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Papel da mitocôndria na homeostase oxidativa e na funcionalidade de espermatozoides ovinos submetidos à criopreservação

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RESUMO

LOSANO, J. D. A. **Papel da mitocôndria na homeostase oxidativa e na funcionalidade de espermatozoides ovinos submetidos à criopreservação**. [Role of mitochondria in oxidative homeostasis and functionality of ram sperm submitted to cryopreservation]. 2016. 111 f. Tese (Doutorado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2016.

Estudos têm demonstrado a importância da mitocôndria para a funcionalidade do espermatozoide, referindo-a como a principal fonte de energia para a motilidade e a homeostase celular. No entanto, para algumas espécies animais, estudos recentes indicam que a glicólise parece ser o principal mecanismo de produção de ATP para a motilidade espermática, superior à fosforilação oxidativa. Em ovinos estudos envolvendo o metabolismo energético do espermatozoide são necessários não apenas pelo seu interesse zootécnico, mas também como modelo experimental para bovino, espécie na qual este mecanismo é também pouco conhecido. Apesar da importância da mitocôndria para o metabolismo celular durante a fosforilação oxidativa, são produzidos metabólitos denominados Espécies Reativas de Oxigênio, as quais possuem um papel fundamental em diversos processos fisiológicos. No entanto, um eventual desequilíbrio entre a produção de EROs e os mecanismos antioxidantes caracteriza o estresse oxidativo, que pode ser letal para as células espermáticas. Ademais, estudos anteriores relacionam as disfunções mitocondriais causadas pela criopreservação espermática ao estresse oxidativo e a diminuição da atividade mitocondrial. Desta forma, acreditamos que injúrias mitocondriais durante a criopreservação são a origem da produção excessiva de fatores pró-oxidativos e, em última análise, causadores dos danos espermáticos pós-descongelação e diminuição da motilidade. Em face do exposto, a hipótese central do presente experimento é que o espermatozoide ovino, após despolarização mitocondrial por desacoplamento da fosforilazação oxidativa e suplementação para a glicólise, é capaz de manter a produção de ATP e, consequentemente, a motilidade espermática. Ainda, um leve desacoplamento mitocondrial é benéfico para os espermatozoides durante a criopreservação por diminuir as crioinjúrias mediadas por disrupções mitocondriais. Em relação aos nossos estudos de fisiologia, observamos no experimento 1 que os espermatozoides ovinos, mesmo apresentando suas mitocôndrias despolarizadas são capazes de manter a motilidade total. Este

resultado nos sugere que a via glicolítica possivelmente é capaz de manter a motilidade espermática. Por outro lado, o desacolpamento mitocondrial alterou os padrões do movimento espermático, nos sugerindo que a mitocôndria possui um papel mais importante na qualidade do movimento espermático do que na motilidade total. Ainda, no experimento 2 observamos que a via glicolítica, após ser estimulada, é capaz de manter os níveis de ATP, os padrões de cinética espermática e a homeostase oxidativa dos espermatozoides epididimários bovinos submetidos ao desacoplamento mitocondrial. Em relação ao nosso estudo aplicado (experimento 3), observamos que os espermatozoides ovinos criopreservados submetidos à um leve desacoplamento mitocondrial concomitantemente à estimulação da via glicolítica apresentaram maior motilidade, menor peroxidação lipídica, menor susceptibilidade da cromatina à denaturação ácida e maior potencial de membrana mitocondrial. Estes resultados nos indicam que um leve desacoplamento mitocondrial durante a criopreservação espermática é capaz de proteger as mitocôndrias contra as crioinjúrias e consequentemente melhorar a qualidade espermática pós-descongelação.

Palavras-chave: Espermatozoides. Ruminantes. Metabolismo espermático. Glicólise. Fosforilação oxidativa

ABSTRACT

LOSANO, J. D. A. **Role of mitochondria in oxidative homeostasis and functionality of ram sperm submitted to cryopreservation**. [Papel da mitocôndria na homeostase oxidativa e na funcionalidade de espermatozoides ovinos submetidos à criopreservação]. 2016. 111 f. Tese (Doutorado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2016.

Studies have demonstrated the importance of mitochondria in the sperm functionality, referring to it as the main source of energy for motility and cellular homeostasis. However, for some animal species, recent studies indicate that glycolysis seems to be the main mechanism ATP production for sperm motility, higher than the oxidative phosphorylation. In ovine studies involving energy metabolism of sperm are required not only for their livestock interest, but also as an experimental model for bovine species in which this mechanism is also unknown. Despite the importance of mitochondria for cellular metabolism during oxidative phosphorylation, they are produced metabolites called reactive oxygen species, which have a key role in many physiological processes. However, any imbalance between ROS and antioxidant mechanisms characterizes oxidative stress, which may be lethal for the sperm cells. Moreover, previous studies relate to mitochondrial dysfunction caused by oxidative stress on sperm cryopreservation and decreased mitochondrial activity. Thus, we believe that mitochondrial injury during cryopreservation are the source of excessive production of pro-oxidative factors and ultimately, causing the post-thaw sperm damage and decrease in motility. In view of the above, the central hypothesis of this experiment is that the ovine sperm after mitochondrial depolarization by uncoupling of oxidative phosphorylation and glycolysis supplementation is capable of maintaining the ATP production and consequently sperm motility. Additionally, a mild mitochondrial uncoupling is beneficial for spermatozoa during cryopreservation by decreasing the cryoinjuries mediated by mitochondrial disruption. Regarding our physiology studies, we observed in experiment 1 that the ovine sperm, even with their depolarized mitochondria are able to maintain total motility. This result suggests that the glycolytic pathway is possibly able to maintain motility. Moreover, the fact that mitochondrial uncoupling altered sperm movement patterns suggests that mitochondria has a more important role in the quality of sperm kinetic than the total motility. Furthermore, in the experiment 2 we observed that glycolytic pathway, after being stimulated, is able to

