Testicular Cancer and Male Fertility: A Meta-Analysis

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Thesis Application to the Degree of Master

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Again to my family and to Catarina,

For shining an eternal light in my life

RESUMO

OBJETIVO: O cancro testicular é das neoplasias diagnosticas em idade fértil com melhor prognóstico. No entanto, o impacto das sequelas do cancro testicular e seu tratamento no potencial reprodutivo masculino permanecem controversos. Desta forma, tivemos como objetivo avaliar e estimar o risco do cancro testicular e seu tratamento na função testicular.

DESENHO: Foi realizada uma procura na PubMed utilizando palavras-chave tópico sobre o assunto. Os estudos foram selecionados de acordo com vários critérios. De seguida, foi realizada uma meta-análise usando um modelo de efeitos aleatórios, e foram utilizadas as seguintes medidas de consistência: I^2, Q de Cochrane e estimativa de τ.

RESULTADOS: Os pacientes incluídos encontravam-se em idade reprodutiva (29,20 ± 6,15) no momento do diagnóstico. A espermatogénese estava alterada antes do tratamento, com menor concentração de espermatozoides e motilidade. O tratamento não teve um impacto negativo na espermatogénese a longo prazo. Antes do tratamento, foi ainda observado um valor superior no dano genómico nos espermatozoides. Em relação ao controlo hormonal da espermatogénese, após tratamento verificou-se um aumento dos níveis das hormonas folículo-estimulante e luteinizante e uma diminuição dos níveis séricos de testosterona.

CONCLUSÃO: Estudos que avaliam a função gonadal em doentes com cancro testicular têm sempre amostras limitadas e informação sobre o doente antes e após tratamento encontra-se em falta. A maioria destes doentes parece recuperar a sua capacidade reprodutiva e/ou são aconselhados a criopreservar esperma antes do tratamento. No entanto a recuperação imprevisível e o risco de transmitir uma patologia aos filhos são motivos de preocupação. No sentido de validar as nossas descobertas, outras investigações devem ser realizadas e avaliada a validação clínica dos resultados.

PALAVRAS CHAVE: Cancro Testicular, Função Gonadal, Preservação da Fertilidade

ABSTRACT

PURPOSE: Testicular cancer (TC) is one of the most treatable malignancies diagnosed at reproductive age. However, the adverse sequelae of TC and its treatment on future male reproductive potential remain controversial. Therefore we aimed to evaluate and estimate the risk of TC and its treatment on male gonadal function.

DESIGN: A PubMed search was performed using topic keywords on the subject. Studies were included according several criteria. Afterwards, a meta-analysis using a random-effect model was conducted, and measures of consistency I^2, Cochrane's Q and estimation of τ were used.

RESULTS: Patients included in this study were on reproductive age (29.20 ± 6.15) at diagnosis. Spermatogenesis was already affected before treatment, with patients showing decreased sperm concentration and motility. Therapy did not have a long-term negative influence on spermatogenesis. A significantly increase of male gamete genomic damage before treatment was also observed. Regarding the hormonal control of spermatogenesis it was found that serum of follicle-stimulating hormone and of luteinizing hormone levels increased after treatment, and serum testosterone levels decreased after treatment.

CONCLUSION: Studies concerning gonadal function in TC patients were based on a limited number of patients, and lacked several information regarding pre-treatment and patients' follow-up post-treatment. Most TC patients seem to recover their ability to conceive and/or are counselled to preserve their fertility before treatment. Nonetheless fertility recovery is difficult to predict and the potential risk of transmitting an alteration to the offspring is of concern. In order to increase the validity of our findings future research should be conducted and clinical outcome significance evaluated.

KEYWORDS: Testicular Cancer, Gonadal Function, Fertility Preservation.

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LIST OF ABBREVIATIONS

βhCG – β-human Chorionic Gonadotrophin

BEP – Bleomycin + Etoposide + Cisplatin

CT – Chemotherapy

FSH – Follicle-Stimulating Hormone

LH – Luteinizing Hormone

NA – Not Available

nsSM – Non-strict Sperm Morphology

PM – Sperm Progressive Motility

RT – Radiotherapy

SC – Sperm Concentration

SCS – Sperm Chromatin Structure

SM – Sperm Morphology

sSM – Strict Sperm Morphology

Surg – Surgery

TT – Total Testosterone

TC – Testicular Cancer

TM – Sperm Total Motility

TSC – Total Sperm Concentration

V – Sperm Volume

VIT – Sperm Vitality

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INTRODUCTION

In recent years, there has been an increase in the incidence of testicular cancer (TC) (1). Although most TC patients will be cured, thousands will still die every year around the world (1), and many challenges remain (2). TC constitutes a group of tumours, of which 95% are germ cell tumours (3). These can be categorized in seminoma and nonseminoma (4). TC is the most common solid malignancy among men between 15 and 35 years (5). An increased risk of TC is associated with cryptorchidism (6), personal (7) and family (8-10) history of TC, testicular microlithiasis (11, 12), diet (13), environmental endocrine disrupting chemicals (14, 15) and genetic factors (16), such as isochromosome i(12)p (17) and mutations on KIT and RAS genes (18), among others. Although subfertile men also have a higher incidence of TC (19), TC can also cause sub/infertility (20). In fact, there is an overwhelming body of evidence that TC patients have lower fertility status, with up to 52% of patients being oligozoospermic and 10% being azoospermic at diagnosis (21). Moreover, there is evidence that TC treatment can diminish even more the fertility potential of these patients (22). Despite this, and unless a bilateral radical orchiectomy is performed, data seem to suggest that: a) these men may convalesce sperm production after orchiectomy (23), and b) when these patients need to resort to chemotherapy (CT) and radiotherapy (RT), the impact of these treatments on spermatogenesis appears to be augmented (20, 23), with those resulting from CT being more injurious (20, 23). Therefore, long-term sequelae on reproductive health and fertility are of great concern. The treatment-related toxicity over spermatogenesis may instigate testicular failure, and an abnormal hormonal status. This is mainly due to proliferating spermatogonial stem cells and their similarity to swiftly dividing malignant cells to damage during treatment. The time required to recover testicular function also remains controversial. Due to the sensitiveness of germinal

epithelium to TC and its treatment, and given that the influence of TC and of treatments on male fertility remains controversial, we have considered relevant to perform an evaluation of the studies that have determined the former associations. Knowledge of the association between testicular cancer and patients' semen parameters, hormonal levels and sperm integrity is of clinical significance as it will enable physicians to take an informed preventive strategy regarding fertility. To the best of our knowledge, there is no meta-analysis on this specific subject. As such, we conducted this meta-analysis to estimate the magnitude of the influence of TC on male reproductive potential before and/or after treatment. Because of studies' scarcity, both retrospective and prospective studies were included in this meta-analysis. No comparisons were made with the condition of other cancer patients.

METHODS

Search Strategies

The search for articles consisted of three initial searches in the PubMed database using the following search words: testicular cancer AND (hormonal profile OR semen parameters OR sexual function). The searches wielded 31, 81 and 319 results, respectively. An additional search was conducted using the words (chemotherapy AND male reproduction) which exerted 22425 results. After filtering for clinical trials and human species, the results were reduced to 1872. All articles' abstracts were read and if the content matched the study design for any parameters of sexual fertility in TC patients (either before and/or after cancer treatment), the article was fully analysed. Articles were included if they provided the necessary data to conduct the meta-analysis. The reference lists of manuscripts of key journals were hand searched for additional articles. A total of 42 articles from 1984 to April 2016 were included in this meta-analysis. We did not include unpublished data, or articles written in languages other than English. Figure 1 depicts the search process.

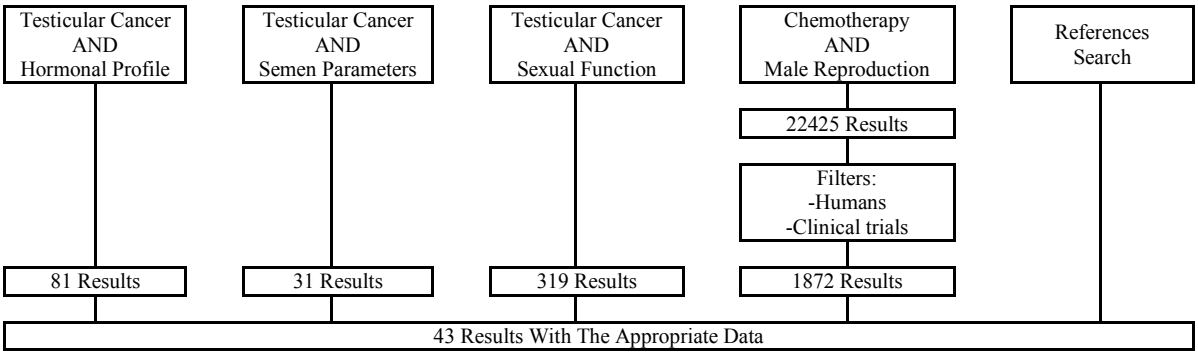


Fig 1 - Study Selection Flowchart.

Statistical analysis

When the studies presented information for the different types of TC but not for the TC group as a whole, this group was recreated using the summary data of the different sub-groups.

The meta-analysis was performed under a random effect model except when estimated τ was zero (24). The choice of using random effect was due because most studies included were observational meaning that different possible factors could influence the assessed measures, making this model the best for the present study (25).

Heterogeneity and dispersion between studies were assessed with Cochran's Q-test and I^2, respectively. However, their values were used only to speculate on factors that could justify the differences observed.

Statistical analyses were conducted using Microsoft Office Excel and the ESCI software (26). Two-tailed t tests were used to assess possible differences (assuming equal variances when differences were calculated within the same studies and not assuming equal variances when differences were calculated with different studies) and a P value less than 0.05 was chosen as the cut-off to consider a statistically significant difference. Information is presented as mean ± standard deviation, or mean (confidence interval 95%).

RESULTS

Studies' selection

Table 1 describes the selected studies. Forty-three studies were included in the present meta-analisys, which enrolled 7511 patients suffering from TC. Among these, 710 and 654 men were identified to have seminoma and nonseminoma, respectively. 2531 were recruited as controls.

Table 1. Characteristics of the studies included.

Publication year	Reference	Type of study	Time (months)	Country	Sample size (Patients/Controls)	Treatment	Mean age	Comments	Parameters evaluated included in the present meta-analysis
1984	(27)	Prospective	At diagnosis	USA	TC: 14	Orchiectomy	TC: 30	NA	V, SC
1991	(28)	Retrospective	At diagnosis	USA	TC: 60 Controls: 20	Prophylactic semen cryopreservation	NA	Orchiectomy not defined	V, SC, TM
1992	(29)	Prospective	At diagnosis	USA	TC: 10	BEP	NA	Orchiectomy not defined	LH, TT
1994	(30)	Prospective	At diagnosis 3, 6, 12, 18, 30	USA	T0: 8 T3: 4 T6: 4 T12: 4 T18: 2 T30: 1	Orchiectomy and RT	TC: 32.9 ± 5.6	Seminoma	SC, TM, VIT, SM
1995	(31)	Retrospective	At diagnosis	USA	TC: 33 Seminoma: 9 Nonseminoma: 24 Controls: 30	Prophylactic semen cryopreservation	TC: 26.0 ± 5.8 Controls: 31.4 ± 7.6	Separated by type of cancer, has stages Orchiectomy not defined	V
1997	(32)	Retrospective	At diagnosis	Israel	TC: 66 Seminoma: 32 Nonseminoma: 22	Prophylactic semen cryopreservation Orchiectomy in some patients	TC: 29.6	Separated by type of cancer	FSH, LH, TT
1997	(33)	Prospective	At diagnosis 3, 6, 12, 24, > 24	Germany	Transversal: 232 Longitudinal: T0: 51 T6: 38 T12: 38 T24: 41 T>24: 37	Orchiectomy and CT (different treatments)	TC: 31.5 ± 7.8	Has a Longitudinal and Transversal Study	FSH, LH, TT
1998	(34)	Retrospective	18	Greece	TC: 16 Controls: 52	RT	TC: 30.4 ± 4.8 Controls: 30.4 ± 4.8	NA	V, SC, TM, SM
1999	(35)	Prospective	At diagnosis	Greece	TC: 12 Controls: 60	Orchiectomy	NA	Seminoma	V, SC, TM, SM
2001	(36)	Prospective	At diagnosis	USA	TC: 20 Controls: 12	Prophylactic semen cryopreservation	NA	NA	V, SC, TM
2003	(37)	Retrospective	At diagnosis	Italy	TC: 222 Seminoma: 118 Nonseminoma: 104	Prophylactic semen cryopreservation Orchiectomy	TC: 28.8 ± 5.6 Controls: NA	Separated by type of cancer No azoospermic patients	V, SC, TSC, PM, SM
2003	(38)	Retrospective	> 24	Norway	TC: 1183 Surg: 251 RT: 515 CT1: 373 CT2: 96 Controls: 200	Surgery, RT, cisplatin (with or without surg and RT)	Surg: 42 ± 8.9 RT: 46 ± 8.3 CT1: 42 ± 8.7 CT2: 38 ± 9.2	Separated by type of treatment	LH, TT
2004	(39)	Prospective	At diagnosis	Germany	TC: 16		NA	NA	V, S, TSC, SM
2004	(40)	Retrospective	At diagnosis	USA	TC: 32	Prophylactic semen cryopreservation	NA	Orchiectomy not defined	SC, SM, SM
2004	(41)	Retrospective	> 24	Japan	TC: 10 Seminoma: 5 Nonseminoma: 5	CT (High dose)	Azoospermic: 36.2 ± 6.53 Non-azoospermic: 28.2 ± 8.76	Separates cases depending on azoospermia	SC, TM FSH, LH, TT
2004	(42)	Prospective	At diagnosis > 24	France	TC: 14	BEP and/or RT	TC: 35.4 ± 6.4	Orchiectomy not defined All over 18 years	SC, PM, SM
2006	(43)	Retrospective	At diagnosis	Taiwan	TC: 10	Prophylactic semen cryopreservation	TC: 26.3 ± 8.5 Controls: NA	Orchiectomy not defined	SC, PM
2006	(44)	Prospective	At diagnosis 3, 6, 9, 12, 24	Italy	T0: 166/ T3: 67 T6: 53 T9: 74 T12: 106 T24: 86	BEP or RT	CH: 26.7 ± 4.4 RT: 29.8 ± 4.9	Separated by type of cancer No azoospermic patients at diagnosis Orchiectomy not defined	V, SC, TSC, PM, SM
2007	(45)	Retrospective	At diagnosis	Canada	TC: 40	Prophylactic semen cryopreservation	NA	Orchiectomy not defined SM not used due to SD value (0) Some pediatric patients	V, SC, PM
2008	(46)	Retrospective	At diagnosis	Spain	TC: 37 Seminoma: 26 Nonseminoma: 11	Prophylactic semen cryopreservation	NA	Separated by type of cancer Orchiectomy not defined	SCS

