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A comparative study on parthenogenetic activation and embryo production from in vitro matured caprine oocytes

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ABSTRACT

The objective of this study was to compare the effectiveness of different activation treatments of in vitro matured oocytes and their developmental potency in potassium simplex optimization medium. Ovaries were collected from the local abattoir and transported within 4 h to the laboratory in warm saline (37 °C) containing 100 IU penicillin-G and 100 μg streptomycin sulphate per ml. A total of 1004 cumulus oocyte complexes (COC's) were collected from 454 ovaries. Oocytes were matured in TCM-199 medium containing FSH (5 μg/ml), LH (10 μg/ml), oestradiol-17β (1 μg/ml) supplemented with 10% fetal bovine serum and 3 mg/ml BSA at 38.5 °C and 5% CO_2 in an incubator under humidified air for 27 h. After 27 h of IVM, oocytes were denuded, washed and selected 933 in vitro matured oocytes were randomly divided into four groups. Group 1 in vitro matured oocytes (n = 579), were exposed to 7% ethanol for 5 min followed by treatment with 2.0 mM DMAP for 4 h in KSOM. Group 2 in vitro matured oocytes (n = 145) were exposed to 7% ethanol for 5 min followed by treatment with 10 μg/ml CHX for 4 h in KSOM. Group 3 in vitro matured oocytes (n = 100) were exposed to 7% ethanol for 5 min followed by treatment with 2.0 mM DMAP and 10 μg/ml CHX for 4 h in KSOM. Group 4 in vitro matured oocytes (n = 109) were cultured for 4 h without any chemical activation treatment in KSOM medium (control). After 4 h of culture in different chemicals, the oocytes were washed five to ten times in the culture medium (KSOM) and cultured in 50 μl drops of KSOM. Development of activated oocytes was observed at every 48 h till day 10 post activation under an inverted phase contrast microscope (200x, Nikon, Japan). The cleavage rate in groups 1, 2, 3 and 4 were 42.83%, 58.62%, 74.0% and 0.00%, respectively and morula production in groups 1, 2 and 3 were 24.59%, 30.58% and 31.08%, respectively. These results indicated that the activation of in vitro matured oocytes by 7% ethanol for 5 min followed by treatment with 2.0 mM DMAP and 10 μg/ml CHX for 4 h in KSOM is most favorable for parthenogenetic caprine embryos production.

1. Introduction

In the last two decades there has been a great revolution in the field of biotechnology. Embryo transfer technology has become a tool for enhancing lifetime productivity of livestock. In vitro embryo production is an 'attractive alternative' for the production of large number of embryos required for the rapid multiplication of superior germplasm of goats, upgrading a commercial or pure bred breeding programme and conservation of endangered breeds of goats.

Problems with infertility and a slow rate of genetic gain hamper livestock breeding programs forced researchers to emphasize more on the development of pure breeds of

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animals. The new innovative idea all together led to a new line of development and a new technique called "Parthenogenesis" which is quite different from the old and usual IVF techniques (Suomalainen, 1950). Parthenogenesis can be a viable alternative method of producing the identical female and thus faster multiplication of a few superior female animals can be achieved. Efficient in vitro procedure for oocyte maturation and parthenogenetic development in small ruminants are important for the development of new biotechnologies such as gene-transfer, gene-expression occurring through different modes of parthenogenetic development i.e. haploid or diploid as well as in vitro multiplication of identical embryos.

Several methods have been developed for induction of parthenogenetic activation, including calcium ionophore (Funahashi et al., 1994), ethanol (Kharche et al., 2013; Loi et al., 1998), A-23187, calcium ionophore and cycloheximide alone (Nussbaum et al. 1995) or combined with a protein phosphorylation inhibitor, (DMAP) (Liu and Yang, 1999), electrical shock (Kono et al., 1989; Kim et al., 1996), $CaCl_2$ (Machaty et al., 1996), protein kinase inhibitors (Mayes et al., 1995), G protein stimulation (Machaty et al., 1996), cycloheximide (Nussbaum and Prather, 1995) ionomycin (Loi et al., 1998), ultrasound (Sato et al., 2005), ethanol (Loi et al., 1998), strontium (Meo et al., 2004), Ca–EDTA (Zae and Ryoo, 2007) and magnetic field (Max et al., 2007). In traditional method for production of cloned goat embryos, electric pulses were applied for activation of reconstructed oocytes (Shen et al., 2006). Oocyte activation by electrical pulse is initiated by an elevation of intracellular Ca^{2+}. Immediately after electrical stimulation, there is an influx of extracellular Ca^{2+} which in turn triggers an increase of intracellular Ca^{2+} (Cheong et al., 2002). Ionophores such as calcium ionophore and ionomycin induces a great single intracellular calcium rise in MII oocytes which originates exclusively from the internal deposits and a likely consequence is the activation of several calcium-dependent proteolytic pathways, leading to the destruction of cyclin B, reduction of MPF activity, and resumption of meiosis (Jellerette et al., 2006). In each method, an initial response in the mature oocyte arrested at metaphase II is an incremental increase in intracellular Ca^{2+} concentration, similar to that produced after activation by the penetrating spermatozoon during the fertilization process. Then, the artificially activated oocyte further responds by initiating the cortical reaction and resuming meiosis, followed by second polar body extrusion. Thus, the sequence of events occurring during parthenogenetic activation mimics the cascade of intracellular activities produced by the penetrating spermatozoon.