maintain ATP levels, sperm kinetics patterns and oxidative homeostasis of bovine epididymal spermatozoa submitted to mitochondrial uncoupling. Regarding our applied study (Experiment 3), we observed that cryopreserved ovine sperm submitted to mild mitochondrial uncoupling concurrently with glycolysis stimulation showed increased motility, lower lipid peroxidation, lower susceptibility of chromatin to acid denaturation and higher mitochondrial membrane potential. These results indicate that a slight mitochondrial uncoupling during sperm cryopreservation can protect mitochondria against cryoinjuries and hence improve the post-thaw spermatozoa quality.

Keywords: Spermatozoa. Ruminants. Sperm metabolism. Glycolysis. Oxidative Phosphorylation

1 INTRODUCTION

The nuclear power plant Chernobyl, located in the Ukraine and considered a worldwide reference on energy production, was capable of generating an amount of four megawatts of electric energy. In 1986, a serious accident in reactor no. 4 led to release of radioactive material equivalent to 400 times than was observed in the atomic bombing of Hiroshima. As a result, approximately 3.900.000 Km^2 of the European and Asian continents were contaminated with cesium - 137 (FAIRLIE; SUMNER, 2006). Despite the obvious difficulties on estimating the casualties directly or indirectly linked to the accident (FAIRLIE; SUMNER, 2006), millions of people were exposed to radioactive material leading to high incidence of mutation, several types of cancer, especially in the thyroid (KAZAKOV; DEMIDCHIK; ASTAKHOVA, 1992; KLUGBAUER et al., 1995), as well as infant leukemia after intrauterine exposure (PETRIDOU et al., 1996). Until now, some areas near the power plant cannot be inhabited due to isotopes still present in the environment.

Similarly to a nuclear power plant, mitochondria exhibit high energy production capacity; however, in situations which the structure of this organelle is compromised, the potential to release extremely toxic products is also injurious. Such toxic substances may lead to damages in the surrounding cells and other tissues. In fact, several studies have linked mitochondrial dysfunction to some pathological conditions such as neurodegenerative diseases (LIN; BEAL, 2006), type 2 diabetes (LOWELL; SHULMAN, 2005) and neoplasia (MODICA-NAPOLITANO; SINGH, 2004).

In relation to the spermatozoa, several studies have referred mitochondria as the main source of energy, also playing important role on the cellular homeostasis maintenance and motility (TRAVIS et al., 1998; ST. JOHN, 2002). However, for some species, evidences suggest that glycolysis may be the main source of ATP production for sperm motility, superior to oxidative phosphorylation (MUKAI; OKUNO, 2004; FORD, 2006; NASCIMENTO et al., 2008).

Despite the importance of mitochondria to sperm metabolism, during oxidative phosphorylation are produced metabolites called reactive oxygen species (ROS), substances with important role on several reproductive physiological mechanisms (DE LAMIRANDE et al., 1997). Nevertheless, an unbalance between ROS

production and mechanisms aiming to avoid their powerful oxidative potential (i.e., antioxidants), may be extremely harmful to the spermatozoa (HALLIWELL, 1999; NICHI et al., 2007b).

As the main source of pro-oxidative factors, mitochondria has been found as crucial on the disruption of oxidative homeostasis (AGARWAL et al., 2014). In fact, several studies have demonstrated correlations between impaired mitochondrial activity with both oxidative stress and sperm DNA fragmentation, indicating a close relationship between these variables on the sperm damage pathogenesis (BARROS, 2007; NICHI et al., 2007a; BLUMER et al., 2012).

Since the Chernobyl accident, the main concern of nuclear energy specialists and the community in general is on the approaches to avoid the destruction caused by an eventual nuclear disaster. If it was possible, the deactivation of the power plant would probably avoid most of the damages prior a predictable stressful event. Similarly, the reversible inhibition of mitochondrial activity in situations where this organelle dysfunction is known (i.e., sperm cryopreservation) (O'CONNELL; MCCLURE; LEWIS, 2002; SARIOZKAN et al., 2009; THOMSON et al., 2009) would probably improve sperm viability by decreasing the amount of pro-oxidative factors available for release. Actually, a few studies have suggested that, for some cellular types, uncouplers of the oxidative phosphorylation are capable of reducing oxidative stress (VINCENT et al., 2004; MAILLOUX; HARPER, 2011).

