MethodsX 12 (2024) 102549

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/methodsx

Fabrication of silastic testosterone enanthate implants to achieve virilizing levels of serum testosterone in swine

Nirvana Mahabir, Annie E. Newell-Fugate*

Department of Veterinary Physiology and Pharmacology, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

A R T I C L E I N F O

Method name:
Fabrication of testosterone enanthate silastic implants for use in large animal models

Keywords:
Silastic implant
Testosterone
Swine
Pigs
Steroids

A B S T R A C T

The aim of this study was to create a continuous drug delivery system that would yield serum testosterone levels in sexually mature, female pigs consistent with serum testosterone levels of human transgender men. Testosterone enanthate was mixed with medical grade silicone at a ratio of 20 % by weight, placed in silastic tubing to cure at room temperature for 24 h, then removed from the tubing mold and stored at 4 °C until surgical implantation. Testosterone enanthate oxidizes at high temperatures which is why the implants had to be stored cold. Each implant was 9 cm long and contained 0.56 g of testosterone enanthate. A minimum of one implant (0.56 g testosterone enanthate) and a maximum of four implants (2.24 g testosterone enanthate) were placed in the cervical subcutaneous fat of each pig. After implantation, serum testosterone was assessed over 40 days. Silastic testosterone enanthate implants increase serum testosterone and maintain it in a relatively constant state in a dose-dependent manner for ~ 21–25 days post-implantation.

- Effective method for subcutaneous delivery of large quantities of testosterone or testosterone metabolites in compact implants to large animal biomedical model species.
- Maintains increased circulating testosterone levels for up to 3, 4 weeks post-implantation in pigs.

Specifications table

Subject area:	Pharmacology, Toxicology and Pharmaceutical Science
More specific subject area:	Comparative Reproductive Endocrinology
Name of your method:	Fabrication of testosterone enanthate silastic implants for use in large animal models
Name and reference of original method:	N. H. Ing, H. Francis, J. J. Mc Donnell, J. F. Amann and R. Michael Roberts. 1989. Progesterone Induction of the Uterine Milk Proteins: Major Secretory Proteins of Sheep Endometrlum1. *Biology of Reproduction.* 41(4): 643–654. DOI: 10.1095/biolreprod41.4.643 https://doi.org/10.1095/biolreprod41.4.643

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* Corresponding author.
E-mail address: Anewell-fugate@cvm.tamu.edu (A.E. Newell-Fugate).

https://doi.org/10.1016/j.mex.2024.102549
Received 10 September 2023; Accepted 3 January 2024
Available online 4 January 2024

Resource availability:	4-androsten-17β-OL-3-one enanthate (Trivial name: testosterone enanthate; Steraloids Cat # A6975–000) Analytical balance (Mettler Toledo Cat # ME54TE) Precision balance (Mettler Toledo Cat # ME103TE) Medical grade silicone adhesive Type A (Fisher Scientific Cat # NC1825601) Weigh boat Plastic knife 60 mL catheter tip syringe 22 scalpel blade Dissecting scissors Tygon PVC Soft Tubing for Chemicals, 6 mm ID, 9 mm OD (McMaster Carr, Cat # E-3603)

Method details

Background

Based on work in primates [1], a silastic implant containing testosterone enanthate (TE) was selected as the system of choice for our experiments due to the sustained, consistent release of TE from this implant. In monkeys subcutaneously placed silicone implants containing 10 mg TE resulted in serum testosterone concentrations of ~ 5 ng/ml in castrated male monkeys for 105 days post-implantation [1]. An implant was chosen as the delivery system for our study, because this route of administration achieves desired serum levels of testosterone without the fluctuations commonly seen with transdermal [2] or intramuscular administration of testosterone [3]. TE was selected as our testosterone formulation, because it is commonly used in transgender male patients and, as a steroid ester, it has a slower release and excretion rate than native testosterone [4]. For our studies, we needed to achieve a serum testosterone level of 5–8 ng/ml, which is 10–15 fold normal serum testosterone levels in female pigs [5,6]. Given the large amount of TE that would be required to increase serum testosterone levels in female pigs by 10–15 fold, a traditional silicone implant in which dry compound is packed into a silastic tube and sealed would be difficult to utilize. The silastic tube would need to be very long or to be very wide to accommodate the amount of TE needed, which would make it difficult to tightly pack steroid into it. Therefore, the fabrication approach used for the TE silicone implant was modified from Ing et al. [7] who used this implant to deliver large doses of progesterone to ewes. By mixing TE with silicone as in the study by Ing et al., a large amount of TE could be incorporated into an implant with the smallest possible size.

