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Effect of honeybee royal jelly on the nuclear maturation of bovine oocytes in vitro

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Introduction

Serum supplementation as a protein source to culture media for in vitro embryo production;

- high blastocyst yield
- disease risks
- detrimental to embryonic development and metabolism
- lipid accumulation affecting viability during cryopreservation
- one of the factors causing LOS following the transfer of in vitro produced bovine embryos

Introduction

Serum free culture systems for IVM, IVF and IVC are desirable for the production of high quality and normal embryos

- BSA
- PVA
- Serum replacers

None is still satisfactory in terms of blastocyst yield, quality and pregnancy rate

Introduction

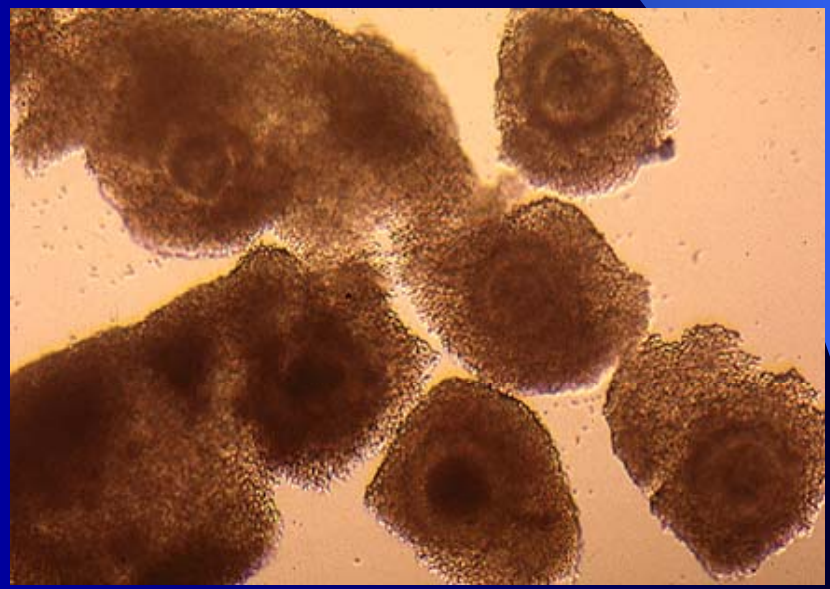
Honeybee royal jelly;

- embryonic development and metamorphosis
- protein content similar to blood
- anti-oxidative effect
- naturally occurring biological product
- 2/3 of protein is albumin
- no disease risk to animals

- may be a good replacer for serum in culture media?

Objectives

To investigate whether bovine oocytes can be matured in vitro in culture media supplemented with honeybee royal jelly as a protein source instead of serum



Material and Methods

- ovaries from slaughterhouse (transported within 2 h at 30-35°C in PBS)
- cumulus oocyte complexes from 2 to 8 mm follicles
- oocytes with homogenous ooplasm and evenly distributed 3 layers of cumulus investment
- search medium; Hepes-buffered TCM-199 supplemented with 0.4% BSA, L-glutamine, penicillin (50 IU/ml) and streptomycin (50 µl/ml)

Material and Methods

Selected oocytes were matured either in;

- ✓ 10% (v/v) FCS (control; n=229 oocytes) or
- ✓ 1% (w/v) honeybee royal jelly (n=239 oocytes)

- ✓ Bicarbonate-buffered TCM-199 containing with antibiotics and Na-pyruvate
- ✓ 50 µl culture media drop (5-10 oocytes/drop) under mineral oil
- ✓ a humidified atmosphere of 5% CO_2 in air
- ✓ at 38.6 °C for 22 h

Material and Methods

At the end of maturation period;

- Oocytes with expaded cumulus were recorded
- oocytes incubated in Hepes buffered TCM 199 containing 100 Unit/ml hyalorudinase for 5 min at room temperature
- Cumulus cells removed by repeated pipetting
- Fixed with acetic acid: ethanol (1:3) for 24 h before staining with aceto orcein (1%)
- Each oocyte was examined (100X) for a first polar body and/or metaphase II choromosome configuration

