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Biotherapeutics from Transgenic Porcine Sources: Bioprocessing Approaches and Challenges

By DARCY BIRSE, DAN LACROIX, MICHAEL DYCK, FRANÇOIS POTHIER, and MARC-ANDRÉ SIRARD

The biopharmaceutical manufacturing sector is rapidly gearing up production capacity to satisfy the steadily escalating global demand for complex biologics to combat a number of treatable illnesses.[1,2] Frequently, the biotherapeutics in demand are too complicated to be chemically synthesized and thus are beyond the reach of traditional pharmaceutical approaches. To effectively address this issue, these products must be developed and produced using viable and robust biological systems.

As the complexity of biopharmaceutical products evolved from single-chain biologics to larger macromolecules and multiple-chain or glycosylated biotherapeutics, it became evident that early host cells (e.g., microbes and yeast) were unable to produce the required biologically active molecules. For complex products, mammalian cells must be utilized as effective hosts to produce specific biochemical modifications.

Mammalian cell culture production technologies have been proven for many types of biologics; however, the mammalian cell line manufacturing industry faces significant challenges due to inefficient expression levels and production capacity limitations. Furthermore, there are inherent limitations to conventional mammalian cell systems, specifically efficiency (cost) and robustness, in production of active biotherapeutics requiring complex post-translational modification patterns (e.g., glycosylation).

As the scale of industrial mammalian cell culture production facilities grows, time constraints and considerable infrastructure costs are assimilated into the overall manufacturing process costs. In many cases, projected cost increases and time delays inhibit or prohibit further product pipeline development. A viable alternative to large-scale mammalian cell culture facilities for manufacturing biologics is the use of transgenic systems as bioreactors.

Transgenic production of recombinant biologics is one alternative to help resolve mammalian cell culture capacity limitations that can prevent ongoing projects or products in the pipeline from being economically feasible or that may tie up valuable resources. Each production system has its advantages and disadvantages, as well its focus applications. Ideally, the entire spectrum of biologics production can be met by applying several production techniques to satisfy global demand for required biotherapeutics without placing an enormous financial burden on the end-user, the patient.

TGN Biotech has developed a transgenic technology platform, SEMENESIS™, to address a specific set of biologic production issues. The SEMENESIS technology platform is based on producing biotherapeutics in the seminal vesicle of transgenic porcine bioreactors. The principle is to make use of specific porcine gene promoters that regulate gene expression in the seminal gland of porcine hosts. The target selection criteria are well defined and complement weaknesses present in conventional production systems. A suitable transgenic manufacturing platform must: (i) express highly complex macromolecules (e.g., glycosylation, disulphide bridges, multimers); (ii) tolerate high potency products and toxic biologics; (iii) produce large, difficult-to-express macromolecules; and (iv) manufacture biologics in the range of 10's to 100's of kilograms per year. TGN Biotech has demonstrated, using three independent biotherapeutic production models, its ability to express biologics in the semen of transgenic hosts. This article presents data on one of the selected biologics, follicle-stimulating hormone (FSH), as a proof-of-concept model.

When evaluating the SEMENESIS platform, it is relevant to assess the effectiveness of transgenic animals as candidate biologic sources. Table 1 lists the advantages inherent to the SEMENESIS platform. There are four critical attributes to consider: (i) biocompatibility of the host animal with humans; (ii) suitability of the host medium; (iii) time frame for target development and

Darcy Birse, Ph.D. *(dbirse@tgnbiotech.com) is director, process development; Dan Lacroix, Ph.D. is director, molecular biology; Michael Dyck, Ph.D. is the former director, transgenesis (presently, assistant professor, department of agriculture, food, and nutritional science, University of Alberta, Edmonton, Alberta, Canada); François Pothier, Ph.D. is vice president, molecular biology; and Marc-André Sirard, Ph.D., DMV is vice president, reproduction, TGN Biotech Inc., Sainte-Foy, Québec, Canada.*

scale-up; and (iv) costs associated with producing commercial quantities of biologics.

Biocompatibility. It is widely accepted that the human and porcine physiological systems are relatively similar and demonstrate a high level of biocompatibility (reduced anti-immunogenicity). As a result, human biotherapeutics derived from porcine sources have been used for decades and global regulatory agencies have approved products from porcine origin; examples include insulin, heparin, pancrelipase, and heart valves. Furthermore, the risk of pathogen transmission from porcine to humans has been assessed as negligible with an unprecedented reduced risk of transmission of transmissible spongiform encephalopathies (TSE's).[3]

Suitability of host medium. The choice to develop the SEMENESIS platform, utilizing seminal fluid as a host medium, is strategic in principle and in practice. In this application, the host medium must be suitable for delivering transgenic product, free of nucleases, and protected from proteolytic attacks. Choosing porcine semen as the host medium is based on seminal fluid as being the natural vehicle responsible for the transport of genetic material from a male to a female to ensure the propagation of a species. This suitability is largely due to the fact that seminal fluid is an inert and hospitable environment for the delivery of intact genetic material. In addition, seminal fluid has been observed to have protease-inhibitor activity.

