# *In vitro* Fertilization of Cumulus-Free Bovine Oocytes in Caffeine and Different Concentrations of Calf Serum



**Tajik P; Niwa K**
[1]*Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, P.O. Box 14155-6453, Tehran, Iran, Fax: +98 21 933222, Email: ptajik@chamran.ut.ac.ir*
[2]*Division of Animal Science and Technology, Faculty of Agriculture, Okayama University, Okayama 700, Japan*

## Introduction

Bovine in vitro fertilization (IVF) has been of great importance and the subject of different studies in animal lab oratories around the world. Different protein supplements have been used for IVF in these laboratories. In our previous study[2] fetal bovine serum (FBS), bovine serum albumin (BSA) and calf serum (CS) had been added to fertilization medium containing caffeine and heparin, and different results were observed. One of the most interesting points was that, in 20% CS, limited penetration rates were observed in media containing caffeine and heparin. In the present study, the same concentrations of CS were added to the fertilization medium, with or without caffeine, to study whether exclusion of heparin can support IVF of cumulus-free bovine oocytes in high concentrations of CS.

## Materials and Methods

Bovine follicular oocytes were aspirated from follicles of 2 to 5 mm in diameter with a 24-gauge needle attached to a disposable syringe, and washed four times with TCM-199 (with Earle's salts) and supplemented with 10% (v/v) heat-treated FBS, 100 IU/ml penicillin G and 100mg/ml streptomycin. Ten oocytes with compact cumulus cells were transferred into a 0.1-ml drop of the same medium under warm paraffin oil. After culture of oocytes at 39°C (102.2°F) in 5% $CO_2$ in air for 22 to 24 hour matured oocytes were freed from cumulus and corona cells by treatment with PBS containing 0.1% hyaluronidase for 10 to 20 minutes and by repeated passage through a fine pipette. Oocytes were then washed twice with BO[1] medium containing different concentrations of CS, and with or without caffeine, and transferred into a 50-ml drop of the same medium. Semen preparations and insemination were according to our previous experiment. After 22-24 hours oocytes were washed and mounted onto slide glasses in aceto-alcohol for three days, stained with 1% aceto orcein and observed under a phase-contrast microscope for evidence of fertilization.

## Results

The table shows effects of caffeine* on different concentrations of CS in IVF of cumulus-free bovine oocytes.

## Conclusion

There was no penetration in the media lacking protein supplement (here CS). On the other hand, caffeine could support penetration *in vitro* of bovine oocytes in 5% CS. However, the concentration of 20% CS in not recommended for bovine IVF.

## References

1. Brackett BG, Oliphant G: *Biology of Reproduction*, 12:260-274, 1975.
2. Tajik P, Niwa K, Murase T: *Theriogenology*, 40:949-958, 1993

**Table 1.**

| Serum concentrations | No. oocytes examined penetrated (%) | No. oocytes oocytes (%)** | No. polyspermic |
|---|---|---|---|
| 0 (Control) | 37 | 0 | |
| 5% | 37 | 34(92) | 8(24) |
| 10% | 42 | 26(62) | 5(17) |
| 20% | 39 | 7(18) | 1(14) |

* Caffeine = 5 mM, ** Percentage of number of oocytes penetrated.