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Applications of antioxidants in reproduction in farm animals

A Graduation Project Submitted to the Department Council of the Internal and Preventive Medicine-College of Veterinary Medicine/ University of Al-Qadisiyah in a partial fulfillment of the requirements for the Degree of Bachelor of Science in Veterinary Medicine and Surgery.

By

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

فَتَعَٰلَى ٱللَّهُ ٱلۡمَلِكُ ٱلۡحَقُّۗ وَلَا تَعۡجَلۡ بِٱلۡقُرۡءَانِ مِن قَبۡلِ أَن يُقۡضَىٰٓ
إِلَيۡكَ وَحۡيُهُۥۖ وَقُل رَّبِّ زِدۡنِي عِلۡمٗا ﴿١١٤﴾

صدق الله العظيم

من سورة طه

Certificate of Supervisor

I certify that the project entitled (**Applications of antioxidants in reproduction in farm animals**) was prepared by **Ibrahim Mowaffaq Ameen** under my supervision at the College of Veterinary Medicine / University of Al-Qadisiyah.

Supervisor

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22 / 5 / 2021

Certificate of Department

We certify that **Ibrahim Mowaffaq Ameen** has finished his Graduation Project entitled (**Applications of antioxidants in reproduction in farm animals**) and candidate it for debating.

Instructor

Dr. Muthanna H. Hussain

-- / -- / 2020

Head of Dept of Int. and Prev. Med.

-- / -- / 2020

Dedication

Every challenging work, needs self-efforts as well as guidance of elders especially those who were very close to our heart.

My humble effort I dedicate to my sweet loving Father and Mother, whose affection, love, encouragement and prays of day and night make me able to get such success and honor, along with all my hard working and respected doctors in the College of Veterinary Medicine at University of Al-Qadisiyah.

Ibrahim

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Summary

This study examines the role of oxidative stress on reproduction of female and male animal, as well as the positive and harmful effects of plant-derived antioxidants. The cattle industry suffers tremendously as a result of oxidative stress caused by free radicals in animal reproduction. Antioxidants medication has been demonstrated to help prevent diseases caused by oxidative stress. Because natural antioxidants have fewer side effects than synthetic antioxidants, the plant extracts or whole have been widely employed in sheep, goats, and cows. Although great improvements have been made in the use of plant-derived anticipants to alleviate oxidative stress, there are still some questions because various opposite effects have been found in the same animal utilizing heerb extracts containing identical bioactive components. As a result, plant-based antioxidants, such as free radicals, can alter semen activities, spermatogenesis, estrous cycles, ovulation, ovary activities, embryonic development, endometrium, and gestation in both positive and negative ways in the reproduction of sheep, goats, and cows. The mechanism of plant-based antioxidant' actions in various reproductive systems, as well as the dose-dependent technique as an rationale for herb extracts' multimodel action, require further investigation.

Chapter two

2.1.Oxidative stress in the reproduction of female animals:

In both in vivo and in vitro culture (IVC), free radicals are produced in several ways in the female reproductive system(Sorelle et al., 2019). Reactive oxygen species, or ROS, are produced directly by oocytes and embryos, as well as by their surroundings, and they mediate the processes of embryonic development(Khazaei and Aghaz, 2017). Reactive nitrogen species, or RNS, are implicated in the oocyte meiotic maturation of sheep, pigs, and rats (Rong-zhen and Dao-wei, 2013), in addition to ROS generation. In the female reproductive system, free radicals have a multiple role, particularly in ovulation. Many in vitro conditions, in addition to the usual creation of free radicals in vivo, generate oxidative stress (OS) and damage to the reproductive systems. For example, increased ambient temperature and different toxins are two major sources of (OS) (Hasanuzzaman et al., 2013). Heat shock causes changes in membrane characteristics, chromatin structure, and meiotic spindle behavior in embryos and oocytes (Ju et al., 2005). Ovarian inefficiency in farm animals has been connected to high blood and milk lead levels, and (OS) has been connected to the pathophysiology of cadmium and lead -induced reproductive disorders (Patra et al., 2011). Free radicals have a pivotal role in the pathophysiology of abortion, birth abnormalities, endometriosis, the hydatid form mole, preeclampsia , and infertility in sheep, goats, and cows reproductive systems. Bovine embryo development was improved by low exposure to H_2O_2 throughout oocyte maturation (Vandaele et al., 2009). On the other side, too many free radicals had harmful consequences. In addition, studies in cattle have revealed that early phases of embryonic development, such as the two-cell and four-cell stage, are more vulnerable to free radical-induced stress than oocytes, morulas, and blastocysts, due to more active mitochondria.

