treatment *Timing of semen analysis* Before cryopreservation	1. Outcome definitions *Testicular volumes* *Semen analysis* (Sperm concentration, total sperm number, motility and morphology of sperm prior to cryopreservation) *Live births* 2. Results *Semen analysis before cryopreservation* 28/29 (97%) patients with successful sample collected and cryopreserved 1/29 (3%) patient did not produce ejaculate (15-year old osteosarcoma patient) *Sperm motility before vs after freezing and thawing* 14-17 years: mean 30 ± 7 vs. mean 18 ± 6 18-20 years: mean 45 ± 5 vs. mean 22 ± 4 *Semen analysis before cryopreservation* *Total sperm number* Group 1: mean 157±94, median 46 Group 2: mean 127±33, median 72 *Total sperm number (mill/ejaculate)* Group 1: mean 157 ± 94, median 46	1. Strengths Adequate semen analysis. 2. Limitations - No long follow-up data on semen quality after thawing and pregnancy rate. - No control group 3. Risk of bias A. Selection bias unclear Reason: unclear how the 239 patients were selected B. Attrition bias Low risk Reason: outcomes assessed for all patients C. Detection bias unclear Reason: unclear if outcome assessors were blinded for important determinants related to the outcome D. Confounding High risk

	29/239 (12%) patients: 14-20 years at study <u>4. Age at cryopreservation</u> 3 groups: Group 1 age 12-17 (12 patients; median age = 15.9 years) Group 2 age 18-20 (17 patients; median age = 19.5 years) Group 3 age > 20 (210 patients; median age = 28.9 years) <u>4. Age at follow-up</u> NM <u>5. Controls (if applicable)</u> - <u>6. Additional study characteristics, if relevant</u> - <u>7. Chemotherapy</u> NM <u>8. Radiotherapy</u> NM <u>9. Surgery</u> NM <u>10. Other treatments</u> NM		Group 2: mean 127 ± 33, median 72 *Sperm concentration (mill/mL)* Group 1: mean 44 ± 21, median 13 Group 2: mean 40 ± 8, median 31 *Testes volume, right and left (mL)* Group 1: mean 29.6 ± 2.2 Group 2: mean 32.6 ± 2.4 *Live births* 5/239(2%) patients' partners had 13 inseminations 3/13 (23%) inseminations resulted in pregnancies 2/13 (15%) inseminations in patients' partners produced live births (twins) 1/13 (7.7%) inseminations in patients' partners resulted in abortion (unclear if these patients were <20 years at study)	Reason: no multivariate analysis performed

Abbreviations: NA: not applicable; NM: not mentioned

What male reproductive preservation methods should be offered?				
Ho et al. A short report on current fertility preservation strategies for boys. Clin Endocrinol (Oxf). 2017;87:279-285				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Retrospective study 2. Treatment era 1987 -2015 3. Follow-up: No long-term follow-up 4 pts died 40 pts still alive	1. Type and Number of Participants 30/44(68%) male patients with malignant diagnosis (various) *Original cohort*: 46 patients (2 declined) 2. Diagnoses 30/44(68%) malignant diagnosis 12/44 (27.2%) non-malignant diagnosis 3. Age at diagnosis NM Range 0.3-16.8 years Prepubertal: 33/44(75%) Pubertal: 11/44(25%) Patients with tissue only stored (n=33, prepubertal) Age at study 0.3-11.3	1. Fertility Preservation method 44 patients had testicular tissue cryopreservation collected *Timing of intervention* Prior to anticancer treatment *Timing of semen analysis* Prior to anticancer treatment	1. Outcome definitions - Tissue dissection in pubertal patients - Complications of intervention 2. Results *Tissue dissection in pubertal patients before cryopreservation* 3/11 (27.2%) azoospermic 8/11 (72.7%) mature sperm found (testicular size >10ml and Tanner stage 3; all CT naive) *Complications of intervention* 1/44 (2.3%) patient suffered scrotal wound dehiscence occurring 2 weeks after procedure (patient with aplastic anaemia) 0/44 patients had delay in treatment	1. Strengths Study present new fertility preservation method 2. Limitations 3. Risk of bias A. Selection bias Unclear Reason: unclear how the selection for the 46 patients was done B. Attrition bias Low risk Reason: outcomes assessed for all 44 patients C. Detection bias Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome D. Confounding bias

