integrated into the larger population of food animal livestock; and (3) determination of the more complex environmental and safety consequences of their release into livestock populations. We previously developed and characterized transgenic swine containing a mammary-specific transgene (bovine α-lactalbumin, bALAC) that results in increased milk production in sows. We currently are determining whether bALAC is expressed in tissues of T swine other than the lactating mammary gland and whether the transgene DNA (Tg) crosses into non-transgenic control (C) swine under various physiological and physical conditions. The specific aims addressed in the present study were to determine: (1) whether the Tg can be transferred directly from T animals to C animals by physical association or contact and (2) whether the Tg can be transferred directly from an adult T animal to an adult C animal via mating. The T animals utilized in these studies are in at least generation 10 and have stable incorporation of the Tg. Comparable age- and weight-matched animals, T and C, were housed together allowing for general contact that is normal within swine production, for either 180, 220, or 250 d of age after weaning. Swine due to their behavior ingest saliva, regurgitated food, and stool or urinary products, as well as other bodily fluids and cells during normal housing. In a second study, vaginal, cervical, uterine, oviductal, and ovarian tissues from C females on 2, 7, or 90 d after mating to T males and penis, bulbourethral gland, urethra, testis, and epididymis tissues from C males on 2 or 7 days after mating to Tg females were collected. The presence of Tg in tissues from all C animals was tested via PCR. We have analyzed for the presence of the Tg in various tissues [including mammary gland, salivary gland, skin (sebaceous gland), muscle, lung, liver, kidney, brain, ovary, oviduct, uterus, cervix, vagina, penis, bulbourethral gland, urethra, testis, epididymis, and intestine]. Results indicate no presence of the Tg in tissues of C animals ($n = 28$) after co-habitation for 180, 220, or 250 d ($n = 305$ samples analyzed) or at 2 ($n = 5$), 7($n = 14$), or 90 ($n = 2$) d post-mating ($n = 60$, 174, or 24 samples analyzed, respectively). The present results suggest that there is no horizontal Tg transmission between T and C pigs due to rearing or mating. This work provides a critical step toward providing rigorous scientific data for risk assessment of transgenic livestock.

This project supported by the USDA BRAG Project #2005-03799.

312 CONSTRUCTION OF SPECIFICALLY EXPRESSED VECTOR IN MAMMARY GLAND FOR *lacS* AND ITS TRANSFECTION INTO BOVINE FETAL FIBROBLASTS MEDIATED BY LIPOSOME

Z. H. Zhou, Z. J. Yan, and L. J. Zhang

Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China

This study was designed to optimize conditions for transfection of a mammary gland specific transgene into bovine fetal fibroblasts. Transfection of *Sulfolobus solfataricus* β-glycosidase gene (*lacS*) was mediated by liposome. Neomycin resistance (Neo^r) and enhanced green fluorescent protein (EGFP) gene were used as genetic markers to screen transgenic somatic cells. A 0.92-kb fragment of bovine β-lactoglobulin gene sequence was obtained from bovine genome by PCR amplification and was inserted into the T site of pMD19-Simple T plasmid. 1.49 kb of *lacS* gene coding sequence was cloned from *Sulfolobus solfataricus* genome by PCR amplification and inserted into the pUC19 plasmid. The coding sequence of Neo^r was derived by PCR amplification from pIRES2-EGFP plasmid and inserted into the *BamHI*/*Nhe*I site of pIRES2-EGFP plasmid. The resultant vector (pNIE) contained a Neo^r and an EGFP gene, which were linked by an internal ribosome entry site sequence downstream of the cytomegalovirus (CMV) promoter. Finally, the vector pNIE was assembled into the pUC19 plasmid, thus creating a pBLI vector, which contained the Neo^r and EGFP gene regulated by CMV promoter for expression in a non-tissue specific mode and the *lacS* gene regulated by bovine β-lactoglobulin promoter for specific expression in mammary gland. Bovine fetal fibroblasts (bFF) were isolated from the ear skin of female fetuses at the age of 2 to 3 months. The cells proliferated well and grew normally in culture, with typical fibroblast morphology and growth curve. The effects of different concentrations of transfection and pBLI were compared on the efficiency of transfection. The passage 4 bFF cells at 70 to 80% confluency were transfected in a 24-well culture plate. 2×10^5 cells were cultured in DMEM with 0.5, 0.75, 1.0, 1.5, 2.0, and 2.5 μg of pBLI using transfection (1, 2, 3, 4, 5, and 6 μL) for 48 h, respectively. The transfected cells were cultured for 48 h before adding G418 at concentrations of 200, 300, 400, 500, 600, 700, 800, and 900 μg mL^{-1} for 14 d, respectively. Positive cell colonies were selected and purified through both the expression of Neo^r and EGFP gene under a fluorescence microscopy. The selected colonies were propagated in DMEM containing 300 μg mL^{-1} G418. The results showed that bright green fluorescence could be detected at 48 h after transfection. 1.0 μg of pBLI plasmid and 3 μL of transfection yielded the desirable efficiency of transfection. More transgenic bFF colonies were selected by G418 at the concentration of 800 μg mL^{-1}. In conclusion, a specifically expressed vector in mammary gland for *lacS* gene was successfully constructed, transfection parameters were developed, and efficient screening measures were established for detecting transgenic somatic cells.

