PERSPECTIVE FOR PROGRESS

Physical Activity and Male Reproductive Function: A New Role for Gamete Mitochondria

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LUNETTI, P., L. CAPOBIANCO, V. ZARA, and A. FERRAMOSCA. Physical activity and male reproductive function: a new role for gamete mitochondria. *Exerc. Sport Sci. Rev.*, Vol. 49, No. 2, pp. 99–106, 2021. *Several studies demonstrated that some types of physical exercise might affect male reproductive potential, even though the potential mechanisms involved in the modulation of sperm quality remain poorly understood. Therefore, we propose a new role for gamete mitochondria as a key hub that coordinates molecular events related to the effects induced by physical exercise.* **Key Words:** physical activity, oxidative stress, testosterone, sperm, mitochondria

Key Points

- Physical activity has beneficial effects on a variety of medical illnesses, but the impact on male fertility is less well established.
- Physical activity impacts the redox balance and testosterone levels. The degree of this effect depends on the duration, type, and intensity of the exercise.
- An increase in oxidative stress or a decline in testosterone concentrations are responsible for the decrease in sperm quality.
- Because gamete mitochondria are a common target of oxidative stress and testosterone levels, their functionality may represent a new perspective for understanding the effects of physical activity on male reproductive potential.

INTRODUCTION

Physical activity is known to have beneficial effects on a variety of medical illnesses, but the impact on male fertility is less well established. In fact, various studies support the hypothesis that exercise may affect male fertility, and the degree of this effect depends on the duration, type, and intensity of the exercise. It is known that intense physical activity is associated with a decrease in male reproductive potential (1). Alternatively, aerobic, resistance, and combined exercise may be a successful therapy for male factor infertility (2).

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Despite a solid body of evidence showing the effects of physical exercise on male reproductive potential, there is little knowledge concerning the potential mechanisms involved in the modulation of sperm quality.

Physical activity is responsible for multiple changes at the endocrine, cellular, and molecular levels; therefore, hormonal abnormalities caused by a deregulation of the hypothalamic-pituitary-gonadal axis, as well as an increase in oxidative stress, usually result in sperm with decreased functionality.

Gamete mitochondria are a common target of oxidative stress and testosterone levels (3). These organelles are involved in several metabolic pathways, even though they are generally known as the energy-generating powerhouses of the cell by virtue of their key role in the production of adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS).

Mitochondria not only play a pivotal role for sperm function but also regulate different aspects of reproductive function (4–8). In fact, the morphological, functional, and molecular development of mitochondria in germ cells reflect changes in the testicular microenvironment during spermatogenesis, when germ cells are moving from the base of the seminiferous epithelium to the lumen (4). During this process, germ cells are classified into four broad categories (spermatogonia, spermatocytes, spermatids, and spermatozoa), and different types of mitochondria are identified (orthodox-type mitochondria, the intermediate form, the condensed form, and a morphology that shifts back to the intermediate form). A parallel association between structural changes, cell localization, and metabolic status during spermatogenesis was also postulated (3,5). For example, a profound change both in the number and size of mitochondrial cristae is accompanied by an increase in respiratory activity (6–8). These adaptations seem to be the results of the substrates available in seminiferous tubules, as well as of the ATP demand. Furthermore, mitochondria play a major role in Leydig cell steroidogenesis, because testosterone is the major androgen in the testis that regulates gamete production (9).

Many studies reported that mitochondrial dysfunction can negatively affect male fertility at distinct levels, including poor OXPHOS activity, changes in mitochondrial DNA (mtDNA), excessive reactive oxygen species (ROS) production, acceleration of apoptosis, or defects in steroidogenesis (5,10–13). Therefore, germ cell mitochondria could be proposed as the hub of cellular events related to energy production, ROS signaling, and steroid hormone biosynthesis. By virtue of this role, gamete mitochondria functionality could be responsible for the effects of physical exercise on male reproductive potential.

