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Trabalho de Conclusão de Curso

Técnicas utilizadas para a avaliação de toxicidade em células espermáticas pelo efeito de drogas antitumorais

Daniel Dâmaso Bertoldi

Pelotas, 2016

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Trabalho de Conclusão de Curso apresentado ao curso de Bacharelado em Biotecnologia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Bacharel em Biotecnologia.

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Daniel Dâmaso Bertoldi

Review of techniques used for the evaluation of toxicity on spermatic cells due to the effect of antitumoral drugs

Trabalho de Conclusão de Curso aprovado, como requisito parcial, para obtenção do grau de Bacharel em Biotecnologia, Centro de Desenvolvimento Tecnológico, Universidade Federal de Pelotas.

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Resumo

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O Câncer é um conjunto de doenças que têm em comum o crescimento desordenado de células que invadem tecidos e órgãos podendo se espalhar para outras partes do corpo. É uma das causas de morte mais comuns globalmente, tanto em termos proporcionais quanto em termos absolutos. As drogas antitumorais são aquelas cujo efeito atua sob um dos pilares do câncer, como a propagação celular descontrolada. Um dos principais problemas apresentados pela utilização dessas drogas são os efeitos adversos onde a droga atua tanto em células tumorais quanto em grupos de células normais de proliferação rápida. Dentre estas se incluem as células espermáticas que sofrem um efeito toxicológico diferente dependendo do estágio de desenvolvimento que se encontram. A utilização de células espermáticas em diferentes estágios da espermatogênese combinado a diferentes técnicas é a maneira mais correta para a realização da avaliação dos efeitos toxicológicos nestas células. Neste trabalho, foi realizado um levantamento das principais técnicas e estágios celulares da espermatogênese utilizados para a avaliação de toxicidade a fim de elaborar um protocolo para a análise do efeito de drogas antitumorais em células espermáticas. Foram identificados 27 artigos sobre o assunto proposto após a busca nas bases de dados Pubmed, Scopus e Web of Science, sendo então elaborado um protocolo utilizando as células espermáticas em diferentes estágios da espermatogênese.

Palavras-chave: drogas antitumorais; células espermáticas; toxicidade.

Abstract

BERTOLDI, Daniel Dâmaso. **Techniques used for the evaluation of toxicity on spermatic cells due to the effect of antitumoral drugs**. 2016. Trabalho de Conclusão de Curso – Curso de Graduação em Biotecnologia. Universidade Federal de Pelotas, Pelotas, 2016.

Cancer is a group of diseases which have in common disordered cell proliferation, are invasive to the tissues and organs, and are able to spread to other body parts. It is one of the most common causes of death globally, both in relative and in absolute terms. Antitumoral drugs are those whose effect lies on targeting one of the hallmarks of cancer, such as the disordered proliferation. One of the main issues with the utilization of these drugs is the possibility of side effects, because the drug acts both on tumor cells and on groups of normal cells with high turnover rate. The spermatic cells are among those cells, and they are affected differently depending on what cell stage of the spermatogenesis they are. The utilization of spermatic cells on different stages combined with the application of different techniques is the most appropriate way to perform a toxicity evaluation on this cellular type. The idea of establishing a protocol with a group of techniques in order to evaluate de spermatic toxicity by the effect of antitumoral drugs directly as a cellular model is approached as one of the objectives of this paper. Alongside, the gathering of information about the main techniques and the cell stages of the spermatogenesis which are utilized on these toxicity evaluations are also topics to be addressed. We conducted a literature review on Pubmed, Scopus and Web of Science, identifying 27 articles for the review. The most frequently used technique was the morphological analysis. A protocol using four spermatogenesis cell stages is proposed.

Key-words: antitumoral drugs; spermatic cells; toxicity.

Lista de figuras

Lista de Tabelas

Sumário

1. Introdução

O Câncer é um conjunto de mais de cem doenças que têm em comum o crescimento desordenado de células que invadem tecidos e órgãos podendo se espalhar para outras partes do corpo formando metástases. É uma das principais causas de morte tanto em termos relativos quanto em termos absolutos, tanto no Brasil quanto globalmente. Por esse motivo, é imperativa a busca por drogas capazes de servir como tratamento, e ao mesmo tempo tendo em vista minimizar os efeitos adversos nocivos.

O objetivo deste trabalho é obter informação sobre quais são as principais técnicas usadas para a avaliação do nível de toxicidade de drogas antitumorais em células espermáticas, e propor um protocolo de avaliação de toxicidade de células espermáticas como modelo de estudo. Foi realizada uma busca sistemática em três bancos de dados: Pubmed, Scopus e Web of Science. Após a seleção de artigos, foi montada uma tabela contendo as seguintes informações: autor e ano, drogas utilizadas, células espermáticas testadas, e técnicas utilizadas. Um protocolo utilizando quatro estágios celulares da espermatogênese foi proposto.

2. Manuscrito

Este trabalho foi realizado em formato de artigo, sendo intitulado “**Techniques used for the evaluation of toxicity on spermatic cells due to the effect of antitumoral drugs”**. Foi redigido em inglês visando a publicação em periódico científico internacional.

2.1. Introduction

Cancer is characterized by the unrestrained cell proliferation, which can invade and spread to other body parts. It is one of the most common causes of non-communicable deaths globally, both in relative and in absolute terms, leading to the constant need for new and better treatments (WORLD HEALTH ORGANIZATION, 2014). Antitumoral drugs are those whose effect lies on either stopping the malignancy to spread, to cause cancer cells death, or both, by targeting one or more of the hallmarks of cancer (HANAHAN; WEINBERG, 2011).

