(age: 8th–19th day) out of which 22 proved to be chimaeric and 3 were adults. Two of these animals were albino but had the triploid component in several internal tissues; both were fertile. The third animal, a male, was an overt chimaera. It turned out to be infertile (no sperm in the ejaculate; testes small and deprived of germ cells). The infertility of this individual is puzzling because the FISH studies with the help of X and Y chromosome painting probes proved that the diploid component was XY and the triploid component was XXX. The results of our study indicate that the rate of postimplantation development of 2n-3n chimaeric embryos is normal or only slightly retarded. Developmental stage of chimaeric embryos was assessed by comparison of their external morphology with normal diploid embryos of equivalent post-coital age according to the descriptions given by Theiler K (1972 *The House Mouse*, Springer-Verlag, Berlin). With the exception of one embryo lacking both eyes (but otherwise looking quite normal) no other morphological abnormalities were observed. Comparison of the contribution of both components to the fetal and extra-embryonic tissues at the consecutive foetal stages has shown that participation of triploid cells slightly but steadily decreased in all tissues examined. However, the presence of triploid cells in mouse chimaeras was compatible with their normal postnatal development to adulthood.

121 HEAT SHOCK TO PIG OOCYTES DOES NOT INDUCE APOPTOSIS BUT REDUCES EMBRYO DEVELOPMENT

J.-K. Tseng, *P.-C. Tang*, *and J.-C. Ju*

Department of Animal Science, National Chung-Hsing University, Taichung, Taiwan, ROC. Email: kaikai0420@yahoo.com.tw

Oocytes are susceptible to heat shock (HS) during the maturation process. It has been demonstrated that HS induces apoptosis and/or the expression of HS protein 70 (hsp 70) in *in vitro*-produced oocytes and embryos. The objectives of this study were to analyze the effects of HS on the development and apoptosis of pig oocytes and embryos. Porcine ovaries were collected from a local slaughterhouse and the cumulus-oocyte complexes (COCs) were aspirated from follicles 3–6 mm in diameter and subjected to standard *in vitro* maturation procedures at 39°C for 42 h. The *in vitro* matured oocytes were then randomly allocated to different HS treatments at 41.5°C for 0 (control, C0h), 1 (HS1h), 2 (HS2h), or 4 h (HS4h). An additional control group of oocytes was cultured for 4 h without HS (C4h). Data were analyzed by chi-square test. In Experiment 1, anti-hsp 70 (SPA-810AP, Stressgen, San Diego, CA, USA) and Western blotting were used to examine the expression of hsp 70. Results indicated that no significant difference of hsp 70 expression in metaphase II porcine oocytes occurred between controls and HS groups ($P > 0.05$, 7 replicates). In Experiment 2, apoptosis of metaphase II oocytes after HS was identified by annexin V-FITC (Sigma, St. Louis, MO, USA) staining and TUNEL (Roche, Indianapolis, IN, USA). No significant apoptotic signal was detected in the HS groups compared to the controls. The intensity of annexin V staining was not affected by HS, but it increased with the time of culture ($P < 0.05$, $n = 24$–37). In Experiment 3, the apoptotic rate and developmental competence of the HS-oocytes were evaluated by TUNEL assay ($n = 123$–137, 4 replicates). Parthenogenetic activation ($n = 123$–137) was performed by an electric pulse (2.2 kV cm^{-1}) combined with 6-dimethyaminopurine treatment (6-DMAP, 2.5 μM, 4 h, Sigma). The cleavage rates in HS2h ($43 \pm 29\%$) and HS4h ($35 \pm 28\%$) decreased ($P < 0.05$) compared to those in C0h ($62 \pm 12\%$) and C4h ($66 \pm 8\%$). In addition, the blastocyst formation rates and total cell numbers reduced ($P < 0.05$) after 2 h ($11 \pm 10\%$, 20 ± 16) and 4 h ($11 \pm 8\%$, 19 ± 8) of HS treatments compared to those in C0h ($23 \pm 14\%$, 32 ± 22) and C4h ($21 \pm 11\%$, 27 ± 17), all respectively. The numbers of blastocysts with TUNEL-positive signals were not significantly different between the HS and control groups, but the signals increased ($P < 0.05$) before the 8-cell stage in HS groups (22–24%) compared to the C0h and C4h controls (16 and 11%), respectively. These results indicate that reduction in developmental competence of *in vitro*-matured pig oocytes after heat shock is not closely correlated to the expression of hsp 70 in the oocytes and to the apoptotic cell numbers in the blastocyst. Whether detection of apoptosis by TUNEL or annexin V-FITC in oocytes is a good indicator requires further investigation.

