Early Pregnancy/Pregnancy Recognition

101 EFFECT OF DIETARY UREA ON EMBRYONIC VIABILITY AND DEVELOPMENT OF TOGGENBURG GOATS

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Urea supplementation in the diet of ruminants may alter the pH and protein concentration in different tissues and may affect the embryo quality and viability. Imbalances in nitrogen metabolism during the periods of oocyte growth and/or fertilization may dispose the zygote, pre-implantation embryo, and surviving conceptus to developmental errors. The aim of this research was to investigate the effect of feeding urea to goats on embryo quality. Eighteen Toggenburg goats, 48.6 ± 7.9 kg of BW, 2.9 ± 0.5 BCS, and 34.3 ± 20.8 months of age, were allocated randomly to 2 treatments: T1 (control, $n = 8$): no urea, and T2 ($n = 10$): 2.4% of dry matter (DM) as urea in the diet. The animals were fed diets based on Coast-cross hay (*Cynodon dactylon*) and concentrate (14% crude protein, DM basis) for 42 ± 2 days before embryo flushing. Estrus was synchronized with intravaginal sponges impregnated with 60 mg of medroxyprogesterone acetate (Progespon®, Syntex S.A., Buenos Aires, Argentina) for 11 days plus i.m. injection of 100 μg of cloprostenol (Ciosin®, Schering-Plough, Cotia, Brazil), on the Day 9. The superovulation protocol consisted of i.m. injections of 200 mg equivalent of NIH-FSH-P-1 (Folltropin®, Bioniche, Athens, GA), in 6 decreasing doses at 12-h intervals, on Days 9, 10, and 11 (Day 0 = sponge insertion). Estrus was monitored twice a day for 30 min from sponge withdrawal by use of teaser bucks, and females were mated by fertile bucks every 12 h during standing estrus. Early luteal regression was preventing by administration of an antiluteolytic (Banamine®, Schering-Plough) 72 h after sponge withdrawal, 1.1 mg kg^{-1} per day i.m. for 3 days. Embryo recovery was done on the seventh day of estrous cycle by a transcervical technique. Recovered embryos were classified by quality (I = excellent, V = degenerated), development stage percent (1 = unfertilized oocytes, 9 = hatched blastocyst), and viability with the embryo grades 1 (excellent), 2 (good), and 3 (fair). The effect of urea supplementation on embryo stage and viability was analyzed by ANOVA. From the 18 goats, 12 (67%) were responsive to the superovulation protocol, 62.5% from T1 and 70.0% from T2. The number of embryos flushed (7.20 ± 2.39 *v.* 6.29 ± 2.93) and the percentage and number of viable embryos [100% (36/36) *v.* 100% (39/39)] from T1 and T2 were not different ($P > 0.01$). Thereafter, embryos in advanced stages of development (7, 8, and 9) were 58.65% from T2 compared with 0% from T1 ($P < 0.01$). In addition to the embryos, 13 unfertilized oocytes were recovered from the T2 goats *v.* none from the T1 goats ($P < 0.01$). These results suggest that urea may cause acceleration of embryo development and may affect oocyte fertilization.

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102 EVIDENCE FOR PRE-IMPLANTATION ORIGIN OF PLACENTAL FAILURE IN BOVINE CLONE PREGNANCIES

