Chapter

Effect of Oxidative Stress on Sperm Cells

Alejando Córdova Izquierdo, Adrian Emmanuel Iglesias Reyes, Alda Roció Ortiz Muñiz, María de Lourdes Juárez Mosqueda, Jesús Alberto Guevara González, Juan Eulogio Guerra Liera, Gustavo Ruiz Lang, Rubén Huerta Crispín, Abel E. Villa Mancera, Pedro Sánchez Aparico and Raúl Sánchez Sánchez

Abstract

Free radicals are unstable molecules that have an unpaired electron in their last orbital, which makes them highly unstable agents. In medicine, it has been discovered that they play an important role in cell signaling and without them some cells such as leukocytes or sperm could not perform their biological functions. To protect itself from these oxidizing agents, the cell has a defense system based on antioxidants; however, when this balance is lost and oxidizing agents exceed the cellular antioxidant capacity, the cell enters oxidative stress, which affects cellular components such as proteins, nucleic acids, lipids, amino acids, and carbohydrates, among others. In the case of spermatozoa, due to their high metabolic rate, they produce large quantities of oxygen reactive species (ROS), decreasing sperm motility, alterations in cytoplasmic components, modifications in genetic material, or sperm death. In this chapter, a review is made of a brief history of how the toxicity of oxygen and free radicals was discovered, the oxidative stress in cells, and the effect of oxidative stress in the cytoplasmic sperm membrane, in the spermatic mitochondria, in the spermatic acrosome, in the sperm DNA, and in the fertility of the female and the male.

Keywords: spermatozoa, oxidative stress, free radicals, reproduction

1. Introduction

Semen freezing is one of the most important procedures in the development of biotechnologies for assisted reproduction. Among the advantages that we can find in artificial insemination is as follows: to keep the biological material viable for an indefinite time, the establishment of gene banks and the exchange of genetic material over very long distances economically rationalize the ejaculate; improve the use of wild boar elite, an adequate available germinal material of economic interest for man; and perform the collection of semen only in the most favorable reproductive seasons. However, the composition of the plasma membrane of the pig sperm, the

large phospholipid layer (the comparison of bull sperm, which has a smaller layer of skin), is the cause of the sperm cell. Free radical changes that occur during freezing, the occasion when the effects of sperm freezing occur in the wild boar, affect the integrity of the plasma membrane, the acrosome, the nucleus, as well as the mitochondrial functions and motility of spermatozoa [1–4]. The purpose of this review is to publicize the main causes of ROS generation in sperm cells, as well as a brief explanation of how ROS is a part of sperm parts.

2. Background

Air is a vital element for any living being and is a mixture of gases based on nitrogen (78%), oxygen (21%), water vapor (variable between 0 and 7%), ozone, carbon dioxide (CO_2), hydrogen, and some noble gases such as krypton, neon, helium, and argon. Of these, oxygen (which appeared approximately 2500 million years ago) plays a vital role in the processes of aerobic life, being the second most abundant element in the atmosphere [5–7].

Antoine Lavoisier in the eighteenth century gives the name to "oxygen" which means "generator of acids," because despite having a therapeutic use, it was already known that it was a toxic substance, due to its great oxidizing power. In 1774, the toxic effects of the gas are demonstrated, and 6 years later (1780) experiments are made of the use of oxygen in newborns; in 1878, the toxic effect of oxygen in the brain is documented by Paul Bert, manifested by the presence of convulsive crises to more than three atmospheres, and in 1899, when trying to replicate the Bert effect, J. Lorrain Smith reports fatal pneumonia in rats exposed to 73% oxygen for 4 days. In 1940, it is reported that babies with periodic breathing pattern improved with the use of oxygen to 70%, beginning the routine use of oxygen in premature babies. Between 1951 and 1956, it is demonstrated that oxygen was safe when it occurred in concentrations lower than 40%. Harman in 1954 stated that the life expectancy increases decreasing the degree of oxidative phenomena. Thus, throughout history, it has been described that the higher the toxicity of O_2 is, the higher is the metabolic rate of the species considered [6, 8].