Early Pregnancy/Pregnancy Recognition

122 USE OF A DAY-14 EMBRYONIC ARRAY TO STUDY THE ELONGATION PHASE OF THE BOVINE EMBRYO

S. Degrelle, *I. Hue*, *and J.P. Renard*

INRA, UMR Biologie du Développement et Reproduction, Jouy En Josas, France. Email: degrelle@jouy.inra.fr

In cattle, more than 30% of embryonic losses observed after artificial insemination (AI) have an early origin, coincident with a marked elongation of the trophoblast which occurs before implantation, between the 13th and 19th days of pregnancy. During this exponential growth phase, physiological interactions essential for pregnancy are established between the embryo and the uterus. Our work focuses on the identification of transcripts that regulate this key developmental period in several domestic species. For that, we generated a nylon membrane that contained 1920 gridded inserts originating from a Day-14 bovine embryo cDNA library (dbEST ID.15979; Hue *et al*., in preparation). Gene expression profiles in trophoblasts of increasing sizes were compared using ovoid (10–18-mm), tubular (50–60-mm), and early filamentous (140–150-mm) stages as complex probes.

Trophoblasts were collected and immediately snap-frozen. RNA extractions were performed using RNAplus (Quantum Appligene, Illkirch 67402, France). Due to the scarce amount of mRNA per embryo, amplified material was used to hybridize the array. For that, antisense-RNA (aRNA) and cDNA were generated starting from 1 μg of total RNA, as described by the MessageAmp aRNA kit instructions (Ambion, Rusin, TX 78744, USA) and according to Revel *et al*. (1995 Zygote 3, 241–250). Five hundred nanograms of aRNA or cDNA were random-primed and labelled with ^{33}P-alpha-dATP [aRNA, according to the procedure of Decraene *et al*. 1999 BioTechniques 27, 962–966; cDNA using the Atlas SMART Probe Amplification kit, (Clontech, Osyme, Saint Quentin Yvelines 78053, France)]. For each protocol, two probes were generated independently and each of these probes was hybridized to four identical membranes according to Clontech instructions. These were then exposed to phosphoscreens and scanned after 7 days. Quantifications were done using ImaGene 5.1 (BioDiscovery, El Segundo, CA 90245, USA) and statistically analyzed

with the AnovArray package freely available for non-commercial use at http://www.jouy.inra.fr/stat/AnovArray (Piot *et al*. 2004 Bioinformatics, submitted). Reproducibility of the two protocols used to amplify material (aRNA and cDNA) was confirmed by slot blot quantifications before labelling. The hybridization profiles generated for each protocol (8 membranes per stage) were also highly reproducible ($0.95 < r < 0.97$), allowing a global statistical analysis with the AnovArray package. The results of the analysis of variance (ANOVA), including the correction for False Discovery Rate (FDR < 0.05), led to the identification of several bovine ESTs with unknown function that are differentially expressed during the rapid phase of trophoblastic elongation. Since genes, already known to be involved during elongation (IFN tau, Kunitz inhibitor), were also found differentially expressed in this study, this genomic approach using amplified complex probes is reliable to search for new markers of early developmental stages in cattle. Additionally, a thorough analysis of those markers may define them as interesting tools to assess the quality of embryonic development after AI, IVF (*in vitro* fertilisation), or SNT (somatic nuclear transfer).