This review aims to provide a brief introduction to cellular respiration, compile literature data about the role of mitochondria in oxidative homeostasis and sperm functionality as well as suggest some tools to assess sperm mitochondrial function.

6 CONCLUSION

In conclusion, we observed that the glycolytic pathway is as important as oxidative phosphorylation for motility and ram sperm functionality. On the other hand, oxidative phosphorylation seems to have more influence in the sperm movement patterns than motility. In addition, we verified that the glycolytic pathway, after stimulation, is able to maintain sperm kinetic patterns, ATP levels and oxidative homeostasis of bovine epididymal spermatozoa submitted to mitochondrial uncoupling. Furthermore, we observed that the mitochondrial uncoupling associated with the glycolysis stimulation during the ovine sperm cryopreservation prevents oxidative injuries and then improving the post-thawing sperm quality.

REFERENCES

AGARWAL, A.; NALLELLA, K. P.; ALLAMANENI, S. S. R.; SAID, T. M. Role of antioxidants in treatment of male infertility: an overview of the literature. **Reproductive BioMedicine Online**, v. 8, n. 6, p. 616-627, 2004.

AGARWAL, A.; VIRK, G.; ONG, C.; DU PLESSIS, S. S. Effect of Oxidative Stress on Male Reproduction. **World J Mens Health**, v. 32, n. 1, p. 1-17, 2014.

AITKEN, R. J.; PATERSON, M.; FISHER, H.; BUCKINGHAM, D. W.; VAN DUIN, M. Redox regulation of tyrosine phosphorylation in human spermatozoa and its role in the control of human sperm function. **Journal of Cell Science**, v. 108, n. 5, p. 2017-2025, 1995.

AITKEN, R. J.; RYAN, A. L.; BAKER, M. A.; MCLAUGHLIN, E. A. Redox activity associated with the maturation and capacitation of mammalian spermatozoa. **Free Radical Biology and Medicine**, v. 36, n. 8, p. 994-1010, 2004.

AITKEN, R. J. Sperm function tests and fertility. **International Journal of Andrology**, v. 29, n. 1, p. 69-75, 2006.

ALVAREZ, J. G.; TOUCHSTONE, J. C.; BLASCO, L.; STOREY, B. T. Spontaneous Lipid Peroxidation and Production of Hydrogen Peroxide and Superoxide in Human Spermatozoa Superoxide Dismutase as Major Enzyme Protectant Against Oxygen Toxicity. **Journal of Andrology**, v. 8, n. 5, p. 338-348, 1987.

AMARAL, A.; LOURENÇO, B.; MARQUES, M.; RAMALHO-SANTOS, J. Mitochondria functionality and sperm quality. **Reproduction**, v. 146, n. 5, p. R163-R174, 2013.

ASKARI, H. A.; CHECK, J. H.; PEYMER, N.; BOLLENDORF, A. Effect of Natural Antioxidants Tocopherol and Ascorbic Acids in Maintenance of Sperm Activity During Freeze-Thaw Process. **Systems Biology in Reproductive Medicine**, v. 33, n. 1, p. 11-15, 1994.

BARROS, P. **Estresse oxidativo e integridade do DNA em sêmen resfriado de gato-do-mato-pequeno (Leopardus tigrinus, SCHREBER, 1775)**. 2007. 120 p. Tese (Doutorado em Reprodução Animal) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2007.

BILODEAU, J.-F.; CHATTERJEE, S.; SIRARD, M.-A.; GAGNON, C. Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing

and thawing. **Molecular Reproduction and Development**, v. 55, n. 3, p. 282-288, 2000.

BILODEAU, J. F.; BLANCHETTE, S.; GAGNON, C.; SIRARD, M. A. Thiols prevent H2O2-mediated loss of sperm motility in cryopreserved bull semen. **Theriogenology**, v. 56, n. 2, p. 275-286, 2001.

BLUMER, C. G.; RESTELLI, A. E.; GIUDICE, P. T. D.; SOLER, T. B.; FRAIETTA, R.; NICHI, M.; BERTOLLA, R. P.; CEDENHO, A. P. Effect of varicocele on sperm function and semen oxidative stress. **BJU International**, v. 109, n. 2, p. 259-265, 2012.

BRAND, M. D.; ESTEVES, T. C. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. **Cell Metabolism**, v. 2, n. 2, p. 85-93, 2005.

BREITBART, H. Intracellular calcium regulation in sperm capacitation and acrosomal reaction. **Molecular and Cellular Endocrinology**, v. 187, n. 1–2, p. 139-144, 2002.

BREWIS, I. A.; MORTON, I. E.; MOHAMMAD, S. N.; BROWES, C. E.; MOORE, H. D. M. Measurement of Intracellular Calcium Concentration and Plasma Membrane Potential in Human Spermatozoa Using Flow Cytometry. **Journal of Andrology**, v. 21, n. 2, p. 238-249, 2000.

CELEGHINI, E. C. C.; DE ARRUDA, R. P.; DE ANDRADE, A. F. C.; NASCIMENTO, J.; RAPHAEL, C. F. Practical Techniques for Bovine Sperm Simultaneous Fluorimetric Assessment of Plasma, Acrosomal and Mitochondrial Membranes. **Reproduction in Domestic Animals**, v. 42, n. 5, p. 479-488, 2007.

