# STRONTIUM CHLORIDE INDUCED PARTHENOGENETIC OOCYTE ACTIVATION AND EMBRYONIC DEVELOPMENT IN BUFFALOES

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**ABSTRACT**

*Artificial oocyte stimulation is essential for assisted reproductive technologies like Somatic Cell Nuclear Transfer (SCNT), Intracytoplasmic Sperm Injection (ICSI). In this study, we examined the ability of strontium Chloride ($Sr^{2+}$) to induce activation and in vitro embryonic development in buffalo oocytes in combination with Cytochalasin B and 6- dimethyl aminopurine (DMAP). Matured buffalo oocytes (n=276) were activated with 10 µM $Sr^{2+}$ and 5 µg/ml cytochalasin B (Group I) for 2.5 hrs. In group II, 266 matured oocytes were activated with 10 µM $Sr^{2+}$ and 5 µg/ml cytochalasin B for 2.5 hrs followed by culture in 2 mM 6-DMAP for 4 hrs. The activation rate for group I was significantly higher ($P < 0.01$) than for group II (38.35±1.00% and 31.29±0.90%, respectively). However, no significant difference was observed in morula development (9.57±0.84% and 6.80±0.59%, respectively). In conclusion, it is practicable to use $Sr^{2+}$ in combination with cytochalasin B for parthenogenetic embryo production in buffalo.*

**Keywords:** Activation; Strontium chloride; 6-Dimethylaminopurine; Embryo development; Buffalo

## INTRODUCTION

Parthenogenetic activation is a prospective means to produce homogeneous embryos with the same ploidy for basic reproductive biotechnological research. In SCNT reconstructed embryos in addition to the transfer of donor genetic material from the karyoplast to the cytoplast, the cytoplast must be 'activated' in order to initiate development.

Fully matured mammalian oocytes could be induced to undergo activation artificially (parthenogenetic) by a variety of physical and chemical treatments in the absence of the male genome (Kaufman and Gardner, 1974). The activation stimuli was designed to mimic closely the events initiated by the sperm factor released upon fertilization that resulted in $Ca^{2+}$ rise in the treated oocyte (Saunders *et al*., 2002). Chemical stimulation is the most common method of activation of embryos in SCNT and ICSI studies that have succeeded in producing animals. To date many artificial activation metods including ethanol, calcium ionophore have been developed.

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However, most activating agents caused a monotonic increase in $Ca^{2+}$, on the other hand, $Sr^{2+}$ containing medium induces repetitive intracellular ca oscillations similar to those that occur during normal fertilization.

Strontium chloride action mimicked the effects of sperm on oocyte and seemed to be mediated through the inositol triphosphate receptors. Molecular pathways of the reprogramming process had yet to be elucidated, but might contain specific pathways that required continuous (or at least more than a single) $[Ca^{2+}]_i$ stimulation. If those pathways failed to be fully or even partially activated by the current activation methods used in SCNT, it might cause epigenetic abnormalities that were deleterious to the developing embryo.

By optimizing the activation methodologies to better mimic that during normal fertilization, it might be possible to decrease the range of methylation patterns observed and potentially increase the rate of production of viable offspring (De Sousa *et al*., 2002). The present study was to assess the ability of strontium chloride to induce activation and *in vitro* embryonic development in buffalo oocytes in combination with cytochalasin B and 6- dimethylaminopurine.

## METHODOLOGY

All chemicals were purchased from Sigma Chemicals Company (St.Louis,Mo,USA) and disposable plastics wares from Nunc (Denmark), unless otherwise stated.

