Reproduction, Fertility and Development

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line (Gyobu *et al.* 2015 Mol. Cell. Biol. **36**, 645-659). To determine whether this is one reason behind *mt*/*mt* bull subfertility, we assessed the ability of sperm from these bulls to migrate through cervical mucus of oestrus cows. In addition, because *mt*/*mt* bulls are homozygous for such an extensive chromosomal segment, this study aimed to determine whether TMEM95 alone is responsible for the loss of sperm function. To test this, sperm from a *wt*/*wt* bull was incubated in the presence of anti-TMEM95 antibody (Ab; 1:20 dilution) 1 h before fertilization. In all experiments, data were assessed for normality of distribution and analysed using a one-way ANOVA. In experiment 1, bull genotype had no effect on the number of sperm present in mucus-filled capillaries or on their distribution along them, following 30-min incubation. In experiment 2, incubation of sperm from *wt*/*wt* bulls with Ab decreased oocyte cleavage rates compared with the untreated control (59 ± 0.3 *v.* 76 ± 0.9%, respectively; $P < 0.05$). However, this percentage was still higher than in the *mt*/*mt* group (59 ± 0.3 *v.* 31 ± 2.9% respectively; $P < 0.05$). Surprisingly, the Ab did not affect the percentage of blastocysts that developed at Day 8 after fertilization. Three hours after fertilization, a lower number of Ab-treated sperm bound to the ZP compared with untreated sperm (4 ± 1.2 *v.* 9 ± 2.1, respectively; $P < 0.05$). This number did not differ between the Ab and *mt*/*mt* groups. Consistent with the previous results, Ab-treated sperm were less able to penetrate ZP-free oocytes than untreated sperm (47 ± 1.5 and 58 ± 4.2%, respectively; $P < 0.05$). However, lower penetration rates were observed in the *mt*/*mt* than in the Ab-treated group (24 ± 3.9 and 47 ± 1.5%, respectively; $P < 0.05$). We concluded that sperm from *mt*/*mt* bulls could traverse cervical mucus from oestrus cows. Transmembrane protein 95 seems to be directly responsible for the lower ZP binding and, partially, for the lower plasma membrane penetration observed. However, how this protein exerts these functions and what other factors might be involved remain to be determined.

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140 Endocrine Profiling in Prepubertal Stallions with Abnormal Testicular Development

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The use of anti-Müllerian hormone (AMH) as a biomarker for assessment of male gonadal activity has become increasingly widespread. Aberrant AMH concentration successfully detects cryptorchids or pathologic testes in postpubertal stallions, but the ability to use AMH to identify stallions with testicular pathologies during their prepubertal life has not been analysed so far. The objectives of this work were to (1) assess AMH, testosterone, LH, and FSH dynamics in male horses with or without testicular pathologies during the first year of life; and (2) find a reliable diagnostic approach that would enable the identification of animals that will develop abnormal testes at an early stage. Warmblood colts ($n = 16$) born from February to May on the same stud farm were used. Blood samples for hormone determinations were collected from birth onwards every 4 weeks until the age of 1 year. At 2 years, testicular development was assessed, total testicular volume calculated. and a blood sample collected. Concentrations of AMH, testosterone, LH, and FSH were determined in all samples; AMH (AL-115, Ansh Laboratories, Webster, TX, USA) and testosterone (DE1559, Demeditec, Kiel-Wellsee, Germany) concentrations were determined by ELISA, whereas LH and FSH concentrations were determined by radioimmunoassay. Statistical analysis (SPSS Statistics 24; IBM/SPSS, Armonk, NY, USA) was performed by ANOVA using a general linear model for repeated measures. In 2 stallions, unilateral cryptorchism, and in other 4 stallions, subnormal total testicular volume (<mean minus SD) were diagnosed at 2 years. Concentrations of AMH, testosterone, and FSH changed over time ($P < 0.001$) but were similar ($P > 0.05$) within the first year of life irrespective of testicular morphology and location. Concentration of LH at birth was lower ($P = 0.05$) in stallions with abnormal testes (0.3 ± 0.2 ng mL^{-1}) than in controls (0.6 ± 0.2 ng mL^{-1}), but did not differ thereafter. At 2 years of age, AMH concentration was higher ($P < 0.01$) in stallions with abnormal testes (39.7 ± 12.7 ng mL^{-1}) than in controls (8.0 ± 0.2 ng mL^{-1}), but no group differences with regard to LH, FSH, and testosterone existed. There was a low but significant negative correlation between AMH and FSH ($P < 0.001$, $r = -0.24$), as well as between AMH and LH ($P < 0.05$, $r = -0.15$). Also, testosterone concentration was positively correlated with FSH ($P < 0.05$, $r = 0.18$) and LH ($P < 0.05$, $r = 0.16$) concentrations. In conclusion, AMH determination can be reliably used from 2 years onwards to identify stallions with abnormal testicular development, but it is inconclusive before puberty. We concluded that LH secretion in the perinatal period is involved in testicular development and descent in the horse.

