EVALUATION OF RISKS ASSOCIATED WITH CLOSE ENVIRONMENTAL CONTACT TO SWINE THAT ARE TRANSGENIC FOR A BOVINE MILK PROTEIN ALPHA-LACTALBUMIN

BY

JONATHON FORREST MOSLEY

THESIS

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Master's Committee:

Professor Walter Hurley, Chair
Professor Matthew Wheeler, Director of Research
Associate Professor Robert Knox

ABSTRACT

Assessment of the risks associated with exposing non-transgenic animal populations to transgenic animals is important to the future contributions of transgenic livestock to society. Evaluation of the potential for transfer of a transgene (Tg) from livestock to a non-transgenic animal during parturition, mating, gestation, or lactation is the initial step in a risk assessment. We previously developed and characterized transgenic swine containing a mammary-specific transgene, bovine α-lactalbumin, (Bα-LA) that results in increased milk production in sows. In this study, we wanted to determine whether Bα-LA is expressed in tissues of transgenic swine other than the lactating mammary gland and if the Tg DNA crosses into non-transgenic swine under various physiological and physical conditions. The specific aims addressed in this study were to determine (1) whether the Tg can be synthesized in any other tissues than the mammary gland of a transgenic sow; (2) whether the Tg can be transferred directly by physical association or contact; (3) whether the Tg can be transferred directly via mating; and (4) whether the Tg can be transferred directly during gestation, parturition, or lactation.

This study included four separate experiments. In the first experiment 4 transgenic (T) and 6 non-transgenic (control; C) pigs were raised to 180, 220, 250 d of age, or 112 days post-breeding and then sacrificed for tissue collection. In the second experiment 41 T and 54 C pigs, of comparable age and weight, were housed together beginning at weaning (d 21) allowing for general contact that is normal in swine production. Pigs were housed together up to either 180, 220, or 250 d of age and then sacrificed for tissue collection. In the first and second experiments blood, brain, jejunum, kidney, liver, lung, mammary gland, muscle, ovary, sublingual salivary gland, skin, and spleen were collected. In the third experiment, the above samples, plus vaginal, cervical, uterine, oviductal, and ovarian tissues were collected from 34 C females on 2, 7, 90 or 112 d after mating to one of 10 T males, and along with above samples, penis, bulbourethral gland, urethra, testis, and epididymis tissues were collected from 12 C

males 7 d after mating to one of 4 Tg females. The fourth experiment was divided into 3 sub-experiments: 4a. fetuses were collected from 4 C sows bred to a C boar and 4 T sows bred to a C boar on d 112 of gestation and sacrificed fetuses were retrieved for tissue collection (n=56); 4b. Fifty-eight newborn piglets were removed from their birth dam before they were allowed to suckle and sacrificed for tissue collection, along with twenty-one C piglets were removed from their birth dam at the time of farrowing and before they were allowed to suckle and fostered to a lactating T sow (n=11) or to a lactating C sow (n=10). Piglets were allowed to suckle for 24 or 72 h and then sacrificed for tissue collection; 4c. nineteen C piglets were allowed to suckle their birth dam until d 3, and then were fostered to a lactating T sow (n= 11) or to a lactating C sow (n= 8). After cross-fostering the piglets were allowed to suckle for 72 or 168 h and then sacrificed for tissue collection. For each of the sub-experiments under experiment four, the tissues harvested were jejunum, liver, lung, muscle, and skin. For all experiments the presence of the Tg or its expression in tissues from C and T animals was determined by PCR analyses. Results indicated that the Tg is not expressed in tissues other than the mammary gland of a T lactating sow (n= 50 samples negative). The Tg was not identified in tissues from C animals after co-habitation for 180, 220, or 250 d (n=571 samples analyzed), nor from animals at 2, 7, 90, or 112 d post-mating (n =89, 166, 71, or 44 samples analyzed, respectively). At day 112 of gestation, all samples (n=127 samples analyzed) from non-transgenic piglets whose dam was transgenic were negative except for the outer placental membrane, which screened positive for the Tg, and which is consistent with the outer placental membrane being derived from the maternal tissue. Newborn piglets (n=58) all samples derived from mating C females to C males were all negative for the Tg. Of those samples taken from piglets derived from mating T females to C males 79 were positive and 52 were negative for Tg. Again, this is consistent with the T females being heterozygous for the Tg. Finally C piglets (n=21) that were cross-fostered before suckling (d 0) or were cross-fostered 3 d after birth and suckled Tg dams (n=19) were negative for the Tg in their tissues. These results strongly indicate that

there is no horizontal transmission of the transgene between transgenic and non-transgenic pigs that occurs during rearing, mating, gestation, or lactation.

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LIST OF ABBREVIATIONS

Bα-LA	Bovine alpha-lactalbumin
C	Control
CO_2	Carbon Dioxide
cohab	Cohabitation
d	Day(s)
DNA	Deoxyribonucleic Acid
h	Hour(s)
kg	Kilograms
mg	Milligram
ml	Milliliter
n	Number
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
T	Transgenic
Tg	Transgene DNA

CHAPTER 1

INTRODUCTION

In the past two decades the increasing use of transgenic animals as models in biomedical sciences and production agriculture has raised questions regarding the risk of the transgene transferring from a transgenic animal to a non-transgenic animal. The assessment of this risk is an important requirement for the future of transgenic livestock. In the present study, the term 'transgenic' will be defined as "one whose genetic make-up has been modified by addition or deletion of a specific DNA sequence" (Wall and Seidel, 1992). Thus, producing what is more commonly known as transgenic plants and/or animals. Transgenic technology provides a method to rapidly introduce new genes into plants and animals. This technology allows for the introduction of new genes into cattle, swine, sheep and goats without crossbreeding (Wheeler, 2003). Current research in swine is devoted to improvement of productivity traits, enhancement of animal health, and production of biomedical models.

Transgenic swine are a critical part to the development of models in research using pigs for biomedical sciences. The completion of the pig genome sequence, gives the ability to produce transgenic animals and the ability to replicate the model (Schook et al., 2005). Owing to the overwhelming physiological similarities between pigs and humans (Tumbleson and Schook, 1996), the pig provides a relevant animal model for biomedical sciences. In addition to bioengineering, imaging, and behavioral studies transgenic animals can be used to enhance productivity traits in swine.

The technology involved in production of transgenic livestock holds great promise for agriculture and biomedical sciences as well as potential risks. The public's perception of biotechnology tends to be accepting when involving the development of new pharmaceuticals; however, it is less accepting when dealing with production traits or animals as a food source.

It is clear that in order to realize the long-term benefits or risks of transgenic technology to society, the impacts of transgenic animals on the environment, producers, consumers and the animals themselves must be carefully evaluated. It is worth pointing out here that the goal of using this technology is for the benefit not the detriment of mankind. Because the use of this technology is complex, inefficient and expensive, a thorough understanding of the risks associated with transgenic animals is needed.

Before 2013 transgenic swine must be housed separated from the rest of the production herd to deter any form of gene transfer. The aim of this research is to determine if bovine alpha-lactalbumin (Bα-LA) is expressed in tissues other than the mammary gland during lactation of transgenic sows and whether the transgene (DNA) crosses over into non-transgenic animals under various physiological and physical conditions. These include housing, mating, gestation, lactation, and suckling.

When evaluating transgenic organisms, the following questions are among those that regulators may ask: Is there a hazard? What is the nature of the hazard? How likely is the hazard to occur? What is the severity and extent of the hazard if it occurs?