EFFECTS OF PHYSICAL ACTIVITY ON REPRODUCTIVE POTENTIAL AND SPERM QUALITY

Over the last decade, several studies proposed the relevance of physical exercise on reproductive function and semen quality. In particular, there is growing evidence that significant changes in semen parameters occur due to sports training of certain types, intensities, and durations.

Different recent studies show that physical activity may affect in a positive way semen quality. For instance, higher physical activity and less TV watching are associated with higher total sperm count and sperm concentration in young healthy men (14). Similarly, a moderate aerobic treadmill training improves semen quality in obese sedentary adults (25–40 yr with body mass index ≥30 $kg \cdot m^{-2}$), with a significant increase in sperm concentration, motility, and morphology (15).

According to this evidence, two randomized clinical trials have been recently published. By comparing the effects of moderate- and high-intensity continuous training, as well as high-intensity interval training, Hajizadeh Maleki *et al.* (16) demonstrated that moderate-intensity continuous training was more beneficial in improving markers of male reproductive function. In particular, sperm progressive motility, morphology, concentration, and number were elevated significantly at both 12 and 24 wk after training.

The second randomized controlled trial was performed on 1026 sedentary men (aged 25–40 yr) attending the infertility clinic with history of more than one year of infertility (17). Authors reported that a moderate aerobic exercise training improved sperm quality (progressive motility, morphology, concentration, and number), thus recommending moderate aerobic exercise training as a treatment option for male factor infertility.

Training at higher intensities and with excessive loads seems to negatively affect male reproductive system and fertility, as well as sperm quality (1,18). Total sperm counts and motility significantly decrease in endurance trained men (19–24), whereas the proportion of abnormal spermatozoa in the semen ejaculate increases in athletes undergoing periods of intense physical efforts, including above all cyclist, mountain trekkers, and runners (16,22,23,25).

Some sports can have a negative effect on fertility, depending not only on the duration and intensity but also on the type of physical activity (26). For example, cycling impairs sperm morphology and motility, probably because the mechanical impact sustained from sitting on the saddle, gonadal overheating, and wearing tight clothes (22,23,27) causes an increase in intrascrotal temperature or a compressive damage to the testes.

Semen quality also is impaired by high-mileage running and triathlon, normally characterized by high-volume endurance activity (1,19,28,29). Finally, physical exercise at high altitude in experienced mountaineers is associated with a testicular dysfunction leading to reduced sperm concentration and motility (24,30).

A general concept emerging from literature analysis is that, whereas in recreational athletes exercise seems to be mainly associated with positive or neutral effects, professional athletes could be exposed to potential risks. However, it is important to underline that the comparison among studies on this topic is a complex matter because of the differences in number of subjects, inclusion and exclusion criteria of participants, experimental design, methodology of the semen analysis, and criteria used to evaluate sperm quality. Moreover, there is little knowledge concerning the potential mechanisms involved in the decrease in sperm quality.

In this context, the aim of the present review is to explore new perspectives to understand how physical activity can be linked to sperm quality. The literature review on this topic suggests that the negative effects of physical activity on male fertility, leading to the impairment of spermatogenesis and general fertility capacity, can be attributed mainly to a state of oxidative stress, along with an impairment of hypothalamic and testicular endocrine functions (1,16,19,22,23,25,28,29,31,32). To this aim, in the next paragraphs, we describe the connections between physical activity, oxidative stress, and testosterone levels, to evaluate possible effects of oxidative stress and testosterone concentrations on sperm quality, with a focus on gamete mitochondria.

OXIDATIVE STRESS MODULATION THROUGH PHYSICAL ACTIVITY

Despite the known health benefits of physical activity, there is some evidence suggesting that high exercise induces oxidative stress (33,34). The mechanisms responsible for this condition could be the increased oxygen consumption during aerobic exercise, which is associated with a greater rate of electrons flowing through the mitochondrial respiratory chain complexes (35–38). Other sources of ROS are catecholamines released during exercise, prostanoid metabolism, xanthine oxidase, and NAD(P)H oxidase.