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Placental abnormalities account for a high proportion of pregnancy loss after transfer of cloned (SCNT) bovine embryos. The high rate of pregnancy failure has been linked to the finding of abnormal placental formation and function. Because bovine SCNT embryos were shown to exhibit altered trophoblast differentiation (Arnold DR *et al.* 2006 Reproduction **132**, 279–290), we hypothesized that placental abnormalities in bovine clone pregnancies may originate from disturbed embryo-maternal communication in the pre-implantation period. To test this hypothesis, we evaluated the response of the endometrium to SCNT *v.* IVF embryos. The SCNT embryos were produced from fibroblast cultures derived from 4 different fetuses to exclude specific effects of a particular donor cell culture. After SCNT or IVF, embryos were cultured under identical conditions. Two SCNT or IVF blastocysts (grade 1) were transferred per recipient heifer (Day 8 of estrous cycle). Ten days later the recipients were slaughtered, the uteri were recovered, and pregnancy was verified by the presence of at least one normally developed embryo. Endometrium samples of 9 SCNT and 10 IVF pregnancies were used for transcriptome profiling with a custom cDNA microarray (BOE array; Bauersachs S *et al.* 2007 J. Dairy Sci. **90**, 4420–4423). In total, 58 transcripts were found to be differently abundant between endometrium samples from SCNT *v.* IVF pregnancies (SAM, FDR 5.24%). Interestingly, for some of them an important role in implantation and/or placentation has already been shown. *NR2F2*, encoding the orphan nuclear receptor superfamily member NR2F2 (COUP-TFII), was downregulated in endometrium from SCNT pregnancies (1.5-fold; $P < 0.01$). Uterine-specific *Nr2f2* mutant mice are infertile due to implantation failure (Kurihara I *et al.* 2007 PLoS Genetics **3**, e102). Another interesting candidate is *GJA1*, encoding connexin 43 (Cx43), with 1.8-fold ($P < 0.001$) downregulated transcript levels in SCNT pregnancies. A striking increase of stromal Cx43 has been observed in the ovine intercaruncular and caruncular endometrium during intensification of the feto-maternal contact (Gabriel *et al.* 2005 Placenta **25**, 287–296), suggesting that reduced *GJA1* mRNA expression in bovine clone pregnancies may negatively affect

placentation. In view of the well-orchestrated spectrum of transcriptome changes in endometrium during the peri-attachment period (Bauersachs S *et al.* 2006 Reproduction **132**, 319–331), these findings suggest that placental failure in bovine clone pregnancies may originate from abnormal embryo-maternal communication already in the pre- or peri-implantation period.

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103 MASS SPECTROMETRY ANALYSIS OF UTERUS ENDOMETRIUM DURING PREGNANCY IN THE PIG

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In general, many important molecular events occur within the female reproductive tract, especially within the uterine endometrium, during periods of pregnancy. The endometrium includes a mucosal lining of the uterus, which functions to provide a suitable site for implantation and development of a fertilized egg and fetus. To date, developmental integrity involves molecular cascades whose interrelationships are not fully understood within the endometrium during pregnancy in pigs. In this study, we explored the functional regulated proteins in endometrium during periods of pregnancy periods (Day 40, $n = 6$; Day 70, $n = 7$; Day 90, $n = 6$ of pregnancy; and nonpregnancy, $n = 1$) using 2-dimensional gel electrophoresis (2-DE) and Western blotting. The functional regulated proteins were identified and discovered from differentially expressed proteins in the endometrium during pregnancy. In the proteomic analysis of the endometrium, 820 protein spots were matched on 2-DE gels. With 98 proteins regulated differentially among nonpregnant and pregnant tissues (matched and unmatched spots), 63 up- or down-regulated proteins have been identified. Interestingly, 6 of these 63 proteins were endothelial growth factor-associated proteins such as transgelin, transferrin, galectin-1, tropomyosin alpha, protein DJ-1, and beta-defensin. We also confirmed the expression levels of these proteins in the endometrium during pregnancy by using Western blotting. Our results suggest that the expressions of these genes involved in endometrial function and development from early to late gestation are associated with the regulation of endothelial growth factor.

104 ESTROUS CYCLE-DEPENDENT CHANGES IN THE BOVINE ENDOMETRIUM PROTEOME

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Among the reproductive tissues, the endometrium plays a central role in the context of embryo-maternal communication and pregnancy recognition. During the estrous cycle, characteristic morphological and functional changes occur in the bovine endometrium, being crucial for uterine receptivity. These changes are mainly regulated by the hormones progesterone, estradiol, and oxytocin. The bovine estrous cycle, with a length of 21 days, can be divided into 4 stages: i) estrus (considered as Day 0, low progesterone level and time of ovulation); ii) metestrus (Days 1 to 5, corpus luteum formation, rising progesterone level); iii) diestrus (Days 6 to 17, high progesterone); and iv) proestrus (Days 18 to 20, corpus luteum degeneration; declining progesterone). The principles of hormonal regulation during the estrous cycle are well understood; however, in-depth knowledge of the detailed molecular mechanisms is still incomplete. To elucidate the underlying biochemical processes, the proteomes of bovine endometrial samples of all 4 stages (cycle Day 0, Day 3.5, Day 12, and Day 18) were compared in a quantitative manner. To maximize the accuracy of protein quantification, sophisticated 2-dimensional (2D) fluorescence difference gel electrophoresis (2D-DIGE) experiments were performed, using internal pooled standards for inter-gel normalization. To enhance the resolution of 2D-polyacrylamide gel electrophoresis separation, the proteins were analyzed by 2 overlapping pH gradients. In total, 28 individual DIGE experiments (14 2D gels $\times$ 2 pH gradients) were performed, corresponding to 84 gel images. With a refined statistical analysis of spot intensities, we were able to identify a total of 91 spots altered by at least a factor of ± 2 ($P < 0.05$) in intensity between at least 2 of the 4 stages. Matrix-assisted laser desorption/ionization tandem mass spectrometry (MALDI-MS/MS) identification of these spots showed that they originated from 66 different proteins. Moreover, for 14 of these proteins, several polymorphic variants could be identified. Gene ontology analysis of the protein IDs revealed a broad diversity of biological and biochemical functions as well as cellular localizations of these proteins. Several proteins detected (e.g. FK506 and 20 alpha-HSD) are crucial components for uterine receptivity and represent interesting targets for further functional studies.

