

Review Article

The Role of Nutrition and Melatonin in Balancing Oxidative Stress and Regulating Reproductive Performance in Cows: A Scientific Review

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Abstract: Reproduction is considered the means through which genetic material is transferred from one generation to another, and since the reproductive rate determines the success or failure of breeding systems, attention must be directed toward the reproductive system of males and females and improving productive and reproductive efficiency, thus it represents a biological and economic challenge. The aim of this article is to clarify the complex relationship between three main factors: nutrition (which leads to negative energy balance), stress (which leads to oxidative stress), and the hormone melatonin and its role as a molecular protective mediator. Research has reported that after calving, negative energy balance negatively affects reproductive hormones. Recently, the resumption of reproduction is associated with metabolic and heat stress that increase the production of reactive oxygen species (ROS), thus causing oxidative stress that leads to oocyte damage. Here we highlight melatonin hormone, due to its antioxidant properties, as a defensive mechanism to reduce oxidative effects and improve oocyte and ovarian function. Understanding the mechanism of these three factors is important for improving modern nutritional and management strategies to enhance reproduction and productivity in the herd.

Keywords: Reproductive Efficiency, Negative Energy Balance (NEB), Oxidative Stress, Melatonin, Oocyte Quality.

1. INTRODUCTION

Improving the reproductive efficiency of cows is considered one of the challenges facing researchers and breeders due to its importance in the economic efficiency of herds (Roch & Diskin, 2020). Despite the development in breeding programs, management systems, care, and artificial insemination, environmental factors still constitute an obstacle to achieving the required reproductive efficiency. Unsuitable environmental and climatic conditions, such as poor nutrition, improper management systems, and unbalanced feeding in nutrients including energy and protein—especially during pregnancy and the postpartum period affect the reproductive efficiency of animals and the activity of sexual hormones. Therefore, a balanced feeding system must be provided, particularly during this period (Roche, 2009; Waldron and Overton, 2004; Yitong Ding *et al.*, 2024). Temperature and humidity also affect the thermal balance of the body, which in turn influences physiological processes, reduces ovulation rate and fertilization percentage (Shehab-El-Deen, 2010), and thus lowers reproductive efficiency. Nutrition and stress are considered major factors that cause disturbance in negative energy balance (NEB) after calving, which negatively affects reproductive efficiency (Roch and Diskin, 2020). Heat stress also increases oxidative stress and thus negatively affects reproduction (Srivastava & Das, 2020). Therefore, there is a need to analyze and review previous research to understand the mechanisms of these influences, with the aim of developing therapeutic and preventive strategies to maintain and improve reproductive and productive efficiency in cows, and thus sustain animal production.

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2. The Fundamental Role of Nutrition in Regulating Reproduction

One of the most important factors affecting the reproductive efficiency of animals is nutrition, which determines the sustainability and success of fertility. Poor nutrition during pregnancy and after calving leads to negative outcomes, most notably increased metabolic disorders (Roche, 2009; Waldron and Overton, 2004) and decreased immunity and fertility (Roche, 2007). Therefore, nutritional management especially during the last period of pregnancy and the beginning of lactation—is considered highly important. Accordingly, feeding strategies have been developed to meet the nutritional requirements of cows to improve productivity. During late pregnancy and the postpartum period, the cow is exposed to the stress of parturition and lactation, resulting in increased requirements for energy and protein for milk production, while feed intake decreases (Mavangira, 2018; O’Hara L. A., Bage R., Holtenius & Sordillo, 2016). Studies recommend providing 17–19% crude protein in the diet on a dry matter basis for cows during lactation to meet their nutritional needs and supply essential amino acids. The animal may compensate for protein from body reserves in the short term; however, long-term protein deficiency negatively affects reproductive efficiency. Dairy cows require adequate protein throughout life, as deficiency leads to delayed puberty, reduced pregnancy rate, decreased appetite, weight loss, and impaired normal development and function of body organs. At the same time, protein should not be supplied in excess of actual requirements, because excess protein is converted into ammonia, absorbed into the bloodstream and not utilized in the rumen. This ammonia is then converted into urea in the blood, and elevated blood urea causes disturbances and reduced pregnancy rates in cows (Cheng *et al.*, 2015). Increased ammonia and urea impair oocyte maturation and fertilization and negatively affect embryos. Since embryo development depends on the uterine environment, elevated urea levels reduce uterine pH, which negatively affects embryo implantation in the uterine lining (Arunvipas *et al.*, 2007) and embryo development during cleavage and blastocyst formation (Humer *et al.*, 2016). However, adequate dietary energy may help the animal overcome these problems. Therefore, reducing excessive protein is recommended because it negatively affects reproductive efficiency by lowering uterine pH during the corpus luteum stage of the estrous cycle, and such excess is also economically costly. Consequently, the cow may enter a period of nutritional deficiency, showing noticeable changes, endocrine and metabolic disorders, and other physiological alterations that increase oxidative stress and weaken immunity (Aitken and Sordillo, 2009). In this condition, the cow becomes susceptible to retained placenta, liver diseases (Doherty and Mulligan, 2008), mastitis, endometrial damage, and genetic damage to the oocyte (Boudjellaba *et al.*, 2018), resulting in reduced reproductive efficiency and significant economic losses (Ouweltjes *et al.*, 1996; Ahmadzadeh *et al.*, 2009). A negative feeding system affects reproductive performance, especially ovarian function (Singh, 2020). Therefore, the ration should contain an energy source to increase insulin concentration (Berry *et al.*, 2016), because low blood insulin levels after calving reduce reproductive efficiency by delaying ovulation and causing other pregnancy-related problems (Rodrigues, 2014). Excess body fat also reduces immune efficiency, decreases productivity, and increases susceptibility to postpartum and metabolic diseases (Bezdicsek *et al.*, 2020). Consequently, ovarian activity decreases after calving due to reduced estrogen secretion, which in turn reduces LH and FSH secretion (Berry, 2016). The presence of fatty acids such as linoleic acid in the ration stimulates ovarian function and thus increases pregnancy rate in cows (Santos, 2008; Rodney *et al.*, 2015).

