



Semen quality from patients affected by seminomatous and non-seminomatous testicular tumor

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ABSTRACT

Testicular cancer is considered a rare disease affecting approximately 1% to 2% of the male population. This neoplasm has a cure rate of over 95%; as a result, a major concern is the future of fertility of carriers from this disease. There are several histological subtypes of testicular tumors; however, the Testicular Germ Cell Tumors (TGCTs), comprising both seminoma and non-seminoma tumors, are considered the main subtypes of testicular neoplasms. TGCT are characterized by being a solid tumor that mostly affects young men aged between 15 and 40 years old. While TGCT subtypes may have an invasive potential, seminoma subtype does not affect other cells rather than germ cells, while non-seminomas have more invasive properties and can achieve somatic cells; thus, having a more aggressive nature. This research intends to review the literature regarding information about sperm parameters, correlating the data found in those studies to the subfertility and infertility of patients with TCGTs. Furthermore, it will also correlate the data to the non-seminoma and seminoma histological subtypes from pre- and post-cancer therapy. PubMed databases were used. Searched keywords included: seminoma AND non-seminoma; male infertility; germ cell tumor; chemotherapy AND radiotherapy. Only articles published in English were considered. Current studies demonstrate that both TGCT subtypes promote deleterious effects on semen quality resulting in decreased sperm concentration, declined sperm total motility and an increase in the morphology alterations. However, findings suggest that the non-seminoma subtype effects are more pronounced and deleterious. More studies will be necessary to clarify the behavior of seminoma and non-seminoma tumors implicating the reproductive health of male patients.

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INTRODUCTION

Testicular cancer is considered a rare disease that affects approximately 1% to 2% of the male population (1). This neoplasm has a cure rate of over 95%, as a result, a major concern is the future of fertility of carriers from this disease. Furthermore, there is a growing body of evidence that

testicular tumor and infertility are deeply related, with infertile men having a higher chance of also presenting testicular cancer (2, 3).

There are several histological subtypes of tumors which may affect the testis including: germ cell neoplasia in situ (GCNIS), previously known as carcinoma *in situ*, stromal tumors (Leydig cell and Sertoli cell tumors), and testicu-

lar germ cell tumors (TGCTs), with seminoma and non-seminoma tumors (Figure-1) considered the main subtypes of testicular neoplasms (3). Importantly, both are derived from a common precursor, the in situ germ cell neoplasia of the testis (4, 5).

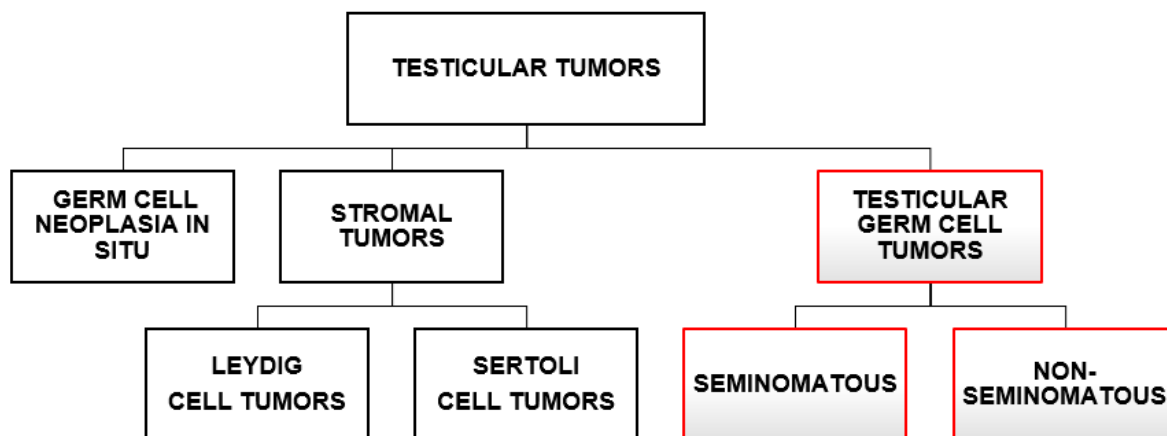
TGCT is characterized by a solid tumor that mostly affects young men aged between 15 and 40 years old. The worldwide incidence of this disease is 1.5 cases per 100.000 men; however, it can differ between countries and breeds, presenting a higher incidence in Scandinavian countries, Switzerland and Germany; yet, in African countries, Asians and Latin populations, it has demonstrated a very low incidence (3, 6, 7).