Table 1. SEMENESIS™ Technology Platform Advantages.

Advantages of Porcine Species as Bioreactors:
• Short generation time (< 1 year)
• No cloning required (embryo microinjection)
• Ten to twelve piglets per litter
• Reduced risk of zoonosis (e.g., virus, prions)
• SPF facilities and disease-free animals
• Low cost to acquire, house, and maintain animals
• Easy to manipulate, long productive life, sexual maturity at 6–7 months
• Well established pre-existing infrastructure (global artificial insemination units)
• Production herd generation by traditional animal husbandry methods
Advantages of Seminal Vesicle as a Bioreactor:
• Well established site of protein synthesis (~30 g/L total protein)
• Semen is harvested on a daily basis, year-round (constant production)
• Harvest volumes range between 250– 1000 mL/day/animal
• Accessory sex glands are a tightly closed physiological system (high potency)
• Minor population of contaminating proteins (facilitates bioprocessing)
• Inherently, semen is the vehicle necessary to transport genetic material
• High concentration of endogenous protease inhibitors (spermadhesins)
• High biocompatibility of porcine glycosylation pattern relative to humans*

** Note: The enzyme α1,3-galactosyltransferase (α1,3GT or GGTA1) synthesizes α1,3-galactose (α1,3Gal) sugar epitopes (Gala1,3Gala1,4GlcNAc-R), which are the major xenoantigens causing hyperacute rejection in porcine-to-human xenotransplantation. To date, α1,3Gal has not been detected on biologics produced using the SEMENESIS platform.*

Further advantages showing the suitability of this host medium include: (i) no need to induce host using hormones and drugs; (ii) high reproductive rates obviate the need for expensive and time-consuming cloning methods (we utilize microinjection); (iii) no documented history of prion contamination; (iv) the ability of a transgenic host to produce high-potency products without compromising the health of the host (i.e., reabsorption of biologic); (v) a reduced risk of proteolytic attack on the target biologic; (vi) complete post-translational modifications that enhance biotherapeutic properties (e.g., bioactivity, bioavailability, stability); and (vii) the ability to use conventional methods to efficiently isolate and purify intact and bioactive biotherapeutic products from seminal fluid.

Time frame for development and scale-up. Following construction of the target transgene, embryo microinjection, and transplantation, a surrogate host's pregnancy is ~114 days. Maturation of a transgenic host occurs after 6–9 months whereby propagation of a selected founder (~114 days) by artificial insemination is completed. Subsequent to this, the maturation of a production herd (6–9 months) can be scaled accordingly, where herd numbers are time-independent (i.e., it requires a similar length of time to propagate a production herd of two transgenic hosts as it would take for a production herd of 200 transgenic hosts). The time frame for the entire process, from target vector construction to full-scale transgenic host production herd, is less than 24 months.

Cost of manufacturing commercial quantities of biotherapeutics. The transgenic porcine production host solution is a low-cost alternative to competing conventional and transgenic animal systems due to the following factors: (i) the rapid reproductive cycle of the porcine host and its high offspring rate allows for reduced costs; (ii) porcine hosts produce economically significant quantities of seminal fluid, processed in batch mode, throughout the entire year; and (iii) the generation of a production herd can be accomplished

without using costly, complicated, and inefficient cloning techniques.

SEMENESIS Platform Development

The use of the male accessory sex gland for the production of proteins has been demonstrated as a viable alternative for biotherapeutic production.[3] The male accessory sex gland of the porcine host possesses several characteristics that make it suitable for the production of biologics, including: a large capacity for protein production (>30 g/L); continuous protein production throughout the reproductive life of the animal (>8 years); the ability to perform complex post-translational modifications; protein secretion by these tissues is uniquely exocrine, minimizing the risk of a biologically active product compromising the host's own physiology; and the porcine host seminal fluid protein profile is well characterized, which facilitates the expression and isolation of target biologics.

Spermadhesins represent a family of proteins found in seminal fluid of various domestic animals.[4–9] A variety of spermadhesins is expressed in porcine seminal fluid; of these, the major abundant proteins are referred to as PSP-I, PSP-II, AQN-1, AQN-3, and AWN. These proteins make up the bulk of proteins observed in porcine seminal plasma, with individual protein expression levels above 7 g/L.[10,11] In-depth studies of spermadhesin gene expression in the male and female reproductive tracts have demonstrated that transcription of these genes is limited to the male reproductive tract. Considering tissue specificity and the robustness of spermadhesin expression in porcine seminal fluid, the regulatory sequences of these genes were selected to drive the expression of biotherapeutics into the semen of a transgenic host. Based on this data, the utilization of selected spermadhesin promoter elements is projected to drive biologic expression up to the 7 g/L range in transgenic porcine hosts.