One of the main issues with the development of new treatments is the thin line which separates the death of the intended cancer cells targeted and the toxic effect on normal cells. It is common for cell types with higher turnover rate, such as hair follicles and spermatic cells, to be affected by antitumor treatments (GOODMAN; GILMAN, 2011). Analyzing the toxicity on those cells is a proxy of how viable the treatment is, and helps figuring out the limiting dosage, to keep the balance between making the most out of the wanted effect and minimizing the collateral damage (GOODMAN; GILMAN, 2011).

The spermatic cell proliferation and differentiation is structured in stages. Firstly, parting from the primordial cell, the multiplication stage is where spermatogonia proliferate, then it differentiates into spermatocyte close to the basal portion of the seminiferous tubes in the growth stage. In the maturing stage the meiotic differentiations to spermatids takes place directing itself across the tubules towards its other extremity. Lastly, the spermiogenesis occurs resulting in the transformation and formation of the spermatozoa (RUSSELL *et al.*, 1990).

Each cell stage is not affected equally when exposed to certain drugs. In fact, it is a characteristic of toxic agents to affect them differently. Drugs such as the doxorubicin target mainly the cells in the G2 division phase intercalating itself with the DNA, affecting the cancer cells and having an effect on normal ones such as the spermatic cells (GEWIRTZ, 1999). Hydroxyurea, another antitumoral drug, presents

its effect on the ones in the S phase on dividing cells. It also has the issue with unwanted cell destruction of normal cells with higher proliferating rates (SHIN *et al.*, 1999).

The aim of this paper was to make a systematic literature review collecting information on which techniques are used for the evaluation of toxicity levels of antitumoral drugs on spermatic cells, and to propose a protocol of toxic evaluation using spermatic cells as model of study.

2.2. Methods

In order to further develop an understanding of the current techniques utilized on the evaluation of antitumoral drug toxicity on spermatic cells, a systematic review was performed. Three databases were accessed: Pubmed, Scopus and Web of Science. Five searches were made on each database to get a grasp of how many papers were showing up until the definite search was made.

On Pubmed, the search included Mesh terms and Abstract or Title terms, on Scopus it was just the Abstract or Title terms, and on Web of Science the search was initially made just with Title terms. However, due to the fact that it did not include Abstract terms and because it found a single paper, the search was changed to include Topic terms.

The first search was made to find all the papers on antitumoral drugs and their synonyms, the second was to find all the papers on spermatic cells and their synonyms, the third was to find all of the papers mentioning toxicity, the forth was a combination of the first and the second, and finally, the fifth was the combination of the first, second and third. This process was the same for each database, with the exception of Web of Science, which was made a sixth search as well (Table 1).

There were no restrictions based on where papers were published, languages used and year of publication. The selection of the papers were carried out by two individuals separately and were compared afterwards to find differences and discuss them, using the following inclusion criteria: “original study which evaluated the toxic effects of a single or a group of antitumoral drugs on spermatic cells”.

The main exclusion criteria were: papers that included just the effects of other drugs or interventions on the antitumoral drug; reviews; papers of non-antitumoral drugs and studies of toxicity on different body parts other than the male reproductive system.

Table 1. Description of the search strategy to find the relevant papers.

Pubmed (Title, abstract and Mesh term) 09/10/2016	**Words used on the search**	**Number of Papers**
1) Antitumoral drugs	"Antineoplastic Agents/toxicity"[Mesh] OR "Antitumor Agents"[Title/Abstract] OR "Antitumor Agent"[Title/Abstract] OR "Anticancer Agents"[Title/Abstract] OR "Anticancer Agent"[Title/Abstract] OR "Anticancer Drug"[Title/Abstract] OR "Anticancer Drugs"[Title/Abstract] OR "Chemotherapeutic Anticancer"[Title/Abstract] OR "Chemotherapeutic Anticancer Agents"[Title/Abstract] OR "Cancer Chemotherapy"[Title/Abstract] OR "Chemotherapy Drugs"[Title/Abstract] OR "Cancer Chemotherapy Drugs"[Title/Abstract] OR "Chemotherapy Agents"[Title/Abstract] OR "Antineoplastic Drug"[Title/Abstract] OR "Antineoplastic Drugs"[Title/Abstract] OR Antineoplastics[Title/Abstract] OR "Chemotherapeutic Anticancer Drug"[Title/Abstract] OR "Chemotherapeutic Anticancer"[Title/Abstract] OR "Antitumor Drugs"[Title/Abstract] OR "Antitumor Drug"[Title/Abstract] OR "Cancer Chemotherapy Agents"[Title/Abstract] OR "Cancer Chemotherapy"[Title/Abstract] OR "Oncological Drugs"[Title/Abstract] OR "Oncological Agents"[Title/Abstract] OR "Oncological Drug"[Title/Abstract] OR "Oncological Agent"[Title/Abstract] OR "Oncologic Drugs"[Title/Abstract] OR "Oncologic Agents"[Title/Abstract] OR "Oncologic Drug"[Title/Abstract] OR "Oncologic Agent"[Title/Abstract]	67276
2) Spermatic cell	Spermatozoon[Title/Abstract] OR Sperm[Title/Abstract] OR Sperms[Title/Abstract] OR Spermatozoid[Title/Abstract] OR Spermatozoids[Title/Abstract] OR "Male Germ Cell" [Title/Abstract] OR "Male Germ Cells" [Title/Abstract] OR "Male Gametes" [Title/Abstract] OR "Male Gamete" [Title/Abstract] OR "Spermatozoa/drug effects"[Mesh]	74242