Although the exact cause of its occurrence is not yet defined, some elements are known as associated risk factors, which are: cryptorchidism (3), infertility, contralateral tumor presence and genetic factors (8). Among the genetic causes, it has already been described that 80% of the TGCTs are featured by an addition in the short arm of 12p chromosome as a isochromosome i (9). Thus, the insertion in the 12p chromosome leads to a relevant functional alteration, promoting the stem cell maintenance and proliferation by the key genes activations, as: POU5F1/OCT3/4, SOX2 e NANOG (10). Mutations in the SRY gene are connected to the gonadal tumor development, gonadal dysgenesis and infertility, which may occur in approximately 52.5% of the individuals presenting an

alteration in this gene (11). Another studied gene is the tumor suppressor p53, which has an important role in spermatogenesis; it is know that p53 mutations leads to a genomic and chromosomal instability that can compromise sperm fecundity ability in the oocyte; further, it is related to TCGTs pathogenesis (12). Studies have shown that genetic factors along with epigenetics plays an important role in the neoplasm development (8, 13). The epigenetics regulatory processes occurs in the beginning and protection pluripotency mechanisms of embryonic stem cells and also in identity maintenance of differentiated cell types (14).

Skakkebaek in 1993 hypothesized that an increase in anomalies incidence of male reproductive function could be related to increased estrogen exposure in utero. Highlights the diethylstilbestrol (DES), a powerful synthetic estrogen indicated to pregnant women with repeat abortion history, being associated with increased risk of hypospadias, cryptorchidism and low quality sperm in the offspring (15). Chemical and xenoestrogens products that promote endocrine dysregulation may constitute a mediation factor that connects the testicular cancer and infertility; these agents are antiandrogenic and cause an increased in estrogen levels inhibiting the hypothalamus-pituitary-gonadal axis (HPG) resulting in decreased Follicle Stimulating hormone (FSH) production and affecting the amount of Sertoli Cells between the fetal

Figure 1 - Testicular tumor types.



The types of cancer that can affect the testicles are in the figure. We can divide them into two major groups: stromal tumors (Leydig cells and Sertoli cell tumors) and germ cell tumors having the germ cell neoplasia in situ (GCNIS) as a common precursor.

phase and pre pubertal phase (12).

The TGCTs initial diagnosis is performed by a doctor through physical examination, ultrasound and analysis of serum tumor marker increase, such as: alpha fetoprotein (AFP), human chorionic gonadotropin (hCG) and lactic dehydrogenase enzyme (LDH) (16, 17). The AFP is a glycoprotein with 70kDa produced by fetal yolk sac, liver and gastrointestinal tract. Higher levels of this glycoprotein are usually found in non-seminoma tumors. When hCG levels are increased, it may indicate the presence of seminoma and non-seminoma tumors and of the above markers, the LDH is the least specific; however, evidence of its association with the tumor has increased (18).

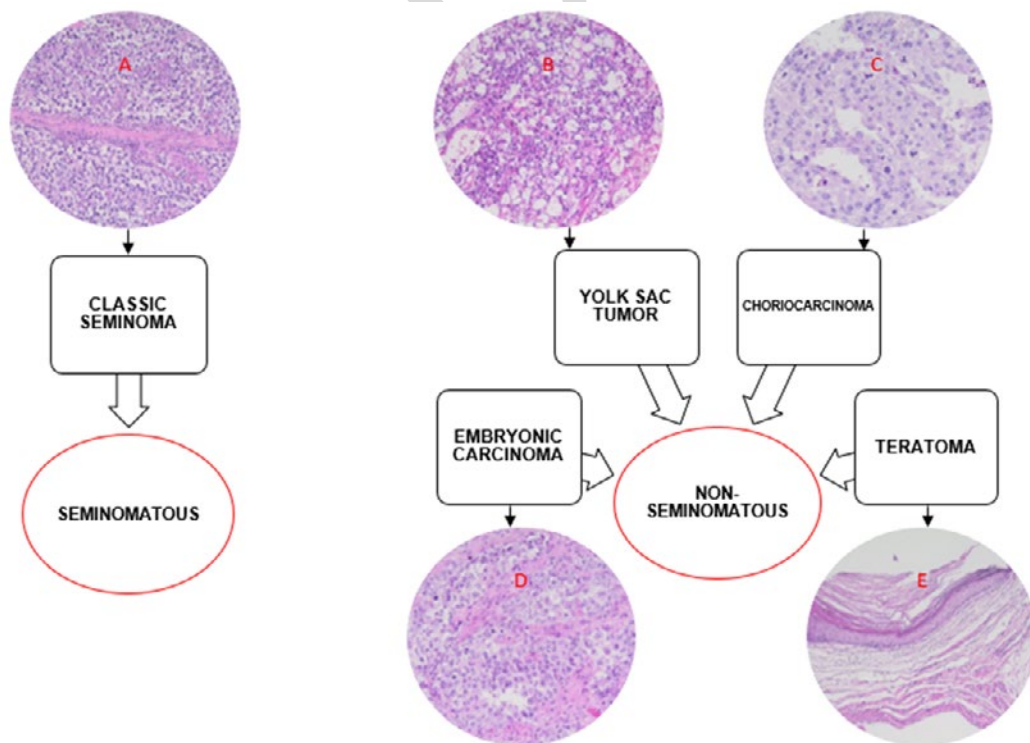
The availability of effective therapies and the discovery of sensitive tumor markers help in disease treatment, raising the healing chances up