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123 PROGESTERONE LEVELS DURING 20 DAYS OF PREGNANCY IN RABBIT TREATED FOR ENDOMETRIOSIS OR WITH ANTI-CD44

M.J. Illera, *P. Bermejo*, *A. Natarajan*, *C. Willingham*, *and J. Hernandez*

Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain. Email: mjillera@vet.ucm.es

Embryo implantation begins when the blastocyst both assumes a fixed position in the uterus and establishes a more intimate relationship with the endometrium. Successful implantation depends upon hormonal synchronization and development and the receptivity of the endometrium. CD44 is a cell surface molecule that has been implicated in the initial attachment of the embryo. The aim of this work was to study the hormonal levels of P4 in three groups of animals which have a normal pregnancy or an induced reduction in the number of implants. Twelve adult New Zealand does ($n = 12$) were naturally inseminated with a buck of proven fertility. Blood samples were obtained daily during 20 days of pregnancy. Hormonal determinations were performed by enzyme immunoassay. Animals were divided into three groups: group A ($n = 4$): control animals; group B ($n = 4$): endometriosis was surgically induced in the right horn a month before the animal was mated; and group C ($n = 4$): animals received an injection of 20 micrograms of anti-CD44 in the right horn via mid-ventral laparotomy on Day 6.5 post-coitum (0.5 mL each, from the ovarian end to the cervix). Each animal served as her own control with the left uterine horn receiving 0.5 mL of saline. Statistical analysis was performed using ANOVA.The number of corpora lutea was similar in all treatments. No statistical differences were found comparing CLs in the right/left ovary. In group A, a mean of 3.4 ± 0.47 (mean $\pm$ SE) implants was found in the right horn while the mean in the left side was 4.6 ± 0.81. In group B, a marked reduction in implantation sites was found, with 1.8 ± 0.60 and 4.66 ± 0.84 on the right and left horns, respectively. With anti-CD44 injected into the uterine horn (Group C), a mean of 0.12 implant was present in the right uterine horn compared with 3.6 implants on the left side ($P < 0.001$). Progesterone levels from Days 1 to 10 are shown in the following table (mean $\pm$ SE). Comparisons in day values are not statistically significant $P > 0.05$. After Day 10 the levels of progesterone were similar in all groups. The results showed an increase of progesterone levels in group B; this could be due to endometriosis and not to the number of implants. The results in the CD44 group reveal that progesterone profiles were similar to those in the control group, and we can conclude that the reduced number of implants found in group C did not affect the progesterone levels.

Table 1. Progesterone levels (ng/mL) from Days 1 to 10 (mean ± SE)

	Group A	Group B	Group C
Day 0	1.53 ± 0.61	2.12 ± 0.66	2.28 ± 0.43
Day 1	0.82 ± 0.30	0.75 ± 0.11	1.16 ± 0.04
Day 2	1.64 ± 0.21	1.66 ± 0.17	1.49 ± 0.11
Day 3	2.20 ± 0.23	3.66 ± 0.60	2.86 ± 0.32
Day 4	3.63 ± 0.23	5.40 ± 0.26	4.14 ± 0.43
Day 5	3.99 ± 0.30	5.95 ± 0.32	4.65 ± 0.54
Day 6	4.55 ± 0.93	7.29 ± 1.33	4.78 ± 0.66
Day 7	6.25 ± 1.42	8.15 ± 0.91	5.30 ± 0.68
Day 8	8.46 ± 1.59	9.16 ± 0.54	8.83 ± 0.52
Day 9	11.66 ± 1.58	10.71 ± 0.28	9.03 ± 0.42
Day 10	13.15 ± 1.94	11.17 ± 1.56	14.93 ± 1.29

124 THE MAJOR GENE EXPRESSION PATTERNS IN ENDOMETRIAL TISSUE OF PIGS DURING EARLY GESTATION

B.-K. Kim[A], *H.J. Chung*[A], *Y.G. Ko*[A], *Y.M. Kim*[A], *H.-H. Seong*[A], *J.K. Jung*[A], *N.-H. Kim*[B], *and W.K. Chang*[A]