The proof-of-concept model developed by TGN Biotech to demonstrate the effectiveness and robustness of the SEMENESIS platform utilized a well-characterized protein hormone, follicle-stimulating hormone (FSH). FSH is a member of the glycoprotein family of pituitary hormones that includes thyroid-stimulating hormone (TSH), luteinizing hormone (LH), chorionic gonadotropin (CG), and pregnant mare serum gonadotropin (PMSG). These hormones, which play a role in fertility, share a common α-subunit (92 amino acids) linked to a hormone-specific β-subunit (111 amino acids).[12] FSHα-

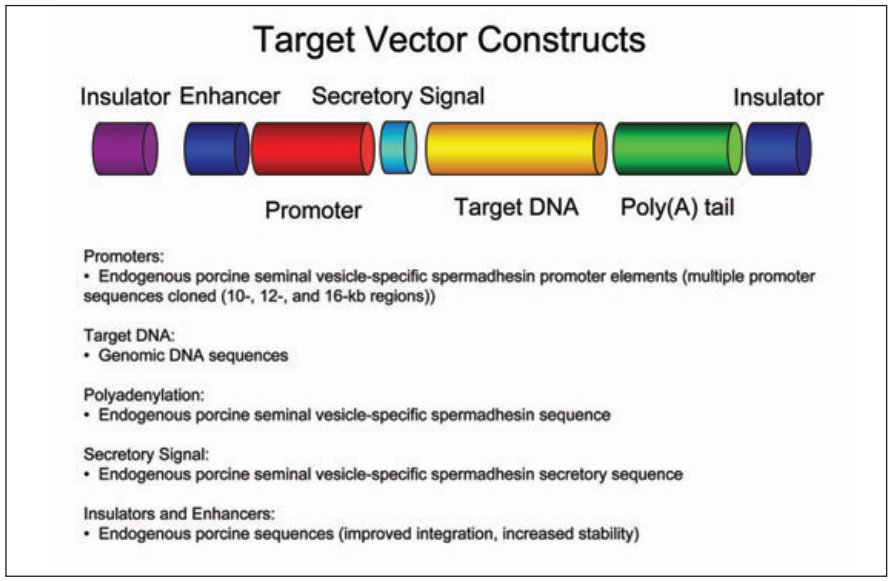

Figure 1.

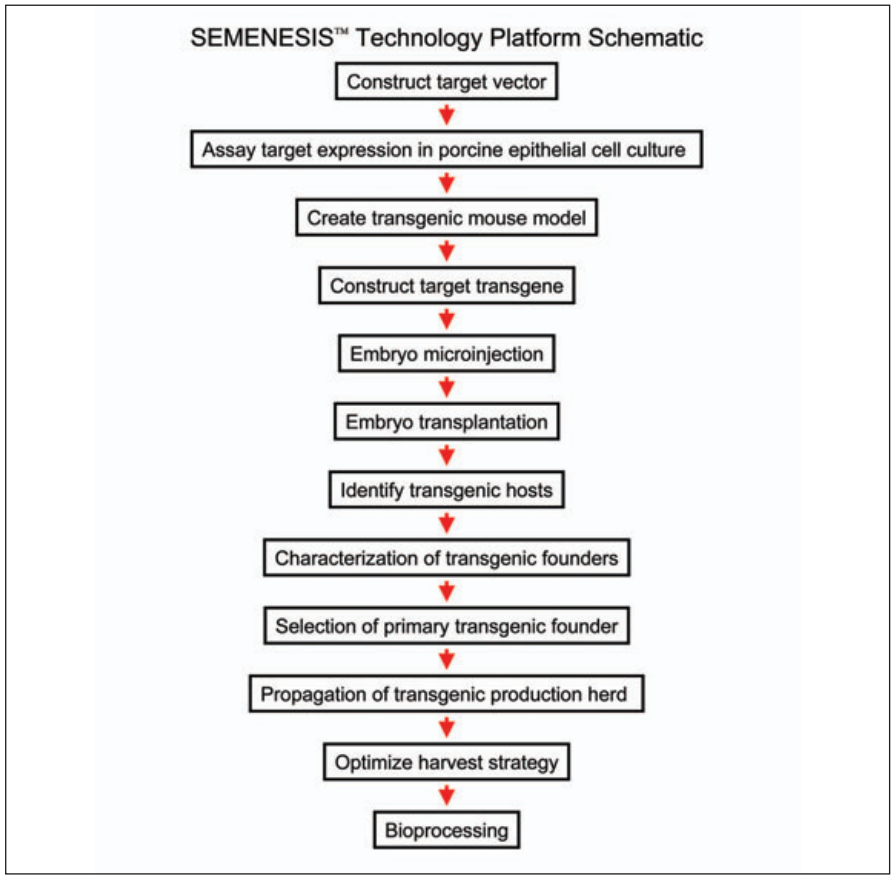

Figure 2.

and β-subunits require post-translational modifications, including glycosylation (two sites per subunit) and disulphide linkages (FSH belongs to the family of cysteine-knot growth factors), which are necessary for appropriate subunit assembly, stability, receptor binding, and biological activity.[13–16]

Following isolation and characterization, the 5' regulatory promoter sequences of the *PSP-I* and *PSP-II* spermadhesin genes were assayed to determine their effectiveness in driving the expression and secretion of FSH into the seminal fluid of a transgenic animal. To challenge the system, four different gene constructs, consisting of different combinations of FSH α- and β-subunit coding sequences linked to either porcine *PSP-I* or *PSP-II* regulatory sequences, were developed and tested. A schematic diagram (Fig. 1) illustrates the architecture of the target vector constructs. Transgenic murine and porcine hosts were generated using these constructs and observations were made by detecting biologically active FSH secreted into their seminal fluid.

