Seminal vesicle production and secretion of growth hormone into seminal fluid

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Production of foreign proteins in the tissues of transgenic animals represents an efficient and economical method of producing therapeutic and pharmaceutical proteins. In this study, we demonstrate that the mouse P12 gene promoter specific to the male accessory sex gland can be used to generate transgenic mice that express human growth hormone (hGH) in their seminal vesicle epithelium. The hGH is secreted into the ejaculated seminal fluids with the seminal vesicle lumen contents containing concentrations of up to 0.5 mg/ml. As semen is a body fluid that can be collected easily on a continuous basis, the production of transgenic animals expressing pharmaceutical proteins into their seminal fluid could prove to be a viable alternative to use of the mammary gland as a bioreactor.

Keywords: transgenic mice, agriculture, genetic engineering, bioreactor

The advent of transgenic technologies has made the production of pharmaceutical proteins by transgenic animals an attractive alternative to the production of such proteins in microbial fermentors or in cultured mammalian cells[1]. Currently, these technologies have advanced to the stage that milk-derived proteins are routinely being expressed by transgenic livestock[2–4]. The mammary gland is generally considered to be the tissue of choice for the production of pharmaceutical proteins because milk is easily collected in large volumes. However, the production of proteins in milk is limited by the relatively long interval from birth to first lactation encountered with domestic livestock, the discontinuous nature of the lactation cycle, and the substantial time and material investments required to produce transgenic cattle[5], the species from which milk is most easily and efficiently collected.

Therefore, other forms of collectable body fluids that could be used for the production of foreign proteins in transgenic animals are being considered. To date, the possibility of isolating foreign proteins from the blood of transgenic pigs has been explored[6], as has the idea of using the bladder as a bioreactor by engineering urethelium production and secretion of a foreign protein into the urine[7].

The seminal fluid of the male ejaculate represents an alternative body fluid that is commonly and easily collected from domestic livestock. Of particular interest is the seminal fluid of the pig, as a boar ejaculates the largest volume of seminal fluid of all domestic livestock, reaches sexual maturity at 110–125 days of age, and is able to produce semen on a continuous basis[8]. The seminal vesicle, prostate, and bulbourethral glands, which are among the accessory sex glands, are responsible for the majority of liquid and proteins found in semen[9].

One type of protein known to be expressed by the accessory sex glands of several mammalian species[10,11], including the boar[12], is a group of protease inhibitors that are believed to protect the genital tract from proteolytic damage[13] and to play a role in fertilization[14,15]. The mouse form of this protease inhibitor, P12, has been well characterized in the male glands and demonstrates testosterone-dependent activity in the accessory sex glands with a lesser constitutive expression in the pancreas[16,17]. The P12 upstream 5′ regulatory sequence has also been identified, and its activity extensively is well characterized[18,19].

In this study, we explore the possibility of using the male accessory sex glands, particularly the seminal vesicle, as a bioreactor using the mouse P12 gene promoter to drive the production of a foreign protein into the seminal fluid of transgenic mice. Human growth hormone (hGH) was chosen as the protein of expression because detection systems for this protein are readily available and the effects associated with ectopic transgene expression are well characterized[20].

Results

Production of transgenic mice. The microinjection of the P12-hGH DNA construct resulted in the production of four transgenic founder mice (three male and one female). The female founder died shortly after producing her first litter, which contained no transgenic pups. One of the male founders did not produce detectable levels of hGH in its semen and failed to pass the transgene on to its offspring, suggesting that this founder was mosaic. The remaining two transgenic males (founders 1027 and 2097) produced detectable levels of hGH in their seminal fluid and were mated to nontransgenic females to produce the P12-hGH-1027 and P12-hGH-2097 lines. These founders proved to be nonmosaic as evidenced by the facts that: (1) they passed the transgene on to approximately 50% of their offspring, and (2) hGH production by founders and their progeny was similar. Reduced libido in the transgenic founders and their transgenic male progeny, a common problem associated with animals overexpressing GH[20,21], made the production of offspring and physiological evaluation somewhat arduous, particularly in the P12-hGH-2097 line.

Expression of the hGH transgene. Transgene expression in the testes, ovaries, uterus, seminal vesicle, pancreas, spleen, kidney, liver, heart, and brain was assessed by northern blot analysis. Production of hGH mRNA was detectable in the seminal vesicles and kidneys of males from both transgenic lines (Fig. 1), and in the kidneys of transgenic females. A degree of variation in the levels of expression was observed between lines (Fig. 2). The unique band observed in the seminal vesicle and kidney was of similar size to the hGH mRNA standard present in the human pituitary. No pancreatic expression of the transgene was detected, despite reported constitutive expression of the endogenous P12 gene in this tissue[22]. Quantification of