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Isolamento bioguiado de compostos com atividade antioxidante para otimizar a maturação *in vitro* de oócitos de mamíferos

Bioguided isolation of compounds with antioxidant activity to improve the *in vitro* maturation of mammalian oocytes

Aislamiento bioguidado de compuestos con actividad antioxidante para mejorar la maduración *in vitro* de ovocitos de mamíferos

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Resumo

Os compostos bioativos são caracterizados como metabólitos secundários derivados de plantas que exercem efeitos farmacológicos e/ou toxicológicos em humanos e/ou animais. Nos últimos anos, tais compostos, especialmente aqueles com ação antioxidante, têm sido utilizados na maturação *in vitro* (MIV) de oócitos de diferentes mamíferos. Tal aplicação tem objetivado aumentar a eficiência da produção *in vitro* de embriões, reduzindo o estresse oxidativo causado pelas condições *in vitro*. Esta revisão tem como objetivo uma visão geral

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dos processos de obtenção de bioativos e sua utilização na MIV de oócitos, destacando os diferentes compostos e seus papéis na redução do estresse oxidativo em oócitos de mamíferos. Trata-se de uma revisão, focada em periódicos nacionais e internacionais, para reunir as melhores informações sobre o isolamento bioguiado de compostos que podem ser utilizados na MIV. Em geral, o caminho percorrido para atingir a molécula final com atividade evidente e reproduzível envolve os ensaios bioguiados e de bioprospecção com a extração da matéria-prima e os estudos de sua atividade química e biológica. Assim, uma série de compostos já foi utilizada em bovinos, ovinos, suínos e caprinos, com resultados promissores, comparados aos dos antioxidantes sintéticos. O uso de estudos bioguiados auxiliados por técnicas de bioprospecção é imprevisível para a descoberta de bioativos com potencial ação no nível tecnológico de uso. A otimização dessas tecnologias é extremamente importante para obter resultados cada vez mais claros e confiáveis, aumentando o sucesso reprodutivo das espécies por meio da aplicação em larga escala de técnicas *in vitro*.
Palavras-chave: Atividade antioxidante; Bioprospecção; Compostos fenólicos; Reprodução.

Abstract

Bioactive compounds are characterized as secondary metabolites, derived from plants, that exert pharmacological and/or toxicological effects on humans and/or animals. In recent years, such compounds, particularly those exhibiting antioxidant activity, have been employed in the *in vitro* maturation (IVM) of oocytes from different mammals. Such an application is aimed at increasing the efficiency of *in vitro* embryo production by reducing the oxidative stress caused by *in vitro* conditions. This review objective is an overview of the processes of obtaining bioactives and their utilization in the IVM of oocytes by highlighting the different compounds and their roles in reducing the oxidative stress in mammalian oocytes. The review is focused on national and international journals, to gather the best information on the bioguided isolation of compounds that can be utilized in IVM. Generally, the path taken to achieve the final molecule with evident and reproducible activity involves the bioguided and bioprospecting assays with the extraction of the raw material and the studies of its chemical and biological activity. Thus, a series of compounds have already been utilized in cattle, sheep, pigs, and goats, with promising results, compared to those of synthetic antioxidants. The benefit of the bioguided studies, aided by the bioprospecting techniques, is unpredictable in the discovery of bioactive with potential action at the technological level of application. The optimization of these technologies is extremely beneficial to obtaining very evident and reliable results, thus increasing the reproductive success of the species through the large-scale application of the *in vitro* techniques.

Keywords: Antioxidant activity; Bioprospecting; Phenolic compound; Reproduction.