The state of oxidative stress is indicated by an increase in oxidized molecules in tissues and body fluids, in dependence on exercise mode, intensity, and duration (39).

The degree of oxidative stress seems to be attenuated by chronic anaerobic training or habitual exercise, which are able to upregulate the endogenous antioxidant defense systems (38,40–51).

Despite this adaptation, several studies suggest that an intense period of training or competition can cause an increase in oxidative stress (38,51–56), because ROS release may exceed the protective capacity of the antioxidant system and lead to dysregulation within the inflammatory and neuroendocrinological systems (57). In fact, although the organism has a complex antioxidant defense system that serves to provide protection against oxidative stress, defenses are often not sufficient to eliminate oxidative damage.

Therefore, it seems that there is no specific exercise type effect in terms of redox balance. In fact, all forms of exercise, both aerobic and anaerobic, have the potential to result in increased oxidative stress (58) and in improving redox balance (50). In particular, a general concept is that a moderate level of ROS production during exercise promotes positive physiological

adaptation in the active skeletal muscles, whereas high levels of ROS production result in damage to macromolecules, such as proteins, lipids, and DNA (59).

We can conclude that the interrelation of exercise and oxidative stress remains an extremely complex and controversial topic, depending on the mode, intensity, and duration of exercise. Moreover, individual susceptibility to oxidative stress injury determined by genetic and lifestyle factors is an important factor that should be also considered in such kind of studies.

ASSOCIATIONS BETWEEN PHYSICAL ACTIVITY AND TESTOSTERONE LEVELS

Proper hormonal signaling is essential for the physiological adaptations to exercise training. Dependent on the magnitude of the training stimulus, hormones elicit specific training adaptations (60). Testosterone is among the key hormones critical to athletes, because it is an important mediator of the adaptive response to endurance training and a biomarker of anabolic and catabolic hormonal control (61). In muscle, it stimulates protein synthesis and inhibits protein degradation, promoting muscle growth and subsequent increase in muscle strength in response to resistance training in men (62).

Even though there is a large degree of interindividual variability in hormone responses to exercise, several studies reported that regular physical activity increases free testosterone levels in men. It has been observed that total and free testosterone levels were higher in physically active subjects, who regularly have been practicing endurance activities for over a year, when compared with sedentary subjects (63). Therefore, a moderate-intensity physical activity performed 3 $d \cdot wk^{-1}$ on alternate days produces an adequate hormonal environment, thus improving semen production (63).

Different exercise intensities cause different responses of free testosterone concentrations in people with different levels of physical fitness (64). In nonathlete healthy young adults, all exercise intensity levels increase free testosterone concentrations, whereas in athletes, only high-intensity exercise is able to increase testosterone levels during and immediately after each exercise session.

Accordingly, only high-intensity interval training has been shown to improve free testosterone in male master athletes (65). Therefore, it has been proposed that not only does the intensity of training induce hormonal alterations, but also the volume of the exercise may exert an influence on hormonal profile alterations.

Recent studies documented testosterone concentrations well below the normal physiological range in elite athletic populations participating in ultraendurance events, such as marathon distance running, long-course triathlons, and Olympic distance race walking (66–68). Other studies reported a decrease in testosterone values in athletes undergoing exhaustive endurance training, such as running or cycling 10–20 or more hours per week (69). This seems to be due to the chronic exposure to high volume and intensity of aerobic exercise, with the athlete coming close to overtraining or with persistent fatigue.

EFFECTS OF OXIDATIVE STRESS AND OF TESTOSTERONE LEVELS ON SPERM QUALITY

As previously reported, physical activity impacts redox balance and testosterone levels. The increase in free testosterone levels has been associated with an improvement in the main semen parameters, such as volume, sperm count, motility, and morphology (63), whereas a reduction has been linked to decreased spermatogenesis and oligospermia (70). In fact, a remarkable aspect related to a decrease in male reproductive potential is the gradual and progressive decline in testosterone levels (71). At the same time, low testosterone levels are related to oxidative stress (72), which, in turn, is associated to a decrease in sperm quality (73) (Fig. 1).