to 97%. The treatment for TGCTs consists of: radical orchiectomy, resection of retroperitoneal lymph nodes, chemotherapy and radiotherapy (3, 19).

The subtypes of TGCT may have an invasive potential but seminoma type does not surpass spermatogenic cells. On the other hand, non-seminomas, apart from invading seminoma germ cells, are capable of targeting somatic cells, thereby presenting a more aggressive nature (8). Embryonic carcinoma, yolk sac tumor, choriocarcinoma and teratoma are part of the non-seminoma group (Figure-2) (20).

It has been reported that semen quality of patients with TGCTs is affected, especially in cases of non-seminoma tumors; however, little is known about the mechanisms leading to this alteration (21, 22). For several reasons, subfertility is common among TGCTs patients since

Figure 2 - Seminoma and non-seminoma tumors subtypes and their histological representations. The histological representation was prepared by hematoxylin and eosin (HE) method. The analysis was done under 200x magnification and the images were provided by the Department of Pathology of the São Paulo Federal University.



A) Presence of inflammatory infiltrate, consisting of uniform cells, divided into thin trabecular septa. B) Microcystic pattern; cells compressed by the presence of vacuoles. C) Presence of syncytio-trophoblast cells with multiple mitoses. D) Presence of neoplastic cells forming a solid array. E) Mature teratoma with predominance of keratinized squamous epithelium; presence of epidermoid cyst containing keratin in the lumen.

50% of them have abnormal sperm counts (23), which also emphasizes that chemotherapy and radiotherapy treatments have a negative impact on fertility (24).

Around 240 reviews were published with this topic that are extremely useful to clinical research; so, the authors are advancing gradually in the different aspects and contributing to new findings about this neoplasm. Nevertheless, it is important to investigate the differences between the histological subtypes seminoma and non-seminoma since how each one affects seminal quality is not understood yet. Therefore, this research intends to review the literature regarding information about sperm parameters, correlating the data found in those studies to the subfertility and infertility of patients with TCGTs. Furthermore, it will also correlate the data to the non-seminoma and seminoma histological subtypes from pre- and post-cancer therapy.

MATERIAL AND METHODS

To construct this narrative review, we have used the PubMed database. The inclusion criteria were: papers that evaluated spermatogenic parameters of seminoma and non-seminoma tumors patients and which made a comparative analysis between the two histological subtypes, pre and post orchiectomy and adjuvant treatment; the exclusion criteria were: papers that did not analyze the seminal parameters and which studied non-germ tumors of the testis. The keywords searched for included: seminoma; non-seminoma; male infertility; germ cell tumor and testicular neoplasms. Only papers published in English were considered.

As a search strategy we have selected four research terms that were combined with the boolean descriptor "AND", for example: "seminoma AND non-seminoma; testicular neoplasms AND infertility and sperm quality AND germ cell tumor". We started the searches in February 2019 and we did not stipulate a publication period of time due to the scarcity of relevant articles according to our inclusion criteria; also, we did not find meta-analyses with the comparison between the histological subtypes; thus, just retrospective studies, and prospective and systematic reviews were selected.

RESULTS

After putting into practice the search strategy using the terms combination, we have found 1511 related papers and through the abstracts we have selected 44 papers that were considered relevant and out of these, only 5 articles match the inclusion and exclusion criteria as described above.

TGCTs and fertility potential

Spermatogenesis is the process through which sperm is produced, so any interference in this process can impair cell division and maturation, affecting or not male fertility potential (25). TGCTs arise from a failure in the maturation of gonocytes, which are primary embryonic cells that give rise to germ cells; thus, it is essential to understand the mechanisms related to spermatogenesis to broaden information on the emergence and distinctions of this malignancy (26).