141 Antioxidant Supplementation Alleviates DNA Damage in Boar Sperm Induced by Tropical Heat Stress

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Seasonal heat stress is known to significantly diminish reproductive performance in pigs, particularly in the tropics, costing the industry millions in annual losses. The boar's reduced capacity to sweat and non-pendulous scrotum, combined with the widespread use of European breeds in the tropics, makes this species particularly vulnerable to heat stress. Although heat stress is traditionally considered a sow problem, recent mouse studies demonstrate that heat stress-induced sperm DNA damage can result in arrested development and loss of early embryos. Our study investigated the

impact of tropical summer heat stress on the quality and DNA integrity of boar sperm, and trialled antioxidant supplementation to alleviate the problem. Data, expressed as mean ± SEM, were analysed by one-way repeated-measures ANOVA with pairwise Bonferroni tests. Motility of sperm obtained from Large White boars ($n = 5$) housed in the dry tropics of Townsville, North Queensland, Australia, was characterised by computer-assisted sperm analysis but did not differ between summer, winter, or spring (total motility: 71.3 ± 8.1 *v.* 90.2 ± 4.2 *v.* 70.8 ± 5.5%, respectively; $P > 0.05$; progressive motility: 35.4 ± 7.0 *v.* 46.6 ± 4.0 *v.* 41.7 ± 2.8%, respectively; $P > 0.05$). Sperm DNA integrity in 20,000 sperm/boar per season, evaluated using TUNEL and flow cytometry, revealed 16-fold more DNA-damaged sperm in summer than winter, and nearly 9-fold more than spring (16.1 ± 4.8 *v.* 1.0 ± 0.2 *v.* 1.9 ± 0.5%, respectively; $P \leq 0.05$). However, boar feed supplemented with 100 g/boar per day of proprietary custom-made antioxidants during summer significantly reduced sperm DNA damage to 9.9 ± 4.5% and 7.2 ± 1.6% ($P \leq 0.05$) after 42 and 84 days of treatment respectively. Total and progressive motility were not altered by the supplement. In summary, sperm DNA integrity is compromised in boars during summer, suggesting that boar factors may contribute to seasonal embryo loss in sows. Moreover, such damage appears undetectable using traditional measures of sperm motility. Antioxidant supplementation during summer appears to mitigate the negative impact of heat stress on sperm DNA integrity.