2008	(47)	Prospective	At diagnosis	Brasil	TC: 48 Seminoma: 19 Nonseminoma: 29 Controls: 50	Prophylactic semen cryopreservation	Seminoma: 26.1 ± 5.1 Nonseminoma: 27 ± 4.8 Controls: 28.9 ± 6.4	All patients achieved paternity Separated by type of cancer Orchiectomy not defined	V, SC, PM, SM
2009	(48)	Retrospective	At diagnosis	Brasil	TC: 55	Prophylactic semen cryopreservation	TC: 28.9 ± 0.9	Orchiectomy not defined	SC, PM, TM, SM
2009	(49)	Retrospective	At diagnosis	Czeck Republic	TC: 270	Prophylactic semen cryopreservation	NA	NA	SC
2009	(50)	Retrospective	At diagnosis	France	TC: 33	Prophylactic semen cryopreservation	NA	Only volume used because of possible patient repetition with Rives et al	V
2009	(51)	Prospective	At diagnosis	Canada?	TC: 39 Controls: 20	Prophylactic semen cryopreservation	TC: 30.7 ± 11.9 Controls: 33.7 ± 4.8	Orchiectomy not defined	SC, PM, SCS
2010	(52)	Prospective	At diagnosis	Brasil	TC: 100 Seminoma: 37 Nonseminoma: 63	Prophylactic semen cryopreservation	Seminoma: 30.4 ± 6.4 Nonseminoma: 25.1 ± 5.1	Has 3 cases of bilateral testicular cancer and 2 of extra-gonadal Orchiectomy not defined Separated by type of cancer	V, SC, PM, SM
2011	(53)	Retrospective	At diagnosis	Israel	TC: 43	Prophylactic semen cryopreservation	TC: 26.0 ± 7.0	Orchiectomy not defined	V, SC, PM, SM
2012	(54)	Retrospective	> 24	Germany	TC: 238	Various	TC: 38.7 ± 9.4	Separated by type of cancer	FSH, LH, TT
2012	(55)	Retrospective	At diagnosis	France	TC: 1149	Prophylactic semen cryopreservation	TC: 29.7 ± 6.57	Some with orchiectomy Compares different groups before and after orchiectomy	SC, TSC, PM
2012	(56)	Prospective	> 24	Turkey	TC: 27	BEP	TC: 34.0 ± 8.9	NA	FSH, LH, TT
2013	(57)	Retrospective	At diagnosis	Israel	TC: 17	Prophylactic semen cryopreservation	NA	Orchiectomy not defined	SC, TM FSH
2013	(58)	Prospective	At diagnosis 3, 6, 12, 24	France	TC: 127 3 months: 106; 6 months: 113; 12 months: 103; 24 months: 91 Controls: 257	BEP or RT	TC: 30.9 ± 4.9	Separated by type of cancer	V, SC, TSC, PM, VIT, SCS
2013	(59)	Retrospective	At diagnosis > 24	China	TC: 6	Prophylactic semen cryopreservation, after treatment (CH or RT)	TC: 27.5 ± 6.8	Orchiectomy not defined	V, SC, TM
2013	(60)	Retrospective	At diagnosis	Italy	TC: 150 Seminoma: 76 Nonseminoma: 14	Prophylactic semen cryopreservation Orchiectomy in some patients	NA	Separated by type of cancer	V, TSC, PM, VIT
2013	(61)	Prospective	At diagnosis 6, 12, 18, 24, 36	Italy	TC: 261 Seminoma: 154 Nonseminoma: 107	BEP or RT	TC: 27.9 ± 0.6	Separated by type of cancer	SC, PM, SM FSH, LH, TT
2013	(62)	Retrospective	At diagnosis	USA	TC: 165 Controls: 104	Prophylactic semen cryopreservation	NA	Used motility stimulants and as such TM was excluded No azoospermic	TSC
2013	(63)	Retrospective	At diagnosis	Australia	TC: 37 Controls: 35	Prophylactic semen cryopreservation	TC: 27.5	Orchiectomy not defined.	V, SC, TM, SCS
2013	(64)	Prospective	At diagnosis	Japan	TC: 49	Orchiectomy	TC: 30.6 ± 5.8	NA	V, SC, TM
2013	(65)	Prospective	At diagnosis	Spain	TC: 37 Seminoma: 15 Nonseminoma: 22 Controls: 35		TC: 28.4 ± 7.68 Seminoma: 31.38 ± 7.95 Nonseminoma: 26.4 ± 7.42 Controls: 30.26 ± 6.9	Separated by type of cancer	TSC, PM, TM, SM, FSH, LH, TT
2015	(66)	Retrospective	At diagnosis	South Korea	TC: 31	Prophylactic semen cryopreservation	TC: 33.4 ± 6.0	Orchiectomy not defined	V, SC, PM, SM, VIT
2015	(67)	Prospective	At diagnosis 3, 6, 9, 12, 24	Italy	T0: 139 T3: 59 T6: 54 T9: 60 T12: 75 T24: 75	CH or RT	NA	Some patients with orchiectomy at diagnosis	TSC, TM, SM, SCS
2015	(68)	Retrospective	At diagnosis	Japan	TC: 7	Orchiectomy	TC: 36.3 ± 9.1	Only seminoma	V, SC, TM
2016	(69)	Retrospective	At diagnosis	France	TC: 2315 Controls: 1656	Prophylactic semen cryopreservation		Most before orchiectomy	V, SC, TSC, PM, SM

BEP, Bleomycin + Etoposide + Cisplatin; CT, chemotherapy; FSH, follicle-stimulating hormone; LH, luteinizing hormone; NA, not available; PM, sperm progressive motility; RT, radiotherapy; SC, sperm concentration; SCS, sperm chromatin structure; SM, sperm morphology; Surg, surgery; TT, total testosterone; TC, testicular cancer; TM, sperm total motility; TSC, total sperm concentration; V, sperm volume; VIT, sperm vitality.

This meta-analysis was implemented to evaluate the association between TC and male infertility. To do this, patients' characteristics, hormonal status or semen features before and/or after treatment were registered. Both retrospective and prospective studies were included. Studies were excluded if there was reason to believe that some parameter would be skewed, if there was the risk of patient repetition between studies or if they did not investigate the outcomes assessed in the present meta-analysis. Data was extracted manually, independently, and in duplicate by two authors (DQA and RS).

The variables collected from the studies were: country (name), pathology (testicular and if available seminoma and nonseminoma), time at evaluation (in months, 0 being after diagnosis and eventually orchiectomy but before other treatments, and then, months after treatment), status of orchiectomy at evaluation (prior, after or unknown), number of patients, type of treatment and dose if available, age at time of evaluation, sperm concentration (SC; 10^6/mL), total sperm concentration (TSC; 10^6), semen volume (V; mL), sperm progressive motility (PM; %), sperm total motility (TM; %), sperm vitality (VIT; %), sperm morphology (SM; %), sperm chromatin structure (SCS; %), follicle stimulating hormone (FSH; IU/L), luteinizing hormone (LH; IU/L), and total testosterone (TT, nmol/L).

Effects on patients' characteristics

Regarding age, patients suffering from TC and selected for this study (n = 7511) were within the expected age interval (5) as they presented a mean age of 29.20 ± 6.15 years old (assessed with 2405 patients). Of these, patients with seminoma (n = 710) presented a statistically increased ($p < 0.0001$) mean age (30.17 ± 5.70 assessed with 308 patients) than patients with nonseminoma (n = 654; 26.32 ± 5.32 assessed with 313 patients).

Effects on sperm characteristics of patients

Sperm characteristics are directly related to male fertility potential and are both commonly associated to sperm ability to successfully fertilize an oocyte and to pregnancy rates (70). TC appears to display a more negative effect on semen than other cancers (21). The results on the influence of TC on the seminal parameters are reported in Table 2.

For seminal volume, before treatment were analysed 21 studies (n = 3529), and 9 for controls (n = 2172). Before treatment, 7 studies were considered to determine the difference between seminoma (n = 424) and nonseminoma (n = 364). After treatment, 3 studies (n = 183) that assessed V after 12 months were included. 2 studies assessed V for 24 months. No alterations are observed in patients suffering from TC even when considering different histopathological groups.

Concerning concentration, both SC and TSC were studied. Although no difference is observed in SC before and after treatment, a statistically significant lower value ($p < 0.0001$) of SC both before and after treatment compared with control groups is observed on TC (28 studies before treatment, n=5167; 9 studies with controls, n=2162; 6 studies after treatment > 12 months, n=468). Regarding the histopathological groups of TC (6 studies), SC is statistically significantly inferior ($p < 0.0001$) in nonseminoma patients (n = 480) compared to seminoma patients (n = 446). After treatment, prospective studies show a statistically significant decrease ($p < 0.0001$) on SC in the first 3 months. SC then remains stable up to 12 months. However, it is also detected a statistically significant increase ($p < 0.0001$) from this period until one year later. As far as for TSC evaluation this was statistically significantly higher ($p =0.0014$) after treatment (3 studies; n = 252) in relation to the time of diagnosis (10 studies; n = 4473). Still, both TSC values at diagnosis and after treatment were statistically significantly lower than the corresponding control group (3 studies; n = 2017). Before treatment, patients experiencing seminoma (n =259) do not present any difference on TSC from the ones experiencing nonseminoma (n = 248). Prospective

studies show a statistically significant lower ($p < 0.0001$) TSC up to 12 months after treatment. Though, with a trivial rise noticed from 3 to 6 months and then with a statistically significant increase ($p < 0.0001$) from 6 to 12 months, returning to values similar at diagnosis after 24 months ($p < 0.0001$).

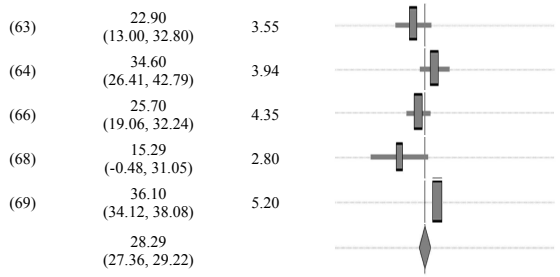
With reference to motility, when analysing patients with TC no differences are observed both for PM (n = 4790) and TM (n =462), either before and after treatment. Nevertheless, both values are statistically significantly decreased comparing to controls (PM: n = 1983, p=0.0016; TM: n= 179; p=0.0164). Seminoma patients (7 studies; n =522) present a statistically significant higher (p =0.0033) PM than nonseminoma ones (n = 494). Prospective studies (3 studies; n = 191) demonstrate that PM statistically significantly decreases ($p < 0.0001$) from diagnosis up to 3 months after treatment but from 6 to 12 months after treatment it statistically significantly increases ($p < 0.0001$) recovering to diagnosis' values.

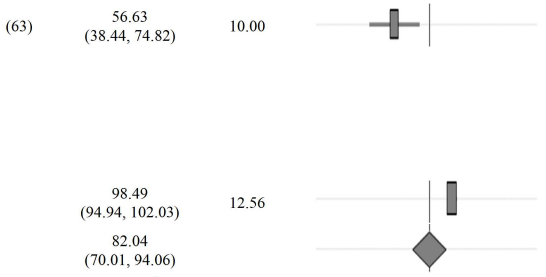
For the parameter VIT, no differences are seen between seminoma (n = 146) and nonseminoma (n = 73) patients. Data for controls and for patients after treatment were not available.

Regarding morphology, since diverse authors use different criteria, studies were divide according to strict or non-strict analyses. When non-strict criteria are used to classify normal SM (nsSM), sperm from seminoma (n = 213) and nonseminoma (n = 175) patients seem to display equal rates of normal morphology. Data for controls and for patients after treatment were not available. However, using strict criteria (sSM) TC patients (n = 725) have similar rates as controls (n = 162). Considering the two histopathological groups of TC, nonseminoma (246) has statistically significantly lower (p =0.0200) sSM than the seminoma (n = 163) one.