In veterinary and human medicine, more and more agents that cause diseases in the body have been discovered; some of them are derived from metabolic processes of oxygen, among which are the production of energy, detoxification of harmful compounds, and defense against pathogens, among which are free radicals (RL), which are highly reactive oxidation agents, which act as short-lived chemical intermediates on lipids, amino acids, carbohydrates, and nucleic acids [5, 7].

The RL can be divided into the following: (i) reactive oxygen species (ROS), which are highly reactive molecules that constantly attack organisms through oxidation-reduction reactions, among which are molecular oxygen (O_2), superoxide anion (O21) hydrogen peroxide (H_2O_2), hydroperoxyl (HO_2), and hydroxyl radical (OH); (ii) the transition metals, which have unpaired electrons and can exist as RL; and (iii) reactive nitrogen species (ERN), which are capable of generating oxidative damage and cell death, among which are nitric oxide (NO), peroxynitrite anion ($ONOO-$), and nitric dioxide (NO_2) [9–11].

The RL must be attenuated by different antioxidant defense systems, which involve enzymes and molecules. Antioxidants are divided into enzymatic, also called endogenous production, which are the first line of defense against the production of RL and are proteins with antioxidant capacity that are not consumed when reacting with the RL. Among the most important of this group are catalase, superoxide dismutase, and glutathione peroxidase. The nonenzymatic ones come mainly from the diet and are small liposoluble molecules, which, unlike the

enzymatic, are consumed during their antioxidant action, so they must be replaced; among the most important in this group are vitamins E and C, beta-carotenes, retinol, uric acid, pyruvate, albumin, carnitine, taurine, hypotaurine, transferrin, ceruloplasmin, polyphenoids, flavonoids, and trace elements [12–16]. These antioxidant defense systems are linked in a cellular buffer system, where they add up and collaborate with each other, to deal with any oxidative aggression in cells, for example, nonenzymatic antioxidants can have synergistic effects in combination with enzymatic antioxidants, regenerating enzymatic antioxidants through the donation of hydrogen, neutralizing molecular oxygen, and catalyzing the synthesis or regeneration of nonenzymatic antioxidants [9].

When there is an imbalance and the amount of RL exceeds the balance between oxidant production and antioxidant capacity, a phenomenon known as oxidative stress (EO) is generated, which has negative consequences on multiple cellular processes [7, 14, 17, 18].

3. Effect of oxidative stress on cells

Due to aerobic conditions, cells maintain a high concentration of oxidant products in their metabolism, such as RL, which are generated as a result of cellular metabolism and in cellular physiological concentrations are related to cell signaling processes or to fulfill their functions biological, including leukocytes that are recruited to the sites of infection by chemotactic factors and are able to eliminate microorganisms through phagocytosis, exposing them to high concentrations of ROS (superoxide and hydrogen peroxide) and other microbicidal products contained in cell granules. However, when EO exists, ROS can mainly affect cellular components such as proteins, nucleic acids, sugars, and lipids [7, 9, 17].

Most of the main diseases that cause the death of animals and people or deteriorate their quality of life are caused by the RL. Each cell of the body suffers about 10,000 impacts of free radicals per day. For this reason, the EO has been the target of intense research in recent years, mainly in the implications on how mitochondria produce ROS, since they are of vital importance to understand their relationship with the pathogenesis of several chronic diseases such as cancer, osteoporosis, Alzheimer's, type 2 diabetes, neurodegenerative diseases, and cardiovascular diseases such as heart failure [7].