CHEN, L. B. Mitochondrial membrane potential in living cells. **Annual review of cell biology**, v. 4, n. 1, p. 155-181, 1988.

CHRISTEN, R.; SCHACKMANN, R. W.; SHAPIRO, B. M. Metabolism of sea urchin sperm. Interrelationships between intracellular pH, ATPase activity, and mitochondrial respiration. **Journal of Biological Chemistry**, v. 258, n. 9, p. 5392-5399, 1983.

COPELAND, W. C. **Mitochondrial DNA**: Springer, 2002
CUMMINS, J. Mitochondrial DNA in mammalian reproduction. **Reviews of Reproduction**, v. 3, n. 3, p. 172-182, 1998.

DE J GARCÍA-RIVAS, G.; CARVAJAL, K.; CORREA, F.; ZAZUETA, C. Ru360, a specific mitochondrial calcium uptake inhibitor, improves cardiac post-ischaemic

functional recovery in rats in vivo. **British Journal of Pharmacology**, v. 149, n. 7, p. 829-837, 2006.

DE LAMIRANDE, E.; CAGNON, C. Human sperm hyperactivation and capacitation as parts of an oxidative process. **Free Radical Biology and Medicine**, v. 14, n. 2, p. 157-166, 1993.

DE LAMIRANDE, E.; JIANG, H.; ZINI, A.; KODAMA, H.; GAGNON, C. Reactive oxygen species and sperm physiology. **Reviews of Reproduction**, v. 2, n. 1, p. 48-54, 1997.

DE LAMIRANDE, E. V. E.; TSAI, C.; HARAKAT, A.; GAGNON, C. Involvement of Reactive Oxygen Species in Human Sperm Arcosome Reaction Induced by A23187, Lysophosphatidylcholine, and Biological Fluid Ultrafiltrates. **Journal of Andrology**, v. 19, n. 5, p. 585-594, 1998.

DORWEILER, B.; PRUEFER, D.; ANDRASI, T.; MAKSAN, S.; SCHMIEDT, W.; NEUFANG, A.; VAHL, C. Ischemia-Reperfusion Injury. **European Journal of Trauma and Emergency Surgery**, v. 33, n. 6, p. 600-612, 2007.

FAIRLIE, I.; SUMNER, D. **The other report on Chernobyl (TORCH)**: secondary title: Altner Combecher Foundation, Berlin, 2006.

FERNÁNDEZ-SANTOS, M. R.; MARTÍNEZ-PASTOR, F.; GARCÍA-MACÍAS, V.; ESTESO, M. C.; SOLER, A. J.; PAZ, P.; ANEL, L.; GARDE, J. J. Sperm Characteristics and DNA Integrity of Iberian Red Deer (Cervus elaphus hispanicus) Epididymal Spermatozoa Frozen in the Presence of Enzymatic and Nonenzymatic Antioxidants. **Journal of Andrology**, v. 28, n. 2, p. 294-305, 2007.

FERREIRA, A.; MATSUBARA, L. Radicais livres: conceitos, doenças relacionadas, sistema de defesa e estresse oxidativo. **Revista da Associação Médica Brasileira**, v. 43, n. 1, p. 61-68, 1997.

FORD, W. C. L. Glycolysis and sperm motility: does a spoonful of sugar help the flagellum go round? **Human Reproduction Update**, v. 12, n. 3, p. 269-274, 2006.

GALLON, F.; MARCHETTI, C.; JOUY, N.; MARCHETTI, P. The functionality of mitochondria differentiates human spermatozoa with high and low fertilizing capability. **Fertility and Sterility**, v. 86, n. 5, p. 1526-1530, 2006.

GARNER, D. L.; THOMAS, C. A.; JOERG, H. W.; DEJARNETTE, J. M.; MARSHALL, C. E. Fluorometric assessments of mitochondrial function and viability in

cryopreserved bovine spermatozoa. **Biology of Reproduction**, v. 57, n. 6, p. 1401-1406, 1997.

GILLAN, L.; EVANS, G.; MAXWELL, W. M. C. Flow cytometric evaluation of sperm parameters in relation to fertility potential. **Theriogenology**, v. 63, n. 2, p. 445-457, 2005.

GIOJALAS, L. C. Correlation between response to progesterone and other functional parameters in human spermatozoa. **Fertility and Sterility**, v. 69, n. 1, p. 107-111, 1998.

GRAHAM, J. K.; KUNZE, E.; HAMMERSTEDT, R. H. Analysis of sperm cell viability, acrosomal integrity, and mitochondrial function using flow cytometry. **Biology of Reproduction**, v. 43, n. 1, p. 55-64, 1990.

GUNTER, T. E.; YULE, D. I.; GUNTER, K. K.; ELISEEV, R. A.; SALTER, J. D. Calcium and mitochondria. **FEBS Letters**, v. 567, n. 1, p. 96-102.

HALLIWELL, B.; GUTTERIDGE, J. **Free radicals in biology and medicine.** secondary title: Pergamon, 1985.