Resumen

Los compuestos bioactivos se caracterizan como metabolitos secundarios, derivados de plantas, que ejercen efectos farmacológicos y/o toxicológicos en humanos y/o animales. En los últimos años, tales compuestos, particularmente aquellos que exhiben actividad antioxidante, se han empleado en la maduración *in vitro* (MIV) de ovocitos de diferentes mamíferos. Dicha aplicación tiene como objetivo aumentar la eficiencia de la producción de embriones *in vitro* al reducir el estrés oxidativo causado por las condiciones *in vitro*. El objetivo de esta revisión es una visión general de los procesos de obtención de bioactivos y su utilización en la MIV de los ovocitos al destacar los diferentes compuestos y su papel en la reducción del estrés oxidativo en los ovocitos de mamíferos. La revisión se centra en revistas nacionales e internacionales, para recopilar la mejor información sobre el aislamiento bioguidado de compuestos que se pueden utilizar en MIV. Generalmente, el camino tomado para lograr la molécula final con actividad evidente y reproducible involucra los ensayos bioguiados y de bioprospección con la extracción de la materia prima y los estudios de su actividad química y biológica. Por lo tanto, una serie de compuestos ya se han utilizado en ganado bovino, ovino, porcino y caprino, con resultados prometedores, en comparación con los de los antioxidantes sintéticos. El beneficio de los estudios bioguiados, ayudado por las técnicas de bioprospección, es impredecible en el descubrimiento de bioactivos con acción potencial a nivel tecnológico de aplicación. La optimización de estas tecnologías es extremadamente beneficiosa para obtener resultados muy evidentes y confiables, aumentando así el éxito reproductivo de la especie a través de la aplicación a gran escala de las técnicas *in vitro*.

Palabras clave: Actividad antioxidante; Bioprospección; Compuesto fenólico; Reproducción.

1. Introduction

Among research practices involving phytochemicals, bioprospecting has been widely studied and applied for years. This process is defined as a purposeful evaluation of wild biological materials, by applying advanced technologies, in an attempt to obtain new valuable products (Cicka, & Quave, 2019). Because of this method of investigation, several molecules, of different chemical groups, have been discovered and isolated for utilization, as antioxidants (Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2017). However, specific studies, also called bioguided studies, can be conducted to obtain pure and biologically active compounds, based on the monitoring of the targeted biological activity.

With these bioguided studies, it is possible to define the chemical functions of interest and explore the results, based on the positive responses, obtained from bioprospecting the products (Bucar, Wube, & Schmid, 2013). These analyses identify the active groups of the compound and determine how modifications can maximize the effect of the compound (Wang, Li, & Bi, 2018). Wherefore, advances in the discovery of molecules with high potentials have attracted the interest of different areas of research in recent years, including reproduction.

Generally, fertility preservation and infertility treatments have emerged at the forefront of reproductive-related research in recent years (Vuong, Ho, Gilchrist, & Smitz, 2019). This exponential growth was due to the increased application of assisted reproductive technologies, such as *in vitro* embryo production (IVEP). The main objective of this technology is to optimize the reproductive potential of females, enhance the gene of animals as well as generate large numbers of embryos (Ealy, Woodridge, & Mccoski, 2019). Although the IVEP protocols have substantially progressed in recent years, the *in vitro* rates are still low, ranging from 20 to 40% for blastocyst production (Santos, Borges, Queiroz Neta, Bertini, & Pereira, 2018).

Therefore, different approaches, such as the optimization of the stages of IVEP to the level of the *in vitro* maturation (IVM), have been employed to improve the efficiency of this technology. IVM consists of an initial step of IVEP, where the oocytes are stimulated, *in vitro*, by the luteinizing hormones, to resume their meiotic progression from the stages of prophase I to metaphase II (MII). However, the oocyte maturation rates continue to vary from species to species. Authors have reported the rates of the MII oocytes in various species as follows: ~70% in porcine (Alvarez et al., 2019), sheep (Zabihi, Shabankareh, Hajarian, & Foroutanifar, 2019), and cattle (An et al., 2019); 45% in goats (Piras et al., 2019); and 65% in humans (Vuong et al., 2020).

These efficiency variations may be due to complications related to the *in vitro* culture environment, as denoted by El-Aziz, Mahrous, Kamel, & Sabek, (2016) who reported a 20–30% difference in the IVM efficiency, compared to IVM of the bovine oocytes. Several parameters can be correlated with IVM efficiency, especially the medium supplementation (Guemra et al., 2013). The imbalance of this feature will result in the phenomenon, called oxidative stress.

Nowadays, the use of different antioxidants, in culture media, have been explored to improve the existing rates of IVM and reduce the injuries caused by oxidative stress. Therefore, the application of bioguided studies in the isolation and evaluation of molecules for the subsequent characterization of their potentials is extremely beneficial to ensuring greater experimental reproducibility as well as obtaining the prospective result for an isolated compound. Thus, this review discusses the application of the bioguided studies in obtaining isolated compounds, with antioxidant potentials, from plants to improve the rates of IVM in mammalian the oocytes and the subsequent embryonic development.

2. Methodology

The study is a bibliographic review, conducted, based on national and international journals. The queries in the online libraries: SciELO, Pubmed, and journals portal CAPES. To search, we utilized the following keywords: “Antioxidant activity,” “bioprospecting in plants,” “IVEP,” and “IVM.”