2.1. Negative Energy Balance and Its Effect on Performance

Negative energy balance (NEB) is considered a common phenomenon during the postpartum period in dairy cows, and it causes an increase in non-esterified fatty acids (NEFA) in the bloodstream. This metabolic problem negatively affects the function of insulin and insulin-like growth factor-1 (IGF-I), and these hormones are fundamental in supporting oocyte growth (Roch and Diskin, 2020). Thus, nutrition becomes synchronized with reproductive metabolism.

2.2. The Role of Minerals and Vitamins

Minerals are major components for the function of enzymes and hormones, and they regulate cell proliferation and differentiation. A decrease in their levels in the ration or their toxicity leads to disturbance in the reproductive efficiency of the animal, due to their important role in animal health and productivity after protein and energy. Therefore, attention must be given to minerals when improving reproductive efficiency, especially in dairy cows, because their deficiency causes hypocalcemia, lameness, mastitis, and retained placenta. Macrominerals include sulfur, potassium, calcium, phosphorus, magnesium, sodium, and chloride. Microminerals include cobalt, iodine, iron, copper, manganese, selenium, and zinc. Zinc is important in repairing the uterine lining, especially after calving, in enzyme function, and in carbohydrate and protein metabolism. It also reduces cases of lameness in addition to its role in reproductive hormone function (Omur *et al.*, 2016). A decrease in zinc levels in cow rations leads to reduced feed intake, decreased growth, skin lesions in the head, neck, and legs, increased salivation, and reduced reproductive efficiency (Smith *et al.*, 2010). Selenium also affects fertility and causes uterine and mammary inflammation and retained placenta (Mehdi & Dufasne, 2016). It protects the cell membrane and milk fat from oxidation, and its deficiency causes dysfunction in antioxidant activity (Mehdi Y. & Dufasne I., 2016). When administered during the first month of pregnancy, it reduces embryonic mortality (Roche J. F., 2006). Calcium is also important for increasing productivity, especially in pregnant and lactating cows. Alteration in the calcium-to-phosphorus ratio affects the secretion of reproductive enzymes and causes postpartum problems such as retained placenta and dystocia (Bachmann *et al.*, 2017). Copper contributes to the elimination of free radicals and tissue protection; its deficiency causes embryonic death, retained placenta, and reduced pregnancy rate (Ermauw *et al.*, 2014). Calcium

deficiency also causes endometritis and increases the risk of retained placenta, thus reducing fertilization rate (Rodney *et al.*, 2015). Therefore, trace elements are essential for the integrity and function of the oocyte and act as antioxidants (Sharma and Singh, 2021). These problems can be overcome nutritionally through a balanced diet, where glucose is provided after calving to stimulate oocyte function and resume ovarian cycles. The ration should also be rich in fats during the breeding season to provide fatty acids that enhance embryo growth and affect oocyte quality (Garnsworthy *et al.*, 2009).

3. Stress and Its Destructive Effect on Reproduction: The Oxidative Stress Axis

Stress affects reproduction in two ways: a direct hormonal effect and a cellular effect through free radicals.