In order to examine the risk or hazard constituted by transgenic livestock they are studied in a setting where the animals are born, raised, and held. It is important to

understand all possible modes of DNA transmission especially in production settings in order to get a handle on the possible risks to the environment associated with the use of genetic engineering technologies in domestic farm livestock.

This brings me to the hypothesis of this experiment, that the alpha-lactalbumin transgene is not horizontally transmitted from transgenic to non-transgenic swine.

CHAPTER 2

LITERATURE REVIEW

The following review highlights several important papers that have played key roles in the development of transgenic animals, and why the risk of the genes transferring from a transgenic animal to a non-transgenic animal is important.

Development of Transgenic Animals

The first technique to produce transgenic animals was by DNA microinjection. It was applied to mice by Gordon and Ruddle (1981). This paper also included the first use of the term "transgenic." This method involves the direct microinjection of a chosen gene construct from another member of the same species or from a different species, into the pronucleus of a fertilized ovum. The introduced DNA may lead to the over-or-under expression of certain genes or to the expression of genes entirely new to the animal species. The insertion of DNA is, however, a random process, and there is a high probability that the introduced gene will not insert itself into the site on the host DNA that will permit its expression. The manipulated fertilized ovum is transferred into the oviduct of a recipient female, or foster mother that has been induced to act as a recipient by mating with a vasectomized male (Gordon and Ruddle, 1981). Microinjection of DNA into the pronucleus of a fertilized ovum has been the most widely used and most successful method for production transgenic mice (Wheeler et al., 2003). A major advantage of this method is its applicability to a wide variety of species.

Another technique that has been developed is retrovirus-mediated transgenesis (Jaenisch, 1976). Retrovirus-mediated gene transfer is used to increase the probability of gene expression. Gene transfer is mediated by means of a carrier or vector, generally a virus or a plasmid. Retroviruses are commonly used as vectors to transfer genetic material into the cell, taking advantage of their ability to infect host cells in this way. Transmission of the transgene is possible only if the retrovirus integrates into some of the germ cells.

A third technique demonstrates that blastocyst-derived embryonic stem cells can be used as a vehicle for transgenesis (Grossler et al., 1986). This method involves prior insertion of the desired DNA sequence by homologous recombination into an in-vitro culture of embryonic stem cells. Stem cells are undifferentiated cells that have the potential to differentiate into any type of cell and therefore to give rise to a complete organism. These cells are then incorporated into an embryo at the blastocyst stage of development (Grossler et al., 1986).

The production of transgenic livestock carries a large investment because of low efficiency steps to produce a few founder animals (Seidel 1993). This founder animal is produced by the injection of DNA into hundreds of embryos, which then are transferred to recipients for gestation to birth if pregnancy occurs. Then after birth the animal, a biopsy is done then the use of a polymerase chain reaction (PCR) to see if the transgene was transferred or not.

Applications of Transgenic Animals

There are numerous applications where transgenic livestock can be used for to improve specific needs to society. Transgenic animals can have great importance to many aspects of

biomedical science, including gene regulation, the immune system, cancer research, developmental biology, biomedicine, manufacturing and agriculture (Wheeler 2007). Applications for production agriculture include: improved milk production and composition, increased growth rate, improved feed usage, improved carcass composition, increased disease resistance, and enhanced reproductive performance.

In transgenic animals that could be used as a food source, the incorporation of a growth hormone into an animal's genome which would make the animal grow faster in a shorter time period. This has been performed with rainbow trout where eggs from a slow growing wild strain of trout were microinjected with a salmon gene construct overexpressing growth hormone (Devlin et al., 2001). These transgenic trout grew much faster than non-transgenic sibling controls. Another example of this type of technology has also been done in swine (Pursel et al., 1997).

Other applications are directed to production of a particular protein in milk that could be used for medications and hormones. These types of animals may produce high volumes milk containing a particular substance, without endangering the animal's own health. One of the first examples of this included mice that express a clot dissolving enzyme, plasminogen activator (Gordon et al., 1987). Other examples include lambs producing the human clotting factor (Schneike et al., 1997) and cows that express human lactoferrin in their milk (Krimpenfort et al., 1991).

Transgenic animals also may be used as disease models. This application is used to study different disease mechanisms so that proper therapy can be developed. For instance the

AIDS Mouse (Reid et., 2001), Alzheimer's Mouse (Duff et al., 1996), Oncomouse (Harvey et al., 1993), and Parkinson's Fly (Feany and Bender, 2000) are all transgenic models to study different diseases.

Another application for transgenic animals is for the production of donor organs. Certain species such as pigs can be altered to produce successful donors of tissues and organs for humans. In 2002, four pigs were produced which had one allele of the α -1,3-galactosyltransferase locus had been knocked out (Lai et al., 2002). This knock out is important because humans produce antibodies to the to protect against the α -1,3-galactosyltransferase. So with it knocked out the organs can be accepted by the human being.

Development of Transgenic Swine

Transgenic lines of pigs have been developed that overproduce the bovine milk protein alpha-lactalbumin (Bα-LA) (Bleck et al., 1998). The method of microinjection was used to produce these pigs containing the Bα-LA gene. The goals of producing these transgenic lines were to improve piglet weaning weight, neonatal health, and to provide a model for study of the role of α-LA on lactose synthesis and lactation in swine.

Transgenic swine containing a mammary specific Bα-LA transgene have been shown to have significantly increased milk production (Noble et al., 2002; Wheeler, 2003; Marshall et al., 2006). This increased milk production has resulted in increased weaning weights in piglets suckling these transgenic sows (Noble et al., 2002). The overall hypothesis evaluated in those previous studies was that higher expression of the rate limiting enzyme involved in milk

synthesis would result in higher milk production and/or higher concentration of milk components. There is evidence that the lactose synthase complex may be limiting in the production of lactose and milk volume (Grimble etal., 1987). This complex is composed of two proteins. β1, 4-galactosyltransferase, the enzymatic subunit of lactose synthase, is the subunit complex that is responsible for producing lactose in the mammary gland. Alpha-lactalbumin is the second component of this complex and is thought to be the rate-limiting subunit. The bovine, murine and porcine α-LA genes have each been sequenced (Brew and Grobler, 1992; Vilotte and Soulier, 1992; Das Gupta et al., 1992). As the primary osmotic component of milk, lactose draws water into mammary secretory vesicles until milk is iso-osmotic. Thus, the amount of lactose produced is proportional to the volume of milk generated. Despite the obvious importance of this complex to milk synthesis and the application to agricultural species, the limitations to increased lactose synthesis are not understood. Based on the initial data in pigs, α-LA is limiting for lactose production in early lactation (Noble et al., 2002). Furthermore, increased lactose production and lactogenesis has led to higher milk production and faster piglet growth (Noble et al., 2002; Marshall et al., 2006).

The idea that the lactose synthase complex is limiting for milk production arose from the observation that the primary osmotic component in milk is lactose. Because lactose cannot diffuse out of secretory vesicles, water is drawn into the vesicles to balance the osmotic pressure. Since lactose synthase is necessary for production of lactose and the subsequent movement of water into the mammary secretory vesicles, it is critical in the lactational control and secretion of milk (Hayssen and Blackburn, 1985; Brew and Grobler, 1992).