The spermatozoon was the first cell type in which the presence of ROS could be identified, because until a few years ago, ROS were considered toxic elements for sperm; however, the RL are currently known (mainly O_2.-) in low concentrations in semen, which play a fundamental role in their biological functions during sperm capacitation, sperm maturation, tyrosine phosphorylation, intergame interaction, and the acrosomal reaction that occurs for fertilization of the oocyte; these phenomena are controlled by the mechanism of defense of enzymatic and nonenzymatic antioxidants that when this balance is broken between the RL and the antioxidant defense system, damages are induced in the nucleic acids, proteins, and lipids present in the membrane of the sperm, causing loss of mobility, decrease in viability, and alterations in the intermediate piece, which finally produce a decrease in seminal quality or sperm death [2, 7, 14, 16, 19–26]. A clear example of this is nitric oxide (NO), which has an important function in the sperm pathophysiology, since in low concentrations it favors the processes of sperm capacitation, the acrosomal reaction, and the union to the zona pelucida; however, in high concentrations it leads to the formation of peroxynitrites, which alters sperm motility [27].

It has been observed that in the ejaculate, the main sources of ROS are leukocytes and abnormal sperm cells, although it has been proposed that there are other

possibilities on the generation of intracellular ROS in the spermatozoon, such as the leakage of electrons from the mitochondrial transport chain, NADPH oxidase as a possible source of ROS, and the generation of RL by means of nitric oxide in the post-acrosomal and equatorial regions, which can generate a change in the basal state of the oxidizing agents and induce changes in sperm activity [7].

4. Effect of oxidative stress on the cytoplasmic sperm membrane

The spermatic membrane is asymmetric in its structure and functions. It is formed by an association of phospholipids, plasmalogens, and sphingomyelins in dynamic equilibrium with membrane proteins making it an easy target of oxidizing agents. Cholesterol and phospholipids are important in maintaining the structural integrity of membrane systems. In particular, the plasma membrane of the sperm possesses a large quantity of polyunsaturated fatty acids (PUFA), which are necessary for the acrosome reaction and the interaction with the oocyte membrane. On the other hand, the high content of polyunsaturated fatty acids in the plasma membranes of sperm makes them very susceptible to lipoperoxidation (LP), making it highly vulnerable to oxidative stress [7, 14, 20, 24].

The low concentrations of antioxidant enzymes (catalases, dismutases, peroxidases, and glutathione reductase) in the plasma membrane also convert sperm into cells susceptible to the attack of the RL (particularly the attack of hydroxyl radical (OH) and hydroperoxyl (HO_2)), on all the post-acrosomal region, causing alterations in its permeability (since ROS induces LP of the phospholipids of the membrane, which causes the appearance of "orifices"), affecting the Na^+ and Ca^{2+} pumps, causing these to enter cations into the sperm, altering the osmolarity, which causes the formation of few soluble calcium phosphates, depletion of ATP, and activation by means of Ca^{2+} of proteolytic and phosphoglycolytic enzymes. It also damages the enzymes lactate dehydrogenase, pyruvate kinase, glyceraldehyde 3 phosphate dehydrogenase, and ATPase, generating loss or reduction in mobility, protein and lipid damage, alterations in deoxyribonucleic acid (DNA), anomalies in its morphology, fertility problems, and cell death [9, 14, 20, 23, 24, 28, 29].

5. Effect of oxidative stress on sperm mitochondria

Mitochondria are considered one of the main cellular sources of ROS, which are responsible for regulating physiological processes such as transduction of intracellular signals, the response to oxidative stress, embryonic development, cell proliferation and adhesion, gene expression, and apoptosis [7].

In the sperm mitochondria provide the highest amount of ATP, through glycolysis and oxidative phosphorylation, contributing to the formation of RL during these processes [7, 30, 31]. However, when there is disruption of the mitochondrial respiratory chain (during freezing), these are responsible for the formation and release of ROS. This interruption causes oxygen to undergo complete reductions producing, instead of water molecules, intermediate molecules such as superoxide anion, hydroxyl radical, and hydrogen peroxide, triggering a phenomenon similar to apoptosis, responsible for both the death of sperm and the sublethal damages that decrease the half-life and fertilizing capacity of the cells (**Figure 1**) [32].