Ultrasound

313 ASSESSMENT OF LUTEAL FUNCTION IN TOGGENBURG GOATS BY COMPUTER-ASSISTED IMAGE ANALYSIS

E. K. N. Arashiro[A], *J. H. M. Viana*[B], *J. F. Fonseca*[C], *L. G. B. Siqueira*[B], *J. H. Bruschi*[B], *L. S. Camargo*[B], *C. A. C. Fernandes*[D], *and F. Z. Brandao*[A]

[A]Federal Fluminense University, Niteroi, Rio de Janeiro, Brazil;
[B]Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil;
[C]Embrapa Goats, Sobral, Ceara, Brazil;
[D]Biotran, Alfenas, Minas Gerais, Brazil

Computer-assisted image analysis is a technological extension of reproductive ultrasonography and allows the quantitative assessment of the luteal echotexture, which is related to changes in histological features and, consequently, to steroidogenesis. The aim of this study was to determine the

efficiency of luteal echotexture evaluation as a tool to assess luteal function in different phases of the estrous cycle in Toggenburg goats. Nulliparous goats ($n = 21$), 8 months in age, 33.52 ± 1.22 kg of body weight, and body score condition of 3.5 ± 0.07 (1 to 5 scale), which showed estrus within a 48-h period during the natural breeding season (March and April), were used. After estrous detection (Day 0) and mating, ovarian sonographic evaluations were performed daily using a portable ultrasound device (Aloka SSD 500, Aloka Co.) equipped with an adapted linear transrectal 5-MHz probe. The examinations were preceded by blood sample collections, which were stored until radioimmunoassay for progesterone (P4). Images were recorded in VHS tapes, then digitized to TIFF files (resolution of 1500×1125 pixels) using a video capture board. A representative elementary area of 5625 pixels (0.31 cm^2) was defined for the luteal tissue according to the criterion proposed by Van den Bygaart and Protz 1999. Computer-assisted analyses were performed using custom-developed software (Quantporo®). Each pixel received a numeric value ranging from 0 (black) to 255 (white). Luteal echotexture and plasma P4 data were analyzed by ANOVA, and differences among means were determined by Tukey's test. Correlations were established by Pearson's correlation method. Results are shown as mean ± SEM. Corpora lutea size increased progressively ($P < 0.001$) until Day 9, when it reached the maximum area (1.26 ± 0.32 cm^2). No increase in size was detected on the subsequent days ($P > 0.05$). Plasma P4 levels increased until a maximum value on Day 9 (6.31 ± 0.46 ng mL^{-1}), and no increase was observed further ($P > 0.05$). In nonpregnant animals ($n = 7$), luteolysis was characterized by an abrupt decrease in plasma P4 concentration, which dropped to values lower than 1 ng mL^{-1} 24 h after the onset of the process, whereas luteal area decreased gradually. Plasma P4 concentration was correlated to luteal area during luteogenesis and luteolysis ($r = 0.63$ and $r = 0.50$, respectively; $P < 0.05$). Mean pixel value showed a progressive increase during luteogenesis and reached the maximum value on Day 13 (54.33 ± 1.83). During corpus luteum (CL) regression, mean pixel value decreased to lower values 48 h after the onset of natural luteolysis ($P < 0.05$). Through both luteogenesis and luteolysis, positive correlations were observed between mean pixel values and luteal area ($r = 0.34$ and $r = 0.26$, respectively; $P < 0.05$) and also between mean pixel values and plasma P4 concentration ($r = 0.24$ and $r = 0.37$, respectively; $P < 0.05$). Pixel heterogeneity was not correlated to luteal area nor plasma P4 levels. These results suggest an association between CL echotexture and steroidogenic function; therefore, the quantitative assessment of the pixel brightness has a potential to be used for luteal function evaluation in goats.

