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Animal Reproduction Science 105 (2008) 226–233

ANIMAL REPRODUCTION SCIENCE

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Parthenogenetic development of in vitro matured porcine oocytes treated with chemical agents

Erika Varga [a,*], Renáta Pataki [a], Zsuzsanna Lőrincz [b], Judit Koltai [b], Ágnes Bali Papp [a]

[a] *Institute of Animal Breeding, University of West Hungary, Faculty of Agricultural and Food Sciences, H-9200 Mosonmagyaróvár, Vár 4, Hungary*

[b] *Institute of Economic Sciences, University of West Hungary, Faculty of Agricultural and Food Sciences, H-9200 Mosonmagyaróvár, Vár 2, Hungary*

Received 24 May 2006; received in revised form 26 February 2007; accepted 2 March 2007
Available online 6 March 2007

Abstract

Parthenogenetic activation is a possible way to produce homogeneous embryos with the same ploidy. These embryos could develop to the blastocyst stage during the cultivation. Probably such embryos could be used in other areas of biotechnology.

The objectives of the present study were first to assess the ability of strontium-chloride to induce activation and parthenogenetic development in porcine oocytes in comparison with cycloheximide and 6-dimethylaminopurine; second to verify whether the combination of the two treatments improved activation and parthenogenetic development rates. At first, the effects of cycloheximide, 6-dimethylaminopurine and strontium-chloride on oocyte activation and embryonic development were compared. Oocytes from slaughterhouse ovaries were matured for 42 h in tissue culture medium (TCM) 199 at 38.5 °C, 5% CO_2 in air. Matured oocytes were activated with 10 mM strontium-chloride (S), 0.04 mM cycloheximide (CX), 2 mM 6-dimethylaminopurine (D) for 5 h. The activation rate was judged by pronuclear formation of oocytes. Following the activation, oocytes were incubated in NCSU 37 medium for 6 days and in all groups more than 45% of oocytes activated. The activation rate for CX treatment was significantly higher ($P < 0.05$) than for D (57.37 ± 4.21% and 48.09 ± 3.43%, respectively).

In a second experiment in vitro matured porcine oocytes were activated using a combined treatment of strontium-chloride with cycloheximide (SCX) and strontium-chloride combined with 6-dimethylaminopurine (SD). In S and SCX groups more than 50% of oocytes were activated (53.29 ± 5.39% and 54.3 ± 7.29%, respectively). However a large portion of embryos stopped their development at the two- or four-cell stage. Significantly higher numbers of embryos could reach the eight-cell stage in SD and SCX

* Corresponding author. Tel.: +36 96 566 613; fax: +36 96 566 695.
E-mail address: vargaera81@yahoo.com (E. Varga).

0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2007.03.004

than for S (7.8 ± 1.0%, 7.2 ± 4.0% and 3.9 ± 3.1%, respectively). Blastocyst formation was only observed in S, CX and SCX.

These results show that porcine in vitro matured oocytes can be artificially activated by cycloheximide, 6-dimethylaminopurine and strontium-chloride.

Keywords: Activation; Strontium; Cycloheximide; 6-Dimethylaminopurine; Porcine oocytes; Embryo development

1. Introduction

Oocyte activation is important to nuclear transfer and could be used for cytogenetic studies of embryos because in the resultant parthenotes maternal chromosomes can be analyzed independent of paternal chromosomes.

There are much published protocols for producing parthenogenetic embryos by artificial activation of in vitro matured mammalian oocytes. Oocytes could be electrically activated (Kure-bayashi et al., 2000; Ozil and Huneau, 2001). A variety of agents activate mammalian oocytes in vitro, including ethanol (Zernicka-Goetz, 1991; Meo et al., 2004), ionophore A23187 (Funahashi et al., 1994; Wang et al., 1998), cycloheximide (Nussbaum and Prather, 1995; Cha et al., 1997), strontium (Fraser, 1987; Kline and Kline, 1992; Okada et al., 2003), 6-dimethylaminopurine (Grupen et al., 2002) and calcium chloride (Machaty et al., 1996). In this study, the effects of strontium-chloride, cycloheximide and 6-dimethylaminopurine on oocyte activation were examined.

The objectives of the present study were first to assess the ability of strontium-chloride to induce activation and parthenogenetic development in porcine oocytes in comparison with cycloheximide and 6-dimethylaminopurine; second, to verify whether the combination of two treatments improves activation and parthenogenetic development rates.

2. Materials and methods

All chemical reagents used for oocyte maturation, activation and embryo culture were purchased from Sigma–Aldrich Chemical Co. (Budapest, Hungary) unless otherwise noted.

2.1. Recovery and in vitro maturation (IVM) of oocytes

Ovaries of Hungarian Large White gilts were collected from a local abbatoir and stored at 38 °C during transportation. At the laboratory, they were washed three times with warmed hexadecyl trimethyl-ammonium bromide (CETAB) solution (H-5882) and saline solution, and then stored in a water bath at 38 °C before use. The cumulus-oocyte complexes (COCs) were collected by aspiration of 3–6 mm non-atretic follicles using an 18-gauge needle attached to a 5 ml disposable syringe. The medium for oocyte collection was Hepes (H-3375) medium. The COCs were selected under a stereomicroscope and washed three times in tissue culture medium (TCM) 199 (M-7528). Oocytes surrounded by three or more compact layers of cumulus cells and with an evenly granulated ooplasm were used for in vitro maturation. Oocytes were matured in groups of 50/500 μl of maturation medium for 42 h at 38 °C, 5% CO_2 in air. The basic medium used for oocyte maturation was TCM199 supplemented with 10% pig follicular fluid, 1.25 mM L-glutamine (G-3126), 0.9 mM Na-pyruvate (S-3362), 100 μM cysteamine (M-9768), 0.1 mg/ml streptomycin sulphate, 10 IU/ml PMSG (Werftt-Chemie GmbH) and 10 IU/ml hCG (Werftt-Chemie GmbH).