Theriogenology 82 (2014) 455–460

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of time and dose of recombinant follicle stimulating hormone agonist on the superovulatory response of sheep

Heloisa M. Rutigliano [a,*,1], Betty M. Adams [a], Albina Jablonka-Shariff [b,c], Irving Boime [b,c], Thomas E. Adams [a]

[a] Department of Animal Science, University of California, Davis, California, USA
[b] Department of Developmental Biology, Washington University School of Medicine, Saint Louis, Missouri, USA
[c] Department of Obstetrics and Gynecology, Washington University School of Medicine, Saint Louis, Missouri, USA

ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form 2 May 2014
Accepted 9 May 2014

Keywords:
Gonadotropin
Ovine
Multiple ovulation
Single-chain gonadotropin
Assisted reproductive technologies

ABSTRACT

The objective of this study was to determine the superovulatory potential of a single-chain analog of human FSH (Fcα) when administered to ewes either 3 days before, or coincident with, simulated luteolysis (pessary removal [PR]). A total of 40 animals were randomly assigned to receive Fcα at doses of 0.62, 1.25, or 2.5 IU/kg of body weight (bwt) 3 days before PR or 0.31, 0.62, 1.25, or 2.5 IU/kg of bwt at PR. Control ewes received protein without FSH activity. Blood samples were collected during the periovulatory period and ovarian tissue was collected 11 days after PR. Ovulation rate did not differ from the control group in ewes receiving the smallest doses of Fcα (0.31 and 0.62 IU/kg). However, a significant superovulatory response was noted in sheep receiving Fcα at doses of 1.25 and 2.5 IU/kg and this response was comparable in animals receiving the largest dose levels of Fcα at, or 3 days before, PR. The interval between PR and the LH surge was significantly extended and the LH surges were less synchronous in animals receiving Fcα at PR when compared with animals receiving the potent FSH agonist 3 days before PR. Taken together, these data indicate that the human single-chain gonadotropin with FSH activity promotes superovulation in ewe lambs in the breeding season. A single injection of the recombinant gonadotropin 3 days before luteolysis synchronizes the LH surge. The use of the single-chain analog of FSH in assisted reproduction for domestic animals is likely to be of practical significance as an alternative to conventional gonadotropins in superovulation protocols in livestock species.

1. Introduction

The reproductive technologies that permit synchronization of estrus, induced ovulation, and multiple ovulation and embryo transfer have been widely used in the livestock industry to increase out-of-season breeding, induce precocious puberty, and accelerate the pace of genetic improvement. Many of these manipulations of the normal reproductive process involve the administration of exogenous gonadotropins that stimulate ovarian follicle development and maturation, and ovulation. These stimulatory hormones are generally isolated from tissues of the pituitary glands of pigs and sheep (pituitary FSH and LH), the plasma of pregnant mares (eCG), and the urine of pregnant women (hCG) [1].

Although potent and effective in modulating ovarian activity in laboratory species, the use of tissue-derived

* Corresponding author. Tel.: +1 559 623 3756, +1 435 797 9877; fax: +1 435 797 2118.
E-mail address: heloisa.rutigliano@usu.edu (H.M. Rutigliano).
[1] Present address: School of Veterinary Medicine, Department of Animal, Dairy and Veterinary Sciences, Utah State University, 4815 Old Main Hill, Logan, UT 84322, USA.

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.05.010

gonadotropins in livestock species often is associated with variable ovarian responses. The inconsistent nature of the response in livestock is ascribed to animal-to-animal variation in sensitivity, and batch-to-batch variation in purity and potency of tissue-derived gonadotropin preparations [1]. For example, Murphy et al. [2] found that the FSH activity and the relative proportion of FSH to LH activity in eCG preparations varied between mares and within mares with stage of gestation. Gonadotropins isolated from animal tissue may also be vectors for infectious diseases [3,4]. Additionally, pituitary-derived gonadotropins are cleared very rapidly from the circulation, and most of the superovulation protocols recommend repeated administration of FSH preparations at 8- to 12-hour-intervals over a period of 3 to 4 days [1,5], increasing animal stress and management cost.