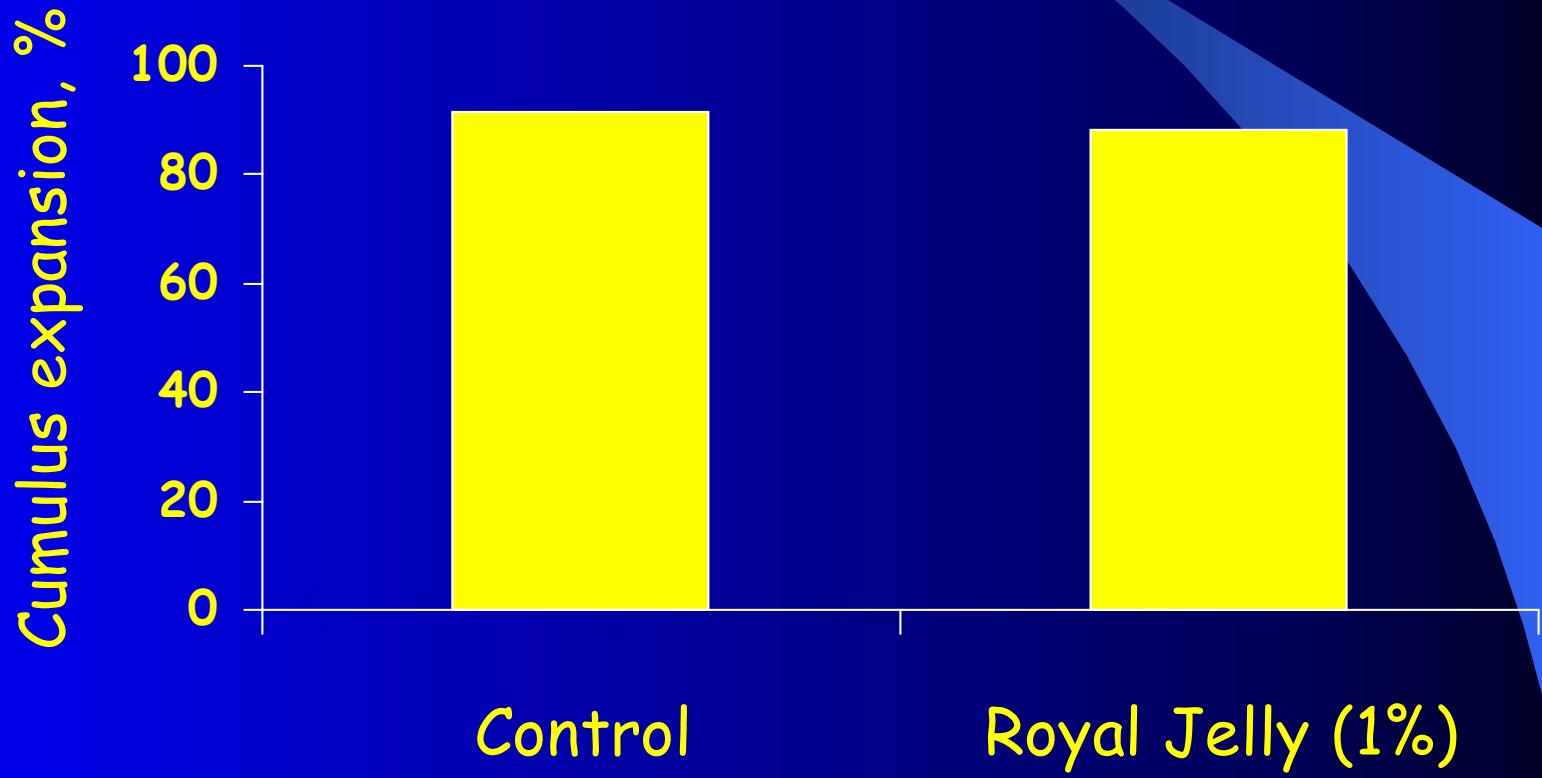
Material and Methods

Parthenogenetic activation: At the end of maturation period, oocytes with first polar body matured in either FCS (n=59) or royal jelly (n=62) supplemented media;

