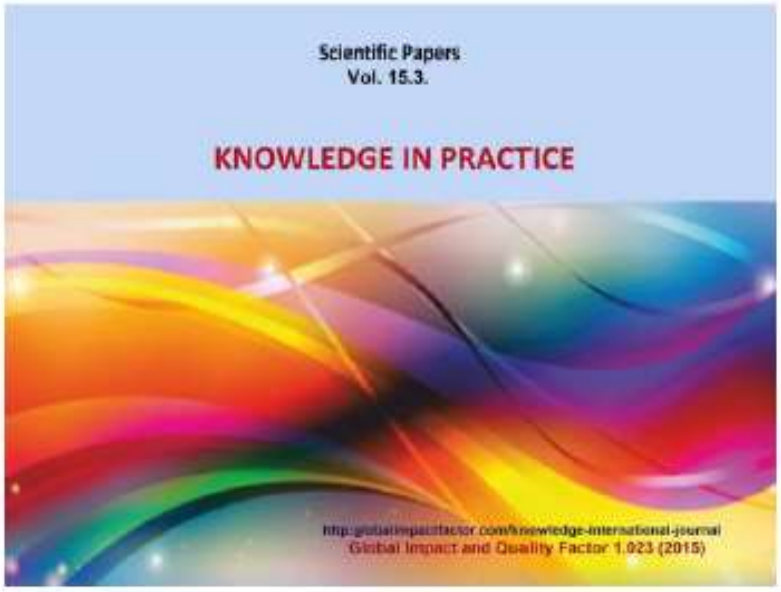
ISSN 1857 - 92

INTERNATIONAL JOURNAL

Institute of Knowledge Management

KNOWLEDGE



KNOWLEDGE

INTERNATIONAL JOURNAL

SCIENTIFIC PAPERS

VOL 15.3

16-18 December, 2016

Bansko, BULGARIA

INSTITUTE OF KNOWLEDGE MANAGEMENT

SKOPJE, MACEDONIA

KNOWLEDGE

International Journal Scientific papers Vol. 15.3

Preparing and correction: Liljana Pushova

Print: GRAFOPROM – Bitola

Editor: IKM – Skopje

For editor
Robert Dimitrovski, PhD

KNOWLEDGE

International Journal Scientific Papers Vol. 15.3

ISSN 1857-92

SCIENTIFIC COMMITTEE

President: Academic, Prof. Vlado Kambovski PhD

- Prof. Robert Dimitrovski PhD, Faculty of Management, MIT University, Skopje (Macedonia)
- Prof. Zivota Radosavljevik PhD, Dean, Faculty FORCUP, Union University, Belgrade (Serbia)
- Prof. Venelin Terziev PhD, University of Rousse (Bulgaria)
- Prof. Paul Sergius Koku PhD, Florida Atlantic University (USA)
- Prof. Cesar Birzea PhD, University of Bucharest (Romania)
- Prof. Rosica Cobanova PhD, Bulgarian Academy of Sciences (Bulgaria)
- Prof. Aleksandar Nikolovski PhD, FON University, Skopje (Macedonia)
- Prof. Anita Trajkovska PhD, Rochester University (USA)
- Prof. Sinisa Zaric, PhD, Faculty of Economics, University of Belgrade (Serbia)
- Prof. Milan Radosavljevic PhD, Dean, Faculty of strategic and operational management, Union University, Belgrade (Serbia)
- Prof. Anka Trajkovska-Petkoska PhD, UKLO, Faculty of technology and technical sciences, Bitola (Macedonia)
- Prof. Predrag Trajkovic PhD, JMPNT, (Serbia)
- Prof. Lidija Tozi PhD, Faculty of Pharmacy, Ss. Cyril and Methodius University, Skopje (Macedonia)
- Prof. Bistra Angelovska, Faculty of Medicine, University "Goce Delcev", Shtip (Macedonia)
- Prof. Misho Hristovski PhD, Faculty of Veterinary Medicine, Ss. Cyril and Methodius University, Skopje (Macedonia)
- Prof. Jove Kekenovski PhD, Faculty of Tourism, UKLO , Bitola (Macedonia)
- Prof. Sasho Korunoski, Dean, Faculty of Tourism, UKLO, Bitola (Macedonia)
- Prof. Cvetko Andreevski, Vise rector, Faculty of Tourism, UKLO, Bitola (Macedonia)
- Prof. Margarita Koleva PhD, Dean, Faculty of Pedagogy, University Neofit Rilski, Blagoevgrad (Bulgaria)
