Physiology and Endocrinology: Male Reproduction, Gamete Cryopreservation and Embryos

T275 Validity of sperm penetration assay in boar fertility testing. S. A. Oh*, Y. J. Park, S. J. Yoon, W. S. Kwon, Y. H. Kim, E. A. Mohamed, Y. A. You, and M. G. Pang, *Department of Animal Science & Technology and BET Research Institute, Chung-Ang University*, *Ansung, Gyeonggi-Do, Korea*.

The prediction of sperm fertility is of paramount importance for breeding animals. Multiple laboratory approaches have been developed for this purpose, but they have yielded equivocal results. The objective of this study was to develop and standardize to a method for predicting fertility in vivo in boars using the in vitro penetration assay. To increase the sensitivity and reduce false-negative results of the assay, each step in the procedure was standardized and quality control was applied. Maximum penetration of hamster zona-free oocytes and immature porcine oocytes was obtained using heparin-treated sperm cells. Hamster zona-free oocytes showed a significantly higher penetration than immature porcine oocytes. To eliminate interassay variability, 2 frozen bull semen samples were applied. All possible variables related to the female were excluded. The SPA (sperm penetration assay using zona-free oocytes) result showed significant correlation with historic average litter size but had no significant correlation with farrowing rates. To determine the normal range for the SPA, lower limits of the sperm fertility index were established as 1.2 for the small litter sizes (<8 piglets) and 2.5 for the large litter sizes (≥10 piglets). The overall accuracy was 93.33% and 93.33% respectively, for the small and large litter sizes. Our laboratory has standardized the procedure for the SPA, resulting in greatly increased sensitivities for small and large litter sizes. The protocol increases the ability to discriminate between good and poor fertility groups and it was highly effective at ranking 30 boars by litter size into large and small litter groups.

Key Words: sperm, fertility, sperm penetration assay

T276 Comprehensive proteomic analysis to defining sperm fertility in bovine. Y. J. Park*, S. A. Oh, W. S. Kwon, S. J. Yoon, Y. H. Kim, E. A. Mohamed, Y. A. You, and M. G. Pang, *Department of Animal Science & Technology and BET Research Institute, Chung-Ang University*, *Ansung, Gyeonggi-Do, Korea*.

The aim of present study was undertaken to determine whether bovine spermatozoa contained protein markers associated with bull fertility, and whether these markers were of value in predicting bull fertility. We undertook differential proteome profiling of spermatozoa from fertile bulls with extreme non-return rates (NRR): a low fertility group (45.10±4.95) and a high fertility group (82.45±6.26). Two-dimensional gel electrophoresis (2-DE) was carried out with triplicate samples of pooled spermatozoa from 3 low and 3 high fertility bulls. Protein expression levels of sperm from low and high fertility were compared using PD-Quest software. The marked difference in spot intensity was arbitrarily set as a >3-fold difference following analysis data from the software manufactures. From the different protein spots expressed in low and high fertility group, only 14 spots showed highly expression in low fertility, conversely, 4 spots were highly expression in high fertility. Six out of 18 spots detected were identified by LC/MS-MS. Metabolism related protein as ATP synthase subunit, protein tyrosin phosphorylation related protein as cytochrome b-c1 complex subunit 2 and sperm motility and cell death pathway related protein as porin were highly expressed in specimen from low fertility group. The other side metabolism related protein as alpha-2-HS glycoprotein, motility related protein as alpha-tubulin and protein oxidation related protein as phospholipid hydroperoxide glutathione peroxide were highly expressed in high fertility group. This study will enable further elucidation of the molecular mechanisms involved in this particular condition and might shed further light on key sperm proteins involved in fertilization. It is also important prerequisite to the development of diagnostic tests for bull fertility.

Key Words: sperm, fertility, proteome

T277 Effects of two egg yolk-free commercial extenders and centrifugation on freezing ability of semen in Mahabadi goat. M. Ansari*[1], A. Towhidi[1], M. Moradi Shahre Babak[1], and M. Bahreini[2], [1]*University of Tehran, Department of Animal Science*, *Karaj, Tehran, Iran*, [2]*Animal Breeding Center of Iran*, *Karaj, Tehran, Iran*.