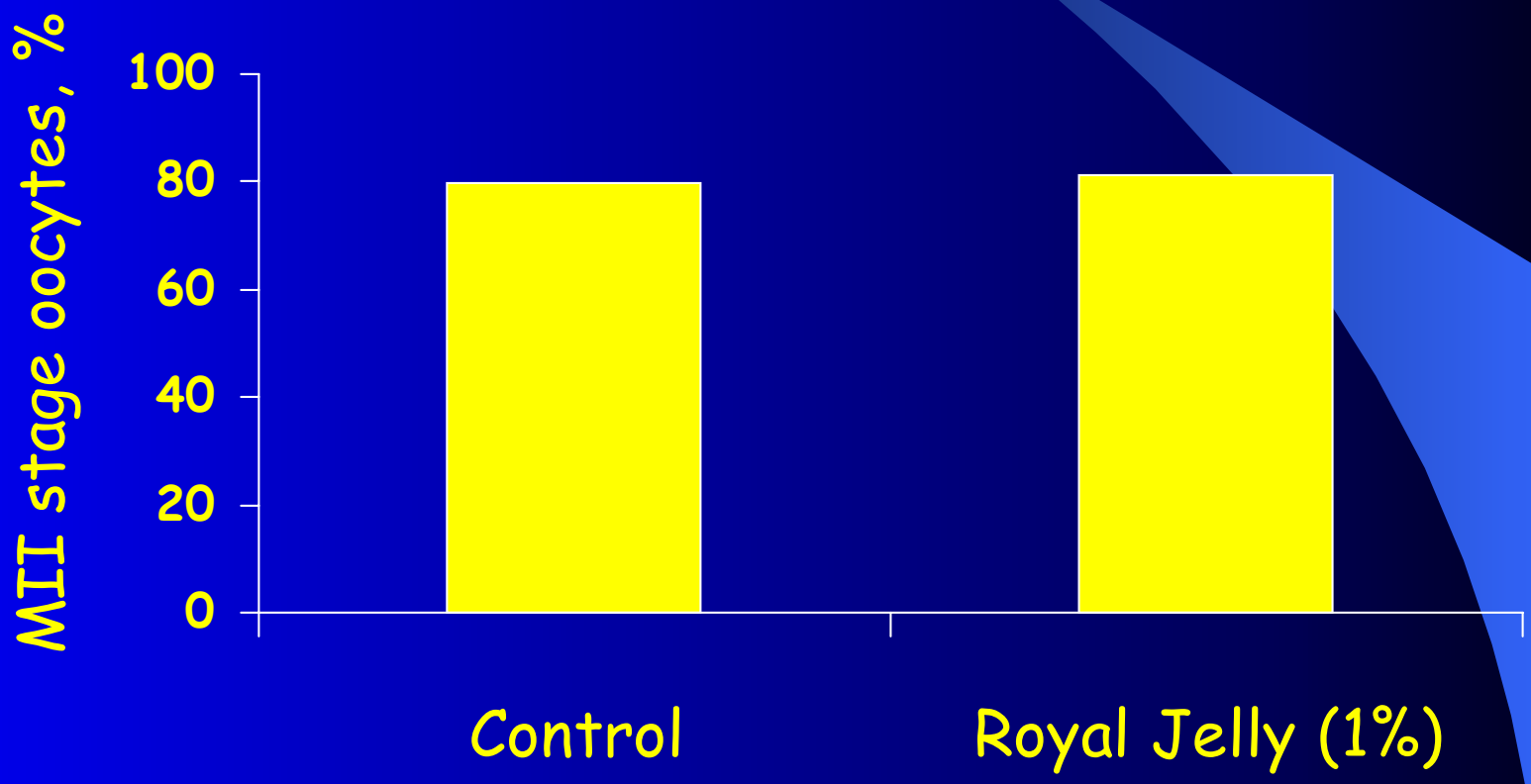
- incubated at room temp. for 5 min in culture media containing 7% ethanol
- Culture in media containing 5 µg/ml cycloheximide and 5 µg/ml cytochalasin B for 6 h
- co-culture with 30 h old granulosa cell monolayers in drops under mineral oil
- cleavage was recorded at 48 h post activation

Results...