[A]Animal Biotechnology Division, National Livestock Research Institute, RDA, Suwon, 441-706, South Korea; [B]Department of Animal Science, Chungbuk National University, Cheongju, Chungbuk, 361-763, South Korea. Email: bkkim@rda.go.kr

Although the expression of important genes in the embryo at pre-implantation stage, which encompasses the period from fertilization to implantation, have been reported for mice and cows, little information relevant to this subject is known in pigs. The objective of this study was to investigate the

changes of importantly expressed genes and proteins in endometrial tissue of pigs from fertilization to implantation. Six genes, including estrogen receptor-α, estrogen receptor-β, LIF, LR (LIF receptor), TGFβ1, and TGFβ2, that may play important roles in regulating uterine receptivity and successful implantation and that show different expression patterns by the stages of pregnancy were selected. As a step toward understanding the role of gene and protein expression changes in endometrial tissue of pigs during the preimplantation stage (Day 2, Day 6, Day 8, Day 12, and Day 17, $n = 3$/group) and the post-implantation stage (Day 21 and Day 33, $n = 3$/group) and Day 0 (estrous), real-time PCR methods for quantitative analysis of genes and immunohistochemistry methods to localize protein expression were utilized. Data from quantitative real-time PCR were analyzed by ANOVA. The results of this experiment indicated that estrogen receptor-β mRNA level was sharply increased to Day 12 of pregnancy, while estrogen receptor-α mRNA did not change drastically during early pregnancy stage. In contrast, levels of LIF and LR mRNA were increased from Day 2 to Day 33. Although TGFβ1 mRNA reached peak on Day 17 and TGFβ2 mRNA showed the highest level on Day 17, TGFβ2 did not appear to change drastically. For the protein expression patterns, estrogen receptor-α and estrogen receptor-β proteins were expressed in both luminal epithelium and glandular epithelium, but they were only partially expressed in some tissues of stroma cells. LIF protein was expressed in all cell types, while TGF β1 protein was high expressed in glandular epithelium. Also, ERs, LIF, and TGF β1 mRNA and protein expression showed stage- and cell-specific expression patterns. We also investigated the gene expression of TGF β1 mRNA and TGF β2 mRNA in early conceptus (Day 12 and Day 17). TGF β1 mRNA expression was low in Day 12 embryos, and increased progressively to Day 17. This indicated that both the maternal uterus and the conceptus represent the same gene expression pattern. These results suggest that estrogen receptor-β could be an important factor in estrogen action in endometrial tissue during early gestation in pigs, and TGF βs function in both autocrine and paracrine interactions. Progressive increase in TGF β1 mRNA expression in conceptus and uterine tissues suggest important roles of TGF β1 in conceptus development and establishment of the uterine receptivity during the peri-implantation period.

125 CHANGES OF PLASMA MACROPHAGE COLONY-STIMULATING FACTOR LEVELS AND ITS GENE EXPRESSION IN PERIPHERAL WHITE BLOOD CELLS DURING PREGNANCY IN JAPANESE BLACK CATTLE

K. Oshima[A], *K. Yoshihara*[B], *H. Watanabe*[C], *T. Kojima*[A], *M. Komatsu*[D], *and N. Yamamoto*[A]

[A]National Agricultural Research Center for Western Region, Fukuyama, Japan; [B]National Institute of Animal Health, Tsukuba, Ibaraki, Japan; [C]Bio-oriented Technology Research Advancement Institution, Saitama, 331-8537, Japan; [D]National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan. Email: tenpoint@naro.affrc.go.jp