2.2.Animal reproduction and exogenous antioxidants:

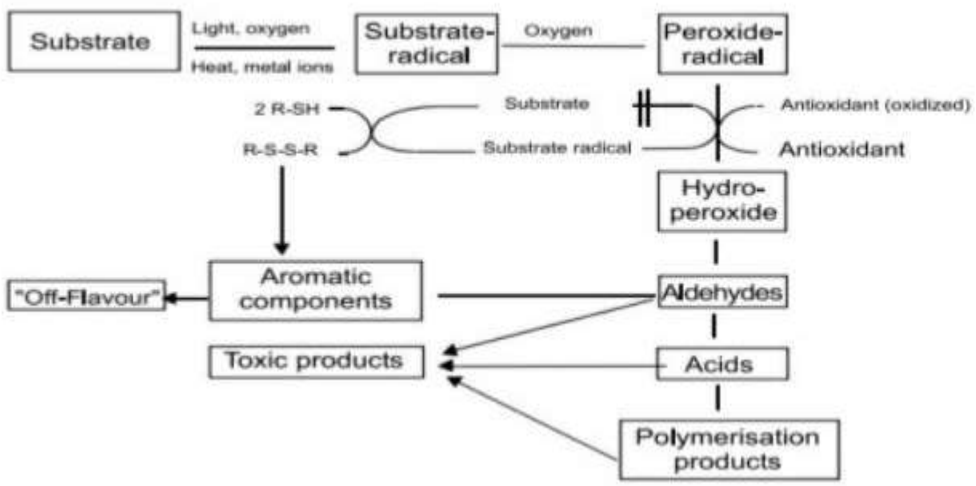
Exogenous antioxidants are important in the delicate balance between oxidation and autoxidation, but they also have a negative impact on living organisms' cellular redox state (Bouayed and Bohn, 2010). The actions of prooxidants and antioxidants are highly dependent on their concentrations(Carocho and Ferreira,2011). Although various synthetized antioxidants, like vitamin E and vitamin C, have been utilized to rescue the ova and embryos from (OS), there are indeed disagreements because of a number of unfavorable impacts on animal reproductive process (Nayyar and Jindal 2010). Some researchers found that 100 mol/ L vitamin E significantly increased development of bovine blastocysts when given antioxidant vitamins (Olson and Seidel). Sudano et al., on the other hand, discovered that adding 200 mol/ L vitamin E to bovine embryo development in vitro had a negative effect. Both investigations indicated that Vitamin C concentrations less than 50 mol/L were ineffective in improving embryo development. Other found that 50 mol/ L vitamin C improved the rate of blastocyst development in vitro in mouse, while 400 to 500 mol/ L with vitamin C considerably slowed embryo formation and development rates (Wang et al., 2002). Natural herbs or their preparations have lately been demonstrated to be healthy and commonly utilized as antioxidants as an alternative. Phenolic substances, such as phenolic acid, hydrolysable tannins, and flavonoids, are the most efficient ingredients essential for antioxidative characteristics (Dai and Mumper, 2010). The antioxidant properties of phenolic compounds are due to their structure, specifically their capacity to donate a H^+ to the peroxy radical generated by lipid peroxidation.

2.3.Overview of the oxidative reaction's role:

Antioxidants have a variety of functions. Antioxidant characteristics research had hitherto been limited to determining the storage stability of lipids and fat-containing meals. Recent study on the effects of antioxidative activities in live organisms has demonstrated that antioxidants are garnering a lot of attention these days in relation to the quality of processed animal products. A healthy body strives to achieve a balance of oxidative and antioxidative activities. Oxidative stress is a condition produced by an increase in the number of oxidative processes in the body. Antioxidant compounds are important throughout the perinatal period in cattle, according to studies, proving the usefulness of antioxidants at periods when physiological performance is at its best (Abuelo et al., 2019). Antioxidants are substances that considerably delay or prevent the oxidation of an oxidizable substrate when present at low concentrations compared to the concentrations of that substrate (Halliwell et al.,1995). These are free-radical chain interactions that are aided through a variable factors and are known as autoxidation (lipid peroxidation) because they happen on their own (Sun et al., 2011). The most important factor in vivo is oxygen. Because of their catalytic activity, metal ions such as iron and copper produce free radicals. Antioxidants halt the chain reaction by trapping reacting substrates and peroxide radicals already when they react with O2 (Williams, 2010).