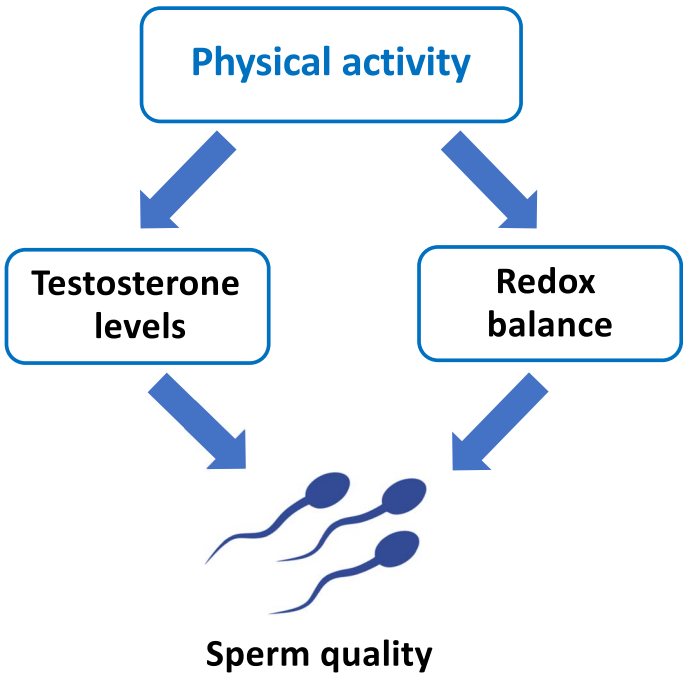

Figure 1. Effect of physical activity on sperm quality. Physical activity impacts the redox balance and testosterone levels, depending on the duration, type, and intensity of the exercise. An increase in oxidative stress or a decline in testosterone concentrations may be responsible for the decrease in sperm quality. Figure 1 can be viewed in full color online at www.acsm-essr.org.

Plasma commonly is used to reveal a condition of oxidative stress induced by physical exercise, and several biomarkers of the redox balance have been proposed (54,74,75). Several studies reported a close relation between semen quality and blood oxidative status (76–79), suggesting that an increase in oxidative stress biomarkers in the blood could be associated with a decrease in sperm quality. This means also that the evaluation of oxidative status in the blood is of diagnostic importance in the evaluation of male infertility.

The exact mechanism by which blood oxidative status may influence sperm quality is still unknown. Plasma ROS can reach by diffusion sperm cells, which are in the lumen of seminiferous tubule, relatively far from blood vessel running within interstitial spaces between tubules.

High ROS levels negatively affect sperm quality and function, even though adequate ROS levels contribute to sperm functionality (73,80,81).

Mature sperm cells are vulnerable to ROS, as they lack proper repair machineries and have inadequate antioxidant capacity. Thus, when a ROS increase damages plasma and acrosomal membranes, mitochondria, and sperm DNA, spermatozoa cannot restore such a damage because of deficiency of cytoplasmic repair enzymes. In fact, although seminal plasma contains protective antioxidants (superoxide dismutase, catalase, and glutathione peroxidase), these defenses are less abundant in sperm (82,83).

Different findings suggest a central role of sperm mitochondria in oxidative damage and related infertility (79,84), because improvement of sperm parameters, such as motility, capacitation, acrosome reaction, and oocyte interaction, involves regulation of ROS levels by mitochondria. This is an intriguing aspect because according the "mitochondrial theory of aging," these organelles are, at the same time, ROS generators and ROS targets (85–87).