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105 DETECTION OF PLACENTAL LACTOGENS IN SWAMP BUFFALO BY RADIOIMMUNOASSAY TECHNIQUE

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Ruminant placental lactogens (PL) are members of the growth factor/prolactin (GH/PRL) family. They are synthesized by trophectodermal binucleate cells. There is evidence to suggest that PL is involved in control of fetal growth, through actions in both the maternal and fetal compartments, as well

as in influencing mammary growth during pregnancy (Byatt JC *et al.* 1992 J. Anim. Sci. **70**, 2911–2923). The structure and biology of PL have been studied in the cow, sheep, goat, human, and mice. The maternal concentration of PL is 100- to 1000-fold greater in pregnant sheep and goats than in cows but no information exists about PL concentration in buffalo. The aim of the present study was to evaluate the ability to detect PL in buffalo fluids by using bovine PL antibody. Samples were collected in the slaughterhouse immediately after animal slaughter. The fetuses were measured after heart blood collection. A bPL RIA system was used to determine the bPL concentrations in the buffalo samples (Alvarez-Oxiley AV *et al.* 2007 Reprod. Fertil. Dev. **19**, 877–885). The rbPL molecules were radio-iodinated with [125]I-Na by using the lactoperoxidase method (Thorell JI and Johansson BG 1971 Biochim. Biophys. Acta **251**, 363–369). Concentrations of buffalo PL are presented in Table 1. In this RIA system, the minimum detected value was 0.068 ng mL^{-1}, and the binding competition curves of bovine PL standard and buffalo fluids dilution using bovine PL antibody were paralleled in all kinds of samples. The lowest concentration was detected in allantoid fluid and the greatest concentration in fetal plasma ($P < 0.05$). Study of the biology of PL in buffalo has proved difficult because the concentration of PL in all buffalo fluids is very low. Furthermore, the research concerning buffalo PL function required *in vivo* experiments. Existing data suggest that at least the concentration of buffalo PL is different from cattle and other smaller domestic ruminants. In conclusion, our results provide preliminary information about concentrations of PL in buffalo fluids.

Table 1. Concentration of placental lactogen in buffalo fluids

Samples	Maternal plasma	Fetal plasma	Amniotic fluid	Allantoid fluid	Maternal urine
No. sample	35	69	57	63	36
Mean ± SD (ng mL^{-1})	0.71 ± 0.14	1.35 ± 2.44	0.54 ± 0.24	0.42 ± 0.28	0.75 ± 0.3

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106 ANGIOGENIC FACTOR mRNA EXPRESSION IN FETAL MEMBRANES IN EARLY PREGNANT SHEEP