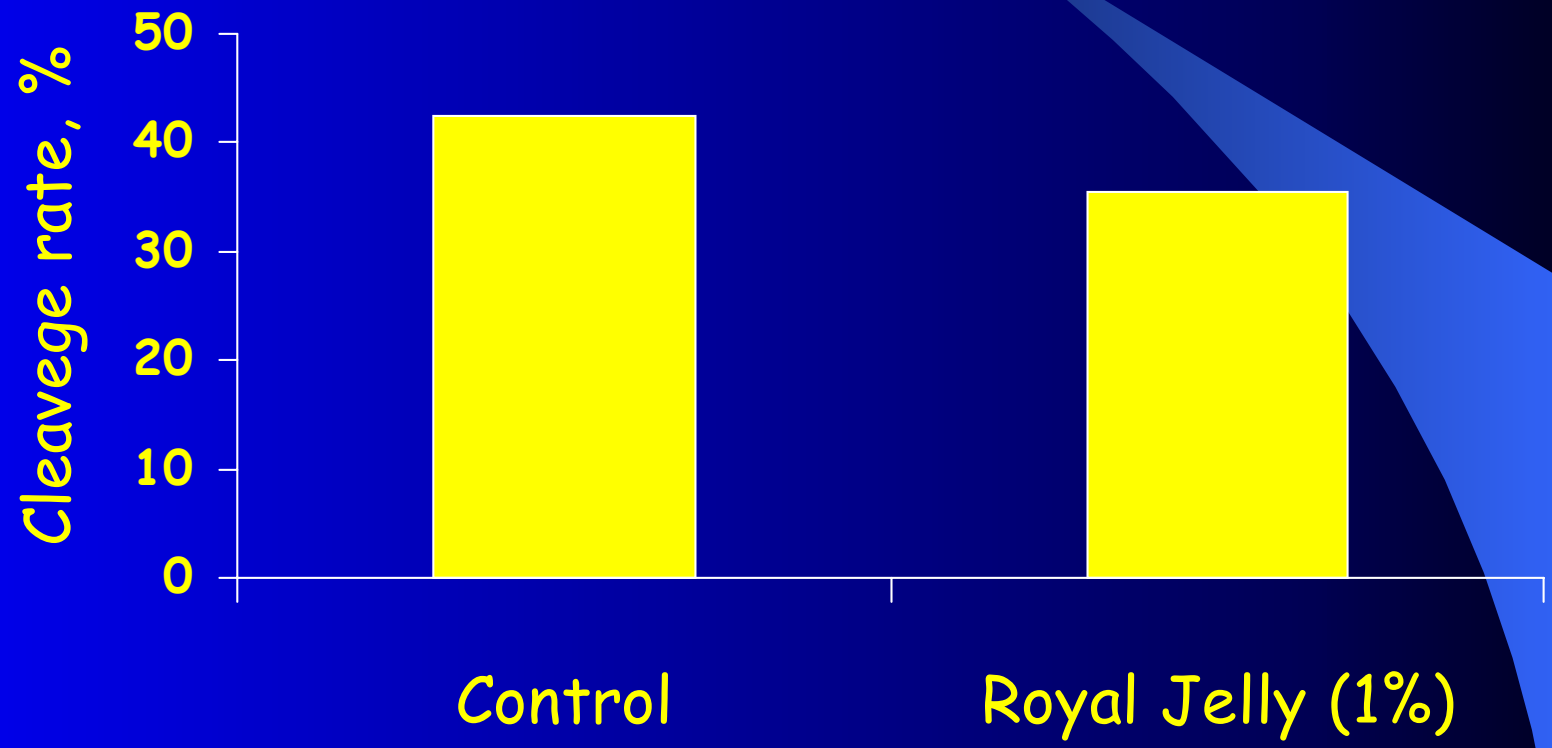
The ratio of bovine oocytes with expanded cumulus in culture systems supplemented with either 10% FCS or 1% Royal Jelly



The ratio of bovine oocytes reaching metaphase II stage of nuclear maturation in culture systems supplemented with either 10% FCS or 1% Royal Jelly



Cleavage rate of parthenogenetic activated bovine oocytes matured in culture systems supplemented with either 10% FCS or 1% Royal Jelly



Conclusions

- ✓ 1% royal jelly can be used successfully as a protein source in the maturation of bovine oocytes in vitro.
- ✓ Although parthenogenetic activated oocytes matured in royal jelly supplemented media have similar cleavage rate to those matured in serum supplemented media, embryonic development to the blastocyst stage in vitro post- fertilization should also be investigated.

Acknowledgements

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Cytoplasmic protein profile (SDS-PAGE) of metaphase II stage oocytes matured in either 10% FCS (S) or 1% royal jelly (RJ) supplemented culture media (n=22)