The freezing of semen also exerts an important damage in the mitochondria, since it has been demonstrated that the EO induces damage in the mitochondrial DNA, observing that the mutation spectrum of said DNA, in the spermatozoon, can

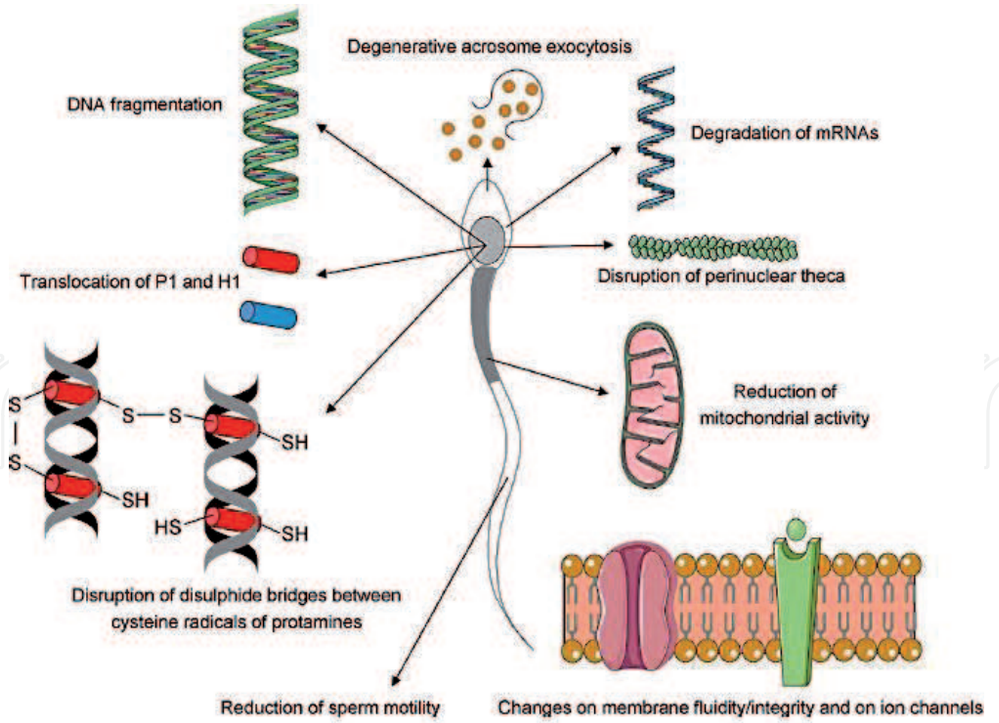

Figure 1.
Lesions resulting from the freezing of pig semen (modified from [4]).

be 10–100 times greater than to nuclear DNA. This can be explained by the cross-linking of DNA proteins that cause RL, exchange of sister chromatids, damage to the structure of deoxyribose phosphate, oxidation of nitrogenous bases, conversion of bases (the deamination of cytosine into uracil and of the 5-methylcytosine in thymidine), ring openings, base release, and chain breaking (one or two strands). This leads directly to a decrease in fertility [4, 7, 9, 24, 33].

6. Effect of oxidative stress on the spermatic acrosome

The acrosome is also affected by the action of the RL during the transport of the sperm through the epididymis, mainly by hydrogen peroxide, since it inhibits the induction of the acrosomal reaction and damages the integrity of the acrosome, producing a malfunction at the time of fertilization of the oocyte [34].

7. Effect of oxidative stress on sperm DNA

Much of the DNA damage in the sperm is generated by the EO. The damage that ROS exerts directly on sperm DNA can induce mutations, affecting the paternal genomics of the embryo, and can be an indication of male fertility [20, 24]. To demonstrate this, in studies where sperm were exposed to high concentrations of artificially produced ROS, a significant increase in DNA damage, decreased sperm motility, and induction in apoptotic processes could be observed [7]. These damages in the chromatic sperm depend on endogenous factors such as in the testicles or the epididymis (during sperm maturation), and exogenous factors as DNA peroxidative damage, infections, immunological factors, or various chemical agents. These may be related to failures in packaging, nuclear maturity, chromatin fragmentation, aneuploidies, or DNA integrity defects [7, 24].