Unlike the ubiquitous β1, 4-galactosyltransferase, α-LA is a mammary–specific protein, the expression of which is regulated by numerous hormones and growth factors (reviewed by Tucker, 1981; Forsyth, 1983; Kuhn et al., 1980; Vonderhaar, 1987; Ziska et al., 1988; Brew and Grobler, 1992). Among the proteins found in milk, it is unique in that its expression is tightly coupled to the onset of lactation after the gland is fully differentiated, suggesting that the regulation of expression of the α-LA gene is fundamentally distinct from that of other most milk proteins (Goodman and Schanbacher, 1991). Most milk proteins (i.e. caseins, β-lactoglobulin) are found in the mammary glands of pregnant animals as soon as secretory cells begin to differentiate in early to mid-pregnancy. However, α-LA is not found in the mammary gland at this time, rather it appears only immediately before birth. Because α-LA is tightly correlated to milk production, it may be limiting for lactose synthesis. Several studies have shown that reducing α-LA reduces milk production. Rats fed low protein diets produced less milk and lactose, but β1, 4-galactosyltransferase content of the glands remained constant (Grimble and Mansaray, 1987).

Additional evidence that α-LA is limiting arises from studies of cows treated with exogenous growth hormone. Cows injected with this hormone produce higher milk volumes and the concentration of α-LA in the milk of these cows is higher compared with controls (Eppard et al., 1985). Other milk proteins and milk components showed no significant changes. This effect of growth hormone on α-LA has also been observed in bovine mammary explants cultured in immune-compromised nude mice (Sheffield et al., 1987). Mammary explants from cows selected for milk production have greater ability to secrete α-LA (McFadden et al., 1989). When stimulated by lactogenic hormones, mammary explants from Holstein (dairy) heifers

secrete 30 times more α-LA than explants from Angus (beef) heifers. However, another group found that a 10% increase in α-LA induced by prolactin injections had only a negligible effect on milk production (Plaut et al., 1987).

Alpha-Lactalbumin

This study makes extensive use of expression of the transgene specifically in mammary tissue, a procedure that is routinely being performed by several laboratories, including our own (Simons et al., 1987; Vilotte et al., 1989; Bleck and Bremel, 1994; Bleck et al. ,1996; Bleck et al., 1998). The 5' flanking regions of many milk protein genes, which have a regulatory function have been used to drive expression of foreign proteins in mammary epithelial cells of transgenic animals (Simons et al., 1987; Vilotte et al., 1989). Of all the bovine milk protein genes, the expression of bovine alpha-lactalbumin is the most tightly regulated and lactation-specific (Goodman and Schanbacher, 1991; Mao et al., 1991). Milk synthesis and lactation are very stage specific developmental processes. The development of the mammary gland and the subsequent secretion of milk and milk proteins are a direct result of pregnancy and the secretion of the hormones and growth factors associated with gestation (Vonderhaar and Ziska, 1989). Many of these hormones regulate the expression of the α-LA gene. The unique expression of the bovine α-LA gene makes its promoter and regulatory elements a useful mammary expression system in transgenic animals. In contrast to the caseins and beta-lactoglobulin, the production of α-LA mRNA and protein shows a dramatic rise at parturition, remains elevated during lactation and drops sharply after the cessation of milk removal and initiation of involution.

Transgenic mice have been produced using the α-LA 5' region to drive the expression of bovine, caprine or guinea pig α-LA transgenes in the mammary gland of mice (Vilotte et al., 1989; Mashio et al., 1991; Soulier et al., 1992; Bleck and Bremel, 1994). Production of exogenous alpha-lactalbumin in the milk of these mice ranged from undetectable levels to up to 3.7 mg/mL in a line of mice producing caprine α-LA. We have previously shown that overexpression of α-LA in swine increases milk production and piglet weaning weight (Noble et al., 2002).

Swine Production Concepts

Current swine production management schemes attempt to maximize the number of piglets born per litter and piglet survival (Hartmann et al., 1984). The first week postpartum is the period of greatest loss for US swine producers. This is mainly because of malnutrition and scours of the baby piglets. In addition, pork producers have continuously reduced lactation lengths in order to maximize the number of piglets born per sow per year. Currently in the swine industry 18-21 day (d) lactation periods are common. In order to get maximum growth from larger litter sizes with shorter lactations, increased milk production in early lactation must occur. Increased litter sizes from selected high genetic merit sows make milk production one of the most important limiting factors in piglets' growth. In fact, studies indicate that milk production and milk composition of the sows accounts for 44% of the growth weight of the piglets (Lewis et al., 1978). Combined, these types of studies illustrate the limiting nature of sow lactation on growth and development of the litter (Boyd and Kensinger, 1998).

The effect of increasing sow milk production on U.S. pork production is dramatic. Using current milk production values (Auldist et al., 1998) we estimate that increasing milk production 10% would result in an additional $2.46 per litter, which would be worth $28.4 million/year in the U.S. due to increased weight gains prior to weaning using a typical hog price of $50/cwt. Modern sows are able to produce about 1 kg of milk/piglet for litter sizes up to 14 piglets (Auldist et al., 1998). This does not consider decreased feed and labor costs associated with rearing pigs with heavier weaning weights.

Risk Assessment

Several key questions remain to be answered with respect to these alpha-lactalbumin transgenic pigs: will the transgene be expressed in the animal in tissues other than the mammary gland, and will the transgene be transferred to other animals in production environments? Regulatory agencies have provided insight into some of the risks that need to be assessed. For example, risks may arise from ingestion of meat or other products from an animal that: contains the transgene in its chromosal DNA, has been reared with transgenic animals, has been bred to or produced litters by an animal containing the transgene, and has ingested the transgene or transgenic milk protein.

Based upon the lack of direct assessment of these types of risks, this study was designed to determine whether Bα-LA is expressed in tissues of transgenic swine other than the lactating mammary gland and if the Tg DNA crosses into non-transgenic swine under various physiological and physical conditions. The specific aims addressed in this study were to determine (1) whether the Tg can be synthesized in any other tissues than the mammary gland

of a transgenic sow; (2) whether the Tg can be transferred directly by physical association or contact; (3) whether the Tg can be transferred directly via mating; and (4) whether the Tg can be transferred directly during gestation, parturition, or lactation.

CHAPTER 3

MATERIALS AND METHODS

Animals and Tissue Collection

Experimental protocols for this study were approved by the University of Illinois Institutional Animal Care and Use Committee. In all experiments, non-transgenic pigs (control = C) and pigs heterozygous for the Bα-LA transgene (transgenic = T) were used. The T pigs were produced as described previously (Bleck et al., 1998). All animals above 25 kg were euthanized by restraining them with a limp rope snare and injecting sodium pentobarbital via a peripheral ear vein (65 mg/ml; equivalent to a dosage of 20-25 mg/kg). All animals less than 25 kg were euthanized by inhalation of CO_2. All carcasses were disposed of by incineration.

All tissue samples were removed using aseptic techniques. Tissues were cut into ~0.5 mm cubes and immediately frozen, and stored in liquid nitrogen until processing. The DNA was extracted from tissues using the method described by Hogan et al. (1986). DNA was amplified by polymerase chain reaction (PCR) using the method described by Monaco et al. (2005). Total cellular RNA was isolated from the tissues as previously described by Monaco et al. (2005) and PCR was performed to identify the transgene was performed using methods of Bleck and Bremel (1994).