3) Toxicity	Toxicity[Title/Abstract] OR Toxic[Title/Abstract]	447311
4) 1 and 2	(("Antineoplastic Agents/toxicity"[Mesh] OR "Antitumor Agents"[Title/Abstract] OR "Antitumor Agent"[Title/Abstract] OR "Anticancer Agents"[Title/Abstract] OR "Anticancer Agent"[Title/Abstract] OR "Anticancer Drug"[Title/Abstract] OR "Anticancer Drugs"[Title/Abstract] OR "Chemotherapeutic Anticancer"[Title/Abstract] OR "Chemotherapeutic Anticancer Agents"[Title/Abstract] OR "Cancer Chemotherapy"[Title/Abstract] OR "Chemotherapy Drugs"[Title/Abstract] OR "Cancer Chemotherapy Drugs"[Title/Abstract] OR "Chemotherapy Agents"[Title/Abstract] OR "Antineoplastic Drug"[Title/Abstract] OR "Antineoplastic Drugs"[Title/Abstract] OR Antineoplastics[Title/Abstract] OR "Chemotherapeutic Anticancer Drug"[Title/Abstract] OR "Chemotherapeutic Anticancer"[Title/Abstract] OR "Antitumor Drugs"[Title/Abstract] OR "Antitumor Drug"[Title/Abstract] OR "Cancer Chemotherapy Agents"[Title/Abstract] OR "Cancer Chemotherapy"[Title/Abstract] OR "Oncological Drugs"[Title/Abstract] OR "Oncological Agents"[Title/Abstract] OR "Oncological Drug"[Title/Abstract] OR "Oncological Agent"[Title/Abstract] OR "Oncologic Drugs"[Title/Abstract] OR "Oncologic Agents"[Title/Abstract] OR "Oncologic Drug"[Title/Abstract] OR "Oncologic Agent"[Title/Abstract])) AND (Spermatozoon[Title/Abstract] OR Sperm[Title/Abstract] OR Sperms[Title/Abstract] OR Spermatozoid[Title/Abstract] OR Spermatozoids[Title/Abstract] OR "Male Germ Cell" [Title/Abstract] OR "Male Germ Cells" [Title/Abstract] OR "Male Gametes" [Title/Abstract] OR "Male Gamete" [Title/Abstract] OR "Spermatozoa/drug effects"[Mesh])	275
5) 1, 2 and 3	((("Antineoplastic Agents/toxicity"[Mesh] OR "Antitumor Agents"[Title/Abstract] OR "Antitumor Agent"[Title/Abstract] OR "Anticancer Agents"[Title/Abstract] OR "Anticancer Agent"[Title/Abstract] OR "Anticancer Drug"[Title/Abstract] OR "Anticancer Drugs"[Title/Abstract] OR "Chemotherapeutic	90

	Anticancer"[Title/Abstract] OR "Chemotherapeutic Anticancer Agents"[Title/Abstract] OR "Cancer Chemotherapy"[Title/Abstract] OR "Chemotherapy Drugs"[Title/Abstract] OR "Cancer Chemotherapy Drugs"[Title/Abstract] OR "Chemotherapy Agents"[Title/Abstract] OR "Antineoplastic Drug"[Title/Abstract] OR "Antineoplastic Drugs"[Title/Abstract] OR Antineoplastics[Title/Abstract] OR "Chemotherapeutic Anticancer Drug"[Title/Abstract] OR "Chemotherapeutic Anticancer"[Title/Abstract] OR "Antitumor Drugs"[Title/Abstract] OR "Antitumor Drug"[Title/Abstract] OR "Cancer Chemotherapy Agents"[Title/Abstract] OR "Cancer Chemotherapy"[Title/Abstract] OR "Oncological Drugs"[Title/Abstract] OR "Oncological Agents"[Title/Abstract] OR "Oncological Drug"[Title/Abstract] OR "Oncological Agent"[Title/Abstract] OR "Oncologic Drugs"[Title/Abstract] OR "Oncologic Agents"[Title/Abstract] OR "Oncologic Drug"[Title/Abstract] OR "Oncologic Agent"[Title/Abstract])) AND (Spermatozoon[Title/Abstract] OR Sperm[Title/Abstract] OR Sperms[Title/Abstract] OR Spermatozoid[Title/Abstract] OR Spermatozoids[Title/Abstract] OR "Male Germ Cell" [Title/Abstract] OR "Male Germ Cells" [Title/Abstract] OR "Male Gametes" [Title/Abstract] OR "Male Gamete" [Title/Abstract] OR "Spermatozoa/drug effects"[Mesh])) AND (Toxicity[Title/Abstract] OR Toxic[Title/Abstract])	
Scopus (Title and abstract) 09/10/2016	**Words used on the search**	**Number of Papers**
1) Antitumoral drugs	TITLE-ABS ("Antitumor Agents" OR "Antitumor Agent" OR "Anticancer Agents" OR "Anticancer Agent" OR "Anticancer Drug" OR "Anticancer Drugs" OR "Chemotherapeutic Anticancer" OR "Chemotherapeutic Anticancer Agents" OR "Cancer Chemotherapy" OR "Chemotherapy Drugs" OR "Cancer Chemotherapy Drugs" OR "Chemotherapy Agents" OR "Antineoplastic Drug" OR "Antineoplastic Drugs" OR antineoplastics OR "Chemotherapeutic Anticancer Drug" OR "Chemotherapeutic Anticancer" OR "Antitumor Drugs" OR "Antitumor Drug" OR "Cancer Chemotherapy Agents" OR "Cancer Chemotherapy"	72195

