nuclear protein present in all phases of the cell cycle except G0). The sections were incubated overnight at 4 °C with the appropriate antibody. The slides were then incubated with an appropriate secondary antibody, visualized with DAB chromagen, and counterstained with Mayer's hematoxylin. GATA-4 stained intensely in all treatments. Statistical differences between treatment groups were detected using paired Student's *t*-tests. Positive staining for Ki-67 was found in $19.9 \pm 0.7\%$ and $20.0 \pm 1.8\%$ of the Sertoli cells from testes in control and FSH treated nude mice, respectively. The percentage of Sertoli cells positive for Ki-67 was increased in PTU ($27.9 \pm 3.4\%$, $p = 0.06$) and slightly increased in the PTU + FSH ($22.1 \pm 5.6\%$, $p = 0.36$) treated nude mice when compared with control treated mice. The results indicate that PTU is a key regulator of Sertoli cell proliferation in pigs, although the interactions between PTU and FSH remain unclear. Further studies of the interaction of these and other regulatory factors are necessary to completely understand the porcine Sertoli cell cycle.

Keywords: Testis; Porcine; Follicle stimulating hormone; Propylthiouracil

CLONED HOLSTEIN BULLS WITH DIFFERING RESPONSES TO HEPARIN-INDUCED IN VITRO SPERM CAPACITATION

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Recent reports of semen analysis, in vitro fertilization (IVF), and breeding trials to compare genetically identical (cloned) bulls generated by either blastomere splitting or somatic cell nuclear transfer (SCNT) have revealed no significant differences in these parameters. As more details of the many molecular changes in sperm required for fertilization are determined, more sensitive and specific assays of sperm function can be added to the standard semen analysis, including assays for sperm capacitation, the complex maturational event required for fertilization. The objective of this study was to determine the similarity of semen parameters for two cloned 3-year old Holstein bulls (A and B) derived via SCNT from a single donor bull. Assays utilized semen collected by artificial vagina and included semen analysis, in vitro fertilization and in vitro sperm capacitation. Data were analyzed by ANOVA and Tukey test using SigmaStat 3.0 (SPSS inc., Chicago, IL). Using Society for Theriogenology guidelines, both bulls were categorized as satisfactory potential breeders at the start of the 6 month study, with no abnormalities of external genitalia or accessory sex glands detected, normal scrotal circumference (A: 38.5 cm, B: 37.5 cm), sperm morphology (A and B: 85% normal), and sperm motility (A and B: 90% motile). Sperm viability (carboxyfluorescein diacetate/propidium iodide fluorescence assay) following three 5 min washes via centrifugation for 8 min at $380 \times g$ and resuspension in capacitation medium (TALP) did not differ between bulls (A: $89.6 \pm 1.7\%$, B: $89.8 \pm 0.8\%$, mean $\pm$ S.E.M., $n = 5$). IVF was performed utilizing in vitro matured bovine oocytes and sperm from four separate ejaculates per bull that was incubated for 4 h in TALP with 10 μg/ml heparin at 39 °C in 5% CO_2/95% air in a humidified environment. No significant differences in fertilization, as assessed by cleavage rate (A: 68.4% of 155 oocytes; B: 62.6% of 195 oocytes), or embryo development, as assessed by the percentage of >4 cell embryos at day 2 of culture (A: 45.8%; B: 41.0%) and the percentage of blastocysts at day 7 of culture (A: 26.5%; B: 28.2%), were detected. Sperm capacitation was assessed via the ability to undergo the lysophosphatidylcholine-induced acrosome reaction (LC AR) and protein tyrosine phosphorylation as detected by antiphosphotyrosine protein immunoblotting before and after incubation for 4 h at 39 °C in 5%CO_2/95% air in TALP with and without 10 μg/ml heparin. In contrast to the lack of detected differences between the two bulls by all other assays, a significant difference in heparin-induced sperm capacitation was noted with Bull A displaying a greater increase than bull B in heparin-induced, capacitation-associated protein tyrosine phosphorylation ($n = 5$). In addition, preliminary LC AR data ($n = 3$) indicates that bull A more consistently (3/3 assays) displays a positive response to heparin than bull B (1/3 assays).

Keywords: Sperm capacitation; Bovine; Cloning; Fertilization; Semen analysis

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