Screening for the Expression of the Bovine α-lactalbumin Tg

Total RNA were isolated from the tissues and subjected to reverse transcriptase polymerase chain reaction using random oligo d (T) primers. Concentrations of cDNA were

determined by spectrophotometry at 260 and 280 nm. The resulting cDNA samples were subjected to RT-PCR using Taqman® Mastermix Reagent Kit (Applied Biosystems, Foster City, CA). Primers sets were designed to detect a 71 base pair DNA sequence within the 3′ un-translated region of the bovine transgene transcript and a 78 base pair DNA sequence within the 3′ un-translated region of the endogenous porcine α-LA gene transcript as a control for the reaction. The forward and reverse primers that were used to amplify the bovine α-LA sequence are 5′-GACATGTAAGGACTAATCTCCAGGG-3′ and 5′AGGGACATCGAGCAA-GGGT-3′, respectively. The forward and reverse primers were be used to amplify the porcine α-LA sequence are 5′-GTAGTGATTGTTATCCGGACACTATTCT-3′ and 5′-GGGCACTGAGCA-AAGGTTAAAA-3′, respectively. Regions of the cDNA amplified by these primer sets have at least 4 to 8 base pair mismatches in the cDNA sequences of the bovine transgene and the porcine α-LA gene transcripts to allow for sequence discrimination. A 20 to 25 base pair sequence that is located between the primer sets was used to design fluorescence reporter probes specific for the bovine transgene and the porcine α-LA transcripts. Each probe contains a fluorescent dye conjugated to the 5′ end of the probe sequence and a quencher dye to suppress probe fluorescence conjugated to the 3′ end of the probe. The sequences of the bovine and porcine α-LA probes are 5′-6FAM-ATGAATGGCGCTCTGGACTT-TAMRA-3′ and 5′-VIC AGAGATGCGTGACTGGTGCAC-TGGA-TAMRA-3′, respectively. The 18 s primers and probe sets were run with all samples to normalize the data. The forward and reverse primers that were used to amplify 18s sequence are 5′-GATCCATTGGAGGGCAAGTCT-3′ and 5′-AACTGCAGCAACTTTAATATACGCTATT-3′, respectively. The 18s probe sequence is 5′-6FAM-TGCCAGCAGCCGCGGTAATTC-TAMRA3′ (Figure 1).

Screening for the Bovine α-lactalbumin Tg

DNA from tissue samples or biopsies was extracted (Hogan et al., 1986). Nested Polymerase chain reaction (PCR) was performed using 10 μl 10x PCR reaction buffer (500 mM KCl, 100 mM Tris-HCl (pH=8.8), 15 mM $MgCl_2$, 1% Triton X-100), 200 mM each dNTP, 1.0 μM each primer (Primers 1 and 2 will span a portion of the α-lactalbumin promoter and coding sequence (5'-AAAAAGGTTGGGTCACTCTT-3' 1160/1630 Forward and 5'-ATTGCTTCACTTGTATTACCC-3' 1160/1630 Reverse) and the nested primers 3 and 4 (5'-ACTCTGAGGCTGTCTACAAG-3' 1198/1473 Forward and 5'-CTTGGTTCCTTGTTGAGTGG-3' 1198/1473 REVERSE) will amplify a target sequence inside of the original primer pair), 1 unit Taq DNA polymerase and 1 μg genomic DNA. Volumes were adjusted to 100 μl with double distilled filter sterilized water and reaction is overlaid with light mineral oil. Samples were subjected to two rounds of 30 cycles (94˚C 2 min., 50˚C 1.5 min., 72˚C 1.5 min.). The first round was with primers 1 and 2 and the second round was with the nested primer pair 2 and 4. This gave us the sensitivity to detect a single copy of the target TG DNA. Products were separated in an 1% agarose gel and stained with ethidium bromide. DNA containing the TG will produce a 700 bp band corresponding to a portion of the bovine α-lactalbumin 5' flanking region from the first round amplification. The second round amplification will produce a 276 bp band corresponding to the nested DNA sequence (Figure 2).

Statistical Analysis

The PROC FREQ procedure of SAS (SAS Inst. Inc., Cary, NC) was used to perform a Fisher's exact test. The association between the binary variable gene expression (yes or no) and

objective evaluated using a generalized logistic model. The association between the binary response variable gene expression (yes or no) and the genetics (non-transgenic or transgenic) was evaluated by using a chi-square test.

The statistical power of the experiment was calculated for a wide range of conservative scenarios (Lipsey, 1990). For these power calculations, a type I error rate equal to 0.05 was used. Binomial and Normal distributions were assumed to test the null hypotheses of no statistical evidence of Tg in the non-transgenic group and no statistical significant difference in Tg quantities between T and non-T groups, respectively. Table 1 summarizes the power for different sample sizes per group, different hypothesis (absence or difference between groups) and magnitude of differences between transgenic and non-transgenic groups.

Experimental Design

The objective of experiment 1 was to determine whether the Tg was expressed in any other tissue than the lactating mammary gland. A total of 4 T pigs (2 males and 2 females) and 6 C pigs (1 male and 5 females) were used. Pigs were raised to 180, 220, 250 d of age, or 112 d post-breeding and then sacrificed for tissue collection. Tissues collected from each pig included blood, brain, jejunum, kidney, liver, lung, mammary gland, muscle, ovary, sublingual salivary gland, skin, and spleen (Table 2). There have been reports of the Tg showing low-level expression in the skin (sebaceous gland) (Maschio et al., 1991). PCR was used to identify the presence or absence of the transgene.

The objective of experiment 2 was to determine whether co-habitation of T and C pigs would result in transfer of the Tg to the C pigs. For this experiment 41 T pigs (24 males and 17

females) and 54 C pigs (29 males and 25 females) were used (Table 3). These pigs were raised in pens of four (2 T pigs and 2 C pigs) from weaning (approx. 21 d of age) until they reached either 180, 220 or 250 d of age. At each time 2 T males, 2 T females, 2 C males, and 2 C females were euthanized. Tissues taken from each pig included the blood, brain, jejunum, kidney, liver, lung, mammary gland, muscle, skin, ovary, sublingual salivary gland, and spleen (Table 4). Presence of the Tg was determined by PCR.

The objective of experiment 3 was to determine if there was transfer of the Tg from T pigs to C pigs as a result of mating. Experiment 3 used 4 T females, 34 C females, and 12 C males (Table 5) for tissue sampling. Pigs were raised in routine production settings until they reached breeding age (~7 months) at which time the C females were mated to one of 10 T boars. Females then were sacrificed at 2, 7, 90, or 112 d post-mating and tissue samples were obtained from brain, cervix, jejunum, kidney, liver, lung, mammary gland, muscle, ovary, oviduct, sublingual salivary gland, skin, spleen, uterus, and vagina (Table 4). The C males were allowed to naturally mate a T female and then the C males were sacrificed at 7 days post-mating. Samples of the blood, bulbourethral gland, epididymis, kidney, liver, lung, muscle, penis, skin, spleen, testis, and urethra were collected from the C males (Table 4). Presence of the Tg was determined by PCR.

The objective of experiment 4 was to evaluate the potential Tg transfer from T pigs to C pigs during parturition, gestation, or lactation. Experiment 4 was divided into three sub-experiments. In sub-experiment 4a, 4 C sows were bred to a C boar and 4 T sows were bred to a C boar. Sows were gestated in a routine production setting and allowed to farrow and piglets

were removed immediately after parturition (13 males and 15 females from C sows and 15 males and 15 females from T sows; (Table 6), and 4 C sows were bred to a C boar and 4 T sows were bred to a C boar. Sows gestated in a normal production setting for 112 d of gestation at which point they were euthanized and fetuses collected (15 males and 10 females from C sows and 17 males and 14 females from T sows; Table 6). For sub-experiment 4b, newborn C piglets were removed from their birth dam before they suckled and immediately cross-fostered to a lactating T sow (5 male and 6 female piglets; Table 7) or to a lactating C sow (6 male and 4 female piglets). The methods used for cross-fostering are described in Figure 3. Piglets were allowed to suckle for 24 h (5 suckling T sow, 5 suckling C sow) or 72 h (6 suckling T sow, 5 suckling C sow) before sacrifice. In sub-experiment 4c, C piglets were allowed to suckle their birth dam until day 3 and then fostered to a lactating T sow (7 male and 4 female piglets; Table 7) or to a lactating C sow (2 male and 6 female piglets) (Figure 3). After cross-fostering, the piglets were allowed to suckle for 72 h (5 suckling T sow, 3 suckling C sow) or 168 h (4 suckling T sow, 4 suckling C sow) before sacrifice. Samples collected from all piglets included blood, jejunum, liver, lung, and muscle (Table 4). Presence of the Tg was determined by PCR.