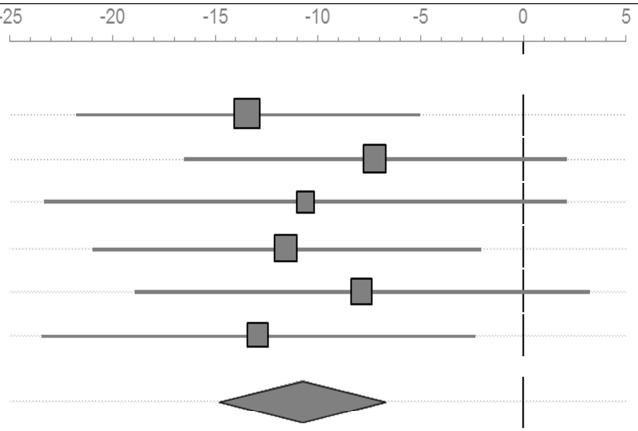
Table 2. Seminal parameters in testicular cancer patients.

SC 10^6/mL	At diagnosis, before treatment			After treatment			Controls		
	Ref.	**Mean (95% CI)**	**Weight %**	**Ref.**	**Mean (95% CI)**	**Weight %**	**Ref.**	**Mean (95% CI)**	**Weight %**
	(27)	42.14 (10.28, 73.99)	0.98						
	(28)	26.70 (21.30, 32.10)	4.60				(28)	73.20 (58.76, 87.64)	10.94
	(30)	53.80 (12.42, 95.18)	0.72						
							(34)	79.90 (72.02, 87.78)	12.07
	(35)	23.80 (9.95, 37.65)	2.91				(35)	77.40 (70.24, 84.56)	12.18
	(36)	29.50 (18.83, 40.17)	3.44				(36)	118.00 (86.31, 149.69)	7.47
	(37)	30.20 (25.93, 34.47)	4.83						
	(39)	18.70 (6.82, 30.58)	3.22						
	(40)	45.20 (28.07, 62.33)	2.15						
				(41)	21.20 (4.46, 37.94)	15.58			
	(42)	36.60 (13.04, 60.16)	1.53	(42)	17.20 (11.59, 22.81)	17.56			
	(43)	41.80 (21.21, 62.37)	1.95						
	(44)	31.32 (26.69, 35.95)	4.76	(44)	46.36 (38.16, 54.56)	17.09			
	(45)	38.40 (28.07, 48.73)	3.44						
	(47)	24.80 (18.45, 31.14)	4.39				(47)	78.10 (63.21, 92.99)	10.71
	(48)	26.10 (19.68, 32.52)	4.37						
	(49)	17.20 (14.64, 19.76)	5.12						
	(51)	34.60 (26.30, 42.90)	3.93				(51)	66.90 (63.76, 70.04)	12.61
	(52)	18.76 (14.08, 23.43)	4.75						
	(53)	38.40 (29.26, 47.54)	3.72						
	(55)	26.18 (24.34, 28.02)	5.20						
	(57)	38.40 (18.71, 58.09)	1.90						
	(58)	31.34 (25.72, 36.96)	4.54	(58)	34.58 (27.71, 41.46)	17.32	(58)	98.67 (87.50, 109.84)	11.46
	(59)	20.00 (4.62, 35.38)	3.04	(59)	2.33 (-0.91, 5.58)	17.80			
	(61)	38.39 (33.15, 43.63)	4.62	(61)	46.64 (28.81, 64.46)	14.65			





Ref.	Seminoma Mean (95% CI)	Seminoma Weight %	Nonseminoma Mean (95% CI)	Nonseminoma Weight %	Difference Mean (95% CI)	Difference Weight %
(30)	53.80 (12.42, 95.18)	2.40				
(35)	23.80 (9.95, 37.65)	10.01				
(37)	36.50 (29.61, 43.39)	14.85	23.09 (18.68, 27.51)	17.68	-13.41 (-21.79, -5.02)	23.42
(44)	34.40 (27.43, 41.37)	14.80	27.20 (21.66, 32.74)	16.68	-7.20 (-16.51, 2.11)	19.05
(47)	31.20 (18.57, 43.83)	10.44	20.60 (13.87, 27.33)	15.68	-10.60 (-23.33, 2.13)	10.59
(52)	25.98 (15.14, 36.82)	11.52	14.46 (10.71, 18.21)	18.25	-11.52 (-20.97, -2.07)	18.70
(58)	34.84 (26.12, 43.57)	13.22	27.01 (20.60, 33.42)	15.85	-7.84 (-18.92, 3.24)	13.52
(61)	46.00 (37.08, 54.92)	12.99	33.10 (26.78, 39.42)	15.86	-12.90 (-23.47, -2.33)	14.72
(68)	15.29 (-0.48, 31.05)	9.79				
	32.51 (26.82, 38.20)		24.02 (18.43, 29.60)		-10.75 (-14.78, -6.71)	
	Q: 22.45; I²: 64.37%; T: 6.68		Q:34.50; I²: 85.50%; T: 6.40		Q:1.41; I²: 0.00%; T: 0	

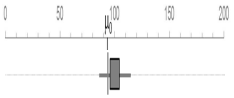


Time after treatment

Ref.	0-3 months Mean (95% CI)	0-3 months Weight %	0-6 months Mean (95% CI)	0-6 months Weight %	3-6 months Mean (95% CI)	3-6 months Weight %	0-12 months Mean (95% CI)	0-12 months Weight %	6-12 months Mean (95% CI)	6-12 months Weight %	0-24 months Mean (95% CI)	0-24 months Weight %	12-24 months Mean (95% CI)	12-24 months Weight %
(30)	-34.10 (16.25, 21.96)	0.87	-41.10 (-98.44, 16.24)	1.31	-7.00 (-42.13, 28.13)	1.47	49.68 (-61.77, 161.12)	0.20	90.78 (-67.28, 248.83)	0.26				
(44)	-24.25 (14.91, 17.68)	42.39	-23.58 (-32.00, -15.16)	33.30	0.67 (-4.05, 5.38)	53.23	-9.91 (-16.64, -3.18)	43.12	13.67 (7.05, 20.29)	39.03	15.04 (6.37, 23.71)	40.04	24.95 (16.19, 33.71)	40.50
(58)	-24.62 (18.13, 26.88)	56.74	-21.73 (-28.95, -14.51)	40.80	2.89 (-2.20, 7.98)	45.30	-12.46 (-19.95, -4.97)	34.80	9.27 (2.90, 15.65)	40.10	3.25 (-5.53, 12.02)	39.61	15.70 (7.60, 23.81)	45.89
(61)			-12.04 (-22.35, -1.74)	24.62			-11.29 (-20.72, -1.85)	21.85	0.76 (-11.10, 12.61)	20.57	9.00 (-6.05, 24.06)	20.34	20.29 (4.14, 36.44)	13.61
	-24.55 (-29.43, -19.66)		-20.21 (-26.02, -14.41)		1.56 (-1.84, 4.97)		-10.98 (-15.38, -6.58)		9.45 (2.97, 15.94)		9.14 (1.23, 17.05)		20.07 (13.93, 26.21)	
	Q:0.13; I²: 0.00%; T: 0		Q:3.87; I²: 22.50%; T: 2.84		Q:0.76; I²: 0.00%; T: 0		Q: 1.72; I²: 0.00%; T: 0		Q: 5.16; I²: 41.86%; T: 4.10		Q: 3.55; I²: 43.63%; T: 4.61		Q: 2.34; I²: 14.38%; T: 2.13	

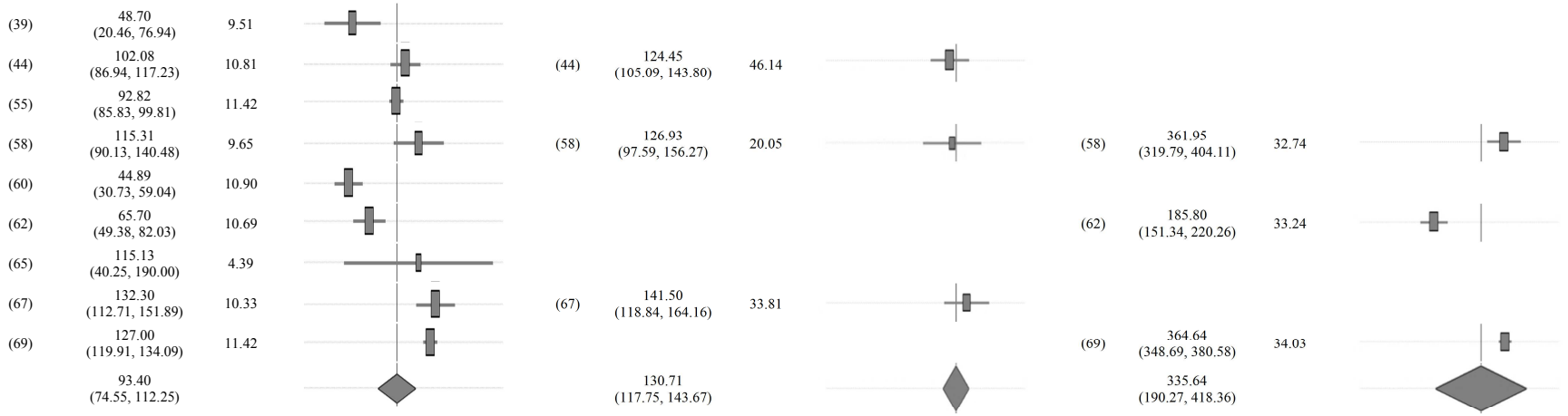
TSC 10^6

At diagnosis, before treatment Ref.	Mean (95% CI)	Weight %
(37)	99.90 (85.59, 114.21)	10.88