- Prof. Aleksandar Donchev, MIT University, Faculty of Law, Skopje (Macedonia)
- Prof. Maria Kavdanska PhD, Faculty of Pedagogy, University Neofit Rilski, Blagoevgrad (Bulgaria)
- Prof. Mirjana Borota-Popovska, PhD, Centre for Management and Human Resource Development, Institute for Sociological, Political and Juridical Research, Skopje (Macedonia)
- Prof. Marija Topuzovska-Latkovic, PhD, Centre for Management and Human Resource Development, Institute for Sociological, Political and Juridical Research, Skopje (Macedonia)
- Prof. Marija Knezevic PhD, Academic, Banja Luka, (Bosnia and Herzegovina)
- Ljupco Naumovski PhD, Forum for Mobility and Research, Bitola (Macedonia)
- Prof. Oliver Iliev PhD , Faculty of Communication and IT, FON University, Skopje (Macedonia)
- Prof. Dimitrija Popovski PhD, Faculty of Sport, Ss. Cyril and Methodius University, Skopje (Macedonia)
- Prof. Yuri Doroshenko PhD, Dean, Faculty of Economics and Management, Belgorod (Russian Federation)
- Prof. Ilija Nasov PhD, Research and Development center PLASMA, Skopje (Macedonia)
- Prof. Sashko Plachkov PhD, Faculty of Pedagogy, University Neofit Rilski, Blagoevgrad (Bulgaria)
- Prof. Ivan Petkov PhD, Rector, European Polytechnic University, Pernik (Bulgaria)
- Prof. Vladimir Lazarov PhD, European Polytechnic University, Pernik (Bulgaria)
- Prof. Tosko Krstev PhD, European Polytechnic University, Pernik (Bulgaria)
- Prof. Antoaneta Hristova PhD, European Polytechnic University, Pernik (Bulgaria)
- Prof. Azra Adjajlic – Dedovic PhD, Faculty of criminology and security, Sarajevo (Bosnia & Herzegovina)

THE INFLUENCE OF REACTIVE OXYGEN SPECIES ON IN VITRO FERTILIZATION SUCCESS

Maryam Shahidi
Hazrat-e Maryam Fertility Center, Mazandaran, Sari, Iran, Department of Biochemistry and Biophysics, Faculty of Medicine, Mazandaran University of Medical Sciences, Mazandaran, Sari, Iran
Fateme Davoodi
Hazrat-e Maryam Fertility Center, Mazandaran, Sari, Iran,
Maryam Rameshgar
Hazrat-e Maryam Fertility Center, Mazandaran, Sari, Iran,
Vaso Taleski
Faculty of Medical Sciences, University „Goce Delchev"-Sthip, Republic of Macedonia
Liljana Simjanovska
Faculty of Medical Sciences, University „Goce Delchev"-Sthip, Republic of Macedonia
lsimjanovska@yahoo.com