Figure 1. Example PCR gel to show the presence or absence of the Tg expression in other tissues than the lactating mammary gland. (Lane 1. Day 12 of lactation control, 2. Day 7 of lactation control, 3. H2O control, 4-13. Transgenic tissue samples, 14-16. Non-transgenic tissue samples, 17-19. Transgenic tissue samples, 20. 10 kb Ladder, 21. Day 15 of lactation control, 22. Day 7 of lactation control, 23. H2O control, 24–26. Non-transgenic tissue samples, 27-30. Transgenic tissue samples, 31-34. Non-transgenic tissue samples, 35-39. Transgenic tissue samples, 40. 10kb Ladder)

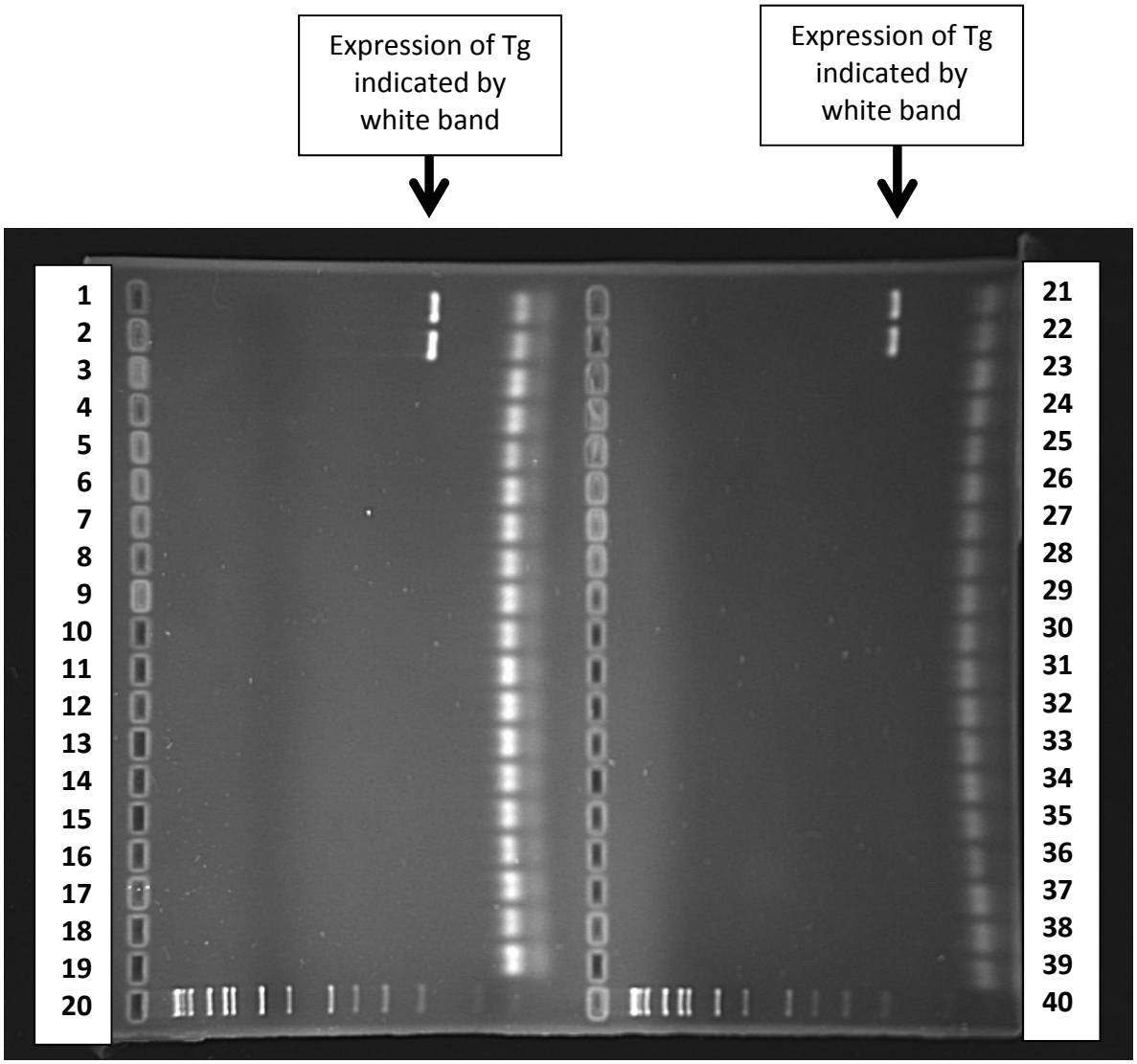

Figure 2. Example PCR gel to show the presence or absence of the Tg of a non-transgenic pig that has close environmental contact to a transgenic pig containing the bovine milk protein alpha-lactalbumin. (Lane 1. + Alpha Lactalbumin control, 2. H2O control, 3. – Alpha Lactalbumin control, 4-13. Non-transgenic pig tissues, 14. 10 kb ladder)

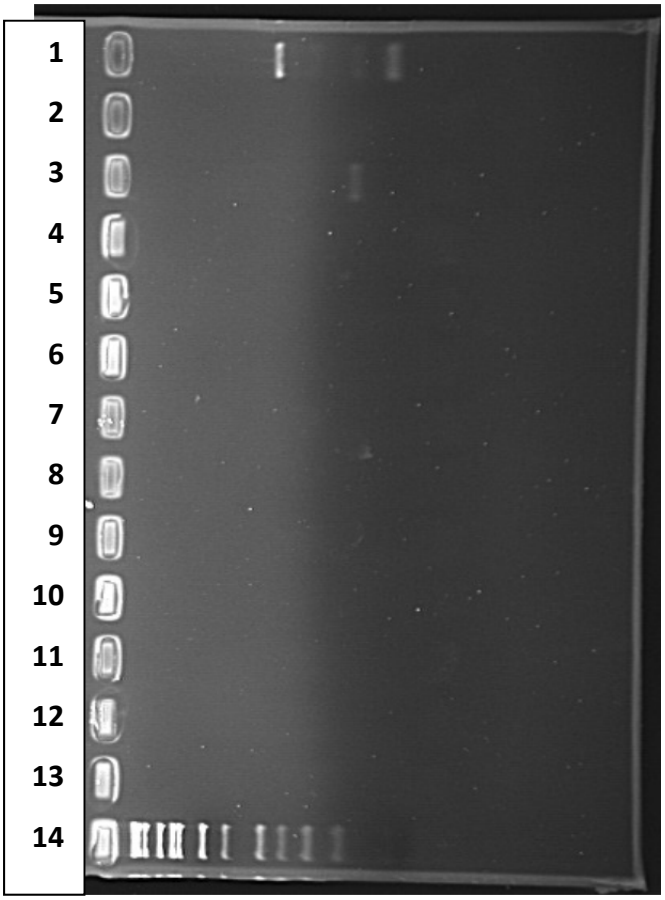

Figure 3. Methods used to cross-foster piglets in experiments 4b and 4c.

