Summary of findings tables, grading of the evidence and detailed conclusions of evidence male fertility preservation

Who should be informed about potential infertility risk?

1. **What is the patient and/or parents/caregivers/partners reported desire and satisfaction with the information about treatment-related fertility risks, fertility preservation and alternative family planning options provided to cancer patients diagnosed before age 25 years?**

Outcome	Study	Participants	Age at patients' diagnosis	Method	Summary of findings
1.1. Satisfaction with information reported by patients and parents (n=1 study)	Wyns 2015	120 pre-pubertal and adolescent males with childhood cancer and their parents 78 responded to the questionnaire related to communication issues	0-18 years 85 (71%) <12 years 35 (29%) 12-18 years	Survey	*Satisfaction with information* 19% was not satisfied with the fertility preservation information content (completeness) Completeness of information provided to patients and parents positively impact decision to preserve fertility (p=0.04) Among boys aged >12 years, 72% considered the information to be clear, 80% complete and 90.9% understandable Among boys aged <12 years, 33.3% were able to comprehend the information, the youngest being 11 years old
GRADE Assessment:					
Methodological limitations:	Some methodological limitations in 1/1				
Coherence:	NA (1 study only)				
Adequacy of data:	Important concerns on adequacy of data: only 1 study investigating satisfaction with information				
Relevance:	No concerns on relevance (all cancer patients)				
Overall assessment of confidence in findings:	**LOW confidence in the evidence**				
Conclusion:	Not all pre- and post-pubertal patients and their families are satisfied with the fertility preservation information content. Satisfaction about completeness of information positively impacts the decision to preserve fertility. *(1 survey; 78 study participants)*				

Abbreviations: NM, not mentioned; NA, not applicable

Outcome	Study	Participants	Age at patients' diagnosis	Method	Summary of findings
1.2. Desire for information reported by patients and parents (n=2 studies)	Gupta 2013	243 cancer patients receiving treatment, or within 5 years of completion of treatment	Age at diagnosis: NM Age at study: median 28 years (17-35 years)	Survey Adapted existing survey to use Likert Scale of importance (1-10) Piloted study with 10 patients and 10 healthcare providers	*Desire for information in fertility preservation discussion* Patients reported information about the effects of cancer treatment on fertility and fertility preservation before cancer treatment as very important (median scores of 9 and 10 in scale 1-10) Female patients rated information on fertility preservation methods (p=0.004) and risk of infertility (p=0.033) as more important than did male patients
	Gupta 2016	153 parents of pre-pubertal males with cancer 77 male survivors of childhood cancer	Parents' child: ≤12 years, median 4 years Survivors: ≤12 years, median 5 years	In-depth interviews	*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
GRADE Assessment:					
Methodological limitations:	Some methodological limitations in 2/2				
Coherence:	No concerns on coherence				
Adequacy of data:	Some concerns on adequacy of data: 1 study investigating desire of information in fertility preservation discussion; 1 study investigating desire of information about testicular biopsy (2 studies; 473 study participants)				
Relevance:	No concerns on relevance (>85% cancer patients in 2/2)				
Overall assessment of confidence in findings:	**LOW confidence in the evidence**				
Conclusion:	Post-pubertal patients have a high desire for information about the effects of cancer treatment on fertility (median score 9) and options for fertility preservation (median score 10) (scale 1-10, includes male and female) *(1 survey; 243 study participants)* Most pre-pubertal patients and their parents would have wanted information on testicular biopsy irrespective of infertility risk (*1 in-depth interview study; 230 study participants)*				

Abbreviations: NM, not mentioned; NA, not applicable

Outcome	Study	Participants	Age at patients' diagnosis	Method	Summary of findings
1.3. Desire for information reported by healthcare providers (n=1 study)	Quinn 2009	24 paediatric oncologists	NM	Semistructured in-depth interviews	*Desire for information about fertility preservation (according to healthcare professionals)* 50% of paediatric oncologists reported that parents and patients want fertility preservation information, but parents and patients are either too embarrassed to discuss it or do not know how to begin a discussion
GRADE Assessment:					
Methodological limitations:	Some methodological limitations in 1/1				
Coherence:	NA (1 study only)				
Adequacy of data:	Important concerns on adequacy of data (1 study; 24 study participants)				
Relevance:	Important concerns on relevance (paediatric oncologists reporting on behalf of patients and parents)				
Overall assessment of confidence in findings:	**VERY LOW confidence in the evidence**				
Conclusion:	Some patients and their parents desire information about fertility preservation but experience difficulties initiating discussions on this topic *(1 semistructured in-depth interview study; 24 study participants*)				

Abbreviations: NM, not mentioned; NA, not applicable

Who should be counselled about fertility preservation?

1. What is the risk of impaired spermatogenesis / testosterone deficiency in male cancer patients diagnosed before age 25 years who will be treated with alkylating agents?

Impaired spermatogenesis

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
1.1.1 Risk impaired spermatogenesis after alkylating agents (any type) and after higher vs. lower doses (n=5 studies)	Lopez Andreu 2000	43 CCS	Mean 13.6 (3.9-25.2) yr after cancer treatment	Cyclophosphamide: 20.9%; Radiotherapy to testes: 2.3%; Abdominal Radiotherapy: 4.7% Cranial radiotherapy: 25.6% Craniospinal radiotherapy: 7.0%	10/43 (23.2%) infertile (azoospermia or severe oligo-asthenozoospermia (<20% progressive motility)	*Risk for infertility* Cumulative cyclophosphamide dose was significantly associated (no effect measure reported)	SB: unclear AB: low risk DB: unclear CF: high risk
	Van Beek 2007	56 childhood Hodgkin lymphoma survivors	Median 15.5 (5.6-30.2) yr after cancer treatment	Alkylating agents: 100%; Radiotherapy to testes: 0%; Cranial radiotherapy: 0%	9/21 (42.9%) azoospermia	*Risk for decreased sperm concentration* Number of MOPP cycles: β -6.25 ($p<0.05$) (Each increase in number of MOPP cycles, mean sperm concentration decreased by 6.25 x 10^6/mL)	SB: high risk AB: high risk DB: unclear CF: low risk
	Jahnukainen 2011	51 childhood ALL survivors vs. 56 age-matched males	Median 20 (11-30) yr after cancer treatment	Cyclophosphamide: 51.0%; Radiotherapy to testes: 35.3%; Cranial radiotherapy: 74.5%	Not reported: median sperm concentration and sperm count	*Median (IQR) sperm concentration (10^6/mL) CCS vs. controls* Controls: 50 (27-66); No cyclophosphamide, no testicular irradiation: 41 (29-74), $p>0.05$; ≤10 g/m² cyclophosphamide, no testicular irradiation: 35 (24-	SB: high risk AB: low risk DB: unclear CF: low risk

						42), p>0.05; >20 g/m² cyclophosphamide, no testicular irradiation: 1 (0-17), p<0.05; Testicular irradiation ± cyclophosphamide: 0, p<0.05	
	Green 2014*	214 CCS	Median 21.0 (10.5-41.6) yr after cancer diagnosis	Alkylating agents: 100%; Radiotherapy to testes: 0%; Cranial radiotherapy: 0%	53/214 (24.7%); azoospermia; 59/214 (27.6%) oligospermia (sperm concentration >0-<15 x 10^6/mL)	*Odds ratio (95% CI) for azoospermia vs. normospermia* CED per 1,000 mg/m²: OR 1.22 (1.11-1.34) *Odds ratio (95% CI) for oligospermia vs. normospermia* CED per 1,000 mg/m²: OR 1.14 (1.04-1.25)	SB: high risk AB: low risk DB: unclear CF: low risk
	Green 2017*	241 childhood ALL survivors	CRT: Mean 26.3 ± 6.3 yr; No CRT: Mean 18.7 ± 6.0 yr	Alkylating agents: 100%; Radiotherapy to testes: 0%; Cranial radiotherapy >26 Gy: 55.6%	65/241 (37.6%) azoospermia; 46/241 (26.6%) oligospermia (sperm concentration >0-<15 x 10^6/mL)	*Relative risk (95% CI) for azoospermia or oligospermia* CED (mg/m²) ≥4000-8000 vs. >0-<4000: RR 1.42 (0.70-2.89); CED (mg/m²) ≥8000-1200 vs. >0-<4000: RR 2.06 (1.08-3.94); CED (mg/m²) ≥12000 vs. >0-<4000: RR 2.12 (1.09-4.12)	SB: high risk AB: high risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 4/5, unclear in 1/5; Attrition bias low in 3/5, high in 2/5; Detection bias unclear in 5/5; Confounding low in 4/5, high in 1/5
Consistency:	0	No important inconsistency, all show effect of (higher doses of) alkylating agents
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊕⊕ HIGH
Conclusion:		Increased risk of impaired spermatogenesis after alkylating agents vs. no alkylating agents in male cancer survivors diagnosed before age 25 years. Increased risk of impaired spermatogenesis after increasing doses of alkylating agents in male cancer survivors diagnosed before age 25 years. (5 studies significant effect; 605 participants; 137 events; 4 multivariable analyses)

Abbreviations: AB, attrition bias; ALL, acute lymphoblastic leukaemia; CCS, childhood cancer survivors; CED, cyclophosphamide equivalence dose; CF, confounding; DB, detection bias; IQR, inter quartile range; MOPP: mechlorethamine, vincristine, prednisone, procarbazine; SB, selection bias; yr, year.
* Overlap in included patients.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
1.1.2 Risk impaired spermatogenesis after cyclophosphamide and after higher vs. lower doses (n=4 studies)	Lopez Andreu 2000	43 CCS	Mean 13.6 (3.9-25.2) yr after cancer treatment	Cyclophosphamide: 20.9%; Radiotherapy to testes: 2.3%; Abdominal Radiotherapy: 4.7% Cranial radiotherapy: 25.6% Craniospinal radiotherapy: 7.0%	10/43 (23.2%) infertile (azoospermia or severe oligo-asthenozoospermia (<20% progressive motility)	*Risk for infertility* Cumulative cyclophosphamide dose was significantly associated (no effect measure reported)	SB: unclear AB: low risk DB: unclear CF: high risk
	Jahnukainen 2011	51 childhood ALL survivors vs. 56 age-matched males	Median 20 (11-30) yr after cancer treatment	Cyclophosphamide: 51.0%; Radiotherapy to testes: 35.3%; Cranial radiotherapy: 74.5%	Not reported: median sperm concentration and sperm count	*Median (IQR) sperm concentration (10^6/mL) CCS vs. controls* Controls: 50 (27-66); No cyclophosphamide, no testicular irradiation: 41 (29-74), p>0.05; ≤10 g/m² cyclophosphamide, no testicular irradiation: 35 (24-42), p>0.05; >20 g/m² cyclophosphamide, no testicular irradiation: 1 (0-17), p<0.05; Testicular irradiation ± cyclophosphamide: 0, p<0.05	SB: high risk AB: low risk DB: unclear CF: low risk
	Green 2014*	214 CCS	Median 21.0 (10.5-41.6) yr after cancer diagnosis	Alkylating agents: 100% (of which 91% cyclophosphamide); Radiotherapy to testes: 0%; Cranial radiotherapy: 0%	53/214 (24.7%); azoospermia; 59/214 (27.6%) oligospermia (sperm concentration >0 -<15 x 10^6/mL)	*Odds ratio (95% CI) for azoospermia vs. normospermia* CED per 1,000 mg/m²: OR 1.22 (1.11-1.34) *Odds ratio (95% CI) for oligospermia vs. normospermia* CED per 1,000 mg/m²: OR 1.14 (1.04-1.25)	SB: high risk AB: low risk DB: unclear CF: low risk
	Green 2017*	241 childhood ALL survivors	CRT: Mean 26.3 ± 6.3 yr; No CRT: Mean 18.7 ± 6.0 yr	Alkylating agents (cyclophosphamide): 100%; Radiotherapy to testes: 0%;	65/241 (37.6%) azoospermia; 46/241 (26.6%) oligospermia (sperm concentration >0-<15 x	*Relative risk (95% CI) for azoospermia or oligospermia* CED (mg/m²) ≥4000-8000 vs. >0-<4000: RR 1.42 (0.70-2.89); CED (mg/m²) ≥8000-1200 vs. >0-	SB: high risk AB: high risk DB: unclear CF: low risk

			Cranial radiotherapy >26 Gy: 55.6%	10^6/mL)	<4000: RR 2.06 (1.08-3.94); CED (mg/m²) ≥12000 vs. >0-<4000: RR 2.12 (1.09-4.12)	
GRADE assessment:						
Study design:	+4	Observational studies				
Study limitations:	-1	Some limitations: Selection bias high in 3/4, unclear in 1/4; Attrition bias low in 3/4, high in 1/4; Detection bias unclear in 4/4; Confounding low in 3/4, high in 1/4				
Consistency:	0	No important inconsistency, all show effect of (higher doses of) cyclophosphamide				
Directness:	0	Results are direct, population and outcomes broadly generalizable				
Precision:	0	No important imprecision, narrow confidence intervals				
Publication bias:	0	Unlikely				
Effect size:	0	No large magnitude of effect				
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses				
Plausible confounding:	0	No plausible confounding				
Quality of evidence:		⊕⊕⊕⊕ HIGH				
Conclusion:		Increased risk of impaired spermatogenesis after cyclophosphamide vs. no cyclophosphamide in male cancer survivors diagnosed before age 25 years. Increased risk of impaired spermatogenesis after increasing doses of cyclophosphamide in male cancer survivors diagnosed before age 25 years. (4 studies significant effect; 549 participants; 128 events; 3 multivariable analyses)				

Abbreviations: AB, attrition bias; ALL, acute lymphoblastic leukaemia; CCS, childhood cancer survivors; CED, cyclophosphamide equivalence dose; CF, confounding; DB, detection bias; IQR, inter quartile range; SB, selection bias; yr, year.
* Overlap in included patients.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
1.1.3 Risk impaired spermatogenesis after procarbazine and mechlorethamine vs. no procarbazine and mechlorethamine and higher vs. lower doses (n=1 study)	Van Beek 2007	56 childhood Hodgkin lymphoma survivors	Median 15.5 (5.6-30.2) yr after cancer treatment	Alkylating agents: 100% ABVD/EBVD, 71% MOPP; Radiotherapy to testes: 0%; Cranial radiotherapy: 0%	9/21 (42.9%) azoospermia	*Risk for decreased sperm concentration* Number of MOPP cycles (including procarbazine and mechlorethamine): β -6.25 (p<0.05) (Each increase in number of MOPP cycles, mean sperm concentration decreased by 6.25 x 10^6/mL)	SB: high risk AB: high risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					

Study limitations:	-2	Important limitations: Selection bias high in 1/1; Attrition bias high in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-2	Important imprecision, only 1 study included and small study population
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⨁⊖⊖⊖ VERY LOW
Conclusion:		Increased risk of impaired spermatogenesis after procarbazine and mechlorethamine (given as part of multi-agent treatment) vs. no procarbazine and mechlorethamine in male cancer survivors diagnosed before age 25 years. Increased risk of impaired spermatogenesis after increasing doses of procarbazine and mechlorethamine (given as part of multi-agent treatment) in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 56 participant, 9 events, 1 multivariable analysis).