EFFECTS OF OXIDATIVE STRESS ON GERM CELL MITOCHONDRIA

Mitochondria usually are considered the "power plant" of the cell because they control all cellular events related to energy metabolism through the sophisticated mechanism of the OXPHOS (88–92). However, this concept is somehow reductive because mitochondria are the hub of cellular events related not only to energy metabolism but also to oxidative stress (89,93). In fact, despite their key role in sperm energetic metabolism, they are highlighted as source of pro-oxidative factors that are crucial in the alteration of oxidative homeostasis (94). Several studies have demonstrated a correlation between impaired mitochondrial activity due to redox unbalance and decrease in sperm quality, therefore indicating a close relation between oxidative stress and sperm damage (95). In fact, the unique architecture of the male germ cell, with an abundance of oxidizable substrates and a limited intracellular antioxidant defenses, renders this cell particularly vulnerable to elevated levels of ROS (82,83).

Nowicka-Bauer and Nixon (80) proposed that the high levels of polyunsaturated fatty acids present in the sperm membrane make spermatozoa particularly vulnerable, by virtue of their ability to act as substrates for lipid peroxidation (LPO) cascades. This pathway also is responsible for the generation of 4-hydroxynonenal, which negatively affects sperm motility, via the dysregulation of proteins involved in sperm bioenergetic pathways.

Several components of the oxidative system are susceptible to oxidative stress: cardiolipin, whose content plays a key role in mitochondrial membrane function (96), the mitochondrial adenine nucleotide translocase (ANT) (97), ATP synthase (98), and the Krebs cycle enzyme aconitase (99). Furthermore, mtDNA (which is localized in the mitochondrial matrix and encodes 13 protein subunits of the respiratory chain) is an easy target of ROS because it has a high turnover rate, lacks protection by histones, and has a limited capacity to repair DNA damage (100,101). As the molecules of sperm mtDNA are very few as compared with mtDNA content in somatic cells (10–100 vs 10^2–10^4 copies), mtDNA mutations in spermatozoa have profound adverse effects on gamete quality (88).

When mitochondria become a target of elevated levels of ROS, proteins and membrane lipids are damaged and, as a consequence, the process of OXPHOS is severely affected. It also has been demonstrated that oxidative stress, along with the concomitant phenomenon of LPO and sperm DNA fragmentation, affects sperm mitochondrial respiration by an uncoupling between electron transport and ATP synthesis (78,79). The reduction in mitochondrial functionality might be one of the causes responsible for the decreased progressive motility of spermatozoa.

Another mechanism by which ROS influence male fertility is an increase in testicular oxidative stress, although the testis has several antioxidant enzymes. This state plays a role in a number of conditions known to be detrimental to testicular dysfunction and, therefore, to male fertility, such as varicocele, cryptorchidism, testicular torsion, or endocrinopathy (102). Moreover, the ROS-induced DNA damage has been suggested to be responsible for the acceleration of germ cell apoptosis, leading to a decrease in sperm count and, thus, a decline in semen quality (82). In fact, high ROS levels are able to disrupt mitochondrial membranes, thus leading to a release from mitochondria of cytochrome *c*, which activates the caspases and induces apoptosis (82). In germ cells, apoptosis seems to be strictly regulated by extrinsic and intrinsic factors during spermatogenesis (103). Moreover, when large amounts of pathogenic mutant mtDNA accumulate in the testis, mitochondrial respiratory dysfunction is induced in spermatogenic cells, causing a decrease in energy production and, therefore, meiotic arrest and the presence of abnormalities in sperm morphology (104).

EFFECTS OF TESTOSTERONE LEVELS ON GERM CELL MITOCHONDRIA

Low testosterone levels are related not only to oxidative stress but also to mitochondrial dysfunction (72).