	Patients with tissue only store (n=3, pubertal) Age at study 12.7-16.8 Patients with tissue and sperm stored (n=4, pubertal): Age at study 12.7-16.2 Patients with sperm stored (n=4, pubertal): Age at study 13.0-15.9 <u>4. Age at follow-up</u> NM <u>5. Controls (if applicable)</u> - <u>6. Additional study characteristics, if relevant</u> - Prior to 2013 only sperm cryopreservation was offered - From October 2013, testicular tissue cryopreservation was offered to boys where a moderate to high risk to future fertility was anticipated (>30% risk) <u>7. Chemotherapy</u> 7/44(15.9) pts received previous CT Rest CT naive			High risk Reason: univariate analysis (not multivariate analysis used) so this study did not control for confounding factors

	8. Radiotherapy NM 9. Surgery NM 10. Other treatments NM			

Abbreviations: NM: not mentioned; CT: chemotherapy

What male reproductive preservation methods should be offered?				
Adank et al. Electroejaculation as a method of fertility preservation in boys diagnosed with cancer: a single-center experience and review of the literature. Fertil Steril. 2014;102:199-205.e1				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
1. Study design Retrospective cohort study 2. Treatment era From January 1998 to March 2013 semen cryopreservation was offered 3. Follow-up: No applicable Electroejaculation was offered from 2003 (when	1. Type and Number of Participants 114 boys diagnosed with cancer had sperm cryopreservation offered: 106 patients with cryopreservation attempt by masturbation 11 patients had cryopreservation attempt by electroejaculation: 8 patients were offered electroejaculation primarily; 3 patients were offered electroejaculation	1. Fertility Preservation method 81 patients produced semen sampling for cryopreservation *Method of sample collection* 78 patients via masturbation 3 patients via electroejaculation (under general anesthesia) *Timing of intervention* Before start of cancer treatment	1. Outcome definitions 1. Successful semen cryopreservation (based on the first attempt, defined as any motile spermatozoa present) 2. Semen analysis 2. Results *1. Successful semen cryopreservation via masturbation* 78/106 (68%) patients with successful sample collected and cryopreserved 18/106 (16%) patients with immotile spermatozoa or absent spermatozoa 10/106 (9%) patients were not able to produce an ejaculate	1. Strengths Focus on electroejacualation in the context of patients who attempt masturbation but fail to produce adequate sample 2. Limitations - Small cohort - No long-term follow-up data 3. Risk of bias A. Selection bias unclear Reason: unclear how many patients were included in original cohort

masturbation was not possible)	secondarily (failed to produce adequate semen by masturbation) <u>2. Diagnoses</u> Various In 11 patients who were offered electroejaculation: Leukaemia/NHL:4 HL: 5 Sarcoma/PNET:1 Diagnosis not reported in 1 patient. <u>3. Age at diagnosis</u> 16.5 years (10.8-18.9) <u>4. Age at follow-up</u> Not applicable <u>5. Controls (if applicable)</u> Not applicable <u>6. Additional study characteristics, if relevant</u> Tanner: Genital development 4.5 (3-5) Pubic hair development 4.5 (3-5) Testicular volume (mL): Left 14.3 (8.9-20.0) Right 13.5 (8.0-20.0) <u>7. Chemotherapy</u>	*Timing of semen analysis* At time of cryopreservation / Before start of cancer treatment	*Successful semen cryopreservation via electroejaculation* 3/11(27%) patients with successful sample collected and cryopreserved *2. Semen analysis in patients with successful sample collected and cryopreserved via electroejaculation* Volume (x10^6 mL): 0.4 (0.4-0.4) Concentration (x10^6/mL): 2.0 (0.1-5.5) Motility (%): 3.0 (2.0-4.0) pH: 7.9 *Semen analysis in patients without successful sample collected and cryopreserved via electroejaculation* Volume (x10^6 mL): 0.4 (0.02-3.0) Concentration (x10^6/mL): 2.0 (0.1-14.5) Motility (%): 0 pH: 7.0 (6.4-8.0)	<u>B. Attrition bias</u> Low risk Reason: outcomes in 106/114 (93%) patients <u>C. Detection bias</u> Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome <u>D. Confounding bias</u> High risk Reason: Descriptive, no multivariate analysis performed

