Improving the IVF efficacy of porcine embryos through the alteration of IVF protocol and medium selection.

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Introduction

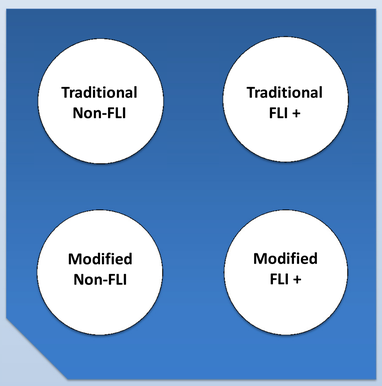
In Vitro Fertilization (IVF) is one of the major steps during embryo in vitro production (IVP) that is important in animal agriculture, particularly in the dairy and beef industries. Although it has played less of a role in the swine industry, it is a necessary technology for introducing direct genetic modifications into animals. The technology requires, however, high quality, in vitro-derived oocytes for its success. In particular, specialized media are needed to allow the immature oocytes to complete meiosis and be fertilized in vitro, and for the resulting zygotes to develop to blastocysts and provide offspring. Recent developments have led to significant increases in the efficiency of producing in vitro-derived piglets. In particular, this laboratory has described a chemically defined maturation medium containing 3 cytokines (FGF2, LIF, and IGF-1) called FLI medium, which doubled the overall efficiency of nuclear maturation of oocytes in cumulus-oocyte complexes (COC's) and their development to blastocysts in vitro.

Objective

The objective of our present study is to compare the efficacy of producing blastocysts after maturing the COC in FLI medium and control medium. At the end of the 42h maturation in medium, half the oocytes in each group will be denuded of their cumulus cells and metaphase II (MII) stage oocytes were selected and subjected to IVF (the traditional method) while the remainder will retain the cumulus cells and undergo IVF without oocyte selection (the modified method).

Experimental Design

Figure 1: Oocytes were arranged in 4-well plates with each well representing a different permutation of the tested variables. Separate plates containing separate media were used for different stages of the experiment (eg. maturation, fertilization, Embryo culture)



Materials and Methods

Prepubertal gilt ovaries were obtained from Smithfield slaughterhouse in Milan, MO. Follicles were aspirated and cumulus oocyte complexes were either matured for 42 hours in M199 supplemented with EGF, LH and FSH, our control medium; or in M199 supplemented with EGF, LH, FSH and 40 ng/ml FGF2, 20 ng/ml LIF, and 20 ng/ml IGF-1, referred to as FLI medium. For the traditionally treated oocytes, cumulus cells were removed at 42 hours and MII oocytes were selected for IVF based on the extrusion of a polar body; for our modified protocol, cumulus cells were kept intact, and all oocytes were selected for fertilization. All groups of oocytes remained in the fertilization media for 5 hours. Post-fertilization, all groups of oocytes were washed in culture media and transferred to a culture maturation media. The traditionally treated oocytes remained in the culture media for the remaining 6 days. For the modified protocol, cumulus cells were mechanically removed and all oocytes were transferred back into the culture media for the remaining 6 days.

