

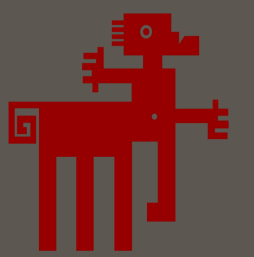
EFFECT OF OOCYTE SECRETED FACTORS (OSFS) DURING IN VITRO MATURATION ON EGF RECEPTOR EXPRESSION AND NUCLEAR MATURATION IN PREPUBERTAL GOAT OOCYTES

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INTRODUCTION

- **Goats** are of great interest as animals that can contribute to medical studies in humans.
- Juvenile in vitro embryo transfer (**JIVET**) provides increased genetic gain, shorten generation intervals and early availability of large number of oocytes per female.
- **Limitation of using prepubertal oocytes:** the majority are from small antral follicles that are underdeveloped.
- EGF-ligands bind in EGF receptor (**EGFR**) on cumulus cells (CCs) → meiotic resumption, oocyte maturation and cumulus expansion.
- CCs differentiation and stimulation of EGFR to support cumulus expansion is **regulated by OSFs: GDF9 and BMP15.**

Oocytes + CC = cumulus-oocyte complex (**COC**)

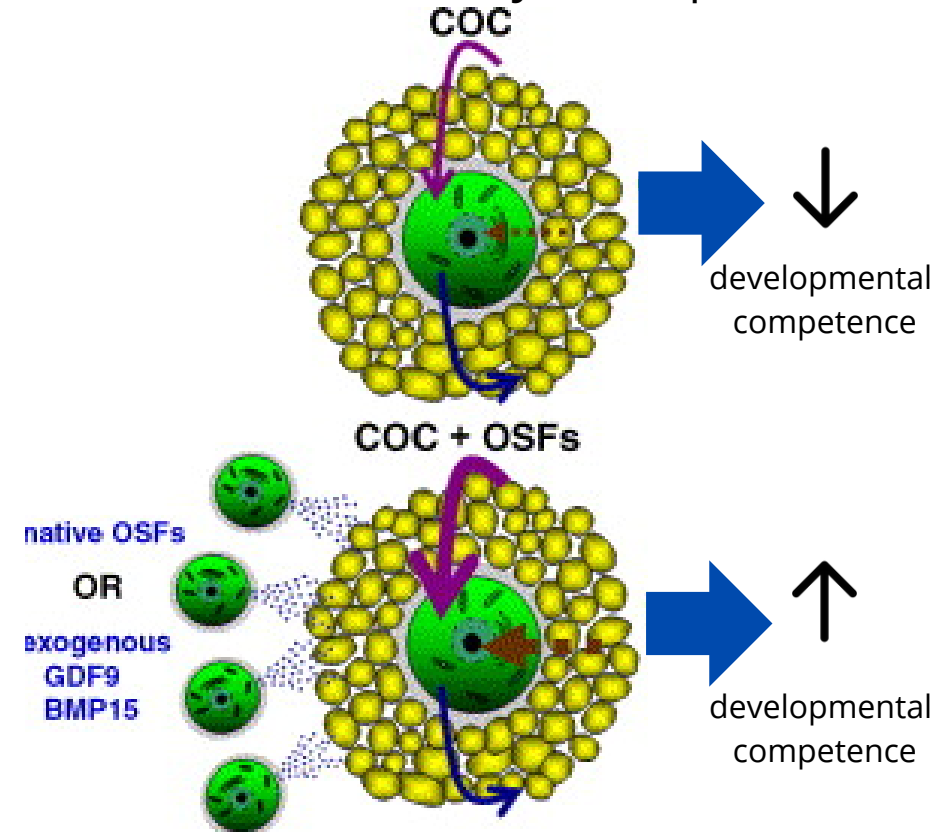


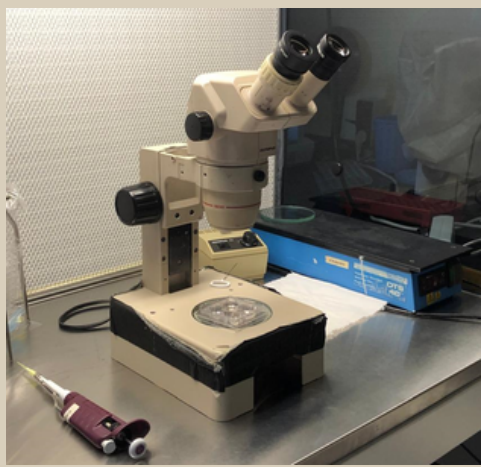
Figure 1. Effect of OSFs in developmental competence in bovine oocytes (figure by Hussein et al. 2006)

OBJECTIVE

To analyze the effect of OSFs on the **expression of EGFR** and **nuclear maturation** of oocytes after IVM with exogenous GDF9 alone or in combination with BMP15.