Abstract: The reactive oxygen species (ROS) are one of the serious factors which negatively affect the in vitro fertilization (IVF) outcome. When the formation of ROS overcome the ability of the biological system to detoxify them, they cause damage of the cells known as oxidative stress. These oxygen derived free radicals normally are produced from mitochondria during normal oxidative respiration. Due to their highly reactive nature, ROS can combine directly with other molecules leading to their structural and functional changes which result in cellular damage. Physiologically ROS can modulate the reproductive processes such as sperm-oocyte interaction, early embryo development and implantation. However the imbalance in their presence can cause oxidative stress affecting the pregnancy outcome. Although the IVF methods are continuously developing and improving there are still not sufficient microenvironment conditions that will avoid ROS accumulation and their negative influence on IVF outcome. The source of ROS during IVF may be due to the absence of endogenous defense mechanisms or due to the use of various manipulative techniques. In that way the ROS can originate from the endogenous production from the gametes and embryos or can be the result of the external factors such as: culturing media, O2 concentration, light or the manipulation technique which has been used. One of the most important endogenous factor is increased oxidative stress in the spermatozoa which is associated with poor fertilization rate, low embryo quality and high rate of pregnancy loss. The other endogenous factors are connected with oocytes and the follicular fluid. It has been shown that higher ROS levels can trigger disruption of the oocyte cytoskeleton, affect the spindle formation, aneuploidy and embryo developmental arrest. Since embryo is a fast developing organism that needs high energy in some cases can also generate excessive amounts of RDS. Pathological levels of ROS during embryo culturing result in a low quality embryos with high fragmentation leading to low clinical pregnancy rates. To avoid the effects of the known external sources of ROS many improvements were made by additions of supplements in the culture media that reduce the ROS formation, culturing under lower O2 concentrations, reduced exposure to light and introduction of vitrification methods for cryopreservation. Although many strategies are implemented so far, there is still a need for further development of new ways to avoid the oxidative stress as much as possible during the IVF treatment. One of the future approaches to prevent the harmful oxidative stress on IVF may be optimization of the environment by using the enriched atmosphere with negative air ions.
Key words: IVF, pregnancy, ROS, fertilization

INTRODUCTION

Free radicals are the molecules which produce oxidative stress whenever they are not neutralized by the antioxidants and they can cause damage of the cells. They are unstable and reactive molecules that acquire an electron from the surrounding molecule. Oxygen radicals, such as the superoxide anion ($O2^{\cdot}$), the hydroxyl radical ($OH^{\cdot}$), Hydrogen peroxide (H2O2), hypochlorite radical ($OHCl^{\cdot}$), and the peroxyl radical ($ROO^{\cdot}$) represent the group named reactive oxygen species (ROS) [1,2]. Also in this group are included the nitrogen radicals, such as nitric oxide (NO), nitric dioxide (NO2), peroxynitrite anion and nitroxyl ion [3]. Normally the physiological processes result in a production of ROS since they are involved in various signal transduction pathways as a second messengers. Usually the life cells maintain the balance between ROS and antioxidants. The process of stabilization of the free radicals is by the addition of an electron by the antioxidants without affecting itself [4]. In the cases of overproduction of ROS damage of the cell constituents such as protein, lipids and DNA occur. According to the intensity of the cell damage the oxidative stress can result in either reconstitution of the cell by repairing process or triggered apoptosis or even necrosis in the worse cases. ROS are known to play role in physiological processes such as phagocytosis, cell proliferation, differentiation and

migration [5]. Concerning the reproductive processes ROS also play an important role. It has been shown that they are involved in sperm-oocyte interaction, implantation and early embryo development [6]. As a consequence of unbalanced ROS production the developed oxidative stress can lead to low pregnancy outcome.

IVF METHODS AND ROS

For more than three decades the assisted reproduction technologies (ART) have been used in many cases of male and female infertility. These manipulative techniques can cause: pH shock, temperature changes, osmotic variations, UV light damage or nutrient and other imbalances [7,8]. However in the recent studies have been shown that in vitro manipulation of the gametes and embryos increase the risk of overphysiological production of ROS affecting the success rate of the treatment. It has been suggested that this occurs mostly due to a lack of gamete and embryo protection by the scavengers needed to neutralize the ROS. The source of ROS in IVF can be from the endogenous origin or external sources during the IVF process.