	OR "Oncological Drugs" OR "Oncological Agents" OR "Oncological Drug" OR "Oncological Agent" OR "Oncologic Drugs" OR "Oncologic Agents" OR "Oncologic Drug" OR "Oncologic Agent")	
2) Spermatic cells	TITLE-ABS (spermatozoon OR sperm OR sperms OR spermatozoid OR spermatozoids OR "Male Germ Cell" OR "Male Germ Cells" OR "Male Gametes" OR "Male Gamete")	109427
3) Toxicity	TITLE-ABS (toxicity OR toxic)	657697
4) 1 and 2	TITLE-ABS ("Antitumor Agents" OR "Antitumor Agent" OR "Anticancer Agents" OR "Anticancer Agent" OR "Anticancer Drug" OR "Anticancer Drugs" OR "Chemotherapeutic Anticancer" OR "Chemotherapeutic Anticancer Agents" OR "Cancer Chemotherapy" OR "Chemotherapy Drugs" OR "Cancer Chemotherapy Drugs" OR "Chemotherapy Agents" OR "Antineoplastic Drug" OR "Antineoplastic Drugs" OR antineoplastics OR "Chemotherapeutic Anticancer Drug" OR "Chemotherapeutic Anticancer" OR "Antitumor Drugs" OR "Antitumor Drug" OR "Cancer Chemotherapy Agents" OR "Cancer Chemotherapy" OR "Oncological Drugs" OR "Oncological Agents" OR "Oncological Drug" OR "Oncological Agent" OR "Oncologic Drugs" OR "Oncologic Agents" OR "Oncologic Drug" OR "Oncologic Agent") AND TITLE-ABS (spermatozoon OR sperm OR sperms OR spermatozoid OR spermatozoids OR "Male Germ Cell" OR "Male Germ Cells" OR "Male Gametes" OR "Male Gamete")	190
5) 1, 2 and 3	TITLE-ABS ("Antitumor Agents" OR "Antitumor Agent" OR "Anticancer Agents" OR "Anticancer Agent" OR "Anticancer Drug" OR "Anticancer Drugs" OR "Chemotherapeutic Anticancer" OR "Chemotherapeutic Anticancer Agents" OR "Cancer Chemotherapy" OR "Chemotherapy Drugs" OR "Cancer Chemotherapy Drugs" OR "Chemotherapy Agents" OR "Antineoplastic Drug" OR "Antineoplastic Drugs" OR antineoplastics OR "Chemotherapeutic Anticancer Drug" OR "Chemotherapeutic Anticancer" OR "Antitumor Drugs" OR "Antitumor Drug" OR "Cancer Chemotherapy Agents" OR "Cancer Chemotherapy" OR "Oncological Drugs" OR "Oncological Agents"	50

	OR "Oncological Drug" OR "Oncological Agent" OR "Oncologic Drugs" OR "Oncologic Agents" OR "Oncologic Drug" OR "Oncologic Agent") AND TITLE-ABS (spermatozoon OR sperm OR sperms OR spermatozoid OR spermatozoids OR "Male Germ Cell" OR "Male Germ Cells" OR "Male Gametes" OR "Male Gamete") AND TITLE-ABS (toxicity OR toxic)	
Web of Science (Title) 09/10/2016	**Words used on the search**	**Number of Papers**
1) Antitumoral drugs	Title: ("Antitumor Agents" OR "Antitumor Agent" OR "Anticancer Agents" OR "Anticancer Agent" OR "Anticancer Drug" OR "Anticancer Drugs" OR "Chemotherapeutic Anticancer" OR "Chemotherapeutic Anticancer Agents" OR "Cancer Chemotherapy" OR "Chemotherapy Drugs" OR "Cancer Chemotherapy Drugs" OR "Chemotherapy Agents" OR "Antineoplastic Drug" OR "Antineoplastic Drugs" OR antineoplastics OR "Chemotherapeutic Anticancer Drug" OR "Chemotherapeutic Anticancer" OR "Antitumor Drugs" OR "Antitumor Drug" OR "Cancer Chemotherapy Agents" OR "Cancer Chemotherapy" OR "Oncological Drugs" OR "Oncological Agents" OR "Oncological Drug" OR "Oncological Agent" OR "Oncologic Drugs" OR "Oncologic Agents" OR "Oncologic Drug" OR "Oncologic Agent")	17930
2) Spermatic cells	Title: (spermatozoon OR sperm OR sperms OR spermatozoid OR spermatozoids OR "Male Germ Cell" OR "Male Germ Cells" OR "Male Gametes" OR "Male Gamete")	41961
3) Toxicity	Title: (toxicity OR toxic)	144684
4) 1 and 2	Title: ("Antitumor Agents" OR "Antitumor Agent" OR "Anticancer Agents" OR "Anticancer Agent" OR "Anticancer Drug" OR "Anticancer Drugs" OR "Chemotherapeutic Anticancer" OR "Chemotherapeutic Anticancer Agents" OR "Cancer Chemotherapy" OR "Chemotherapy Drugs" OR "Cancer Chemotherapy Drugs" OR "Chemotherapy Agents" OR "Antineoplastic Drug" OR "Antineoplastic Drugs" OR antineoplastics OR "Chemotherapeutic	9