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Maternal and fetal placental development may be compromised by use of assisted reproductive techniques, including cloning, resulting in poor placental angiogenesis and subsequent high embryonic/fetal loss (Palmieri *et al.* 2007 Placenta **28**, 577–584). Before changes in vascular development in placenta from compromised pregnancies can be understood, a detailed knowledge of regulation of angiogenesis in placental tissues from normal pregnancies is necessary. Therefore, this study determined the expression pattern of mRNA for several angiogenic factors and their receptors: vascular endothelial growth factor (VEGF) and receptor (R) 1 and 2; basic fibroblast growth factor (FGF2) and FGFRIIIc; angiopoietin (ANGPT) 1 and 2 and ANGPTR (Tie2); endothelial nitric oxide synthase (eNOS) and NO receptor GUCY1B3 in fetal membranes (FM; fetal placenta) collected on Days 16, 18, 20, 22, 24, 26, 28, and 30 after mating ($n = 5$ to 8/day). Fetal membranes were snap frozen for evaluation of gene expression using quantitative, real-time RT-PCR. VEGF mRNA was increased ($P < 0.05$) 2-fold on Days 28 and 30 compared with Days 16, 18, and 20, whereas VEGFR1 mRNA increased ($P < 0.05$) 25- to 50-fold on Days 28 and 30 compared with Day 16, and VEGFR2 mRNA was greatest ($P < 0.05$) on Day 22 compared with Days 16, 18, 28, and 30. FGF2 mRNA was 4-fold greater ($P < 0.05$) on Day 22 compared with Day 16; however, FGF2RIIIc was unchanged from Day 16 through 30. eNOS mRNA was greatest ($P < 0.05$) on Days 22 and 24 compared with Days 16 and 18, but GUCY1B3 mRNA was greatest ($P < 0.05$) on Day 18 compared with Days 20, 24, and 28. ANGPT1 mRNA increased ($P < 0.05$) 40-fold by Days 28 and 30 compared with Days 16 and 18. ANGPT2 mRNA was undetectable on Day 16, and increased ($P < 0.05$) 5-fold from Days 18 through 30. ANGPTR mRNA was greatest ($P < 0.05$) on Days 22 and 24 compared with Days 16 and 18. This description of expression of factors potentially regulating early placental angiogenesis during normal pregnancy in sheep will provide the foundation for understanding the dramatic increases in capillary cell proliferation and capillary size we have previously observed (unpublished) and for determining whether placental vascular development is altered in compromised pregnancies.

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107 EARLY EMBRYONIC SURVIVAL IN THE PIG IS HIGHER THAN CONVENTIONALLY REPORTED

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It is generally accepted that 30% of the embryos in a porcine litter die within the first 40 days of pregnancy (Pope WP and First NL 1985 Theriogenology **23**, 91–105). The aim of the study was to investigate the dynamics of embryonic mortality from the 2nd to the 7th week of pregnancy in a homogeneous pig population in order to test whether this dogma holds true. A total of 141 pregnant Danish Landrace × Yorkshire gilts were divided into three groups dependent on gestational length: Group 1 (Days 9 to 24 post insemination (p.i.)): At Days 9 to 18 p.i., embryos were collected by flushing the uterine horns with PBS containing 1% serum. At Days 19 to 24, embryos were identified *in situ* by opening of the horns along the anti-mesometrial side. All embryos were staged according to the morphological appearance of embryo proper. Pre-somite stage embryos were categorized as either: Hatched blastocysts, pre-streak 1, pre-streak 2, primitive streak, or neural groove stage embryos (Vejlsted M *et al.* 2006 Mol. Reprod. Dev. **73**,

709–718). Somite stage embryos were staged according to the number of somites. All embryos in Group 2 (Days 24.5 to 33 p.i.) and Group 3 (Days 40.5 to 47 p.i.) were identified in situ by opening the uterine horns as described above. The localization in the uterus and the Crown Rump Length (CRL) was recorded for all embryos in these groups. The average embryo recovery rate, (i.e. the ratio between the numbers of recovered embryos and the CL numbers) was 82%. Moreover, there were no significant differences between the groups with respect to the embryo recovery rate, signaling the absence of continued embryonic mortality. No significant correlations were obtained between the location of the embryos in the uterus and the CRL (only measured for Groups 2 and 3). Our data indicate that (1) the level of embryonic mortality was less than 10 to 15% and (2) there was no continued embryonic mortality occurring between Days 9 to 47 p.i. This is in great contrast to previous reports. Furthermore, there is no evidence that the location in the uterine horn has any influence on the embryonic development.

Table 1. The average numbers of corpora lutea (CL), embryos, and the embryo recovery rates in gilts at different time points after insemination

Group	1 (Days 9 to 24) $n = 84$	2 (Days 24.5 to 33) $n = 25$	3 (Days 40.5 to 47) $n = 32$
CL (n)	19.3 ± 3.1^{a}	19.1 ± 2.3^{a}	17.9 ± 2.8^{b}
Embryos (n)	15.4 ± 5.4	16.8 ± 4.8	15.1 ± 3.4
Embryo recovery rates (%)	81 ± 26	88 ± 24	84 ± 16

[a,b]Values with different superscript within rows are significantly different ($P < 0.05$).

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