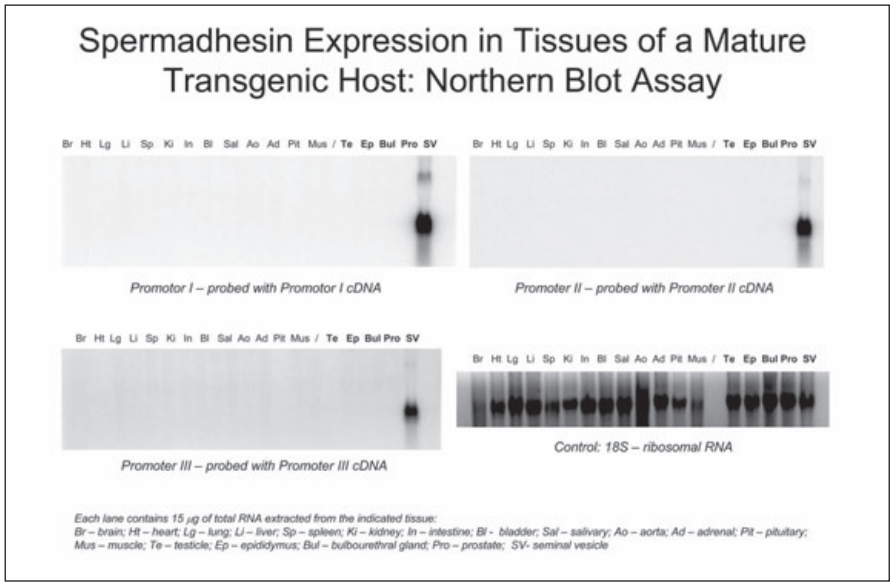

Figure 3.

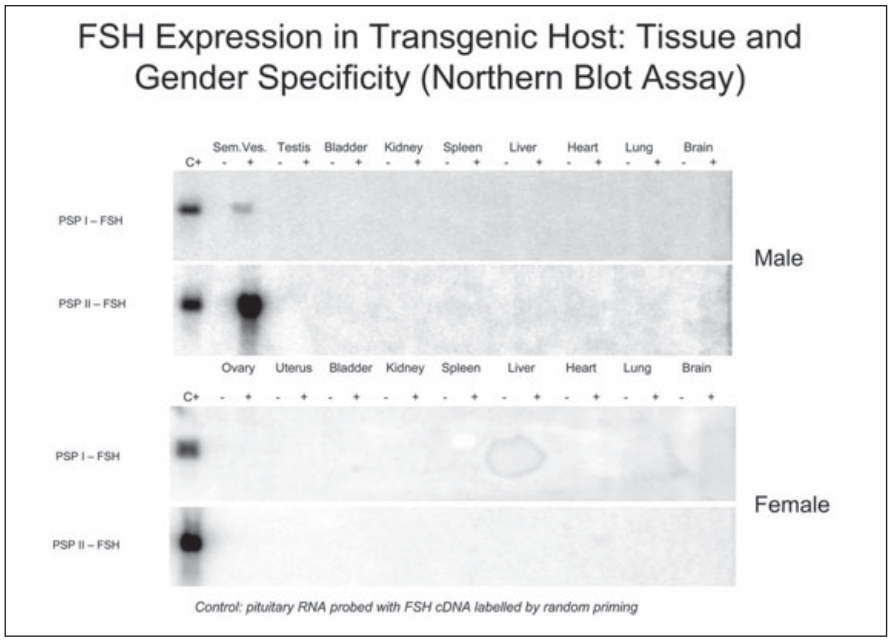

Figure 4.

The SEMENESIS platform executes a rational and systematic evaluation according to a series of checkpoints defined in the flow scheme (Fig. 2). Progression through the many stages of product development is performed according to this path. At the initiation stage, the target biologic nucleic acid sequence is integrated into a vector and verified by sequencing. Following confirmation of sequence integrity, the vector is transiently transfected into cell culture to provide a rapid go/no-go response concerning target expression and secretion. This assay is semi-quantitative in terms of level of expression of biologic, but is invaluable for optimizing the throughput of assays for different constructs or targets. Once the target has been verified to be both expressed and secreted in mammalian cells, a transgenic murine model can be produced. The levels of biologic expression in the murine model are indicative of the levels of biologic production expected to be observed in the transgenic porcine host. The scale factor is not linear, but does give a quantitative indication of the predicted expression rates in the final production host.