Ratio of testosterone enanthate to medical grade silicone

Silastic implants were 20 % TE (Steraloids Inc, Newport, RI, cat # A6975–000) by weight with the remaining weight comprised of medical grade silicone (Fisher Scientific, Waltham, MA, cat # NC1825601). Implants were made at doses of 0.56 g, 1.12 g, and 2.24 g TE. Therefore, implants were comprised of: 0.56 g TE + 2.25 g silicone (S; 1 implant), 1.12 g TE + 4.5 g S (2 implants), and 2.24 g TE + 8.978 g S (4 implants). Twenty percent TE by weight was chosen, because this is the optimal ratio used to make melengestrol acetate-silicone implants for contraception of zoological species [8].

Preparation of silicone tubing mold for the testosterone enanthate implant

Tygon PVC soft tubing with 6 mm inner diameter (ID) and 9 mm OD (McMaster Carr, Elmhurst, IL, cat # E-3603) was cut with a 22-scalpel blade into 9 cm lengths. This tubing length was chosen, because our lowest dose of TE (0.56 g) resulted in a ~ 9 cm long implant once the silicone and TE were mixed and the tubing mold was filled with the mixture. For doses larger than 0.56 g TE, multiple 9 cm long implants were made from the TE-silicone mixture.

Preparation of the testosterone enanthate and silicone mixture

TE was weighed on an analytical balance with a readability of 0.0001 g (Mettler Toldeo, Columbus, OH, cat #ME54TE). Medical grade silicone (Fisher Scientific cat #NC1825601) was weighed on a precision balance with a readability of 0.001 g (Mettler Toldeo, cat #ME103TE). Each dose of TE was mixed with its corresponding amount of silicone in a large weigh boat with a plastic knife (Fig. 1). The entirety of the mixture was scraped off the weigh boat and into a 60 mL catheter tip syringe by using two plastic knives. After the mixture was placed in the 60 mL catheter tip syringe, the plunger for the syringe was attached and the mixture was pushed to the end of the catheter tip (Fig. 1). The 9 cm Tygon PVC tubing was snugly attached to the catheter tip syringe and the mixture was deposited into the Tygon PVC tubing. When putting the mixture into the tubing, it was critical not to overfill the tubing. Leaving about 0.5 cm of space on either end of the TE + silicone mixture in the silicone tubing was optimal. After the mixture was deposited into the tubing mold, the implant was kept at room temperature for 24 h to cure. After 24 h, the tubing around the cured implant was cut with a pair of dissecting scissors to remove the solidified implant. Due to the instability of testosterone enanthate at room temperature (27, 28 ˚C), implants were stored at 4 ˚C until they were surgically implanted.

When making the implants using 60 ml catheter tip syringes, we noted that some of the mixture was left behind in the syringe tip. To account for this loss, the tip of an unused syringe was cut off and weighed. The tip of a used syringe containing the TE and silicone mixture was also weighed. The difference in weights were calculated (an empty syringe tip weighed 1.024 8 g and a filled