The objective of this study was to determine the effects of 2 egg yolk-free extenders (Bioxcell and AndroMed) with or without semen centrifugation in 2 × 2 factorial design on quality of frozen-thawed semen in goat. Five mature Mahabadi bucks were selected for semen collection using an artificial vagina. Semen samples were collected, pooled and divided to 4 groups after quality evaluation. The groups consisted of Andromed (AC) or Bioxcell (BC) with centrifuged semen; and Andromed (A) and Bioxcell (B) with whole semen (without centrifugation). Percentage of motility and progressive motility of sperm were evaluated and recovery rate was calculated using post thaw motility divided by before freezing motility. Data was analyzed using proc GLM of SAS. Effect of extender, centrifugation and their interaction was significant ($P \leq 0.05$). Motility and progressive motility percentage of B (50.0% ± 3.5; 36.0% ± 3.6) was significantly higher than BC (28.0% ± 3.5; 17% ± 3.6) and AC (40.0% ± 3.5 ; 26.0% ± 3.6) ($P \leq 0.05$) and tended to be higher than A (45.0% ± 3.5; 26.0 ± 3.6) ($P = 0.07$). Recovery rate percentage in B (71.4 ± 5.0) was significantly higher than BC (40.0% ± 5.0) and AC (57.1% ± 5.0) ($P \leq 0.05$), with no significant difference between B and A (64.3% ± 5.0). It was concluded that extender Bioxcell with whole semen (without centrifugation) was more efficient for cryopreservation of goat semen.

Key Words: sperm cryopreservation, extender, centrifugation

T278 The effect of ethanol supplemented extender on freezing ability of goat semen. M. Ansari*[1], A. Towhidi[1], M. Moradi Shahre Babak[1], and M. Bahreini[2], [1]*University of Tehran, Department of Animal Science*, *Karaj, Tehran, Iran*, [2]*Animal Breeding Center of Iran*, *Karaj, Tehran, Iran*.

Inclusion of fat soluble material to the semen extender or embryo in vitro culture involves their solution into an appropriate solvent such as ethanol. Ethanol might have detrimental effects on sperm characteristics. Thus, the objective of this study was to investigate the effects of adding ethanol to the Bioxcell extender on goat semen freezing ability. Five mature Mahabadi bucks were selected and semen samples were collected using an artificial vagina during 4 weeks. Semen samples were pooled and divided into 2 groups after evaluation for qualitative characteristics. Treatment groups were including 1) basal extender as a control and 2) basal extender+ ethanol (0.05% v/v). Motility, progressive motility were evaluated by standard methods and recovery rate was calculated using post thaw motility divided by before freezing motility. Data was analyzed using Proc GLM of SAS. Motility percentage and recovery rate were

significantly higher in control than those in the treated group (44% ± 1.58, 58.01% ± 0.02 vs. 37% ± 1.58, 50.76% ± 0.02, respectively) ($P \leq 0.05$) but progressive motility percentage was not affected by treatment (33% ± 2.12 vs. 28% ± 2.12, respectively). The result indicated that ethanol had a toxic effect on sperm characteristics.

Key Words: sperm, ethanol, extender

T279 Natural non-synonymous mutations in the ovine leptin gene affect leptin binding affinity and biological activity. S. Reicher*[1,2], A. Gertler[2], E. Seroussi[1], and E. Gootwine[1], [1]*Institute of Animal Science, ARO, The Volcani Center*, *Bet Dagan, Israel*, [2]*The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem*, *Rehovot, Israel*.