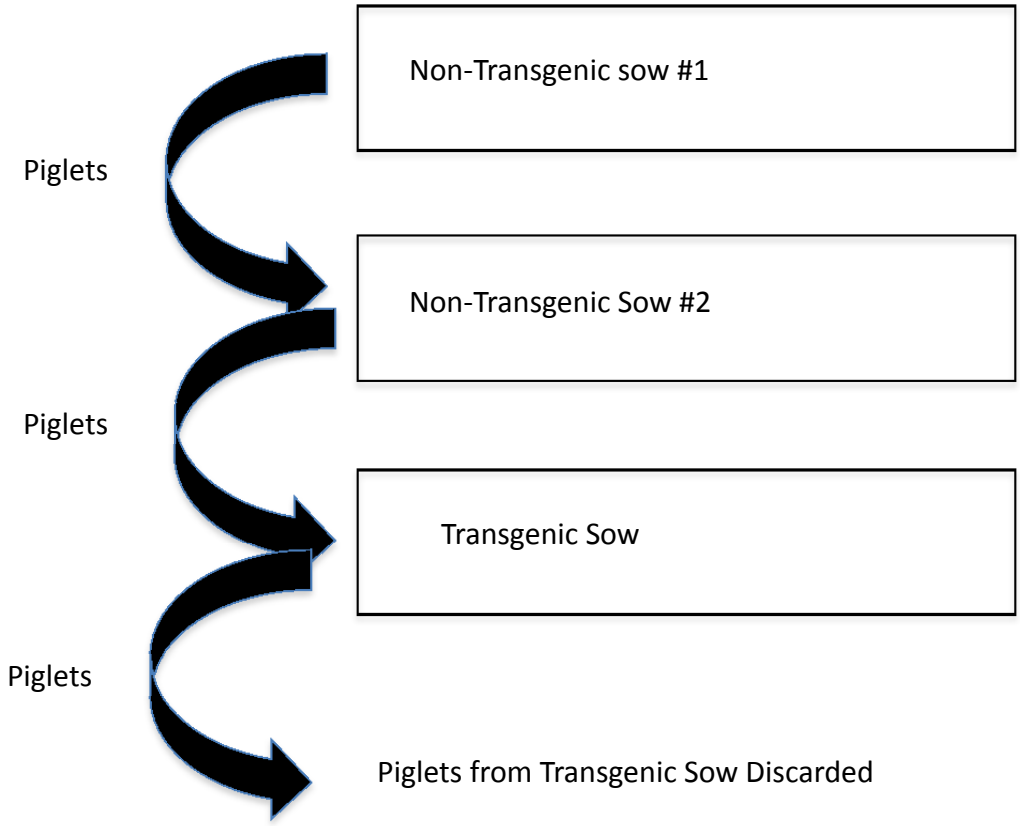

Table 1. Power Calculations

Sample size/group	Probability of positive Tg test			Standardized differences between group		
	1%	2.5%	5%	1	5	10
2	0.7826	0.7076	0.6101	0.0573	0.7268	0.9877
4	0.9826	0.9435	0.8863	0.175	0.9982	0.9999
10	0.9999	0.9999	0.9988	0.5532	0.9999	0.9999

Table 2. Tissue samples used for Experiment 1.

180 d cohab. **220 d cohab.** **250 d cohab.** **112 d post-breeding**	blood, brain, jejunum, kidney, liver, lung, mammary gland, muscle, ovary, sublingual salivary gland, skin, spleen

Table 3. Animals used for cohabitation (Experiment 2).

				Non-Transgenic		Transgenic[1]	
	# of Animals[2]	# Non-transgenic Animals[3]	# Transgenic Animals[4]	Males	Females	Males	Females
180 d cohab.	28	16	12	8	8	5	7
220 d cohab.	27	16	11	8	8	6	5
250 d cohab.	40	22	18	13	9	13	5
Total	**95**	**54**	**41**	**29**	**25**	**24**	**17**

[1] DNA was isolated from ear biopsies to determine that the Tg was present at birth of these animals.
[2] Total number of animals that were T and non-T combined used in experiment 2.
[3] Number of animals that were non-T used in experiment 2.
[4] Number of animals that were T used in experiment 2.

Table 4. Tissue samples collected in Experiments 2, 3, and 4.

Experiment	Objective	Tissues collected
Experiment 2[1]	**180 d cohab.** **220 d cohab.** **250 d cohab.**	blood, brain, jejunum, kidney, liver, lung, mammary gland, muscle, ovary, sublingual salivary gland, skin, spleen
Experiment 3[2]	**2 d post-breeding** **7 d post-breeding** **90 d post-breeding** **112 d post-breeding**	brain, cervix, jejunum, kidney, liver, lung, mammary gland, muscle, ovary, oviduct, sublingual salivary gland, skin, spleen, uterus, vagina, bulbourethral gland, epididymis, penis, testis
Experiment 4[3]	**112 d piglets**	external placenta, internal placenta, jejunum, liver, lung, muscle, spleen
	Newborn/Non-suckle **d 0 cross-foster** **d 3 cross-foster**	Blood, jejunum, liver, lung, muscle

[1] Experiment to determine if cohabitation of T and non-T pigs would result in transfer of the Tg to the non-T pigs.
[2] Experiment to determine if the Tg would transfer from T pigs to non-T pigs as a result of mating.
[3] Experiment to determine if Tg would transfer from T pigs to non-T pigs during partition, gestation, or lactation.

Table 5. Non-transgenic animals used for mating (Experiment 3).

	# of Animals[1]	Males	Females
2 d post-breeding	10	0	10
7 d post-breeding[2]	22	12	10
90 d post-breeding	10	0	10
112 d post-breeding	4	0	4
Total	**46**	**12**	**34**

[1] Total number of animals that were non-T used in experiment 3.
[2] Includes the males and the females from which tissues were collected at 7 d post-breeding.

Table 6. Animals used for parturition and gestation (Experiment 4a).

	# of Animals[2]	# Non-Transgenic Animals[3]	# Transgenic Animals[4]	Non-Transgenic		Transgenic[1]	
				Males	Females	Males	Females
Newborn/Non-suckle[5]	58	28	30	13	15	15	15
112 d Fetuses[6]	56	25	31	15	10	17	14
Total	**114**	**53**	**61**	**28**	**25**	**32**	**29**

[1] DNA was isolated from ear biopsies to determine that the Tg was present at birth of these animals.
[2] Total number of animals that were T and non-T used in sub-experiment 4a.
[3] Number of animals that were non-T used in sub-experiment 4a.
[4] Number of animals that were T used in sub-experiment 4a.
[5] Piglets that were allowed to farrow normally, but were not allowed to suckle their birth dam.
[6] Fetuses collected at 112 d post-breeding.

Table 7. Animals used to cross-foster (Experiments 4b and 4c).

	# of Animals[1]	Males	Females
0 d cross-foster[2]	21	11	10
3 d cross-foster[3]	19	9	10
Total	**40**	**20**	**20**

[1] Number of animals that were non-T and used in experiments 4b and 4c .
[2] Non-T piglets that were fostered to either a lactating T sow or a non-T sow immediately after birth before and they were allowed to suckle their birth dam.
[3] Non-T piglets that were fostered to either a lactating transgenic sow or a non-T sow at 3 d after birth.