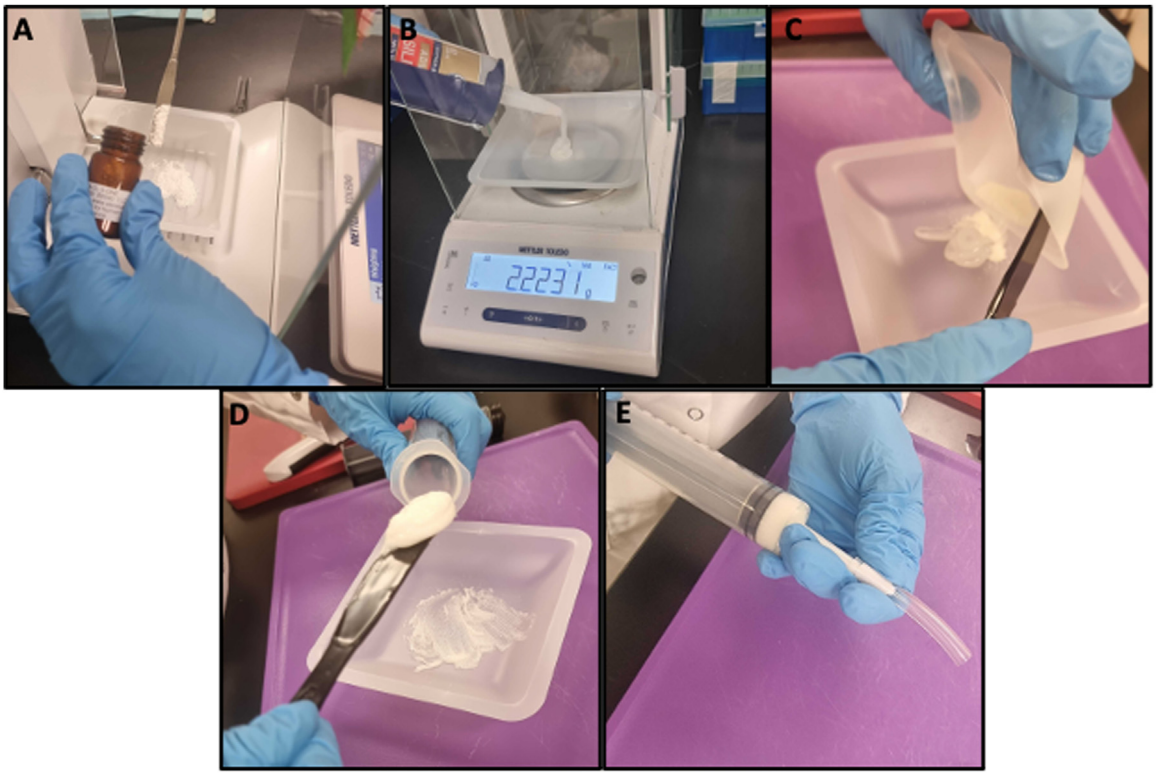

Fig. 1. Fabrication of testosterone enanthate silastic implants. (A) Weighing powdered testosterone enanthate (B) Weighing medical grade silicone (C) Mixing medical grade silicone and testosterone enanthate (D) Filling 60 mL catheter tip syringe with silicone-testosterone enanthate mixture (E) Filling the silastic tubing mold with the silicone-testosterone enanthate mixture.

one weighed 1.9897 g), and the number of grams of TE-silicone mixture was then subtracted from the final implant doses contained in each implant.

In vivo validation of testosterone enanthate silastic implants

Prior to surgical placement, implants were sterilized overnight in chlorohexidine followed by storage in 100 % ethanol for 1, 2 h. For this study, TE silastic implants were inserted in the subcutaneous fat in the lateral cervical region of each female pig. Based on previous studies in male primates [1], we expected serum testosterone levels in response to the maximum TE dosage to be maintained in the desired range of ~5 ng/ml for up to 100 days (~ 3.5 months). After implant insertion, blood was collected from pigs every three

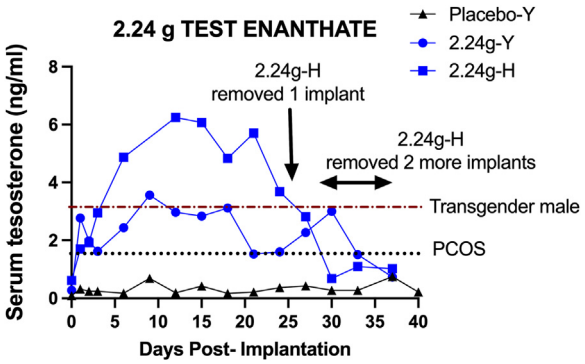

Fig. 2. Serum testosterone levels in female pigs in response to implantation with a silastic implant containing 2.24 g of testosterone enanthate. The Hampshire pig manually removed one implant around 25 days post-implantation and another two implants from day 27 to day 35 post-implantation. The Yorkshire pig's implants remained in place throughout the duration of the study. Y: Yorkshire pig; H: Hampshire pig. This pilot study included one pig per breed*treatment combination. Therefore, statistical comparison of serum testosterone concentrations over time was not performed.

days for 40–42 days for the assessment of serum testosterone. We expected stably elevated serum testosterone levels throughout the duration of our study. As shown in Fig. 2, the 2.24 g TE silastic implant in the Hampshire pig maintained serum testosterone levels ~10 fold the serum testosterone of the placebo treated female pig for 22 days. In contrast, the serum testosterone in the Yorkshire pig receiving the 2.24 g TE silastic implant was ~4 fold the serum testosterone of the placebo treated female pig. Although the Hampshire pig manually removed some of her implants by rubbing on the bars in her enclosure, her serum testosterone was beginning to drop prior to these incidents. In the Yorkshire pig, serum testosterone remained relatively elevated until ~ 30 days post-implantation.

Ethics statements

All animal experiments complied with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 8023, revised 1978) [9] and were conducted under an Institutional Animal Care and Use Protocol through Texas A&M University (IACUC-2022–0259).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Nirvana Mahabir: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Annie E. Newell-Fugate:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Acknowledgment

We would like to Kadden Kothmann, Michael Grizzaffi, and the staff members of Texas A&M University Veterinary Medical Park for their help with the surgical placement of implants and blood collection from the pigs. This work was funded by the President's Excellence Fund from Texas A&M University.

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