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In vitro maturation of bovine oocytes: beneficial effects of cysteamine

Abstract

Antioxidant substances used at any stage of *in vitro* embryo production increase intracellular glutathione synthesis (GSH), improve nuclear maturation rates and protect embryos against endogenous or exogenous oxidative stresses, making embryos resistant to freeze, as well as increasing cell quality and number of embryos reaching blastocysts. Production of GSH depends on the availability and uptake of cysteine in the medium. However, cysteine is very unstable outside the cell and is auto-oxidized to cystine. Cysteamine reduces cystine to cysteine and promotes the uptake of cysteine by cells thereby enhancing the GSH synthesis. Consequently, cysteamine plays an important role in the synthesis of GSH and is a key factor in the defense mechanism against ROS.

Keywords: bovine, oocyte, maturation, culture, cysteamine

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Introduction

Antioxidant and cryoprotectant substances has often been supplemented to media for *in vitro* production and vitrification of bovine embryos in the last ten years.[1–3] It has been established that oxygen pressure in oviduct and uterus is less than atmospheric oxygen pressure.[4] *In vitro* embryo production of mammals under atmospheric oxygen pressure is being used routinely but during embryo culture, this high pressure leads to the formation of reactive oxygen species (ROS).[5–7] Harmful effects of ROS are DNA damage, lipid peroxidation, oxidative modifications of proteins, spermatozoon and oocyte fusion inhibition.[8] As well as known negative effects of ROS in some circumstances cell apoptosis is another important physiological factor.[5,9] One of the most important endogen sources of ROS is oxidative phosphorylation. Inhibition of oxidative phosphorylation decreases ROS and has a positive effect on *in vitro* embryo development.[10] The most important factor that leads to an increase in the formation of ROS is the exogenous oxygen pressure. Oxygen pressure in the oviduct is only 1/4th of atmospheric oxygen pressure. In *in vitro* produced bovine embryos under low oxygen tension (5-7%) it has been reported to increase resistance to freezing and non-protein structure one of the sulfhydryl compounds glutathione (GSH) shynthesis.[11,12] Oxidative stress is mediated by reactive oxygen species and results in an imbalance of the intracellular redox potential.[13] Reactive oxygen species are highly reactive and unstable. They can react with nucleic acids, lipids, proteins, carbohydrates to acquire an electron and become stable. These reactions induce a cascade of subsequent chain reactions eventually resulting in cellular damage.[14-16] Antioxidants such as β-mercaptoethanol, cysteamine, cystine, cysteine, N-acetyl-L-cysteine (NAC), superoxide dismutase (SOD) and resveratrol are used frequently in order to protect *in vitro* produced bovine embryos against oxidative stress.[6,17,18] It is known that antioxidants have positive effects on embryo development but some researchers advocate these positive effects can be effective under certain conditions.[9,19] Studies revealed that positive effects of antioxidants can be occurred fewer than 20% oxygen tension.[19] Various oxygen pressures have been tested in different culture conditions by researcher's *in vitro* production of bovine embryos. For example, oviductal or granulosa cells used in the co-culture environments 20% O_2 pressure, non-co-culture that does not contain somatic cells environments 5% oxygen pressure was found to increase results.[9,20] So many researchers advocate antioxidant substances have positive effects on oocyte maturation also embryo development but the mechanism of antioxidant action is not well known.[21,22] In the studies without supplementing antioxidant substances maturated and cultured embryos in different animal species and medium reaching to blastocyst stage in bovine in TCM-199 medium is 7.2%, in SOF medium 6% also in bovine, in pigs it was detected 23.3% as in SOF medium.[11,17,23,24] Gasparrini et al.,[24] detected cleavage rate of bovine embryos as 56.92% that was supplemented with 100µM cysteamine in TCM-199 medium for maturation. Singhal et al.,[23] reported in their study the cleavage rate of the buffalo oocytes as 60.7% supplemented with 50µM cysteamine. Oyamada & Fukui[11] investigated the effect of epidermal growth factor and cysteamine on the maturation of bovine oocytes, in the group that they added 100µM cysteamine, they found the cleavage rate as 62.4%, in the group they added epidermal growth factor and cysteamine, and they found the cleavage rate as 63.2%. Low molecular weight thiol components such as cysteine and cystine are precursors of glutathione (GSH), which plays an important protective role in relation to ROS generated by normal oxidative metabolism in the cell. The cellular content of GSH is regulated by the gamma-glutamyl cycle as reviewed.[25] Production of GSH depends on the availability and uptake of cysteine in the medium. However, cysteine is very unstable outside the cell and is auto-oxidized to cystine. Cysteamine reduces cystine to cysteine and promotes the uptake of cysteine by cells thereby enhancing the GSH synthesis. Consequently, cysteamine plays an important role in the synthesis of GSH and is a key factor in the defense mechanism against ROS.

Antioxidant substances used at any stage of *in vitro* embryo production increase intracellular glutathione synthesis (GSH), improve nuclear maturation rates and protect embryos against endogenous or

exogenous oxidative stresses, making embryos resistant to freeze, as well as increasing cell quality and number of embryos reaching blastocysts. Increased cell quality, more resistant to freeze, and an increase in the number of embryos that can be transferred are undoubtedly desirable. Scientists are doing a myriad of research to ensure that *in vitro* produced embryos can both be transferred and frozen. They work to standardize or improve existing outcomes using different chemistries, methods, either in the *in vitro* production phase, transfer, pregnancy and birth, as well as freezing stages. In some studies, it has been reported that antioxidants and transfer methods used in the *in vitro* production stage of the embryo may contribute to the development of genetically superior embryos and also to the spread of the embryo transfer technique. In contrast to these results, the embryos from oocytes exposed to cysteamine did not appear to be different with respect to cryotolerance, post-transfer embryo survival, and calf, ovine characteristics as measured by gestation length, birth weight, perinatal mortality, and sex ratio.[26,27]

Conclusion

Low molecular weight thiol compounds, such as cysteamine, can increase cysteine uptake by oocytes during IVM. This subsequently can increase GSH content, which is a major anti-oxidant system that protects the cells against the deleterious effects of oxidative stress by scavenging ROS.

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Conflict of interest

Authors declare that there is no conflict of interest.

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