Once the murine data has been analyzed, the product can be developed further by constructing a transgene to be microinjected into a donor porcine embryo. The modified embryos are transplanted into a surrogate host and allowed to come to term. Following birth, transgenic progeny are immediately identified (transgenic males destined for production, transgenic females destined to cross-breed) and segregated. Upon male maturity, hosts are trained and evaluated for selection of the primary founder. Criteria used to select founders are based on: phenotype (e.g., behavior, aggression, trainability); harvest volume; biologic concentration; frequency of harvest; and reproducibility of production. After making a critical selection of a single founder (F_0), the founder is propagated using conventional artificial insemination methods to create a production herd (F_1) of the required size. Following the matura-

tion of the herd, harvest strategies are optimized and scaleable bioprocessing methods can be applied to recover and purify the target biologic.

Materials and Methods

Transgene Construction

PCR: All PCR reactions were performed with Expand High Fidelity enzyme mix and buffers under conditions recommended by the manufacturer (Roche Diagnostics, Laval, Quebec, Canada).

Promoters. A 3.3-kb porcine genomic fragment was PCR-amplified using primers based on the *PSP-I* cDNA sequence (GenBank U02626) supplemented with internal 5'-RACE data. A 1.3-kb *Eco*RI-*Hin*dIII sub-fragment was used to generate radiolabelled probes to screen a porcine genomic library (PL1010j; Clontech, Palo Alto, CA). Clones were selected, purified, and analyzed by comparing their regulatory and transcribed regions. Following PCR amplification and cloning, the regulatory regions were assembled into clones *G*, *I*, and *J3*, which contained sequences from the porcine spermadhesin genes *PSP-I*, *AQN-3*, and *PSP-II*, respectively. The regulatory regions from clones *G* and *J3* were PCR-amplified and ligated into *pCR-XL-TOPO*, generating *pTOPO-GP* and *pTOPO-J3P* plasmids. These plasmids were subjected to random transposon insertion (GPS-1 kit; New England Biolabs, Beverly, MA) and derivative clones were analyzed to identify insertion positions. Primers based on 5' untranslated region (UTR) conserved regions and transposon ends were used to amplify promoters of different lengths from specific transposon derivatives of plasmids *pTOPO-GP* and *pTOPO-J3P*, generating promoters *GP8* (7.0 kb) and *J3P8* (7.3 kb), which encode regulatory regions for the *PSP-I* and *PSP-II* genes, respectively.

FSH coding sequences. Genomic fragments of FSHα and FSHβ genes (including leader peptide sequences) were PCR-amplified from genomic DNA with primers designed from published sequences (GenBank D00621 and D00766) and cloned into *pCR3.1-Uni* (Invitrogen, Carlsbad, CA). The plasmid coding for FSHβ was treated to excise an intronic *Bgl*II-*Eco*NI fragment and generate the *FSHβ*Δ minigene. The plasmids *pCR3.1-FSHα* and *pCR3.1-FSHβ*Δ were used to generate amplicons with flanking restriction sites that were subsequently manipulated to generate genomic coding sequences for individual FSH subunits terminated by a polyadenylation signal. The amplicons containing the coding units were ligated into *pGEM-8C*, a modified *pGEM* plasmid.

Transgenes. To generate plasmids *pGP8-FSHα* and *pGP8-FSHβ*Δ, FSHα- and FSHβ-coding amplicons were ligated into *pGEM-8C* where both amplicon plasmids were digested with *AscI-Sda*I and ligated upstream to coding sequences, creating *GP8*. To generate plasmids *pJ3P8-FSHα* and *pJ3P8-FSHβ*Δ, a similar procedure was followed using

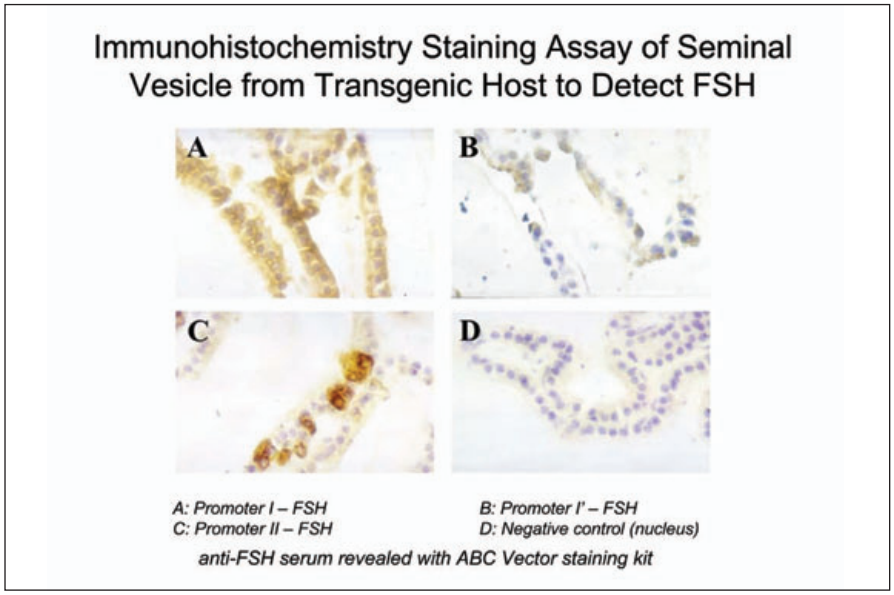

Figure 5.