3. Literature Review

3.1 Extraction of the bioactives from plants: extracts *versus* essential oil

To obtain the benefits of plant phytochemicals, it is necessary to employ adequate, cost-effective, and low-toxicity extractive methods, for the extractions of either plant extracts or essential oils, and those are the objectives of this study. The form of extraction, chemical composition, and success of isolating the components from these solutions differ in several aspects, thereby necessitating the desire to assess the best method to complete the bioguided study of interest (Altemimi et al., 2017).

The extracts are concentrated solutions, obtained from vegetal materials, which were exsiccated and crushed. Generally, the process aims to identify, isolate, purify, and extract the phytochemicals through the addition of solvents. The quality and quantity of the extracted metabolites require the following standards for adoption: visual inspection, desiccation, shredding, granulometry, solvent determination, and extraction methods (Ingle et al., 2017). The choice of solvents depends on the chemical affinity, which is related to the pH and polarity of the molecules present in the extract. The most utilized solvents include water, ethanol, methanol, chloroform, ether, and acetone (Ingle et al., 2017).

Moreover, there are several standard procedures for the extraction of plant metabolites, to obtain the extracts: i) maceration, which consists of maintaining a plant, with the bioactive of interest, in contact with the solvent for a period ranging from 3 h to 3 wk, at 25 °C (Jovanović et al., 2017); ii) infusion, which is a process, employed to obtain volatile substances, by diluting the solute in a boiling solvent (Herrera et al., 2018); iii) ultrasound extraction, which requires a device that emits waves in the frequency of 20–2.0 × 10^3 KHz, thus increasing the penetration of the solvent to the solute matrix through the fragmentation of vegetal cell membranes (Nora, & Borges, 2017); and iv) decoction, which is employed to obtain only thermo-resistant substances. Here, a solvent is added to the solute and both are heated and boiled (Hussain, Saquib, & Khan, 2019).

Contrarily, essential oils are products of a more complex general composition than vegetal extracts that contain volatile principles present in the plants with less modification during their preparation. Generally, they are produced from specialized secretory structures, such as oil channels and differentiated parenchymal cells; they are predominantly colorless or slightly yellow; and exhibit slight instabilities in the presence of light and heat (Satyal, & Setzer, 2019). Essential oils are mainly found in superior plants, and it is estimated that there are ~17,500 aromatic species (Satyal, & Setzer, 2019).

The methods of extracting oil consist partially of four types. The first type is steam distillation, which is the most widely employed method for extracting essential oils, worldwide. Through it, it is possible to obtain oils from leaves, roots, grasses, branches, seeds, and even some flowers. The process consists of subjecting the plant material to water vapor, which extracts the oil by "steam dragging." The extracted oil vaporizes thereafter and is subjected to thermal shock, which condenses, cools, and returns the oil to the liquid phase (Božović, Navarra, Garzoli, Pepi, & Ragno, 2017). The second method is cold pressing, which is widely employed for citrus fruits (Ferhat, Boukhatem, Hazzit, Meklati, & Chemat, 2016). Hydrodistillation, the third method, is a variation of steam distillation but it does not involve high temperatures and is widely employed in laboratories (Ferhat et al., 2016). Finally, enfleurage, also called enfloration, is a technique for the extraction of essential oils from delicate materials whose compounds can suffer alterations if extracted by the other methods (Hanif, Nisar, Khan, Mushtaq, & Zubair, 2019).

However, although there are several extraction methods for the bioactive materials of interest, some factors must be considered, based on the intended results. It is evident that plant extracts, obtained with solvents, despite being the most widely used method, exhibit a significant disadvantage when compared to essential oils although both the extract and essential oils possess similar major components (Xu, Liu, Hu, & Cao, 2016), the utilization of solvents, for obtaining the extracts, critically influences the composition and concentration of their constituents (Azwanida, 2015). Essential oils, however, afford a purer combination of the bioactive substances, without the interference of different solvents, thereby maintaining greater similarity with their plant sources (El-Maati, Mahgoub, Labib, Al-Gaby, & Ramadan, 2016). It was observed by Chaicouski, Silva, Trindade, & Canteri (2014), utilizing Yerba mate (*Ilex paraguariensisa*), that hydroalcoholic extraction was more efficient, compared to an aqueous one (which totally changed the composition of the extract) because the alcohol extracted both the polar and nonpolar compounds. Additionally, it was seen that when the temperature of the solvent was increased, as in the extraction by infusion, the composition of the extract changed drastically because several chemical reactions could occur between the solute and the solvent (Herrera et al., 2018).