	Anticancer Drug" OR "Chemotherapeutic Anticancer" OR "Antitumor Drugs" OR "Antitumor Drug" OR "Cancer Chemotherapy Agents" OR "Cancer Chemotherapy" OR "Oncological Drugs" OR "Oncological Agents" OR "Oncological Drug" OR "Oncological Agent" OR "Oncologic Drugs" OR "Oncologic Agents" OR "Oncologic Drug" OR "Oncologic Agent") AND Título: (spermatozoon OR sperm OR sperms OR spermatozoid OR spermatozoids OR "Male Germ Cell" OR "Male Germ Cells" OR "Male Gametes" OR "Male Gamete")	
5) 1, 2 and 3	Title: ("Antitumor Agents" OR "Antitumor Agent" OR "Anticancer Agents" OR "Anticancer Agent" OR "Anticancer Drug" OR "Anticancer Drugs" OR "Chemotherapeutic Anticancer" OR "Chemotherapeutic Anticancer Agents" OR "Cancer Chemotherapy" OR "Chemotherapy Drugs" OR "Cancer Chemotherapy Drugs" OR "Chemotherapy Agents" OR "Antineoplastic Drug" OR "Antineoplastic Drugs" OR antineoplastics OR "Chemotherapeutic Anticancer Drug" OR "Chemotherapeutic Anticancer" OR "Antitumor Drugs" OR "Antitumor Drug" OR "Cancer Chemotherapy Agents" OR "Cancer Chemotherapy" OR "Oncological Drugs" OR "Oncological Agents" OR "Oncological Drug" OR "Oncological Agent" OR "Oncologic Drugs" OR "Oncologic Agents" OR "Oncologic Drug" OR "Oncologic Agent") AND Título: (spermatozoon OR sperm OR sperms OR spermatozoid OR spermatozoids OR "Male Germ Cell" OR "Male Germ Cells" OR "Male Gametes" OR "Male Gamete") AND Título: (toxicity OR toxic)	1
6) 1, 2 e 3 (Topic)	Topic: ("Antitumor Agents" OR "Antitumor Agent" OR "Anticancer Agents" OR "Anticancer Agent" OR "Anticancer Drug" OR "Anticancer Drugs" OR "Chemotherapeutic Anticancer" OR "Chemotherapeutic Anticancer Agents" OR "Cancer Chemotherapy" OR "Chemotherapy Drugs" OR "Cancer Chemotherapy Drugs" OR "Chemotherapy Agents" OR "Antineoplastic Drug" OR "Antineoplastic Drugs" OR antineoplastics OR "Chemotherapeutic Anticancer Drug" OR "Chemotherapeutic Anticancer" OR "Antitumor Drugs" OR "Antitumor Drug" OR "Cancer Chemotherapy Agents" OR "Cancer Chemotherapy" OR "Oncological Drugs" OR "Oncological Agents" OR "Oncological Drug" OR	56

	"Oncological Agent" OR "Oncologic Drugs" OR "Oncologic Agents" OR "Oncologic Drug" OR "Oncologic Agent") AND Tópico: (spermatozoon OR sperm OR sperms OR spermatozoid OR spermatozoids OR "Male Germ Cell" OR "Male Germ Cells" OR "Male Gametes" OR "Male Gamete") AND Tópico: (toxicity OR toxic)	

2.3. Results

Each database search terms were slightly different in order to work properly and in a similar manner on each of the different search engines. The 196 papers - Pubmed (n=90) + Scopus (n=50) + Web of Science (n=56) - were exported into EndNote™ X5 Program in order to eliminate duplicates (47 were excluded) and assist with the sorting to the next stage. Afterwards, the 149 papers' titles were analyzed (58 were excluded). Following, the 91 papers' abstracts were read (37 were excluded). The remaining 54 papers were then read closely to identify the final number of papers included, finishing with a total of 27 papers (Figure 1).