The hormone leptin is involved in diverse biological processes, including regulation of feed intake, body-weight homeostasis and energy balance. Polymorphism in the bovine leptin gene has been found to be associated with variations in carcass fat content and average daily gain, as well as in milk yield, milk somatic cell count and several traits governing reproduction. In the current study, we sequenced genomic DNA and cDNA samples of individuals from 5 divergent sheep breeds and revealed synonymous as well as novel non-synonymous mutations at the third exon of the ovine leptin gene *(oLEP)*. In addition, 2 alternatively spliced *(oLEP)* transcripts were found in the abdominal fat tissue. The biochemical and biological significance of these naturally occurring mutations was examined by generating recombinant oLEP muteins which carried the corresponding mutations. Surface plasmon resonance experiments revealed reduced affinity of all oLEP muteins examined, namely: Q28 deletion, N78S, R84Q, P99Q, V123L and R138Q, to chicken leptin-binding domain (chLBD) relative to the wild-type hormone (0.54, 0.38, 0.36, 0.55, 0.69 and 0.60, respectively). In competitive binding assays between biotinylated oLEP and the leptin muteins, N78S and R84Q exhibited the lowest affinity to both chLBD (0.19 and 0.08, respectively) and human LBD (0.18 and 0.41, respectively) as compared with the wild-type hormone. We then tested the muteins' ability to induce proliferation in Baf-3 cells stably transfected with the long form of the human leptin receptor: significant differences in proliferative activity were only found for N78S (1.8-fold higher) and R138Q (4.2-fold lower) relative to wild-type oLEP. It is speculated that under artificial selection in farm animals, reduced affinity of leptin for its receptor may confer a selective advantage.

Key Words: leptin, polymorphism, ovine

T280 Effect of different aspiration pressure on the number and quality of ovine oocyte. A. Abedini*, H. Kohram, and R. Salehi, *Tehran University*.

This study was designed to characterize the effects of different aspiration pressure (20, 70, 110 mmHg) during oocyte collection on the number and quality of ovine oocytes. Ovine ovaries were collected exactly after slaughter and placed them in a 0.9% NaCl aqueous solution containing penicillin-streptomycin. Cumulus-oocyte complex (COCs) were aspirated almost from 300 ovaries by aspiration pump with a 20-g needle. After aspiration per ovary, with same needle was sucked TCM 199 that supplemented with penicillin-streptomycin. The oocytes were observed under microscope. COCs were categorized to 3 groups: A: denature oocytes, B: oocyte with damaged cumulus, C: qualify oocytes (had more than 3 layers with health cumulus layer). The data of oocytes were investigated separately in each group of oocytes with different aspiration pressure. The total numbers of oocytes and grade A, B, C oocytes per group (20, 70, 110 mmHg) were analyzed by ANOVA. Statistical differences showed that in group with 20 mmHg pressure, the number of oocytes was low but the number of grade C oocytes was more than other groups ($P < 0.05$). In 110 pressure treatment, the total number of oocytes was high but the quality was low. The percentage of grade A and B oocytes in 110 treatment was higher than other groups ($P < 0.05$). This results showed that the high pressure treatment destroyed the cumulus layer and decreased the qualify oocytes. In group with 70 pressure treatment the number and quality of oocytes were in average. The difference between the numbers of oocyte in 110 and 70 mmHg pressure was not significant however, the qualify oocyte was higher in 70 pressure treatment ($P < 0.05$). The results demonstrated that vacuum pressure during ovine oocyte aspiration affects the number and quality of oocytes. High pressure increase the number of oocytes harvested however reduced the qualify oocyte, while low pressure decrease the number of oocyte and increase the quality. To sum up, it seems that the average pressure (70 mmHg) could harvest the qualify oocytes with reasonable numbers.

Key Words: aspiration pressure, number and quality, ovine oocyte

T281 The effect of poly-L-lysine as a new cryoprotectant for ovine oocyte vitrification. N. Li*[1], T. Wuliji[1], A. Qi[1], S. H. Hyon[2], K. Matsumura[2], L. Shi[1], and W. Chen[1], [1]*University of Nevada, Reno*, [2]Kyoto University, Kyoto, Japan.