Abbreviations: AB, attrition bias; ABVD/EBVD: adriamycin or epirubicin, bleomycin, vinblastine, dacarbazine; CF, confounding; DB, detection bias; MOPP: mechlorethamine, vincristine, prednisone, procarbazine; N/A, not applicable; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
1.1.4 Risk impaired spermatogenesis after higher vs. lower doses dacarbazine (n=1 study)	Van Beek 2007	56 childhood Hodgkin lymphoma survivors	Median 15.5 (5.6-30.2) yr after cancer treatment	Alkylating agents: 100% ABVD/EBVD, 71% MOPP; Radiotherapy to testes: 0%; Cranial radiotherapy: 0%	9/21 (42.9%) azoospermia	*Risk for decreased sperm concentration* Number of ABVD/EBVD cycles: p>0.05 (no effect measures reported)	SB: high risk AB: high risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-2	Important limitations: Selection bias high in 1/1; Attrition bias high in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1					
Consistency:	0	N/A (1 study)					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	-2	Important imprecision, only 1 study included and small study population					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:		⨁⊖⊖⊖ VERY LOW					

Conclusion:	No significant effect of dacarbazine dose on the risk of impaired spermatogenesis in male cancer survivors diagnosed before age 25 years. (1 study non-significant effect, 56 participant, 9 events, 1 multivariable analysis).

Abbreviations: AB, attrition bias; ABVD/EBVD: adriamycin or epirubicin, bleomycin, vinblastine, dacarbazine; CF, confounding; DB, detection bias; MOPP: mechlorethamine, vincristine, prednisone, procarbazine; N/A, not applicable; SB, selection bias; yr, year.

Testosterone deficiency

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
1.2.1 Risk testosterone deficiency after alkylating agents (any type) (n=4 studies)	Siimes 1993	41 childhood ALL survivors	Mean 15.2 (4.0-25.0) yr after cancer diagnosis	Cyclophosphamide: 56.1%; Radiotherapy to testes: 0%; Cranial radiotherapy: 41.5%	Not reported: median testosterone levels	*Risk for lower* (*but not necessarily abnormal) testosterone levels* Cyclophosphamide was not significantly associated (no effect measure reported)	SB: unclear AB: low risk DB: unclear CF: low risk
	Jahnukainen 2011	51 childhood ALL survivors vs. 56 age-matched males	Median 20 (11-30) yr after cancer treatment	Cyclophosphamide: 51.0%; Radiotherapy to testes: 35.3%; Cranial radiotherapy: 74.5%	Not reported: median testosterone levels	*Median (IQR) testosterone levels (pmol/L) CCS vs. controls* Controls: 18.4 (14.7-24.0); No cyclophosphamide, no testicular irradiation: 18.3 (13.6-20.1), p>0.05; ≤10 g/m² cyclophosphamide, no testicular irradiation: 12.7 (12.2-16.6), p<0.05; >20 g/m² cyclophosphamide, no testicular irradiation: 13.4 (7.7-17.5), p<0.05; Testicular irradiation ± cyclophosphamide: 1.4 (0.9-8.9), p<0.05	SB: high risk AB: low risk DB: unclear CF: low risk
	Tromp 2011	565 CCS	Median 15 (range 5.0-39.0) yr after cancer diagnosis	Alkylating agents: at least 59.5%; Radiotherapy to testes: 9.7% pelvic abdominal irradiation, 1.9% TBI; Cranial radiotherapy:	57/460 (12.4%) ↓ testosterone	*Risk for lower* (*but not necessarily abnormal) testosterone levels* Cyclophosphamide, procarbazine and other alkylating agents were not significantly associated (no effect measure reported)	SB: high risk AB: low risk DB: unclear CF: low risk

				21.9%			
	Chemaitilly 2019	1,516 CCS	Median 22.0 (range 7.5-49.8) yr after cancer diagnosis	Alkylating agents: 59.2% Radiotherapy to testes: 8.1% Unilateral orchiectomy: 2.3%	104/1516 (6.9%) Leydig cell failure: morning serum levels of total testosterone <250 ng/dL (or 8.67 nmol/L) and LH > 9.85 IU/L	*Odds ratio (95% CI) for Leydig cell failure* CED >0->4,000 mg/m² vs. none: OR 0.5 (0.2-1.7); CED 4,000-<8,000 mg/m² vs. none: OR 3.4 (1.7-6.8); CED 8,000-<12,000 mg/m² vs. none: OR 2.9 (1.4-6.0); CED ≥12,000 mg/m² vs. none: OR 5.6 (2.8-10.9)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 3/4, unclear in 1/4; Attrition bias low in 4/4; Detection bias unclear in 4/4; Confounding low in 4/4
Consistency:	-1	Some inconsistency, 2 studies show a significant effect of alkylating agents and 2 studies show no significant effect of cyclophosphamide (unclear which direction)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, large number of patients and events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Increased risk of testosterone deficiency after alkylating agents vs. no alkylating agents in male cancer survivors diagnosed before age 25 years. (2 studies significant effect, 2 studies non-significant effect; 2,173 participants; 161 events; 4 multivariable analyses)

Abbreviations: ALL, acute lymphoblastic leukaemia; AB, attrition bias; CCS, childhood cancer survivors; CED, cyclophosphamide equivalent dose; CF, confounding; DB, detection bias; IQR, inter quartile range; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
1.2.2 Risk testosterone deficiency after higher vs. lower alkylating agent dose (any type) (n=3 studies)	Mackie 1996	58 childhood Hodgkin disease survivors	Median 6 (range 2.5-11.1) years after cancer diagnosis	Alkylating agents: 100%; Radiotherapy to testes: 0%; Cranial radiotherapy: 0%	5/37 (13.5%) ↓ testosterone; 10/41 (24.4%) ↑ LH	*Risk for Leydig cell dysfunction* Higher amount of ChlVPP chemotherapy (including chlorambicil and procarbazine) was not significantly associated (no effect measure reported)	SB: high risk AB: high risk DB: unclear CF: low risk
	Jahnukainen 2011	51 childhood ALL survivors vs. 56 age-	Median 20 (11-30) yr after cancer treatment	Cyclophosphamide: 51.0%; Radiotherapy to	Not reported: median testosterone levels	*Median (IQR) testosterone levels (pmol/L) CCS vs. controls* Controls: 18.4 (14.7-24.0);	SB: high risk AB: low risk DB: unclear

		matched males		testes: 35.3%; Cranial radiotherapy: 74.5%		No cyclophosphamide, no testicular irradiation: 18.3 (13.6-20.1), p>0.05; ≤10 g/m² cyclophosphamide, no testicular irradiation: 12.7 (12.2-16.6), p<0.05; >20 g/m² cyclophosphamide, no testicular irradiation: 13.4 (7.7-17.5), p<0.05; Testicular irradiation ± cyclophosphamide: 1.4 (0.9-8.9), p<0.05	CF: low risk
	Chemaitilly 2019	1,516 CCS	Median 22.0 (range 7.5-49.8) yr after cancer diagnosis	Alkylating agents: 59.2% Radiotherapy to testes: 8.1% Unilateral orchiectomy: 2.3%	104/1516 (6.9%) Leydig cell failure: morning serum levels of total testosterone <250 ng/dL (or 8.67 nmol/L) and LH > 9.85 IU/L	*Odds ratio (95% CI) for Leydig cell failure* CED >0->4,000 mg/m² vs. none: OR 0.5 (0.2-1.7); CED 4,000-<8,000 mg/m² vs. none: OR 3.4 (1.7-6.8); CED 8,000-<12,000 mg/m² vs. none: OR 2.9 (1.4-6.0); CED ≥12,000 mg/m² vs. none: OR 5.6 (2.8-10.9); 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)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 3/3; Attrition bias low in 2/3, high in 1/3; Detection bias unclear in 3/3; Confounding low in 3/3
Consistency:	-1	Some inconsistency, 1 study shows significant effect of alkylating agent dose and 2 studies show non-significant effect of alkylating agent dose
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, large study population and number of events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Low quality evidence for a dose-response relationship, so not totally certain
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Increased risk of testosterone deficiency after increasing doses of alkylating agents in male cancer survivors diagnosed before age 25 years (1 study significant effect, 2 studies non-significant effect; 1,625 participants;109 events; 3 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CED, cyclophosphamide equivalent dose; ChlVPP, clorambucil, vinblastine, prednisolone, procarbazine; CF, confounding; DB, detection bias; NM, not mentioned; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
1.2.3 Risk testosterone deficiency after cyclophosphamide (n=4 studies)	Siimes 1993	41 childhood ALL survivors	Mean 15.2 (4.0-25.0) yr after cancer diagnosis	Alkylating agents: 51.0%; Radiotherapy to testes: 0%; Cranial radiotherapy: 41.5%	Not reported: median testosterone levels	*Risk for lower (but not necessarily abnormal) testosterone levels* Cyclophosphamide was not significantly associated (no effect measure reported)	SB: unclear AB: low risk DB: unclear CF: low risk
	Jahnukainen 2011	51 childhood ALL survivors vs. 56 age-matched males	Median 20 (11-30) yr after cancer treatment	Cyclophosphamide: 51.0%; Radiotherapy to testes: 35.3%; Cranial radiotherapy: 74.5%	Not reported: median testosterone levels	*Median (IQR) testosterone levels (pmol/L) CCS vs. controls* Controls: 18.4 (14.7-24.0); No cyclophosphamide, no testicular irradiation: 18.3 (13.6-20.1), p>0.05; ≤10 g/m² cyclophosphamide, no testicular irradiation: 12.7 (12.2-16.6), p<0.05; >20 g/m² cyclophosphamide, no testicular irradiation: 13.4 (7.7-17.5), p<0.05; Testicular irradiation ± cyclophosphamide: 1.4 (0.9-8.9), p<0.05	SB: high risk AB: low risk DB: unclear CF: low risk
	Tromp 2011	565 CCS	Median 15 (range 5.0-39.0) yr after cancer diagnosis	Alkylating agents: at least 59.5%; Radiotherapy to testes: 9.7% pelvic abdominal irradiation, 1.9% TBI; Cranial radiotherapy: 21.9%	57/460 (12.4%) ↓ testosterone	*Risk for lower (but not necessarily abnormal) testosterone levels* Cyclophosphamide was not significantly associated (no effect measure reported)	SB: high risk AB: low risk DB: unclear CF: low risk
	Chemaitilly 2019	1,516 CCS	Median 22.0 (range 7.5-49.8) yr after cancer	Alkylating agents: 59.2% Radiotherapy to	104/1516 (6.9%) Leydig cell failure: morning serum levels	*Odds ratio (95% CI) for Leydig cell failure* CED >0->4,000 mg/m² vs. none: OR 0.5 (0.2-1.7);	SB: high risk AB: low risk DB: unclear

			diagnosis	testes: 8.1% Unilateral orchiectomy: 2.3%	of total testosterone <250 ng/dL (or 8.67 nmol/L) and LH > 9.85 IU/L	CED 4,000-<8,000 mg/m^2 vs. none: OR 3.4 (1.7-6.8); CED 8,000-<12,000 mg/m^2 vs. none: OR 2.9 (1.4-6.0); CED ≥12,000 mg/m^2 vs. none: OR 5.6 (2.8-10.9)	CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Limitations: Selection bias high in 2/3, unclear in 1/3; Attrition bias low in 3/3; Detection bias unclear in 3/3; Confounding low in 3/3					
Consistency:	-1	Some inconsistency, 2 studies show no significant effect of cyclophosphamide (unclear which direction), 1 study shows a significant effect of cyclophosphamide					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	0	No important imprecision, large study population and number of events					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	No dose-response relationship					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:		⊕⊕⊖⊖ LOW					
Conclusion:		Increased risk of testosterone deficiency after cyclophosphamide vs. no cyclophosphamide in male cancer survivors diagnosed before age 25 years. (2 studies significant effect, 2 studies non-significant effect; 2,173 participants; 161 events; 4 multivariable analyses)					

Abbreviations: ALL, acute lymphoblastic leukaemia; AB, attrition bias; CCS, childhood cancer survivors; CED, cyclophosphamide equivalent dose; CF, confounding; DB, detection bias; IQR, inter quartile range; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Cranial radiotherapy	Events	Effect size	Risk of bias
1.2.4 Risk testosterone deficiency after higher vs. lower cyclophosphamide dose (n=2 studies)	Jahnukainen 2011	51 childhood ALL survivors vs. 56 age-matched males	Median 20 (11-30) yr after cancer treatment	Cyclophosphamide: 51.0%; Radiotherapy to testes: 35.3%; Cranial radiotherapy: 74.5%	Not reported: median testosterone levels	*Median (IQR) testosterone levels (pmol/L) CCS vs. controls* Controls: 18.4 (14.7-24.0); No cyclophosphamide, no testicular irradiation: 18.3 (13.6-20.1), p>0.05; ≤10 g/m^2 cyclophosphamide, no testicular irradiation: 12.7 (12.2-16.6), p<0.05; >20 g/m^2 cyclophosphamide, no testicular irradiation: 13.4 (7.7-17.5), p<0.05; Testicular irradiation ± cyclophosphamide: 1.4 (0.9-8.9), p<0.05	SB: high risk AB: low risk DB: unclear CF: low risk

	Chemaitilly 2019	1,516 CCS	Median 22.0 (range 7.5-49.8) yr after cancer diagnosis	Alkylating agents: 59.2% Radiotherapy to testes: 8.1% Unilateral orchiectomy: 2.3%	104/1516 (6.9%) Leydig cell failure: morning serum levels of total testosterone <250 ng/dL (or 8.67 nmol/L) and LH > 9.85 IU/L	*Odds ratio (95% CI) for Leydig cell failure* CED >0->4,000 mg/m^2 vs. none: OR 0.5 (0.2-1.7); CED 4,000-<8,000 mg/m^2 vs. none: OR 3.4 (1.7-6.8); CED 8,000-<12,000 mg/m^2 vs. none: OR 2.9 (1.4-6.0); CED ≥12,000 mg/m^2 vs. none: OR 5.6 (2.8-10.9); 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)	SB: high risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Limitations: Selection bias high in 2/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2					
Consistency:	-1	Some inconsistency, 1 study shows significant effect of cyclophosphamide dose and 1 study show non-significant effect of cyclophosphamide dose					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	0	No important imprecision, large study population and number of events					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	Low quality evidence for a dose-response relationship, so not totally certain					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:		⊕⊕⊖⊖ LOW					
Conclusion:		Increased risk of testosterone deficiency after increasing doses of alkylating agents in male cancer survivors diagnosed before age 25 years (1 study significant effect, 1 study non-significant effect; 1567 participants; 104 events; 1 multivariable analysis)					

Abbreviations: ALL, acute lymphoblastic leukaemia; AB, attrition bias; CED, cyclophosphamide equivalent dose; CF, confounding; DB, detection bias; IQR, inter quartile range; N/A, not applicable; SB, selection bias; yr, year.