Macrophage colony-stimulating factor (M-CSF) is a hemopoietic cytokine that plays a primary role in placental physiology. Gene expression of M-CSF in bovine intercaruncular endometrium shows an upward trend in mid-pregnancy. The objective of this study was to determine the plasma M-CSF levels and the M-CSF gene expression levels in maternal peripheral white blood cells (PWBCs) during pregnancy using ELISA and quantitative RT-PCR. In Experiment 1, the plasma M-CSF levels in 112 Japanese Black heifers or cows were determined. Animals were divided into four groups according to pregnancy stage: first- ($n = 29$), second- ($n = 33$), third- ($n = 26$) trimester, and non-pregnant ($n = 24$). ELISA for bovine M-CSF established by Yoshihara *et al*. (2003 Vet. Immunol. Immunopathol. 95, 103–111) was used according to their instructions. The absorbance was measured at 405 nm in the Biomek Plate Reader (Beckman Coulter, Fullerton, CA, USA). In Experiment 2, the plasma M-CSF levels and M-CSF gene expression levels in PWBCs during pregnancy were determined. The plasma samples for ELISA were obtained from 8 heifers and 3 cows every 1 and/or 2 weeks. The PWBCs samples for quantitative RT-PCR were obtained from 4 heifers every 1 and/or 4 weeks. All quantitative RT-PCR protocols were carried out according to the previous report (Oshima *et al*. 2003 Theriogenology 60, 1217–1226). The quantitative PCR assay used an ABI Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA, USA). Signals were detected according to the manufacturer's instructions. The relative level of M-CSF expression was calculated on the basis of glyceraldehyde-phosphate-dehydrogenase (GAPDH) quantity (in the method of calculation, the relative level = M-CSF quantity/GAPDH quantity). Data were analyzed by Kruskal-Wallis test. In Experiment 1, the plasma M-CSF level in second-trimester cows was significantly higher than those in other stages ($P < 0.05$). In Experiment 2, the plasma M-CSF levels were significantly higher in gestational age from −4 to 1 weeks compared with the last stage of pregnancy ($P < 0.05$). The levels decreased until 6 weeks, appeared to temporarily increase, and were relatively constant until 35 weeks. Macrophage colony-stimulating factor genes were expressed in all samples examined; the levels were relatively constant in early pregnancy, and then were widely varied until parturition. These results suggest that plasma M-CSF levels may be related to the maternal condition of pregnancy and to a slight extent to M-CSF gene expression in PWBCs.

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126 EFFECTS OF THE REPRODUCTIVE STATUS ON DEVELOPMENTAL COMPETENCE OF RECIPIENT OOCYTES AFTER SOMATIC CELL NUCLEAR TRANSFER IN CAT

T. Otoi, *N.W.K. Karja*, *M. Fahrudin*, *B. Agung*, *and P. Wongsrikeao*

Laboratory of Animal Reproduction, Department of Veterinary Sciences, Yamaguchi University, Yamaguchi, 753-8515, Japan. Email: otoi@yamaguchi-u.ac.jp

The reproductive status of donor cat has been suggested to influence developmental competence of the oocytes after IVM/IVF (Karja *et al*. 2002 Theriogenology 57, 2289–2298). This study was conducted to examine the effect of the reproductive cycle stage of cat ovaries supplying recipient oocytes for nuclear transfer (NT) on the developmental competence of the oocytes after somatic cell nuclear transfer. Cat ovaries were collected at local veterinary clinics and stored at 35°C for a short period (1–6 h). Based on the presence or absence of follicles and corpora lutea, the ovarian