Figure 1 explain the mechanism described forward.

Figure 1: Schematic representation of oxidation processes (from EDER, 2001)



One of the most potent antioxidants in the human body is vitamin E. Vitamin E cannot be produced by the body, so it must be obtained through food. Vitamin E requirements are influenced by the body's fatty acid profile, selenium status, metal ion concentration like iron and copper, amount amino acids of sulphur content, and concentration of retinol. Vitamin E (8-tocopherols) naturally found in ration contents (natural fats, forage, cereals and oils) is sometimes low to meet the body's demands, and intakes can vary greatly depending on the composition of the ration (Agarwal et al.,2005). The amount and type of unsaturated fatty acids given affects vitamin E requirements as well.

2.4.What are antioxidants?

A substance that shields the body from the negative effects of oxygen, such as free radicals. Well-known antioxidants include enzymes and other chemicals capable of counteracting the damaging effects of oxidation, such as vitamin C, vitamin E, and beta carotene. Antioxidants are frequently added to meals such as vegetable oils and processed foods to prevent or delay degradation caused by air exposure. Antioxidants have been shown to lower cancer risks and to slow the progression of age-related macular degeneration (Hajhashemi et al., 2010).

2.5.Type of antioxidants:

Vitamins, phytochemicals and enzymes are the three basic forms of antioxidants found in nature, with phytochemicals being the most effective antioxidants(Nimse and Pal, 2015). Because plants are exposed to UV light throughout the day and produce a large number of free radicals, they have a natural built-in protection system that prevents free radicals from causing cellular damage, which would eventually cause the plant to wither and die; this protection is provided by naturally occurring antioxidants (Hasanuzzaman et al., 2020).

2.5.1. Vitamins with antioxidant properties:

Because the human body does not naturally create antioxidant vitamins, it is critical to include dietary sources of them in our regular diet, whether by supplements or foods. Beta-carotene, vitamins E, C,A, and folic acid are examples of antioxidant vitamins(Poljsak et al., 2013). Vitamin A is very beneficial to the immune system, eye health, tissue healing, and cholesterol levels. Vitamin C protects the skin from UV damage (Poljsak et al., 2013), promotes improved iron absorption (Lynch et al., 1980), increases infection resistance, and aids in blood cholesterol regulation. Vitamin E helps to keep blood vessels healthy, improve skin conditions, and protect the body's membranes (Agarwal et al, 2008). In the meanwhile, folic acid is essential for women of childbearing age, especially in preventing neural tube abnormalities in the fetus. Beta-carotene is a powerful carotenoid (a type of phytochemical) that offers the best protection against singlet oxygen and free radicals(Shankaranarayanan et al., 2018). This vitamin is abundant in orange-colored foods such as carrots, pumpkins, and sweet potatoes, as well as dark green vegetables such as spinach, kale, and collards (Shankaranarayanan et al., 2018). Coenzyme Q10 (or CoQ10) is a vitamin-like molecule that is created by the body and has been shown to be an important component of cell functioning(Bonakdar and Guarneri, (2005). Our bodies generate less of this molecule as we age, which has been linked to the development of a number of age-related disorders and disorders.

2.5.2.Enzymatic antioxidants:

Enzymes are antioxidants that are produced in the human body and include glutathione peroxidase, superoxide dismutase (SOD), catalases, and glutathione reductase (Hasanuzzaman et al., 2020). Antioxidant enzymes require co-factors such as iron, copper, selenium, magnesium, and zinc to offer optimal antioxidant activity; nevertheless, the quality of the protein supply has an impact on the antioxidant enzymes' quality.

2.5.3. Phytochemical antioxidants:

Plants use phytochemicals as antioxidants to protect themselves from free radicals in the environment(Hasenuzzaman et al., 2020). Humans that eat phytochemical-rich foods reap the benefits of the plant's antioxidant characteristics, according to research(Rodriguez et al., 2006). The following are the different types of phytochemicals. Carotenoids, flavonoids, allyl sulfides, and polyphenols are all types of carotenoids. Phytochemicals are found in most natural whole foods, such as whole grains, fruits, and vegetables (Rodriguez et al., 2006), whereas processed or refined meals have little to no phytochemicals.