Table 2. Total recombinant protein and FSH protein as a percentage of total protein in the seminal vesicle lumen contents from transgenic male offspring.

Assaying FSH Protein Expression Levels in Seminal Vesicle of Transgenic Murine Model

		Seminal vesicle contents		
Transgenic line	*n*	**FSH protein µg/ml** *mean ± S.E*	**Total protein mg/ml** *mean ± S.E*	**FSH as % of total protein** *mean ± S.E*
Promoter I – FSH	3	110.06 ± 5.61	46.28 ± 15.56	0.34 ± 0.16
Promoter I' – FSH Promoter II – FSH	4	180.55 ± 8.34	74.15 ± 10.43	0.26 ± 0.03
Promoter II' – FSH Promoter I'' – FSH	3	335.31 ± 13.02	65.11 ± 10.43	0.71 ± 0.13

*Pac*I-*Fse*I restriction sites. The resulting plasmids were digested to generate linear transgenes for pronuclear microinjection.

Production of Transgenic Murine and Porcine Models

Microinjection of the gene construct into murine and porcine embryos. Production of transgenic murine models by introduction of DNA constructs into the pronucleus of fertilized oocytes was performed following established protocols.[17] Donor females (strain B6C/3F1; Charles River Laboratories, Wilmington, MA) were superovulated using PMSG (Vetrepharm, Belleville, Ontario, Canada) and hCG (Intervet, Whitby, Ontario, Canada), mated to fertile males, and sacrificed the following day. Embryos at the pronuclear stage were harvested from the oviducts and a single nuclei from each was micro-injected with the DNA construct. The injected embryos were implanted into pseudo-pregnant surrogate females (strain *CD1*; Charles River Laboratories) that had been mated with vasectomized males.

Porcine embryos were produced using cycling Landrace-Yorkshire x Duroc gilts (weight: ~100 kg; age: ~6 months). Embryo donors were synchronized and superovulated by the following steps: 17 days of oral altrenogest treatment (20 mg Regumate; Hoechst Canada, Québec, Montreal, Canada); one day following the end of the altrenogest treatment, injection of 1200–1400 IU PMSG (Vetrepharm); 78 hours later, injection of 1000-IU hCG (Intervet); 24 and 36 hours later, artificial insemination; 52 to 57 hours later, embryos were isolated from oviducts. The collection and transfer of embryos was performed by mid-ventral laparotomy, with gilt under general anesthesia as approved by the institutional animal care and use committee of the Université Laval, Québec, Canada.

Embryos were flushed from oviducts using two 15-ml volumes (37° C) phosphate-buffered saline (PBS) with 0.4% (w/v) bovine serum albumin (BSA; Sigma-Aldrich Canada, Oakville, Ontario, Canada) and 50 mg/ml gentamycin (Sigma-Aldrich Canada). For visualization of pronuclei, embryos were centrifuged at 15,000 x g for 10 minutes.[18] Embryos at the pronuclear stage were identified, separated, and one of the nuclei was microinjected with target DNA at concentration of 4 ng/µl. Surviving embryos were implanted into the oviduct of a surrogate female. Surrogates were prepared in a similar manner to the donors; however, insemination was not performed. A mid-ventral laparotomy was performed on the surrogates, and those which were identified by ovarian morphology as having ovulated had injected embryos transplanted into their oviducts.

Offspring resulting from the recipient surrogates were evaluated by PCR and Southern blot for the presence of the transgene to identify transgenic founder animals.

Identification of transgenic animals. Murine host tail tissue was collected and immediately digested with proteinase K (0.4 mg/ml) in 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 1% sodium dodecyl sulphate (SDS) overnight at 55° C. Porcine host tail and ear tissue were collected, frozen, and digested as previously described. High-molecular weight DNA was isolated by phenol-chloroform extraction and precipitated in 95% ethanol. Isolated genomic DNA was washed twice in 70% ethanol. The DNA pellet was dissolved in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and stored at 4° C.

PCR identification of transgenic animals was performed using primers designed to specifically amplify a DNA fragment of predetermined size from each transgene. Amplification products were separated by electrophoresis on a 1.0% agarose gel and the corresponding predicted bands were analyzed to verify and confirm transgenic animals.