HALLIWELL, B. **Free radicals in biology and medicine.** Oxford: University Press, 1999.

HAMMERSTEDT, R. H.; GRAHAM, J. K.; NOLAN, J. P. Cryopreservation of Mammalian Sperm: What We Ask Them to Survive. **Journal of Andrology**, v. 11, n. 1, p. 73-88, 1990.

HARRISON, R. A. P.; MAIRET, B.; MILLER, N. G. A. Flow cytometric studies of bicarbonate-mediated Ca2+ influx in boar sperm populations. **Molecular Reproduction and Development**, v. 35, n. 2, p. 197-208, 1993.

HOLT, W. V. Fundamental aspects of sperm cryobiology: The importance of species and individual differences. **Theriogenology**, v. 53, n. 1, p. 47-58, 2000.

HRUDKA, F. Cytochemical and ultracytochemical demonstration of cytochrome c oxidase in spermatozoa and dynamics of its changes accompanying ageing or induced by stress. **International Journal of Andrology**, v. 10, n. 6, p. 809-828, 1987.

IRVINE, D. S.; AITKEN, R. J. Measurement of intracellular calcium in human spermatozoa. **Gamete research**, v. 15, n. 1, p. 57-71, 1986.

JOHN, J. C. S.; JOKHI, R. P.; BARRATT, C. L. R. The impact of mitochondrial genetics on male infertility. **International Journal of Andrology**, v. 28, n. 2, p. 65-73, 2005.

KASAI, T.; OGAWA, K.; MIZUNO, K.; NAGAI, S.; UCHIDA, Y.; OHTA, S.; FUJIE, M.; SUZUKI, K.; HIRATA, S.; HOSHI, K. Relationship between sperm mitochondrial membrane potential, sperm motility, and fertility potential. **Asian journal of andrology**, v. 4, n. 2, p. 97-104, 2002.

KASIANOWICZ, J.; BENZ, R.; MCLAUGHLIN, S. The kinetic mechanism by which CCCP (carbonyl cyanidem-Chlorophenylhydrazone) transports protons across membranes. **The Journal of Membrane Biology**, v. 82, n. 2, p. 179-190, 1984.

KAZAKOV, V. S.; DEMIDCHIK, E. P.; ASTAKHOVA, L. N. Thyroid cancer after Chernobyl. **Nature**, v. 359, n. 6390, p. 21, 1992.

KLUGBAUER, S.; LENGFELDER, E.; DEMIDCHIK, E.; RABES, H. High prevalence of RET rearrangement in thyroid tumors of children from Belarus after the Chernobyl reactor accident. **Oncogene**, v. 11, n. 12, p. 2459-2467, 1995.

KOPPERS, A. J.; DE IULIIS, G. N.; FINNIE, J. M.; MCLAUGHLIN, E. A.; AITKEN, R. J. Significance of mitochondrial reactive oxygen species in the generation of oxidative stress in spermatozoa. **The Journal of Clinical Endocrinology and Metabolism**, v. 93, n. 8, p. 3199-3207, 2008.

KRZYZOSIAK, J.; MOLAN, P.; VISHWANATH, R. Measurements of bovine sperm velocities under true anaerobic and aerobic conditions. **Animal Reproduction Science**, v. 55, n. 3–4, p. 163-173, 1999.

LARDY, H.; WINCHESTER, B.; PHILLIPS, P. The respiratory metabolism of ram spermatozoa. **Archives of Biochemistry**, v. 6, n. 1, p. 33-40, 1945.

LIN, M. T.; BEAL, M. F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. **Nature**, v. 443, n. 7113, p. 787-795, 2006.

LOWELL, B. B.; SHULMAN, G. I. Mitochondrial Dysfunction and Type 2 Diabetes. **Science**, v. 307, n. 5708, p. 384-387, 2005.

MACHADO-OLIVEIRA, G.; LEFIÈVRE, L.; FORD, C.; HERRERO, M. B.; BARRATT, C.; CONNOLLY, T. J.; NASH, K.; MORALES-GARCIA, A.; KIRKMAN-BROWN, J.; PUBLICOVER, S. Mobilisation of Ca2+ stores and flagellar regulation in human

sperm by S-nitrosylation: a role for NO synthesised in the female reproductive tract. **Development**, v. 135, n. 22, p. 3677-3686, 2008.

MAILLOUX, R. J.; HARPER, M.-E. Uncoupling proteins and the control of mitochondrial reactive oxygen species production. **Free Radical Biology and Medicine**, v. 51, n. 6, p. 1106-1115, 2011.

MARCHETTI, C.; OBERT, G.; DEFFOSEZ, A.; FORMSTECHER, P.; MARCHETTI, P. Study of mitochondrial membrane potential, reactive oxygen species, DNA fragmentation and cell viability by flow cytometry in human sperm. **Human Reproduction**, v. 17, n. 5, p. 1257-1265, 2002.

MARCHETTI, C.; JOUY, N.; LEROY-MARTIN, B.; DEFOSSEZ, A.; FORMSTECHER, P.; MARCHETTI, P. Comparison of four fluorochromes for the detection of the inner mitochondrial membrane potential in human spermatozoa and their correlation with sperm motility. **Human Reproduction**, v. 19, n. 10, p. 2267-2276, 2004.