Subgroup	Study	No. of participants	Follow up (median/mean, range) yr	Cranial radiotherapy	Events	Effect size	Risk of bias
1.2.5 Risk testosterone deficiency after procarbazine	Tromp 2011	565 CCS	Median 15 (range 5.0-39.0) yr after cancer diagnosis	Alkylating agents: at least 59.5%; Radiotherapy to testes: 9.7% pelvic	57/460 (12.4%) ↓ testosterone	*Risk for lower (but not necessarily abnormal) testosterone levels* Procarbazine was not significantly associated (no effect measure	SB: high risk AB: low risk DB: unclear CF: low risk

(n=1 study)			abdominal irradiation, 1.9% TBI; Cranial radiotherapy: 21.9%	reported)
GRADE assessment:				
Study design:	+4	Observational study		
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1		
Consistency:	0	N/A (1 study)		
Directness:	0	Results are direct, population and outcomes broadly generalizable		
Precision:	-2	Important imprecision, only 1 study included and small study population and number of events unclear		
Publication bias:	0	Unlikely		
Effect size:	0	No large magnitude of effect		
Dose-response:	0	No dose-response relationship		
Plausible confounding:	0	No plausible confounding		
Quality of evidence:		⊕⊖⊖⊖ VERY LOW		
Conclusion:		No significant effect of procarbazine vs. no procarbazine on the risk of testosterone deficiency (analyzed as lower, but not necessarily abnormal testosterone levels) in male cancer survivors diagnosed before age 25 years. (1 study non-significant effect, 565 participants, 57 events, 1 multivariable analysis)		

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; N/A, not applicable; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
1.2.6 Risk testosterone deficiency after higher vs. lower procarbazine and chlorambucil dose (n=1 study)	Mackie 1996	58 childhood Hodgkin disease survivors	Median 6 (range 2.5-11.1) years after cancer diagnosis	Alkylating agents: 100%; Radiotherapy to testes: 0%; Cranial radiotherapy: 0%	5/37 (13.5%) ↓ testosterone; 10/41 (24.4%) ↑ LH	*Risk for Leydig cell dysfunction* Higher amount of ChlVPP chemotherapy (including procarbazine and chlorambucil) was not significantly associated (no effect measure reported)	SB: high risk AB: high risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational study					
Study limitations:	-2	Important limitations: Selection bias high in 1/1; Attrition bias high in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1					
Consistency:	0	N/A (1 study)					
Directness:	0	Results are direct, population and outcomes broadly generalizable					

Precision:	-2	Important imprecision, only 1 study included and low number of events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊖⊖⊖ VERY LOW
Conclusion:		No significant effect of procarbazine and chlorambucil dose (given as part of multi-agent treatment) on the risk of testosterone deficiency in male cancer survivors diagnosed before age 25 years. (1 study non-significant effect, 58 participants, 5 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; ChlVPP: clorambucil, vinblastine, prednisolone, procarbazine; CF, confounding; DB, detection bias; LH, luteinizing hormone; N/A, not applicable; SB, selection bias; yr, year.

Combined outcomes referring to hypogonadism

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
1.3.1 Risk hypogonadism after alkylating agents (n=2 studies)	Brignardello 2016	199 CCS	Median 14.01 (inter quartile range 10.08-17.76) yr	Alkylating agents: 85.5%; Radiotherapy to testes: 16.6%; Cranial radiotherapy: 19.1%	68/199 (34.2%) spermatogenesis damage (↑ FSH, ↓ inhibin B) confirmed in 41 patients in whom semen analysis was performed; 13/199 (6.5%) primary hypogonadism (↓ testosterone)	*Odds ratio (95% CI) for spermatogenesis damage and primary hypogonadism* Alkylating + platinum agents vs. alkylating agents only: OR 9.22 (2.17-39.23); Other chemotherapy or none vs. alkylating agents only: OR 0.19 (0.05-0.76)	SB: unclear AB: low risk DB: unclear CF: low risk
	Isaksson 2018	125 CCS	Mean 24.3 (±7.1) years after cancer treatment	Alkylating agents: 13%; CED >4000 mg/m^2: 10 (8.0%) Radiotherapy to testes: 4.0%; Cranial radiotherapy: 9.6%; Cranial radiotherapy and chemotherapy: 13%	31/121 (25.6%) hypogonadism (primary hypogonadism: ↓ testosterone, ↑ LH and FSH with FSH > LH or ↓ testosterone, ↓ LH and ↑ FSH; secondary hypogonadism: ↓ testosterone, ↓LH and FSH; compensated hypogonadism: ↑ testosterone, ↑ LH; or ongoing androgen	*Odds ratio (95% CI) for hypogonadism survivors vs. controls* CED >4000 mg/m^2: OR 2.0 (0.36-11.0)	SB: high risk AB: low risk DB: unclear CF: low risk

		replacement therapy)
GRADE assessment:		
Study design:	+4	Observational study
Study limitations:	-1	Limitations: Selection bias high in 1/2, unclear in 1/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2
Consistency:	-1	Some inconsistency, 1 study shows significant effect of alkylating agents and 1 study shows no significant effect
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, only 1 study showed a significant effect and with broad confidence interval
Publication bias:	0	Unlikely
Effect size:	0	Unclear if large magnitude of effect, as the confidence intervals are broad
Dose-response:	0	No dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊖⊖⊖ VERY LOW
Conclusion:		Increased risk of hypogonadism after alkylating agents vs. no alkylating agents only in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 1 study non-significant effect, 324 participants, 112 events, 2 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; LH, luteinizing hormone; N/A, not applicable; SB, selection bias; yr, year.

2. What is the risk of impaired spermatogenesis / testosterone deficiency in male cancer patients diagnosed before age 25 years who will be treated with antimetabolites (cytarabine, fludarabine, methotrexate)?

No studies identified investigating the risk of impaired spermatogenesis in childhood cancer survivors treated with antimetabolites.

Testosterone deficiency

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
2.1 Risk testosterone deficiency after antimetabolites (n=2 studies)	Siimes 1993	41 childhood ALL survivors	Mean 15.2 (4.0-25.0) yr after cancer diagnosis	Alkylating agents: 51.0%; Radiotherapy to testes: 0%; Cranial radiotherapy: 41.5%	Not reported: median testosterone levels	*Risk for lower (but not necessarily abnormal) testosterone levels* Cytosine arabinoside was not significantly associated (no effect measure reported)	SB: unclear AB: low risk DB: unclear CF: low risk
	Tromp 2011	565 CCS	Median 15 (range 5.0-39.0) yr after	Alkylating agents: at least 59.5%;	57/460 (12.4%) ↓ testosterone	*Risk for lower (but not necessarily abnormal) testosterone levels*	SB: high risk AB: low risk

			cancer diagnosis	Radiotherapy to testes: 9.7% pelvic abdominal irradiation, 1.9% TBI; Cranial radiotherapy: 21.9%	Antimetabolites were not significantly associated (no effect measure reported)	DB: unclear CF: low risk
GRADE assessment:						
Study design:	+4	Observational studies				
Study limitations:	-1	Limitations: Selection bias high in 1/2, unclear in 1/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2				
Consistency:	0	No important inconsistency, both studies show non-significant effect of antimetabolites				
Directness:	0	Results are direct, population and outcomes broadly generalizable				
Precision:	-1	Some imprecision, low number of events				
Publication bias:	0	Unlikely				
Effect size:	0	No large magnitude of effect				
Dose-response:	0	No dose-response relationship				
Plausible confounding:	0	No plausible confounding				
Quality of evidence:		⊕⊕⊖⊖ LOW				
Conclusion:		No significant effect of antimetabolites on the risk of testosterone deficiency (analyzed as lower, but not necessarily abnormal testosterone levels) in male cancer survivors diagnosed before age 25 years. (2 studies non-significant effect, 606 participants, 57 events, 2 multivariable analyses)				

Abbreviations: AB, attrition bias; ALL, acute lymphoblastic leukaemia; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

3. What is the risk of impaired spermatogenesis / testosterone deficiency in male cancer patients diagnosed before age 25 years treated with platinum compounds (cisplatin, carboplatin)?

No studies identified investigating the risk of impaired spermatogenesis in childhood cancer survivors treated with platinum compounds.

Testosterone deficiency

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
3.1 Risk testosterone deficiency after	Tromp 2011	565 CCS	Median 15 (range 5.0-39.0) yr after cancer diagnosis	Alkylating agents: at least 59.5%; Radiotherapy to	57/460 (12.4%) ↓ testosterone	*Risk for lower (but not necessarily abnormal) testosterone levels* Carboplatin/cisplatin was not	SB: high risk AB: low risk DB: unclear

platinum compounds (n=1 study)			testes: 9.7% pelvic abdominal irradiation, 1.9% TBI; Cranial radiotherapy: 21.9%	significantly associated (no effect measure reported)		CF: low risk
GRADE assessment:						
Study design:	+4	Observational study				
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1				
Consistency:	0	N/A (1 study)				
Directness:	0	Results are direct, population and outcomes broadly generalizable				
Precision:	-2	Important imprecision, only 1 study included and low number of events				
Publication bias:	0	Unlikely				
Effect size:	0	No large magnitude of effect				
Dose-response:	0	No dose-response relationship				
Plausible confounding:	0	No plausible confounding				
Quality of evidence:		⊕⊖⊖⊖ VERY LOW				
Conclusion:		No significant effect of platinum compounds on the risk of testosterone deficiency (analyzed as lower, but not necessarily abnormal testosterone levels) in male cancer survivors diagnosed before age 25 years. (1 study non-significant effect, 565 participants, 57 events, 1 multivariable analysis).				

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; N/A, not applicable; SB, selection bias; yr, year.

Combined outcomes referring to hypogonadism

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
3.2 Risk hypogonadism after alkylating agents and platinum compounds (n=1 study)	Brignardello 2016	199 CCS	Median 14.01 (inter quartile range 10.08-17.76) yr	Alkylating agents: 85.5%; Radiotherapy to testes: 16.6%; Cranial radiotherapy: 19.1%	68/199 (34.2%) spermatogenesis damage (↑ FSH, ↓ inhibin B) confirmed in 41 patients in whom semen analysis was performed; 13/199 (6.5%) primary hypogonadism (↓ testosterone)	*Odds ratio (95% CI) for spermatogenesis damage and primary hypogonadism* Alkylating + platinum agents vs. alkylating agents only: OR 9.22 (2.17-39.23)	SB: unclear AB: low risk DB: unclear CF: low risk
GRADE assessment:							

Study design:	+4	Observational study
Study limitations:	-1	Limitations: Selection bias unclear in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-2	Important imprecision, only 1 study included and small patient population and low number of events
Publication bias:	0	Unlikely
Effect size:	0	Unclear if large magnitude of effect, as the confidence intervals are broad
Dose-response:	0	No dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⨁⊖⊖⊖ VERY LOW
Conclusion:		Increased risk of hypogonadism after alkylating agents and platinum compounds vs. alkylating agents only in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 199 participants, 81 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; N/A, not applicable; SB, selection bias; yr, year.

4. What is the risk of impaired spermatogenesis / testosterone deficiency in male cancer patients diagnosed before age 25 years who will be treated with radiotherapy to volumes exposing the testes?

Impaired spermatogenesis

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
4.1 Risk impaired spermatogenesis after radiotherapy to volumes exposing testes (n=2 studies)	Jahnukainen 2011	51 childhood ALL survivors vs. 56 age-matched males	Median 20 (11-30) yr after cancer treatment	Cyclophosphamide: 51.0%; Radiotherapy to testes: 35.3%; Cranial radiotherapy: 74.5%	Not reported: median sperm concentration and sperm count	*Median (IQR) sperm concentration (10^6/mL) CCS vs. controls* Controls: 50 (27-66); No cyclophosphamide, no testicular irradiation: 41 (29-74), p>0.05; ≤10 g/m² cyclophosphamide, no testicular irradiation: 35 (24-42), p>0.05; >20 g/m² cyclophosphamide, no testicular irradiation: 1 (0-17), p<0.05; Testicular irradiation ± cyclophosphamide: 0, p<0.05	SB: high risk AB: low risk DB: unclear CF: low risk
	Wilhelmsson 2014	106 childhood HSCT survivors	Mean 13 (4-28) yr from HSCT	Alkylating agents: 100%; Radiotherapy to testes: 12%; TBI:	21/31 (67.7%) azoospermia	*Risk for azoospermia* TBI (10-12 Gy) not significantly associated with azoospermia as compared to survivors treated without	SB: low risk AB: high risk DB: unclear CF: high risk

			67%; TNI: 4.7%; Cranial radiotherapy: 22%	TBI (but treated with cyclophosphamide, or busulfan, or both, or cyclophosphamide and total nodal irradiation) (no effect measures reported); Association significant in a univariable regression analysis (OR 30.0; 95% CI 2.8-322.1)		
GRADE assessment:						
Study design:	+4	Observational studies				
Study limitations:	-2	Serious limitations: Selection bias low in 1/2, high in 1/2; Attrition bias low in 1/2, high in 1/2; Detection bias unclear in 2/2; Confounding low in 1/2, high in 1/2				
Consistency:	0	No important inconsistency, 1 study shows significant effect of testicular irradiation, 1 study shows non-significant effect of TBI, but significant in univariable analysis				
Directness:	0	Results are direct, population and outcomes broadly generalizable				
Precision:	-1	Some imprecision, small study population and low number of events				
Publication bias:	0	Unlikely				
Effect size:	0	No large magnitude of effect				
Dose-response:	0	Unclear if dose-response relationship				
Plausible confounding:	0	No plausible confounding				
Quality of evidence:		⊕⊖⊖⊖ VERY LOW				
Conclusion:		Increased risk of impaired spermatogenesis after radiation to volumes including the testes vs. no radiation to the testes in male cancer survivors diagnosed before age 25 years. (2 studies significant effect, 157 participants, 21 events, 1 multivariable analysis).				

Abbreviations: AB, attrition bias; ALL, acute lymphoblastic leukaemia; CF, confounding; DB, detection bias; HSCT, hematopoietic stem cell transplant; SB, selection bias; yr, year.

