# FORSKOLIN IMPACTS ON MATURATION AND LIPID CONTENT OF SWINE OOCYTES.

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## 1. INTRODUCTION

*In vitro* embryo production (IVP) are not as efficient in pigs compared to other domestic species, such as cattle (KIKUCHI et al., 2016). That is attributed to several factors: insufficient cytoplasmatic development of oocytes and embryos; the high lipid content in their cytoplasm; the increase in the production of reactive oxygen species (ROS); and a high rate of polyspermy (ZHANG et al., 2012).

Events such as oocyte maturation and incorporation of intracytoplasmic lipids occurs simultaneously during the growth of ovarian follicles (CONTI; FRANCIOSI, 2018). Physiologically, *in vivo*, antral follicles produce molecules of Cyclic Adenosine Monophosphate (cAMP), responsible for inhibiting the maturation-promoting factor (MPF). These molecules are transferred to the oocyte through the *cumulus-oophorus* cells, preventing meiotic progression, and ensuring synchronous cytoplasmic and nuclear maturation (CONTI; FRANCIOSI, 2018; MEHLMANN, 2005). Thus, when the oocyte is removed from the follicular environment for *in vitro* maturation (IVM), there is a decrease in cAMP levels, causing spontaneous resumption of meiosis through MPF activation (MEHLMANN, 2005).

Additionally, the asynchronous maturation can alter the energetic metabolism, leading to an accumulation of lipid droplets (KRISHER et al., 2007). One of the alternatives to improve the quality of oocytes in IVM is the use of cAMP modulators such as Forskolin (FSK) (APPELTANT et al., 2016), which is able to activate cAMP and stimulate lipolysis, providing an improvement in cytoplasmic and nuclear maturation synchronization (PARK et al., 2016).

This study aimed to evaluate the effect of FSK addition during the last 22h (PARK et al., 2016) of IVM on the nuclear maturation rates and lipid droplet contents of swine oocytes, in an attempt to synchronize oocyte maturation and reduce oocyte lipid content.

## 2. METHODOLOGY

The ovaries were collected in a local slaughterhouse and transported to the laboratory in saline solution (0.9% NaCl), at a temperature between 30°C - 35°C. With the aid of a vacuum pump, follicles from 3-6 mm were recovered, and the *cumulus-oophorus* complexes (COCs) were washed and selected in manipulation medium TCM-HEPES (TCM-199 with 0.1% PVA, 2.0 mM $C_3H_4O_3$ and 2.5 mM $NaHCO_3$). Only COCs with homogeneous cytoplasm and at least 3 layers of *cumulus-oophorus* cells were selected.

After washing and selection as descripted above, the COCs were matured in a plate (Nunc, Roskilde, Denmark) containing 400 µL of medium, distributed among the following groups: 1) Control - TCM-MIV (TCM-199 supplemented with 0.1% PVA and 2.5 mM NaHCO3, 0.57 mM cysteine, 0.91 mM C3H3NaO3, 0.001 g/mL EGF, 0.05 g/mL streptomycin, 0.065 g/mL penicillin and 25% pFF); 2) FSK - TCM-IVM + 10 µM de FSK during the last 22 h.

After maturation, the oocytes were denuded by vortex for 5 min, washed in PBS, fixed in paraformaldehyde for 30 min and stored in PBS at 4°C, until staining to evaluate maturation and lipid content.

To assess maturation, the oocytes were fixed as described above, and stained with 7.5 µg/mL of Hoestch for 10 min. The structures were stained and placed on a slide with a Mowiol drop and covered with a coverslip and evaluated using epifluorescence microscopy (Nikon 80i), oocyte maturation was evaluated according to (UHM et al., 2007) by the same technician.

To measure lipid content, fixed oocytes were stained with 1 µg/mL Nile Red for 30 min and washed in PBS. After being staining, they were placed on a slide with a drop of Mowiol and covered with coverslips for evaluation. As described by Barceló-Fimbres and Seidel (2011), the images were obtained by epifluorescence microscope (Nikon 80i), using a G2A filter with 1920 X 1080 resolution and 80 ms exposure. For fluorescence intensity measurements, only the oocyte cytoplasm was selected with the free hand drawing tool Image J, where the values were adjusted by Corrected Total Cell Fluorescence (CTCF): CTCF = Density Integrated - (Specific cell area x Average fluorescence of background readings (Fitzpatrick, 2014).

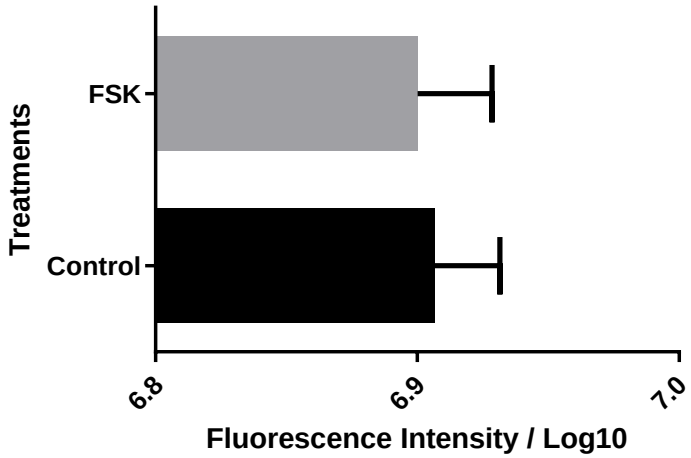
The statistical analyses were performed using the Statistix 10 software. The maturation rates were compared by chi-square tests. The lipid contents were compared by ANOVA, with comparison of means through the Tukey test.

## 3. RESULTS AND DISCUSSION

Maturation rates (n = 248) were similar (P > 0.05) for the Control (55.2%) and FSK (50.5%) treatments.

There was no difference (P > 0.05) in lipid content between the control and FSK groups (Figure 1).

Figure 1- Fluorescence intensity of lipid droplets (log10) of oocytes stained with Nile Red after maturation in control (n = 68) and FSK (n = 62).



Forskolin was chosen because it functions as a cAMP modulator and influences meiotic arrest, leading to a better synchrony between nuclear and cytoplasmic maturation (SUN; NAGAI, 2003). Additionally, Forskolin stimulates lipolysis (PARK et al., 2016) and increases mitochondrial function, oxygen consumption and ATP levels (HASHIMOTO et al., 2019).

However, these benefits were not present in this study. Other studies shown that FSK should be administrated during pre-IVM or during the first half of swine IVM (GRUPEN, 2014) to achieve better synchronization in nuclear and cytoplasmic maturation (APPELTANT et al., 2016). On the other hand, one study suggests that FSK addition impair maturation whatsoever (FU et al., 2011).

## 4. CONCLUSION

Forskolin neither improved nuclear maturation, nor decreased oocyte lipid content.

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