In any part of the spermatogenesis, a damage to the spermatic DNA can be induced, which despite is being a multifactorial phenomenon and not being completely delimited; some of the factors that can produce irreversible damage is the generation of ROS, which come from the respiratory chain, since these oxidative molecules react with the nitrogenous bases and with deoxyribose, causing DNA fragmentation, problems in the compaction and winding of the DNA inside the chromatin, deletions, mutations, translocations, degradation of purine or pyrimidic bases, rupture of chains, and cross-links between proteins and DNA. The magnitude of damage induced by RL during sperm transit through the epididymis depends on the levels of these produced by immature sperm, the presence of epithelial cells or activated leukocytes in the epididymis, and the levels of antioxidant enzymes present in the epididymis lumen [2, 4, 21, 23, 24, 34–37].

It is important to note that there are mainly two RL that affect the DNA strand. The first is the OH radical, which results in the formation of 8-OH-guanine and 8-OH-2 deoxyguanosine at the first stage, attacking the purines as pyrimidines, causing fragmentation of double-stranded DNA, and the second is the radical O21, which generally produces only guanine adducts, especially 8-hydroxyguanine, which affect sperm motility [4, 7, 9, 24]. If a sperm with fragmentation of double-stranded DNA manages to fertilize an oocyte, it is incompatible and may affect the normal development of pregnancy [24].

8. Effect of oxidative stress on female and male fertility

Infertility is defined as the inability of a couple to conceive after a year of sexual intercourse without contraceptive measures [24]. There are multiple causes of male infertility, which may be congenital or acquired; of all of them, idiopathic infertility is caused by multiple factors such as endocrine alterations, oxidative stress, and genetic or epigenetic alterations [38].

In particular, the role of EO as one of the main causes of male infertility has been well established, since ROS can affect all cellular components, including the AGP of membranes, proteins, and nucleic acids, causing in males oligozoospermia, prostate carcinoma, cryptorchidism, varicocele, low seminal quality, low motility of spermatozoa, decreased sperm concentration, and acceleration in the process of apoptosis of geminous cells [24, 27].

In a study conducted by Pérez [27], it was observed that in asthenozoospermic patients have an overexpression of the enzyme inducible nitric oxide synthase (iNOS), compared with the normospermic, which results in a sperm dysfunction and in the decrease of the fecundate capacity of sperm. It has also been shown that in sperm of individuals whose partners have recurrent early embryonic death, there is a significant increase in aneuploidies, abnormal chromatin condensation, DNA fragmentation, apoptosis, and abnormal sperm morphology [19].

It is important to highlight the importance of antioxidants in semen, since it has been observed that the low levels or deficiency of antioxidants in the seminal plasma leaves the sperm unprotected to the EO [20]. So the use of antioxidants has been proposed as a tool to protect sperm from oxidative damage, and it has even been proven that the addition of antioxidants (vitamin C, E or glutathione), at the time of the seminal conservation, produces better results in the seminal evaluation at the time of insemination [4, 7, 29, 39].

In the case of females, it has been suggested that ROS can participate in the formation of adhesions associated with endometriosis, decreasing its fertility. There are also alterations of folliculogenesis caused by ROS, which can deteriorate the quality of the oocyte and have been proposed as a cause of subfertility associated

with endometriosis. The EO has also been associated with numerous pathologies among which we can mention mastitis, edema of the udder, higher incidence of diseases in the peripartum period, deficit in the synthesis of steroid horns in cows, and degenerative nutritional myopathy in sheep. In the case of sows, the EO can cause postweaning inflammatory states, modifying the status of selenium and vitamin E affecting the growth rate of piglets [33, 40–42].

9. Conclusions

The effect of EO on sperm cells significantly affects the fecundating capacity of sperm, causing infertility in males and/or low reproductive parameters in females so that the issue of EO in the fertilizing capacity of spermatozoa mammals is of utmost importance at present.

Declaration of conflict of interest

The authors declare that there are no conflicts of interest.