CHAPTER 4

RESULTS

Experiment 1

A total of 50 tissue samples were analyzed to determine if the Tg was expressed only in the mammary gland of a T lactating sow. All the samples from C and T pigs were negative for the expression of the transgene, including mammary tissue (Table 8).

Experiment 2

A total of 365 samples were tested from the C animals that had direct association or physical contact with T animals. All samples were negative for the Bα-LA transgene (Table 9). Conversely, all samples from T pigs (n=206) were positive for the Tg.

Experiment 3

A total of 343 samples were analyzed from C sows mated to T boars or from C boars mated to T sows. All samples tested were negative for the Bα-LA transgene (Table 10).

Experiment 4

To determine if the Tg can be transferred to C fetuses during gestation, 285 samples were analyzed (Table 11). Samples from 112 day fetuses 127 samples were derived from mating C females to C males were all negative for Tg. Of samples derived from 112 day fetuses from mating a T female to a C male 99 were positive and 59 were negative for Tg. This is consistent with the T females being heterozygous for the Tg. The exception was the outer placental

membrane, derived from the maternal reproductive tract, which was positive for all fetuses from the T sows (Table 12).

To assess whether the Tg can be transferred through the birthing process, 243 tissue samples were collected from newborn piglets prior to suckling the dam (Table 11). All the samples derived from mating C females to C males were all negative for the Tg. Of those samples taken from piglets derived from mating T females to C males 79 were positive and 52 were negative for Tg. Again, this is consistent with the T females being heterozygous for the Tg.

To assess whether the Tg can be transferred through ingestion of colostrum or milk, tissue samples were collected from C piglets that were cross-fostered to T sows either at 0 d (prior to consuming the birth sow's colostrum; n=87 samples) or at 3 d post-partum (after consuming the birth sow's colostrum (n=70 samples). All the samples were negative for the Tg (Table 13).

Table 8. Presence or absence of Tg expression in tissue samples of non-T and T swine (Experiment 1).

				Non-Transgenic		Transgenic[1]	
	# of tissue samples[2]	Non-Transgenic tissue samples[3]	Transgenic tissue samples[4]	Present[5]	Absent[6]	Present[5]	Absent[6]
180 d cohab.	6	3	3	0	3	0	3
220 d cohab.	4	4	0	0	4	0	0
250 d cohab.	12	4	8	0	4	0	8
112 d post-breeding	28	17	11	0	17	0	11
Total	**50**	**28**	**22**	**0**	**28**	**0**	**22**

[1] DNA was isolated from ear biopsies to determine that the Tg was present at birth of these animals.
[2] Total number of animals that were T and non-T combined used in experiment 1.
[3] Number of animals that were non-T used in experiment 1.
[4] Number of animals that were T used in experiment 1.
[5] Number of tissue samples that had the presence of Tg expression.
[6] Number of tissue samples that had the absence of the Tg expression.

Table 9. Number of tissue samples analyzed for cohabitation (Experiment 2).

				Non-Transgenic		Transgenic[1]	
	# of tissue samples[2]	# Non-Transgenic tissue samples[3]	# Transgenic tissue samples[4]	Present[5]	Absent[6]	Present[5]	Absent[6]
180 d cohab.	282	169	113	0	169	113	0
220 d cohab.	127	80	47	0	80	47	0
250 d cohab.	162	116	46	0	116	46	0
Total	**571**	**365**	**206**	**0**	**365**	**206**	**0**

[1] DNA was isolated from ear biopsies to determine that the Tg was present at birth of these animals.
[2] Total number of animals that were T and non-T combined used in experiment 2.
[3] Number of animals that were non-T used in experiment 2.
[4] Number of animals that were T used in experiment 2.
[5] Number of tissue samples that had the presence of Tg.
[6] Number of tissue samples that had the absence of the Tg.

Table 10. Number of tissue samples analyzed for mating (Experiment 3).

	# of tissue samples[1]	Present[2]	Absent[3]
2 d post-breeding	89	0	89
7 d post-breeding[4]	166	0	166
90 d post-breeding	71	0	71
112 d post-breeding	17	0	17
Total	**343**	**0**	**343**

[1] Number of samples that were analyzed for the Tg in experiment 3 from non-T animals.
[2] Number of tissue samples that had the presence of Tg.
[3] Number of tissue samples that had the absence of the Tg.
[4] The 7 d post-breeding number includes the male and the female samples from experiment 3.

Table 11. Number of tissue samples analyzed for parturition and gestation (Experiment 4a).

				Non-Transgenic		Transgenic[1]	
	# of tissue samples[2]	# Non-Transgenic tissue samples[3]	# Transgenic tissue samples[4]	Present[5]	Absent[6]	Present[5]	Absent[6]
Newborn /Non-suckle[7]	243	112	131	0	112	79	52
112 d fetuses[8]	285	127	158	0	127	99	59
Total	**528**	**239**	**289**	**0**	**239**	**178**	**111**

[1] DNA was isolated from ear biopsies to determine that the Tg was present at birth of theseanimals.
[2] Total number of tissue samples that were T and non-T combined used in sub-experiment 4a.
[3] Number of tissue samples that were non-T used in sub-experiment 4a.
[4] Number of tissue samples that were T used in sub-experiment 4a.
[5] Number of tissue samples that had the presence of Tg.
[6] Number of tissue samples that had the absence of the Tg.
[7] Piglets that were allowed to farrow normally, but not allowed to suckle their birth dam.
[8] Fetuses that were collected at 112 d post-breeding.

Table 12. Distribution of Tg among tissue samples from 112 d fetuses from heterozygous transgenic sows mated to non-transgenic boars (Experiment 4).

		Tg Present[1]	Tg Absent[2]
Fetal Tissue[3]	Muscle	13	10
	Lung	13	10
	Liver	13	10
	Jejunum	13	10
	Internal Placenta	13	10
Maternal Tissue[4]	External Placenta	23	0

[1] Number of tissue samples that had the presence of Tg.
[2] Number of tissue samples that had the absence of the Tg.
[3] Tissue samples that were derived from the fetus.
[4] Tissue samples that were derived from the dam.

Table 13. Number of tissue samples analyzed for cross-fostering (Experiments 4b and 4c).

	# of tissue samples[1]	Present[2]	Absent[3]
d 0 cross-foster[4]	87	0	87
d 3 cross-foster[5]	70	0	70
Total	**157**	**0**	**157**

[1] Number of samples that were from non-T animals and analyzed for experiments 4b and 4c.
[2] Number of tissue samples that had the presence of Tg.
[3] Number of tissue samples that had the absence of the Tg.
[4] Non-T piglet samples that were analyzed from piglets that were fostered to either a lactating T sow or a non-T sow immediately after birth before they were allowed to suckle their birth dam.
[5] Non-T piglet samples that were analyzed from piglets that were fostered to either a lactating T sow or a non-T sow at 3 d after birth.

CHAPTER 5

Discussion

The present study demonstrates that the Bα-LA Tg 1) is not expressed in tissues other than the mammary gland of lactating transgenic sows; 2) is not transferred from transgenic animals to the genome of non-transgenic animals through direct contact; 3) is not transferred to the genome of control females or males through mating with a transgenic animal; and 4) is not transferred to the genome of a non-transgenic fetus during gestation in a transgenic mother, nor transferred to the genome of a non-transgenic neonate through suckling a transgenic sow. These results indicate that the transfer of genetic material from a transgenic animal to a non-transgenic animal does not result in alteration of the genome of the non-transgenic animal.