2.6.Comparison of in vitro and in vivo antioxidant:

In vitro, a flavonoid isolated from mulberry fruit has a high antioxidant activity and prevents H,O-induced hemolysis of mouse red blood cells as well as lipid peroxidation in the liver, mitochondria, and microsomal of mice (Agarwal and Prabakaran, 2005). Flavonoids' antioxidant properties are thought to be influenced by structural diversity. Flavonoids' antioxidant properties are determined by their degree of polymerization. Flavonoids' antioxidant properties are influenced by the presence of a 4-carbonyl group and a C2=C3 double bond. (Ahmed et al., 2010) Glycosylate reduces flavonoids' antioxidant properties.

2.7.The role of natural antioxidants in reproductive physiology:

Application of antioxidants in male AI and IVP (IVM,IVF, IVC) treatment of infertility: handling, preparation and manipulation of gametes during the procedure of assisted reproductive technologies (ART) exposes them to a number of ROS-inducing factors (Fig.). Superoxide anion radicals, hydroxyl radicals, and nitric oxide radicals, as well as non-radical derivatives such as hydrogen peroxide, peroxy nitrite, and hypochlorous acid, are all members of the ROS family(Combelles et al.,2009). ROS have beneficial effects at physiologic levels, allowing male germ cells to operate normally and potentiating their fertilizing powers. Incubation of spermatozoa with hydrogen peroxide has been demonstrated to enhance sperm capacitation, hyperactivation, acrosome response, and oocyte fusion (Bansal and Bilaspuri 2010).

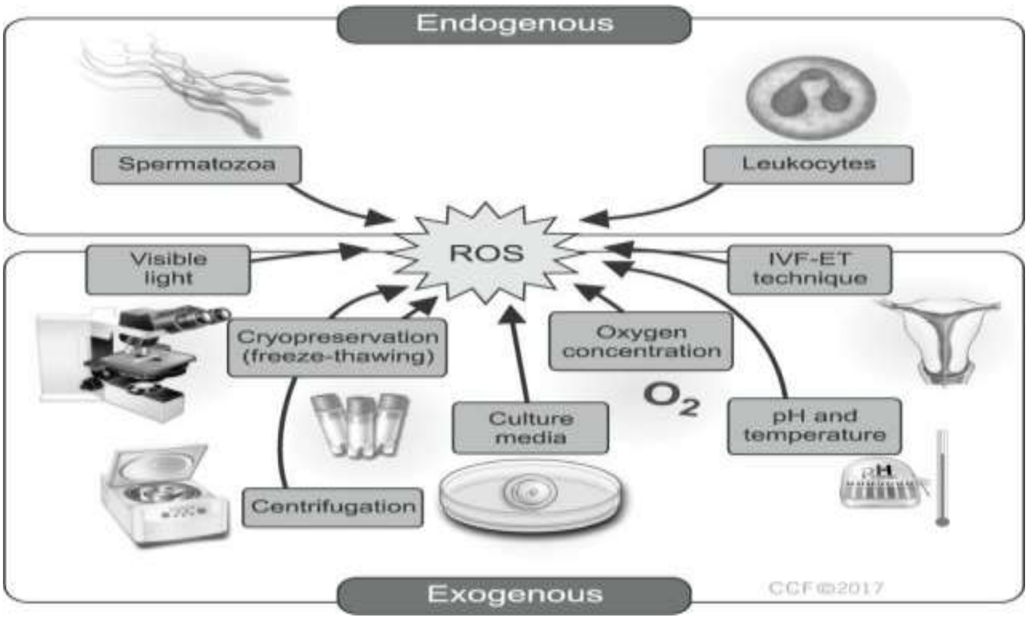


Figure .Sources of reactive oxygen species (ROS) in the ART. in vitro fertilization-embryo transfer. Data from Cleveland Clinic Foundation (CCF) with CCF's permission(Agarwal and Majzob, 2017).

Antioxidants found in follicular and seminal fluid are capable of stabilizing or deactivating free radicals, ensuring that they exist at ideal physiologic levels. The enzymatic system, which contains SOD, catalase, and GHS, and the non-enzymatic system, which includes vitamin C, urate, tocopherol (vitamin E), pyruvate, glutathione, and others, have been identified(Starlin et al., 2013).