Testosterone deficiency

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
4.2.1 Risk testosterone deficiency after radiotherapy to volumes exposing the testes (n=4 studies)	Romerius 2009	144 CCS vs. 141 healthy fertile men	Mean 20 yr (± 7.3) after cancer diagnosis	Alkylating agents: at least 14.6%; Radiotherapy to testes: 4.2%; Cranial radiotherapy: NM	33/144 (22.9%) hypogonadism (↓ testosterone and/or ↑ LH; or receiving androgen replacement therapy)	*Odds ratio (95% CI) for hypogonadism* Radiotherapy to testes yes vs. no (controls): OR 110.0 (11.0-1100.0)	SB: high risk AB: low risk DB: unclear CF: high risk
	Jahnukainen 2011	51 childhood ALL survivors vs. 56 age-	Median 20 (11-30) yr after cancer treatment	Cyclophosphamide: 51.0%; Radiotherapy to	Not reported: median testosterone levels	*Median (IQR) testosterone levels (pmol/L) CCS vs. controls* Controls: 18.4 (14.7-24.0);	SB: high risk AB: low risk DB: unclear

		matched males		testes: 35.3%; Cranial radiotherapy: 74.5%		No cyclophosphamide, no testicular irradiation: 18.3 (13.6-20.1), p>0.05; ≤10 g/m² cyclophosphamide, no testicular irradiation: 12.7 (12.2-16.6), p<0.05; >20 g/m² cyclophosphamide, no testicular irradiation: 13.4 (7.7-17.5), p<0.05; Testicular irradiation ± cyclophosphamide: 1.4 (0.9-8.9), p<0.05	CF: low risk
	Tromp 2011	565 CCS	Median 15 (range 5.0-39.0) yr after cancer diagnosis	Alkylating agents: at least 59.5%; Radiotherapy to testes: 9.7% pelvic abdominal irradiation, 1.9% TBI; Cranial radiotherapy: 21.9%	57/460 (12.4%) ↓ testosterone	*Beta for lower (but not necessarily abnormal) testosterone levels* TBI yes vs. no adjusted for age at diagnosis and follow-up duration: -3.53 (p=0.036); TBI yes vs. no also adjusted for other cancer treatment: not significant (no effect measure reported)	SB: high risk AB: low risk DB: unclear CF: low risk
	Chemaitilly 2019	1,516 CCS	Median 22.0 (range 7.5-49.8) yr after cancer diagnosis	Alkylating agents: 59.2% Radiotherapy to testes: 8.1% Unilateral orchiectomy: 2.3%	104/1516 (6.9%) Leydig cell failure: morning serum levels of total testosterone <250 ng/dL (or 8.67 nmol/L) and LH > 9.85 IU/L	*Odds ratio (95% CI) for Leydig cell failure* Testicular radiation dose >0-11.9 Gy vs. none: OR 3.1 (1.4-7.2); Testicular radiation dose 12-19.9 Gy vs. none: OR 97.3 (29.2-323.6); Testicular radiation dose ≥20 Gy vs. none: OR 220.0 (26.0-1,858.8)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:

Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 4/4; Attrition bias low in 4/4; Detection bias unclear in 4/4; Confounding low in 3/4, high in 1/4
Consistency:	0	No important inconsistency, all studies show effect of radiotherapy to volumes exposing the testes
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, large study population and number of events
Publication bias:	0	Unlikely
Effect size:	+1	Large magnitude of effect
Dose-response:	0	No dose-response relationship
Plausible confounding:	0	No plausible confounding

Quality of evidence: ⨁⨁⨁⨁ HIGH

Conclusion: Increased risk of testosterone deficiency after radiation to volumes including the testes vs. no radiation to the testes in male cancer survivors diagnosed before age 25 years.

	(4 studies significant effects, 2,276 participants, 161 events, 3 multivariable analyses)

Abbreviations: AB, attrition bias; ALL, acute lymphoblastic leukaemia; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; LH, luteinizing hormone; NM, not mentioned; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
4.2.2 Risk testosterone deficiency after higher vs. lower doses of radiotherapy to volumes exposing the testes (n=1 study)	Chemaitilly 2019	1,516 CCS	Median 22.0 (range 7.5-49.8) yr after cancer diagnosis	Alkylating agents: 59.2% Radiotherapy to testes: 8.1% Unilateral orchiectomy: 2.3%	104/1516 (6.9%) Leydig cell failure: morning serum levels of total testosterone <250 ng/dL (or 8.67 nmol/L) and LH > 9.85 IU/L	*Odds ratio (95% CI) for Leydig cell failure* Testicular radiation dose >0-11.9 Gy vs. none: OR 3.1 (1.4-7.2); Testicular radiation dose 12-19.9 Gy vs. none: OR 97.3 (29.2-323.6); Testicular radiation dose ≥20 Gy vs. none: OR 220.0 (26.0-1,858.8)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational study
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	Not applicable, only one study performed
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, large study population and number of events but only one study performed
Publication bias:	0	Unlikely
Effect size:	0	Magnitude of effect unclear
Dose-response:	0	Low quality evidence for a dose-response relationship, so not totally certain
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Increased risk of testosterone deficiency after higher vs. lower doses of radiation to volumes including the testes in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 1,516 participants, 104 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; LH, luteinizing hormone; NM, not mentioned; SB, selection bias; yr, year.

Combined outcomes referring to hypogonadism

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
4.3 Risk hypogonadism after radiotherapy to volumes exposing the testes (n=2 studies)	Brignardello 2016	199 CCS	Median 14.01 (inter quartile range 10.08-17.76) yr	Alkylating agents: 85.5%; Radiotherapy to testes: 16.6%; Cranial radiotherapy: 19.1%	68/199 (34.2%) spermatogenesis damage (↑ FSH, ↓ inhibin B) confirmed in 41 patients in whom semen analysis was performed; 13/199 (6.5%) primary hypogonadism (↓ testosterone)	*Odds ratio (95% CI) for spermatogenesis damage and primary hypogonadism* Any radiation vs. none: OR 8.72 (3.94-19.30)	SB: unclear AB: low risk DB: unclear CF: low risk
	Isaksson 2018	125 CCS	Mean 24.3 (±7.1) years after cancer treatment	Alkylating agents: 13%; CED >4000 mg/m²: 10 (8.0%) Radiotherapy to testes: 4.0%; Cranial radiotherapy: 9.6%; Cranial radiotherapy and chemotherapy: 13%	31/121 (25.6%) hypogonadism (primary hypogonadism: ↓ testosterone, ↑ LH and FSH with FSH > LH or ↓ testosterone, ↓ LH and ↑ FSH; secondary hypogonadism: ↓ testosterone, ↓LH and FSH; compensated hypogonadism: ↑ testosterone, ↑ LH; or ongoing androgen replacement therapy)	*Odds ratio (95% CI) for hypogonadism survivors vs. controls* Radiotherapy to testes: OR 28.0 (2.9-279.0)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational study
Study limitations:	-1	Limitations: Selection bias high in 1/2, unclear in 1/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2
Consistency:	0	No important inconsistency, both studies show significant effect of radiotherapy to the testes
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, broad confidence intervals
Publication bias:	0	Unlikely
Effect size:	+1	Large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊕⊖ MODERATE
Conclusion:		Increased risk of hypogonadism after radiotherapy to volumes exposing the testes vs. no radiotherapy in male cancer survivors diagnosed before age 25 years.

					(2 studies significant effect, 324 participants, 112 events, 2 multivariable analysis)		

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; LH, luteinizing hormone; N/A, not applicable; SB, selection bias; yr, year.

5. **What is the risk of impaired spermatogenesis / testosterone deficiency in male cancer patients diagnosed before age 25 years who will be treated with gonadotoxic chemotherapy combined with radiotherapy to volumes exposing the testes?**

   No studies identified investigating the risk of impaired spermatogenesis or testosterone deficiency in childhood cancer survivors treated with gonadotoxic chemotherapy combined with radiotherapy to volumes exposing the testes.

6. **What is the risk of impaired spermatogenesis / testosterone deficiency in male cancer patients diagnosed before age 25 years who will be treated with unilateral orchiectomy combined with radiotherapy to volumes exposing the testes or gonadotoxic chemotherapy?**

   No studies identified investigating the risk of impaired spermatogenesis or testosterone deficiency in childhood cancer survivors treated with unilateral orchiectomy combined with radiotherapy to volumes exposing the testes or gonadotoxic chemotherapy.

7. **What is the risk of impaired spermatogenesis / testosterone deficiency in male cancer patients diagnosed before age 25 years who will be treated with novel agents, tyrosine kinase inhibitors, demethylating agents, oxaliplatin used in early phase studies?**

   No studies identified investigating the risk of impaired spermatogenesis in childhood cancer survivors treated with novel agents, tyrosine kinase inhibitors, demethylating agents, oxaliplatin.

Testosterone deficiency

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Treatment	Events	Effect size	Risk of bias
7.1 Risk testosterone deficiency after imatinib (n=1 study)	Tauer 2014	13 CML patients	mean 39 (range 0-89) weeks	Imatinib: 100%	Not reported: Testosterone levels compared to age-related reference ranges	*Testosterone levels after imatinib* All patients testosterone levels within the normal range	SB: unclear AB: low risk DB: unclear CF: high risk
GRADE assessment: Study design:	+4	Observational study					

Study limitations:	-1	Limitations: Selection bias unclear in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding high in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-2	Important imprecision, only 1 study included and small patient population and low number of events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊖⊖⊖ VERY LOW
Conclusion:		No significant effect of imatinib on the risk of testosterone deficiency in male cancer patients diagnosed before age 25 years. (1 study, 13 participants, 0 events, 0 multivariable analyses).

Abbreviations: AB, attrition bias; CML, chronic myeloid leukaemia; CF, confounding; DB, detection bias; N/A, not applicable; SB, selection bias; yr, year.

8. **What is the risk of ejaculation disorders (anejaculation, retrograde ejaculation) in male cancer patients diagnosed before age 25 years who will be treated with orchiectomy, retroperitoneal lymph node dissection or** genitourinary surgery **(exenteration, prostate/bladder/bladder neck surgery, rectum surgery)?**

   No studies identified investigating the risk of ejaculation disorders in childhood cancer survivors treated with orchiectomy, retroperitoneal lymph node dissection or genitourinary surgery.

9. **What is the risk of obstructive azoospermia after orchiectomy in male cancer patients diagnosed before age 25 years who will be treated with retroperitoneal lymph node dissection or** genitourinary surgery **(exenteration, prostate/bladder/bladder neck surgery, rectum surgery)?**

   No studies identified investigating the risk of obstructive azoospermia after orchiectomy in childhood cancer survivors treated with treated with retroperitoneal lymph node dissection or genitourinary surgery.

10. **What is the risk of central hypogonadism in male cancer patients diagnosed before age 25 years who will be treated with radiotherapy to volumes exposing the hypothalamic-pituitary axis?**
    - **What is the risk in younger vs older patients?**
    - **What is the risk after higher doses vs lower doses?**
    - **What is the risk after conventional vs proton therapy?**

    Evidence from IGHG hypothalamic-pituitary dysfunction surveillance guideline; note this is evidence for both males and females

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Cranial radiotherapy Alkylating agents	Events	Effect size	Risk of bias
10.1 Risk hypogonadotropic hypogonadism in males and females after cranial radiotherapy (n=1 study)	Gan 2015	116 male and female childhood optic glioma survivors	Median 8.3 yr (range 0.04-26.8)	Cranial radiotherapy: 59.5%; Alkylating agents: NM	21/103 (20.4%) central hypogonadism (boys: testicular volume <4mL at age 14 yr or failure to progress through puberty after normal onset; girls: tanner breast stage B1 at age 13 yr or pubertal arrest or primary amenorrhea at age 16 yr	*Hazard ratio (95% CI) for central hypogonadism* Primary radiotherapy yes vs. no: HR 3.27 (1.35-7.94)	SB: low risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	0	No important limitations: Selection bias low in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-2	Important imprecision, only 1 study included and low number of events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Increased risk of hypogonadotropic hypogonadism after cranial radiotherapy vs. no cranial radiotherapy in male brain tumour survivors diagnosed before age 25 years. (1 study significant effect, 116 participants, 21 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; NM, not mentioned; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Cranial radiotherapy Alkylating agents	Events	Effect size	Risk of bias
10.2 Risk hypogonadotropic hypogonadism in males and females after higher vs. lower doses of cranial radiotherapy (n=1 study)	Chemaitilly 2015	748 male and female CCS treated with cranial radiotherapy	Mean 27.3 yr (range 10.8-47.7) after cancer diagnosis	Cranial radiotherapy: 100%; Alkylating agents: NM	79/731 (10.8%) central hypogonadism (males: ↓ testosterone and ↓ LH; females: amenorrhea or ↓ estradiol and ↓ FSH)	*Odds ratio (95% CI) for central hypogonadism* Cranial radiotherapy dose 22-29.9 Gy vs. ≤21.9 Gy: OR 3.02 (1.3-7.0); Cranial radiotherapy dose ≥30 Gy vs. ≤21.9 Gy: OR 9.71 (4.2-22.3)	SB: high risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1					
Consistency:	0	N/A (1 study)					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	-1	Some imprecision, only 1 study included but high number of events					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:		⊕⊕⊕⊖ MODERATE					
Conclusion:		Increased risk of hypogonadotropic hypogonadism after increasing doses of cranial radiotherapy in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 748 participants, 79 events, 1 multivariable analysis)					

Abbreviations: AB, attrition bias; ALL, acute lymphoblastic leukaemia; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; LH, luteinizing hormone; NM, not mentioned; SB, selection bias; yr, year.

Combined outcomes referring to hypogonadism

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
10.3 Risk hypogonadism after cranial	Isaksson 2018	125 CCS	Mean 24.3 (±7.1) years after cancer treatment	Alkylating agents: 13%; CED >4000	31/121 (25.6%) hypogonadism (primary hypogonadism: ↓	*Odds ratio (95% CI) for hypogonadism survivors vs. controls* Cranial radiotherapy: OR 4.4 (1.1-	SB: high risk AB: low risk DB: unclear

radiotherapy (n=1 study)			mg/m²: 10 (8.0%) Radiotherapy to testes: 4.0%; Cranial radiotherapy: 9.6%; Cranial radiotherapy and chemotherapy: 13%	testosterone, ↑ LH and FSH with FSH > LH or ↓ testosterone, ↓ LH and ↑ FSH; secondary hypogonadism: ↓ testosterone, ↓LH and FSH; compensated hypogonadism: ↑ testosterone, ↑ LH; or ongoing androgen replacement therapy)	18.0)	CF: low risk
GRADE assessment:						
Study design:	+4	Observational study				
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1				
Consistency:	0	N/A (one study)				
Directness:	0	Results are direct, population and outcomes broadly generalizable				
Precision:	-2	Important imprecision, only 1 study included with small number of events				
Publication bias:	0	Unlikely				
Effect size:	+1	Large magnitude of effect				
Dose-response:	0	Unclear if dose-response relationship				
Plausible confounding:	0	No plausible confounding				
Quality of evidence:		⊕⊕⊖⊖ LOW				
Conclusion:		Increased risk of hypogonadism after cranial radiotherapy vs. no cranial radiotherapy in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 125 participants, 31 events, 1 multivariable analysis)				

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; LH, luteinizing hormone; N/A, not applicable; SB, selection bias; yr, year.

