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Aminopeptidase activity in seminal plasma and effect of dilution rate on rabbit reproductive performance after insemination with an extender supplemented with buserelin acetate

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ABSTRACT

Ovulation induction in artificially inseminated rabbits by adding GnRH synthetic analogues in the seminal doses is a welfare-orientated method to induce ovulation in rabbits and could have some advantages in field practice. This study was conducted to determine the effect of male genotype on the aminopeptidase activity in rabbit seminal plasma and the effects of dilution rate of semen on availability and reproductive performance when buserelin acetate is added to the seminal dose. To study the aminopeptidase activity, 12 mature bucks belonging to a paternal line and 12 from a maternal line were used. The bucks from the paternal line were used to study the effect of dilution rate on the availability of buserelin acetate after 2 hours of dilution and on the reproductive performance of the doses after artificial insemination of 389 commercial crossbreed does. Aminopeptidase activity in seminal plasma is dependent on the male genotype. The paternal line resulted 27% more aminopeptidase activity than the maternal line ($P < 0.05$). On the other hand, semen diluted 1:20 exhibited a marked increase in the availability of buserelin acetate and the fertility in this group was significantly higher than females from dilution rate 1:5 group, which showed similar results to that of the negative control group (does inseminated with semen diluted 1:20 in non–GnRH-supplemented extender). We conclude that the bioavailability of buserelin acetate when added to the seminal dose appears to be determined by the activity of the existing aminopeptidases and is consequently affected by the dilution rate used to prepare the artificial insemination doses.

1. Introduction

Ovulation in rabbits is induced by sensory stimulation associated with mating; therefore, artificial insemination (AI) requires an effective control of ovulation. Because the first assays with GnRH synthetic analogues applied to ovulation induction in rabbit does took place 25 years ago [1,2], the use of AI has become a common practice in the rabbit meat–producing areas of European Countries. The most frequent method used for ovulation induction in AI is intramuscular administration of GnRH analogue. Recently, several authors reported the possibility of ovulation induction after supplementation of semen extender with GnRH synthetic analogues [3–8]. The addition of GnRH

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analogues to the seminal dose fulfils the need for a welfare-orientated method to inducing ovulation in rabbits [9]. There are clear breeding advantages of intravaginal administration of GnRH analogue (less treatment distress, labor for the farmers, and operating time), but unfortunately, the concentration of GnRH analogue to be added to the seminal dose to achieve fertility results similar to those of intramuscular administration is much higher than the amount administered intramuscularly.

Vaginal absorption of GnRH may be influenced by the characteristics and state of the mucosa, the extender composition, and probably by the enzymes present in the seminal plasma. A common practice in rabbit AI is dilution of the semen pool with extenders for handling and storage of sperm samples. Because of the relatively low sperm concentration of this species (200–500 million/mL) and the sperm count used per insemination dose (10–20 million of sperm are ordinarily used per insemination), the dilution rate used may fluctuate from 1:5 to 1:20 (vol:vol).

It was suggested that the dilution rate of semen had a great effect on GnRH activity when hormone is added to the extender. Results from Vicente et al. [8] showed that when buserelin acetate was added to seminal plasma diluted 1:5, a more marked decrease in ovulation frequency occurred than if it was diluted 1:20, which could indicate that the availability of synthetic analogue of GnRH was increased as a consequence of the reduction of the existing aminopeptidases.

No study on rabbit seminal plasma aminopeptidase activity was found in the literature, although they may play an important role in AI when the hormone to induce ovulation is added to the seminal dose. Thus, this work was undertaken to quantify the aminopeptidase activity in rabbit seminal plasma of two male genotypes and evaluate their possible involvement in the bioavailability of acetate buserelin and in the fertilizing capabilities of fresh semen diluted at different rates when the GnRH analogue is added to the seminal dose.

2. Material and methods

Unless stated otherwise, all chemicals in this study were purchased from Sigma–Aldrich Química S.A (Madrid, Spain).

2.1. Animals

All animals were handled according to the principles of animal care published by Spanish Royal Decree 53/2013 (BOE, 2013; BOE = Official Spanish State Gazette).

To study the effect of the male genotype on aminopeptidase activity, a total of 24 adult bucks, 12 belonging to a paternal rabbit line (Line R) and 12 from a maternal rabbit line (Line A) were used. Line R was selected for daily weight gain between 28 and 63 days of age by individual selection [10] and Line A (New Zealand White line) has been selected since 1980 for litter size at weaning with a family index [11]. All males were kept individually in flat deck cages under 16 hours light:8 hours dark conditions at the experimental farm of the Animal Technology and Research Center (CITA-IVIA, Segorbe, Castellón, Spain) and fed with the same commercial diet (17.5% crude protein, 2.3% ether extract, 16.8% crude fiber, 2.600 kcal DE/kg) and had free access to water.

To study the effect of dilution rate on their reproductive performance, 389 commercial crossbreed does were inseminated on one commercial farm (Altura, Castellón, Spain) with semen from the 12 Line R adult males. Females were classified by physiological status as multiparous lactating does (more than two delivered births and eight or nine young rabbits suckled) and multiparous non–lactating does (females with more than one parity without suckling any young). Multiparous lactating does were inseminated 10 to 12 days after delivery.

2.2. Aminopeptidase activity determination

2.2.1. Seminal plasma preparation

Semen samples were collected over 14 weeks. Each week, two ejaculates per male were collected, with a minimum of 30 minutes between ejaculate collections, on a single day using an artificial vagina. Sperm evaluation was performed to assess the initial seminal quality. Only ejaculates exhibiting a white color and possessing greater than 70% motile sperm were used in the experiment (minimum requirements commonly used in AI centers); all other ejaculates were discarded. All ejaculates from the same line were pooled (14 pools for Line R and 10 pools for Line A, 24 pools in total).

Semen samples were centrifuged at 10,000× *g* for 10 minutes at 22 °C. The resulting supernatants were collected and centrifuged again (10,000× *g* for 10 minutes) to remove residual spermatozoa and cell debris. The resulting pellets were discarded, whereas the supernatants were stored at −80 °C until use.

2.2.2. Measurement of aminopeptidase activity

Aminopeptidase activity was fluorometrically measured by a modification of the Greenberg method [12] using alanine-β-naphthylamide as substrate. The assay is on the basis of the fluorescence of β-naphthylamide (BNA) released from the hydrolysis of an appropriate substrate by the enzyme. This release is the result of the incubation in a 96-well white polystyrene microplate (30 minutes incubation at 37 °C) of 25 μL of seminal plasma sample with 100 μL of substrate solution. After 30 minutes, 100 μL of 0.1 M sodium acetate buffer (pH = 4.2) was added to terminate the reaction. The released BNA was determined by measuring the fluorescence intensity at 460 nm with excitation at 355 nm. Wells without sample were used to determine the background fluorescence. The relative fluorescence was converted to pmol of released BNA by comparison with a standard curve, previously obtained with increasing concentrations of product and decreasing concentrations of substrate. Protein concentration was measured using the bicinchoninic acid assay using bovine serum albumin as the standard [13]. Enzyme activity and protein concentration were measured in triplicate. The peptidase activity was expressed as pmol of BNA released per milligram of protein per minute. The existence of a linear relation between time of hydrolysis and protein content in the fluorometric assay was a previous condition.