Therefore, the utilization of essential oils is outstanding for maintaining the closest characteristics observed in the raw material, thereby affording a substance, rich in natural bioactive products and ready to be explored (Hanif et al., 2019). However, the concentration and predominance of these bioactive substances can be altered by several inherent factors of the plant, such as the part utilized, place of origin, and season of the year (Affonso, Rennó, Slana, & Franca, 2012). Thus, it is necessary to advance to the next step in the bioguided study of the isolation of the materials of interest to obtain the bioactive molecules and evaluate their action potential.

3.2 Types of bioactives and their applications in oocyte IVM

Active principles are responsible for global plant protection, nutrition, and defense against predators and pathogens. These bioactive compounds assume different biological roles, depending on their classification. They are broadly divided into primary and secondary metabolites. The primary metabolites are organic compounds, such as carbohydrates, amino acids, fatty acids, nucleotides, and their polymeric derivatives (polysaccharides, proteins, lipids, RNA, and DNA), which can be employed as sources of energy for respiration, growth in photosynthesis, and as precursors for the synthesis of the secondary metabolites, through enzymatic reactions (Cheng, 2017). However, although some studies reported the extraction of these molecules, they are not commonly isolated as the final bioactive compounds as per the bioguided studies.

Further, the secondary metabolites are complex structures, in small quantities, with different molecular weights, which can exhibit different polarities (Shitan, 2016). The disadvantage of utilizing these molecules is related to the quantity and quality of these metabolic compounds because they are directly influenced by leaf development, dilution, increase or decrease in biomass, seasonality, time of the day, rainfall, temperature, and altitude (Böttger, Vothknecht, Bolle, & Wolf, 2018). However, the standardized extraction, through the production of biological exsiccates, and analysis of the composition, by chromatographic techniques, aid the acquisition of similar bioactives molecules, during the bioguided study chain (Böttger et al., 2018).

Among the secondary metabolites most exploited for their biological potentials, are the alkaloids, phenols, tannins, flavonoids, and saponins (Kabera, Semana, Mussa, & He, 2014). These are widely explored by several areas of research, including the optimization of human and animal reproduction. In IVM studies with oocytes, the compounds, with antioxidant potentials, are the most explored because of the desire to supplement the *in vitro* culture media to reduce the oxidative stress (Santos et al., 2018). Therefore, several bioguided studies have been developed over the years to reduce the negative effects caused by oxidative stress, thus increasing efficiency and productivity, linked to the technique (**Table 1**).

Different approaches can be considered when evaluating the isolated compounds in IVM. The first is to obtain the compounds, by isolating them from the extracts themselves, as was conducted by Mesalam et al. (2017). They produced a methanolic extract from the roots of *Polygonum cuspidatum*. The fractions were chromatographed to obtain the required isolate (2-methoxystypandrone). They utilized this bioactive-of-interest as an antioxidant for IVM of a bovine oocyte and successfully improved the blastocyst quality, by decreasing the apoptotic cells and increasing the total cell number, compared to that of the control groups. Contrarily, other studies demonstrated the utilization of commercially acquired isolates (Piras et al., 2019; Zhao et al., 2020).

Another characteristic, found in the bioguided studies with antioxidants in IVM, would be the investigation of the most abundant antioxidant molecules present in the extracts and oils (Nie et al., 2020; Chen et al., 2019; Mesalam et al., 2017). In all the works, the studied molecules were the most abundant components of their plant extracts and all yielded positive results, by increasing the rates of IVM and embryonic development in addition to decreasing cell apoptosis in different species. Therefore, with the application of established isolated bioactive agents, it was necessary to differentiate these molecules, through their biological activities, so that the best class of antioxidants, among all the tested ones, could be established at the end of the studies. Currently, the main groups-of-interest are the phenolic compounds, ascorbic acid, α-tocopherol, and carotenoids.

Table 1 – Bioguided studies with antioxidant potential bioactives on IVM of mammalian oocytes.