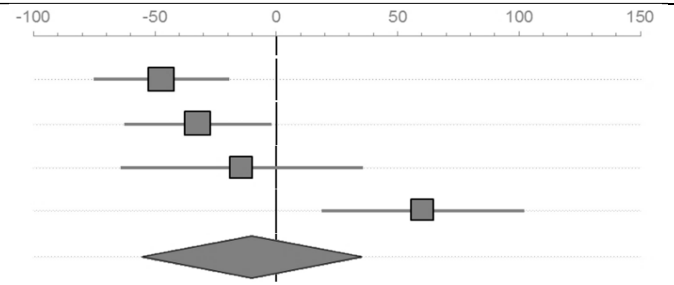


After treatment Ref.	Mean (95% CI)	Weight %

Controls Ref.	Mean (95% CI)	Weight %

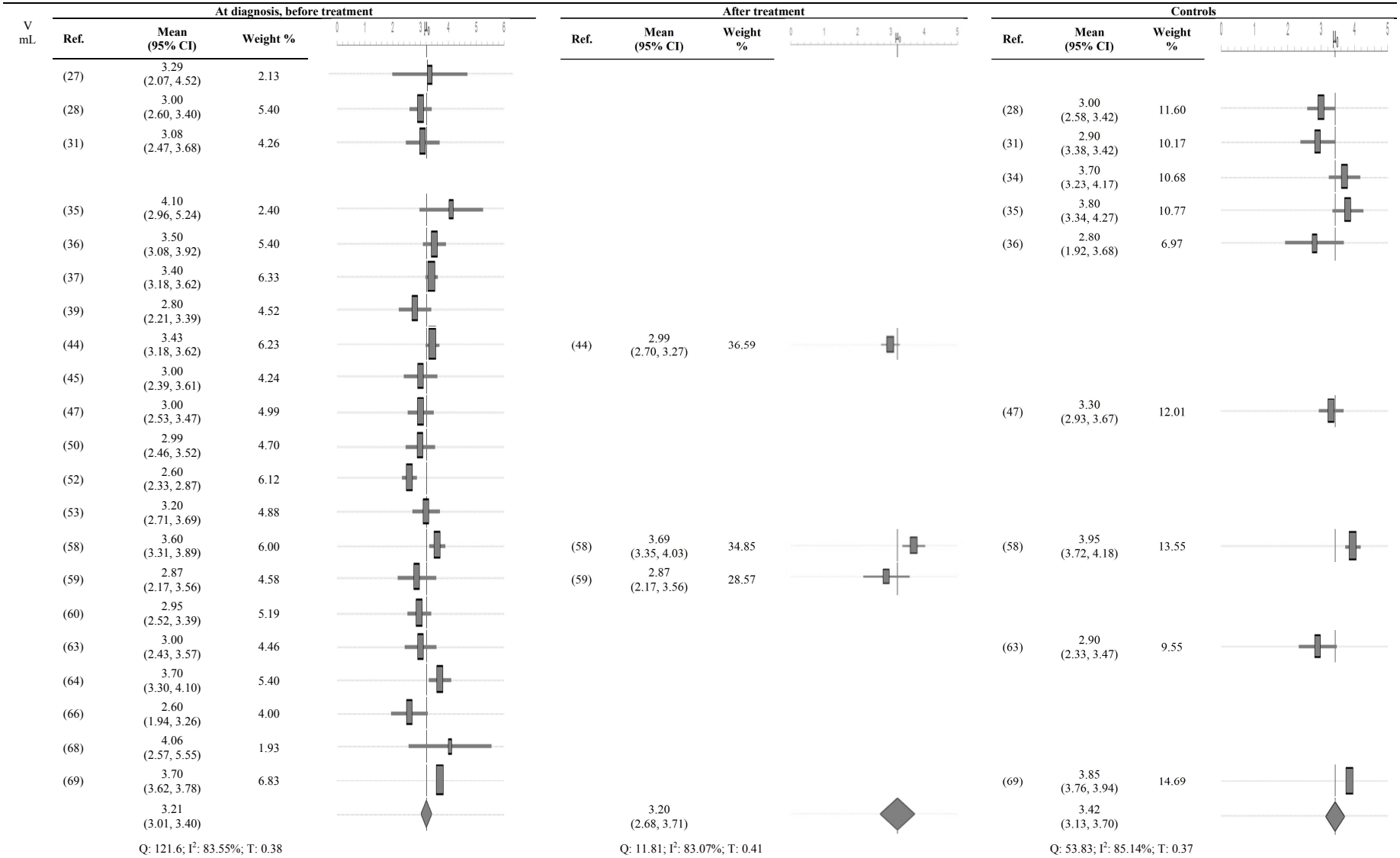


Ref.	Seminoma Mean (95% CI)	Seminoma Weight %	Nonseminoma Mean (95% CI)	Nonseminoma Weight %	Difference Mean (95% CI)	Difference Weight %
(37)	122.00 (99.87, 144.13)	25.43	74.73 (58.28, 91.19)	43.42	-47.27 (-75.30, -19.23)	27.08
(44)	115.90 (93.47, 138.33)	25.29	83.60 (65.07, 102.13)	36.10	-32.30 (-62.60, -2.01)	26.63
(58)	121.54 (85.40, 157.69)	23.50	107.59 (73.26, 141.91)	12.04	-13.96 (-63.92, 36.01)	22.15
(60)	34.00 (17.74, 50.26)	25.89	94.50 (49.86, 139.14)	8.44	60.50 (18.82, 102.18)	24.15
	97.56 (47.05, 148.07)		82.92 (71.94, 93.90)		-9.88 (-54.98, 35.22)	
	Q: 61.15; I²: 95.09%; T: 49.98		Q:3.36; I²: 10.75%; T: 4.20		Q:19.11; I²: 84.30%; T: 41.90	

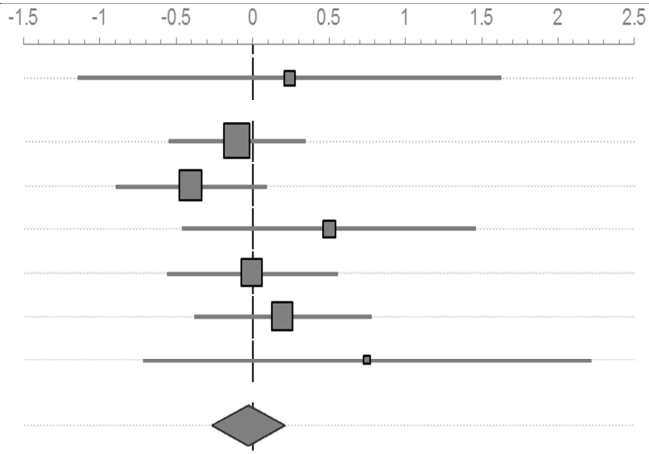


Ref.	0-3 months Mean (95% CI)	0-3 months Weight %	0-6 months Mean (95% CI)	0-6 months Weight %	3-6 months Mean (95% CI)	3-6 months Weight %	0-12 months Mean (95% CI)	0-12 months Weight %	6-12 months Mean (95% CI)	6-12 months Weight %	0-24 months Mean (95% CI)	0-24 months Weight %	12-24 months Mean (95% CI)	12-24 months Weight %
(44)	-79.21 (-103.91, -54.51)	58.14	-77.58 (-105.1, -50.06)	59.97	1.63 (-13.74, 17.01)	72.56	-33.99 (-56.45, -11.53)	48.78	43.59 (20.61, 66.57)	61.41	22.36 (-2.76, 47.48)	48.27	56.35 (32.18, 80.53)	45.24
(58)	-90.58 (-119.68, -61.47)	41.86	-76.68 (-110.35, -43.01)	40.03	13.90 (-10.98, 38.78)	27.44	-46.96 (-79.36, -14.56)	23.49	29.72 (0.79, 58.65)	38.59	11.62 (-26.90, 50.15)	20.55	58.59 (24.92, 92.25)	23.33
(67)							-37.80 (-67.63, -7.97)	27.73			9.20 (-22.08, 40.48)	31.18	47.00 (17.94, 76.06)	31.43
	-83.97 (-102.70, -65.24)		-77.22 (-98.41, -56.03)		5.00 (-7.96, 17.96)		-38.09 (-53.71, -22.48)		38.24 (20.37, 56.11)		16.05 (-1.32, 33.42)		53.93 (37.78, 70.09)	
	Q:0.34; I²: 0.00%; T: 0		Q:0.00; I²: 22.50%; T: 2.84		Q:0.68; I²: 0.00%; T: 0		Q: 0.42; I²: 0.00%; T: 0		Q: 0.55; I²: 0.00%; T: 0		Q: 0.48; I²: 0.00%; T: 0		Q: 0.34; I²: 0.00%; T: 0	

Time after treatment


V
mL
At diagnosis, before treatment
Ref.
Mean (95% CI)
Weight %
(27) 3.29 (2.07, 4.52) 2.13
(28) 3.00 (2.60, 3.40) 5.40
(31) 3.08 (2.47, 3.68) 4.26
(35) 4.10 (2.96, 5.24) 2.40
(36) 3.50 (3.08, 3.92) 5.40
(37) 3.40 (3.18, 3.62) 6.33
(39) 2.80 (2.21, 3.39) 4.52
(44) 3.43 (3.18, 3.62) 6.23
(45) 3.00 (2.39, 3.61) 4.24
(47) 3.00 (2.53, 3.47) 4.99
(50) 2.99 (2.46, 3.52) 4.70
(52) 2.60 (2.33, 2.87) 6.12
(53) 3.20 (2.71, 3.69) 4.88
(58) 3.60 (3.31, 3.89) 6.00
(59) 2.87 (2.17, 3.56) 4.58
(60) 2.95 (2.52, 3.39) 5.19
(63) 3.00 (2.43, 3.57) 4.46
(64) 3.70 (3.30, 4.10) 5.40
(66) 2.60 (1.94, 3.26) 4.00
(68) 4.06 (2.57, 5.55) 1.93
(69) 3.70 (3.62, 3.78) 6.83
3.21 (3.01, 3.40)
Q: 121.6; I2: 83.55%; T: 0.38
After treatment
Ref.
Mean (95% CI)
Weight %
(44) 2.99 (2.70, 3.27) 36.59
(58) 3.69 (3.35, 4.03) 34.85
(59) 2.87 (2.17, 3.56) 28.57
3.20 (2.68, 3.71)
Q: 11.81; I2: 83.07%; T: 0.41
Controls
Ref.
Mean (95% CI)
Weight %
(28) 3.00 (2.58, 3.42) 11.60
(31) 2.90 (3.38, 3.42) 10.17
(34) 3.70 (3.23, 4.17) 10.68
(35) 3.80 (3.34, 4.27) 10.77
(36) 2.80 (1.92, 3.68) 6.97
(47) 3.30 (2.93, 3.67) 12.01
(58) 3.95 (3.72, 4.18) 13.55
(63) 2.90 (2.33, 3.47) 9.55
(69) 3.85 (3.76, 3.94) 14.69
3.42 (3.13, 3.70)
Q: 53.83; I2: 85.14%; T: 0.37

Ref.	Seminoma Mean (95% CI)	Seminoma Weight %	Nonseminoma Mean (95% CI)	Nonseminoma Weight %	Difference Mean (95% CI)	Difference Weight %
(31)	2.90 (2.13, 3.67)	10.14	3.14 (2.33, 3.96)	4.61	0.24 (-1.15, 1.63)	3.20
(35)	4.10 (2.96, 5.24)	6.42				
(37)	3.40 (3.09, 3.71)	14.82	3.30 (2.97, 3.63)	25.91	-0.10 (-0.55, 0.35)	28.37
(44)	3.60 (3.27, 3.93)	14.63	3.20 (2.82, 3.58)	19.87	-0.40 (-0.90, 0.10)	23.48
(47)	2.70 (2.07, 3.33)	11.03	3.20 (2.52, 3.88)	6.41	0.50 (-0.46, 1.46)	6.45
(52)	2.60 (2.17, 3.03)	13.32	2.60 (2.25, 2.95)	23.03	0.00 (-0.56, 0.56)	18.53
(58)	3.51 (3.10, 3.91)	13.61	3.71 (3.29, 4.13)	16.36	0.20 (-0.38, 0.78)	17.25
(60)	2.60 (1.99, 3.21)	10.82	3.35 (2.41, 4.29)	3.81	0.75 (-0.72, 2.22)	2.71
(68)	4.06 (2.57, 5.55)	5.20				
	3.20 (2.88, 3.53)		3.20 (3.01, 3.34)		-0.03 (-0.27, 0.21)	
	Q: 29.79; I^2: 73.15%; T: 0.40		Q:17.89; I^2: 66.45%; T: 0.33		Q:5.39; I^2: 0.00%; T: 0	

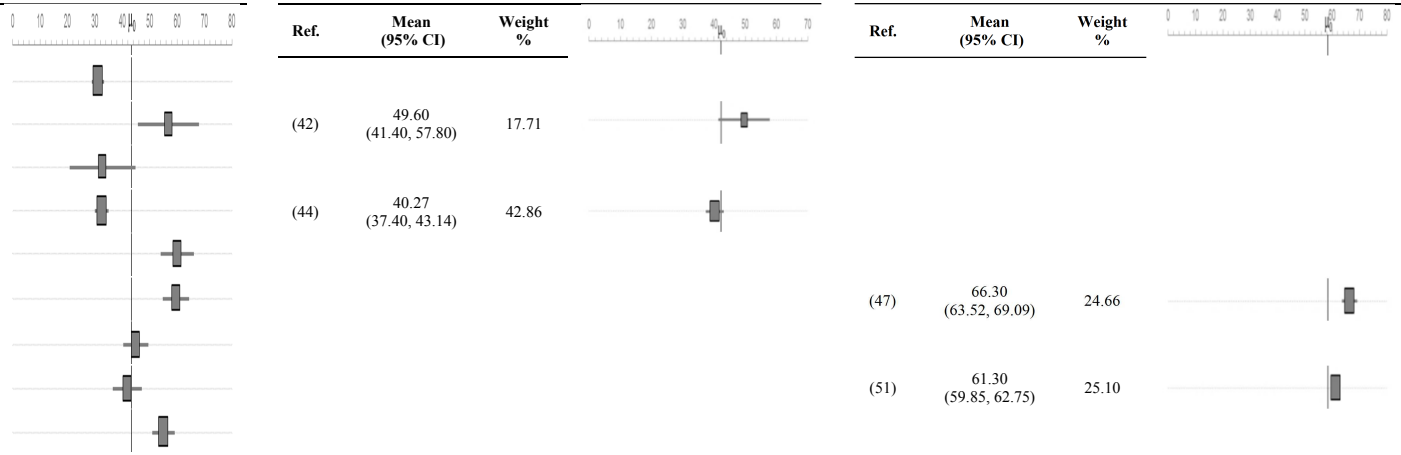


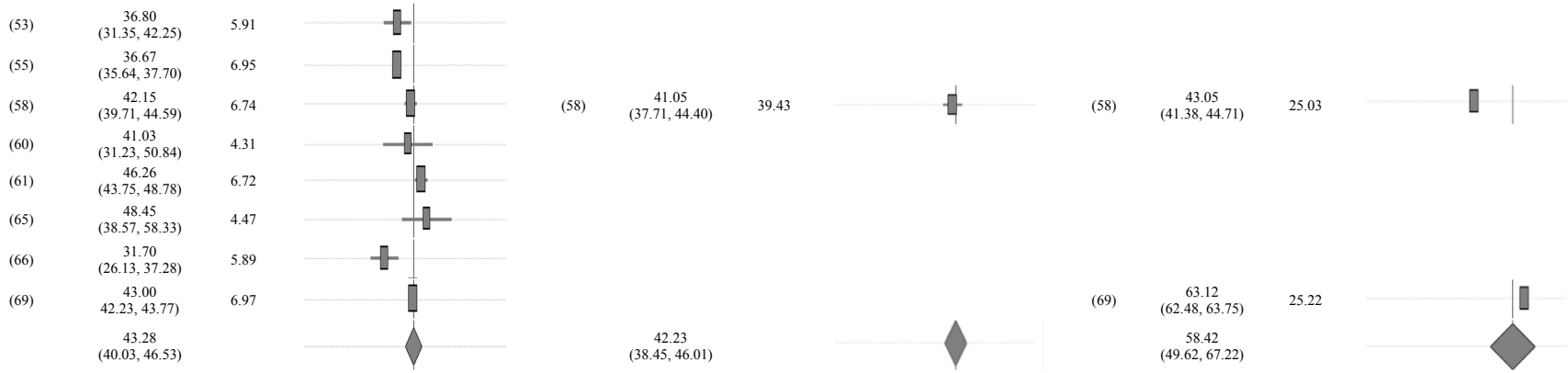
Time after treatment

Ref.	0-3 months Mean (95% CI)	0-3 months Weight %	0-6 months Mean (95% CI)	0-6 months Weight %	3-6 months Mean (95% CI)	3-6 months Weight %	0-12 months Mean (95% CI)	0-12 months Weight %	6-12 months Mean (95% CI)	6-12 months Weight %	0-24 months Mean (95% CI)	0-24 months Weight %	12-24 months Mean (95% CI)	12-24 months Weight %
(44)	-0.04 (-0.49, 0.41)	45.36	-0.07 (-0.58, 0.43)	41.54	-0.03 (-0.62, 0.56)	34.98	-0.13 (-0.52, 0.27)	56.69	-0.06 (-0.61, 0.50)	42.59	-0.44 (-0.84, -0.05)	51.95	-0.31 (-0.75, 0.12)	57.15
(58)	-0.21 (-0.62, 0.20)	54.64	0.01 (-0.42, 0.44)	58.46	0.23 (-0.20, 0.65)	65.02	0.18 (-0.27, 0.64)	43.31	0.17 (-0.31, 0.65)	57.41	0.09 (-0.36, 0.53)	48.05	-0.10 (-0.60, 0.40)	42.85
	-0.14 (-0.44, 0.17)		-0.02 (-0.35, 0.30)		0.14 (-0.21, 0.48)		0.01 (-0.30, 0.31)		0.07 (-0.29, 0.43)		-0.19 (-0.71, 0.33)		0.07 (-0.55, 0.10)	
	Q:0.30; I^2: 0.00%; T: 0		Q:0.06; I^2: 0.00%; T: 0		Q:0.48; I^2: 0.00%; T: 0		Q: 1.04; I^2: 3.50%; T: 0.04		Q: 0.38; I^2: 0.00%; T: 0		Q: 3.05; I^2: 67.17%; T: 0.31		Q: 0.41; I^2: 0.00%; T: 0	