The objective of this study is to evaluate a polyampholyte, poly-L-lysine (PLL), as a new cryoprotectant for ovine oocytes vitrification. The PLL was experimented as an alternative cryoprotective reagents to the traditional dimethyl sulfoxide (DMSO) protocol. Recently, Matsumura et al. (2009) had demonstrated that PLL effectively protecting L929 stem cells during cryopreservation. Oocytes were screened and collected from fresh ovine tissue, then randomly distributed into 2 developmental stages, namely, germinal vesicle (GV) stage oocytes (immature) and metaphase 2 (M2) stage oocytes (mature). GV stage oocytes vitrification was carried out immediately upon collection, while M2 stage oocytes vitrification was made after 24h in vitro culture for oocyte maturation. Each developmental group was subjected to comparison of 2 different vitrification solutions: (1) PLL based formula (5% PLL for 45s, 10% for 25s) (2) DMSO based formula (10% DMSO, ethylene glycol, and holding medium for 45s, 20% for 25s), Therefore, following 4 groups comparison were conducted: Group I GV stage, vitrification solution (VS) (1); Group II M2 stage, VS (1); Group III GV stage VS (2) and Group IV M2 stage, VS (2). There are 5 replicated batches for each group, and each batch was processed on the same day under the same condition. The normality of developing oocytes was examined under microscope. Normal oocytes showed the characteristics of homogeneous cytoplasm and intact zona pellucida, while abnormal oocytes showed low density of granular cells. Under confocal microscope, the spindle and microtubules of normal oocytes are intact and dispersed within plasma uniformly; the abnormal oocytes displayed a reduced amount of microtubules. The group mean were compared by *t*-test procedure. The preliminary results showed that normal oocyte in group II(82.1) are significantly ($P < 0.05$) higher than that of group IV (58.0), the normal oocytes in group IV are significantly ($P < 0.05$) higher than group III (48.1). However, group I (53.6) and II did not differ. Although encouraging, the advantage of using PLL as an alternative cryoprotectant requires future investigation.

Key Words: poly-L-lysine, ovine oocyte, vitrification

T282 Administration of human chorionic gonadotropin (hCG) to embryo transfer (ET) recipients increased ovulation, progesterone,

and transfer pregnancy rates. L. D. Wallace*[1], C. A. Breiner[2], R. M. Breiner[1], and J. S. Stevenson[1], [1]*Kansas State University*, *Manhattan*, [2]*Cross Country Genetics North Inc.*, *Westmoreland, KS*.

We hypothesized that administration of hCG at ET would induce accessory corpora lutea (CL), increase circulating progesterone concentrations, and reduce early embryonic loss. At 2 locations, mature lactating (94%) and nonlactating (6%) purebred and crossbred Angus, Simmental, and Hereford recipients (n = 471) were assigned alternately to receive i.m. 1,000 IU hCG or 1 mL saline (control) at ET. Fresh or frozen-thawed embryos were transferred on d 5.5 to 8.5 (median = d 7) of the estrous cycle to recipients having a palpable CL (CL grade = 1 or 2). Recipients received a BCS of 1 to 9 at ET. Pregnancy diagnoses occurred by transrectal ultrasonography 28 to 39 d (median = d 36) and reconfirmed 58 to 77 d (median = d 74) post-estrus. At one location (n = 108), ovaries were examined to count the number of CL at pregnancy diagnoses. More ($P < 0.001$) pregnant hCG-treated cows (69.0%) had multiple CL than pregnant controls (0%). Serum progesterone (ng/mL) determined at both pregnancy diagnoses in pregnant cows was greater ($P \leq 0.05$) after hCG treatment than in controls (first: 8.1 ± 0.9 vs. 6.1 ± 0.8; second: 8.8 ± 0.9 vs. 6.6 ± 0.7), respectively. Transfer pregnancy rates (PR) were analyzed using logistic regression. Unadjusted PR at the first diagnosis was 62.0 vs. 55.6% for hCG vs. controls. At the second diagnosis, PR was 59.0 vs. 53.0%, respectively. Factors affecting PR were CL grade ($P = 0.12$), number of previous transfers ($P = 0.03$), and BCS ($P = 0.02$). Odds ratios indicated that greater PR occurred in recipients having a CL grade 1 vs. 2 (63.8 vs. 58.2%), fewer previous transfers 1 vs. > 1 (61.3 vs. 43.1%), and when BCS > 5 vs. ≤ 5 (67.1 vs. 54.0%). An interaction ($P = 0.11$) was detected between treatment and BCS in which hCG tended to improve PR in recipients having BCS ≤ 5 than in controls (60 vs. 48%), whereas no treatment effect occurred in recipients having BCS > 5 (65.5 vs. 68.5%), respectively. We concluded that hCG at ET increased incidence of accessory CL, increased progesterone in pregnant recipients, and tended to increase PR in thinner recipients (BCS ≤ 5).

Key Words: embryo transfer, hCG, pregnancy rates

T283 Effect of addition of cAMP regulators to bovine in vitro oocyte maturation medium. C. Burroughs* and G. Seidel, *Colorado State University*.