It is believed that testicular tumors promote a negative effect on the spermatogenesis due to disruptions in the blood-testicular barrier, followed by antibodies formation against the sperm and long lymphocytic infiltrates into the parenchyma adjacent to tumor germ cells; it is known that antisperm antibodies are associated with count decrease, low motility and morphological anomalies of the sperm (27).

It is essential to consider the fertile potential of these patients, since the occurrence of testicular cancer coincides in most cases with the male reproductive phase. Adjuvant treatment is often used, and some patients will become azoospermic after chemotherapy or radiotherapy (24). However, it was also reported that even before treatment, some patients already had poor semen quality, which leads us to believe that the presence of the tumor interferes with spermatogenesis. Indeed, half of the patients in this study revealed oligozoospermia or azoospermia, as described by Ping et al. (28). A possible explanation to spermatogenesis decline in the ipsilateral and contralateral testicle, before the tumor treatment, is not well established. Some causes indicate the inclusion of paracrine and autocrine factors produced by the tumor, cryptorchidism or antispermatic antibodies

(29). Besides that, several studies have already demonstrated that the semen quality of the patients with TCGT tends to be damaged in relation to men considered normozoospermic by the WHO (2010) (30).

The adjuvant treatment utilization is common; thus, a high rate of patients will become azoospermic (10 to 24%) (31). Andrade et al. (2019) investigated the semen quality of patients before and after orchiectomy, and in both groups, there was a decrease in ejaculated volume (54.2%) and sperm concentration (50.0%), motility (50.0%) and morphology (20.8%), but it is necessary to consider the low sample size used (total of 24 men: 15 with seminoma tumor and 9 with non-seminoma tumor) (32).

It is important to note that most carriers of this disease are not aware that adjuvant treatment for testicular cancer may lead to persistent azoospermia. Therefore, it is recommended that these patients receive a correct medical advice in order to preserve their fertility for a future parenthood (31). Thus, patients who wants to attain parenthood should be advised to adhere to semen cryopreservation for its later use in assisted reproduction techniques (32).

One study showed that when the tumor affects more than 50% of the testis, the probability of these patients to present a normal spermatogenesis in the affected testis is less than 50%. However, when sperm production occurs, it is located far from the neoplasia (33). Despite the relevance of these tumors in human reproduction, no studies were performed to analyze testicular tumor effects on semen quality, considering the seminomatous and non-seminomatous subtypes separately.

Seminoma tumor

Considered a malignant germ cell tumor, developed from primordial germ cells (gonocytes), this neoplasia corresponds to about 50% of all TGCTs (3). It tends to grow and spread slowly when compared to the non-seminoma subtype (34). This tumor subtype affects the HPG; thus, disturbing spermatogenesis and promoting and worsening of the patient's fertile potential (35).

Dias et al. (2019) evaluated the semen of seminoma tumor patients and control individuals

(fertile volunteers); 15 samples per group were included. To seminoma group, the analysis was performed before the adjuvant treatment. Their results for semen volume were nearly identical between groups. On the other hand, a decrease in motility ($P=0.019$), sperm concentration ($P=0.003$), total sperm count ($P=0.001$), and total motility ($P=0.001$) was observed in patients with seminoma. However, both groups are still considered normozoospermic, according to WHO 2010 (36).

Sposito et al. (2017) evaluated semen of 52 patients, 12 with seminoma tumor, 12 with non-seminoma tumor and 26 belonging the control group (healthy men) and did not find significant differences in the parameters of sperm motility and volume. However, sperm concentration, morphology and total count showed a significant decrease compared to the control group (37).

Non-seminoma Tumor

This tumor subtype presents characteristics of embryonic and extraembryonic differentiation. It has had a worse prognosis and a higher metastatic potential in the short term compared to the seminoma subtype (38). Approximately half of the cases of TGCT are diagnosed as this subtype (39).

Fraietta et al. (2010) analyzed semen samples of 100 patients, out of these 37 samples had been detected with seminoma tumor and 63 with non-seminoma tumor, and it was observed that the values for total motile morphologically normal sperm were lower ($P=0.022$) in non-seminoma patients. Accordingly, Liguori et al. (2008) evaluated the semen quality of 30 patients in the moment pre and post orchiectomy, and found that sperm concentration was significantly lower in patients with non-seminoma tumor ($17.9 \times 10^6/\text{ml}$ and $8.16 \times 10^6/\text{ml}$) compared to the seminoma subtype ($35.47 \times 10^6/\text{ml}$ and $23.99 \times 10^6/\text{ml}$) ($P=0.004$). Therefore, non-seminoma tumors have had a negative effect on semen quality which is more pronounced than in seminoma tumors (21, 22).

