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Mini Review

Melatonin and Canthaxanthin Ameliorate Oxidative Stress and Improve Semen Quality: A Special Reference to Ram

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ABSTRACT

Success in the outcomes of applied assisted reproductive techniques mainly relies on the quality of the semen. Oxidative stress created by Reactive Oxygen Species (ROS) and Lipid Peroxidation (LPO) generated during different steps of semen preservation reduces semen quality. Melatonin and canthaxanthin are potent antioxidants involved in many biological processes. They have excellent capacity to scavenge free radicals, combat against oxidative stress and improve endogenous antioxidant defenses. Melatonin is well established antioxidant in semen preservation to protect against oxidative stress-induced damage; however, little literature is available on use of canthaxanthin as semen extender additive. Melatonin and its metabolites act as direct or indirect scavenger of free radicals arrest LPO and reduce generation of ROS, hence oxidative stress, thereby shielding seminal quality to prolong sperm morphological and functional attributes. Similar to melatonin, canthaxanthin by virtue of its antioxidant potential has shown promising results in preserving the seminal quality as well as their efficiency to reduce and/or prevent sperm damages during storage. This brief review encapsulated new uncovering related to the beneficial antioxidant effects of melatonin and canthaxanthin on semen preservation along with future perspective in respect of different combination and/or concentration along with fertility trails.

Keywords: Reactive oxygen species; Lipid peroxidation; Canthaxanthin

INTRODUCTION

Artificial Insemination (AI) is considered as irreplaceable, most pragmatic approach and capable biotechnological arsenal to overcome hurdles associated with low productive potential and scarcity of elite germplasm especially in the unorganized animal husbandry sector of developing countries. Therefore, AI can be used to provide superior germplasm even at distant remote location to accelerate rate of genetic improvement [1,2]. Sperm quality is particularly altered by oxidative stress, which is defined as an imbalance between the generation of Reactive Oxygen Species (ROS) and the protective action of antioxidant systems responsible for their neutralization and removal [3,4]. Imbalance between production and utilization of ROS leads to damage of many cell structures, especially phospholipids of cellular membranes. Oxidative damage to lipids, called Lipid Peroxidation (LPO), triggers, in turn, signaling cascades of the inflammatory processes which promote peroxidation of lipids, resulting in intracellular oxidative burden. During semen freezing-thawing procedures, ROS and LPO generated mainly

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attributed to presence of Polyunsaturated Fatty Acid (PUFA) in sperm membrane and smidgen endogenous antioxidants due to little amount of cytoplasm leading to oxidative stress to sperm [5,6]. Oxidative stress results in axonemal damage, decreased sperm viability, and increased midpiece sperm morphological defects. These dysfunctions may contribute to decreased sperm motility and decrease fertilizing potential of spermatozoa by altering oxidative metabolism and sperm physiological functions [7-9]. Such situation paved the way for addition of antioxidant in semen extender to counteract ROS and LPO during semen storage [10].

LITERATURE REVIEW

A flawless semen extender must not only maintain an environment with an appropriate pH and buffering capacity, but it must also stop the production of excess ROS or scavenge it. Recently, antioxidants have been launched to conquer the drawback of freezing-thawing process and play a key role in maintaining seminal quality by equalizing the effects induced by these procedures [11,12]. Melatonin (MLT) is a terminal, potent non-enzymatic and non-oxidative antioxidant, synthesized and secreted in the pineal gland of all mammalian species. In recent years, researchers have unveiled the antioxidant capability of MLT and its metabolic derivatives to act collegially with other classic antioxidants to safeguard cells from oxidative stress at physiological and pharmacological concentrations [13,14]. In addition to melatonin, carotenoids are also acknowledged as methodical quenchers of ROS and inhibitors of LPOand are important in many biological processes through their antioxidant activity [15,16]. Canthaxanthin, a carotenoid, naturally produced by algae, fungi, and bacteria, is a superior free radical scavenger and antioxidant other than carotenoids due to presence of keto group [17,18]. The antioxidant role of this compound and its function in clearing ROS and preventing LPO has been little explored in semen biology [19,20]. In this review, findings have been highlighted on antioxidant ability of melatonin and canthaxanthin for enhancing seminal quality during preservation and their future perspectives.