Thanks

To CONACYT, for the support to the second author as a fellow with registration number 624422.

Implications

In this paper, a review is made of a brief history of how the toxicity of oxygen and free radicals was discovered, the oxidative stress in cells, and the effect of oxidative stress in the cytoplasmic sperm membrane, in the spermatic mitochondria, in the spermatic acrosome, in the sperm DNA, and in the fertility of the female and the male.

Author details

Alejando Córdova Izquierdo[1*], Adrian Emmanuel Iglesias Reyes[2], Alda Roció Ortiz Muñiz[3], María de Lourdes Juárez Mosqueda[4], Jesús Alberto Guevara González[1], Juan Eulogio Guerra Liera[5], Gustavo Ruiz Lang[1], Rubén Huerta Crispín[6], Abel E. Villa Mancera[6], Pedro Sánchez Aparico[7] and Raúl Sánchez Sánchez[8]

1 Department of Agricultural and Animal Production, University Autonomous Unit Xochimilco, Mexico City, Mexico

2 Master's Degree in Agricultural Sciences, University Autonomous Unit Xochimilco, Mexico City, Mexico

3 University Autonomous Metropolitan Unit Iztapalapa, Mexico City, Mexico

4 Department of Morphology, FMVZ-UNAM, Mexico City, Mexico

5 Faculty of Agronomy, Autonomous University of Sinaloa, Mexico

6 Faculty of Veterinary, Benemérita Autonomous University of Puebla, Mexico

7 Faculty of Veterinary, Autonomous Mexico State University, Mexico

8 Department of Reproduction, INIA, Madrid, Spain

*Address all correspondence to: acordova@correo.xoc.uam.mx

References

[1] Restrepo BG, Pizarro LE, Albero RB. Estrés oxidativo en el semen equino criopreservado. Revista Lasallista de Investivación. 2012;**9**(1):128-136

[2] Williams S. Criopreservación de semen porcino: Desafíos y perspectivas. Revista Brasileira de Reprodução Animal. 2013;**37**(2):207-2012

[3] Gallardo BJO, Vargas SCA. Evaluacion de tres diluyentes para criopreservar semen bovino de toros cruce Sahiwal (Bos Taurus) En el trópico Húmedo [Tesis previa a la obtención del título de Ingeniero Agropecuario]. Universidad de las Fuerzas Armadas. Departamento de Ciencias de la Vida y la Agricultura; 2015. pp. 1-89

[4] Yeste M, Rodríguez GJE, Bonet S. Artificial insemination with frozen-thawed boar sperm. Molecular Reproduction and Development. 2017;**2017**:1-12

[5] Villa ANA, Ceballos MA. Radicales libres e infertilidad en el macho. Veterinaria e Zootecnia. 2007;**1**(2):87-97

[6] Sánchez I, Torres V, Moreno O, Rodríguez A. Determinación del estrés oxidativo mediante peroxidación lipídica en cristalinos humanos con cataratas. Revista de Facultad de Medicina, Universidad de Los Andes. 2011;**20**(1):42-45

[7] Mayorga TJM, Camargo M, Cadavid Ángela P, Cardona Maya Walter D. Estrés oxidativo: ¿un estado celular defectuoso para la función espermática? Revista Chilena de Obstetricia y Ginecología. 2015;**80**(6):486-492

[8] Martínez SG. Especies reactivas de oxígeno y balance redox, parte 1: Aspectos básicos y principales especies reactivas de oxígeno. Revista Cubana de Farmacia. 2005;**39**(3):1-11

[9] Quintanar EMA, Calderón, Víctor SJ. La capacidad antioxidante total. Bases y Aplicaciones. REB. 2009;**28**(3):89-101

[10] Berzosa S. Estudio del daño oxidativo, niveles de defensa antioxidantes y efecto ergogénico de la melatonina en pruebas de esfuerzo físico agudo [Tesis doctoral para optar al grado de doctor europeo]. Universidad de Zaragoza. Facultad de Medicina. Departamento de Farmacología y Fisiología; 2011. pp. 11-15