MARGULIS, L. Origin of eukaryotic cells: evidence and research implications for a theory of the origin and evolution of microbial, plant, and animal cells on the Precambrian earth. Yale: University Press New Haven, 1970.

MCCORMACK, J. G.; HALESTRAP, A. P.; DENTON, R. M. Role of calcium ions in regulation of mammalian intramitochondrial metabolism. **Physiology Reviews**, v. 70, n. 2, p. 391-425, 1990.

MCCORMACK, J. G.; DENTON, R. M. Mitochondrial Ca²⁺ Transport and the Role of Intramitochondrial Ca²⁺ in the Regulation of Energy Metabolism. **Developmental Neuroscience**, v. 15, n. 3-5, p. 165-173, 1993.

MEIJER, J.; FENTENER VAN VLISSINGEN, J. Gross structure and development of reproductive organs. In: KING, G. J. **Reproduction in domesticated animals.** Amsterdam: Elsevier Science Publishers, v., n., p. 9-26, 1993. (Wold Animal Science-B9).

MODICA-NAPOLITANO, J. S.; SINGH, K. K. Mitochondrial dysfunction in cancer. **Mitochondrion**, v. 4, n. 5–6, p. 755-762, 2004.

MUKAI, C.; OKUNO, M. Glycolysis plays a major role for adenosine triphosphate supplementation in mouse sperm flagellar movement. **Biology of Reproduction**, v. 71, n. 2, p. 540-547, 2004.

NASCIMENTO, J. M.; SHI, L. Z.; TAM, J.; CHANDSAWANGBHUWANA, C.; DURRANT, B.; BOTVINICK, E. L.; BERNS, M. W. Comparison of glycolysis and oxidative phosphorylation as energy sources for mammalian sperm motility, using the

combination of fluorescence imaging, laser tweezers, and real-time automated tracking and trapping. **Journal of Cellular Physiology**, v. 217, n. 3, p. 745-751, 2008.

NELSON, D. L.; COX, M. M. **Principles of biochemistry.** 5. ed. New York, NY: W.H. Freeman and Company, 2008.

NEVO, A. C.; RIKMENSPOEL, R. Diffusion of ATP in sperm flagella. **Journal of Theoretical Biology**, v. 26, n. 1, p. 11-18, 1970.

NICHI, M.; BOLS, P. E. J.; ZÜGE, R. M.; BARNABE, V. H.; GOOVAERTS, I. G. F.; BARNABE, R. C.; CORTADA, C. N. M. Seasonal variation in semen quality in Bos indicus and Bos taurus bulls raised under tropical conditions. **Theriogenology**, v. 66, n. 4, p. 822-828, 2006.

NICHI, M.; GOOVAERTS, I. G. F.; CORTADA, C. N. M.; BARNABE, V. H.; DE CLERCQ, J. B. P.; BOLS, P. E. J. Roles of lipid peroxidation and cytoplasmic droplets on in vitro fertilization capacity of sperm collected from bovine epididymides stored at 4 and 34 °C. **Theriogenology**, v. 67, n. 2, p. 334-340, 2007a.

NICHI, M.; GOOVAERTS, I. G.; CORTADA, C. N.; BARNABE, V. H.; DE CLERCQ, J. B.; BOLS, P. E. Roles of lipid peroxidation and cytoplasmic droplets on in vitro fertilization capacity of sperm collected from bovine epididymides stored at 4 and 34 degrees C. **Theriogenology**, v. 67, n. 2, p. 334-340, 2007b.

NORDBERG, J.; ARNÉR, E. S. J. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. **Free Radical Biology and Medicine**, v. 31, n. 11, p. 1287-1312, 2001.

O'CONNELL, M.; MCCLURE, N.; LEWIS, S. E. M. The effects of cryopreservation on sperm morphology, motility and mitochondrial function. **Human Reproduction**, v. 17, n. 3, p. 704-709, 2002.

PAUL, C.; TENG, S.; SAUNDERS, P. T. K. A Single, Mild, Transient Scrotal Heat Stress Causes Hypoxia and Oxidative Stress in Mouse Testes, Which Induces Germ Cell Death. **Biology of Reproduction**, v. 80, n. 5, p. 913-919, 2009.

PENEFSKY, H. S. Mechanism of inhibition of mitochondrial adenosine triphosphatase by dicyclohexylcarbodiimide and oligomycin: relationship to ATP synthesis. **Proceedings of the National Academy of Sciences**, v. 82, n. 6, p. 1589-1593, 1985.

PERCHEC, G.; JEULIN, C.; COSSON, J.; ANDRE, F.; BILLARD, R. Relationship between sperm ATP content and motility of carp spermatozoa. **Journal of Cell Science**, v. 108, n. 2, p. 747-753, 1995.