The first step of synthesis of testosterone, which has a pivotal role in spermatogenesis and expression of male secondary characteristics, occurs in the mitochondria of Leydig cells, located in the connective tissue surrounding the seminiferous tubules. Leydig cell steroidogenic function, which is regulated by luteinizing hormone, needs functional mitochondria (105) and is regulated by proteins located in the outer mitochondrial membrane in the transduceosome complex (106). This complex mediates cholesterol transfer in the mitochondria and includes a translocator protein, the voltage-dependent anion channel,

ANT, and steroidogenic acute regulatory protein (StAR). Cholesterol delivered into the mitochondrial matrix is further converted to pregnenolone by cytochrome P450 side-chain cleavage enzyme (107). All other steps of steroidogenesis take place outside the mitochondria, where pregnenolone then is converted to testosterone.

Leydig cells produce ROS from several sources, such as mitochondrial electron transport chain and microsomal cytochrome P450 enzyme reactions (108), but they contain also enzymes and molecules that can neutralize or scavenge ROS.

Little is known about the control of ROS production in Leydig cells. Based on the observations that ROS are capable of damaging cell components and that ROS are produced during steroidogenesis itself, it has been suggested that the long-term suppression of Leydig cell steroidogenesis prevents Leydig cell damage due to aging (109). This suggests that the decrease in testosterone levels could be a mechanism to protect Leydig cells from ROS damages. Accordingly, one of the major factors contributing to the low levels of testosterone is an increase in ROS in Leydig cells (110).

Therefore, a decrease in blood testosterone levels could be not only the consequence of mitochondrial dysfunctions in Leydig cells but also a physiological response to an increase in oxidative stress.

Anyway, a positive correlation has been found between blood testosterone levels and sperm motility (111), whereas a negative correlation was observed between testosterone concentration and sperm DNA fragmentation, which is a marker of oxidative damage (112). This condition can negatively affect sperm mitochondrial respiration (78,79,113,114), which also is closely correlated with sperm motility (91,115,116).

Interestingly, experiments performed in isolated rat liver mitochondria showed that testosterone concentration is able to regulate organelle function (117), suggesting a possible role for this hormone also in the regulation of sperm mitochondria bioenergetic.

It is important to underline that, in the context of physical activity, the decrease in testosterone levels could be also the result of the gonadotropin releasing hormone releasing from hypothalamus, even if there is no clear consensus on this matter (118). In particular, it has been suggested that the exercise-induced suppression of serum testosterone is associated with two effects: suppressed endogenous gonadotropin releasing hormone stimulation of gonadotrophin release during exercise and decreased testicular capacity to secrete testosterone during recovery period (119).

At the present, there are no studies showing dysfunctional mitochondrial activities in Leydig cells due to oxidative stress upon exercises. However, the results obtained in cellular models suggest that ROS-mediated perturbation of Leydig cell mitochondria (resulting in inhibition of StAR protein expression and function) may well be an important contributing factor to the decrease in testosterone levels (120,121).

GAMETE MITOCHONDRIA: A LINK BETWEEN PHYSICAL EXERCISE EFFECTS AND MALE FERTILITY

Several studies demonstrated that some types of physical exercise have the potential to result in increased oxidative stress, depending on the type, the intensity, or the duration of the exercise. At the same time, low testosterone levels are doubly related to oxidative stress: the decrease in testosterone levels could be the result of mitochondrial defects in Leydig cells (as a consequence of oxidative damages) or a physiological response to reduce oxidative stress, because ROS are produced during steroidogenesis itself.

The common target of physical exercise effects, such as oxidative stress and testosterone levels, are gamete mitochondria, which also are the main ROS source. The development of studies aimed at the prevention of mitochondrial dysfunction in sperm cells is important as these organelles are susceptible to oxidative damage.