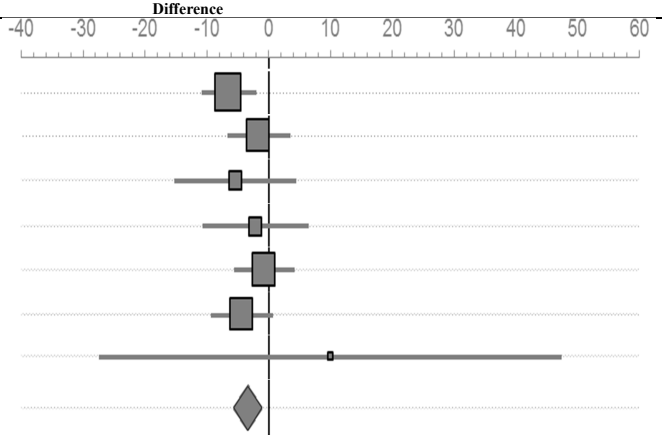
PM %

At diagnosis, before treatment Ref.	Mean (95% CI)	Weight %	After treatment Ref.	Mean (95% CI)	Weight %	Controls Ref.	Mean (95% CI)	Weight %
(37)	31.10 (28.85, 33.35)	6.78						
(42)	56.80 (45.71, 67.89)	4.19	(42)	49.60 (41.40, 57.80)	17.71			
(43)	32.80 (20.81, 44.79)	4.09						
(44)	32.52 (30.01, 35.02)	6.73	(44)	40.27 (37.40, 43.14)	42.86			
(45)	60.00 (53.92, 66.08)	5.69						
(47)	59.54 (54.73, 64.35)	6.11				(47)	66.30 (63.52, 69.09)	24.66
(48)	44.90 (40.29, 49.51)	6.17						
(51)	41.80 (36.52, 47.08)	5.97				(51)	61.30 (59.85, 62.75)	25.10
(52)	54.98 (50.88, 59.08)	6.32						





	Seminoma		Nonseminoma		Difference	
Ref.	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %
(37)	34.10 (31.15, 37.05)	15.56	27.69 (24.31, 31.07)	15.41	-6.41 (-10.85, -1.97)	25.80
(44)	33.20 (29.94, 36.46)	15.49	31.60 (27.60, 35.60)	15.25	-1.60 (-6.68, 3.48)	19.82
(47)	62.80 (55.91, 69.69)	14.30	57.40 (50.63, 64.17)	14.32	-5.40 (-15.21, 4.41)	5.51
(52)	56.30 (49.67, 62.94)	14.31	54.20 (48.86, 59.54)	14.81	-2.10 (-10.62, 6.42)	14.81
(58)	42.39 (38.83, 45.95)	15.41	41.71 (38.44, 44.98)	15.44	-0.68 (-5.53, 4.18)	15.44
(61)	48.80 (45.03, 52.57)	15.34	44.50 (41.14, 47.86)	15.41	-4.30 (-9.39, 0.79)	15.41
(60)	39.50 (23.75, 55.25)	9.59	49.50 (31.31, 67.69)	9.37	10.00 (-27.45, 47.45)	9.37
	45.29 (37.68, 52.90)		43.26 (35.37, 51.16)		-3.37 (-5.62, -1.13)	
	Q: 127.50; I²: 95.29%; T: 9.73		Q:135.01; I²: 95.56%; T: 10.12		Q:4.39; I²: 0.00%; T: 0	



	Time after treatment									
	0-3 months		0-6 months		3-6 months		0-12 months		6-12 months		0-24 months		12-24 months	
Ref	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %
(44)	-16.95 (-21.53, -12.38)	49.42	-17.17 (-22.41, -11.93)	47.11	-0.22 (-6.29, 5.86)	44.57	-2.43 (-6.50, 1.63)	51.95	14.74 (8.93, 20.54)	48.37	7.75 (3.72, 11.79)	49.98	10.19 (5.75, 14.62)	51.05
(58)	-14.55 (-19.07, -10.02)	50.58	-10.89 (-15.2, -6.58)	52.89	3.66 (-1.77, 9.08)	55.43	-3.50 (-7.73, 0.74)	48.05	7.39 (2.22, 12.56)	51.63	-1.10 (-5.12, 2.92)	50.02	2.40 (-2.56, 7.36)	48.95
	-15.74 (-18.94, -12.53)		-13.85 (-19.99, -7.70)		1.93 (-2.08, 5.95)		-2.94 (-5.86, 0.03)		10.95 (3.75, 18.14)		3.33 (-5.35, 12.00)		6.38 (-1.26, 14.01)	
	Q:0.54; I²: 0.00%; T: 0		Q:3.33; I²: 69.94%; T: 3.72		Q:0.88; I²: 0.00%; T: 0		Q: 0.13; I²: 0.00%; T: 0		Q: 3.48; I²: 71.26%; T: 4.38		Q: 9.38; I²: 81.23%; T: 5.92		Q: 5.33; I²: 81.23%; T: 4.96	

TM %

At diagnosis, before treatment

Ref.	Mean (95% CI)	Weight %
(28)	47.50 (41.30, 53.70)	8.62
(30)	59.40 (50.41, 68.39)	8.18
(35)	35.20 (20.71, 49.69)	6.28
(36)	47.40 (37.35, 57.45)	7.50
(40)	55.30 (47.75, 62.85)	8.25
(48)	58.02 (54.01, 62.03)	9.19
(57)	35.70 (25.47, 45.93)	7.49
(59)	34.33 (16.91, 51.75)	6.15
(63)	51.95 (44.81, 59.09)	8.36
(64)	32.50 (26.21, 38.79)	8.60
(65)	63.55 (52.40, 74.70)	7.14
(67)	42.20 (39.95, 44.45)	9.50
(68)	47.00 (24.68, 69.32)	4.75
	47.71 (42.97, 52.46)	

Q: 108.64; I^2: 88.95%; T: 8.98

After treatment

Ref.	Mean (95% CI)	Weight %
(34)	35.20 (25.29, 45.11)	34.76
(59)	14.83 (-8.00, 37.67)	24.60
(67)	44.90 (42.42, 47.39)	40.64
	34.13 (20.53, 47.74)	

Q: 14.83; I^2: 86.51%; T: 10.82

Controls

Ref.	Mean (95% CI)	Weight %
(28)	68.80 (65.03, 72.57)	20.80
(34)	60.90 (58.23, 63.57)	22.42
(35)	60.60 (58.20, 63.00)	22.82
(36)	71.90 (65.30, 78.50)	16.20
(63)	60.48 (55.22, 65.74)	17.77
	61.81 (56.48, 67.13)	

Q: 70.22; I^2: 92.88%; T: 6.34

Seminoma

Ref.	Mean (95% CI)	Weight %
(30)	53.80 (12.42, 95.18)	13.78
(35)	23.80 (9.95, 37.65)	43.44
(68)	15.29 (-0.48, 31.05)	42.78
	24.29 (9.91, 38.67)	

Q: 4.47; I^2: 55.23%; T: 6.68

VIT %

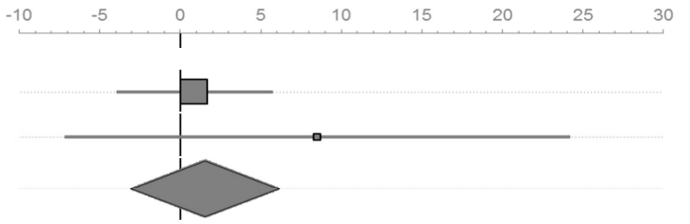
At diagnosis, before treatment

Ref.	Mean (95% CI)	Weight %
(30)	64.00 (55.49, 72.51)	23.71
(58)	67.44 (64.99, 69.88)	26.17
(60)	71.69 (67.83, 75.55)	25.64
(66)	42.20 (36.04, 48.36)	24.49

61.53
(51.00, 72.06)

Q: 72.26; I^2: 95.85%; T: 10.43

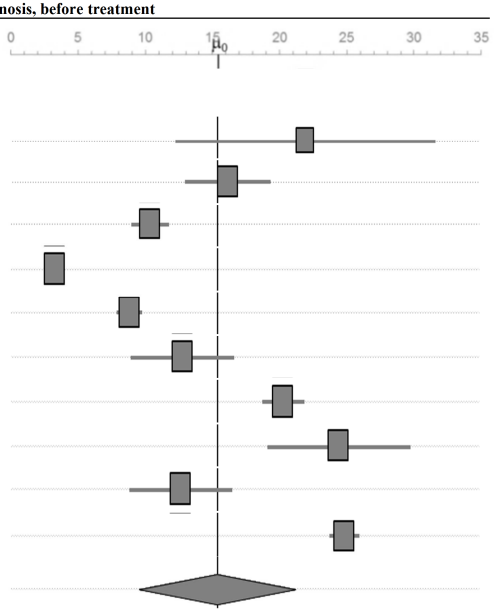
Ref.	Seminoma Mean (95% CI)	Seminoma Weight %	Nonseminoma Mean (95% CI)	Nonseminoma Weight %	Difference Mean (95% CI)	Difference Weight %
(30)	64.00 (55.49, 72.51)	16.50				
(58)	66.98 (63.33, 70.62)	64.06	67.85 (64.72, 70.98)	56.15	0.87 (-3.08, 5.73)	91.20
(60)	68.50 (61.89, 75.11)	19.44	77.00 (69.28, 84.72)	43.85	8.50 (-7.21, 24.21)	8.80
	66.78 (63.92, 69.65)		71.86 (62.96, 80.76)		1.54 (-3.05, 6.14)	
	Q: 0.88; I^2: 0.00%; T: 0		Q: 5.51; I^2: 81.85%; T: 5.85		Q: 0.85; I^2: 0.00%; T: 0	



sSM %

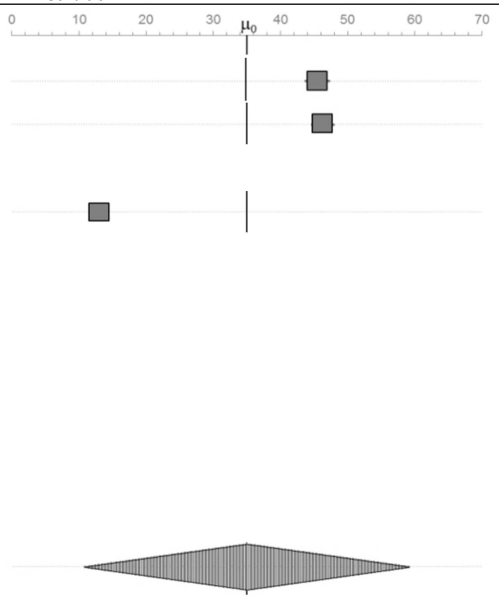
At diagnosis, before treatment Ref.	Mean (95% CI)	Weight %
(35)	22.00 (12.28, 31.72)	8.46
(39)	16.20 (13.00, 19.40)	10.13
(47)	10.40 (8.99, 11.80)	10.34
(48)	3.30 (2.70, 3.90)	10.39
(52)	8.87 (7.91, 9.82)	10.37
(53)	12.81 (8.96, 16.66)	9.98
(61)	20.34 (18.78, 21.91)	10.32
(65)	24.50 (19.14, 29.86)	9.66
(66)	12.70 (8.85, 16.55)	9.98
(67)	24.90 (23.78, 26.02)	10.36
	15.43 (9.60, 21.27)	