pairs collected were classified into the inactive, follicular or luteal stages. Cumulus-oocyte complexes (COCs) were obtained from ovaries at each stage of the reproductive cycle by mincing/dissection and matured *in vitro* for 24 h, as previously described (Karja *et al*. 2002 Theriogenology 57, 2289–2298). *In vitro* matured oocytes from ovaries at the inactive ($n = 114$), follicular ($n = 124$), and luteal ($n = 126$) stages were mechanically enucleated in PBS supplemented with 5 μL mL^{-1} of cytochalasin B and 3 mg mL^{-1} BSA, and reconstructed with fibroblast cells derived from uterus tissue. The couplets were fused in Zimmerman medium with a single DC pulse of 1.5 kV cm^{-1} for 50 μs. The successfully fused couplets were activated by a 5-min exposure to 10 μg mL^{-1} calcium ionophore A23187 in MK1 medium (Kanda *et al*. 1998 J. Vet. Med. Sci. 60, 423–431) followed by 5 h of incubation in MK1 medium supplemented with 10 μg mL^{-1} cycloheximide. The NT embryos were cultured in MK1 medium supplemented with 4 mg mL^{-1} BSA at 38.0°C in a humidified atmosphere of 5% CO_2 in air. At 72 h of culture, all cleaved NT embryos were transferred to fresh MK1 medium supplemented with 5% fetal calf serum for an additional 4 days to evaluate their ability of development to the blastocyst stage. Data were analyzed by ANOVA. There were no significant differences ($P > 0.05$) among the fused oocytes derived from ovaries at the inactive, follicular, and luteal stages with respect to the percentages of cleavage (64.4%, 69.4%, and 74.5%, respectively) and blastocyst formation (17.4%, 21.0%, and 12.0%, respectively). These results indicate that the reproductive cycle stage of cat ovaries has no apparent effect on the development at competence of recipient oocytes after somatic cell nuclear transfer.

127 VILLOUS ARCHITECTURE AND FETO-MATERNAL INTERDIGITATION IN THE AFRICAN BUFFALO (*SYNCERUS CAFFER*) DURING DIFFERENT GESTATION STAGES

S. Schmidt[A], *J.T. Soley*[B], *T.A. Aire*[B], *and D. Gerber*[A]

[A]Section of Reproduction, Department of Production Animals Studies, University of Pretoria; [B]Department of Anatomy and Physiology, University of Pretoria, Onderstepoort, South Africa. Email: susanne@op.up.ac.za

Anchorage of the ruminant placenta is achieved by complementary indentation of chorionic villi into endometrial crypts. Geometrical patterns of interdigitation vary greatly among ruminants. Fetal villosity has been studied in other bovid species (*Bos taurus*, *Bubalus bubalis*) closely related to the African buffalo. The present study provides first information on villous architecture and feto-maternal anchorage in the African buffalo. Tissue samples from six pregnant cows (crown/rump length in cm: 2, 3, 14, 17, full-term) were collected during disease eradication programs in the Hluhluwe Game Reserve and from a buffalo breeding centre at Phalaborwa (afterbirths, $n = 3$). Tissue was fixed with 2.4% glutaraldehyde in Millonig's phosphate buffer by immersion or, if possible, via vascular perfusion through uterine and umbilical arteries prior to separation of feto-maternal tissue for scanning electron microscopy (SEM) preparation. Samples were further prepared for light microscopy and SEM using standard techniques. SEM samples were examined in a Philips XL 20 Microscope operated at 7 kV. Fetal villi consisted of a vascularized mesenchymal core covered by trophoblast epithelium. At a crown/rump length of 2 and 3 cm, the cotyledons comprised numerous short finger-like villi (length = 0.15–0.46 mm) originating from the chorionic plate. Some of these primary (stem) villi divided longitudinally a short distance from their origin. These relative simple villi were accommodated within corresponding caruncular crypts. At later gestation (crown/rump length: 14 and 17 cm), fetal villi had lengthened (length = 2–7.5 mm) and lateral (secondary) branching had started. The latter appeared in the form of bulbous or elongated structures projecting at approximately right angles from the stem villus. During the last month of gestation and in post-partum samples, primary (8–12 mm) and secondary villi had developed considerably in length. Secondary villi displayed a complex array of tertiary (terminal) villi of variable shape and size. A rich capillary network was observed within the connective tissue core of the tertiary villi as well as in the corresponding endometrial septae forming the walls of the caruncular crypts. Fully developed, each primary villus with its attendant secondary and tertiary villi formed a slender conical-shaped unit, also referred to as a cotyledonary villous tree. This paper provides the first description of villous architecture and placentome development in the African buffalo. The specific branching pattern of cotyledonary villi and complementary caruncular crypts in the African buffalo placenta, as described in this study, appears less complex when compared to that in the placenta of related *Bos* and *Bubalus* species.