Therefore, we propose a role for these organelles as a key hub that coordinates molecular events related to sperm quality, by

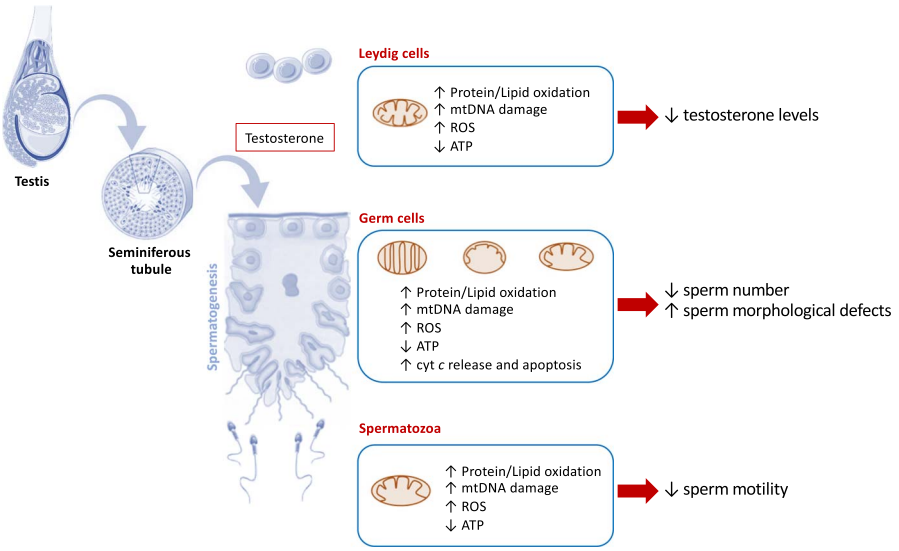

Figure 2. Proposed effects of oxidative stress and testosterone levels on gamete mitochondria. Testosterone, synthesized and released by Leydig cells, regulates spermatogenesis. Increased oxidative stress and decreased testosterone could be responsible for oxidative damages in macromolecules (lipids, proteins, and mtDNA) in Leydig cells, germ cells, and mature spermatozoa. These damages led to defects in gamete mitochondrial function, which could be responsible for the decrease in testosterone levels (Leydig cells) or sperm quality (germ cells and mature spermatozoa). Figure 2 can be viewed in full color online at www.acsm-essr.org.

controlling energy metabolism and oxidative homeostasis. We are conscious that oxidative stress, along with testosterone levels, does not just affect mitochondrial activities but also many other cellular processes. However, the purpose of this review is to focus the attention on gamete mitochondria, because it is known that mitochondrial dysfunctions are negatively correlated with, and may affect, several reproductive parameters (10,12,84,89,95,104,115).

The proposed effects of oxidative stress and testosterone levels on gamete mitochondria are described in Figure 2.

Mitochondria of Leydig cells, located in the connective tissue surrounding the seminiferous tubules, must be functional to synthesize testosterone. Defects in Leydig cell mitochondria are responsible for oxidative damages in macromolecules (lipids, proteins, and mtDNA) and cause a decrease in ATP levels. The consequence is a decrease in testosterone levels, which may result in low sperm production, because this hormone is the major androgen in the testis that regulates spermatogenesis.

Mitochondria of germ cells are susceptible to oxidative stress: proteins and membrane lipids are damaged, and mtDNA is fragmented. As a consequence, the process of OXPHOS is severely affected, causing a decrease in energy production and, therefore, meiotic arrest and the presence of abnormalities in sperm morphology. High ROS levels also are able to disrupt mitochondrial membranes, thus leading to a release from mitochondria of cytochrome *c*, which induces apoptosis, causing a decrease in sperm number.

Finally, in mature spermatozoa, mitochondria are the target of elevated levels of ROS. The reduction in mitochondrial functionality might be one of the causes responsible for the decreased progressive motility of spermatozoa.

FUTURE PERSPECTIVES

We suggest that the study of gamete mitochondria functionality may represent a new perspective for understanding the effects of physical activity on male fertility.

The modulation of mitochondrial function is a complex matter because several mechanisms, numerous proteins, and protein complexes are involved in specific pathways allowing crosstalk between mitochondrial metabolism and oxidoreductive homeostasis. Therefore, further investigation of the role of gamete mitochondria during physical activity is necessary to develop new therapeutic approaches that, by improving mitochondrial function and by restoring redox homeostasis, are able to improve sperm quality and reproductive potential in athletes.

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