Q: 1418.7; I^2: 99.37%; T: 9.23

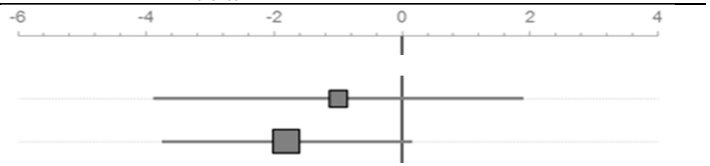


Controls Ref.	Mean (95% CI)	Weight %
(34)	45.70 (43.81, 47.59)	33.32
(35)	46.30 (44.49, 48.11)	33.32
(47)	13.10 (11.96, 14.24)	33.36
	35.02 (10.86, 59.19)	

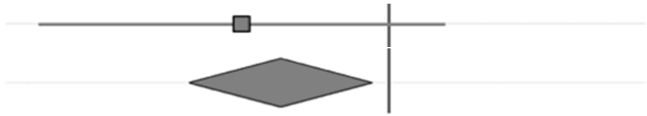
Q: 1452.9; I^2: 99.86%; T: 21.34



Ref.	Seminoma Mean (95% CI)	Seminoma Weight %	Non-seminoma Mean (95% CI)	Non-seminoma Weight %	Difference Mean (95% CI)	Difference Weight %
(35)	22.00 (12.28, 31.72)	17.28				
(47)	11.00 (9.02, 12.98)	27.72	10.00 (7.98, 12.02)	33.14	-1.00 (-3.89, 1.89)	25.59
(52)	10.00 (8.43, 11.57)	27.98	8.20 (6.99, 9.41)	33.74	-1.80 (-3.76, 0.16)	54.22

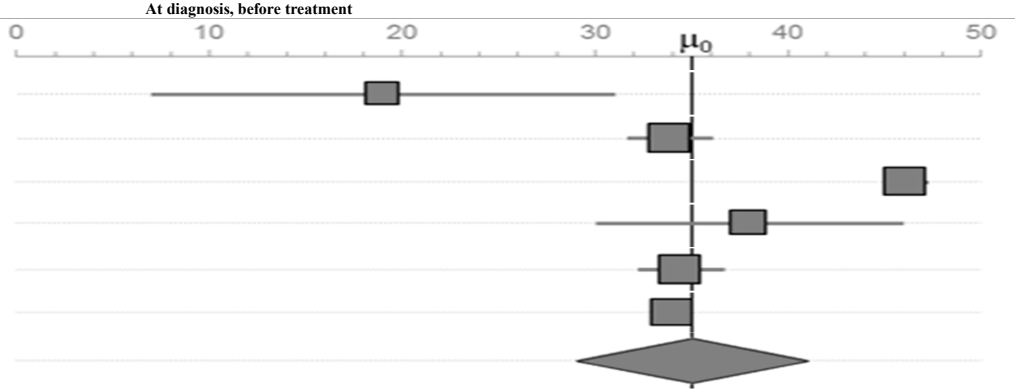


(61)	21.70 (19.12, 24.28)	27.02	19.40 (17.42, 21.38)	33.11	-2.30 (-5.48, 0.88)	20.20
	15.51 (9.78, 21.24)		12.51 (5.94, 19.07)		-1.70 (-3.12, -0.27)	
	Q: 67.72; I^2: 95.57%; T: 5.47		Q: 93.01; I^2: 97.85%; T: 5.74		Q: 0.39; I^2: 0.00%; T: 0	

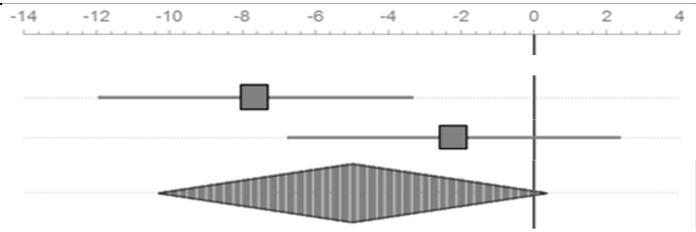


At diagnosis, before treatment

nsSM %	Ref.	Mean (95% CI)	Weight %
	(30)	19.00 (6.94, 31.06)	12.23
	(37)	33.90 (31.69, 36.11)	18.12
	(40)	46.10 (44.91, 47.29)	18.44
	(42)	38.00 (30.03, 45.97)	14.61
	(44)	34.46 (32.19, 36.72)	18.09
	(69)	34.00 (33.23, 34.77)	18.51
		35.05 (29.07, 41.03)	
		Q: 165.98; I^2: 97.59%; T: 8.10	



	Seminoma		Nonseminoma		Difference	
Ref.	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %
(30)	19.00 (6.94, 31.06)	20.26				
(37)	37.50 (34.53, 40.47)	40.18	29.86 (26.69, 33.03)	49.71	-7.64 (-11.96, -3.32)	50.97
(44)	35.40 (32.18, 38.62)	39.56	33.20 (30.05, 36.35)	50.29	-2.20 (-6.78, 2.38)	49.03
	32.92 (26.81, 39.03)		31.54 (28.27, 34.81)		-4.97 (-10.30, 0.36)	
	Q: 12.19; I^2: 83.60%; T: 4.68		Q: 2.21; I^2: 54.77%; T: 1.75		Q: 2.91; I^2: 65.58%; T: 3.12	



nsSM, non-strict sperm morphology; PM, sperm progressive motility; SC, sperm concentration; sSM, strict sperm morphology; TSC, total sperm concetration; TM, total sperm motility; V, sperm volume; VIT, vitality.

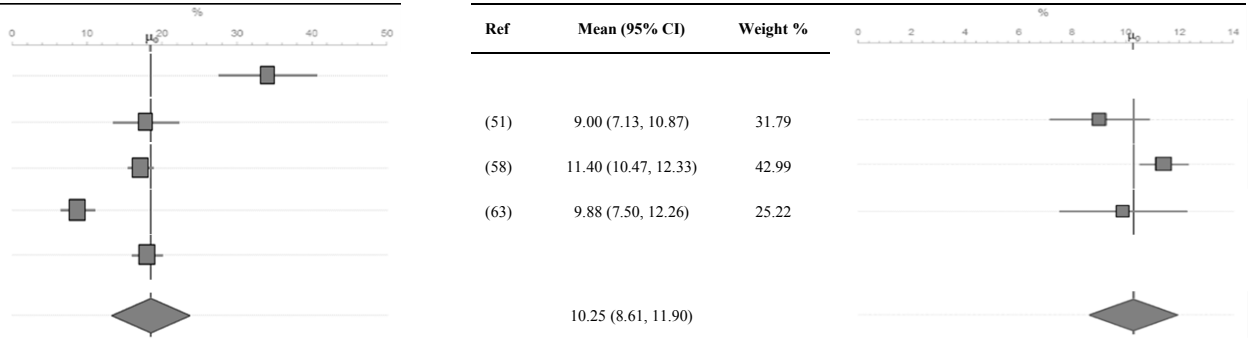
Effects on sperm chromatin structure

Sperm chromatin is a highly complex but organized structure. The routine analysis of semen does not detect defects in this structure. However, the normal sperm chromatin structure is important for sperm fertilizing ability as any form of sperm DNA damage may result in male infertility (71). Moreover, whether sperm DNA damage is augmented in men with cancer remains controversial (72, 73).

To determine the influence of TC on the risk of the sperm genetic damage, were included in this meta-analysis 5 studies that assessed SCS on patients with TC before treatment (n=379) and 3 studies that assessed SCS in men without TC (n=312). The results of the present meta-analysis indicate that SCS is statistically significantly higher ($p = 0.0032$) among men who suffer from TC (Mean: 18.46, 95% CI: 13.27, 23.64). This effect is observed among studies that involved patients identified only with TC without discriminating among seminoma and nonseminoma (Table 3).

Table 3. Sperm chromatin structure in testicular cancer patients.

Patients			Controls		
Ref	**Mean (95% CI)**	**Weight %**	**Ref**	**Mean (95% CI)**	**Weight %**
(46)	34.04 (27.44, 40.64)	16.57			
(51)	17.80 (13.36, 22.24)	19.20	(51)	9.00 (7.13, 10.87)	31.79
(58)	17.10 (15.38, 18.82)	21.61	(58)	11.40 (10.47, 12.33)	42.99
(63)	8.73 (6.41, 11.05)	21.25	(63)	9.88 (7.50, 12.26)	25.22
(67)	18.00 (15.90, 20.10)	21.37			
	18.46 (13.27, 23.64)			10.25 (8.61, 11.90)	
	Q: 77.03; I^2: 94.81%; T: 5.62			Q: 6.26; I^2: 68.04%; T: 1.19	



Effects on reproductive hormonal characteristics of patients

Normal spermatogenesis depends on appropriate hormonal secretion of FSH and LH by the pituitary, and of testosterone by the testis (74). These are key hormones in male fertility health as FSH will influence sperm production, LH will affect the production of testosterone, which in turn will act on the Sertoli cell and stimulate spermatogenesis.

To determine the influence of TC on the regulation of reproductive hormones, 5 studies were included that assessed FSH (n = 432), LH (n = 425) and TT (n = 425) on patients with TC before treatment; 3 studies that assessed FSH, LH and TT (n = 154 for each hormone) on patients presenting seminoma before treatment; 3 studies that assessed FSH, LH and TT (n = 198 for each hormone) on patients presenting nonseminoma before treatment; 3 studies that determined the difference between seminoma (n = 154) and nonseminoma (n = 198) for FSH, LH and TT before treatment; 5 studies that assessed FSH (n = 778) and LH (n = 1700), and 4 studies that assessed TT (n = 1673) on patients after treatment (> 12 months); and 2 studies that assessed FSH for 24 months. We observed that while FSH ($p = 0.042$) and LH ($p < 0.0001$) are statistically significantly increased after treatment, TT ($p = 0.0346$) is statistically significantly decreased. For TT, when differences are determined in relation to controls no difference is noted ($p = 0.1492$). When the TC is discriminated between seminoma and nonseminona, it is noticed that, before treatment, non-seminoma patients feature significantly lower FSH values ($p < 0.0001$) than seminoma patients. No statistical difference between seminoma and nonseminoma patients is observed when LH values are analysed. In relation to TT, it is significantly higher ($p = 0.0057$) in nonseminoma. Moreover, when prospective studies were analysed it is observed an increase of FSH up to 6 months after treatment, and that after this period and up to 24 months it remains stable (Table 4).

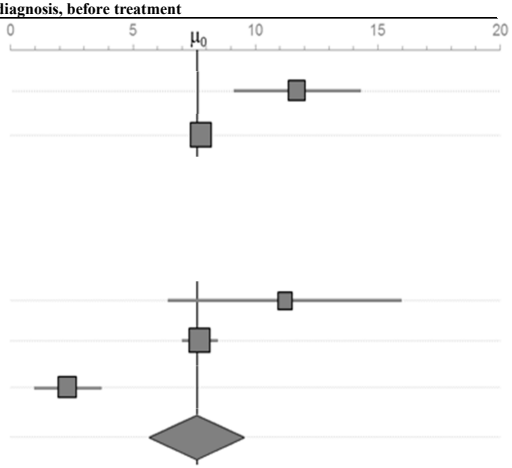
Table 4. Hormonal profile in testicular cancer patients.

FSH IU/L

At diagnosis, before treatment

Ref.	Mean (95% CI)	Weight %
(32)	11.70 (9.10, 14.30)	17.48
(33)	7.79 (7.53, 8.05)	25.00
(57)	11.20 (6.42, 15.98)	10.85
(61)	7.73 (7.00, 8.46)	24.25
(65)	2.34 (0.96, 3.72)	22.4
	7.61 (5.67, 9.54)	

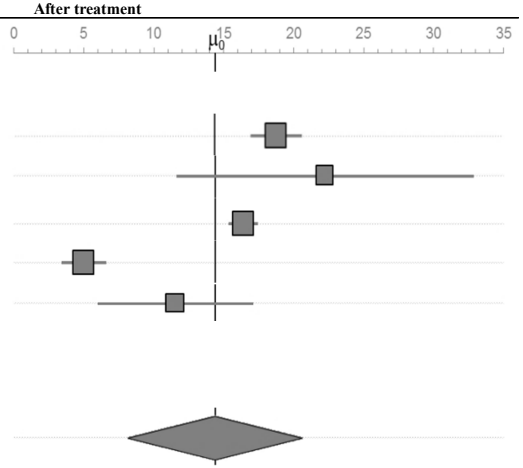
Q:74.35; I^2: 94.61%; T: 1.97



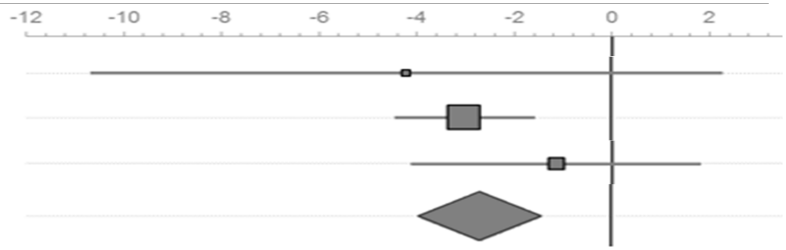
After treatment

Ref.	Mean (95% CI)	Weight %
(33)	18.80 (16.97, 20.63)	21.89
(41)	22.25 (11.62, 32.88)	14.96
(54)	16.40 (15.36, 17.44)	22.18
(56)	5.00 (3.38, 6.62)	22.01
(61)	11.53 (5.98, 17.09)	19.00
	14.37 (8.16, 20.57)	