	Not mentioned Patients with previous gonadotoxic therapy as well as patients diagnosed with a brain tumour were excluded <u>8. Radiotherapy</u> Not mentioned <u>9. Surgery</u> Not mentioned <u>10. Other treatments</u> Not mentioned			

What male reproductive preservation methods should be offered?				
Müller et al. Cryopreservation of semen from pubertal boys with cancer. Med Pediatr Oncol. 2000;34:191-4				
Study design Treatment era Years of follow-up	**Participants**	**Intervention**	**Main outcomes**	**Additional remarks**
<u>1. Study design</u> Cohort study <u>2. Treatment era</u> January 1 1995 – July 31 1998 <u>3. Follow-up:</u> Not applicable	<u>1. Type and Number of Participants</u> 21 patients delivered semen sample 45 patients with cancer eligible for cryopreservation (4 patients were not able to produce sperm; 20 patients time did not allow attempt of cryopreservation, or patient was not assessed to	<u>1. Fertility Preservation method</u> 21 patients produced semen sampling for cryopreservation *Method of sample collection* 18 patients via masturbation	<u>1. Outcome definitions</u> 1. Successful semen cryopreservation 2. Semen analysis <u>2. Results</u> *1. Successful semen cryopreservation via masturbation* 17/19 (89.5%) patients with successful sample collected and cryopreserved *Successful semen cryopreservation via electroejaculation*	<u>1. Strengths</u> Focus on electroejaculation as well as masturbation <u>2. Limitations</u> - Small cohort - No long-term follow-up data <u>3. Risk of bias</u> <u>A. Selection bias</u> Unclear

	be mature enough to deliver semen sample or the procedure was not accepted) <u>2. Diagnoses</u> ALL: 7 (2 with relapse) Non-Hodgkin lymphoma: 2 AML: 1 Hodgkin lymphoma: 6 Osteosarcoma: 2 Testicular cancer: 1 CNS tumour: 1 Wilms tumour: 1 <u>3. Age at diagnosis</u> Mean 14.5 years (13-18) <u>4. Age at follow-up</u> Not applicable <u>5. Controls (if applicable)</u> Not applicable <u>6. Additional study characteristics, if relevant</u> 2 patients suffered from relapse and had received before chemotherapy for standard-risk ALL (vincristine, daunorubicin, MTX, and prednisolone) <u>7. Chemotherapy</u> Not mentioned	2 patients via electroejaculation (under general anesthesia) 1 patient via vibration *Timing of intervention* Before start of cancer treatment (2 patients had received chemotherapy before) *Timing of semen analysis* Before start of cancer treatment (2 patients had received chemotherapy before)	2/2 (100%) patients with successful sample collected and cryopreserved *2. Semen analysis in patients with successful sample collected and cryopreserved via masturbation* Median percentage of motile sperm: 50% (range 9-86%) *Semen analysis in patients with successful sample collected and cryopreserved via electroejaculation* Patient 1: Volume 0.8 mL Concentation 75 x 10^6/mL Motility 38% Patient 2: Volume 3.2 mL Concentration 4.0 x 10^6/mL Motility 10%	Reason: unclear how the 45 patients were selected <u>B. Attrition bias</u> High risk Reason: outcomes in 21/45 (47%) patients <u>C. Detection bias</u> Unclear Reason: unclear if the outcome assessors were blinded for important determinants related to the outcome <u>D. Confounding bias</u> High risk Reason: Descriptive, no multivariate analysis performed

	8. Radiotherapy Not mentioned 9. Surgery Not mentioned 10. Other treatments Not mentioned			