Lactogenesis involves the changes in the mammary epithelial cells that allow the development from the relatively undifferentiated mammary gland in pregnancy to full lactation sometime after parturition (Neville et al., 2001). Lactogenesis in the porcine mammary gland, like other species, can be divided into two stages. Stage I occurs between days 90 and 105 of gestation and stage II between d 112 of gestation and the onset of early lactation (Kensinger et al., 1982; Kensinger et al., 1986). During stage II of lactogenesis, the onset of copious milk secretion begins when the release of inhibitory effects of progesterone on lactogenesis and the stimulation by the very high blood concentrations of prolactin and glucocorticoids associated with parturition occur. Interestingly, in this study there was no evidence shown that the Bα-LA Tg was expressed in the mammary gland of a sow at d 112 of gestation.

In swine production, pigs are regularly housed in groups, so it is imperative to determine if cohabitation of transgenic animals with non-transgenic animals results in transfer of Tg among the animals. In this study, the animals were able to perform normal behaviors like ingesting bodily fluids, licking, rubbing, and biting during cohabitation. This was done to look at the horizontal transmission of the Bα-LA Tg from one animal to the other though things like oral fluids, skin cells, tears, urine, and other bodily fluids. There is evidence that pigs group housed have a higher percentage of transmission of diseases such as Salmonella (Wilkins et al. 2010) than those that are individually housed. In contrast to transmission of contagious pathogenic organisms, this study demonstrated that Bα-LA Tg was not horizontally transmitted among animals during cohabitation. This study also evaluated the horizontal transmission of the Bα-LA Tg through reproduction and exposing the control animals to Bα-LA Tg animals during mating. This is a concern because it is likely that during copulation there is an exchange of cells from the genitals that could of 'contaminated' the female reproductive tract with the Tg. Interestingly, this study did not find any evidence for horizontal transmission of the Bα-LA Tg. Ejaculated sperm and leukocytes found in semen apparently are rapidly cleared from the reproductive tract and DNA from those cells does not become integrated as a result of horizontal transmission.

We observed that the external part of the placenta of non-transgenic fetuses carried by Tg female tested positive for the transgene, while the rest of the tissues tested negative. This is not surprising because the external part of the placenta, which is also referred to as the basal plate represents the maternal surface of placenta. The basal plate contains fetal trophoblasts and several of maternal cell types that include the decidual stroma cells, natural killer cells,

macrophages, and other immune cells (Huppertz, 2008). These maternal cells from a T female are detected by the PCR analysis.

The fourth experiment examined whether the Tg was transferred to the piglet while suckling colostrum from a T sow or suckling milk from a T sow. Two features of this early neonatal period are important for considering the results of this experiment. First, intestinal macromolecular closure occurs in newborn piglets between 18 and 36 h after birth (Westrom et al., 1984), coinciding with the period of high immunoglobulin concentrations in sow mammary secretions (Jackson et al., 1995), thereby defining the colostrum phase of lactation. And, second, both colostrum and milk of the sow contain concentrations of leukocytes (Hurley and Grieve, 1988). In spite of the presence of leukocytes in the colostrum and milk ingested by piglets, the Tg was not identified in any tissues of cross-fostered piglets in Experiment 4, indicating that ingested leukocytes do not transfer the Tg to the piglet's tissues.

Animals can be genetically modified to improve food animal production. Transgenic livestock can be used to improve milk production and composition, growth rate, feed utilization, disease resistance, reproductive performance, and prolificacy (Wheeler 2003).

The improvement of the composition and nutrients of milk may have a great influence on the survival and growth of newborns in both humans and animals. Milk production in sows is limiting to piglet growth and therefore pig production (Boyd and Kensinger, 1998; Wheeler 2003). A way to increase milk production in pigs may be achieved by changing the milk composition. We have previously shown that it is possible to increase milk production and piglet growth with these transgenic swine (Noble et al., 2002; Marshall et al., 2006; Wheeler, 2003). Lactose is a major osmole in milk. Lactose is formed inside the vesicles of the Golgi

apparatus of mammary secretory cells, transported to the apical membrane of the epithelial cells via secretory vesicles and secreted into the lumen. Lactose draws water into the vesicles by osmosis. More lactose means more volume of milk volume produced. Lactose is synthetized by the lactose synthase complex which is composed of the mammary-specific protein α-LA and the enzyme β-1,4 galactosyltransferase. Because the complex is necessary for the production of lactose and lactose is necessary for the movement of the water into the secretory vesicles and so into the lumen of the gland, it becomes evident that the complex is critical in the control of milk secretion. In particular, it was demonstrated that milk volume is directly related to the expression of the α-LA gene: α-LA gene expression correlates with the induction of copious milk secretion at the beginning of lactation (Goodman and Schanbacher, 1991).

With the purpose of increasing milk production in pigs we have previously produced two lines of transgenic pigs containing the Bα-LA gene (Bleck et al., 1998). The Bα-LA gene was chosen for a variety of reasons, such as: its expression is the most strictly regulated and lactation specific of all the bovine milk protein genes; it is produced in milk of most animals; and the bovine and porcine α-LA proteins have similar molecular weights (Wheeler, 2003). We have shown that the Bα-LA gene can be expressed in the pig and the protein can be secreted into the milk. The concentration of Bα-LA was highest on d 0 of lactation and decreased as lactation progressed (Bleck et al., 1998). Because Bα-LA was being produced at higher levels at the beginning of lactation while porcine α-LA had not yet reached its maximum concentration, total α-LA of transgenic sows was dramatically elevated in early lactation milk. The production of the bovine protein caused approximately a 50 % increase in the total α-LA concentration of pig milk throughout lactation. Interestingly, the level of milk protein and total solids was not

significantly affected by the increased lactose concentration on d 0. If a higher lactose concentration would lead to increased milk production, one may expect that the concentration of protein and total solids would be lower due to added water being drawn into milk by osmosis, but this was not the case. The mammary gland machinery appeared to be able to re-equilibrate the milk composition in the right amount of solids, protein and water.

It is clear that for the long-term benefit of society and the area of transgenic technology, the impacts on the environment, producers, consumers and especially the animals must be carefully evaluated. This opinion has already been presented to the scientific community and it is important for scientists using this technology to become engaged and be willing participants in the discussion and consideration of ethical issues and concerns surrounding the implementation of this work (Mench, 1999).

It is worth pointing out here that the goal of using this technology is for the benefit not the detriment of mankind. The use of this technology is not simple, efficient or inexpensive. Scientists using this technology are trying to develop models to study disease, produce bio-pharmaceuticals and produce more wholesome, healthy and economical food. These studies are difficult and great care must be taken before such investigations begin. Such considerations are critical due to the time, cost, welfare, ethics, concerns, risks and benefits involved in these kinds of investigations. Realize none of these groups, producers, consumers or scientists, are motivated to produce inappropriate medical models, ineffective or dangerous pharmaceuticals, or un-safe food. None of these groups would survive the political and economical repercussions if this were the case. Therefore, although this type of research is expensive and difficult, concern for animal welfare, ethics, societal benefit and vigilance should be emphasized that

much more during the development of research objectives. Consideration of these as well as scientific issues, will lead us forward to reaping the benefits from this important technology.

CHAPTER 6

Conclusion

For as many advantages the transgenic technology can bring in the modern world it also carries many concerns and oppositions. The future use of transgenic animals for the improvement of livestock production will depend upon the safety and effectiveness of the transgenic technology. Results of this study provide strong evidence that the Bα-LA transgene is not transferred from transgenic swine to non-transgenic swine through environmental contact, mating, gestation, parturition, or lactation. The importance of this work is in that it provides evidence of the safety of this technology for the conditions here explored.

CHAPTER 7

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