11. What is the likelihood of a pregnancy/live birth among partners of male cancer patients diagnosed before age 25 years who will be treated with alkylating agents?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
11.1 Likelihood pregnancy and live birth after cyclophosphamide and higher vs.	Chow 2016	5640 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 54%; Radiotherapy to pelvis, brain or total body: 0%	1694/5640 (30.0%) reported at least 1 pregnancy; 1425/5640 (25.3%) reported at least 1	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Cyclophosphamide lower tertile dose (<3625 mg/m²) vs. 0: HR 1.22 (1.07-1.40); Cyclophosphamide middle tertile dose	SB: high risk AB: low risk DB: unclear CF: low risk

lower doses (n=2 studies)					live birth	(3625-7411 mg/m²) vs. 0: HR 0.89 (0.77-1.03); Cyclophosphamide upper tertile dose (>7411 mg/m²) vs. 0: HR 0.60 (0.51-0·71); Cyclophosphamide equivalent lower tertile dose (<4897 mg/m²) vs. 0: HR 1.14 (1.00-1.30); Cyclophosphamide equivalent middle tertile dose (4897-9638 mg/m²) vs. 0: HR 0.79 (0.68-0.91); Cyclophosphamide equivalent upper tertile dose (>9638 mg/m²) vs. 0: HR 0.55 (0.47-0.64); Cyclophosphamide equivalent linear dose per 5000 mg/m²: HR 0.82 (0.79-0.86) *Hazard ratio (95% CI) for likelihood of reporting first live birth* Cyclophosphamide lower tertile dose (<3625 mg/m²) vs. 0: HR 1.15 (0.99-1.34); Cyclophosphamide middle tertile dose (3625-7411 mg/m²) vs. 0: HR 0.90 (0.77-1.05); Cyclophosphamide upper tertile dose (>7411 mg/m²) vs. 0: HR 0.58 (0.48-0·69); Cyclophosphamide equivalent lower tertile dose (<4897 mg/m²) vs. 0: HR 1.08 (0.72-0.97); Cyclophosphamide equivalent middle tertile dose (4897-9638 mg/m²) vs. 0: HR 0.84 (0.72-0.97); Cyclophosphamide equivalent upper tertile dose (>9638 mg/m²) vs. 0: HR 0.53 (0.44-0.62) Cyclophosphamide equivalent linear dose per 5000 mg/m²: HR 0.82 (0.78-0.86)	
	Green 2009	6224 CCS	>5 yr	Alkylating agents:	941/6224 (16.7%)	*Hazard ratio (95% CI) for likelihood of*	SB: high risk

			37.1%; Radiotherapy to testes: 56.2%; Cranial radiotherapy: 57.5%	reported at least 1 pregnancy	*reporting first pregnancy* Cyclophosphamide lower tertile dose vs. 0: HR 1.03 (0.76-1.39); Cyclophosphamide middle tertile dose vs. 0: HR 0.82 (0.63-1.07); Cyclophosphamide upper tertile dose vs. 0: HR 0.42 (0.31-0.57) *Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Alkylating agent dose score 1 vs. 0: HR 0.95 (0.68-1.33); Alkylating agent dose score 2 vs. 0: HR 0.67 (0.51-0.88); Alkylating agent dose score 3 vs. 0: HR 0.48 (0.36-0.65); Alkylating agent dose score 4 vs. 0: HR 0.34 (0.22-0.66); Alkylating agent dose score 5 vs. 0: HR 0.38 (0.22-0.66); Alkylating agent dose score 6-11 vs. 0: HR 0.16 (0.08-0.32)	AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 2/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2
Consistency:	0	No important inconsistency, however, both studies are from the same cohort
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, both studies are from the same cohort, but there is a high total number of included patients and events and narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⨁⨁◯◯ LOW
Conclusion:		Decreased likelihood of pregnancy and live birth after (increasing doses of) cyclophosphamide in male cancer survivors diagnosed before age 25 years. (2 studies from 1 cohort significant effect, 11,864 participants, 2,635 events, 2 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
11.2 Likelihood pregnancy and live birth after ifosfamide (n=1 study)	Chow 2016	5640 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 54%; Radiotherapy to pelvis, brain or total body: 0%	1694/5640 (30.0%) reported at least 1 pregnancy; 1425/5640 (25.3%) reported at least 1 live birth	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Ifosfamide lower tertile dose (<26853 mg/m²) vs. 0: HR 0.90 (0.56-1.45) Ifosfamide middle tertile dose (26853-52999 mg/m²) vs. 0: HR 0.61 (0.36-1.01) Ifosfamide upper tertile dose (>52999 mg/m²) vs. 0: HR 0.42 (0.23-0.79) *Hazard ratio (95% CI) for likelihood of reporting first live birth* Ifosfamide lower tertile dose (<26853 mg/m²) vs. 0: HR 0.90 (0.64-1.30) Ifosfamide middle tertile dose (26853-52999 mg/m²) vs. 0: HR 0.61 (0.36-1.04) Ifosfamide upper tertile dose (>52999 mg/m²) vs. 0: HR 0.46 (0.24-0.89)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, only 1 study, but high total number of included patients and events and narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Decreased likelihood of pregnancy and live birth after higher doses ifosfamide in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 5,640 participants, 1,694 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
11.3 Likelihood pregnancy and live birth after busulfan (n=1 study)	Chow 2016	5640 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 54%; Radiotherapy to pelvis, brain or total body: 0%	1694/5640 (30.0%) reported at least 1 pregnancy; 1425/5640 (25.3%) reported at least 1 live birth	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Busulfan lower dose (<450 mg/m²) vs. 0: HR 0.46 (0.15-1.42) Busulfan upper dose (≥450 mg/m²) vs. 0: HR: 1.39 (0.76-2.52) *Hazard ratio (95% CI) for likelihood of reporting first live birth* Busulfan lower dose (<450 mg/m²) vs. 0: HR 0.58 (0.19-1.80) Busulfan upper dose (≥450 mg/m²) vs. 0: HR: 1.58 (0.87-2.88)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, only 1 study, but high total number of included patients and events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship (low level evidence for dose-response)
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		No significant effect of busulfan on the likelihood of pregnancy and live birth in male cancer survivors diagnosed before age 25 years. (1 study no significant effect, 5,640 participants, 1,694 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
11.4 Likelihood pregnancy and live birth after lomustine (n=2 studies)	Chow 2016	5640 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 54%; Radiotherapy to pelvis, brain or total body: 0%	1694/5640 (30.0%) reported at least 1 pregnancy; 1425/5640 (25.3%) reported at least 1 live birth	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Lomustine lower dose (<411 mg/m^2) vs. 0: HR 1.13 (0.58-2.20) Lomustine upper dose (≥411 mg/m^2) vs. 0: HR: 0.82 (0.26-2.60) *Hazard ratio (95% CI) for likelihood of reporting first live birth* Lomustine lower dose (<411 mg/m^2) vs. 0: HR 0.82 (0.36-1.85) Lomustine upper dose (≥411 mg/m^2) vs. 0: HR: 0.94 (0.28-3.14)	SB: high risk AB: low risk DB: unclear CF: low risk
	Green 2009	6224 CCS	>5 yr	Alkylating agents: 37.1%; Radiotherapy to testes: 56.2%; Cranial radiotherapy: 57.5%	941/6224 (16.7%) reported at least 1 pregnancy	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Lomustine yes vs. no: HR 0. 67 (0.33-1.33)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, only 1 study, but high total number of included patients and events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship (low level evidence for dose-response)
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		No significant effect of lomustine on the likelihood of pregnancy and live birth in male cancer survivors diagnosed before age 25 years. (2 studies no significant effect, 11,864 participants, 2,635 events, 2 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
11.5 Likelihood pregnancy after mechlorethamine (n=1 study)	Green 2009	6224 CCS	>5 yr	Alkylating agents: 37.1%; Radiotherapy to testes: 56.2%; Cranial radiotherapy: 57.5%	941/6224 (16.7%) reported at least 1 pregnancy	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Mechlorethamine yes vs. no: HR 0.69 (0.40-1.21)	SB: high risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1					
Consistency:	0	N/A (1 study)					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	-1	Some imprecision, only 1 study, but high total number of included patients and events					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	Unclear if dose-response relationship (low level evidence for dose-response)					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:		⊕⊕⊖⊖ LOW					
Conclusion:		No significant effect of mechlorethamine on the likelihood of pregnancy in male cancer survivors diagnosed before age 25 years. (1 study no significant effect, 6,224 participants, 941 events, 1 multivariable analysis)					

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
11.6 Likelihood pregnancy and live birth after procarbazine (n=2 studies)	Chow 2016	5640 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 54%; Radiotherapy to pelvis, brain or total body: 0%	1694/5640 (30.0%) reported at least 1 pregnancy; 1425/5640 (25.3%) reported at least 1 live birth	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Procarbazine lower tertile dose (<3352 mg/m^2) vs. 0: HR 0.63 (0.44-0.91); Procarbazine middle tertile dose (3352-5059 g/m^2) vs. 0: HR 0.83 (0.24-0.60); Procarbazine upper tertile dose (>5059 mg/m^2) vs. 0: HR 0.30 (0.20-0.46) *Hazard ratio (95% CI) for likelihood of reporting first live birth*	SB: high risk AB: low risk DB: unclear CF: low risk

						Procarbazine lower tertile dose (<3352 mg/m²) vs. 0: HR 0.61 (0.40-0.91); Procarbazine middle tertile dose (3352-5059 g/m²) vs. 0: HR 0.45 (0.29-0.71); Procarbazine upper tertile dose (>5059 mg/m²) vs. 0: HR 0.30 (0.20-0.46)	
	Green 2009	6224 CCS	>5 yr	Alkylating agents: 37.1%; Radiotherapy to testes: 56.2%; Cranial radiotherapy: 57.5%	941/6224 (16.7%) reported at least 1 pregnancy	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Procarbazine lower tertile dose vs. 0: HR 0.56 (0.29-1.11); Procarbazine middle tertile dose vs. 0: HR 0.48 (0.26-0.87); Procarbazine upper tertile dose vs. 0: HR 0.17 (0.07-0.41)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 2/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2
Consistency:	0	No important inconsistency, however, both studies are from the same cohort
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, both studies are from the same cohort, but there is a high total number of included patients and events and narrow confidence intervals.
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Decreased likelihood of pregnancy and live birth after procarbazine in male cancer survivors diagnosed before age 25 years. (2 studies from 1 cohort significant effect, 11,864 participants, 2,635 events, 2 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

12. What is the likelihood of a pregnancy/live birth among partners of male cancer patients diagnosed before age 25 years who will be treated with antimetabolites (cytarabine, fludarabine, methotrexate)?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
12.1 Likelihood pregnancy after cytarabine (n=1 study)	Green 2009	6224 CCS	>5 yr	Alkylating agents: 37.1%; Radiotherapy to testes: 56.2%; Cranial radiotherapy: 57.5%	941/6224 (16.7%) reported at least 1 pregnancy	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Cytarabine yes vs. no: HR 1.80 (1.35-2.40)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, only 1 study, but high total number of included patients and events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship (low level evidence for dose-response)
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Increased likelihood of pregnancy after cytarabine in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 6,224 participants, 941 events, 1 multivariable analysis)

13. What is the likelihood of a pregnancy/live birth among partners of male cancer patients diagnosed before age 25 years who will be treated with platinum compounds?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
13.1 Likelihood pregnancy and live birth after cisplatin	Chow 2016	5640 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 54%; Carboplatin: 3%; Cisplatin: 8%; Radiotherapy to	1694/5640 (30.0%) reported at least 1 pregnancy; 1425/5640 (25.3%) reported at least 1	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Cisplatin lower tertile dose (<355 mg/m^2) vs. 0: HR 0.85 (0.58-1.27) Cisplatin middle tertile dose (355-487	SB: high risk AB: low risk DB: unclear CF: low risk

(n=1 study)			pelvis, brain or total body: 0%	live birth	g/m²) vs. 0: HR 0.74 (0.52-1.07) Cisplatin upper tertile dose (>487 mg/m²) vs. 0: HR 0.56 (0.39-0.82) *Hazard ratio (95% CI) for likelihood of reporting first live birth* Cisplatin lower tertile dose (<355 mg/m²) vs. 0: HR 0.95 (0.63-1.44) Cisplatin middle tertile dose (355-487 g/m²) vs. 0: HR 0.64 (0.43-0.97) Cisplatin upper tertile dose (>487 mg/m²) vs. 0: HR 0.53 (0.36-0.79)

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, only 1 study, but high total number of included patients and events and narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Decreased likelihood of pregnancy and live birth after cisplatin in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 5,640 participants, 1,694 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

14. What is the likelihood of a pregnancy/live birth among partners of male cancer patients diagnosed before age 25 years who will be treated with radiotherapy to volumes exposing the testes?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
14.1 Likelihood pregnancy after radiotherapy to volumes exposing the testes	Green 2009	6224 CCS	>5 yr	Alkylating agents: 37.1%; Radiotherapy to testes: 56.2%; Cranial	941/6224 (16.7%) reported at least 1 pregnancy	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Radiotherapy to testes >0-7.5 Gy vs. 0: HR 1.62 (0.39-6.71); Radiotherapy to testes >7.5 Gy vs. 0:	SB: high risk AB: low risk DB: unclear CF: low risk

(n=1 study)				radiotherapy: 57.5%	HR 0.12 (0.02-0.64)
GRADE assessment:					
Study design:	+4	Observational studies			
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1			
Consistency:	0	N/A (1 study)			
Directness:	0	Results are direct, population and outcomes broadly generalizable			
Precision:	-1	Some imprecision, only 1 study, but high total number of included patients and events			
Publication bias:	0	Unlikely			
Effect size:	0	No large magnitude of effect			
Dose-response:	0	Unclear if dose-response relationship (low level evidence for dose-response)			
Plausible confounding:	0	No plausible confounding			
Quality of evidence:		⊕⊕⊖⊖ LOW			
Conclusion:		Decreased likelihood of pregnancy after radiotherapy to volumes exposing the testes (>7.5 Gy) in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 6,224 participants, 941 events, 1 multivariable analysis)			

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

15. What is the likelihood of a pregnancy/live birth among partners of male cancer patients diagnosed before age 25 years who will be treated with radiotherapy to volumes exposing the hypothalamic-pituitary axis?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
15.1 Likelihood pregnancy after radiotherapy to volumes exposing the hypothalamic-pituitary axis	Green 2009	6224 CCS	>5 yr	Alkylating agents: 37.1%; Radiotherapy to testes: 56.2%; Cranial radiotherapy: 57.5%	941/6224 (16.7%) reported at least 1 pregnancy	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Hypothalamic/pituitary radiation >0-40.0 Gy vs. 0: HR 0.52 (0.13-2.16); Hypothalamic/pituitary radiation >40.0 Gy vs. 0: HR 0.29 (0.06-1.28)	SB: high risk AB: low risk DB: unclear CF: low risk