Southern analysis was performed using established methods.[19] Genomic DNA was digested overnight with *Pst*I restriction endonuclease under appropriate conditions. DNA fragments were separated by electrophoresis on 0.8% agarose gel and transferred to nylon membranes (Roche). ^{32}P-radiolabelled probes specific to each transgene were prepared by random priming. Pre-hybridization and hybridiza-

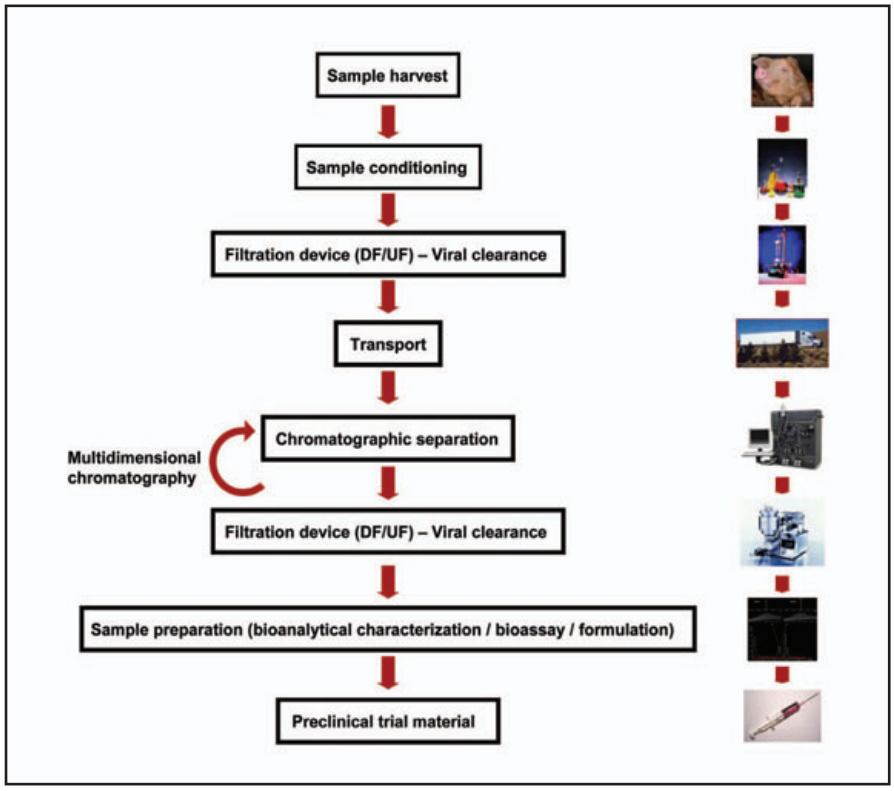

Figure 6. Downstream process development strategies for TGN Biotech biologics

tion with probes were performed in a standard hybridization solution at 42° C. Membranes were washed 2 x 15 minutes at 55–65° C in 0.5% SDS, 0.1% standard saline citrate (SSC). Bound probe was detected using a Phosphor Imager (Molecular Dynamics/Amersham Biosciences, Piscataway, NJ).

Characterization of Transgenic Murine and Porcine Models

Evaluation of transgene expression. RNA was extracted from frozen tissues using the TRIzol Reagents extraction system (Life Technologies, Burlington, Ontario, Canada). RNA was fractionated by electrophoresis on 1.0% agarose gel containing formaldehyde, then transferred to nylon membranes (Qiagen, Valencia, CA). ^{32}P-radiolabelled cDNA probes prepared by random priming of FSHα and FSHβ were applied. Pre-hybridization, hybridization, and imaging was performed as previously described.

Immunohistochemistry. Tissues were fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin, sectioned at 6 μm and stained using Vectastain ABC kit (Vector Laboratories, Burlington, CA). FSH was detected using FSHβ antibody (Biogenesis, Poole, UK).

Detection of recombinant FSH by radioimmunoassay (RIA). The concentration of FSH in murine vaginal plug extracts and seminal vesicle contents, as well as in porcine seminal fluid, was determined by RIA methods. Vaginal plug extracts were produced by placing transgenic and non-transgenic males with PMSG-hCG-synchronized females overnight and collecting the vaginal plugs the next morning. The vaginal plugs were incubated in physiological buffer so that the FSH present in the plugs could diffuse into the solution. Seminal vesicle contents were collected from mature transgenic and non-transgenic male mice following dissection of the coagulating gland. Porcine semen was collected and seminal fluid was separated from the sperm by centrifugation at 800 x g. Samples were incubated at –20° C until the time of assay. Concentrations of FSH were determined using a double-antibody RIA protocol as described by the supplier of the FSHβ primary antibody (Biogenesis).

***In vitro* FSH bioassay.** Bioactivity of FSH was determined based on the induction of progesterone production by a primary culture of HEK 293CHO cells following standard methods.[20]

Results

Transgenic Murine Hosts

Microinjection of the *PSPI FSHα/PSPII-FSHβ*Δ and *PSPII FSHα/PSPI-FSHβ*Δ constructs resulted in transgenic male and female founders that were mated to establish transgenic lines for experimentation.

Transgene expression, detected by Northern blot analysis (Fig. 3), revealed that for both transgenic lines, of all the tissues tested (testes, ovaries, uterus, seminal vesicle, bladder, spleen, kidney, liver, heart and brain), transgene transcription is observed exclusively in the seminal vesicles of the transgenic males (Fig. 4). Transgenic females demonstrated no FSH expression in any of the tissues tested. Immunohistochemical staining revealed that FSHβ secretion was restricted to the seminal vesicle epithelium-independent transgene promoters *PSP-I* or *PSP-II* (Fig. 5). No FSHβ production was detected in the seminal vesicle epithelium of non-transgenic males.