142 Isolation of the Quail Spermatogonia

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Spermatogonia are testicular stem cells, the precursors of male sex cells. They are target cells for introduction of recombinant DNA and suitable for creation of cryobanks to preserve biological materials. The aim of our research was to optimize the individual stages culturing quail spermatogonia. In an initial study, dynamics of change in the composition of spermatogenic cells in the seminiferous tubules were assessed histologically, at weekly intervals from 1 week to 1.5 months of age. Thereafter, spermatogonia were isolated from quail testes. Disaggregation of the testis tissue was carried out by consecutive enzymatic treatment in 0.25% trypsin and 0.1% collagenase solution. Purification of spermatogonia from other types of spermatogenic cells was conducted by separation of the cells by adhesion. The duration and conditions of cultivation of spermatogenic cells were selected experimentally. Cultivation of spermatogonia was performed on feeder layers, including quail primary Sertoli cells, STO cell line, and transplanted porcine Sertoli cells. Growth medium for culturing spermatogonia was DMEM HG medium supplemented with 5% FCS, 2 mM α-glutamine, MEM (10 μL mL^{-1}), antibiotic (100×), insulin-transferrin-selenium (ITS, 10 μL mL^{-1}), 2-mercaptoethanol (5×10^{-5} M), albumin (5 mg mL^{-1}), epidermal growth factor (EGF, 20 ng mL^{-1}), basic fibroblast growth factor (bFGF, 10 ng mL^{-1}), and leukemia inhibitory factor (LIF, 2 ng mL^{-1}). For identification of spermatogonia colonies, SSEA-1 antibodies were used. The maximum number of spermatogonia in seminiferous tubules of quail occurred at 3 weeks of age; there were mainly spermatogonia and Sertoli cells at this time. The percentage of spermatogonia from the total number of spermatogenic cells in the seminiferous tubule reached 76 ± 2%. In view of this, spermatogonia were isolated from the testes of 2-week-old quail. Spermatogenic cells were cultured for 24 h, after which the supernatant with unattached cells, mainly spermatogonia, was transferred to a new dish and cultured. Maximum homogeneity of the cell population was detected by dividing the cells by 3-fold transfer of the cell supernatant at an interval of 24 h; the proportion of spermatogonia in the suspension reached 88%. Quail Sertoli cells were the optimal feeder layer for cultivation of quail spermatogonia. Formation of spermatogonia colonies was observed on Day 5 to 7 of cultures, and their identity confirmed by immunohistochemical staining for SSEA-1.

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143 The Dynamics of Spermatogenesis in Guinea Fowls

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Male gonads are valuable genetic material for creation of biomaterial cryobanks to preserve the genes of various animals, including poultry. Spermatogonia, which are stem cells of the testes, are of greatest interest. For effective selection of spermatogenic cells, including spermatogonia, it is necessary to know the specific features of spermatogenesis of the species of interest. In this regard, the aim of this study was to investigate the dynamics of spermatogenesis in guinea fowl. Histological examinations of guinea fowl testes ($n = 90$ birds) were done for 9 age categories, from 2 wk to 6 months. For each individual, at least 30 seminiferous tubules were examined. Seminiferous tubule diameters and numbers and types of spermatogenic cells (based on morphology) were determined. Overall, the histologic structure of guinea fowl testes was similar to that of mammals. Cell populations of the seminiferous tubules included Sertoli cells and generative cells, including spermatogonia, spermatocytes, spermatids, and sperm, at various stages of differentiation. Diameter of seminiferous tubules was (mean ± SEM) 36 ± 1, 58 ± 1, 64 ± 1, 65 ± 1, 110 ± 3, 178 ± 4, 233 ± 4, 274 ± 6, and 295 ± 5 μm at 2 wk, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 months, respectively. Furthermore, at those ages, the number of spermatogenic cells per tubule was 18 ± 1, 20 ± 1, 29 ± 2, 30 ± 2, 68 ± 5, 114 ± 8, 186 ± 10, 400 ± 20, and 447 ± 24. Maximum percentage of spermatogonia was 72 ± 2% at 6 wk. Primary and secondary spermatocytes were first observed at 10 and 12 wk of age, respectively, whereas spermatids were first apparent at 4 months. Sperm were first identified at 5 months, with more present at 6 months. We concluded that the optimal age for retrieving testicular germ cells in guinea fowl was no later than 8 wk, as that represented the age when seminiferous tubules were dominated by spermatogonia.

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