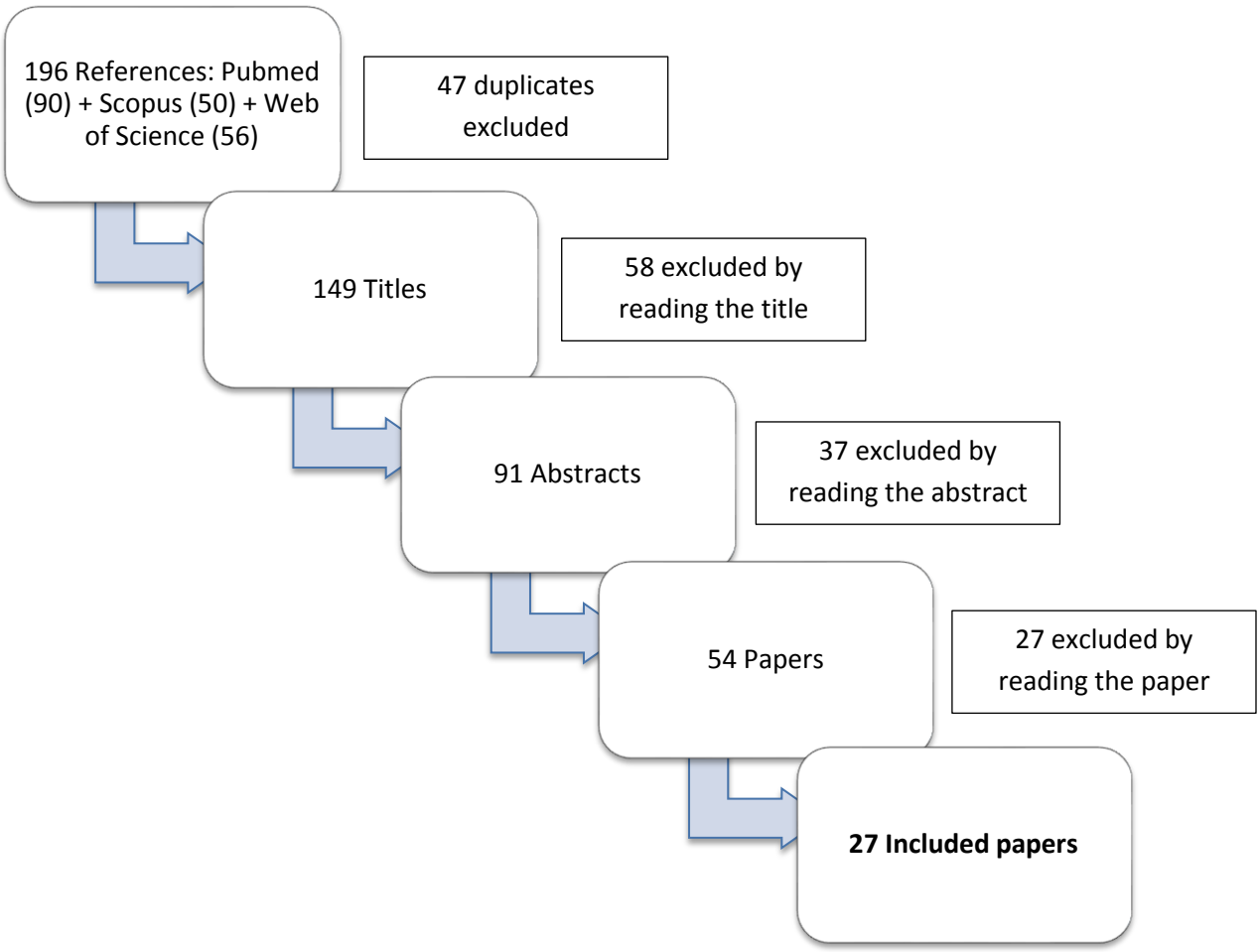

Figure 1. Flowchart showing the progression on the selection of the papers to be included.

The extraction of information from the 27 final papers had a summarizing purpose - trying to assemble and classify the techniques on groups by similarity. The management of the information fit accordingly into a table containing the first author, year, antineoplastic drug evaluated or group of more than one drug, stage of the spermatogenesis assessed on the experiment, and the techniques utilized in order to evaluate the toxicity (Table 2).

Table 2. Description of the included papers (n=27) on the antineoplastic drugs used, cell stages evaluated and a summary of the techniques.

Author	Drug	Cell stage	Technique
(ETTLIN *et al.*, 1984)	vincristine, procarbazine	each stage (rats)	microscopy, testis weight
(DA CUNHA *et al.*, 1985)	amsacrine	each stage (mice)	morphology, microscopy
(ADLER; EL TARRAS, 1990)	cisplatin	spermatogonia, primary spermatocyte (mice)	microscopy (chromosomal aberration), testis weight
(IMAHIE *et al.*, 1995)	doxorubicin	spermatozoa (rats)	microscopy, testis weight
(MATSUI *et al.*, 1995)	cyclophosphamide	each stage (rats)	morphology, testis weight
(SHIN *et al.*, 1999)	hydroxyurea	spermatogonia, spermatocyte, spermatids (mice)	TUNEL assay, LM-PCR, testis weight
(SHINODA *et al.*, 1999)	doxorubicin	spermatogonia, spermatocyte, spermatids (rats)	TUNEL
(CHOUDHURY *et al.*, 2000)	cisplatin	spermatogonia, primary spermatocyte, spermatozoa (mice)	morphology, microscopy (chromosomal aberration)
(CHOUDHURY *et al.*, 2001)	cyclophosphamide, methotrexate	spermatogonia, primary spermatocyte, spermatozoa (mice)	morphology, microscopy (chromosomal aberration)
(JYOTHI *et al.*, 2001)	teniposide	each stage (rats)	flow cytometry
(KATO *et al.*, 2001)	doxorubicin	each stage (rats)	morphology, microscopy
(PLASSMANN; URWYLER, 2001)	doxorubicin	each stage (rats)	morphology, microscopy, testis weight
(CHOUDHURY *et al.*, 2002)	vincristine	spermatogonia, primary spermatocyte, spermatozoa (mice)	morphology, microscopy (chromosomal aberration)
(SUKHACHEVA *et*	etoposide	each stage (mice)	microscopy