FAPEMIG and CAPES.

314 EARLY PREGNANCY DIAGNOSIS USING A NOVEL TRANSRECTAL ULTRASONOGRAPHY PROTOCOL IN JAPANESE BLACK COWS

A. Gaja[B], *C. Kubota*[A], *and T. Kojima*[A]

[A]Kagoshima University, Kagoshima, Japan;
[B]Yamaguchi University, Yamaguchi, Japan

The present study aims to establish a novel practical protocol for early pregnancy diagnosis in cows by using transrectal ultrasonography. The protocol is based on measurements of corpus luteum (CL) cross-sectional area (CL c-s area) change performed at 2 separate days before the coming estrus after AI. Fourteen cows were inseminated artificially, and transrectal ultrasonographical observation of the ovaries and blood collection for measurement of peripheral plasma progesterone (P4) concentration were carried out daily from Days 12 to 23 (Day 0 = the day of onset of estrus). Thereafter, cows were routinely diagnosed for pregnancy at Day 30 by transrectal ultrasonography. The largest CL c-s area was obtained at Day 14 in both pregnant and non-pregnant cows. Seven out of 8 non-pregnant cows showed significant CL c-s area regression between Days 14 and 20 (422 ± 112 *v.* 249 ± 63 mm^2), whereas no regression was observed between Days 14 and 20 in pregnant cows (416 ± 65 *v.* 402 ± 78 mm^2). The regression in the CL c-s area between pregnant and non-pregnant cows was significantly different during Day 18 (424 ± 65 *v.* 288 ± 88 mm^2) to Day 23 (402 ± 71 *v.* 139 ± 64 mm^2). P4 concentration was significantly low (less than 1 ng mL^{-1}) at Day 20 in 3 out of 8 non-pregnant cows, whereas the pregnant cows showed significant increase of P4 between Days 14 and 20 (2.6 ± 0.2 *v.* 3.4 ± 0.5 ng mL^{-1}). The pregnant cows showed significantly higher P4 concentration starting from Day 18 than non-pregnant cows. However, in non-pregnant cows, 4 cows returned to estrus on Day 20 or after, 3 cows showed no signs of estrus, and 1 cow came in estrus as early as Day 18 after AI. In conclusion, the results of the present study suggest that measuring the change in the CL c-s area at Days 14 and 20 makes it possible to detect the non-pregnant cows at Day 20 after AI. However, it was also indicated that measuring the change of P4 concentrations on the same days did not always successfully detect non-pregnant cows. The new protocol based on CL c-s area regression rate can detect almost certainly non-pregnant cows at Day 20 after AI. It is suggested that this method is advantageous in research and industrial breeding.

315 USE OF PERIFOLLICULAR BLOOD FLOW TO PREDICT THE DEVELOPMENTAL COMPETENCE OF BOVINE CUMULUS–OOCYTE COMPLEXES COLLECTED DURING REPEATED OVUM PICKUP SESSIONS ONCE OR TWICE WEEKLY