(n=2 studies)	Reulen 2009	5350 CCS	>5 yr	Not reported	2021/2521 (80.2%) singleton pregnancies among partners of male survivors resulted in a live birth	*Odds ratio (95% CI) for likelihood of live birth* Cranial radiotherapy vs. no radiotherapy: 1.1 (0.7-1.7)	SB: high AB: low DB: unclear CF: low

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 2/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2
Consistency:	0	No important inconsistency, both studies showed no significant effect of CRT
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, high total number of included patients and events and narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship (low level evidence for dose-response)
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊕⊖ MODERATE
Conclusion:		No significant effect of radiotherapy to volumes exposing the pituitary-hypothalamic axis on the likelihood of pregnancy and live births in male cancer survivors diagnosed before age 25 years. (2 studies no significant effect, 11,574 participants, 2,962 events, 2 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

16. What is the influence of age at treatment on the risk of impaired spermatogenesis or testosterone deficiency?

Impaired spermatogenesis

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
16.1 Risk impaired spermatogenesis after younger vs. older age at treatment (n=3 studies)	Van Beek 2007	56 childhood Hodgkin lymphoma survivors	Median 15.5 (5.6-30.2) yr after cancer treatment	Alkylating agents: 100%; Radiotherapy to testes: 0%; Cranial radiotherapy: 0%	9/21 (42.9%) azoospermia	*Risk for decreased sperm concentration* Age at diagnosis: β -6.18 (p<0.05)	SB: high risk AB: high risk DB: unclear CF: low risk
	Green 2014*	214 CCS	Median 21.0 (10.5-41.6) yr after cancer diagnosis	Alkylating agents: 100%; Radiotherapy to testes: 0%;	53/214 (24.7%); azoospermia; 59/214 (27.6%) oligospermia (sperm	*Odds ratio (95% CI) for azoospermia vs. normospermia* Age at diagnosis per yr: OR 0.97 (0.91-1.05)	SB: high risk AB: low risk DB: unclear CF: low risk

				Cranial radiotherapy: 0%	concentration >0-<15 x 10^6/mL)	*Odds ratio (95% CI) for oligospermia vs. normospermia* Age at diagnosis per yr: OR 0.95 (0.89-1.02)	
	Green 2017*	241 childhood ALL survivors	CRT: Mean 26.3 ± 6.3 yr; No CRT: Mean 18.7 ± 6.0 yr	Alkylating agents: 100%; Radiotherapy to testes: 0%; Cranial radiotherapy >26 Gy: 55.6%	65/241 (37.6%) azoospermia; 46/241 (26.6%) oligospermia (sperm concentration >0-<15 x 10^6/mL)	*Relative risk (95% CI) for azoospermia or oligospermia* Age at diagnosis 5-9 vs. <4 yr: 1.3 (1.05-1.61); Age at diagnosis ≥10 vs. <4 yr: 0.92 (0.69-1.23)	SB: high risk AB: high risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Limitations: Selection bias high in 3/3; Attrition bias low in 1/3, high in 2/3; Detection bias unclear in 3/3; Confounding low in 3/3					
Consistency:	-1	Some inconsistency, 1 study shows significant effect of age at diagnosis and 2 studies show non-significant effects					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	0	No important imprecision, narrow confidence intervals					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	No dose response relationship					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:		⊕⊕⊖⊖ LOW					
Conclusion:		Increased risk of impaired spermatogenesis after older age at cancer treatment vs. younger age in male cancer survivors diagnosed before age 25 years. (1 study significant effect, 2 studies non-significant effect; 511 participants; 173 events; 3 multivariable analyses)					

Abbreviations: AB, attrition bias; ALL, acute lymphoblastic leukaemia; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.
* Overlap in included patients.

Testosterone deficiency

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
16.2 Risk testosterone deficiency after younger vs. older age at treatment	Mackie 1996	58 childhood Hodgkin disease survivors	Median 6 (range 2.5-11.1) years after cancer diagnosis	Alkylating agents: 100%; Radiotherapy to testes: 0%; Cranial radiotherapy: 0%	5/37 (13.5%) ↓ testosterone; 10/41 (24.4%) ↑ LH	*Risk for Leydig cell dysfunction* Age at treatment was not significantly associated (no effect measure reported)	SB: high risk AB: high risk DB: unclear CF: low risk

(n=3 studies)	Siimes 1993	41 childhood ALL survivors	Mean 15.2 (4.0-25.0) yr after cancer diagnosis	Alkylating agents: 51.0%; Radiotherapy to testes: 0%; Cranial radiotherapy: 41.5%	Not reported: median testosterone levels	*Risk for lower (but not necessarily abnormal) testosterone levels* Age at treatment was not significantly associated (no effect measure reported)	SB: unclear AB: low risk DB: unclear CF: low risk
	Chemaitilly 2019	1,516 CCS	Median 22.0 (range 7.5-49.8) yr after cancer diagnosis	Alkylating agents: 59.2% Radiotherapy to testes: 8.1% Unilateral orchiectomy: 2.3%	104/1516 (6.9%) Leydig cell failure: morning serum levels of total testosterone <250 ng/dL (or 8.67 nmol/L) and LH > 9.85 IU/L	*Odds ratio (95% CI) for Leydig cell failure* Age at diagnosis 5-9.9 vs. 0-4.9 yr: 1.8 (1.0-3.3) Age at diagnosis 10-14.9 vs. 0-4.9 yr: 1.1 (0.6-2.2) Age at diagnosis ≥15 vs. 0-4.9 yr: 0.8 (0.4-1.8)	SB: high risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Limitations: Selection bias high in 2/3, unclear in 1/3; Attrition bias low in 2/3, high in 1/3; Detection bias unclear in 3/3; Confounding low in 3/3					
Consistency:	0	No important inconsistency, all show non-significant effect of age at treatment					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	0	No important imprecision, large study population and number of events					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	No dose response relationship					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:		⨁⨁⨁◯ MODERATE					
Conclusion:		No significant effect of age at treatment on testosterone deficiency in male cancer survivors diagnosed before age 25 years. (3 studies non-significant effect; 1,615 participants; 109 events; 3 multivariable analyses)					

Abbreviations: AB, attrition bias; ALL, acute lymphoblastic leukaemia; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; LH, luteinizing hormone; SB, selection bias; yr, year.

Combined outcomes referring to hypogonadism

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
3.2 Risk hypogonadism	Brignardello 2016	199 CCS	Median 14.01 (inter quartile	Alkylating agents: 85.5%;	68/199 (34.2%) spermatogenesis damage	*Odds ratio (95% CI) for spermatogenesis damage and*	SB: unclear AB: low risk

after alkylating agents and platinum compounds (n=1 study)		range 10.08-17.76) yr	Radiotherapy to testes: 16.6%; Cranial radiotherapy: 19.1%	(↑ FSH, ↓ inhibin B) confirmed in 41 patients in whom semen analysis was performed; 13/199 (6.5%) primary hypogonadism (↓ testosterone)	*primary hypogonadism* Age at cancer diagnosis 5-9 vs. 0-4 yr: 1.08 (0.40-2.93) Age at cancer diagnosis ≥10 vs. 0-4 yr: 0.64 (0.25-1.68)	DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational study
Study limitations:	-1	Limitations: Selection bias unclear in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-2	Important imprecision, only 1 study included and small patient population and low number of events
Publication bias:	0	Unlikely
Effect size:	0	Unclear if large magnitude of effect, as the confidence intervals are broad
Dose-response:	0	No dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⨁⊖⊖⊖ VERY LOW
Conclusion:		No significant effect of age at treatment on hypogonadism in male cancer survivors diagnosed before age 25 years. (1 study non-significant effect, 199 participants, 81 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; N/A, not applicable; SB, selection bias; yr, year.

What male reproductive preservation methods are appropriate to offer in counselling?

1. What is the quality and yield of sperm after sperm cryopreservation via masturbation or vibration in male patients[a] diagnosed with cancer before 25 years?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Sperm cryopreservation via masturbation	Sperm quality and yield	Risk of bias
1. Sperm quality and yield after sperm cryopreservation via masturbation (n=5 studies)	Hagenäs 2010	80/86 (93%) male patients with malignant disease (various)	Median 16.2 years (12.2 - 17.9)	No long-term follow up (successful semen sampling)	86 patients produced semen sampling for cryopreservation *Method of sample collection* 74 (86%) patients by masturbation; 11 (13%) patients by electroejaculation; 1 (1.2%) patients by penile vibration *Timing of intervention* Cryopreservation before treatment	*Semen analysis before cryopreservation by masturbation* 65/74 (87.8%) patients with successful sample collected and cryopreserved 6/74 (8.1%) patients with azoospermia 3/74 (4%) patients with immotile sperm	SB: unclear AB: low risk DB: unclear CF: high risk
	Kamischke 2004	300 male patients with malignant disease <25 years	NM <25 years 111/300 (37%) patients <20 years at cryopreservation	NM	300 patients produced semen sampling for cryopreservation *Method of sample collection* Unclear (?masturbation) *Timing of intervention* - Cryopreservation before initiation of anticancer treatment - Except: 61% patients with testicular cancer had unilateral ablation of the	*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	SB: unclear AB: low risk DB: unclear CF: high risk

					testis before cryopreservation		
	Kliesch 1996	28/239 (11.7%) male patients with malignant diseases(various)	NM 29/239 (12%) patients: 14-20 years at study[b]	NM	239 patients produced semen sampling for cryopreservation, of which 29 <20 years *Method of sample collection* Unclear (?masturbation) *Timing of intervention* Before cancer treatment	*Semen analysis before cryopreservation* 28/29 (97%) patients with successful sample collected and cryopreserved 1/29 (3%) patient did not produce ejaculate (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 (p >0.05 for differences between the age groups)	SB: unclear AB: low risk DB: unclear CF: high risk
	Adank 2014	106 male patients with malignant diseases (various)	Median 16.5 years (10.8-18.9)	No long-term follow up (successful semen sampling)	81 patients produced semen sampling for cryopreservation *Method of sample collection* 78 patients via masturbation; 3 patients via electroejaculation *Timing of intervention* Cryopreservation before treatment	*Semen analysis before cryopreservation by 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	SB: unclear AB: low risk DB: unclear CF: high risk
	Müller 2000	21 male patients with malignant diseases (various)	Median 14.5 years (13-18)	No long-term follow up (successful semen sampling)	21 patients produced semen sampling for cryopreservation *Method of sample collection* 18 patients via masturbation; 2 patients via electroejaculation; 1 patient via vibration	*Semen analysis before cryopreservation by masturbation* 17/19 (89.5%) patients with successful sample collected and cryopreserved *Semen analysis in patients with successful sample collected and cryopreserved via masturbation* Median percentage of motile sperm: 50% (range 9-86%)	SB: unclear AB: high risk DB: unclear CF: high risk

			Timing of intervention Cryopreservation before treatment (2 patients had chemotherapy before)
GRADE assessment:			
Study design:	+4	Observational studies	
Study limitations:	-1	Some limitations: Selection bias unclear in 5/5; Attrition bias low in 4/5, high in 1/5; Detection bias unclear in 5/5; Confounding high in 5/5	
Consistency:	0	No important inconsistency	
Directness:	0	Results are direct, population and outcomes broadly generalizable (>85% cancer patients in 4/5)	
Precision:	0	No important imprecision	
Publication bias:	0	Unlikely	
Effect size:	0	No large magnitude of effect	
Dose-response:	0	No dose-response	
Plausible confounding:	0	No plausible confounding	
Quality assessment:	⊕⊕⊕⊖ MODERATE		
Conclusion:	Sufficient sperm quality and yield for successful cryopreservation in male patients who produced s*emen sampling via masturbation or vibration. (5 studies; 639 patients)* Sperm motility decreases after *sperm freezing and thawing* for cryopreservation. *(2 studies; 329 patients)*		

Abbreviations: NM, not mentioned; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

[a] If evidence allows, distinction between pre-pubertal/low Tanner stage and post-pubertal/high Tanner stage

[b] Out of patients aged <20 years, 1 patient with non-malignant disease

2. What is the quality and yield of sperm after sperm cryopreservation via electroejaculation in male patients diagnosed with cancer before 25 years?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Sperm cryopreservation via vibration or electro-ejaculation	Sperm quality and yield	Risk of bias
2. Sperm quality and yield after sperm banking via vibration or electro-ejaculation (n=4 studies)	Hagenäs 2010	80/86 (93%) male patients with malignant disease (various)	Median 16.2 years (12.2 - 17.9)	No long-term follow up (successful semen sampling)	86 patients produced semen sampling for cryopreservation *Method of sample collection* 74 (86%) patients by masturbation	*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	SB: unclear AB: low risk DB: unclear CF: high risk

					12/86 (13.4%) patients unable to collect semen by masturbation: 11 (13%) patients by electroejaculation 1 (1.2%) patients by penile vibration		
	Hovav 2001	6 male patients with malignant diseases (various)	Mean 18 ±3 years Range 15-22 years	No long-term follow up (successful semen sampling)	6 patients produced semen sampling for cryopreservation *Method of semen collection* Electroejaculation under general anesthesia (antegrade and retrograde semen collected) *Timing of intervention* Before anticancer therapy	*Semen analysis before cryopreservation* *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%	SB: unclear AB: low risk DB: unclear CF: high risk
	Adank 2014	106 male patients with malignant diseases (various)	Median 16.5 years (10.8-18.9)	No long-term follow up (successful semen sampling)	81 patients produced semen sampling for cryopreservation *Method of sample collection* 78 patients via masturbation; 3 patients via electroejaculation	*Semen analysis before cryopreservation by electro-ejaculation* 3/11 (27%) patients with successful sample collected and cryopreserved *Semen analysis in patients with successful sample collected and cryopreserved* 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* Volume (x10^6 mL): 0.4 (0.02-3.0) Concentration (x10^6/mL): 2.0 (0.1-14.5)	SB: unclear AB: low risk DB: unclear CF: high risk

						Motility (%): 0 pH: 7.0 (6.4-8.0)	
	Müller 2000	21 male patients with malignant diseases (various)	Median 14.5 years (13-18)	No long-term follow up (successful semen sampling)	21 patients produced semen sampling for cryopreservation *Method of sample collection* 18 patients via masturbationl; 2 patients via electroejaculation; 1 patient via vibration *Timing of intervention* Cryopreservation before treatment (2 patients had chemotherapy before)	*Semen analysis before cryopreservation by electroejaculation* 2/2 (100%) patients with successful sample collected and cryopreserved PT1: Volume 0.8 mL Concentration 75 x 10^6/mL Motility 38% PT2: Volume 3.2 mL Concentration 4.0 x 10^6/mL Motility 10%	SB: unclear AB: high risk DB: unclear CF: high risk
GRADE assessment:							
Study design:	+4	Observational study					
Study limitations:	-1	Some limitations: Selection bias unclear 5/5; Attrition bias low in 4/5, high in 1/5; Detection bias unclear in 5/5; Confounding high in 5/5					
Consistency:	0	No important inconsistency					
Directness:	0	Results are direct, population and outcomes broadly generalizable (>85% cancer patients in 5/5)					
Precision:	-2	Important imprecision, small number of events with case series studies					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	No dose -response					
Plausible confounding:	0	No plausible confounding					
Quality assessment:	⊕⊖⊖⊖ VERY LOW						
Conclusion:	*Diminished sperm count and motility for cryopreservation with semen sampling via electro-ejaculation (4 studies, 31 patients)*						

Abbreviations: SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding; PT: patient

3. What is the quality and yield of sperm after testicular sperm extraction (TESE) male patients diagnosed with cancer before 25 years?