128 SERUM TRANSAMINASE ACTIVITIES IN ENDOMETRITIC, REPEAT BREEDER AND ESTRUS CROSSBRED COWS

K.D. Tareen[A], *M.A. Kakar*[A], *Z.I. Qureshi*[B], *L.A. Lodhi*[B], *and H.A. Samad*[B]

[A]Livestock and Dairy Development Department Quetta, Balochistan, Pakistan; [B]Department of Animal Reproduction, Faculty of Animal Sciences, University of Agriculture Faisalabad, Pakistan. Email: adagul@hotmail.com

This study was carried out to determine the serum transaminase activities in endometritic, repeat breeder, and anestrus crossbred (Sahiwal Friesian) cows. A total of 60 blood samples were studied for enzyme activity in serum, with a Spectronic 20 using the commercial diagnostic kit adjusted at 546 nm wavelengths. The performance of liver and the intensity of liver damage were studied by iodine flocculation test. The highest mean (142.2 ± 2.10 μ/L) value of (GOT) activity was observed in the anestrus group, while the lowest mean (58.36 ± 2.1 μ/L) value of GOT was seen in the control group. Analysis of variance revealed a significant ($P < 0.01$) difference for GOT activity in anestrus compared to control, repeat breeder, and endometritic groups. The highest mean (18.45 ± 3.05 μ/L) value of GPT activities was recorded in repeat breeders while the lowest mean (11.81 ± 1.99 μ/L) value was noted in the control. The occurrence of moderate liver damage was 41.67%, whereas the prevalences of mild and normal damage were 41.67 and 16.66%, respectively. The highest activity (134.80 ± 1.27 μ/L) of GOT was recorded in cows with moderate liver damage, while the lowest activity (50.54 ± 2.72 μ/L) of GOT was observed in cows with normal livers. Mild liver-damaged animals had lower mean (62.13 ± 3.14 μ/L) GOT values compare to moderate liver-damaged animals. Analysis of variance revealed that the moderate liver-damaged group differed significantly in GOT activity from the normal and mild liver-damaged groups. The highest mean values of GOP (91.42 ± 2.8 μ/L) and GPT (20.83 ± 3.40 μ/L) activities were seen in cows with weak body condition, while lowest activities of GOP (82.56 ± 6.42 μ/L) and GPT (13.25 ± 1.55 μ/L) activities were recorded in animals with satisfactory body condition. When the milk yield was considered, the highest mean value of

GOT (91.73 ± 3.59 μ/L) and GPT (16.09 ± 3.23 μ/L) activities were recorded in high yielder and the lowest mean values of GOP (72.58 ± 4.79 μ/L) and GPT (13.05 ± 1.99 μ/L) activities were observed in low yielder cows. Based on the findings of this study, it was concluded that although the occurrence of moderate fatty liver was 41.67%, this could be associated with the infertility condition of crossbred dairy cows. It is quite possible that cows showing mild liver damage were in recovery stage after severe or moderate liver damage, as the postpartum period in cross bred cows under study varied from 1.5 to 8 months.

Embryo Culture

129 RESPIRATION ACTIVITY OF BOVINE EMBRYOS CULTURED IN SERUM-FREE AND SERUM-CONTAINING MEDIA

H. Abe[A], *H. Shiku*[B], *S. Aoyagi*[C], *T. Matsue*[B], *and H. Hoshi*[D]

[A]Tohoku University Biomedical Engineering Research Organization, Sendai, Japan; [B]Graduate School of Environmental Studies, Tohoku University, Sendai, Japan; [C]Hokuto Denko Corporation, Tokyo, Japan; [D]Research Institute for the Functional Peptides, Shimojo, Yamagata, Japan. Email: abe@tubero.tohoku.ac.jp

