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Parthenogenetic activation of domestic cat oocytes using ethanol, calcium ionophore, cycloheximide and a magnetic field

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Abstract

The objective of this study was to evaluate parthenogenetic activation of domestic cat oocytes after being exposed to either ethanol, magnetic field, calcium ionophore A23187, or cycloheximide and a combination of these agents. We also wished to evaluate the usefulness of the magnetic field for oocyte activation. In vitro matured oocytes subjected to artificial activation were randomly assigned into eight groups according to activating agents: (1) 10% ethanol; (2) the magnetic field (slow-changing, homogenous magnetic field with low values of induction); (3) 10% ethanol plus magnetic field; (4) 10 μM calcium ionophore A23187; (5) 10 μM calcium ionophore A23187 plus magnetic field; (6) 10% ethanol and 10 μg/mL of cycloheximide; (7) 10% ethanol and 10 μg/mL of cycloheximide plus magnetic field; (8) oocytes were not exposed to any of the activating agents. After activation oocytes were stained with Hoechst 33258 and parthenogenetic activation was defined as oocytes containing pronuclei and second polar bodies or two to four or six nuclei (embryonic cleavage). The total activation rate by using different activation treatments was 40%. The addition of the magnetic field to ethanol or calcium ionophore treatments resulted in increased parthenogenetic activation rates from 47% to 75%, and from 19% to 48%, respectively ($P < 0.001$). Instead, when the magnetic field was added to ethanol and cycloheximide treatment, activation rate decreased from 48% to 30%. Oocytes activated with magnetic field only gave the lowest activation rate (12%).

We concluded that a magnetic field can be used as an activating agent, and the combination of ethanol and magnetic field is an effective method for domestic cat oocyte activation.

Keywords: Cat; Oocyte; Activation; Parthenogenesis; Magnetic field

1. Introduction

In the process of fertilization interaction between a sperm cell and a secondary oocyte triggers off a series of morphological and biochemical transformations, known as oocyte activation. The key mechanism is a calcium signal, shared by most animal species. Several minutes after the penetration of a sperm cell into an oocyte a quick and transitory drawing occurs from intracellular reserve of calcium. This is the calcium collected in endoplasmic reticulum, although extracellular calcium can also be used to supplement the reserve, thus enabling continuation of the calcium signal [1]. The activation leads to meiosis resumption and extrusion of the second polar body. Consequently, pronuclei are formed, DNA synthesis begins and embryonic cleavage is initiated of artificial activation,

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caused by external stimuli. Parthenogenesis phenomenon does not occur naturally in mammals, although very rarely spontaneous can take place. Artificial activation is a necessary step in the cloning procedure, while somatic cloning can become one of the methods for saving endangered species of animals (including felids).

Although, several publications on artificial activation of feline oocytes exist, most of them are related to activation during cloning procedure. Only few studies had compared different methods of activation and their effectiveness, as measured by the number of activated oocytes and parthenogenetic embryos [2–5]. The stimulus which triggers oocyte activation, can be a physical, mechanical or chemical agent. Combination of these agents is also applied. One common, universal method has not been developed because the process is highly specific for each species. Cat oocyte activation by electric current or chemical agents (ethanol, calcium ionophore) has been reported, along with the inhibitors of protein synthesis, protein phosphorylation or histone kinases (kinases PF/MAP; cycloheximide, cytochalasine B) [6–9]. A single electrical stimulation seems to be insufficient to activate domestic cat oocytes, but their exposure to cycloheximide following electrical stimulation improved the efficacy of parthenogenetic development [3].

Domestic cat oocytes used for nuclear transfer have been activated 1–2 h post-fusion by exposing them to 7% ethanol and then culturing in cycloheximide + cytochalasin D. The results in this study demonstrated that 90% of oocytes cleaved to two to six cells and above 13% reached blastocyst stage [10]. In similar study, reconstructed cat oocytes were activated with 7% ethanol (5 min) followed by incubation in cycloheximide for 4 h. Cleavage rates (55–80%) and embryonic development to the blastocyst stage (5–8%) were similar to those observed with the presence of cytochalasin D [8].

Ethanol alone, at concentration 10% has not been used in cats to trigger parthenogenetic activation, while calcium ionophore has been mainly used in combination with other agents.

Cleavage and blastocyst development of in vivo and in vitro matured domestic cat oocytes exposed to two ionophores (A23187 or ionomycin) at two different concentrations (5 or 10 μM) for 3 or 5 min have been evaluated. In that study, higher activation rates were observed when in vivo matured oocytes were used (60% versus 47%). The cleavage on day 2 was higher for oocytes exposed to the ionophore for 5 min rather than for 3 min. Instead cleavage was not affected by the type of ionophore or its concentration [11]. Protein synthesis and protein phosphorylation inhibitors in combination with ionophores have been used for cat oocyte activation. Oocytes were exposed to calcium ionophore A23187 (5 μM) for 5 min, followed by incubation either with cycloheximide (10 μg/mL) plus cytochalasin D (2.5 μg/mL), or with 6-dimethylaminopurine (2.5 mM) for 4–5 h resulting in similar cleavage rates of 88% versus 82%, and blastocyst development 45% versus 28%, respectively [2]. It has been also demonstrated that in vitro matured cat oocytes can be activated after being exposed to ionomycin (5 μM) followed by 5 h incubation in 10 μg/mL cycloheximide. The overall rate of activation for the treated oocytes was 90% [12].

Magnetic field has not been used to induce artificial activation in oocytes of any animal species. The magnetic field can influence movement of calcium ions in the cell. Studies carried out on isolated pituitary cells had demonstrated that calcium concentration in the cells had increased almost twice from the normal concentration after being exposed to the magnetic field at frequency of 50 Hz and induction of 50 μT [13].

Most of the authors point out, however, that the results of experiments are closely connected with the parameters of magnetic fields applied and also depend on the experimental object (cellular model, animals), subjected to magnetic field. The results vary, sometimes very significantly, even for slightly different values of induction, frequency and field form. Biological effects observed may be stronger for weaker fields and inversely, some of them are proportional to the induction value or do not depend on it. Therefore, the choice of parameters is the product of many trials and the final result depends on the field values chosen.

The aim of this study was to activate in vitro matured domestic cat oocytes using ethanol, magnetic field, calcium ionophore A23187, ethanol and cycloheximide and combinations of these agents, as well as to compare their effectiveness and to evaluate magnetic field usefulness for oocyte activation.

2. Materials and methods

2.1. Oocyte recovery and maturation

Ovaries from domestic cats were obtained after ovariohysterectomy at local veterinary clinics. Oocytes selected for in vitro maturation were surrounded by compact layers of cumulus cells and dark ooplasm. Ten to fifteen cumulus–oocyte complexes (COCs) were cultured in four-well tissue culture dishes containing 700 μL of TCM-199 supplemented with 0.3% BSA, 1 UI/mL hCG, 0.5 UI/mL eCG (Intervet International