ENDOGENOUS SOURCES OF ROS

Under the normal conditions in aerobic cells the most important source of ROS are the oxygen-derived radicals formed in mitochondria during the intermediate steps of their reduction. Since the gametes and embryos as the other cells have energy needs they use the ATP created through mitochondrial oxidative phosphorylation and glycolysis. These processes are the physiological source of ROS [9,10]. The internal sources of ROS during IVF can originate either from spermatozoa, oocytes or embryos. ROS formation occurs during the capacitation and acrosome reaction [11]. The major source of overphysiological amounts of ROS in the ejaculate are the morphologically abnormal spermatozoa and leucocytes [12]. Also the pathological effects result from the loss of plasma membrane fluidity of spermatozoa affecting the sperm motility. Increase of free radicals can produce DNA damage of the sperm cells. Many different types of mutations such as deletions, frameshifts, DNA cross links and chromosomal rearrangements have been described [13]. Also increased ROS decreases the mitochondrial membrane potential triggering the apoptosis of the sperm cells. Consequently during the IVF process many of these factors can lead to low fertilization rate, impaired embryo development and high pregnancy loss [14]. In contrast to the male gamete the situation with the oocyte ROS investigation is more complex. So far several studies have shown negative correlation of ROS production with the pregnancy outcome in IVF patients. Excessive ROS levels can be a cause of oocyte cytoskeleton disruption which result in microtubule dysfunction and aneuploidy. Any alteration of the meiotic spindle function can affect the normal fertilization, development of the embryos and consequently the IVF outcome [15]. The fast developing embryo is the third endogenous source of ROS during IVF. The high energy needs of the early embryo is supplied by the ATP production through mitochondrial phosphorylation and glycolysis. Excessive generation of ROS usually are the result of increased energy demands during the processes of compaction and hatching. It has been found that in vitro conditions facilitate the ROS production when compared to in vivo conditions. It is clear that abnormal levels of ROS have negative impact on embryo quality recorded as slow development, high fragmentation and impaired blastocyst formation [16]. Studies of the day three levels of ROS have shown that increase of ROS ultimately affect the clinical pregnancy rates.

EXOGENOUS SOURCES OF ROS

The manipulative methods used in the IVF processes are one of the exogenous sources of ROS. However many other external factors also exist, such as media and supplements, excess of glucose, oxygen concentration, visible light and volatile organic compounds (VOC) [17]. Comparing the IVF methods which has been used, it seems that the IVF conventional process of spontaneous fertilization give different increase of the ROS levels compared to the intracytoplasmic sperm injection (ICSI) [18]. Also the cryopreservation methods for the gametes and embryos cause damage of the cells as a result of a decrease of the natural defense mechanisms. However the two known methods for cryopreservation, vitrification or slow freezing result in different levels of damage of the cells by ROS [19]. The culture media as a factor for increased ROS formation is mostly connected with the presence of metallic ions which accelerate ROS generation. This is solved by the addition of transferrin and ethylenediamine tetra-acetic acid as a chelators [20]. For the O2 as a factor in IVF procedures it is known that compared to the in vivo conditions it is three times more concentrated. Lowering the percentage of O2 in the incubators better development of the embryos to the blastocyst stage have been achieved [21]. To avoid the visible light exposure of the cells many IVF laboratories work under decrease light intensity or even some of them using the fluorescent light filters [22].

SOLUTION STRATEGIES TO AMELIORATE THE ROS FORMATION IN IVF

There are several solution strategies that have been applied to ameliorate the negative influence of over creation of ROS. One of the implemented approaches were various sperm preparation methods that lower the ROS from teratogenic sperm cells and leucocytes [23]. Double density gradient centrifugation and glass wool filtration were recommended [24]. The high quality semen samples were obtained by the combination of

migration [5]. Concerning the reproductive processes ROS also play an important role. It has been shown that they are involved in sperm-oocyte interaction, implantation and early embryo development [6]. As a consequence of unbalanced ROS production the developed oxidative stress can lead to low pregnancy outcome.

