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Rabbit seminal plasma proteome: The importance of the genetic origin

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ABSTRACT

The present study was conducted to characterise rabbit seminal plasma proteins (SP proteins) focusing on the influence of the genetic origin and seasonality. In addition, β-NGF protein quantity in SP was determined. Semen samples were recovered from January to December 2014 using 6 males belonging to genotype A and six from genotype R. For each genotype, one pooled sample at the beginning, middle and end of each season was selected to develop the experiment. A total of 24 pools (3 for each season and genetic line) were analysed. SP proteins of the two experimental groups were recovered and subjected to in-solution digestion nano LC–MS/MS and bioinformatics analysis. The resulting library included 402 identified proteins validated with ≥95% Confidence (unused Score ≥ 1.3). These data are available via ProteomeXchange with identifier PXD006308. Only 6 proteins were specifically implicated in reproductive processes according to Gene Ontology annotation. Twenty-three proteins were differentially expressed between genotypes, 11 over-expressed in genotype A and 12 in genotype R. Regarding the effect of season on rabbit SP proteome, results showed that there is no clear pattern of protein variation throughout the year. Similar β-NGF relative quantity was observed between seasons and genotypes. In conclusion, this study generates the largest library of SP proteins reported to date in rabbits and provides evidence that genotype is related to a specific abundance of SP proteins.

1. Introduction

The control of rabbit reproduction has experienced great changes in the last decade, mainly as a consequence of the development of new techniques such as commercially applicable artificial insemination (AI) (Safaa et al., 2008). The use of AI in intensive meat rabbit production is currently a common practice (Piles et al., 2013), like in the vast majority of livestock (Hansen, 2014), and it utilisation has contributed to improve the knowledge of rabbit spermatozoa and bucks' management (Boiti et al., 2005; Castellini, 2008; Lavara et al., 2005; Pascual et al., 2016; Safaa et al., 2008; Theau-Clement et al., 2015; Theau-Clément et al., 2016; Viudes-de-Castro et al., 2014). Rabbit ejaculates present some peculiarities that should be taken into account, for instance, they present occasionally gel plug or gelatinous mass and contain several vesicles that have been related to modulate different sperm functions such as motility, capacitation and acrosome reaction (Castellini et al., 2006, 2012, 2013; Collodel et al., 2012). In addition, rabbit belongs to the few species in which ovulation is induced by copulation (Fischer et al., 2012), like cats, camelids, koala, voles and sumatran

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rhinos (McGraw et al., 2015). In these species, a specific protein named β-NGF has been studied in seminal plasma because of its potential role in inducing ovulation in camelids (Adams and Ratto, 2013; Belardin et al., 2016; Druart et al., 2013; Kershaw-Young et al., 2012; Li et al., 2010; Silva et al., 2011). Nevertheless, in rabbits, the intramuscular administration of seminal plasma did not provoke ovulation (Silva et al., 2011), but plays a role in promoting the formation and development of the testis and the differentiation, maturation, and movement of the spermatozoa (Li et al., 2010).

Many factors influence the production and quality of rabbit semen such as the genetic origin (growth lines have worse seminal qualities and fertility rates than maternal lines) (Mocé et al., 2003; Vicente et al., 2000), the season (Marai et al., 2002; Pascual et al., 2004; Schneidgenová et al., 2011; Theau-Clement et al., 2015), the photoperiod (Ain-Baziz et al., 2012; Roca et al., 1992; Sabés-Alsina et al., 2015) and the collection frequency (Nizza et al., 2003). The production of fertile doses is determined by several components: i) male libido and characteristics of the ejaculate which form part of the criterion for ejaculate rejection; ii) volume and sperm concentration of the ejaculate (determining the amount of doses that can be obtained); and iii) the quality of sperm (determining the minimum sperm dosage required to ensure fertilization) (Piles et al., 2013). Subjective estimation of motility and evaluation of sperm morphology are the two laboratory assays most widely used for the rabbit semen evaluation in insemination centers (Lavara et al., 2005). However, the ability of these seminal characteristics to predict reproductive performance is very low (Piles et al., 2013). In line with the greater number of livestock species, the prediction of ejaculates of high fertility or good cryopreservation remains unresolved. However, while most of these previous studies have been focused on the sperm cell, little attention has been paid to the seminal plasma in rabbit. To date, a limited number of studies have performed an analysis of rabbit seminal plasma proteins (Arruda-Alencar et al., 2012; Casares-Crespo et al., 2016a; Davis and Davis, 1983; de Lamirande et al., 1983; Lavon, 1972; Minelli et al., 2001; Okabe et al., 1993; Thomas et al., 1986; Viudes-de-Castro et al., 2004) in comparison to the main commercially relevant domestic mammalian species (Rodríguez-Martínez et al., 2011; Druart et al., 2013; Bromfield, 2016).

