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L-arginine affects the IVM of cattle cumulus-oocyte complexes

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ABSTRACT

Nitric oxide (NO) is identified as a signaling molecule involved in many cellular or physiological functions, including meiotic maturation of cattle oocytes. This study aimed to evaluate the effect of supplementation of culture medium with the L-arginine (L-arg, NO synthesis precursor) in nuclear maturation of oocytes, concentrations of nitrate/nitrite, progesterone (P_4), and 17β-estradiol (E_2) in the culture medium; and the cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) intracellular concentrations in the cumulus-oocyte complexes (COCs) during the first hours of maturation in the presence of hemisections (HSs) of the follicular wall (control −ve). The addition of 5.0-mM L-arg increased ($P < 0.05$) the percentage of oocytes at the germinal vesicle breakdown stage after 7 hours of cultivation compared with control −ve. All concentrations of L-arg (2.5, 5.0, and 10.0 mM) increased the percentage of oocytes that reached the metaphase I (MI) at 15 hours ($P < 0.05$) but do not affect the progression from MI to metaphase II ($P > 0.05$) at 22 hours. All concentrations of L-arg tested increased ($P < 0.05$) the percentage of cumulus cells with plasma membrane integrity at 22 hours of cultivation. L-arginine did not change ($P > 0.05$) the nitrate/nitrite, P_4, and E_2 concentrations in relation to control −ve at any of the times tested. In immature COCs, immediately after being removed from the follicles (0 hours), the intracellular concentration of cGMP in the control −ve and treatment with 5-mM L-arg progressively decreased ($P < 0.05$) after the first hour of cultivation; however, COCs treated with 5.0-mM L-arg had higher concentrations of cGMP at 1 hour of cultivation ($P < 0.05$). The cAMP concentration of COCs supplemented or not with 5.0-mM L-arg progressively increased until 3 hours of cultivation and at, 6 hours, decreased ($P < 0.05$). The results show, in using this system, that (1) the mechanisms that give the oocyte the ability to restart the meiosis until MI after adding 5.0-mM L-arg do not involve changes in the concentration of nitrate/nitrite, P_4, and E_2 in the culture medium and (2) L-arg acts on a pathway that involves changing the cGMP concentration but does not involve changing cAMP concentration. More studies are needed to assess whether the observed effects of L-arg during IVM using this system are via NO or not and what the role is in increasing the viability of cumulus cells in the resumption and progression of meiosis until MI.

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1. Introduction

The signaling pathways and associated substances that promote the resumption and progression of meiosis of bovine oocytes *in vivo* after the LH peak are not completely understood [1]. One of the substances whose involvement in oocyte maturation has been studied is nitric oxide (NO). It is produced by the activity of the nitric oxide synthase (NOS) enzyme, which catalyzes the reaction that converts the amino acid L-arginine (L-arg) to L-citrulline and NO [2,3]. Availability of L-arg for NO synthesis in the medium used to culture cells is one of the key points in controlling the synthesis of NO *in vitro* [3].

Most studies use artificial NO donors compounds (sodium nitroprusside [SNP] and S-nitroso-*N*-acetylpenicillamine) rather than natural precursor, L-arginine, to ascertain the role of this signaling molecule on *in vitro* oocyte maturation in rodents [4,5], pigs [6,7], sheep [8], buffalo [9], and cattle [10,11]. Supplementation of the medium with L-arg instead of NO donors to assess their role in the IVM of oocytes may have the advantage of controlling the amount of NO synthesized by the cells themselves in culture, whereas the L-arg availability can play important roles in determining rates of NO synthesis [3]. The L-arg is a semi-essential amino acid, consumed by metabolic pathways other than NO synthases—for instance, the synthesis of protein, urea, creatine, agmatine, and L-ornithine [12].

The importance of NO in oocyte maturation in cattle has been demonstrated by the presence of different isoforms of NOS, especially endothelial (eNOS) and inducible NOS (iNOS), in oocytes and in ovarian somatic cells [13,14]. Likewise, changes in NO concentration in the maturation medium due to supplementation with NO donor [10,15,16] or blocking NOS activity [17,18] affect the resumption and progression of meiosis.