***al.*, 2003)**			
(VAISHEVA *et al.*, 2007)	CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone)	spermatozoa (rats)	morphology, microscopy, TUNEL, Acridine Orange Assay
(PADMANABHAN *et al.*, 2008)	methotrexate	each stage (mice)	morphology, microscopy, comet, halo assay, TUNEL assay, testis weight
(BAKER, 2009)	gemcitabine	each stage (mice)	morphology, microscopy
(SHARMA *et al.*, 2009)	boswelic acids	spermatozoa (rats)	morphology
(CERIBASI *et al.*, 2010)	cyclophosphamide	spermatozoa (rats)	morphology, microscopy
(MARCON *et al.*, 2010)	bleomycin, etoposide, cisplatin	spermatogonia (rats)	flow cytometry, immunofluorescence
(TRIVEDI *et al.*, 2010)	doxorubicin	each stage (rats)	morphology, microscopy, comet assay, halo assay
(CIFTCI *et al.*, 2012)	ruthenium(II) and gold(I)-NHC complexes	spermatozoa (rats)	morphology, microscopy, testis weight
(KILARKAJE *et al.*, 2012)	bleomycin, etoposide, cisplatin	each stage (rats)	morphology, microscopy
(COBURN *et al.*, 2012)	sunitinib	spermatozoa (rats)	morphology, microscopy, testis weight
(SHETTY; BAIRY, 2015)	sorafenib	spermatozoa (mice)	microscopy
(GUTIERREZ *et al.*, 2016)	doxorubicin	spermatozoa (mice)	morphology, microscopy, qRT-PCR, western blot
(ATTIA *et al.*, 2016)	doxorubicin	spermatocyte (mice)	FISH assay, meiotic delay assay

Parting from the information gathered on the Table 2, the number of papers utilizing each technique was put into a bar graph in order to improve the demonstration of most common techniques and the variety amongst them (Figure 2). The technique most commonly used was the evaluation through morphological analysis, which in most of the cases was a head and tail abnormality examination. There were around four studies more histologically and morphologically oriented checking it on the whole tissue and on cell stages prior to the spermatozoa, as well as one of those presenting a quantitative morphometry approach. Even though this analysis utilizes the microscope, it was not classified as microscopy for analytical purposes.

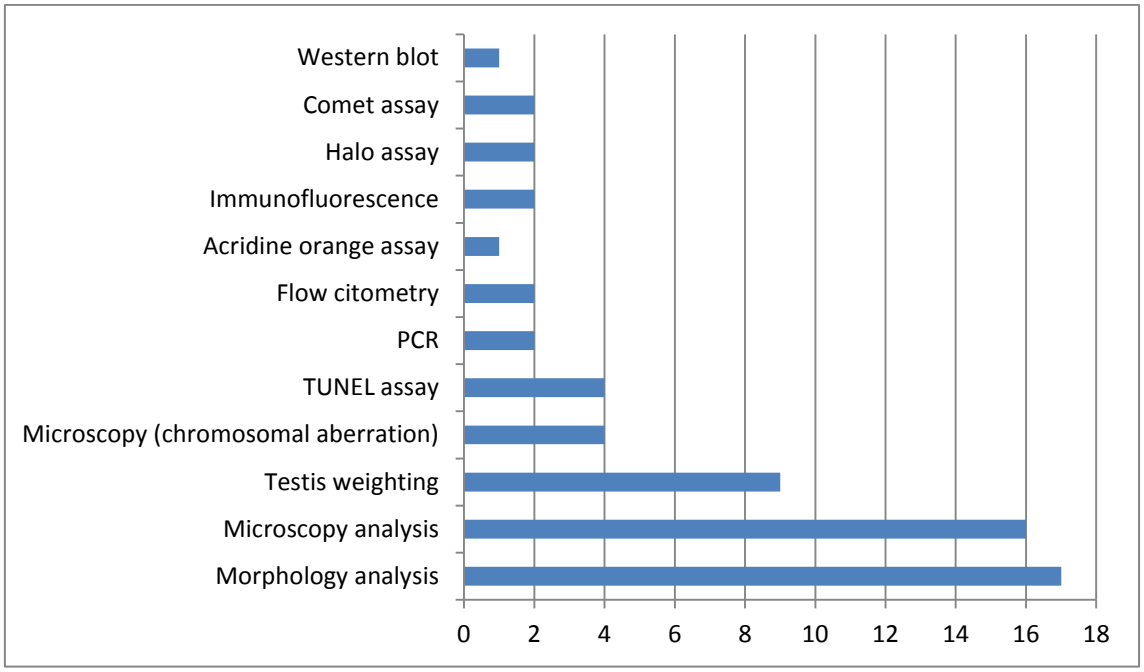

Figure 2. Number of papers using each type of technique to evaluate the toxicity.

Following, the spermatic parameters generally available by microscopy techniques were considered as a group of basic techniques such as motility, vigor, total head count. Among the cell viability and kinetic tests it also included the observation of disintegration of the spermatogenic layer, disorganization in germinal cells, multinucleated giant cell formation, and degeneration, desquamation, and vacuolization of spermatogenic cells, just as it was used by Cifti (CIFTCI *et al.*, 2012). The microscopy directed to the chromosomal aberration analysis was classified differently due to the fact that its approach was considered somewhat specific, utilizing cells on the metaphase to analyze the chromosomes directly.

After comparing the techniques among the selected papers, the next step would be to propose a protocol for the evaluation of toxicity by antitumoral drugs utilizing the spermatic cells as model. The schematics on Figure 3 show the utilization of four stages of the spermatogenesis as model, evaluating the toxicity after drug exposure through cell kinetic evaluation just on the spermatozoa cell stage, as well as viability evaluation on each stage.