[11] García TBE, Saldaña BA, Saldaña GL. El estrés oxidativo y los antioxidantes en la prevención del cáncer. Revista Habanera de Ciencias Médicas. 2012;**12**(2):187-196

[12] Villa NA, Sánchez LE, Ceballos A. Actividad de glutatión peroxidasa y superóxido dismutasa en plasma seminal y sangre en cerdos reproductores. Veterinaria e Zootecnia. 2009;**3**(1):9-51

[13] Gašparovic AC, Lovakovic T, Zarkovic N. Oxidative stress and antioxidants: Biological response modifiers of oxidative homeostasis in cancer. Periodicum Biologorum. 2010;**112**(4):433-439

[14] Flores C, Márquez Y, Vilanova L, Mendoza C. Dienos conjugados y malondialdehído como indicadores de lipoperoxidación en semen de toros "Carora". Revista Veterinaria. 2011;**22**(2):91-94

[15] Villalba MC. Implicaciones del estrés oxidativo en la infertilidad masculina: Análisis de marcadores bioquímicos en plasma seminal y su asociación con parámetros del seminograma y la capacitación espermática [Mediora presentada por Celia Villalba Martínez para optar al grado de Doctor]. Universitat d' Alacant. Departamento de Biotecnología; 2014. pp. 1-321

[16] Gumbao B, David D. Efecto antioxidante del glutatión aplicado en el medio de descongelación seminal de tres especies con interés. Universidad de Murcia. Departamento de Fisiología; 2015. pp. 1-154

[17] Zepeda AB, Farías JB. Antioxidantes frente a estrés oxidativo inducido por hipoxia hipobária en testículo y epidídimo. Revista de Farmacología de Chile. 2013;**6**(1):31-36

[18] Cota MAI. Actividad de las enzimas antioxidantes: Superóxido dismutasa, catalasa y glutatión peroxidasa, en el espermatozoide y líquido seminal de conejo nueva Zelanda y su relación con el sobrepeso [tesis para obtener el grado de Maestra en Biología de la Reproducción Animal]. Universidad Autónoma Metropolitana Unidad Iztapalapa; 2014. pp. 1-71

[19] Rodríguez E, Gil VAM, Aguirre ADC, Cardona MW, Cadavid AP. Evaluación de parámetros seminales no convencionales en individuos cuyas parejas presentan muerte embrionaria temprana recurrente: En busca de un valor de referencia. Biomédica. 2011;**31**:100-107

[20] Villa NA, Castaño D, Duque PC, Ceballos A. Glutatione peroxidase and superoxide dismutase activities in blood and seminal plasma in colombian stallions. Revista Colombiana de Ciencias Pecuarias. 2012;**25**:64-70

[21] Santiani A. Uso de Antioxidantes para mejorar la calidad de semen criopreservado. Spermova. 2013;**3**(2):154-157

[22] Zhong RZ, Zhou DW. Oxidative stress and role of natural plant derived antioxidants in animal reproduction. Journal of Integrative Agriculture. 2013;**12**(10):1826-1838

[23] Orozco BMG, Navarrete MR, Murray NR, Curiel PE. Efecto de la temperatura en el proceso de criopreservación, sobre la motilidad progresiva del espermatozoide de cerdo. Revista Educatecnociencia. 2014;**4**(59):53-64

[24] Paparella CV, Pavesi AB, Feldman RN, Bouvet BR. Importancia de la evaluación del estrés oxidativo en el semen humano. Archivio di Medicina Interna. 2015;**37**(1):7-14

[25] Álvarez Rodríguez M, Vicente Carrillo A, Rodríguez Martínez H. Exogenous individual lecthin-phospholipids (Phosphatidylchoine and Phosphatidylglycerol) cannot prevent the oxidative stress imposed by criopreservation of boar sperm. Journal of Veterinary Medicine and Surgery. 2017;**1**(2):1-11