MATERIALS AND METHODS

1. Oocyte collection in an abattoir and slicing, COCs selected:
 - Homogeneous cytoplasm
 - Round shape
 - Three or more layers of compact cumulus cells (CC)
2. In vitro maturation (IVM). Three groups:
 1. Control group
 2. GDF9 group
 3. GDF9 with BMP15 group
3. Immunostaining and mounting



4. Nuclear stage assesment. Divided in three stages:
 - Germinal vesicle (GV)
 - Meiosis I (MI)
 - Meiosis II (MII)
5. Microscope analysis and EGFR quantification by ImageJ



Figure 2. EGFR-stained prepubertal goat oocyte in vitro matured

6. Statistical analyses using two-way ANOVA followed by Tukey's multiple-comparison test.

RESULTS AND DISCUSSION

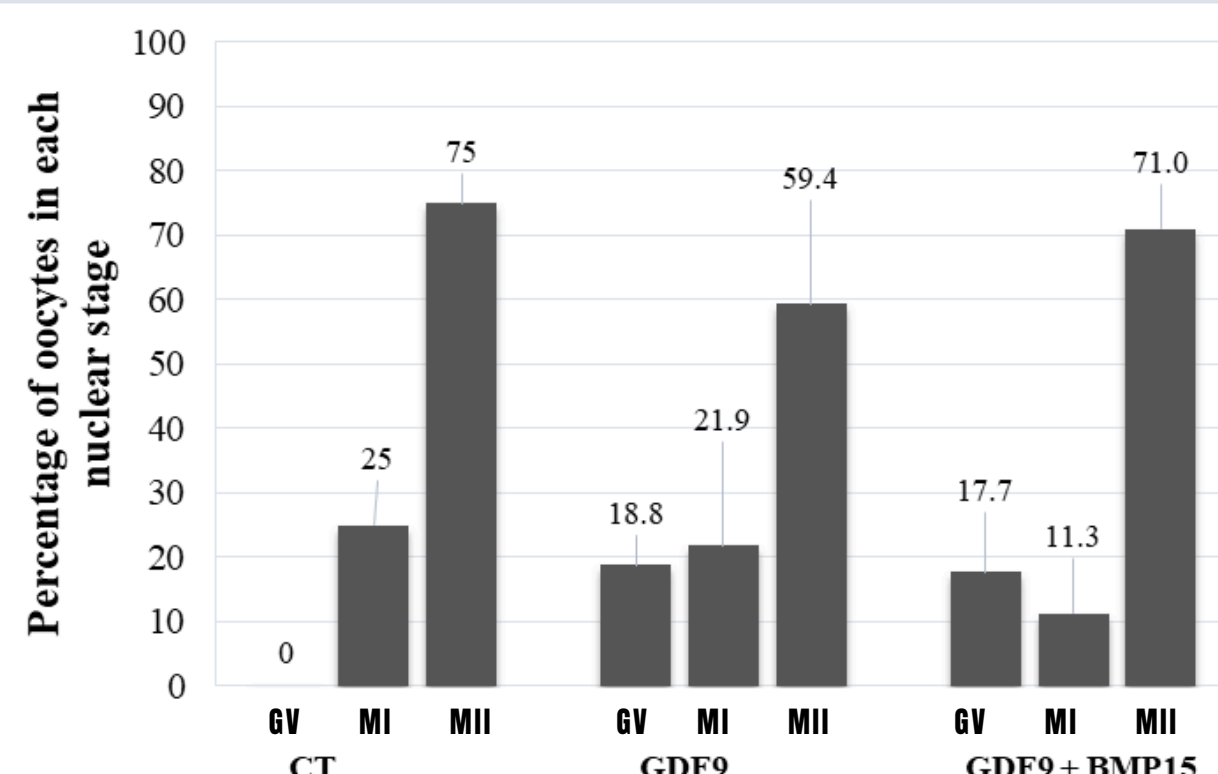


Figure 3. Percentage of oocytes in each nuclear stage. **No significant differences** were observed among the percentages of GV, MI and MII obtained in the 3 experimental groups. ($P > 0.05$).