2.8.Some applications of antioxidants in the female treatment and some reproductive failure:

2.8.1. IVM:

The findings in the field of in IVEP were made possible by research into animal physiological parameters in vivo. Several studies refer to the positive activities of vitamin antioxidants when used throughout IVM programs at specific concentrations (Combelles et al., 2009). Abdulnabi and Daham (2021) recorded that the use of Royal jelly in 10mg/ ml as supplement to IVM media was improving the rate of in vitro maturation of bovine oocytes and refer that this iprovment may be due to the antioxidant activity of royal jelly. Eshtiyaghi et al (2016) recorded that the royal jelly positively impacted in vitro maturation of oocytes by improving the redox state and glucose metabolism. Isolated murine oocyte–granulosa cell complexes were grown in a serum-free Waymouth medium with increasing doses of ascorbic acid, demonstrating an antiapoptotic effect for vitaminC (Combelles et al., 2009).

2.8.2. IVC:

Despite IVEP is currently actually successful in majority of domestic animals, our understanding of the mechanisms that control embryonic development is still limited, and an optimum media for IVC has yet to be developed. In fact, after IVF and embryo culture, just 30-45 percent of developed oocytes reaching the blastocyst stages, with pregnancy chances near to 40–60 percent after embryo transfer program (Hasler et al., 1995). Ammonia, oxygen radicals, growth hormones, and other variables are known to alter in vitro embryo development. Increased (OS) is one of the key factors impacting mammalian IVEP (Gasparrini et al., 2000).

Defined culture media still need to be refined in order to standardize embryo production procedures; and despite numerous attempts to improve IVC programs in various species animals, the oviduct still indispensable for embryos growth. Antioxidant supplements such as SOD and thioredoxin (Nonogaki et al., 1991), catalase, vitamins C and E (Nasr-Esfahani and Johnson, 1992), EDTA (Nasr-Esfahani et al., 1992. The impacts of , vitamins C and E, EDTA on IVEP cattle embryo culture were studied at Seidel's lab; vitamin E significantly increased blastocyst growth, but vitamin C and EDTA had no discernible benefit (Olson and Seidel, 2000). Other than medium ingredients are among the elements that are hazardous to embryo culture.

2.8.3 Treatment of some reproductive infections:

Excess reactive oxygen radicals can cause male and female infertility, as well as pregnancy complicatios. As a result, antioxidants, often known as "scavengers," are commonly employed to detoxify excess (ROS) (Merve and Elmas, 2016). (OS) can also cause pregnancy difficulties such as recurrent pregnancy loss and spontaneous abortion (Agarwal et al.,2012). Some researchers testing the therapeutic and protective activity of the pumpkin seed ethanolic extract on experimental induced prostatic hyperplasia in rats and they recorded a significant positive activity on both therapeutic and protective level and they suggested that these effects might be due to the antioxidant activity of phenolic contents of pumpkin (Daham et al., 2021). Another researchers also studying the antioxidant activity of propolis on prostatic hyperplasia in vitro and in vivo and they found a significant positive antioxidative effects on both in vivo and in vitro levels (Abd-Alhassen et al., 2020). During the sperm maturation, selenium protects spermatozoa from oxidative damage (Merve and Elmas, 2016). The combination of Alpha-Lipoic Acid and NAcetylcysteine is utilized to protect against testicular damage caused by oxidative stress (Jana et al., 2014). In diabetic rats produced by streptozotocin, a combination of 300 mg/kg green tea and 0,4 mg/kg vitamin E exhibited a protective effect in the testicular tissue (Kaplanoglu et al., 2013) due to its high content of catchine which have antioxidant activity (Sato et al., 2010). Exogenously delivered melatonin during pregnancy may help protect the mother and her fetus from oxidative stress (Reiter et al., 2014) because of antioxidant activity of it and its metabolites (Gobbo et al., 2015). Aqueous catechin extract also causing improvement of sperm motility in mice (daham and Al-Hilfi, 2010).

3.Conclusions and recommendations:

Conclusions

1-Antioxidants are A substance that shields the body from the negative effects of oxygen, such as free radicals.

2-There are natural and industrial antioxidants and some are enzymatic and other are non-enzymatic.

3-There are many Antioxidants useful in reproduction and reproductive disorders and assisted reproductive technologies.

Recommendations

1- Studying the antioxidant activities of different plant and herbal products on reproduction.
2- Studying the different activities and effects of antioxidants on ART.

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