3.1. Heat Stress and Oxidative Stress

Heat stress causes more harmful effects on reproductive efficiency than other environmental factors. It raises body temperature, which leads to increased secretion of the hormone cortisol. At the cellular level, heat stress causes excessive production of reactive oxygen species (ROS) (Putney and Hansen, 2022), which negatively affects lipids in the cell membrane, proteins, and DNA in oocytes and follicles, thereby reducing oocyte quality in an irreversible manner (Srivastava and Das, 2020).

3.2. The Effect of Metabolic Stress on Reproductive Efficiency

When metabolic stress resulting from negative energy balance (NEB) overlaps with oxidative stress, high levels of NEFA and increased ketone bodies impose additional burden on the mitochondria, leading to increased ROS production. This creates a vicious cycle, as metabolic stress induces oxidative stress, which further increases damage to reproductive efficiency (Putney and Hansen, 2022). Heat stress represents a group of factors that affect the animal, causing elevated body temperature and physiological problems (Dikmen and Hansen, 2009). Heat stress has become a threat to productivity due to reduced reproductive efficiency, deterioration of physiological status, and economic losses resulting from stress (Liam *et al.*, 2017). In addition to the deterioration of productivity and animal health, in extreme cases it may cause death (Mader, 2006), unless the animal is able to use physiological adaptation strategies such as panting, drinking water, feed intake adjustments, sweating, increased respiration rate, reduced milk production, and reduced reproductive efficiency (West, 2003; Shutz, 2008). Exposure of cows to heat stress causes reduced appetite and feed intake, and thus decreased body weight compared to cows not exposed to stress, especially during the first period after calving (Shehab-El-Deen, 2010). This leads to decreased concentrations of glucose, IGF-1, and cholesterol, while concentrations of NEFA and urea increase in blood and follicular fluid (Shehab-El-Deen, 2010). Therefore, appropriate housing systems must be selected to achieve animal welfare and high productivity (Krueger, 2020), and to avoid psychological or physical harm to the animal and prevent lameness and stress (Thomsen and Houe, 2018). These problems negatively reflect on reproductive efficiency and productivity (Polsky and Von Keyserlingk, 2017). Lameness resulting from stress and poor housing systems may negatively affect delayed pregnancy and fertilization rate (Blackie & Maclaurin, 2019). Stress also causes retained placenta and postpartum uterine inflammation (Wakayo *et al.*, 2015), and milk from stressed cows contains lower fat and protein content (Liu *et al.*, 2017). Heat stress associated with climate change may negatively affect body weight, milk production, and reproductive efficiency, resulting in significant economic losses in dairy and beef cattle herds (Philip, 2022). Hussam *et al.*, (2019) reported that a 1% increase in the heat stress index causes approximately a 24% decrease in milk production, an approximately 15% increase in the average calving interval, an increase in the number of insemination attempts by about 4.4%, and a decrease in fertility rate by approximately 9.8%. The use of stress mitigation strategies improves productive and reproductive efficiency and increases farmers' economic returns.

4. Melatonin: A Molecular Mediator of This Shared Interaction

The hormone melatonin is considered a pivotal factor linking stress and nutrition.

4.1. Melatonin as an Antioxidant and Defensive Mechanism

Melatonin is primarily classified as a regulator of the circadian rhythm, but its most important role in this article is as an antioxidant available to the body (Aydin & Demirel, 2021).

Mechanism of Action:

Melatonin acts directly by scavenging reactive oxygen species (ROS) and indirectly by stimulating endogenous antioxidant enzymes such as glutathione peroxidase, glutathione, and superoxide dismutase (Aydin and Demirel, 2021). Therefore, it is considered an excellent defensive mechanism against oxidative stress resulting from metabolic and heat stress. Regarding its role in reproduction, scientific studies have shown that melatonin is very important for oocyte maturation. It reduces oxidative damage in the oocyte, leading to increased fertilization and earlier embryonic development (Garcia and Rodriguez, 2020). Melatonin is secreted from the pineal gland and is also produced in the digestive system, skin, retina, and immune cells. It was discovered by the scientist Aron Lerner in 1958, who identified its important role in regulating circadian rhythm (Yasmin *et al.*, 2021; Kvetnoy *et al.*, 2022). Later, melatonin was used in regulating circadian rhythm (Herxheimer & Petrie, 2002) and sleep regulation. Melatonin affects various organs of the body, including the testes (Yasmin *et al.*, 2021; Liz *et al.*, 2020; Frungieri *et al.*, 2005), ovarian function (Sundaresan *et al.*, 2009), and the