Extraction source	Type of extract	Major bioactive compounds	Isolated bioactive	Concentrations tested	Species	Best results	Reference
Papaver rhoeas L.	Ethanolic extract	Rhoeadine, rhoeadic acid, papaveric acid, anthocyanins	X	0, 25, 50, 100 and 200 µg/mL extract	Caprine	50 µg/mL extract	Rajabi-Toustani, Motamedi-Mojdehi, Mehr, & Motamedi-Mojdehi, (2013)
Olea europea	Essential oil	α-pinene, 2,6-dimethyloctane, verbascoside	Verbascoside	0, 1 and 10 nM verbascoside	Caprine	1 nm verbascoside	Martino et al. (2016)
Auxemma oncocalyx	Ethanolic extract	Oncocalyxone A, oncocalyxone C	Oncocalyxone A (Onco A)	1.2 µg/mL extract 1 µg/mL onco A	Caprine	1 µg/mL onco A	Leiva-Revilla et al. (2017)

Polygonum cuspidatum	Methanolic extract	2-methoxystypandrone, stypandrone, emodin, methoxyemodin	2-methoxystypandrone (2-MS)	0, 0.1, 0.5, 1 and 1.5 µM 2-MS	Bovine	1 µM 2-MS	Mesalam et al. (2017)
Syzygium aromaticum	Essential oil	Eugenol, β -cariophylene, acetyl eugenol	X	0, 10, 15 and 20 µg/mL essential oil	Bovine	20 µg/mL essential oil	Santos et al. (2019)
Croton zehntneri	Essential oil	Anethole, estragole	Anethole	0, 30, 300 and 2000 µg/mL anethole	Bovine	300 µg/mL anethole	Sá et al. (2019)
Crocus sativus	Water extract	Crocin, α-crocin, crocetin, picrocrocin	Crocin	0, 300, 400 and 500 µg/mL crocin	Porcine	400 µg/mL crocin	Chen et al. (2019)
Siraitia grosvenorii	Hydroalcoholic extract	Mogroside V, 11-omogroside V, siamenoside I, mogroside IV	Mogroside V	0, 1, 5, 10, 20 and 40 µM mogroside V	Porcine	20 µM mogroside V	Nie et al. (2020)

X: not evaluated. Source: Authors.

3.2.1 Phenolic compounds

Phenolic compounds are the most studied antioxidants. Their structure includes the aromatic rings and hydroxyl groups that are very stable and can inhibit the oxidation of biologically active compounds (Tabart, Kevers, Pincemail, Defraigne, & Dommes, 2009). These antioxidants exhibit two action mechanisms: the hydrogen atom transfer (HAT) or single-electron transfer (SET). In the first (HAT), the free radical removes a hydrogen atom from the antioxidant. This antioxidant is, therefore, more stable, and more efficient than the pristine antioxidant because its hydrogen bonds, conjugation, and resonance make it a non-reactive phenoxyl radical. In the second mechanism (SET), the antioxidant can donate an electron to the free radical, thereby forming, among other products, a radical cation of the antioxidant, which is stable and does not react with substrates. Both mechanisms can regularly occur simultaneously, although they exhibit different reaction rates (Torres-Osorio, Urrego, Echeverri-Zuluaga, & López-Herrera, 2019).

Some examples of widely explored phenolic compounds, as antioxidants, are resveratrol, eugenol, eugenyl acetate, catechins, rosmarinic acid, hesperetin, anthocyanins, quercetin, verbascoside, and quinones (Table 1). Some examples of the demonstration of the compounds in IVM of different species are as follows: caprine oocytes, which were supplemented with 1 μM of resveratrol, manifested higher blastocyst development (28.3% *vs*. 13.0%), and the glutathione levels were higher in the resveratrol groups than in the control groups (36,554.6 *vs*. 27,624.0 pixels/oocyte) (Piras et al., 2019). Utilizing the same bioactive, Zabihi et al. (2019) observed that the supplementation of the IVM medium with 0.25 and 0.5 μM resveratrol can improve the meiotic competence and early embryonic development of sheep oocytes.