In vivo, the LH surge before ovulation stimulates resumption of oocyte meiosis, but in vitro, resumption occurs due to removal of the oocyte from inhibition in follicular fluid. The objective of this experiment was to create an in vitro system with greater resemblance to the in vivo situation to produce greater numbers of bovine blastocysts. The adenylate cyclase activator forskolin (100 μ*M*) and the phosphodiesterase inhibitors caffeine (2 μ*M*) and cilostamide (10 μ*M*), were added to maturation media to increase and maintain cAMP levels. Bovine oocytes were aspirated from abattoir-collected ovaries and immediately placed into medium containing forskolin and caffeine. Oocytes were incubated with different combinations of cAMP regulators during maturation as shown in Table 1 for all 6 replicates. All oocytes were moved to new medium at each time point. Half of the oocytes in each treatment were fertilized at 23 h of incubation and half at 28 h. Cleavage rates were recorded at 2.5 d post-fertilization and blastocyst rates at 7 d post-fertilization. Cleavage rates in treatment D were lower than the control (A) and those in C ($P < 0.05$, Table 1), indicating that prolonged exposure to cilostamide was detrimental to fertilization. There was no effect of time on cleavage rate, and no treatments had higher blastocyst rates per oocyte than the control ($P > 0.1$). Under the conditions used, there were no added benefits to blastocyst production from the treatments studied.

Table 1. Effects of cAMP regulators during in vitro maturation of oocytes

Trt	n	0-2h cult	2-6h cult	6-23/28h cult	Time of fert	Cleav %	Blast %
A	168	No add	No add	No add	23h	83.8±5.8	22.0±4.3
B	187	F+C*	No add	No add	23h	73.3±6.5	19.4±5.0
B	188	F+C	No add	No add	28h	75.6±10.2	11.3±3.5
C	181	F+C	Cilostamide	No add	23h	78.7±4.7	19.0±2.1
C	176	F+C	Cilostamide	No add	28h	87.2±2.4	18.0±3.1
D	187	F+C	Cilostamide	Cilostamide	23h	63.9±9.4	13.9±2.4
D	188	F+C	Cilostamide	Cilostamide	28h	62.7±6.1	11.9±2.0

*F+C = Forskolin+Caffeine.

Key Words: oocyte maturation, cAMP, bovine

T284 Testicular abnormalities in *Gallus gallus* var. *domesticus* males. J. R. Moyle*, S. M. Whipple, F. D. Clark, and R. K. Bramwell, *University of Arkansas*, *Fayetteville*.

In conducting research on testicular development on *Gallus gallus* var. *domesticus* males it was noticed that several the males had nodules on their testes. Further histological investigation showed that these nodules were composed of seminiferous tubules that were contained within the tunica albuginea of the testicular capsule. The seminiferous tubules within these nodules contained sperm as well as spermatids at various stages of development; however, no direct outlet of the sperm into the testes was identified or observed. These testicular nodules were found in all primary breeder males that were investigated (n = 8 flocks), with occurrences for individual males ranging from 42% to 93%. The flocks that were investigated consisted of commercial breeder flocks, individually caged males, as well as males used in pen trail. Nodules were also found in a pure line of French Mottled Houdan chickens, as well as a random bred broiler breeder line from the 1980s. These nodules occurred only on the left testes in 92% of males, with 1% only on the right, and 7% had nodules on both testes. The number of visible nodules on the testes ranged from 1 to as many as 18, with the size ranging up to 4.5mm. Using histological preparations these testicular abnormalities were detected in males that were 18 weeks of age, and had not been light stimulated. No conclusion about the affect of testicular nodules on fertility was apparent, as all of the flocks investigated had normal or higher fertility. Therefore, at this time the effects of testicular nodules, if any, are unknown.

Key Words: testes, males, broiler breeders

T285 Effects of hypothermic storage of striped bass (*Morone saxatilis*) sperm on intracellular calcium, reactive oxygen species formation, mitochondrial function, motility, and viability. H. D. Guthrie*[1], L. C. Woods III[2], and G. R. Welch[1], [1]*Animal Biosciences and Biotechnology Laboratory, Agricultural Research Service, USDA*, *Beltsville, MD,* [2]*Department of Animal and Avian Sciences, University of Maryland, College Park*.