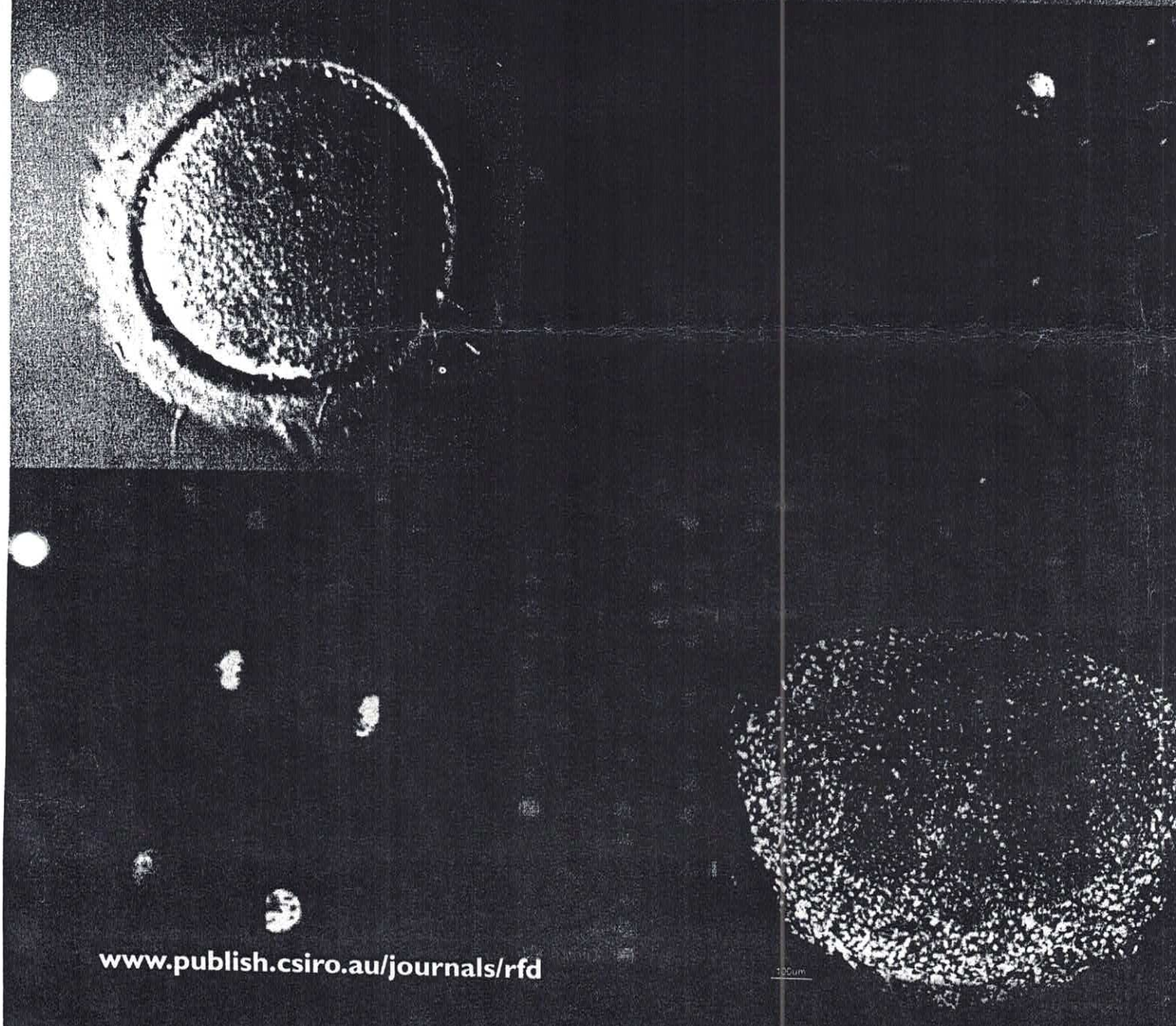
A. Hanstedt[A], *K. Höffmann*[A], *Ä. Honnens*[A], *H. Bollwein*[A], *and C. Wrenzycki*[A,B]

[A]University of Veterinary Medicine Hannover, Clinic for Cattle, Hannover, Germany;
[B]University of Veterinary Medicine Hannover, Reproductive Medicine Unit, Hannover, Germany

On average, only 20% of the cumulus–oocyte complexes (COC) develop to the blastocyst stage (Merton *et al.* 2003 Theriogenology **59**, 651–674). An increase in the blood supply to individual follicles appears to be associated with follicular growth rates, whereas a reduction seems to be closely related to follicular atresia (Acosta *et al.* 2003 Reproduction **125**, 759–767). The purpose of this study was to determine whether qualitative perifollicular blood flow changes can be used to predict the developmental competence of COC collected during repeated ovum pickup (OPU) sessions once or twice weekly. Lactating Holstein cows ($n = 20$) were used as oocyte donors. After dominant follicle removal, OPU was performed twice (group 1, for 3 weeks) or once (group 2, for six weeks) weekly employing a 7.5-MHz transducer (GE 8C-RS) of an ultrasound scanner (GE Logiq Book).

Reproduction, Fertility and Development

Volume 21(1) 2009



www.publish.csiro.au/journals/rfd