Cumulus oocytes complexes (COCs) retrieved from buffalo ovaries by slicing method were morphologically graded based on the cumulus cells investment and homogeneity of ooplasm (Nandi *et al.,* 1998). Only COCs of A, B, and C grades were chosen for *in vitro* parthenogentic activation. Oocytes were matured in Tissue Culture Medium (TCM) 199 medium with 10 per cent fetal bovine serum (FBS) 40 IU/ml pregnant mare serum gonadotrophin (PMSG) and 10 ng/ml epidermal growth factor for 24 hrs. *In vitro* maturation was assessed based on cumulus cell expansion after 24 hrs of incubation (Kobayashi *et al.,* 1994). Oocytes with degree 1 and 2 expansion were considered as mature. Further after denuding ,oocytes were evaluated for the presence of first polar body.

Matured oocytes were activated with 10 µM Strontium Chloride and 5 µg/ml cytochalasin B (Group I) in TCM with 2 per cent FBS for 2.5 hrs. In group II, matured oocytes were activated with 10 µM Strontium Chloride and 5 µg/ml cytochalasin B in TCMh with 2 per cent FBS for 2.5 hrs followed by culture in 2 mM 6-DMAP for 4 hrs.

Activated oocytes were incubated in $G_1$ medium and cultured at 38.5°C in a humidified atmosphere of 5 per cent $CO_2$ in air. After 72 hrs of culture, the parthenogenetic embryos were transferred to $G_2$ medium. *In vitro* embryo development was assessed every 24 hrs up to 7 days. Approximately at every 48 hrs, embryos were fed with fresh i*n vitro* culture medium. Cleavage was assessed at 48 hrs post activation and monitored every 24 hrs, upto 5 days for subsequent developmental stages. The statistical analysis of the data was done as per Snedecor and Cochran (1994).

## RESULT AND DISCUSSION

The activation rate for group I (n=276) was significantly higher ($P < 0.01$) than for group II (n= 266; 38.35 ± 1.00% and 31.29 ± 0.90%, respectively). Significantly higher numbers of embryos reached to 4 cell and 8-cell stages in group I than in group II (26.71 ± 0.85% vs 16.45 ± 0.75% and 18.60 ± 0.94% vs 12.74 ± 0.57, respectively). However, no significant difference was observed in morula development (9.57 ± 0.84% and 6.80 ± 0.59%, respectively). The cleavage and blastocyst percent were very low compared to Shen *et al*. (2008) who got the cleavage and blastocyst per centage of 95 and 33, respectively.

Strontium chloride action mimicked the effects of sperm on oocyte and seemed to be mediated through the ionositol triphosphate receptors (Zhang *et al*. 2005). Strontium chloride was indeed effective to induce oocyte activation in various species (Tomashov - Mater *et al*., 2005), and was used for oocyte activation in mouse cloning (Wakayama *et al*., 1998). It was further demonstrated that the concentration of 10 mM and the exposure time of 2.5 to 3 hrs were the best conditions for the activation of mouse oocytes (Loren and Kaplan, 2006).

This was apparently the first report regarding the use of strontium in combination with 6-DMAP and with cytochalasin B to activate buffalo oocytes and we found that the above combination was not suitable for artificial activation of oocytes. Strontium was more appropriate for single treatment in early matured bovine oocytes (*In vitro* maturation for 22 hrs), might be because it promotes multiple calcium increases in bovine (Meo *et al*., 2007) as observed in mice (Bos-Mikich *et al*., 1995). The combination of strontium and 6-DMAP achieved higher blastocyst rates and ICM cell number in younger oocytes but were harmful for aged oocytes (Meo *et al*., 2007).

## CONCLUSION

In conclusion, the current study demonstrated that strontium chloride in combination with cytochalasin B can be used for parthenogenetic embryo production in buffalo. Furthermore, since calcium oscillations were important for normal embryonic development, the stimulus promoted by strontium chloride could have been efficient for early events of activation, but was not enough for later events. Hence, future experiments are necessary for the comprehension of the effect of the strontium treatment on intracellular calcium response in buffalo oocytes. In addition, to improve development rates, it would be interesting to verify the efficiency of the combination of strontium to treatment that act by different mechanisms, such as protein synthesis and phosphorylation inhibitors.

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