Seminal plasma contributes to the safe environment for sperm maturation, sperm viability and fertilization in mammals (Muiño-Blanco et al., 2008; Rodríguez-Martínez et al., 2011; Manjunath et al., 2007; Bromfield, 2016). Moreover, seminal plasma is a promising source for the study of potential reproductive biomarkers, because it is a complex mixture of secretions from testis, epididymis and male accessory sex glands (González-Cadavid et al., 2014). Sperm maturation is acquired during the transit of the spermatozoa through the epididymis, where its plasma membrane undergoes intense changes in protein composition and in localization of their components (Dacheux et al., 2003). The protein composition of mammalian seminal plasma varies among species, and has important effects on sperm function (Rodríguez-Martínez et al., 2011). Even though seminal plasma contains hundreds of proteins, their functions are not completely understood. In rabbits, seminal plasma has a positive effect in maintaining sperm motility and viability during *in vitro* storage (Castellini et al., 2000).

Against this background, the present study was conducted to characterise rabbit seminal plasma proteins through nano LC–MS/MS analysis, focusing on the influence of the genetic origin and seasonality. In addition, β-NGF protein quantification was done.

2. Materials and methods

Unless stated otherwise, all chemicals in this study were purchased from Sigma-Aldrich Química S.A (Madrid, Spain). All the experimental procedures used in this study were performed in accordance with Directive 2010/63/EU EEC for animal experiments.

2.1. Localization and animals

The experiment was carried out with 24 males from two Spanish commercial rabbit genetic lines (genotypes A and R) from January to December 2014. All bucks were of proven fertility and subjected to a weekly pattern of ejaculate collection. Line A is based on New Zealand White rabbits selected since 1980 by a family index for litter size at weaning over 45 generations (Fig. 1 right). Line R comes from the fusion of two lines, one founded in 1976 with Californian rabbits reared by Valencian farmers and another founded in 1981 with rabbits belonging to specialised paternal lines (Fig. 1 left). The selection method was individual selection on post-weaning daily gain, with weaning taking place at 28 days and the end of the fattening at 63 days. All animals were housed at the Animal Technology and Research Centre (CITA-IVIA, Segorbe, Castellón, Spain) experimental farm in flat deck indoor cages

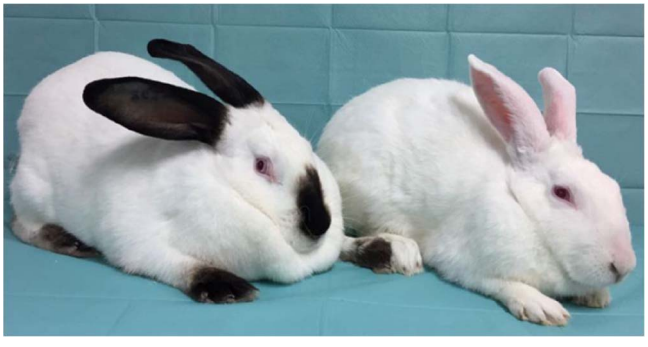

Fig. 1. Picture of rabbit genotypes R (left) and A (right).