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Journal of Embryology & Stem Cell Research
ISSN: 2640-2637

Mitochondria Targeted Antioxidants can Improve *In Vitro* Embryo Production in Buffalo

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Opinion

Volume 6 Issue 1

Received Date: January 31, 2022

Published Date: February 28, 2022

DOI: 10.23880/jes-16000160

Opinion

Buffalo is a large-ruminant dairy animal, mostly found in the Asian continent, considered as 'Black Gold' due to its immense contribution to milk, meat, and draft power. The buffalo contributing significantly to the human food chain by efficiently converting poor nutritional value feed into meat and milk for human consumption. They also have a high capacity for adaptation and survival in different environments with distinct climate, topography, and vegetation. The consumption of buffalo milk and meat are healthier due to presence of low cholesterol than that of cattle milk and meat. The calcium and protein content also found higher (58 %) in buffalo than cow's milk [1]. These beneficial inherent characteristics of the species need to be explored at cellular and molecular level, so that buffalo could be established as the best model for producing humanized products through genetic engineering.

However, despite the importance of buffalo to the socioeconomic status, the reproductive efficiency of buffalo is often compromised, partly due to poor reproductive performance, climatic stress, poor nutrition and seasonal fluctuations in the availability and quality of feed. Management factors such as the system of grazing and suckling by calves also modulate reproductive functions. Besides these, another serious problem associated with buffalo is the poor availability of superior germplasm. Only 0.1% of buffaloes produce 3500 to 4000 kg of milk in a 305-days lactation period. In addition, female buffaloes have few primordial follicles and a high rate of follicular atresia. These limiting factors also limit the embryo transfer technology in buffalo [2].

Therefore, the emphasis has now shifted to in vitro embryo production (IVEP). IVEP by means of in vitro fertilization (IVF) has drawn the interest of innumerable researchers since not only it can be used for faster multiplication of superior germplasm, but also have an integral part of a number of other important reproductive technologies like embryo or somatic cell cloning, production of embryonic stem cells, production of transgenic animals, creation of oocyte or embryo banks for conservation of germplasm, etc. IVEP involves a combination of the techniques of in vitro maturation (IVM), fertilization (IVF) and culture (IVC) of oocytes. Although IVEP has been successfully used for the production of buffalo embryos and live offspring [3], the application of this technology is severely limited due to very low blastocyst yields around 10-to 20% [4-6] of the oocytes subjected to IVM, are much lower than the ~30 to 40% observed in cattle [7]. Any improvements in the IVEP protocols, which could increase the blastocyst yields, could be expected to be highly beneficial for all the reproductive technologies mentioned above.

During IVEP, various factors such as light, ambient temperature and high oxygen pressure effect the growth of oocytes and embryos. Generally, the oocytes and embryos are cultured in an environment consisting of 5% CO_2 and 95% air. Such an environment contains about 20% O_2 which is nearly 3-4 times higher than the O_2 concentration in the female reproductive tract. This high O_2 concentration induce oxidative stress through increasing production of reactive oxygen species (ROS) in the mitochondria (the key site of ROS production) of oocytes and embryos which

decreasing intracellular antioxidants and ultimately reduces the developmental ability of embryos. The major generated ROS such as hydrogen peroxide (H_2O_2), superoxide anions (O^{2-}) and hydroxyl radicals (OH^-) are accumulated in the cytoplasm of the developing oocytes and embryos and cause lipid peroxidation, amino acids and nucleic acids oxidation, and mitochondrial dysfunction, and result in reducing the quality for IVEP [8].

Although the developmental rates of embryos can be increased by culturing them in 5%, instead of 20% O_2, the equipment required for this are very expensive. An alternative to this is to supplement the culture media with antioxidants for neutralizing the effects of ROS. A number of non-enzymatic antioxidants, such as reduced glutathione (GSH), cysteamine, vitamin C, vitamin E, taurine, hypotaurine, resveratrol, melatonin and Coenzyme Q10, and enzymatic antioxidants, like superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR), etc. have been supplemented with culture media either in IVM or IVC or in combination shown to increase the developmental competence of oocytes by reducing oxidative stress [9]. However, the beneficial effects of adding these traditional antioxidants in IVM and IVC media have been inconsistent, possibly due to unable to penetrate to mitochondria and fail to reach the intracellular site(s) of ROS production. It has been reported that large proteins, like catalase and SOD, are not able to cross the membranes, and also highly lipophilic antioxidants, such as vitamin E and coenzyme Q, tend to remain in the cell membranes [10]. Consequently, these antioxidants are not effective in eliminating intracellular ROS produced in mitochondria. Therefore, it seems necessary to facilitate access of antioxidants to the intracellular sites, specifically to mitochondria, to protect cells from intracellular free radicals.

Mitochondrial-targeted antioxidants consist of lipophilic triphenylphosphonium (TPP) covalently linked to ubiquinol, able to cross the mitochondrial phospholipid bilayer and accumulates in hundred-fold higher concentrations inside mitochondria and act as an inhibitor of ROS that can reduce the generation of mitochondria-specific ROS or superoxide [11]. Ubiquinol is an antioxidant that is regenerated by the mitochondria following oxidation, thus mitochondrial-targeted antioxidant is recyclable [12]. There are two types of mitochondrial-targeted antioxidants namely MitoQ or mitoquinol (10-(2,5-dihydroxy-3,4-dimethoxy-6-methylphenyl)decyl] triphenyl-phosphonium, monomethanesulfonate) and MitoT or mitotempol (2,2,6,6-Tetramethyl-4-[[5-(triphenylphosphonio) pentyl] oxy]-1-piperidinyloxy bromide) are specific inhibitors of mitochondrial reactive oxygen that can reduce the generation of mitochondria-specific ROS or superoxide [13]. These antioxidants supplementation in culture media showed improvement in the developmental competence of oocyte and enhance blastocyst production in porcine, bovine and murine embryos. Consequently, we can state that this approach can improve the developmental competence of oocyte and enhance blastocyst production in buffalo. These antioxidants supplementation can increase mitochondrial performance by preventing mitochondrial uncoupling, regulating cellular stress, minimising apoptosis and lowering lipid peroxidation. Mitochondrial-targeted compounds may offer an advantage as well as the keys to future research lines that could aid in the translation of this therapy into clinical practise.

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