placenta (Reiter *et al.*, 2014). It also acts on granulosa cells and functions as an antioxidant (Tamura *et al.*, 2012). It regulates granulosa cell activity and inhibits apoptosis (programmed cell death). Granulosa cells support and surround the oocyte inside the follicle and nourish it during its growth period (Wang *et al.*, 2018; Yang *et al.*, 2019). Studies have reported that melatonin supports antioxidant enzymes (Yuan *et al.*, 2018), improves oocyte growth both in vivo and in vitro, and enhances mitochondrial function (Sananmuang *et al.*, 2020; Yaacobi *et al.*, 2020). Melatonin regulates reproductive hormones through its action on the hypothalamus, where it stimulates the secretion of GnRH, which in turn stimulates the pituitary gland to secrete LH and FSH. These hormones affect ovarian function by regulating estrogen and progesterone secretion in a dose-dependent manner (Zhang *et al.*, 2022). A decrease in melatonin levels due to increased light exposure increases prolactin secretion, leading to increased milk production (Murphy *et al.*, 2021). Melatonin also improves semen characteristics and is an important factor in spermatogenesis through its action on the hypothalamus, pituitary gland, and testes (Frungeri *et al.*, 2017; Shahat *et al.*, 2022). Other studies have shown that melatonin injection in bulls led to increased levels of LH, FSH, and testosterone, thereby stimulating reproductive efficiency in bulls (Perumal *et al.*, 2020). It also enhances sperm motility, reduces the percentage of dead sperm, and preserves acrosome integrity (Gutiérrez-Añez *et al.*, 2022).

4.2. Stress Mitigation

Melatonin dietary supplementation works to reduce the effects of heat stress and negative energy balance (NEB) occurring after calving. This leads to the restoration of oxidative stress balance, thereby providing a suitable environment for the return of reproductive activity and consequently improving oocyte quality (Garcia and Rodriguez, 2020). When oxidative stress occurs, it negatively affects ovarian cells, renal epithelial cells, and cells of the brain and spinal cord (Lan *et al.*, 2018; He *et al.*, 2018). Here, melatonin enhances and regulates antioxidant factors such as the Nrf2 protein, where it activates the nuclear factor associated with Nrf2, stimulating the cell to resist oxidative stress (Huang *et al.*, 2022; Shah *et al.*, 2017). Melatonin also supports the interaction between glutathione (GSH) and reactive oxygen species and other harmful oxidizing agents. It converts free radicals from harmful compounds into non-harmful compounds (He *et al.*, 2018). In addition, melatonin protects mitochondria by stabilizing their membrane potential and enhancing their function, thereby reducing the likelihood of programmed cell death resulting from mitochondrial dysfunction (Murphy *et al.*, 2013; Tan *et al.*, 2016). In summary, melatonin acts to remove oxidative free radicals that cause cell death or damage. It also preserves cell membrane integrity by protecting it from lipid peroxidation, thus maintaining membrane flexibility and functionality. Studies have also demonstrated the ability of melatonin to enhance oocyte and embryo development in vitro in cattle (La *et al.*, 2023) by stimulating meiotic division during oocyte maturation and promoting blastocyst formation, which is the stage at which the embryo is ready for implantation in the uterus (Rodrigues *et al.*, 2016). The presence of the enzyme ASMT and the melatonin receptor (MTNR1A) on the oocyte and surrounding cells in cattle has been confirmed. Administration of 10 and 50 ng/mL of melatonin stimulated nuclear maturation, induced mitochondrial changes, and reduced levels of reactive oxygen species (ROS) that cause oxidative stress, thereby acting as a strong cellular antioxidant (El-Raey *et al.*, 2011). Melatonin also protects embryonic cells exposed to oxidative stress caused by mycotoxins such as zearalenone and prevents apoptosis by increasing antioxidant enzymes (SOD and GSH-px) and by inhibiting mitogen-activated protein kinase signaling pathways (P38 MAPK) and reducing the Bax/Bcl-2 ratio (Yang *et al.*, 2019).

5. CONCLUSION

Through this article or review, it has been shown that the reproductive efficiency of cows is the result of a complex interaction between nutrition and stress, and this interaction ultimately translates at the cellular level into oxidative stress. Melatonin is considered a molecular mediator and regulator that can mitigate the shared negative effects. Therefore, future studies are recommended to focus on the following: Determining the optimal timing and dosage of melatonin administration as practical nutritional strategies to stimulate high-producing cows during the postpartum period. Conducting molecular studies that detail the mechanism of melatonin action on reproduction-related genes under heat stress conditions. By linking these findings, nutrition specialists and even breeders can develop more important and effective strategies to support reproductive efficiency and sustain the meat and dairy industry.

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