Elucidating the Cellular and Molecular Mechanisms Driving Testicular Dysfunction Within *In Utero* Growth Restricted (IUGR) Boars

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To meet the increased global demand for pork, the industry has emphasized sow prolificacy. However, a direct consequence of greater litter size is an increased proportion of *in utero* growth restricted (IUGR) piglets due to uterine capacity limitations. Recent data demonstrates that IUGR negatively affects gonadal development and lifetime reproductive potential of breeding boars. For example, adult IUGR boars have smaller testes, reduced numbers of testicular somatic cells (e.g., Sertoli and Leydig), produce less sperm, and, therefore, generate fewer artificial insemination doses compared with normal birthweight (NBW) boars. However, the cellular/molecular mechanisms mediating this effect are poorly defined. Therefore, the objective of this study was to compare the testicular proteome and testis composition between neonatal NBW and IUGR boars. White crossbred littermates from nine litters were selected based on birth weight, resulting in two experimental groups: NBW (1.56 ± 0.03 kg; n = 28) and IUGR (1.01 ± 0.04 kg; n = 14). At 3 days of age, boars were castrated, and testis samples were processed for histological analysis and a subset (n = 8/treatment) were preserved for proteomics. A total of 1,494 proteins were identified. Of those, 491 proteins were affected by treatment; more specifically, 440 were significantly ($P \leq 0.05$) different between experimental groups, while the remaining 51 proteins tended to differ ($P \leq 0.10$). In total, 222 proteins were upregulated and 269 were downregulated in testes of IUGR boars compared with their NBW littermates. Upregulated proteins in IUGR testes included those involved in apoptosis (BAX, CASP3), fetal gonadal development (NRB0B1), and steroidogenesis (CYP19A3, HSD17B11). Other proteins with important reproductive functions, such as testis descent (INSL3), steroid metabolism (HSD11B2), and antioxidant functions (GSTK1), were downregulated in IUGR testes. Histological analysis revealed that IUGR testes had reduced Leydig cell numbers (per field) compared with NBW boars (82.04 ± 3.39 μm^2 versus 102.30 ± 2.06 μm^2; $P < 0.01$), which is consistent with previous data from mature IUGR boars. Interestingly, Leydig cells from IUGR boars were hypertrophic compared with NBW boars (143.17 ± 2.52 μm^2 versus 125.35 ± 1.52 μm^2; $P < 0.01$), suggesting a potential compensatory mechanism. The following did not differ between experimental groups: average seminiferous tubule area, interstitial area, number of seminiferous tubules (per field), or total seminiferous tubule area ($P > 0.10$). Notably, the concurrent downregulation of INSL3 and upregulation of BAX and CASP3 in IUGR boar testes may reveal a possible biological mechanism driving hypogonadism in adult IUGR boars. Others have reported that when INSL3, a protein secreted by Leydig cells, is neutralized in boars, an upregulation in apoptotic proteins such as BAX and CASP3 occurs within germ cells. Upregulation of these apoptotic proteins may be a causative agent of reduced germ cell numbers; thus, decreasing total sperm production in adult IUGR boars. Overall, these data suggest that neonatal IUGR boars have distinct cellular and molecular alterations within the testis that may

drive poor reproductive function in adulthood. Ultimately, this study elucidates porcine testis biology and exposes possible therapeutic targets to improve boar reproductive performance.