Q:181.31; I^2: 97.79%; T: 6.70



Ref.	Seminoma Mean (95% CI)	Seminoma Weight %	Non-seminoma Mean (95% CI)	Non-seminoma Weight %	Difference Mean (95% CI)	Difference Weight %
(32)	14.60 (9.50, 19.70)	28.01	10.40 (7.49, 13.31)	30.62	-4.20 (-10.70, 2.30)	3.92
(61)	9.50 (8.51, 10.49)	37.09	6.50 (5.51, 7.49)	35.57	-3.00 (-4.44, -1.56)	76.91
(65)	3.28 (0.73, 5.83)	34.89	2.13 (0.27, 3.99)	33.81	-1.15 (-4.12, 1.82)	19.18
	8.76 (3.62, 13.89)		6.22 (2.42, 10.02)		-2.69 (-3.95, -1.44)	
	Q:28.84; I^2: 93.07%; T: 4.27		Q:29.50; I^2: 93.22%; T: 3.21		Q:1.51; I^2: 0.00%; T: 0	



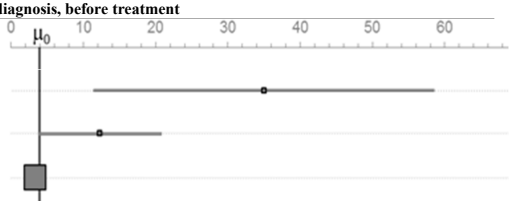
Time after treatment

Ref.	0-6 months Mean (95% CI)	0-6 months Weight %	0-12 months Mean (95% CI)	0-12 months Weight %	6-12 months Mean (95% CI)	6-12 months Weight %	0-24 months Mean (95% CI)	0-24 months Weight %	12-24 months Mean (95% CI)	12-24 months Weight %
(33)	16.61 (15.96, 17.27)	53.60	12.71 (12.04, 13.38)	53.30	-3.90 (-4.86, -2.94)	94.30	9.21 (8.56, 9.86)	76.20	-3.50 (-4.46, -2.54)	95.80
(61)	10.83 (7.72, 13.94)	46.40	7.94 (5.43, 10.45)	46.70	-2.88 (-6.75, 0.98)	5.70	6.51 (2.62, 10.40)	23.80	-1.43 (-5.94, 3.08)	4.20
	13.93 (8.27, 19.58)		10.49 (5.82, 15.15)		-3.84 (-4.76, -2.93)		8.57 (6.31, 10.82)		-3.41 (-4.34, -2.49)	
	Q:12.80; I^2: 92.19%; T: 3.93				Q: 0.25; I^2: 0.00%; T: 0		Q: 1.81; I^2: 44.71%; T: 1.28		Q: 0.78; I^2: 0.00%; T: 0	

LH UI/L

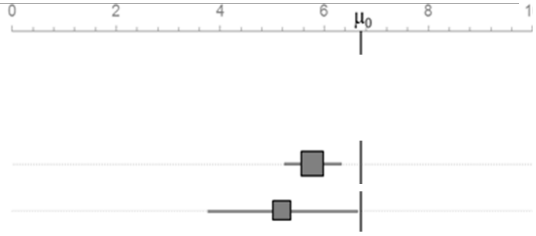
At diagnosis, before treatment

Ref.	Mean (95% CI)	Weight %
(29)	35.00 (11.39, 58.61)	0.13
(32)	12.30 (3.71, 20.89)	0.74
(33)	3.45 (3.35, 3.55)	43.99

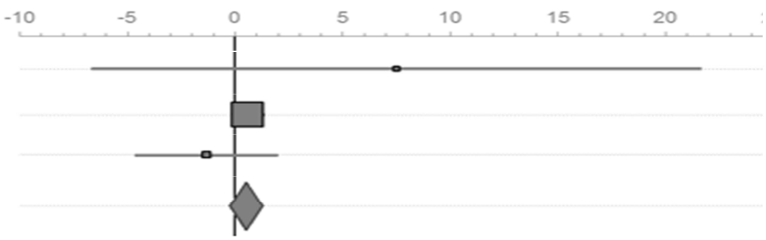


After treatment

Ref.	Mean (95% CI)	Weight %
(33)	5.78 (5.23, 6.33)	24.05
(38)	5.20 (3.75, 6.65)	15.28

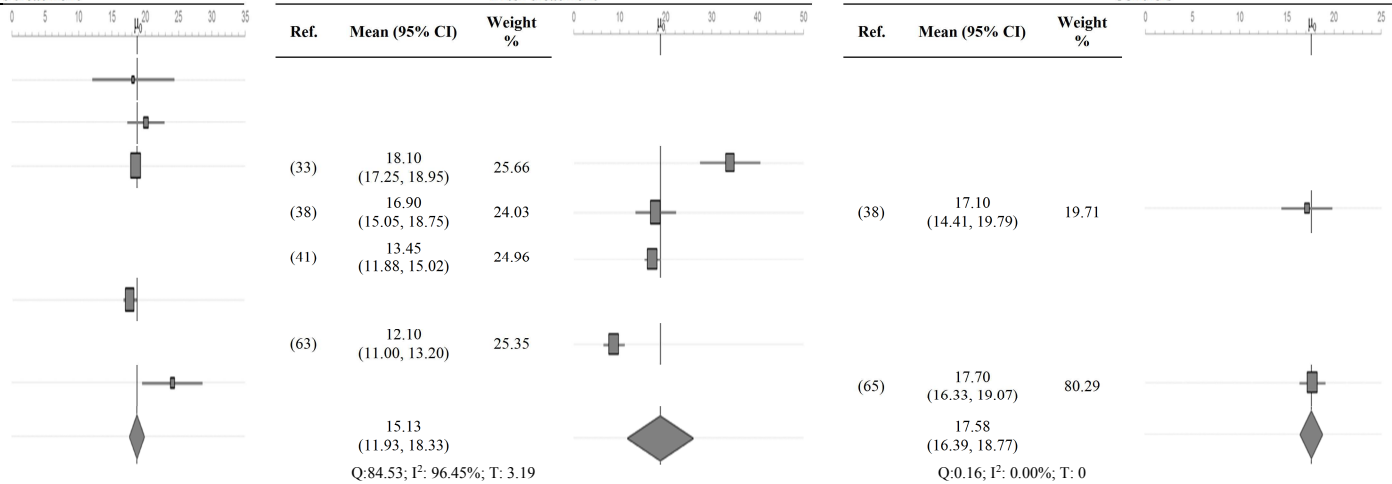


	Seminoma		Nonseminoma		Difference	
Ref.	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %
(32)	6.70 (5.27, 8.13)	33.78	14.2 (-3.48, 31.88)	0.65	7.50 (-6.68, 21.68)	0.88
(61)	3.40 (3.00, 3.80)	36.83	4.00 (3.41, 4.59)	72.98	0.60 (-0.18, 1.38)	84.61
(65)	6.70 (4.25, 9.15)	29.38	5.38 (3.07, 7.69)	26.38	-1.32 (-4.65, 2.01)	14.51
	5.48 (2.83, 8.14)		4.43 (3.09, 5.77)		0.38 (-0.92, 1.69)	
	Q:27.50; I²: 92.73%; T: 2.22		Q:2.86; I²: 29.96%; T: 0.74		Q: 2.27; I²: 11.97%; T: 0.61	

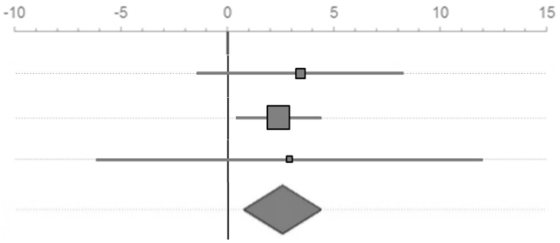


TT nmol/L

	At diagnosis, before treatment		After treatment		Controls	
Ref.	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %
(29)	18.20 (12.05, 24.35)	4.09				
(32)	20.10 (17.30, 22.90)	12.37				
(33)	18.60 (18.26, 18.94)	43.95	18.10 (17.25, 18.95)	25.66		
(38)			16.90 (15.05, 18.75)	24.03	17.10 (14.41, 19.79)	19.71
(41)			13.45 (11.88, 15.02)	24.96		
(61)	17.72 (16.72, 18.71)	33.77				
(63)			12.10 (11.00, 13.20)	25.35		
(65)	24.10 (19.57, 28.63)	5.8			17.70 (16.33, 19.07)	80.29
	18.79 (17.66, 19.92)		15.13 (11.93, 18.33)		17.58 (16.39, 18.77)	
	Q:10.19; I²: 60.76%; T: 0.85		Q:84.53; I²: 96.45%; T: 3.19		Q:0.16; I²: 0.00%; T: 0	



Ref.	Seminoma		Nonseminoma		Difference	
	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %	Mean (95% CI)	Weight %
(32)	19.10 (16.25, 21.96)	33.33	22.5 (18.13, 26.87)	32.17	3.40 (-1.46, 8.26)	14.47
(61)	16.30 (14.91, 17.68)	39.96	18.70 (17.32, 20.08)	47.74	2.40 (0.39, 4.41)	81.28
(65)	22.50 (18.13, 26.88)	26.71	25.40 (18.31, 32.49)	20.09	2.90 (-6.18, 11.98)	4.25
	18.89 (15.52, 22.26)		21.27 (17.41, 25.12)		2.57 (0.76, 4.37)	
	Q:10.19; I^2: 80.37%; T: 2.63		Q:10.19; I^2: 80.37%; T: 2.63		Q: 0.15; I^2: 0.00%; T: 0	



FSH, follicle stimulating hormone; LH – luteinizing hormone; TT – total testosterone.

Impact of radiotherapy and chemotherapy on fertility

Both chemo- and radiotherapy can be adopted to treat TC patients, and presently Bleomycin, Etoposide and Cisplatin (BEP) regimen is the most commonly used (2). We were able to conduct an evaluation of the evolution of four seminal parameters (V, SC, TSC and PM) over follow-up by treatment. In terms of V, there are some significant variations, with radio having a lower trend, but they are no statistically significant. Regarding SC, both treatments, radiotherapy ($p = 0.0004$) and BEP ($p < 0.0001$), significantly reduce SC up until 12 months. 24 months after treatment, while radiotherapy patients return to get diagnostic levels of SC, the BEP patients present a statistically significant higher SC ($p = 0.0004$). There were no statistically significant fluctuations between the reductions of SC among the two treatments. In terms of TSC the picture is different from the SC. Radiotherapy and BEP present a similar effect trend over TSC up to 12 months. Though, radiotherapy treated patients reacquire TSC levels at diagnostic only at 24 months while BEP treated patients at 12 months. At 24 months, TSC in BEP treated patients is higher than at diagnosis ($p=0.0014$). PM is decreased in the first 6 months after treatment for both radiotherapy ($p<0.0001$) and BEP ($p<0.0001$) and returns to diagnostic levels at 12 months. The initial decrease of PM is steeper for BEP than for radiotherapy ($p = 0.0032$).

Table 5. Semen parameters evaluated for testicular cancer patients with BEP regimen or radiotherapy (RT).