PETRIDOU, E.; TRICHOPOULOS, D.; DESSYPRIS, N.; FLYTZANI, V.; HAIDAS, S.; KALMANTI, M.; KOLIOUSKAS, D.; KOSMIDIS, H.; PIPEROPOULOU, F.; TZORTZATOU, F. Infant leukaemia after in utero exposure to radiation from Chernobyl. **Nature**, v. 382, n. 6589, p. 352-353, 1996.

PICCOLI, C.; SCRIMA, R.; D'APRILE, A.; RIPOLI, M.; LECCE, L.; BOFFOLI, D.; CAPITANIO, N. Mitochondrial dysfunction in hepatitis C virus infection. **Biochimica et Biophysica Acta (BBA)-Bioenergetics**, v. 1757, n. 9, p. 1429-1437, 2006.

POOT, M.; ZHANG, Y. Z.; KRÄMER, J. A.; WELLS, K. S.; JONES, L. J.; HANZEL, D. K.; LUGADE, A. G.; SINGER, V. L.; HAUGLAND, R. P. Analysis of mitochondrial morphology and function with novel fixable fluorescent stains. **Journal of Histochemistry & Cytochemistry**, v. 44, n. 12, p. 1363-1372, 1996.

RAMALHO-SANTOS, J.; VARUM, S.; AMARAL, S.; MOTA, P. C.; SOUSA, A. P.; AMARAL, A. Mitochondrial functionality in reproduction: from gonads and gametes to embryos and embryonic stem cells. **Human Reproduction Update**, v. 15, n. 5, p. 553-572, 2009.

REYES, J. G.; FARIAS, J. G.; HENRÍQUEZ-OLAVARRIETA, S.; MADRID, E.; PARRAGA, M.; ZEPEDA, A. B.; MORENO, R. D. The hypoxic testicle: physiology and pathophysiology. **Oxidative Medicine and Cellular Longevity**, v. 2012, n., p., 2012.

ROWE, M.; LASKEMOEN, T.; JOHNSEN, A.; LIFJELD, J. T. Evolution of sperm structure and energetics in passerine birds. **Proceedings of the Royal Society B: Biological Sciences**, v. 280, n. 1753, p., 2013.

RUIZ-PESINI, E.; LAPEÑA, A.-C.; DÍEZ-SÁNCHEZ, C.; PÉREZ-MARTOS, A.; MONTOYA, J.; ALVAREZ, E.; DÍAZ, M.; URRIÉS, A.; MONTORO, L.; LÓPEZ-PÉREZ, M. J.; ENRÍQUEZ, J. A. Human mtDNA Haplogroups Associated with High or Reduced Spermatozoa Motility. **The American Journal of Human Genetics**, v. 67, n. 3, p. 682-696, 2000.

SAMIZO, K.; ISHIKAWA, R.; NAKAMURA, A.; KOHAMA, K. A Highly Sensitive Method for Measurement of Myosin ATPase Activity by Reversed-Phase High-Performance Liquid Chromatography. **Analytical Biochemistry**, v. 293, n. 2, p. 212-215, 2001.

SARASTE, M. Oxidative Phosphorylation at the fin de siècle. **Science**, v. 283, n. 5407, p. 1488-1493, 1999.

SARIOZKAN, S.; BUCAK, M. N.; TUNCER, P. B.; ULUTAS, P. A.; BILGEN, A. The influence of cysteine and taurine on microscopic-oxidative stress parameters and fertilizing ability of bull semen following cryopreservation. **Cryobiology**, v. 58, n. 2, p. 134-138, 2009.

SCHOBER, D.; AURICH, C.; NOHL, H.; GILLE, L. Influence of cryopreservation on mitochondrial functions in equine spermatozoa. **Theriogenology**, v. 68, n. 5, p. 745-754, 2007.

SHERER, T. B.; BETARBET, R.; TESTA, C. M.; SEO, B. B.; RICHARDSON, J. R.; KIM, J. H.; MILLER, G. W.; YAGI, T.; MATSUNO-YAGI, A.; GREENAMYRE, J. T. Mechanism of toxicity in rotenone models of Parkinson's disease. **The Journal of Neuroscience**, v. 23, n. 34, p. 10756-10764, 2003.

SIMÕES, R.; FEITOSA, W. B.; SIQUEIRA, A. F. P.; NICHI, M.; PAULA-LOPES, F. F.; MARQUES, M. G.; PERES, M. A.; BARNABE, V. H.; VISINTIN, J. A.; ASSUMPÇÃO, M. E. O. Influence of bovine sperm DNA fragmentation and oxidative stress on early embryo in vitro development outcome. **Reproduction**, v. 146, n. 5, p. 433-441, 2013.

SLATER, E. C. The mechanism of action of the respiratory inhibitor, antimycin. **Biochimica et Biophysica Acta (BBA) - Reviews on Bioenergetics**, v. 301, n. 2, p. 129-154, 1973.

ST JOHN, J.; BOWLES, E. J.; AMARAL, A. Sperm mitochondria and fertilisation. **Society of Reproduction and Fertility Supplement**, v. 65, p. 399-416, 2006.

ST. JOHN, J. C. The transmission of mitochondrial DNA following assisted reproductive techniques. **Theriogenology**, v. 57, n. 1, p. 109-123, 2002.