Oxygen consumption is a ubiquitous parameter which can provide valuable information about metabolic mechanisms and embryo quality. Recently, we succeeded in non-invasively and quantitatively determining oxygen consumption of individual bovine embryos by the scanning electrochemical microscopy (SECM). The aim of this study was to assess by SECM the oxygen consumption of individual bovine embryos at different developmental stages cultured in serum-free and serum-supplemented media. Bovine oocytes were matured in IVMD101 medium [Research Institute for the Functional Peptides (IFP), Shimojo, Yamagata, Japan] and inseminated in BO-based medium. For serum-free culture, inseminated ooocytes were cultured to the blastocyst stage in IVD101 medium in an atmosphere of a low oxygen condition (5% CO_2/5% O_2/90% N_2) at 38.5°C. For serum-supplemented culture, inseminated oocytes were cultured in HPM199 medium (IFP) supplemented with 5% calf serum (HPM199 + CS) in the presence of bovine cumulus/granulosa cells in a humidified atmosphere of 5% CO_2 in air. Oxygen consumption by individual bovine embryos was non-invasively quantified by the SECM measuring system. Some embryos were prepared for transmission electron microscopy. The oxygen consumption rates are presented in the table. Oxygen consumption rates (F) of the single embryos were low from 2-cell to 8-cell stages (0.45–0.52 × 10^{-14} mol s^{-1}). In serum-free culture, an increase in oxygen consumption rate was found at the morula (1.03 × 10^{-14} mol s^{-1}) stage, and blastocysts showed an even higher oxygen consumption rate (1.86 × 10^{-14} mol s^{-1}). On the other hand, the oxygen consumption of morulae and blastocysts produced in serum-supplemented medium was lower than that of embryos cultured in serum-free medium. Electron microscopic study demonstrated that many of the mitochondria of morulae and blastocycts cultured in HPM199 + CS medium were an immature form, indicating a correlation between respiration activity and development of mitochondria. These results suggest that the culture conditions affect the respiration activity of bovine embryos. The SECM procedures may have a wide application for judging embryo quality and culture conditions for embryos.

Table 1. Oxygen consumption rates (F × 10^{-14} mol s^{-1}) of the bovine embryos at various developmental stages

Embryonic stage	IVD101	HPM199 + CS
2 cell	0.46 ± 0.05 (17)	0.52 ± 0.04 (6)
4 cell	0.45 ± 0.03 (17)	0.47 ± 0.04 (6)
8 cell	0.46 ± 0.02 (10)	0.52 ± 0.04 (10)
Morula	1.03 ± 0.05 (27)	0.70 ± 0.05 (12)*
Blastocyst	1.86 ± 0.07 (21)	1.33 ± 0.10 (12)*

* Significance of differences compared with IVD101 ($P < 0.05$).
The numbers in parentheses represent the numbers of embryos examined.

130 THE EFFECT OF ALTERED ENERGY SUBSTRATE CONCENTRATIONS ON THE DEVELOPMENT OF DIPLOID PARTHENOGENETIC PORCINE EMBRYOS CREATED FROM OOCYTES FROM GILTS AND SOWS

L. Beebe, *S. McIlfactrick*, *and M. Nottle*

Department of Obstetrics and Gynaecology, The University of Adelaide, Adelaide, South Australia, Australia. Email: luke.beebe@adelaide.edu.au

There is building evidence that altering the concentration of energy substrates in the culture medium to more closely approximate the concentrations thought to be present in the reproductive tract improves porcine embryo development. The aim of this experiment was to examine the development of porcine parthenogenetic embryos in such a modified version of NCSU23. The embryos were created from both sow- and gilt-derived oocytes to see whether the source of oocytes influences how the embryos respond. Ovaries from slaughtered sows or prepubertal gilts were collected, follicles (3–6 mm) were aspirated and oocytes surrounded by at least three layers of compact cumulus cells were collected and matured in TCM199 containing cysteamine, insulin, FSH, EGF, and 10% sow follicular fluid, for approximately 40 h. Cumulus cells were removed and good quality mature oocytes