		References	0-3 months		0-6 months		0-12 months		0-24 months	
V			**Mean (95% CI)**	**Weight %**	**Mean (95% CI)**	**Weight %**	**Mean (95% CI)**	**Weight %**	**Mean (95% CI)**	**Weight %**
mL	BEP	(44)	0.50 (-0.25, 1.25)	42.99	0.40 (-0.37, 1.17)	39.47	0.10 (-0.51, 0.71)	51.87	0.10 (-1.30, -0.30)	48.73
		(58)	-0.16 (-0.73, 0.40)	57.01	0.20 (-0.42, 0.82)	60.53	-0.09 (-0.73, 0.54)	48.13	0.17 (-0.64, 0.66)	51.27
			0.12 (-0.52, 0.76)		0.28 (-0.20, 0.75)		0.01 (-0.43, 0.44)		0.14 (-0.30, 0.58)	
			Q: 1.95, I^2: 48.68%, T: 0.33		Q: 0.17, I^2: 0.00%, T: 0		Q: 0.19, I^2: 0.00%, T: 0		Q: 0.03, I^2: 0.00%, T: 0	
	RT	(44)	-0.40 (-0.97, 0.17)	52.92	-0.40 (-1.08, 0.28)	43.84	-0.30 (-0.83, 0.23)	54.86	-0.80 (-1.30, -0.30)	53.15
		(58)	-0.26 (-0.86, 0.34)	47.08	-0.15 (-0.75, 0.45)	56.06	0.40 (-0.25, 1.05)	46.14	0.01 (-0.64, 0.66)	46.85
			-0.33 (-0.74, 0.07)		-0.26 (-0.71, 0.19)		0.02 (-0.66, 0.71)		-0.42 (-1.21, 0.37)	
			Q: 0.11 ,I^2: 0.00%, T: 0		Q: 0.30, I^2: 0.00%, T: 0		Q: 2.72, I^2: 63.29%, T: 0.39		Q: 3.82, I^2: 73.81%, T: 0.49	
SC	BEP	(44)	-24.20 (-33.61, -14.79)	34.70	-19.70 (-30.23, -9.17)	29.79	-4.30 (-12.82, 4.22)	35.44	25.00 (11.98, 38.02)	35.80
10^6/mL		(58)	-24.23 (-31.08, -17.38)	65.30	-18.85 (-26.72, -10.97)	53.01	-10.60 (-20.09, -1.10)	32.13	5.75 (-4.43, 15.92)	41.21
		(61)			-13.60 (-27.33, 0.13)	17.19	-17.60 (-26.93, -8.27)	32.43	9.70 (-11.34, 30.74)	22.98
			-24.22 (-29.69, -18.75)		-18.20 (-23.87, -12.53)		-10.64 (-18.30, -2.98)		13.55 (0.30, 26.8)	
			Q: 0.00, I^2: 0.00%, T: 0		Q: 0.54, I^2: 0.00%, T: 0		Q: 4.32, I^2: 53.69%, T: 4.96		Q: 5.43, I^2: 63.17%, T: 9.2	
	RT	(30)	-34.10 (-93.76, 25.56)	1.98	-41.10 (-98.44, 16.24)	2.71	49.68 (-61.77, 161.12)	0.48		
		(44)	-24.90 (-35.81, -13.99)	46.73	-26.50 (-38.82, -14.18)	34.93	-14.00 (-23.83, -4.17)	48.30	8.50 (-3.12, 20.12)	49.63
		(58)	-25.34 (-35.76, -14.92)	51.29	-24.35 (-36.01, -12.69)	37.87	-14.47 (-25.68, -3.26)	37.31	0.88 (-12.96, -14.72)	35.12
		(61)			-9.80 (-25.22, 5.62)	24.48	-2.20 (-20.47, 16.07)	13.92	8.00 (-12.90, 28.90)	15.25
			-25.31 (-32.7, -17.92)		-21.99 (-30.39, -13.60)		-12.23 (-19.01, -5.45)		5.75(-2.37, 13.86)	
			Q: 0.11, I^2: 0.00%, T: 0		Q: 3.66, I^2: 18.07%, T: 3.71		Q: 2.99, I^2: 0.00%, T: 0		Q: 0.75, I^2: 0.00%, T: 0	
TSC	BEP	(44)	-72.70 (-104.1, -41.30)	46.73	-60.00 (-94.97, -25.03)	49.52	-13.90 (-42.21, -14.41)	55.85	63.20 (26.85, 99.55)	55.07
10^6		(58)	-88.29 (-117.66, -58.93)	53.27	-64.80 (-99.35, -30.25)	50.48	-44.61 (-79.78, -9.44)	44.15	24.02 (-19.97, 68.00)	44.93
			-81.01 (-102.20, -59.82)		-62.42 (-86.70, -38.14)		-27.46 (-57.35, 2.43)		45.60 (7.39, 83.80)	
			Q: 0.52, I^2: 0.00%, T: 0		Q: 0.04, I^2: 0.00%, T: 0		Q: 1.82, I^2: 45.02%, T: 14.6		Q: 1.86, I^2: 46.12%, T: 18.80	
	RT	(44)	-85.90 (-121.16, 50.64)	64.38	-90.80 (-130.52, -51.08)	66.31	-48.90 (-81.59, -16.21)	71.33	-4.70 (-38.59, 29.19)	76.53
		(58)	-94.01 (-141.46, -46.56)	35.62	-87.45 (-143.17, -31.73)	33.69	-50.87 (-102.54, 0.80)	28.67	0.13 (-61.22, 61.48)	23.47
			-88.79 (-116.83, -60.75)		-89.67 (-121.70, -57.64)		-49.46 (-76.86, -22.07)		-3.57 (-32.97, 25.83)	
			Q: 0.07, I^2: 0.00%, T: 0		Q: Q: 0.01, I^2: 0.00%, T: 0		Q: 0.00, I^2: 0.00%, T: 0		Q: 0.02, I^2: 0.00%, T: 0	
PM	BEP	(44)	-19.80 (-27.17, -12.43)	45.45	-17.10 (-25.50, -8.70)	37.54	0.80 (-5.66, 7.26)	50.01	9.80 (3.01, 16.59)	50.52
%		(58)	-22.46 (-29.18, -15.75)	54.55	-11.60 (-17.97, -5.23)	62.46	-6.68 (-13.14, -0.21)	49.99	0.78 (-5.32, 6.88)	49.48
			-21.25 (-26.16, -16.35)		-13.66 (-18.89, -8.44)		-2.94 (-10.27, 4.39)		5.16 (-3.67, 14.00)	
			Q: 0.28, I^2: 0.00%, T: 0		Q: Q: 1.07, I^2: 6.85%, T: 1.02		Q: 2.63, I^2: 62.01%, T: 4.16		Q: 6.02, I^2: 74.02%, T: 5.49	
	Radio	(44)	-15.40 (-21.30, -9.50)	48.90	-17.30 (-24.12, -10.48)	46.95	-4.70 (-9.98, 0.58)	51.68	6.40 (1.34, 11.46)	50.52
		(58)	-8.11 (-13.61, -2.61)	51.10	-10.28 (-16.17, -4.39)	53.05	-1.08 (-6.55, 4.39)	48.32	-2.77 (-8.17, 2.63)	49.48
			-11.67 (-18.82, -4.53)		-13.58 (-20.44, -6.71)		-2.95 (-6.72, 0.81)		1.86 (-7.12, 10.85)	
			Q: 3.20, I^2: 68.74%, T: 4.27		Q: 2.38, I^2: 57.97%, T: 3.78		Q: 0.89, I^2: 0.00%, T: 0		Q: 6.02, I^2: 83.38%, T: 5. 92	

BEP, bleomycin + etoposide + cisplatin regimen; PM, progressive motility; SC, sperm count; TSC, total sperm count; V, sperm volume.

DISCUSSION

The main goal of cancer treatment is to reduce treatment-related long-term toxicities, in which sub/infertility may be included. Concerning male patients, sperm banking (75) or testicular tissue cryopreservation in the case of prepubertal boys (76) should be offered prior the initiation of the treatment. In the case of TC, because the organ in question, the preservation of fertility becomes a prominent concern. Accordingly to the European Association of Urology testis cancer guidelines, among the tests for staging at diagnosis and follow-up schedules after treatment recommended are fertility investigations, which include FSH, LH, TT and semen analyses (77). We have, therefore, evaluated in the present meta-analysis these outcomes along with sperm genomic integrity among men with TC both at diagnosis and after treatment.

Regarding the age of patients, our results demonstrate that TC affects young males, and are in agreement with the peaks of incidence at 25 (26.32 ± 5.32) and 35 (30.17 ± 5.70) for nonseminoma and seminoma tumors, respectively (78). An increase in average of paternal age is a direct consequence of human reproductive behavior (79). Although the effect of male age is less prominent than of the female, our results suggest that TC patients' age becomes significant since TC diagnostic is at their fertility potential peak (80), patients have to delay their fatherhood planning, and as TC survivors present reduced paternity (81).

Standard seminal parameters are routinely used as an indirect measure of male fertility (82). A decrease trend in TC semen characteristics is apparent comparing to matched controls, though these are above the WHO lower reference limits. Since no alterations are observed in TC's V, this may indicate that reproductive accessory glands are not affected. In terms of motility, we found that TM was the only parameter affected beyond the WHO lower reference limit after the treatment, and in seminoma patients. Strangely, TM is inferior to PM. Another, peculiar finding is a substantial increase of TSC after treatment in relation to SC. These results may be attributed to measurement errors, a smaller sample studied in TSC after treatment that

may increase bias or to increased volumes in these specific samples. These results are contradictory to the hormonal results as it would be expected that the semen parameters were most affected. However, when an analysis based on the two histopathological types of TC is performed, the aggressiveness of the nonseminoma type is reflected both by a decrease on FSH, SC, TSC, PM and SM, and on the other hand an increase on LH and TT. Contradictory results may mirror the urgency to adopt a standardized protocol to measure semen parameters. This urgency is alarming on the determination of morphology. We have had, indeed, the need to separate studies using strict criteria from those using non-strict criteria. Even tough, the variability between studies is a huge obstacle that we were not able to disclose if this is due to observer variability or in the absence of this it appears that there are other factors affecting sperm morphology rather than TC per se or its treatment. Both CT and RT pose a high risk of decreased patients' reproductive potential, still conflicting results about which treatment is more detrimental to this potential remain. We have found that patients receiving BEP regimen may expect a recovery of semen parameters to values higher than the ones determined at diagnosis after 24 months of treatment, while the ones subjected to radiotherapy may only expect similar results. Nevertheless, according to the European Association of Urology testis cancer guidelines follow-up should at least be ensued once a year up to 5-10 after treatment (77). Besides, it would be of importance to determine if there are differences between patients already presenting azoospermia at diagnosis from the ones that are not, and the ones that progress to it.

Endocrine system is a crucial regulator of organ system functions, and the testes are no exception. For long that is recognised that FSH, LH and testosterone regulate spermatogenesis. Previous studies indicated that alterations of serum levels of these hormones occur in a significant proportion of TC patients at diagnosis and after treatment, irrespective of the histological type or oncological treatment strategies adopted (83). Serum hormone levels were found to be within the normal range for the majority of patients analysed. Our results agree with

those that have already suggested that an elevation of LH concentration and a decrease of testosterone concentration are indicative of disturbed Leydig cell function. Moreover, the elevated FSH concentrations observed after treatment (almost the double of higher limit of the normal range) may also be suggestive of a disturbance of spermatogenesis. This disturbance appears to be increased in the first 6 months after treatment and for a period of two years no recovery seems to happen. These findings seem to be more expressive for nonseminoma patients in the case of LH and TT, and for seminoma ones in the case of FSH. Our results support the knowledge that patients with TC have abnormal spermatogenesis, which deteriorates even further after treatment. However, recent research has demonstrated that FSH, LH and testosterone are not the only hormones regulating spermatogenesis. Therefore, other hormones should be measured in future prospective studies as they may be better markers for testicular injury. For instance, hormones that reflect the Sertoli cell function, a key player in spermatogenesis, such as AMH (Anti-Mullerian Hormone, already used to predict cryptorchism a known risk factor for TC, (84)), and thyroid hormones (triiodothyronine, T3 and thyroxin, T4 (85)). Likewise, hormones that regulate the activity of FSH, LH or testosterone should also be studied. For example, inhibin B which controls FSH secretion via a negative feedback (86), is of utmost importance. Although undetectable inhibin B levels have been associated with an absence of spermatogenic activity (87), Di Bisceglie and colleagues did not found alterations on inhibin B levels during two years after treatment, though levels were described to be lower in patients treated by CT than by RT (61). Other hormones that may also be studied are prolactin which regulates testosterone synthesis on Leydig cells (88), estradiol which regulates both the activity of LH and testosterone (89), and β-human Chorionic Gonadotrophin (βhCG) which pathological levels were correlated with abnormalities in semen quality of TC patients (37). Tovar-Rodriguez and colleagues have also demonstrated that these two latter hormones are increased in TC

patients comparing to controls, with nonseminoma presenting increased levels compared with the seminoma cases (65).

Delaying fatherhood is a preoccupation of TC patients, but the impact of their condition and treatment that they will be subjected to in the health and well-being of their offspring is perhaps more important (90). Several functional tests are being used to measure sperm DNA integrity in these patients, yet in the present analysis only studies that evaluated the SCS were eligible (46, 51, 58, 63, 67). A progressive decrease of sperm genomic integrity was observed from sperm donors to TC patients. We were unable to study the difference among histopathological types of TC and among different treatments, specifically CT and RT. Regarding the difference between the two TC histological types, only Paoli and colleagues have studied it. They found no differences in pre-treatment, but uncovered a significant increase in impaired SCS after 3-6 months of treatment with 3-4 CT cycles which was dependent on the pathological and clinical stage (67). Concerning the impact of treatment option, results are conflicting with few observing that chemotherapy is more injurious to the sperm DNA than radiotherapy, and others finding opposite outcomes. Nevertheless, both CT and RT can induce other genome alterations like aneuploidy (91), epi- (92) and structural (93) genetic alteration (94, 95), spermatozoa methylation patterns (96), among others. Although, studies of children of TC survivors show no evidence of more frequent abnormalities in offspring (97, 98), we suggest counselling of patients about probable risks. Indeed, sperm DNA integrity has already been correlated to semen parameters and proved to be reduced either with cell sorting (99) and sequential sperm preparation procedures (100), still the mechanism behind it remains unclear. A standardized method to determine sperm DNA integrity is also lacking and further detailed studies of offspring from patients with cancer are necessary. Additionally, literature regarding follow-up is scarce and were not eligible for this study, though no comparisons to post-treatment

patients' sperm genomic integrity were ensued. We thus recommend future prospective studies. Meanwhile, patients should be advised to preserve their fertility prior the initiation of treatment.

In summary, our results point towards a decrease of reproductive potential of TC patients which seems to improve on the long run. Nevertheless, the inability to define thresholds for TC patients or to find correlations between the parameters evaluated are limitations of this study. These limitations arise essentially from the divergences in the previous published studies. Incongruities may directly be related to differences in a) characteristics of patients studied (number, age, time of abstinence before ejaculate collection, discrimination between patients accordingly to their histological type of TC or stage of disease, among others (in)direct-influenceable); b) antineoplastic treatment adopted and dose; c) parameters evaluated, and criteria and methods adopted to assess it (for example cryopreserved samples display abnormal parameters (62, 101, 102); d) variances of the follow-up protocols and time of evaluation; and e) the selection bias of the controls (it is debatable even in ART programs if comparisons should be done against proven fertile patients or population-based controls of age-matched).

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