Animal Reproduction

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Embriology, developmental biology and physiology of reproduction

Effect of FGF2, LIF, and IGF1 supplementation on pregnancy success following embryo transfer of IVP embryos

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Resumo

Culture environment during IVP can affect embryo phenotype and pregnancy outcomes, making culture modifications a logical approach for improving embryo competence. Previously, the addition of the growth factors FGF2 (40ng/ml), LIF (20ng/ml), and IGF1 (20ng/ml), termed FLI, to the culture medium improved bovine embryo development, and re-expansion following cryopreservation (39% to 82%). The objective of this study was to investigate the survival of cryopreserved FLI treated embryos at days 15 and 30 and evaluate conceptus transcriptomes. Embryos were produced using abattoir-derived oocytes, fertilized using standard procedures, and cultured to the blastocyst stage with or without FLI (+/- FLI). Embryos with a quality grade of 1 (6-1) were loaded into straws with ethylene glycol + sucrose and cryopreserved by slow-rate freezing. For experiment 1, 65 -FLI and 65 +FLI embryos were transferred into non-lactating recipient beef cows (n = 26 / 5 embryos each) 7 days after the last GnRH of a 5-day CO-Synch protocol. Eight days later, females were euthanized, uteri were collected, flushed, and conceptuses were recovered and flash frozen. For a subset (n = 4 per treatment) whole transcriptome analysis was performed using the NovaSeq 6000 (NGS platform of Illumina). Sequencing depth was 50 million reads per sample. After quality control, transcriptome of samples was aligned to the cow genome using Hisat2 and FeatureCounts was used to determine the read counts per gene. EdgeR was used to identify differentially expressed genes (FDR < 0.05). In experiment 2, a single frozen-thawed embryo was transferred to recipient females (n = 130) 7 days following detection of estrus. Pregnancy diagnosis was performed on day 30 using transrectal ultrasonography. Data for embryo length and average embryos recovered were analyzed by ANOVA using the GLM procedure of the Statistical Analysis System (SAS V9.4). Data for embryo recovery and day 30 pregnancy were analyzed by logistic regression using the Glimmix procedure. In experiment 1, there was no difference (P > 0.05) in conceptus recovery (-FLI 33.8% ± 5.87 vs +FLI 32.3% ± 5.8) or average conceptus length (-FLI 3.33cm ± 0.73 vs +FLI 4.18cm ± 0.75). There were 32 differentially expressed genes, 23 upregulated and 9 down regulated in the +FLI group compared to -FLI. Genes were involved in interferon signaling, prostaglandin synthesis, and the MAP kinase pathway. The +FLI group had increased expression of genes involved in trophoblast formation. In experiment 2, pregnancies per ET were 30.88 ± 5.6% in the -FLI group and 30.65 ± 5.9% in the +FLI group (P = 0.98). We conclude that embryos cultured +/- FLI and cryopreserved by slow-rate freezing have similar developmental competence up to day 30 of pregnancy. Differences in gene expression show an effect of FLI on conceptus signaling during elongation.

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