Secretion of FSH into the seminal vesicle lumen contents and ejaculated seminal fluids was determined by RIA (Table 2). Total protein in vaginal plug extracts and seminal vesicle contents did not differ between the transgenic lines and was similar to values previously observed for non-transgenic mice.[3] FSH in the fluids tested represents, on average, 0.44% of total protein and does not dramatically alter the total amount of protein present. FSH concentration for the transgenic murine model has been observed to be expressed in the range of 110–335 μg/ml. The fertility of the male and female transgenic mice from these lines was unaffected.

Transgenic Porcine Hosts

Evaluation of transgenesis rates. The *PSPI-FSHα/PSPII-FSHβ*Δ construct was microinjected into 1122 embryos, resulting in 27 transgenic progeny identified (55% male / 45% female) which have since generated 185 offspring. The current rate of transgenesis is at 2.4% (the benchmark within the scientific community is ~1.0%). Transgenic males have reached sexual maturity and have successfully been trained for semen collection. Semen is currently collected on a regular basis and assayed for FSH expression levels. The porcine hosts are scored and evaluated as a function of harvest volumes (250–1000 ml) and FSH concentrations (50–350 mg/L). Attempts to assay FSH in the semen of non-transgenic boars revealed that no endogenous FSH is detectable in porcine seminal fluid.

Bioprocessing. One of the most critical steps toward commercializing a recombinant biotherapeutic product is to demonstrate efficient and effective recovery and purification of the target biologic. This bioprocessing stage represents a significant percentage of the overall cost of manufacturing the final product. TGN Biotech has developed bioprocesses to address specific and multi-target production, involving both upstream and downstream applications, to purify target biologics from transgenic porcine hosts.

Upstream processing. The transgenic porcine host system allows for rapid and flexible manufacturing scale-up for biotherapeutics production. A given biologic product can be expressed within a 10–12 month time frame (from gene to primary founder), while industrial manufacturing capacity requirements (i.e., a production herd) can be propagated in less than 24 months. Upstream, the scale-up process is considered to be linear, whereby traditional animal husbandry practices to propagate the production herd are applied. This process is rapid and significantly more economically feasible than the construction and commissioning of a conventional cell culture production facility. In addition, this production methodology has distinct advantages for

complying with regulatory requirements concerning the conditions mandated for the production herd, namely: specific pathogen-free (SPF) animal housing; current Good Manufacturing Practices (cGMP) and Good Laboratory Practices (GLP) installations for analysis, quality assessment, and pre-processing facilities; Hazard Analysis and determination of Critical Control Points (HACCP); as well as general environmental, animal, and food regulations.

Downstream processing. Currently, bioprocessing strategies are being developed, implemented, and optimized to produce biologics from transgenic porcine sources. The current objective is to drive projects through the pipeline, producing material for early research to the preclinical phase. The bioprocessing methodology applied for the harvesting and purification of biotherapeutics from transgenic porcine sources implements multiple modular processing steps in an integrated manner, providing a stable, robust, and scaleable process (Fig. 6). Presently, upstream and downstream processes have been developed to process products in an aseptic manner, where the downstream processing stage supports viral inactivation and additive viral clearance concerns in addition to integrating the bioprocess with a formulation development window.

The current strategy to process biologics produced from transgenic porcine sources involves the following steps: (i) harvest sample from production hosts and process material in batch mode; (ii) collect, pool, and condition material (viral inactivation step); (iii) utilize hollow-fiber tangential-flow filtration (TFF) devices to condition product in diafiltration (DF) mode, then clarify sample, removing bulk contaminants, microbial burden, and spermatozoids in ultrafiltration (UF) mode (first viral clearance step); (iv) aseptically process and store concentrated sample in biobags as a clarified, microbially intact, stabilized product; (v) transport sample to bioprocessing facility; (vi) condition sample and subject it to iterative serial chromatographic separations (additive viral clearance steps); (vii) condition fractionated sample (formulation window); (viii) and process through dead-end filtration device (viral clearance step); (ix) and load onto a cassette-format TFF device for final DF/UF applications; (x) bioanalytically and biochemically characterize protein sample and assay bioactivity. To date, the model biologic FSH can be produced and purified to a high level of purity in commercially viable quantities using linear scaleable methods and equipment. Currently, FSH is being manufactured for pre-clinical trials.

In summary, TGN Biotech has demonstrated, through utilization of the SEMENESIS platform, that the production of biotherapeutics in the seminal vesicle of transgenic porcine bioreactors is a viable, robust alternative to producing complex biotherapeutics in mammalian cell culture. In addition, a rational and logistic approach has been presented to address the many challenges and hurdles in producing a biotherapeutic product from a novel transgenic source. The proof-of-concept model, FSH, illustrates the potential of this transgenic system to manufacture commercially feasible amounts of bioactive biologic product in a controlled and quality supported manner.

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