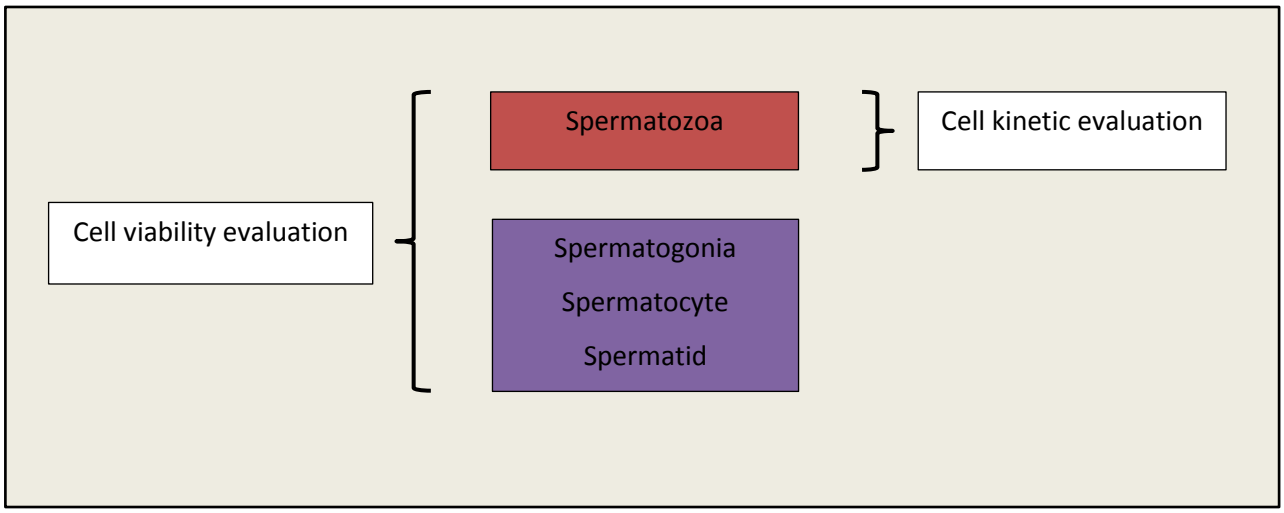

Figure 3. Schematic proposal of a toxicity evaluation protocol after drug exposure.

2.4. Discussion

It is known that the effect of chemical mutagens acts differently on each of the spermatogenesis stages. The spermatocytes are supposed to be more sensitive in general, and spermatogonia a little less sensitive in comparison to spermatocytes (ADLER; EL-TARRAS, 1989). Other than that, to properly identify the toxicity of the drug or agent, it is necessary more than just a singular test (COLENBRANDER *et al.*, 2003). Therefore, it's important to verify the effect on different stages and using different techniques.

Through this review it was observed that in most of the papers, a whole animal model was used – either mice or rats – an issue also stated by Marcon, demonstrating the ongoing concern to find alternative ways to the use of animal as experimental model. This concern goes according to the 3R's idea (replacement, reduction or refinement of animal testing) (RUSSELL; BURCH, 1959). One proposition, for instance, was the development of spermatic stem cells of rodents for long-term cellular maintenance and proliferation as a model replacement (MARCON *et al.*, 2010). Usually, the studies expose the drugs to the animals and collect the cells afterwards, checking the effect on the spermatic cells indirectly. A step prior to the utilization of animals could be the utilization of spermatic cells as model to verify a direct effect and if it is the case, proceed to the animal model experiment. Also, rats and mice are required to be sacrificed for the extraction of the sufficient amount of spermatic cells for an evaluation, therefore the utilization of more accessible spermatic cells from other species, such as bovine sperm, could avoid unnecessary sacrifices.

Toxicity evaluation experiments avoiding to use the conventional drug exposure *in vivo* have been presented such as an experiment by Goldstein, which aimed to test an *ex vivo* model for the toxicity assessment of diverse compounds, utilizing a culture of the seminiferous tubules followed by the application of flow cytometry for toxic effect verification (GOLDSTEIN *et al.*, 2016). There are similar studies with the objective of establishing the gamete cellular model usage instead of

animal models, for instance, Beker van Woudenberg experiment whose effort was to demonstrate a protocol for the evaluation of toxicity directly on class 1 and 2 oocytes, after initially verifying if the effect was just on cumulus cells or not (BEKER VAN WOUDENBERG *et al.*, 2012).

On this paper, a protocol for the toxicity verification using four stages of the spermatogenesis (spermatogonia, spermatocytes, spermatids and spermatozoa), cell kinetic, cell viability tests in combination on different stages after direct exposure could be sufficient to detect it before the usage of animal model come to be necessary. For example, Waseem Asghar analyzed the potential toxicity of single-walled carbon nanotubes directly onto spermatozoa, testing viability, velocity, and generation of superoxide and nitric oxide stresses as a function of nanoparticle concentration and incubation period (ASGHAR *et al.*, 2016).

2.5. Conclusion

Through this review it was observed that the proper way for the evaluation of the toxicity by antitumoral drugs on spermatic cells is the utilization of more than just a single cell stage of the spermatogenesis, as well as the assessment by more than just a singular technique. The evaluation techniques for the assessment of the toxic effect due to the exposure of antitumoral drugs on spermatic cells are yet to be improved and simplified efficiently, in a way that it measures accurately without the necessity of an *in vivo* experimentation before it is shown that it really is required.

3. Considerações finais

Através dessa revisão foi observado que o modo mais apropriado de se avaliar a toxicidade de drogas antitumorais utilizando células espermáticas é através da utilização de células em diferentes estágios da espermatogênese, além de mais de uma técnica. As técnicas de avaliação do efeito tóxico pela exposição de drogas antitumorais em células espermáticas ainda precisam ser aprimoradas e simplificadas eficientemente, de forma que elas meçam adequadamente, sem a necessidade da utilização de experimentação animal antes que se mostre indispensável.

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