Historical perspective of the melatonin and the canthaxanthin as antioxidant

Melatonin or 5-methoxy-N-acetyltryptamine was first described from bovine pineal in the year of 1958 [21]. Initially, it was thought that it is unique to the pineal gland, but now it is known to be produced in many other tissues all over the body. Original expectations of melatonin were related to its capability of influencing reproductive function in photoperiod dependent seasonally breeding mammals [22]. Later on, it was reported that melatonin was not only associated with reproductive function but also related to broad functions including anti-inflammatory, antioxidant, circadian rhythm regulation etc. and was followed by a series of research trails to document melatonin capability to quell oxidative stress and protect cell [13,23]. Later on ,it was uncovered that antioxidant ability of melatonin to diminish oxidative stress was directly related to its concentration and would also be anticipated to reduce more oxidative injury than regularly require lower amplitude rhythm [24,25]. Researchers studied antioxidant ability of melatonin in semen extender and observed promising results in preventing injury due to oxidative stress [26,27]. Carotenoids are natural pigments and have been proved essential in regulation of membrane fluidity or their function as antioxidants [28,29]. Canthaxanthin is a reddish-orange keto-carotenoid found in plants, invertebrates and vertebrates, is well acknowledged as an antioxidant in cell cultures, poultry feed and in semen extender [6,16,20,30,31].

Effects of melatonin and canthaxanthin on oxidative stress in semen

During semen preservation, semen quality might be lost by 40% to 50% due to excessive ROS formation leading to oxidative stress [32]. Many studies have reported that melatonin had ability for counteracting free radicals and reducing ROS concentrations during semen preservation, thereby reduced oxidative stress-induced cell death. During the process, melatonin metabolites are generated which are also evidenced of having antioxidant capacity, and aid in nullifying free radicals, sometimes faster than melatonin [26,33-35]. In addition to this, melatonin also increases the activities of other classic antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase etc.), thereby enriching endogenous ant oxidative enzymatic defense also [36]. Melatonin has lipophilic properties, therefore can easily cross sperm plasma membrane and present in the cytosol, mitochondria, and nuclei and in other body fluids [34,37]. Melatonin can directly bind to specific receptor particularly MT1 and MT2 in sperm plasma membrane, regulate endogenous antioxidants and neutralize apoptotic-like changes generated during the freezing-thawing process by virtue of antioxidant activity [38]. Melatonin interacts with the mitochondrial lipid bilayers, stabilizes membrane integrity, prevent leakage of intracellular enzymes (aspartate transaminase, alanine transaminase etc.) resulting in lowering malondialdehyde (MDA) and increase in total antioxidant capacity (TAC), thereby, decreasing oxidative stress in semen [27,39,40]. Melatonin also interacts with calmodulin, an intracellular regulator of Ca^{2+} functions in sperm which causes improvement in frozen-thawed sperm motility and velocity [41]. Canthaxanthin enhances sperm viability, motility, preserve sperm membrane and acrosome integrity, prevent sperm DNA damage and apoptosis by reducing generation of ROS and LPO [6,16,19,20]. It is speculated that presence of ketone group and conjugated double bonds in its chemical structure make canthaxanthin more powerful antioxidant than other carotenoids [18,42].

Secondly polar part of canthaxanthin is placed on lipid membrane and interacts with polar head of fatty acid of the membrane, thereby smoothly absorbed *via* inactive transmission or by scavenger receptors [19,43].

In addition, canthaxanthin also expand the activity of other antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase etc.) and vanquish the activity of the caspase - 3 pro - apoptotic factor and tumor necrosis factor alpha, thereby maintaining seminal quality by antioxidant ability [19,44].