No studies identified that investigated the quality and yield of sperm after Testicular sperm extraction (TESE) in male patients diagnosed with cancer before 25 years.

4. What is the quality and yield of sperm after testicular tissue cryopreservation/spermatogonial stem cell and spermatogonial stem cell transplantation in male patients[a] diagnosed with cancer before 25 years?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Testicular tissue cryopreservation	Quality and yield of sperm	Risk of bias
4. Quality and yield sperm after testicular tissue cryopreservation (n=4 studies)	Ho 2017	30/44 (68%) male patients with malignant diagnosis (various) Pre-pubertal: 33/44 (75%) Pubertal: 11/44(25%)	NM 0.3-16.8 years at study	No long-term follow up (successful tissue sampling)	44 patients had testicular tissue collected *Transplantation* NM	*Tissue dissection in pubertal patients before cryopreservation* 3/11 (27%) azoospermic 8/11 (73%) mature sperm found	SB: unclear AB: low risk DB: unclear
	Uijldert 2017	64/64 (100%) male patients with malignant diagnosis (various) Pre-pubertal: 64/64 (100%)	Mean 8.3 (range 0.5-15.5 years)	Range 0.08-1 year	64 patients had testicular tissue collected (unilateral biopsy never exceeding 50% of the testicular volume) *Transplantation* NM	*Tissue dissection in pre-pubertal patients before cryopreservation* 1 (1.9%) no spermatogonia 44 (68.8%) spermatogonia only 9 (14.1%) up to spermatocytes 10 (14.1%) up to spermatids	SB: low risk AB: low risk DB: unclear
	Stukenborg 2018	18/32 (56%) male patients with malignant diagnosis (various) Pre-pubertal: 32/32 (100%) Controls: 14 testicular samples without testicular	Range 0.7-13.1 years	No long-term follow up (successful tissue sampling)	32 patients had testicular tissue collected (unilateral open biopsy; <20% of testicular volume of one testes sampled)	*Tissue dissection in pre-pubertal patients before cryopreservation* Spermatogonia per transverse tubular cross-section: Mean 4.1 ± 4.6 in controls; Mean 1.7 ± 1.0 in patients treated with non-alkylating agents (NS 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	SB: unclear AB: low risk DB: unclear

		pathology				compared to controls); Among 5 boys exposed to CED ≥4000 mg/m^2 spermatogonia values were close to zero	
	Corkum 2019	21/23 (91%) male patients with malignant diagnosis (various) Tanner stage 1: 18 (78%) Tanner stage 2: 3 (13%) Tanner stage ≥3: 2 (9%)	Median 10 (range 0.42-18) years	Median 1.4 years (interquartile range 0.9-2.2 years) since testicular tissue cryopreservation	23 patients had testicular tissue collected (unilateral wedge biopsy) Transplantation NM	*Tissue dissection in pubertal patients before cryopreservation* 22/23 (96%) had normal testicular tissue with the presence of germ cells on histopathological analysis	SB: unclear AB: low risk DB: unclear
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Some limitations: Selection bias low in 1/4, unclear in 3/4; Attrition bias low in 4/4; Detection bias unclear in 4/4					
Consistency:	0	No important inconsistency					
Directness:	-1	Some indirectness (<85% cancer patients in 2/4 studies)					
Precision:	0	No important imprecision, large total number of patients					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	No dose -response					
Plausible confounding:	0	No plausible confounding					
Quality assessment:	⊕⊕⊖⊖ LOW						
Conclusion:	*Mature sperm, spermatogonia and spermatogonial germ cells found in testicular tissue dissection before cryopreservation (4 studies; 163 patients)*						

Abbreviations: NM, not mentioned; SB, selection bias; AB, attrition bias; DB, detection bias

[a] If evidence allows, distinction between in pre-pubertal and post-pubertal

5. What is the quality and yield of sperm after Radiation shielding of the testes in male patients diagnosed with cancer before 25 years?

No studies identified that investigated the quality and yield of sperm after radiation shielding of the testes.

6. What is the quality and yield of sperm after hormonal gonadoprotection in male patients[a] diagnosed with cancer before 25 years?

No studies identified that investigated the quality and yield of sperm after hormonal gonadoprotection.

7. Is there evidence for pregnancies and live births after sperm cryopreservation via masturbation in male patients[a] diagnosed with cancer before 25 years?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Sperm cryopreservation via masturbation	Pregnancy and live births	Risk of bias
7. Live births after sperm cryopreservation via masturbation (n=2 studies)	Kliesch 1996	28/239 (11.7%) male patients with malignant diseases(various)	NM 29/239 (12%) patients: 14-20 years at study[b]	NM	239 patients produced semen sampling for cryopreservation, of which 29 <20 years *Method of sample collection* NM (?masturbation) 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)	SB: unclear AB: low risk DB: unclear
	Kamischke 2004	300 male patients with malignant disease <25 years	NM <25 years 111/300 (37%) patients <20 years at cryopreservation	NM	300 patients produced semen sampling for cryopreservation *Method of sample collection* Unclear (?masturbation) 1/111 (0.9%) patient <20 years used cryopreserved sperm for ART	*Pregnancy in patients <20 years* 1/1 (100%) patient who had IVF-ICSI achieved pregnancy but resulted in early abortion	SB: unclear AB: low risk DB: unclear

GRADE assessment:

Study design:	+4	Observational study (retrospective cross-sectional study)
Study limitations:	-1	Some limitations: Selection bias unclear in 2/2; Attrition bias low in 2/2; Detection bias unclear in 2/2
Consistency:	0	No important inconsistency
Directness:	-1	Some indirectness (<85% cancer patients in 1/2)
Precision:	-1	Some imprecision
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect

Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality assessment:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*2 live births after inseminated cryopreserved sperm produced via masturbation (unclear if in patient with cancer diagnosis) (1 study; 2 out of 13 inseminations in partners of male patients produced 2 live births)*	

Abbreviations: NM, not mentioned; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; ART, assisted reproduction; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

[a] If evidence allows, distinction between pre-pubertal/low Tanner stage and post-pubertal/high Tanner stage

[b] Out of patients aged <20 years, 1 patient with non-malignant disease

8. Is there evidence for pregnancies outcome and live births after sperm cryopreservation via vibration or electro-ejaculation in male patients[a] diagnosed with cancer before 25 years?

No studies identified pregnancy outcomes (including live births) after sperm cryopreservation via vibration or electro-ejaculation.

9. Is there evidence for pregnancies and live births after sperm cryopreservation via testicular sperm extraction in male patients[a] diagnosed with cancer before 25 years?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Sperm cryopreservation via Testicular sperm extraction	Pregnancy and live births	Risk of bias
9. Pregnancy and live births after testicular sperm extraction (n=1 study)	Chan 2001	16/17 (94%) male patients with malignant disease (various)	NM Age at 37.4 years at study	Mean 16.3 years after CT completion	17 patients produced semen sampling *Method of semen collection* Testicular sperm extraction Combined with intracytoplasmic sperm injection 9 patients who underwent TESE-ICSI had sperm retrieval	*Clinical pregnancy rate* 3/9 (33%) patients who had TESE-ICSI achieved pregnancy *Live births* 2/9 (22%) patients who had TESE-ICSI fathered 3 live births 1/9 (11%) pregnancies in patients partners' of patients who had TESE-ICSI did not result in live birth	SB: unclear AB: low risk DB: unclear

GRADE assessment:		
Study design:	+4	Observational study (retrospective study)
Study limitations:	-1	Some limitations: Selection bias unclear in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1

Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable (>85% cancer patients in 1/1)
Precision:	-2	Important imprecision, only 1 study included with small study sample
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality assessment:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*3 live births after testicular sperm extraction combined with intracytoplasmic sperm injection (1 study; 2 out of 9 patients fathered 3 live births)*\	

Abbreviations: NM, not mentioned; TESE, Testicular sperm extraction; ICSI, intracytoplasmic sperm injection; SB, selection bias; AB, attrition bias; DB, detection bias

[a] If evidence allows, distinction between pre-pubertal/low Tanner stage and post-pubertal/high Tanner stage

10. Is there evidence for pregnancies and live births after testicular tissue cryopreservation/spermatogonial stem cell and spermatogonial stem cell transplantation in pre-pubertal and post-pubertal male patients diagnosed with cancer before 25 years?

No studies investigating pregnancy outcomes (including live births) after testicular tissue cryopreservation/ spermatogonial stem cell and spermatogonial stem cell transplantation.

11. Is there evidence for pregnancies and live births after radiation shielding of the testes in pre-pubertal and post-pubertal male patients diagnosed with cancer before 25 years?

No studies investigating pregnancy outcomes (including live births) after radiation shielding of the testes.

12. Is there evidence for pregnancies and live births after hormonal gonadoprotection in pre-pubertal, peri-pubertal and post-pubertal male patients diagnosed with cancer before 25 years?

No studies investigating pregnancy outcomes (including live births) after hormonal gonadoprotection.

13.1. In male patients diagnosed (pre- peri- post- pubertals) with cancer before 25 years, what are the complications after sperm cryopreservation via masturbation or vibration?

No studies investigating complications after sperm cryopreservation via masturbation or vibration.

13.2. In male patients[a] diagnosed with cancer before 25 years, what are the complications after sperm cryopreservation via electro-ejaculation?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Sperm banking via electro-ejaculation	Complications	Risk of bias
13.2. Complications after electro-ejaculation (n=1 study)	Hovav 2001	6 male patients with malignant diseases (various)	Mean 18 ±3 years Range 15-22 years	No long-term follow up (successful semen sampling)	6 patients produced semen sampling for cryopreservation *Method of semen collection* Electroejaculation under general anesthesia (antegrade and retrograde semen collected)	0/6 patients with complications	SB: unclear AB: low risk DB: unclear

GRADE assessment:		
Study design:	+2	Observational study (case series)
Study limitations:	-1	Some limitations: Selection bias unclear in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable (>85% cancer patients in 1/1)
Precision:	-2	Important imprecision, only 1 study included with small study sample
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality assessment:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*No complications after sperm cryopreservation via electro-ejaculation (1 study; 6 patients)*	

Abbreviations: SB, selection bias; AB, attrition bias; DB, detection bias

[a] If evidence allows, distinction between pre- peri- post- pubertals

13.3. In male patients[a] diagnosed with cancer before 25 years, what are the complications after testicular sperm extraction?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Testicular sperm extraction	Complications	Risk of bias
13.3. Complications after testicular sperm extraction (n=1 study)	Chan 2001	16/17 (94%) male patients with malignant disease (various)	NM Age at 37.4 years at study	Mean 16.3 years after CT completion	16 patients produced semen sampling *Method of semen collection* Testicular sperm extraction Combined with intracytoplasmic sperm injection	0/16 patients with complications	SB: unclear AB: low risk DB: unclear

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Some limitations: Selection bias unclear in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable (>85% cancer patients in 1/1)
Precision:	-2	Important imprecision, only 1 study included with small study sample
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality assessment:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*No complications after sperm cryopreservation via testicular sperm extraction (1 study; 16 patients)*	

Abbreviations: NM, not mentioned; SB, selection bias; AB, attrition bias; DB, detection bias

[a] If evidence allows, distinction between pre- peri- post- pubertals

13.4. In male patients[a] diagnosed with cancer before 25 years, what are the complications after testicular tissue cryopreservation/spermatogonial stem cell and spermatogonial stem cell transplantation?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Testicular tissue cryopreservation	Complications	Risk of bias
13.4. Complications after testicular tissue cryopreservation (n=3 studies)	Ho 2017	30/44(68%) male patients with malignant diagnosis (various)	NM 0.3-16.8 years at study	No long-term follow up (successful tissue sampling)	44 patients had testicular tissue cryopreservation collected *Transplantation* NM	0/30 cancer patients had complications 0/30 cancer patients had delay in treatment 1 patient with aplastic anaemia suffered scrotal wound dehiscence occurring 2 weeks after procedure	SB: unclear AB: low risk DB: unclear
	Uijldert 2017	64/64 (100%) male patients with malignant diagnosis (various) Pre-pubertal: 64/64 (100%)	Mean 8.3 (range 0.5-15.5 years)	Range 0.08-1 year	64 patients had testicular tissue collected (unilateral biopsy never exceeding 50% of the testicular volume) *Transplantation* NM	*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 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 *Testicular growth* Biopsy had no significant impact on testicular growth (p=0.519)	SB: low risk AB: low risk DB: unclear

	Ming 2018	27/34 (79%) male patients with malignant diagnosis (various) Pre-pubertal: 32 (94%) Post-pubertal: 2 (6%)	Mean 6.9 ± 4.4 years (range 0.7-15 years)	No long-term follow up (successful tissue sampling)	34 patients had testicular tissue collected (unilateral open biopsy) *Transplantation* NM	2/34 (5.9%) developed complications after biopsy: ipsilateral epididymo-orchitis (resolved with antibiotics) and an 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 0/34 (0%) had bleeding complications nor return visits to the operating room	SB: low risk AB: low risk DB: unclear
	Corkum 2019	21/23 (91%) male patients with malignant diagnosis (various) Tanner stage 1: 18 (78%) Tanner stage 2: 3 (13%) Tanner stage ≥3: 2 (9%)	Median 10 (range 0.42-18) years	Median 1.4 years (interquartile range 0.9-2.2 years) since testicular tissue cryopreservation	23 patients had testicular tissue collected (unilateral wedge biopsy) *Transplantation* NM	0/23 had intraoperative complications related to testicular wedge biopsy occurred 1/23 (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 Median time from TTC to start of cancer therapy: 7 days with no unanticipated delays in treatment initiation	SB: unclear AB: low risk DB: unclear

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Some limitations: Selection bias low in 2/4, unclear in 2/4; Attrition bias low in 4/4; Detection bias unclear in 4/4
Consistency:	0	No important inconsistency
Directness:	-1	Some indirectness (<85% cancer patients in 2/4 studies)
Precision:	0	No important imprecision, large total number of patients
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality assessment:	⊕⊕⊖⊖ LOW	
Conclusion:	*Three male patients with wound infection, 1 with post-operative bleeding, 1 with ipsilateral epididymo-orchitis, 1 with ipsilateral torsed appendix testis, 1 with scrotal cellulitis after collection of testicular tissue for cryopreservation (4 studies; 165 patients; 7 reported complications)*	

Abbreviations: NM, not mentioned; SB, selection bias; AB, attrition bias; DB, detection bias
[a] If evidence allows, distinction between pre- peri- post- pubertals

13.5. In male patients diagnosed (pre- peri- post- pubertals) with cancer before 25 years, what are the complications after radiation shielding of the testes?

