FOXO3 Expression and Function in the Pig Oocyte and Embryo

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Summary and Implications

Forkhead box O3 (FOXO3), a member of the FOXO subfamily of Forkhead transcription factors, has been shown to play critical roles in apoptosis, oxidative stress, cell cycle and DNA repair. The objective of this study was to characterize FOXO3 expression and its function during oocyte maturation and early embryo development in the pig. We found: (1) FOXO3 is dynamically expressed at both mRNA and protein level in the maturing oocyte and early *in vitro* fertilized embryos. (2) FOXO3 protein is localized in the cytoplasm of pig maturing oocytes. (3) Co-culture with Doxorubicin (DOX, 2 μM) significantly altered the total FOXO3 protein level during *in vitro* maturation. (4) *in vitro* maturation with DOX (2μM) numerically increased maturation rate, but significantly decreased embryo development to the blastocyst stage after oocyte parthenogenetic activation. Our work provides useful data for functional study of FOXO3 protein in female gametogenesis and the potential impact on developing pig embryos, which could benefit animal reproductive health and provide foundational knowledge for improving swine reproductive efficiency.

Introduction

A role for ovarian FOXO3 in controlling the rate of primordial follicle activation into the growing follicular pool has been established. Forkhead box O3-deficient mice demonstrate global activation of the primordial follicle pool, while oocyte-specific over-expression of FOXO3 prevented activation of primordial follicles. In both cases, altered FOXO3 was associated with impaired fertility. Forkhead box O3 protein activity is regulated via phosphorylation by kinases, such as the serine/threonine protein kinase (AKT), extracellular signal-regulated kinase (ERK) and IκB kinase (IKK) and can be activated by Doxorubicin (DOX), an anti-neoplastic agent. The objective of this study was to characterize FOXO3 expression and its function during oocyte maturation and early embryo development in the pig.

Materials and Methods

Sow ovaries were obtained from an abattoir and 3-6 mm antral follicles were aspirated. Oocytes at germinal vesicle stage were collected and matured *in vitro* for 42-44 hours with or without 2 μM DOX. The maturated oocytes with the first polar body (MII stage) were fertilized with fresh boar semen or electrically activated. Oocyte and embryo samples were collected at the germinal vesicle (GV) stage, metaphase II arrest (MII) and at the 4-8 cell stage of embryonic development for quantitative RT-PCR. GV and MII oocytes were used for immunocytochemistry and Western blotting. Electrically activated embryos were cultured in PZM3 medium for 7 days and development rates were recorded. Immunostaining images were captured using a fluorescence microscope system. The quantitative analyses of staining and Western blotting were performed using Image J software.

Results and Discussion

Relative quantification of *FOXO3* mRNA demonstrated abundance was greatest in MII-arrested oocytes although not statistically different compared to GV oocytes. Forkhead box O3 mRNA expression was 9.1-fold ($P < 0.05$) less in 4-8 cell stage embryos compared to MII oocytes (Figure 1). Western blot analysis demonstrated FOXO3 protein was present in all oocyte samples and was not different ($P > 0.05$). By immunocytochemistry, FOXO3 was located throughout the cytoplasm albeit slightly enriched in the nuclear area of GV oocytes.

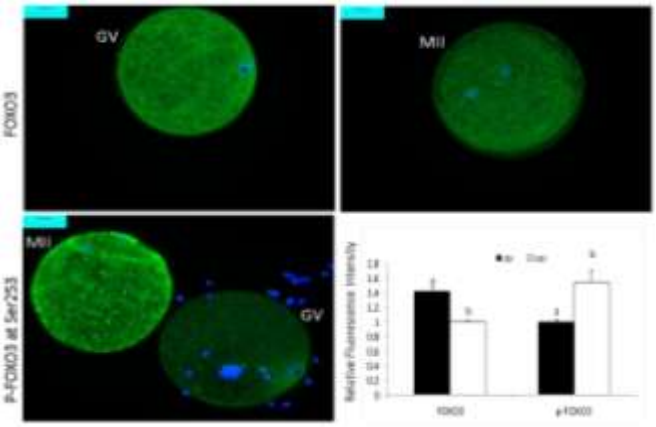

Figure 1. FOXO3 and phosphorylated FOXO3 protein localization in maturing pig oocyte. FOXO3 protein is mainly located in the cytoplasm of GV oocytes, and cytoplasmic staining intensity decreased after oocytes matured to MII stage. The phosphorylated FOXO3 protein (Ser253) is predominantly- located in the nucleus of GV oocytes, and cytoplasmic staining intensity significantly increased by MII stage.

Following *in vitro* maturation, FOXO3 cytoplasmic staining was much less in MII arrested oocytes ($P < 0.01$). Immunostaining of phosphorylated FOXO3 at Ser253 was strongly enriched in the nuclear area and slightly stained throughout the cytoplasm in GV oocytes, and by the MII oocyte stage, cytoplasmic staining was significantly increased ($P < 0.05$; Figure 1). Co-culture with DOX (2 µM) during *in vitro* maturation significantly increased total FOXO3 staining ($P < 0.01$), and decreased the phosphorylated FOXO3 (Ser253) staining ($P < 0.05$) in MII oocytes as compared to controls. *In vitro* maturation with DOX (2µM) increased maturation rate (control = 57.1±1.6%; DOX = 69.8±1.7%; $P < 0.05$, n=8 per treatment), but all electrical activated embryos failed to develop to blastocyst stage. Collectively, these data demonstrate dynamic FOXO3 expression and localization in the pig oocyte and early embryo and suggest a role for FOXO3 in oocyte maturation with subsequent implications for embryo development in the pig.

Acknowledgements

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