

## Sperm Immobilization Prior to Intracytoplasmic Sperm Injection (ICSI) and Oocyte Activation Improves Early Development of Microfertilized Goat Oocytes

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**ABSTRACT.** A preliminary goat oocyte activation and intracytoplasmic sperm injection was evaluated in this study. Immature goat oocytes were cultured in TCM-199 supplemented with 10% goat serum for 27 hours for maturation at 38.5°C in an atmosphere of 5% CO<sub>2</sub> in humidified air. In vitro matured oocytes were used for activation and intracytoplasmic sperm injection. In the first experiments, calcium ionophore were used at final concentration 0, 1, 50, 100 and 200 µM to evaluate sham-injected goat oocyte activation. In the second experiment, the effect of immobilized of goat sperm cell on the cleavage and the development of sperm-injected goat oocyte was evaluated. Results indicated that in vitro matured goat oocyte did not activate spontaneously. Exogenous stimuli such as Calcium ionophore A23187 at certain level (in this experiment > 50 µM) can be used to activate in vitro matured goat oocytes. In this study, results indicated that sperm injected as well as sham injected goat oocyte can initiate first several cycles of cleavage after activation with calcium ionophore. Results in the current study also indicated that immobilization of goat sperm cells increase the percentage of early embryo development to cleavage after intracytoplasmic sperm injection.

**Key words:** ICSI, sperm immobilization, oocyte activation, embryo development

### Introduction

Over the years, live births have been reported with injection of sperm cells into the cytoplasm of oocytes (ICSI) in mice (Roknabadi et al, 1994), rabbits (Hosoi et al, 1988), cows (Goto et al, 1990) as well as in humans (Palermo et al, 1992). Now ICSI is being used successfully as an effective technique for treating couples with severe male factor infertility (Kuramoto et al, 2000). One advantage of ICSI over other assisted fertilization techniques (zona drilling and subzonal fertilization) is that sperm cells are need not to be motile with ICSI (Goto et al, 1990; Palermo et al, 1992). ICSI may be especially useful in farm animals in cases where a premium male has a physical injury so testicle sperm can be collected and used for sperm injection.

Hosoi et al, (1988) reported the birth of live rabbit offspring following transfer of ova fertilized by injection of a live sperm cell into the ooplasm without exogenous activation. Goto et al, (1990) reported that the birth of live calves after intracytoplasmic injection of frozen-thawed bovine sperm cells followed by subsequent activation of oocytes with exogenous calcium ionophore A23187. These results indicated that species difference in the

needed for exogenous stimuli after sperm are injected into cytoplasm of the oocyte to activate the oocyte.

During fertilization, oocyte activation is initiated after the sperm cell membrane fuses with the membrane of the egg. The sperm receptor on the oocyte surface interacts with an oocyte-binding protein located on the sperm surface to form a complex resulting in species-specific adhesions between the sperm cell and the oocyte (Kinsey et al, 1980). However intracytoplasmic sperm injection (ICSI) bypasses the normal sequence of the fertilization event.

It has been suggested that oocytes may be activated by the injection of high-calcium medium during intracytoplasmic sperm injection (Edwards and Van Steirteghem, 1993). However, recent data showed that oocyte activation is started after a considerable lag period following sperm injection, and activation is probably caused by a soluble factor released from the exogenous sperm cell (Tesarik et al, 1994). Tesarik and Sousa (1995) reported that the absence of human oocyte activation is the cause of fertilization failure in most cases when intracytoplasmic sperm injection fails, and