No studies investigating complications after radiation shielding of the testes.

13.6. In male patients[a] diagnosed with cancer before 25 years, what are the complications after hormonal gonadoprotection?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Hormonal gonadoprotection	Complications	Risk of bias
13.6. Complications after hormonal gonadoprotection (n=1 study)	Thomson 2002	7 CCS azoospermic secondary to treatment	Mean 10.4(4.4-13.3)years	Median disease free survival 8.8(3.14.7)years	7 patients underwent suppression of HPG axis with MPA testosterone	0/7 patients with complications	SB: unclear AB: low risk DB: unclear

GRADE assessment:		
Study design:	+2	Observational study (case series)
Study limitations:	-1	Some limitations: Selection bias unclear in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable (>85% cancer patients in 1/1)
Precision:	-2	Important imprecision, only 1 study included with small study sample
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality assessment:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*No complications after hormonal gonadoprotection via MPA testosterone (1 study; 7 patients)*	

Abbreviations: NM, not mentioned; CCS, childhood cancer survivors; HPG, hypothalamic-pituitary-gonadal; MPA, medroxyprogesterone acetate; SB, selection bias; AB, attrition bias; DB, detection bias
[a] If evidence allows, distinction between pre- peri- post- pubertals

14. What are the complications among offspring of male patients (pre- peri- post- pubertals) diagnosed with cancer before 25 years after:

a. Sperm cryopreservation via masturbation or vibartion?

b. Sperm banking via electro-ejaculation?

c. Testicular sperm extraction?

d. Testicular tissue cryopreservation/spermatogonial stem cell and spermatogonial stem cell transplantation?

e. Radiation shielding of the testes?

f. Hormonal gonadoprotection?

No studies identified investigating complications among offspring after reproductive (preservation) methods.

15.1. In male patients[a] diagnosed with cancer before 25 years of age, what is the association between quality of sperm and timing of collection (before and during treatment, including novel agents) for sperm cryopreservation?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Sperm cryopreservation via masturbation	Timing of collection and sperm quality	Risk of bias
15.1. Quality of sperm and timing of collection after sperm cryopreservation via masturbation (n=5 studies)	Hagenäs 2010	80/86 (93%) male patients with malignant disease (various)	Median 16.2 years (12.2 - 17.9)	No long-term follow up (successful semen sampling)	86 patients produced semen sampling for cryopreservation *Method of sample collection* 74 (86%) patients masturbation 11 (13%) patients electroejaculation 1 (1.2%) penile vibration	*Timing of collection* Before treatment *Semen analysis before cryopreservation by masturbation* 65/74 (87.8%) patients with successful sample collected and cryopreserved 6/74 (8.1%) patients with azoospermia 3/74 (4%) patients with immotile sperm	SB: unclear AB: low risk DB: unclear CF: high risk
	Kamischke 2004	300 male patients with malignant disease <25 years	NM <25 years 111/300 (37%) patients <20 years at cryopreservation	NM	851 patients produced semen sampling for cryopreservation *Method of sample collection* Unclear (?masturbation)	*Timing of collection* 39% patients had intervention before treatment 61% patients with testicular cancer had cryopreservation after unilateral ablation of the testis *Semen analysis before cryopreservation in patients <20 years*	SB: unclear AB: low risk DB: unclear CF: high risk

						110/111 (99.1%) patients with successful sample collected and cryopreserved 1/111 (0.9%) patient with azoospermia	
	Kliesch 1996	28/239 (11.7%) male patients with malignant diseases(various)	NM 29/239 (12%) patients: 14-20 years at study	NM	239 patients produced semen sampling for cryopreservation, of which 29 <20 years *Method of sample collection* Unclear (?masturbation)	*Time of collection* Before treatment *Semen analysis before cryopreservation* 28/29 (97%) patients with successful sample collected and cryopreserved 1/29 (3%) patient did not produce ejaculate (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	SB: unclear AB: low risk DB: unclear CF: high risk
	Adank 2014	106 male patients with malignant diseases (various)	Median 16.5 years (10.8-18.9)	No long-term follow up (successful semen sampling)	81 patients produced semen sampling for cryopreservation *Method of sample collection* 78 patients via masturbation 3 patients via electroejaculation *Timing of intervention* Cryopreservation before treatment	*Semen analysis before cryopreservation by 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	SB: unclear AB: low risk DB: unclear CF: high risk
	Müller 2000	21 male patients with malignant diseases (various)	Median 14.5 years (13-18)	No long-term follow up (successful semen sampling)	21 patients produced semen sampling for cryopreservation *Method of sample*	*Semen analysis before cryopreservation by masturbation* 17/19 (89.5%) patients with successful sample collected and cryopreserved	SB: unclear AB: high risk DB: unclear CF: high risk

					collection 18 patients via masturbation; 2 patients via electroejaculation; 1 patient via vibration *Timing of intervention* Cryopreservation before treatment (2 patients had chemotherapy before)	*Semen analysis in patients with successful sample collected and cryopreserved via masturbation* Median percentage of motile sperm: 50% (range 9-86%)	
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Some limitations: Selection bias unclear in 5/5; Attrition bias low in 5/5, high in 1/5; Detection bias unclear in 5/5; Confounding high in 5/5					
Consistency:	0	No important inconsistency					
Directness:	0	Results are direct, population and outcomes broadly generalizable (>85% cancer patients in 4/5)					
Precision:	0	No important imprecision					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	No dose -response					
Plausible confounding:	0	No plausible confounding					
Quality assessment:	⨁⨁⨁⊖ MODERATE						
Conclusion:	*Sufficient sperm quality and yield for successful cryopreservation in male patients who produced semen sampling via masturbation or vibration before cancer treatment (5 studies; 639 patients)* *Sperm motility decreases after sperm freezing and thawing for cryopreservation (2 studies; 329 patients)*						

Abbreviations: NM, not mentioned; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

[a] If evidence allows, distinction between pre-pubertal/low Tanner stage and post-pubertal/high Tanner stage

15.2. In male patients[a] diagnosed with cancer before 25 years of age, what is the association between quality of sperm and timing of collection (before and during treatment, including novel agents) for sperm banking via vibration, electro-ejaculation or testicular sperm extraction?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Sperm cryopreservation via vibration or electro-ejaculation	Timing of collection and Sperm quality and yield	Risk of bias
15.2. Sperm quality and yield after	Hagenäs 2010	80/86 (93%) male patients with malignant	Median 16.2 years (12.2 - 17.9)	No long-term follow up (successful semen sampling)	86 patients produced semen sampling for cryopreservation	*Timing of collection* Before treatment	SB: unclear AB: low risk DB: unclear

sperm banking via vibration or electro-ejaculation (n=4 studies)		disease (various)			*Method of sample collection* 74 (86%) patients by masturbation 12/86 (13.4%) patients unable to collect semen by masturbation: 11 (13%) patients by electroejaculation 1 (1.2%) patients by penile vibration	*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	CF: high risk
	Hovav 2001	6 male patients with malignant diseases (various)	Mean 18 ±3 years Range 15-22 years	No long-term follow up (successful semen sampling)	6 patients produced semen sampling for cryopreservation *Method of semen collection* Electroejaculation under general anesthesia (antegrade and retrograde semen collected) *Timing of intervention* Before anticancer therapy	*Timing of collection* Before treatment *Semen analysis before cryopreservation* *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%	SB: unclear AB: low risk DB: unclear CF: high risk
	Adank 2014	106 male patients with malignant diseases (various)	Median 16.5 years (10.8-18.9)	No long-term follow up (successful semen sampling)	81 patients produced semen sampling for cryopreservation *Method of sample collection* 78 patients via masturbation 3 patients via electroejaculation	*Semen analysis before cryopreservation by electro-ejaculation* 3/11 (27%) patients with successful sample collected and cryopreserved *Semen analysis in patients with successful sample collected and cryopreserved* 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	SB: unclear AB: low risk DB: unclear CF: high risk

						Semen analysis in patients without successful sample collected and cryopreserved 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)	
	Müller 2000	21 male patients with malignant diseases (various)	Median 14.5 years (13-18)	No long-term follow up (successful semen sampling)	21 patients produced semen sampling for cryopreservation *Method of sample collection* 18 patients via masturbationl; 2 patients via electroejaculation; 1 patient via vibration *Timing of intervention* Cryopreservation before treatment (2 patients had chemotherapy before)	*Semen analysis before cryopreservation by electroejaculation* 2/2 (100%) patients with successful sample collected and cryopreserved PT1: Volume 0.8 mL Concentration 75 x 10^6/mL Motility 38% PT2: Volume 3.2 mL Concentration 4.0 x 10^6/mL Motility 10%	SB: unclear AB: high risk DB: unclear CF: high risk

GRADE assessment:		
Study design:	+4	Observational study
Study limitations:	-1	Some limitations: Selection bias unclear 5/5; Attrition bias low in 4/5, high in 1/5; Detection bias unclear in 5/5; Confounding high in 5/5
Consistency:	0	No important inconsistency
Directness:	0	Results are direct, population and outcomes broadly generalizable (>85% cancer patients in 5/5)
Precision:	-2	Important imprecision, small number of events with case series studies
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality assessment:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*Diminished sperm count and motility for cryopreservation with semen sampling via electro-ejaculation before cancer treatment (4 studies, 31 patients)*	

Abbreviations: SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding; PT, patient

[a] If evidence allows, distinction between pre-pubertal/low Tanner stage and post-pubertal/high Tanner stage

15.3. In male patients[a] diagnosed with cancer before 25 years of age, what is the association between quality of sperm and timing of collection (before and during treatment, including novel agents) for testicular tissue cryopreservation/spermatogonial stem cell and spermatogonial stem cell transplantation?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Testicular tissue cryopreservation	Timing of collection and sperm quality	Risk of bias
15.3. Quality of sperm and timing of collection after testicular tissue cryopreservation (n=4 studies)	Ho 2017	30/44(68%) male patients with malignant diagnosis (various)	NM 0.3-16.8 years at study	No long-term follow up (successful tissue sampling)	44 patients had testicular tissue cryopreservation collected *Transplantation* NM	*Timing of collection* Before treatment *Tissue dissection in pubertal patients before cryopreservation* 3/11 (27%) azoospermic 8/11(73%) mature sperm found	SB: unclear AB: low risk DB: unclear CF: high risk
	Uijldert 2017	64/64 (100%) male patients with malignant diagnosis (various) Pre-pubertal: 64/64 (100%)	Mean 8.3 (range 0.5-15.5 years)	Range 0.08-1 year	64 patients had testicular tissue collected (unilateral biopsy never exceeding 50% of the testicular volume) *Transplantation* NM	*Timing of collection* Before treatment *Tissue dissection in pre-pubertal patients before cryopreservation* 1 (1.9%) no spermatogonia 44 (68.8%) spermatogonia only 9 (14.1%) up to spermatocytes 10 (14.1%) up to spermatids	SB: low risk AB: low risk DB: unclear
	Stukenborg 2018	/32 (56%) male patients with malignant diagnosis (various) Pre-pubertal: 32/32 (100%)	Range 0.7-13.1 years	No long-term follow up (successful tissue sampling)	32 patients had testicular tissue collected (unilateral open biopsy; <20% of testicular volume of one testes sampled)	*Timing of collection* 20 (62.5%) testicular biopsy performed 1-45 days after a previous dose of chemotherapy *Tissue dissection in pre-pubertal patients before cryopreservation* Spermatogonia per transverse tubular cross-section: Mean 4.1 ± 4.6 in controls; Mean 1.7 ± 1.0 in patients treated with non-alkylating agents (NS compared to controls); Mean 0.2 ± 0.3 in patients treated with alkylating agents (p<0.05 compared to controls and non-	SB: unclear AB: low risk DB: unclear

						alkylating agent group); Mean 0.8 ± 0.9 in patients treated without chemotherapy (p<0.05 compared to controls); Among 5 boys exposed to CED ≥4000 mg/m² spermatogonia values were close to zero	
	Corkum 2019	21/23 (91%) male patients with malignant diagnosis (various) Tanner stage 1: 18 (78%) Tanner stage 2: 3 (13%) Tanner stage ≥3: 2 (9%)	Median 10 (range 0.42-18) years	Median 1.4 years (interquartile range 0.9-2.2 years) since testicular tissue cryopreservation	23 patients had testicular tissue collected (unilateral wedge biopsy) Transplantation NM	*Timing of collection* 5 (21.7%) received 1 or 2 rounds of chemotherapy prior to biopsy 6 (26%) underwent biopsy at the time of disease relapse *Tissue dissection in pubertal patients before cryopreservation* 22/23 (96%) had normal testicular tissue with the presence of germ cells on histopathological analysis	SB: unclear AB: low risk DB: unclear

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Some limitations: Selection bias low in 1/4, unclear in 3/4; Attrition bias low in 4/4; Detection bias unclear in 4/4
Consistency:	0	No important inconsistency
Directness:	-1	Some indirectness (<85% cancer patients in 2/4 studies)
Precision:	0	No important imprecision, large total number of patients
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality assessment:	⊕⊕⊖⊖ LOW	
Conclusion:	*Mature sperm, spermatogonia and spermatogonial germ cells found in testicular tissue dissection before cryopreservation collected before cancer treatment (4 studies; 132 patients)* *Spermatogonia and spermatogonial germ cells found in testicular tissue dissection before cryopreservation collected after cancer treatment (2 studies; 31 patients)*	

Abbreviations: NM, not mentioned; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

[a] If evidence allows, distinction between pre-pubertal/low Tanner stage and post-pubertal/high Tanner stage

16. **In male patients diagnosed with cancer before 25 years of age, is there an association between length of sperm storage and possibility to use the stored material and/or increased risk of chromosomal abnormalities?**

No studies identified investigating association between length of sperm storage and possibility to use the stored material and risk of chromosomal abnormalities.

17. **In male patients diagnosed with cancer before 25 years of age, is there an association between length of storage for testicular extracted sperm and the possibility to use stored material and/or increased risk of chromosomal abnormalities?**

No studies identified investigating association between length of storage of testicular extracted sperm and possibility to use the stored material and risk of chromosomal abnormalities.

18. **In male patients diagnosed with cancer before 25 years of age, is there an association between length of storage for spermatogonial stem cells storage and the possibility to use the stored material and/or increased risk of chromosomal abnormalities?**

No studies identified investigating association between length of storage of spermatogonial stem cells and possibility to use the stored material and risk of chromosomal abnormalities.