Experiments were conducted to determine the effect of hypothermic 24 h storage of striped bass sperm cells on viability, intracellular Ca^{2+}[Ca^{2+}]$_i$, mitochondrial membrane potential ($\Delta\psi_m$), and reactive oxygen species (ROS) formation (oxidation of hydroethidine to ethidium) as determined by flow cytometry; motion activation; and ATP concentration as determined by Luciferin-Luciferase bioluminescence assay. Semen was stored for 1 or 24 h at 4°C in an O_2 atmosphere undiluted (raw); or diluted 1:4 (one volume semen with 3 volumes) with T350 (20 mM

TRIS base-NaCl, 350 mOsm/mL, pH = 8) or with seminal plasma (SP) in the presence of various treatments. Viability (% cells excluding propidium iodide) approached 100% after 1 h storage raw or in T350 and SP. After 1 h of storage Fluo-3 fluorescence (marker for $[Ca^{2+}]_i$) was detected in only 3% of sperm cells in raw and T350 or SP extended semen. In contrast to storage for 1 h, after 24 h the incidence Fluo-3 fluorescence intensity was increased in >50% of the viable cells in raw and in T350 or SP extended semen along with increased cell death; the presence of 1 mM EGTA prevented increased Fluo-3 fluorescence and attenuated cell death. Activation of sperm motility was 82% after 1 h in T350 and decreased to 30% after 24 h. However, activation failed in the presence of EGTA at 1 or 24 h. During storage $\Delta\psi_m$ and ATP did not change significantly between 1 and 24 h; however in the presence of EGTA ATP, but not $\Delta\psi_m$ decreased between 1 and 24 h. While ROS formation was induced by menadione treatment, there was no evidence of storage-induced ROS formation in the absence of menadione. The increased intracellular calcium found after 24 h indicates a storage-induced defect in the maintenance of cellular calcium homeostasis which may be detrimental to sperm activation.

Key Words: flow cytometry, Fluo-3, sperm viability

T286 Renin message is up-regulated in spermatogonia and testes of male mice in response to treatment with aflatoxin B1. K. J. Austin*, K. L. Speiser, A. M. Kaiser, R. R. Cockrum, and K. M. Cammack, *University of Wyoming*, *Laramie*.

Aflatoxin B1 (AFB1) has been shown to affect fertility in male mice and numerous livestock species. However, the exact genetic mechanisms associated with the disruption are not known. The objective of these experiments was to examine the genetic response to AFB1 and determine which male reproductive cell types are affected by treatment with AFB1. In previous experiments, male mice ≥4 wks of age were administered 50 µg/ kg BW AFB1 by IP injection daily for 45 d. Testes were collected and stored at −80°C until analysis. Mice were further characterized as being tolerant or intolerant to the effects of AFB1 based on TUNEL staining and the number of pups sired. An Affymetrix array was used to initially test for gene expression differences between tolerant and intolerant males; follow-up gene expression was assessed using real-time RT-PCR. Differences in gene expression were tested for effect of treatment (tolerant versus intolerant; control versus AFB1 treatment) using the GLM procedures of SAS. Messenger RNA for *Renin* was found to be upregulated ($P = 0.05$) in tolerant mice (n = 3) compared with intolerant mice (n = 3) by both microarray and real-time RT-PCR analyses. Further experiments using real time RT-PCR to analyze testicular RNA showed that *Renin* expression increased ($P = 0.02$) 10-fold in AFB1-treated mice (n = 8) compared with control (placebo) treated mice (n = 8). Spermatogonia treated with 0, 5, 10 or 20 µg/ mL AFB1 (n = 6 per treatment) resulted in a 10-fold upregulation of *Renin* mRNA at the 20 µg/ mL level, while Leydig tumor cells treated similarly showed no difference ($P > 0.05$) in mRNA for *Renin* in treated versus control cell cultures. These results demonstrate a genetic response to AFB1 in the testes and spermatogonia through upregulation of *Renin* and may lead to a better understanding of the mechanisms by which AFB1 disrupts fertility in male mice as well as livestock species.