IVF METHODS AND ROS

For more than three decades the assisted reproduction technologies (ART) have been used in many cases of male and female infertility. These manipulative techniques can cause: pH shock, temperature changes, osmotic variations, UV light damage or nutrient and other imbalances [7,8]. However in the recent studies have been shown that in vitro manipulation of the gametes and embryos increase the risk of overphysiological production of ROS affecting the success rate of the treatment. It has been suggested that this occurs mostly due to a lack of gamete and embryo protection by the scavengers needed to neutralize the ROS. The source of ROS in IVF can be from the endogenous origin or external sources during the IVF process.

ENDOGENOUS SOURCES OF ROS

Under the normal conditions in aerobic cells the most important source of ROS are the oxygen-derived radicals formed in mitochondria during the intermediate steps of their reduction. Since the gametes and embryos as the other cells have energy needs they use the ATP created through mitochondrial oxidative phosphorylation and glycolysis. These processes are the physiological source of ROS [9,10]. The internal sources of ROS during IVF can originate either from spermatozoa, oocytes or embryos. ROS formation occurs during the capacitation and acrosome reaction [11]. The major source of overphysiological amounts of ROS in the ejaculate are the morphologically abnormal spermatozoa and leucocytes [12]. Also the pathological effects result from the loss of plasma membrane fluidity of spermatozoa affecting the sperm motility. Increase of free radicals can produce DNA damage of the sperm cells. Many different types of mutations such as deletions, frameshifts, DNA cross links and chromosomal rearrangements have been described [13]. Also increased ROS decreases the mitochondrial membrane potential triggering the apoptosis of the sperm cells. Consequently during the IVF process many of these factors can lead to low fertilization rate, impaired embryo development and high pregnancy loss [14]. In contrast to the male gamete the situation with the oocyte ROS investigation is more complex. So far several studies have shown negative correlation of ROS production with the pregnancy outcome in IVF patients. Excessive ROS levels can be a cause of oocyte cytoskeleton disruption which result in microtubule dysfunction and aneuploidy. Any alteration of the meiotic spindle function can affect the normal fertilization, development of the embryos and consequently the IVF outcome [15]. The fist developing embryo is the third endogenous source of ROS during IVF. The high energy needs of the early embryo is supplied by the ATP production through mitochondrial phosphorylation and glycolysis. Excessive generation of ROS usually are the result of increased energy demands during the processes of compaction and hatching. It has been found that in vitro conditions facilitate the ROS production when compared to in vivo conditions. It is clear that abnormal levels of ROS have negative impact on embryo quality recorded as slow development, high fragmentation and impaired blastocyst formation [16]. Studies of the day three levels of ROS have shown that increase of ROS ultimately affect the clinical pregnancy rates.

EXOGENOUS SOURCES OF ROS

The manipulative methods used in the IVF processes are one of the exogenous sources of ROS. However many other external factors also exist, such as media and supplements, excess of glucose, oxygen concentration, visible light and volatile organic compounds (VOC) [17]. Comparing the IVF methods which has been used, it seems that the IVF conventional process of spontaneous fertilization give different increase of the ROS levels compared to the intracytoplasmic sperm injection (ICSI) [18]. Also the cryopreservation methods for the gametes and embryos cause damage of the cells as a result of a decrease of the natural defense mechanisms. However the two known methods for cryopreservation, vitrification or slow freezing result in different levels of damage of the cells by ROS [19]. The culture media as a factor for increased ROS formation is mostly connected with the presence of metallic ions which accelerate ROS generation. This is solved by the addition of transferrin and ethylenediamine tetra-acetic acid as a chelators [20]. For the O2 as a factor in IVF procedures it is known that compared to the in vivo conditions it is three times more concentrated. Lowering the percentage of O2 in the incubators better development of the embryos to the blastocyst stage have been achieved [21]. To avoid the visible light exposure of the cells many IVF laboratories work under decrease light intensity or even some of them using the fluorescent light filters [22].