Dias et al. (2019) compared semen samples of 15 healthy men with 15 cryopreserved samples from patients with non-seminoma tumors prior to cancer therapy and the results were: volume and motility were similar between groups; on the

other hand, sperm concentration and total count decreased significantly compared to the control group ($P=0.0015$ and $P=0.0084$, respectively). These findings confirm the hypothesis of sperm quality deterioration in this group of patients (40).

Table-1 shows studies that executed the semen parameters comparison between the tumor subtypes of seminoma and non-seminoma.

Treatment for TGCTs

There are evidences from the literature that after unilateral orchiectomy, sperm concentration becomes reduced (average sperm concentration: $16.6 \times 10^6/\text{mL}$), compared to the results from the pre-surgery time (average sperm concentration: $26.7 \times 10^6/\text{mL}$) (22, 29); at the same time, the resection of retroperitoneal lymph nodes promotes a dry ejaculation in virtually 100% of the patients (41). Radiation therapy and chemotherapy are considered cytotoxic

and can have a significant effect on the fertile potential. However, radiotherapy has a higher eradication rate, while chemotherapy varies according to dose and cycles (42). Nevertheless, the latter is considered the least deleterious to fertility.

Approximately 50% of testicular tumor patients tend to have an impaired semen quality at the onset of the disease (23). However, after the adjuvant treatment for cancer, the condition can aggravate and 30% of patients present a negative effect on fertility (43).

CONCLUSIONS

More studies are necessary to clarify the behavior of seminoma and non-seminoma tumors implicating the reproductive health of male patients. Current findings show that both subtypes promote deleterious effects on the sperm parameters and

Table 1 - Studies with seminal parameters analysis according the histological tumor types seminoma and nonseminoma.

Study group			Parameters									
Authors	Histological tumor subtypes	Comparator	Semen volume (ml)	Sperm motility (%)	Total motile count ($10^6/\text{mL}$)	Non-progressive motility (%)	Total motile morphologically normal sperm ($10^6/\text{mL}$)	Sperm concentration ($10^6/\text{mL}$)	Total sperm count ($10^6/\text{mL}$)	Morphology (% normal)	Round cells $10^6/\text{mL}$	Neutrophils $10^6/\text{mL}$
Dias et. al (2019) (35)	Seminoma	Control	$p=0.541$	$p=0.019 \downarrow$	$p=0.001 \downarrow$	X	X	$p=0.003 \downarrow$	$p=0.001 \downarrow$	X	X	X
Sposito et. al (2017) (36)	Seminoma/ Nonseminoma	Control	$p=0.478$	$p=0.098$	X	$p=0.340$	X	$p<0.0001 \downarrow$	$p<0.0001 \downarrow$	$p<0.0001 \downarrow$	$p=0.304$	$p=0.577$
Fraietta et. al (2010) (20)	Nonseminoma	Seminoma	$p=0.0943$	$p=0.0644$	X	X	$p=0.022 \uparrow$	$p=0.576$	X	$p=0.105$	$p=0.868$	$p=0.822$
Liguori et. al (2008) (21)	Nonseminoma	Seminoma	X	X	X	X	X	$p=0.004 \downarrow$	$p=0.001 \downarrow$	X	X	X
Dias et. al (2019) (39)	Nonseminoma	Control	$p=0.7044$	$p=0.6936$	$p=0.0136 \downarrow$	X	X	$p=0.0015 \downarrow$	$p=0.0084 \downarrow$	X	X	X

Statistically significant decrease; \uparrow Statistically significant increase; X Parameter not analyzed.

some reports show that the non-seminoma subtype may lead to more impaired semen quality. Therefore, a better understanding of the molecular and pathophysiological mechanisms of TGCTs is necessary to enable the distinct measurement of how these subtypes interfere with the patient's fertility potential.

ABBREVIATIONS

TGCT = Testicular Germ Cell Tumor
 DES = Diethylstilbestrol
 HPG = Hypothalamus-pituitary-gonadal axis
 FSH = Follicle Stimulating hormone
 AFP = Alpha fetoprotein
 hCG = Human chorionic gonadotropin
 LDH = Lactic dehydrogenase
 WHO = World Health Organization
 GCNIS = Germ cell neoplasia in situ

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CONFLICT OF INTEREST

None declared.

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