- In **prepubertal goats**, OSFs in IVM does **not** seem to have a **beneficial effect on meiotic resumption.**
- In mice, porcine and cattle, only BMP15 was associated with increased cumulus cell expansion.
- GDF9 with BMP15 increased cumulus expansion in buffalo.
- Specie-specific roles of GDF9.

- Native OSFs improved blastocyst rate, in mice, gilts and prepubertal goats. GDF9 did not seem to be part of this improvement in goats. **Hypothesis is confirmed.**
- Exogenous GDF9 increased embryonic development in mice, blastocyst stage in cattle. Not in sheep.
- GDF9-lack of effect could be due to donor's age (small follicle size in prepubertal goats).
- BMP15 in IVM of prepubertal goat oocytes, increased EGFR expression in MII-oocytes, yet combined with GDF9, the positive impact is no longer detected. Reported also in sheep.

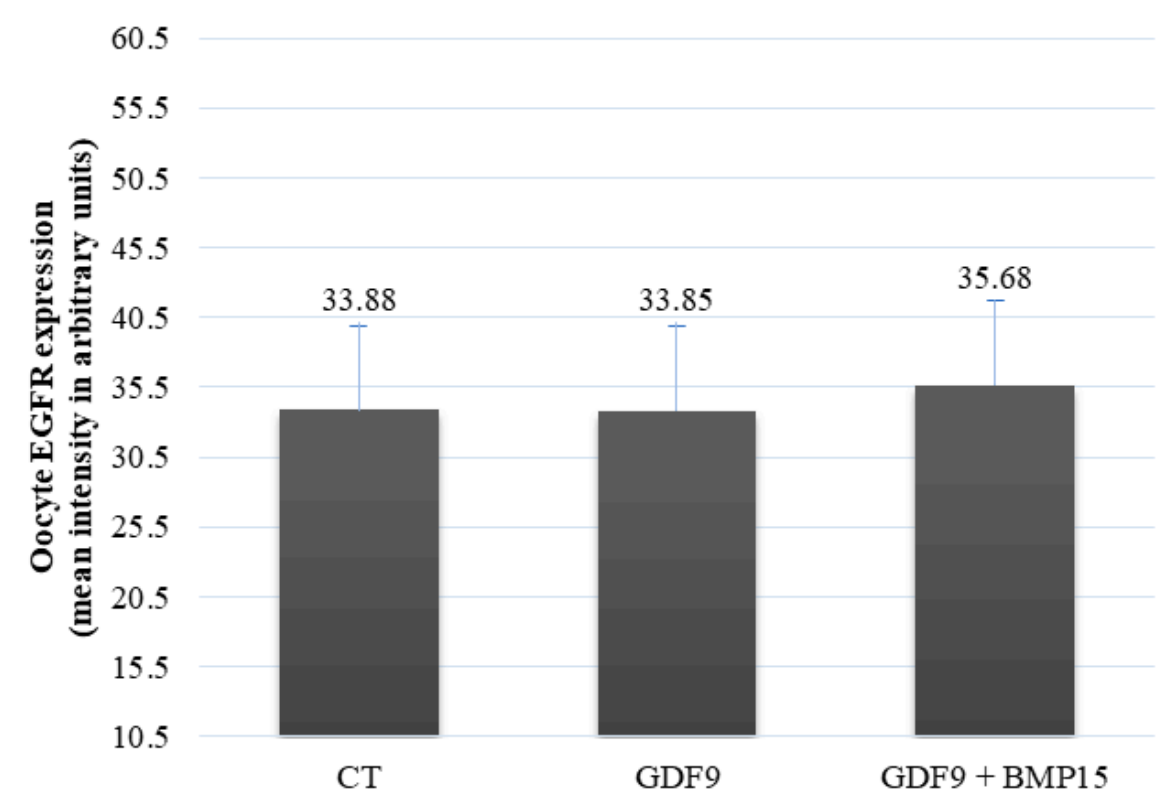


Figure 4. Oocyte EGFR expression. Mean intensity in arbitrary units in each group. **Results did not show any significant differences** in oocyte EGFR expression between groups ($P > 0.05$).

CONCLUSIONS

In conclusion, the present results indicate that the **addition of GDF9**, alone or in combination with BMP15, to IVM of prepubertal goat oocytes **does not have any beneficial effects** neither on **nuclear maturation** nor **EGFR expression**, and hence on oocyte competence.

REFERENCES