Results

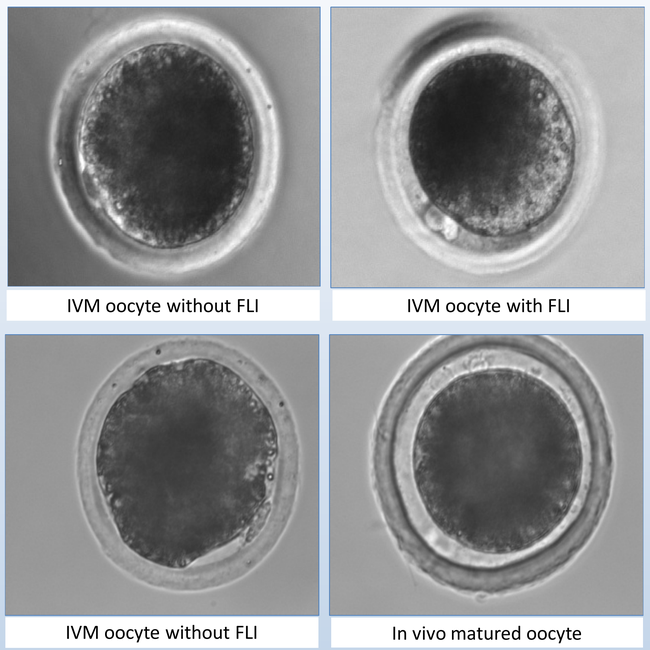


Figure 2: FLI-treated oocytes become morphologically similar to in vivo oocytes, A combination of three cytokines enhances porcine oocyte in vitro maturation and developmental competence

Results (continued):

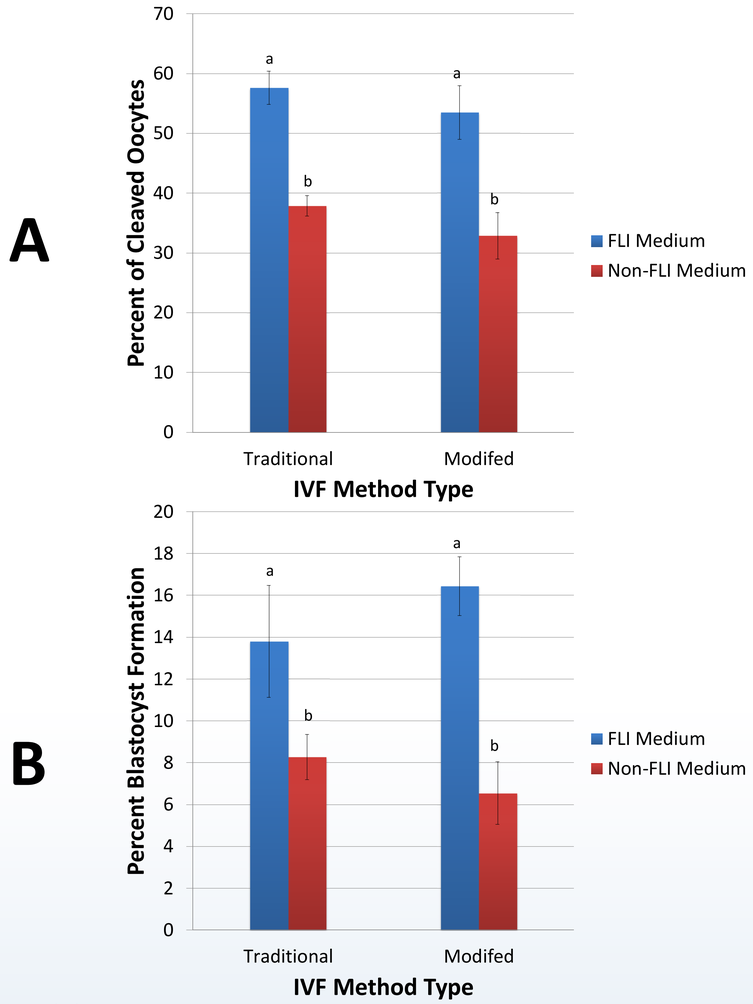


Figure 3: (A) Oocyte cleavage percentages on day 2 of embryo culture among various combinations of IVF methods and mediums (a,b denotes significance at P<.05). (B) Blastocyst percentages on day 6 of embryo culture among various combinations of IVF methods and media (a,b, denotes significance at P<.05).

Conclusion

This initial attempt of using the FLI matured oocyte in modified IVF protocol yielded ~17% overall efficiency of blastocyst development. P-values denoted significance between cleavage and blastocyst percentages of FLI vs Non-FLI oocytes (P<0.05), while failing to denote significance between methods in both cleavage and blastocyst percentages (P>0.05). By eliminating the step of oocyte selection prior to IVF, the modified protocol requires less time to perform and is less technically challenging, therefore, fits better for large-scale embryo production. Optimization of IVF methods in the presence of cumulus cells (modified) could improve cleavage and blastocyst percentages and enhance overall efficiency in swine IVF techniques.

Acknowledgements and Funding

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