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Effects of breed on kinetics of ovine FSH and ovarian response in superovulated sheep

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Abstract

Embryo production is a useful tool for ex situ conservation of endangered species and breeds, despite a high variability in the ovarian response to superovulatory treatments. The current study evaluated the incidence and mechanisms of genetic factors in such variability, by determining the pharmacokinetics and pharmacodynamics of a standard treatment with ovine FSH (oFSH) in two endangered Spanish sheep breeds (Rubia del Molar, R, and Negra de Colmenar, N) in comparison to Manchega ewes (M, control group). In the first experiment, pharmacokinetics of an i.m. single dose of 1.32 mg of oFSH was evaluated in seven animals of each breed. Plasma FSH concentrations reached their maximum at 4 h post-administration in all the ewes, but several of the kinetic parameters (plasma FSH concentration at 4 h post-administration, maximum plasma FSH concentration, C_{max}, and both the area under the plasma concentration-time curve extrapolated to the infinite, AUC_{inf}, and to the last moment of sampling, AUC_{last}) were higher in the N group. In the second trial, 10 animals of each breed were superovulated using eight decreasing doses of oFSH (3× 1.32 mg, 2× 1.10 mg, and 3× 0.88 mg). The R group, when compared to N and M, showed both a higher number of corpora lutea (13.7 ± 0.6 versus 10.0 ± 0.4 in N and 9.8 ± 0.6 in M, $P < 0.05$ for both) and embryos (7.9 ± 0.8 versus 4.3 ± 0.4 in N, $P < 0.05$, and 6.7 ± 0.5 in M, n.s.). Evaluation of pharmacokinetic and dynamic parameters showed that, although there was a trend for a higher hormone availability in R sheep, mean FSH plasma concentrations were similar between breeds (0.54 ± 0.08 ng/ml for R, 0.45 ± 0.05 ng/ml for N and 0.35 ± 0.05 ng/ml for M). However, differences were found in the number of preovulatory follicles growing in response to the FSH treatment between R (24.4 ± 2.2), M (18.9 ± 1.5, n.s.) and N sheep (14.1 ± 1.4; $P < 0.01$). Thus, differences in embryo yields between breeds would be related to differences in the pattern of follicular growth in response to FSH treatment.

Keywords: Breed; FSH kinetics; Follicular dynamics; Sheep; Superovulation

1. Introduction

Protocols for superovulation and embryo transfer are widely used to increase progeny produced in sheep, like in other ruminants, thereby providing an alternative tool both for spread of selected animal genetics

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and for ex situ preservation of endangered breeds. However, despite the improvements of the last few years, the ovarian response to exogenous gonadotrophin treatments remains highly variable. Factors causing such high variability are the major constraint for multiple ovulation and embryo transfer (MOET) protocols [1,2], being classified either as extrinsic (depending on the protocol of treatment used for multiple ovulation) or intrinsic factors (derived from species, breed, age, nutrition, reproductive and lactational status of donors).

Breed is widely recognized as a major factor of variation [3]. In a very exhaustive study using over 9000 superovulated sheep, the breed factor accounted for approximately 30% of the variability in the embryo yields obtained in response to FSH treatments [4]. Early studies established that most of the differences in superovulatory response were related to the different prolificacy of the breeds used in MOET [5], with highly prolific breeds having a greater response to exogenous stimulation [6–8]. These differences were also found when comparing non-prolific breeds, where an interaction between the type of gonadotrophin used and the breed was also described [9]. Thus, due to the variation in superovulatory responses derived from genetic factors [10], it has become important to establish a reliable method of superovulation for each individual breed as the first step toward the establishment of reliable least variable embryo transfer programmes, particularly when this involves the potential conservation of endangered species.

Rubia del Molar and Negra de Colmenar are two native non-prolific Spanish breeds listed as endangered species (FAO DAD-IS; http://dad.fao.org/en/Home.htm) and currently included in conservation programmes by freezing of semen and embryos. Most of the work on superovulation of sheep has been carried out in highly-producing breeds, while only limited information concerning the superovulatory response of other breeds is currently available [11]. The first aim of the present study was to determine the pharmacokinetic behaviour of ovine FSH (oFSH) administered in single and multiple doses in Rubia del Molar and Negra de Colmenar sheep. The second objective was to analyze in practice the pharmacodynamics, in terms of effects on follicular growth, ovulation rate and embryo yields. The second objective would also help to verify a previous hypothesis suggesting that differences in rates of follicular growth and function [12,13] may be the underlying reason for the different responses to exogenous hormone observed between breeds.

2. Material and methods

2.1. Animals

This study included 2 consecutive experiments, conducted during the breeding season (November 2004 and February 2005), which involved a total of 30 ewes, distributed in 3 groups according to their breed (10 Manchega, 10 Negra de Colmenar and 10 Rubia del Molar sheep), without differences in age distribution (range: 4–7 years old). Manchega sheep were used as control, being a breed in which the follicular response to the FSH treatment is well known [14]. Animals were in good body condition, and were part of the herd, maintained outdoors with access to indoor facilities, of the experimental farm of the Centro Nacional de Seleccion y Reproduccion Animal (CENSYRA, Madrid, Spain, latitude 40°N). The farm meets the requirements of the European Union for Scientific Procedure Establishments and the experiment was performed under Project Licence from the INIA Scientific Ethic Committee. None of the sheep had been previously used in superovulatory protocols.

2.2. Experiment 1

Breed differences in the pharmacokinetics of FSH after the administration of a single intramuscular dose of oFSH.

2.2.1. Experimental procedure

A total of 21 sheep were used, 7 ewes from each breed. Animals were treated with an intravaginal progestagen impregnated sponge (40 mg fluorogestone acetate, FGA, Chronogest®, Intervet International, Boxmeer, The Netherlands) for 14 days in a similar way as for our routine embryo production protocol [14]. In order to reduce the endogenous secretion of FSH to its nadir levels [15], a subcutaneous dose of 1.5 mg of the GnRH antagonist (GnRHa) teverelix (Antarelix®, Zentaris, AG, Frankfurt, Germany), was administered 4 days after sponge insertion. On the day after the GnRHa administration (considered Day 0 of the experimental design), all the animals were treated with a single dose of 1.32 mg of oFSH (Ovagen™, equivalent to 17.6 mg NIADDK/20 ml, ICP, Auckland, NZ); administered deeply in the semitendinous muscle, like all the other i.m. treatments used in current study. This dose corresponds to the one used in first injection of oFSH in multiple decreasing dose regimes in superovulatory protocols used in our laboratory. Serial sampling was carried out for determination of FSH kinetics at −24,