[26] Domínguez CO, Toledano OÁ, Ávalos RA. Efecto del suplemento de astaxantina sobre la calidad seminal en *Moenkhausia sanctaefiloenae* (Teleostei: Characidae). Latin American Journal of Aquatic Research. 2015;**43**(1):215-221

[27] Pérez MS. Óxido nítrico sintetasa y nitración en tirosina en la astenozoospermia: Un estudio inmunológico. Revista Saegre. 2012;**19**(3):57.59

[28] Pulgar PEA. Sistemas transportadores de vitamina C en células espermatogénicas y espermatozoides [tesis de grado presentada como parte de los requisitos para optar al grado de Licenciado en bioquímica y al título profesional de bioquímico]. Facultad de Ciencias, Universidad Austral de Chile; 2009. pp. 1-192

[29] Thongrueang N, Chaibangyang N, Chanapiwat P, Kaeoket K. Effects of adding melatonin on the quality of frozen-thawed boar semen. Journal of Applied Animal Science. 2017;**10**(2):47-56

[30] Ramón MMO. Estudio de la actividad aminopeptidásica en espermatozoide astenozoospérmicos. Comparación clínica [tesis doctoral]. Universidad del País Vasco, Facultad de Medicina y Odontología, Departamento de Fisiología; 2014. pp. 1-249

[31] Flores C, Vilanova L. Metabolismo espermático. Gaceta de Ciencias Veterinarias. 2015;**20**(1):23-32

[32] Ortega FC. Factores implicados en la variabilidad individual en la respuesta a la congelación del eyaculado equino: Estructura de subpoblaciones, estrés oxidativo y cambios apoptóticos [tesis doctoral]. Universidad de Extremadura, Facultad de veterinaria, Departamento de Medicina Animal; 2011. pp. 1-176

[33] Gupta S, Goldberg Jeffrey M, Aziz N, Goldberg E, Krajcir N, Agarwal A. Mecanismos patogénicos de la infertilidad asociada con endometriosis. Revista Mexicana de Medicina de la Reproducción. 2010;**3**(2):83-97

[34] Córdova JCA. Control de la peroxidación lipídica del semen refrigerado y criopreservado de verraco mediante antioxidantes (α-Tocoferol/ Glutatión reducido) y su repercusión sobre la calidad espermática [tesis doctoral]. Universidad de León. Facultad de Veterinaria. Departamento de Sanidad Animal; 2010. pp. 1-290

[35] Birben E, Murat SU, Sackesen C, Erzyrum S, Kalayci O. Oxidative stress and antioxidant defense. The World Allergy Organization Journal. 2012;**5**(1):9-19

[36] Mayorga TJM, Peña B, Cadavid ÁP, Walter CM. La importancia clínica del ADN espermática en el análisis seminal cotidiano. Revista Chilena de Obstetricia y Ginecología. 2010;**80**(3):256-268

[37] Leyland F. Assessment of ageing-dependent effects on sperm functions following semen cryopreservation. Veterinary Medicine—Open Journal. 2017;**2**(2):1-2

[38] Palma C, Vinay BJI. Infertilidad masculina. Revista Médica Clínica Las Condes. 2014;**25**(1):122-128

[39] Córdova IA, Iglesias RAE, Espinosa CR, Guerra LJE, Villa MAE, Huerta CR, et al. Effect of addition of antioxidants in the extender to freeze boar in two types of straws on sperm quality. International Journal of Recent Scientific Research. 2017;**8**(6):17466-17468

[40] Castro C, Márquez A. Uso de antioxidantes en animales domésticos. Gaceta de Ciencias Veterinarias. 2006;**12**(1):5-12

[41] Quiles A. Efecto del selenio en la producción porcina. Mundo Ganadero. 2008;**8**:42-44

[42] Reinoso V, Soto C. Importancia de la vitamina E y el selenio en vacas lecheras. In: Artigas, Uruguay. Sitio Argentino de Producción Animal, 1-3. 2009. Obtenido de: http://www.produccion-animal.com.ar/suplementacion_mineral/104-Vit_E_y_Se.pdf