Melatonin and canthaxanthin as antioxidant in semen extender

ROS and LPO production during the semen preservation process is partly accountable for the poor quality of semen. Melatonin and canthaxanthin have been acknowledged excellent antioxidant as semen extender additive. Supplementation of melatonin in semen extender of ram, equine, bull, mithun, boar and human had multiple positive effects and improved sperm functional parameters *via*., live dead percentage, sperm abnormalities, plasma membrane integrity, mitochondrial activity, protected spermatozoa acrosome, reduced the number of apoptotic sperm, enhanced ant oxidative enzyme system, TAC, decreased MDA and ROS production, there by a valuable tool to extend semen storage life span and preventing premature ageing by antioxidant capacity [27,39,45-52]. In respect to canthaxanthin as semen additive, little research trials have been documented with mixed type of observations [16]. Observed positive effects on curvilinear velocity and amplitude of lateral head displacement however no effect was observed on ROS and LPO in ram semen [20]. Observed positive effect on semen quality in terms of progressive sperm motility, viability, hypo-osmotic swelling test and showed potent antioxidant effect by counteracting oxidative stress in terms of MDA and TAC in ram semen. A study conducted in human semen by also reported significantly improvement in total sperm motility, viability, normal morphology, chromatin packaging, acrosome integrity, DNA denaturation and fragmentation by addition of canthaxanthin in semen extender [6].

DISCUSSION

The present review emphasizes the positive effects of Melatonin and canthaxanthin on sperm quality during semen storage. It is now well-established fact that pineal hormone is not only regulate seasonal reproductive activity in photoperiod dependent seasonal breeding animals but also have positive effects on semen quality during preservation in non-seasonal and seasonal breeders by reducing oxidative stress. This phenomenon suggests antioxidant capacity of melatonin to counteract adverse effect of oxidative stress during semen storage. Mammalian sperm membrane possesses more PUFA and scanty cytoplasm leading to less endogenous antioxidant capacity, thereby more sensitive to oxidative stress. Melatonin and canthaxanthin have low toxicity and commonly accepted for antioxidant activity, therefore, could be perfect to improve semen quality during preservation. Peer reviewing of literature showed that addition of all melatonin and canthaxanthin concentrations in semen extender are not excellent for the properties of sperm preservation and supplementation of passable concentrations of antioxidants improve quality of stored semen. However, the concentration of each antioxidant exclusively depends on species, semen extender or preservation medium composition, storage type, and *in vitro* stress conditions.

Therefore, future research on optimizing dose of these antioxidants for semen storage, composition of the semen extender for optimum seminal quality is warranted. Moreover, studies regarding the fertility trial of stored spermatozoa both *in vivo* and *in vitro* are necessary to advance the field of AI which would be helpful in reinforcing intensive breeding programs at field level, hence will be useful in acceleration of genetic improvement through AI. Specific studies investigating the precise mechanisms of action of the over mentioned antioxidants during semen cryopreservation are also imperative and needs of the day particularly in ram, buck and boar to confirm the findings of liquid semen storage. Till date, there is no study on the combined antioxidant effect of melatonin and canthaxanthin as well as different concentrations on either liquid storage or cryopreserved semen; therefore this virgin area should be focused. It is evidenced that systemic administration of melatonin accelerates plasma testosterone concentration, thereby improve sperm attributes, testicular echotexture etc. Therefore, future studies can be focused on fertility trial of both systemic administered melatonin and semen extender supplemented groups to gain a better understanding of the effect of melatonin treatment on seminal quality in terms of fertility in livestock species particularly in ram. Simultaneously, multiplying on-field fertility tests and shedding light on the underlying mechanisms by which melatonin beneficial effects occur, will allow optimal use as additive to extenders, thus better sperm quality and fertility rates. The role and contribution of melatonin in these protocols as an antioxidant or cryoprotectant remain to be explored with the important help of researchers in the topics of genetics, cryobiology and physiology of reproduction [53].

CONCLUSION

In Human beings, melatonin alleviate oxidative stress through biochemical and epigenetic pathways, is well cryopreserved and cancer related cells. Whereas in farm animal studies, available evidence largely focuses on the positive effects of MLT on sperm quality markers, without a clear insight into the underlying mechanisms by which MLT induces ameliorative effects at ultralow temperature. More research needs to be devoted to probing the signal pathways. In the last, the field of sperm cryobiology would benefit from studies on altered expression patterns of specific genes in persevered spermatozoa following antioxidant supplementation and their potential use as markers for sperm quality.

CONFLICT OF INTEREST

The authors declare no competing interests.

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