In turn, NO is involved in various events affecting the IVM of bovine oocytes as the regulation of the integrity, viability, and activity of follicular cells [19]. The NO can protect follicular cells from apoptosis [20,21] and, in suitable *in vitro* concentrations, NO improves the integrity of the plasma membrane of granulosa cells [22], cumulus cells (CCs) [15,17], and oocytes [15]. There is evidence that NO may participate in the regulation of steroidogenic activity of the follicular cells [20,22]. Depending on the concentration in the culture medium, NO may promote or inhibit the synthesis of progesterone (P_4) and 17β-estradiol (E_2) by the granulosa cells [9,22], these being involved in IVM [23,24].

Nitric oxide exerts its effects mainly by the activation of the soluble isoform of guanylate cyclase enzyme (sGC), resulting in the production of cyclic guanosine monophosphate (cGMP) [3]. This nucleotide inhibits the phosphodiesterase 3A in rodent oocytes [25], an enzyme that degrades cAMP, keeping high levels intraoocyte of cAMP [26]. cAMP produced by adenylate cyclase present in the CCs and oocyte has been described as a major modulator of the resumption and progression of meiosis in oocytes [27]. Thus, NO participates in oocyte maturation, regulating the levels of cyclic nucleotides intraoocyte [25,28]. The role of cGMP in the modulation of cAMP levels has been well described in rodents [25,28], but there are few studies on the role of NO/cGMP pathway in the meiosis and this pathway in the modulation of cAMP concentration in cattle during this process [11].

The events that modulate the nuclear maturation of meiotically competent bovine oocytes have been difficult to study in conditions outside a physiological environment because the oocytes spontaneously resume meiosis as soon as they are removed from the follicles and cultured in a suitable medium [29]. The IVM of oocytes in coculture with hemisections (HSs) of the follicular wall is an attempt to mimic the conditions of the follicular environment *in vivo*. The cultured cells of the follicular wall, especially the theca cells, produce a factor of an unknown nature that is soluble in the culture medium and has the ability to partially block the nuclear maturation of oocytes [30,31].

Considering that NO can influence oocyte maturation and the enzymatic activity of NOS is tightly controlled and dependent on the availability of substrate, we therefore used L-arg supplementation to investigate the role of NO on nuclear maturation, on CC plasma membrane integrity, on nitrate/nitrite (NO_3^-)/(NO_2^-), and steroid (P_4 and E_2) production during the IVM of cumulus-oocyte complexes (COCs) in coculture with follicular wall HSs in cattle.

2. Materials and methods

All reagents used in these experiments were obtained from Sigma–Aldrich BrasilLtda (São Paulo, Brazil), unless otherwise indicated.

2.1. Obtaining the follicular wall HSs

Bovine ovaries were collected from adult cows, irrespective of stage of the estrous cycle, at a local abattoir immediately after slaughter and carried to the laboratory within 2 hours in sterile saline solution with antibiotics (100-IU/mL potassium penicillin G, and 50-μg/mL streptomycin sulfate) at 30 °C. In the laboratory, the ovaries were washed three times with saline. Follicles were isolated from ovary, dissected from the stromal tissue, and were selected based on size (3–5 mm) and transparency [32]. These follicles were cut into equal halves with a scalpel, and their respective oocytes were discarded [30]. The follicular walls (HSs) were washed three times in washing medium (tissue culture medium 199 [TCM 199—HEPES], in addition to 0.4% fatty acid–free BSA, 0.2-mM pyruvate, 100-IU/mL potassium penicillin G, and 50-μg/mL streptomycin sulfate). Two hours before the addition of COCs, the HSs were transferred to four-well plates (NUNC, Rochester, NY, USA) containing 200 μL of maturation medium (TCM 199 supplemented with 0.4% BSA [fatty acid free], 0.5-μg/mL FSH [Folltropin-V, Bioniche Life Science Inc., Canada], 5-μg/mL LH [Lutropin-V, Bioniche Life Science Inc., Canada], and 0.2-mM pyruvate, and antibiotics) with the desired treatment [31].

2.2. Collection and cultivation of COCs

Three- to 8-mm follicles were aspirated, and the collected COCs were immediately placed in TCM washing medium supplemented with 3-isobutyl-1-methylxanthine

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