SOLUTION STRATEGIES TO AMELIORATE THE ROS FORMATION IN IVF

There are several solution strategies that have been applied to ameliorate the negative influence of over creation of ROS. One of the implemented approaches were various sperm preparation methods that lower the ROS from teratogenic sperm cells and leucocytes [23]. Double density gradient centrifugation and glass wool filtration were recommended [24]. The high quality semen samples were obtained by the combination of

magnetic-activated separation with gradient centrifugation [25]. Additional supplementation of the media with antioxidants such as pentoxifylline, glutathione or albumin have been implemented [26]. Since the introduction of IVF procedures as a treatment for infertility, there is continuous attempt to mimic the natural conditions. Therefore different supplementations of the media to maintain a stable antioxidant balance have been used. During the time culture media have evolved and sequential culture media were created for the benefit of better embryo development [27]. Although there are certain achievements, there is still a need for further research with an aim to get higher pregnancy rate. To ameliorate the oxidative stress is a serious and very important challenge for further studies in the IVF field. Implementation of enriched environment with negative air ions (NAI) may have some influence on ROS creation. It has been shown that inhalation of NAI by rats influence the energy reactions of mitochondria. The study of liver and brain cells after inhalation of NAI showed decrease in the energy demand after the induced hyperactivation with epinephrine. This study demonstrated the sensitivity of mitochondrial processes in the brain and liver cells [28]. We hypothesize that NAI may have a physiological effect on the cells such as gametes or developing embryos as well, which has to be experimentally investigated.

REFERENCES

[1] G. Spiteller Review: on the chemistry of oxidative stress, Journal of Lipid Mediators, vol 7, pp. 199–221, 1993
[2] H. Sies, Strategies of antioxidant defense, European Journal of Biochemistry, vol. 215,pp.213–219, 1993
[3] M.K. O'Bryan, A. Zini, C.Y. Cheng, P.N. Schlegel, Human sperm endothelial nitric oxide synthase expression: correlation with sperm motility, Fertility and Sterility, vol. 70, pp.1143–1147, 1998
[4] S.G. Rhee, Cell signaling. H_2O_2, a necessary evil for cell signaling, Science, vol.312, pp.1882–1883,2006
[5] Y.M. Janssen, B. Van Houten, P.J. Borm, B.T. Mossman, Cell and tissue responses to oxidative damage, Laboratory Investigation, vol. 69, pp.261–274, 1993.
[6] C. Taylor, Antioxidants and reactive oxygen species in human fertility, Environmental Toxicology and Pharmacology, vol.10, pp.189–196, 2001
[7] Y. Goto, Y. Noda, T. Mori, M. Nakano, Increased generation of reactive oxygen species in embryos cultured *in vitro*, Free Radical Biology and Medicine, vol.15, pp.69–75, 1993
[8] A. Agarwal, S. Gupta, H. Abdel-Razek, N. Krajcir, K. Athayde, Impact of oxidative stress on gametes and embryos in an ART Laboratory, Clinical Embryology, vol. 9, pp.5–22, 2006
[9] R.J. Aitken, Molecular mechanisms regulating human sperm function, Molecular Human Reproduction, vol.3, pp.169–173, 1997
[10] M. Attaran, E.Pasqualotto, T.Falcone, J.M. Goldberg, K.F. Miller, A. Agarwal, R.K. Sharma , The effect of follicular fluid reactive oxygen species on the outcome of *in vitro* fertilization, International Journal of Fertility and Womens Medicine, vol.45, pp. 314–320, 2000
[11] E. de Lamirande , C. Gagnon, A positive role for the superoxide anion in triggering hyperactivation and capacitation of human spermatozoa, International Journal of Andrology, vol.16,pp. 21–25, 1993
[12] M. Ollero, E. Gil-Guzman, M.C. Lopez , R.K. Sharma, A. Agarwal, K.Larson, D. Evanson, A.J. Thomas, J.G. Alvarez, Characterization of subsets of human spermatozoa at different stages of maturation: implications in the diagnosis and treatment of male infertility, Human Reproduction, vol.16, pp. 1912–1921, 2001
[13] R.J. Aitken, C. Krausz, Oxidative stress, DNA damage and the Y chromosome, Reproduction, vol. 122, pp.497–506, 2001
[14] A. Agarwal , T.M. Said, M.A. Bedaiwy, J. Banerjee, J.G. Alvarez, Oxidative stress in an assisted reproductive techniques setting, Fertility and Sterility, vol.86, pp. 503–512, 2006
[15] W.J. Choi, J. Banerjee, T. Falcone, J. Bena, A. Agarwal, R.K. Sharma,Oxidative stress and tumor necrosis factor-α-induced alterations in metaphase II mouse oocyte spindle structure. Fertility and Sterility, vol.88, pp. 1220–1231, 2007
[16] M.A. Bedaiwy, T. Falcone, M.S. Mohamed, A.A. Aleem, R.K. Sharma, S.E. Worley, J. Thornton, A. Agarwal, Differential growth of human embryos *in vitro*: role of reactive oxygen species, Fertility and Sterility vol.82, pp. 593–600, 2004
[17] M.H. Nasr-Esfahani, N.J. Winston, M.H. Johnson, Effects of glucose, glutamine, ethylenediaminetetraacetic acid and oxygen tension on the concentration of reactive oxygen species and on development of the mouse preimplantation embryo *in vitro*, Journal of Reproduction and Fertility, vol. 96, pp. 219–231, 1992
[18] A. Agarwal, T.M. Said, M.A. Bedaiwy, J.Banerjee, J.G. Alvarez, Oxidative stress in an assisted reproductive techniques setting, Fertility and Sterility, vol. 86, pp.503–512, 2006
[19] J.F. Bilodeau, S Chatterjee, M.A. Sirard , C.Gagnon, Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing, Molecular Reproduction Development, vo.55, pp. 282–288, 2000