3.2.2 Ascorbic acid

Ascorbic acid exists in two forms: L-ascorbic acid (the reduced form) and dehydro-L-ascorbic acid (the oxidized form). Although in nature, the vitamin is primarily present as ascorbic acid, both forms are biologically active; the L-isomer of ascorbic acid is biologically active. It possesses the potential to protect both the cytosolic and membrane components of cells from oxidative stress. In the cytosol, ascorbate acts as a primary antioxidant to scavenge free-radical species that are generated as by-products of cellular metabolism. In the cellular membranes, it may indirectly reduce the α-tocopheroxyl radical to α-tocopherol, as an antioxidant (Ardjani, & Alvarez-Idaboy, 2018). Moreover, compelling evidence has been presented for free radical quenching and a glutathione peroxidase–like mechanism of α-tocopherol: the activity of α-tocopheroxyl, rationalized with the peroxyl radicals, yields a radical cation, which is susceptible to oxidation and hydrolysis, whereas α-tocopherol removes H_2O_2 in a reaction requiring glutathione or ascorbate as the ultimate electron donor (Ardjani, & Alvarez-Idaboy, 2018).

Khanday, Ahmed, Nashiruddullah, Sharma, & Chakraborty (2019) employed ascorbic acid (100 µM), as an antioxidant, to supplement the goat oocyte IVM medium. Ascorbic acid improved the developmental competence of the oocytes during heat stress (41 °C) and they demonstrated higher maturation rates, compared to the non-supplemented groups.

3.2.3 α-tocopherol

The main biological function of tocopherols and the related chromanols is antioxidation, i.e., the inhibition of the autocatalytic lipid oxidation reactions, by scavenging the chain-propagating lipid peroxyl radicals (Kamal-Eldin, 2019). This role is facilitated by certain structural features of these molecules, specifically their lipid solubility and ability to donate their phenolic hydrogens to the peroxyl radicals, forming relatively stable chromanoxyl radicals that will not further propagate lipid oxidation reactions (Kamal-Eldin, 2019).

This isolated antioxidant has already been employed in IVM of bovine oocytes, at a standardized concentration of 100 µM as well as in synergy with Epigallocatechin Gallate (EGCG) of up to 10 µM. The combination produced a higher percentage of IVM bovine oocytes, compared to the percentage produced by α-tocopherol alone. These results suggest that α-tocopherol obtains positive results, through positive synergistic mechanisms, in IVM and the subsequent fertilization of bovine oocytes (Singh, Barua, & Sonowal, 2019).

3.2.4 Carotenoids

Carotenoids are colored fat-soluble pigments that are produced by plants and are present in a wide range of food (Eghbaliferiz, & Iranshahi, 2016). The antioxidant activity of carotenoids is associated with their radical scavenging abilities and the quenching of the lipid peroxyl radicals. β-carotene, as an efficient chain-breaking antioxidant, yields the carotenoid peroxyl radical. Carotenoids behave as antioxidants, at low oxygen partial pressures, but they may act as prooxidants at high pressures, producing carotenoid peroxyl radicals, which can act as prooxidants and cause lipid peroxidation. Thus, the relative antioxidant activity of carotenoids is like those of other antioxidants, such as α-tocopherol (Eghbaliferiz, & Iranshahi, 2016).

Some widely utilized carotenoids are crocin, β-caryophyllene, β-carotene, lycopene, lutein, zeaxanthin, α-carotene, and β-cryptoxanthin. The utilization of isolated astaxanthin (500 μM) on bovine oocytes resulted in a lower reactive oxygen species levels than in the controls and better blastocyst rates, by increasing the total cell number (Abdel-Ghani et al., 2019). Further, Park et al. (2018) observed that the utilization of 1 μM isolated β-cryptoxanthin resulted in the improved polar body extrusion and expression of maturation-related genes in *cumulus* cells and oocytes, compared to those of the control groups. Moreover, the total number of cells, per blastocyst and relative mRNA levels of the pluripotency marker and antioxidant genes, were quite higher, significantly, thus demonstrating the efficiency of this antioxidant.

4. Conclusion

Despite involving concepts, such as ethnobotany, bioprospecting, natural bioactive, and bioguided studies, that have been coined long ago, the line of research, focused on the discovery of new bioactive molecules, is constantly expanding, updating, and exploring. Due to the need for constant improvement in assisted reproduction techniques, the application of strategies to optimize the steps involved in IVEP is of utmost benefit. Thus, the supplementation of oocytes with antioxidant substances, to reduce oxidative stress, is one of the main focuses of research today, in which several authors have emphasized the improvement of oocyte quality.

However, to obtain concrete results, the utilization of bioguided studies, aided by bioprospecting techniques, must be unpredictable in the discovery of new bioactives compounds with potential action, at a technological level of application. Therefore, the optimization of these technologies is extremely beneficial to obtaining very evident and reliable results, thus increasing the reproductive success of the species, through the large-scale application of the techniques developed, *in vitro*.

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