Pituitary and placental gonadotropins are heterodimers consisting of noncovalently linked α and β subunits. Within a given species, the sequence of the α subunit is identical among gonadotropins, whereas the amino acid composition of the β subunits of LH, FSH, and CG is unique. Proper post-translational processing of the subunits is essential not only for efficient secretion [6] but also to establish the full biological activity of the gonadotropins [7]. Establishment of the αβ heterodimer is the rate-limiting step in formation of functional gonadotropins [8]. Novel recombinant gonadotropins bypass this limiting step by including α and β subunit domains in a single peptide chain [9]. These unique single-chain analogs of the gonadotropins are created by joining complementary DNA encoding the genomic sequences of the α and the β subunits using a linker sequence consisting of the portion of the exon encoding the C-terminal peptide (CTP) of the β subunit of hCG. The CTP is a 28 amino acid peptide that undergoes extensive O-linked glycosylation and contributes to the longevity and potency of hCG. Indeed, analogs of LH and FSH that contain the CTP domain have extended half-lives and exhibit prolonged activity after administration [10].

Single-chain analogs of the human gonadotropins are effective in inducing an ovulatory response in sheep and may be an effective alternative to tissue-derived gonadotropins in superovulation protocols for domestic species. In addition to the ease and consistency of production by transfected cells, recombinant gonadotropins are likely to involve reduced risk of disease transmission and enhanced biopotency and duration of action relative to tissue-derived gonadotropins. Our recent studies have found that the single-chain human gonadotropins induce a profound follicular and ovulatory response in sheep made deficient in endogenous gonadotropins by passive immunization against GnRH [11].

The success of the response to superovulation protocols is also negatively affected by variation in the interval between luteolysis and the LH surge [12]. The temporal relationship between follicle development, full follicular maturation, and the LH surge is essential for production of viable and transferable embryos. Inappropriate delay in the timing of the LH surge is highly correlated with a reduction in the number of transferable embryos [12]. Conversely, premature onset of the LH surge is associated with reduced rates of ovulation because of inadequate oocyte maturation or insufficient concentration of follicular LH receptors [13,14]. In addition, simulation of luteolysis by abrupt removal of progestogen-containing vaginal inserts is associated with more precise synchronization of ovulation than is induction of luteolysis using exogenous PGF2α [12].

In the present study, we determine the dose of the single-chain human FSH agonist required to induce a superovulatory response in ewe lambs during the breeding season. We also examine the ovarian response of sheep to the FSH agonist when administered 3 days before, or concurrent with, simulated luteolysis.

2. Materials and methods

2.1. Animals

Forty yearling Suffolk ewes displaying regularly estrous activity were used to evaluate the superovulatory potential of the single-chain analog of human FSH. The studies were conducted in late November and early December, a period of active reproductive function in sheep at this latitude. The ewes were housed in an open-sided barn with free access to food and water. Cannulae were inserted 1 day before experimentation using the procedure described previously [11]. After cannulation, animals were freely mobile at the end of a 1 m lead rope. Blood collection and hormone administration were made from the exterior of the animal holding area using the cannula. All experimental procedures involving the use of animals were conducted in accordance with the National Institutes of Health guidelines and were reviewed and approved by the Animal Use and Care Administrative Advisory Committee for the University of California, Davis.

2.2. Construction of single-chain gonadotropins

The single-chain analog of human FSH (hFSHβ-CTP-α; Fcα) was constructed as described previously [10,15]. The complementary DNA encoding the genomic sequences of the α and β subunits of human FSH were joined through a linker sequence coding for the carboxyl terminal 28 amino acids of hCGβ. Chinese hamster ovary (CHO) cells were transfected with constructs encoding Fcα. Cell lines were grown in Ham's F-12 media supplemented with 5% fetal bovine serum, 100 IU/mL penicillin and streptomycin, and 125 μg/mL neomycin analog (G418). Nontransfected (control) cells were grown in similar media without the G418. All cells were grown in T-150 flasks at 37 °C, in air containing 5% CO_2. Conditioned media was collected from control and transfected cells and the protein was concentrated by centrifugation (Centricon Plus-70; Millipore Corp., Billerica MA, USA) and stored at −20 °C.

2.3. Estrus synchronization

Stage of the estrous cycle of the ewes was synchronized using two injections of PGF2α administered 13 days apart. Progestogen (40 mg flugestone acetate) containing vaginal pessaries (Chronogest; Interevet International, Boxmeer, Holland) were inserted 9 days after the second PGF2α injection and removed 10 days later.