[20] M. Ozawa, T. Nagai, M. Fahrudin, N.W. Karja, H. Kaneko, J. Noguchi, K. Ohnuma, K. Kikuchi, Addition of glutathione or thioredoxin to culture medium reduces intracellular redox status of porcine IVM/IVF embryos, resulting in improved development to the blastocyst stage, Molecular Reproduction Development, vol. 73, pp. 998–1007, 2006
[21] G.G. Leoni, I. Rosati, S. Succu, L. Bogliolo, D. Bebbere, F. Berlinguer, S. Ledda, A low oxygen atmosphere during IVF accelerates the kinetic of formation of *in vivo* produced ovine blastocysts, Reproduction in Domestic Animals, vol. 42, pp.299–304, 2007
[22]. T. Nakayama, Y. Noda, Y. Goto, T.Mori, Effects of visible light and other environmental factors on the production of oxygen radicals by hamster embryos, Theriogenology, vol. 41,pp. 499–510, 1994
[23] R.R. Henkel, W.B. Schill, Sperm preparation for ART, Reproductive Biology and Endocrynology, vol.1, pp.108, 2003
[24] S. Grunewald, W Miska, G. Miska, M.Rasch, M. Reinhardt, H.J. Glander, U. Paasch, Molecular glass wool filtration as a new tool for sperm preparation, Human Reproduction, vol.22, pp. 1405–1412, 2007
[25] S. Miltenyi, W. Muller, W. Weichel, A. Radbruch, High gradient magnetic cell separation with MACS, Cytometry, vol.11, pp.231–238, 1990
[26] A. Borini, C. Lagalla, R. Sciajno, V. Distratis, M.A. Bonu, M. Cattoli, G. Coticchio, Artificial reproductive technology achievements for optimizing embryo quality, Annual New York Academy of Science, vol. 1034, pp. 252–261, 2004
[27] A. Mercader, D. Valbuena, C. Simon, Human embryo culture, Methods in Enzymology, vol.420, pp.3–18, 2006
[28] I.G. Stavrovskaia, T.V. Sirota, I.R. Saakian, M.N. Kondrashova, Optimization of energy-dependent processesin mitochondria from rat liver and brain after inhalation of negative air ions, Biofizika, vol.43, pp.766-771, 1998