Key Words: aflatoxin, *Renin*, spermatogonia

T287 Testicular development of breeder males reared on an accelerated growth schedule. W. D. Berry*, S. H. Oates, L. M. Stevenson, and J. B. Hess, *Auburn University Department of Poultry Science*, *Auburn, AL*.

Broiler breeders are reared using feed restriction to control body weight and delay sexual maturation. Earlier maturation of breeder males has not been explored with respect to effects on reproductive development. The objective of this study was to determine how rearing on a relatively accelerated growth schedule affects broiler breeder testicular development. In this study, male breeder chicks reared using a conventional feeding/growth schedule (CON) were compared with males reared on a growth schedule accelerated by 6 weeks (ACCEL). The ACCEL males were grown on a linear growth line designed to reach the CON 22-week body weight at 16 weeks. Male broiler breeder chicks in both treatments were started on a standard starter diet and full fed for 3 weeks. ACCEL male chicks were started 5 weeks after CON. The birds were placed in 3 replicates containing 14 chicks per rep at 4 weeks of age. Both treatments were then fed 15% protein grower diet for the remainder of the rearing period. The birds received 8 h light/day during rearing. The birds were transferred to breeder housing at 22 weeks of age (CON) or 17 weeks of age (ACCEL). Light was increased to 12 h/day to stimulate sexual maturation. Birds in both treatments were then fed to maintain the same body weight until termination of the experiment. Body and testes weights were recorded throughout the experiment. Testes samples were formalin fixed, sectioned, and stained for morphology. Testes areas and cell numbers were obtained from photomicrographs using Image J software. Body weights did not differ at photostimulation. Body weight uniformity was the same for the 2 treatments at photostimulation. However, ACCEL birds tended to be less uniform. Testes weight at initial sexual maturity was higher for ACCEL vs. CON (7.87 ± 0.67 vs. 7.16 ± 0.62). Sertoli cell and interstitial cell numbers as a percent of total cell numbers were not different between the treatments. It was concluded that accelerating sexual maturity by 6 weeks does not adversely affect testicular development of breeder males.

Key Words: breeder, testes, seminiferous

T288 Hypoxic conditions during the CAM development (E5-E12) effect on embryo development. S. Druyan*, *Institute of Animal Science, ARO The Volcani Center*, *PO Box 6, Bet Dagan, Israel*.

Hypoxia is a common situation that vertebrate face during fetal life. It plays an essential role in embryo development, inducing vasculogenesis, angiogenesis, hematopoiesis and chondrogenesis. Hypoxic conditions at different time points during embryonic development were found to affect both anatomical and physiological morphogenesis. The literature is unclear about the actual effect of hypoxic conditions on embryo development. Different levels of hypoxia were found to have conflicting effects on development, depending on time point and duration of exposure. It is still unknown whether chronic, alternating or acute hypoxia will induce some degrees of adaptation to hypoxia as a long lasting effect. Fine-tuning (critical period, level and duration) is required. This study was aimed to elucidate the effect of daily exposure to 17% O_2 for 6 or 12 hours during the chorioallantoic membrane (CAM) development on embryogenesis, angiogenesis and hematopoiesis. Data including hatch time, heart rate, oxygen consumption, embryo weight, hematocrit level and hemoglobin concentration were collected from E13 to hatch. Angiogenesis response was measured in the CAM from E6 to E13 by analyzing the blood vessels coverage area. The hypoxic exposure had a mild insignificant effect on embryo weight and relative yolk weight. However, hypoxic exposure was found to affect vascular area that was significantly higher in the 12h embryos compare to control (e.g. 24.7 mm^2 vs. 23.3 mm^2, measured on E12, $P \leq 0.01$). Oxygen consumption was similar for all 3 treatments although it was slightly lower in the 12h embryos. Heart rate was found to be relatively constant while hematocrit and hemoglobin concentration were affected by hypoxia on

several key developmental days (e.g. 41.6%, 41.9% vs. 38.6% for 6h, 12h and control embryos measured on E16, $P \leq 0.01$). These observations indicate that hypoxic exposure during the CAM development may improved its vascular system. Further exploration of this phenomenon may lead to an alternative management scheme to improve the quality of the neonatal chick, which is associated with superior performance during post hatch.

Key Words: hypoxia, embryogenesis, CAM