STOREY, B. T. Mammalian sperm metabolism: oxygen and sugar, friend and foe. **International Journal of Developmental Biology**, v. 52, n. 5, p. 427, 2008.

SZALAI, G.; KRISHNAMURTHY, R.; HAJNÓCZKY, G. Apoptosis driven by IP3-linked mitochondrial calcium signals. **The EMBO Journal**, v. 18, n. 22, p. 6349-6361, 1999.

TAYLOR, K.; ROBERTS, P.; SANDERS, K.; BURTON, P. Effect of antioxidant supplementation of cryopreservation medium on post-thaw integrity of human spermatozoa. **Reproductive BioMedicine Online**, v. 18, n. 2, p. 184-189, 2009.

TERADA, H. Uncouplers of oxidative phosphorylation. **Environmental Health Perspectives**, v. 87, n., p. 213, 1990.

TERRITO, P. R.; FRENCH, S. A.; DUNLEAVY, M. C.; EVANS, F. J.; BALABAN, R. S. Calcium activation of heart mitochondrial oxidative phosphorylation: rapid kinetics OFMV˙ O2 , NADH, and light scattering. **Journal of Biological Chemistry**, v. 276, n. 4, p. 2586-2599, 2001.

THOMSON, L. K.; FLEMING, S. D.; AITKEN, R. J.; DE IULIIS, G. N.; ZIESCHANG, J. A.; CLARK, A. M. Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis. **Human Reproduction**, v. 24, n. 9, p. 2061-2070, 2009.

THU, L. T.; AHN, J. R.; WOO, S.-H. Inhibition of L-type Ca2+ channel by mitochondrial Na+–Ca2+ exchange inhibitor CGP-37157 in rat atrial myocytes. **European Journal of Pharmacology**, v. 552, n. 1–3, p. 15-19, 2006.

TRAVIS, A. J.; FOSTER, J. A.; ROSENBAUM, N. A.; VISCONTI, P. E.; GERTON, G. L.; KOPF, G. S.; MOSS, S. B. Targeting of a Germ Cell-specific Type 1 Hexokinase Lacking a Porin-binding Domain to the Mitochondria as Well as to the Head and Fibrous Sheath of Murine Spermatozoa. **Molecular Biology of the Cell**, v. 9, n. 2, p. 263-276, 1998.

TROIANO, L.; GRANATA, A. R. M.; COSSARIZZA, A.; KALASHNIKOVA, G.; BIANCHI, R.; PINI, G.; TROPEA, F.; CARANI, C.; FRANCESCHI, C. Mitochondrial Membrane Potential and DNA Stainability in Human Sperm Cells: A Flow Cytometry Analysis with Implications for Male Infertility. **Experimental Cell Research**, v. 241, n. 2, p. 384-393, 1998.

TURNER, R. M. Tales From the Tail: What Do We Really Know About Sperm Motility? **Journal of Andrology**, v. 24, n. 6, p. 790-803, 2003.

VERNET, P.; AITKEN, R. J.; DREVET, J. R. Antioxidant strategies in the epididymis. **Molecular and Cellular Endocrinology**, v. 216, n. 1–2, p. 31-39, 2004.

VINCENT, A. M.; OLZMANN, J. A.; BROWNLEE, M.; SIVITZ, W. I.; RUSSELL, J. W. Uncoupling Proteins Prevent Glucose-Induced Neuronal Oxidative Stress and Programmed Cell Death. **Diabetes**, v. 53, n. 3, p. 726-734, 2004.

WANG, G.; GUO, Y.; ZHOU, T.; SHI, X.; YU, J.; YANG, Y.; WU, Y.; WANG, J.; LIU, M.; CHEN, X.; TU, W.; ZENG, Y.; JIANG, M.; LI, S.; ZHANG, P.; ZHOU, Q.; ZHENG, B.; YU, C.; ZHOU, Z.; GUO, X.; SHA, J. In-depth proteomic analysis of the human

sperm reveals complex protein compositions. **Journal of Proteomics**, v. 79, p. 114-122, 2013.

WANG, X.; SHARMA, R. K.; GUPTA, A.; GEORGE, V.; THOMAS JR, A. J.; FALCONE, T.; AGARWAL, A. Alterations in mitochondria membrane potential and oxidative stress in infertile men: a prospective observational study. **Fertility and Sterility**, v. 80, p. 844-850, 2003. Supplement 2.

WHITE, I. G.; WALES, R. G. COMPARISON OF EPIDIDYMAL AND EJACULATED SEMEN OF THE RAM. **Journal of Reproduction and Fertility**, v. 2, n. 3, p. 225-237, 1961.

WOJCIK, C.; SAWICKI, W.; MARIANOWSKI, P.; BENCHAIB, M.; CZYBA, J. C.; GUERIN, J. F. Cyclodextrin enhances spermicidal effects of magainin-2-amide. **Contraception**, v. 62, n. 2, p. 99-103, 2000.

ZAPZALKA, D. M.; REDMON, J. B.; PRYOR, J. L. A survey of oncologists regarding sperm cryopreservation and assisted reproductive techniques for male cancer patients. **Cancer**, v. 86, n. 9, p. 1812-1817, 1999.