Limited literature on activation protocols for goat oocytes is available, creating the need for effective oocyte activation protocols that can be used during nuclear transfer in goats. In the present study attempts were made to compare different activation protocol on the development competence of parthenogenetic goat embryos. Therefore, the present study was undertaken to determine the effect of different chemical activation protocols on cleavage rate of in vitro matured goat oocytes and to compare the development of parthenogenetic embryos produced from different chemical activation protocols.

2. Materials and methods

All chemicals used in this study were from Sigma–Aldrich (St. Louis, MO, USA), except where otherwise indicated.

2.1. Collection of ovaries

For oocyte collection, a total of 454 goat ovaries were obtained within 4 h. of slaughter from a local abattoir located at Agra and were transported to the laboratory in a Thermos flask containing sterile warm (35–37 °C) physiological normal saline solution (NSS) supplemented with antibiotics (100 IU/ml penicillin G and 100 μg/ml streptomycin sulphate) and transported to the laboratory. In the laboratory, the working area was cleaned with 70% alcohol and ovaries were handled aseptically at room temperature. All ovaries were cleared off the attached tissue and mesovarium (trimming). The trimmed ovaries were then subject to washings (5–6 times) with warm saline fortified with antibiotics and then transferred into laminar flow. Subsequently, all experimental procedures were conducted in laminar flow.

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

A total of 1004 oocytes were recovered from ovaries by using follicle puncture technique; the follicles located on the surface of each ovary were punctured separately in a sterile disposable Petri-dish containing OCM (oocyte collection medium) using a sterile 18-gauge needle. The oocytes were retrieved and placed in another petri-dish containing oocyte holding medium. The collected oocytes were finally graded under the inverted phase contrast as per the method of Kharche et al. (2008). Only grade A, B, C quality oocytes were chosen. The cumulus oocyte complexes (COCs) were selected and placed in a sterile disposable culture dish containing oocyte holding medium (TCM-199) containing L-glutamine (100 μg/ml), sodium pyruvate (0.25 mmol) and gentamycin (50 μg/ml). The selected oocytes subjected to serial drop washing. The oocytes were washed in 3 to 4 drops (100 μl each) of oocyte holding medium (OHM). Further washings were given 3 to 4 times in drops (100 μl each) of maturation media (TCM-199) containing L-glutamine (100 μg/ml), sodium pyruvate (0.25 mmol), gentamycin (50 μg/ml), FSH (5 μg/ml), LH (10 μg/ml) and oestradiol-17β (1 μg/ml) supplemented with 10% FBS and 3 mg/ml BSA). Selected cumulus oocyte complexes (COCs) were matured in 100 μl droplets of maturation media covered with sterile mineral oil for 27 h in humidified atmosphere of 5% CO_2 at 38.5 °C in a CO_2 incubator. The maturation rate of selected COC's was 95.01%.

2.3. Activation of oocytes

After removal of cumulus cells, denuded oocytes were selected for parthenogenetic activation. A total of 954 in vitro matured oocytes were collected, out of which 933 in vitro matured oocytes were randomly divided into following treatment groups:

Group 1 comprised in vitro matured oocytes ($n = 579$) after 27 h of in vitro maturation, activated with 7% ethanol for 5 min followed by treatment with 2.0 mM DMAP for 4 h in KSOM.

Group 2 comprised in vitro matured oocytes ($n = 145$) after 27 h of in vitro maturation, activated with 7% ethanol for 5 min followed by treatment with 10 μg/ml CHX for 4 h in KSOM.

Group 3 comprised in vitro matured oocytes ($n = 100$) after 27 h of in vitro maturation, activated with 7% ethanol for 5 min followed by treatment with 2.0 mM DMAP and 10 μg/ml CHX for 4 h in KSOM.

Group 4 comprised in vitro matured oocytes ($n = 109$) after 27 h of in vitro maturation, cultured without any chemical activation treatment as control for 48 h in KSOM.

2.4. In vitro culture of activated oocytes

After parthenogenetic activation of oocytes for 4 h, activated oocytes were washed in embryo development medium (KSOM) and transferred in 100 μl embryo culture drops placed in a CO_2 incubator at 38.5 °C and 5% CO2 in a humidified atmosphere. For cleavage rate, activated oocytes were observed after 48 h under an inverted phase contrast microscope. The culture media was replenished after every 48 h and observations