Animal Reproduction Science 157 (2015) 33–38

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Some factors that influence semen characteristics in rabbits

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ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form 11 March 2015
Accepted 13 March 2015
Available online 24 March 2015

Keywords:
Rabbit
Sperm production
Semen traits
Age, Season

ABSTRACT

The aim of this study, based on a five-year-long experiment, was to analyse some of the factors that influence rabbit sperm production. A total of 174 bucks between 23 and 44 weeks of age from five successive groups were used for semen collection one day per week, two times, at a 15 min interval (ejaculates of rank 1 and 2), over a period of 21 weeks. Immediately after collection, pH, mass motility, volume and concentration were measured using classical methods, and a set of motility parameters were recorded by a computer-assisted semen analysis system. Between groups, the number of motile sperm per ejaculate, considered as a synthetic criterion combining both qualitative and quantitative aspects of semen characteristics, varied from simple to double (from 150 to 326 $\times 10^6$), reflecting the strong influence of uncontrolled environmental factors. Adult (37–43 weeks old) expressed a higher number of motile sperm/ejaculate than younger bucks (300 vs. 205 $\times 10^6$). In autumn the number of motile sperm/ejaculate was higher than in summer (287 vs. 188 $\times 10^6$). Sperm production was higher on average for the first ejaculate compared to the second one (270 vs. 167 $\times 10^6$). For several semen characteristics, the effect of the collector was significant but without any repercussion on sperm production. Bucks born to nulliparous or primiparous does had higher performances. This study highlights the high variability of rabbit semen characteristics and the multitude of factors involved, either controlled or uncontrolled.

1. Introduction

With the widespread use of artificial insemination (AI) on rabbit farms, farmers generally buy semen from production centres. In recent years, some studies have therefore focused on factors that influence rabbit sperm production with the aim to improve its mean value and reduce its variability. The genetic type of the males, their age, health status, environmental conditions (season, lighting, etc.), diet and collection conditions all affect sperm production (Alvariño, 2000; Castellini, 2008; Piles et al., 2013). The purpose of this study, based on 5 years of observations of semen characteristics, was to improve our knowledge about the factors that may influence the quantitative and qualitative components of rabbit sperm production.

2. Materials and methods

All procedures were conducted in accordance with the guidelines for the Care and Use of Animals in Agricultural Research and Teaching (French Agricultural Agency and

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http://dx.doi.org/10.1016/j.anireprosci.2015.03.011
0378-4320/

Table 1
Periods of semen evaluation for the five successive groups of bucks.

Group of bucks	Number of bucks	Semen evaluation periods
1	36	March to July 2004
2	30	September 2004 to January 2005
3	36	February 2006 to June 2006
4	36	August 2006 to December 2006
5	36	November 2007 to March 2008

Scientific Research Agency; approval number of the PECTOUL Experimental Farm: A 31 113 16).

2.1. Animals

The experiment was conducted between 2004 and 2008, on five successive groups of 30–36 males each, tested at different seasons and representing three generations of the INRA1001 strain (Table 1). This strain, characterised by a high body weight, was provided by a private breeder (Hypharm).

2.2. Experimental design

For each group, at the age of 21 weeks, bucks were submitted to a training phase (contact with a teaser female, one day per week). At 23 weeks of age, from 30 to 36 males were selected on the basis of ability to be collected and their genealogy. Ejaculates were collected once a week over 21 weeks with two collections at 15 min intervals (ejaculate rank 1 and 2, Bencheikh, 1995). The absence of ejaculation within 2 min was considered as failed collections. The second ejaculate when collected after the first failed was originally considered separately (false 2). Semen recording was performed only 1 week out of 2, resulting in 11 weeks of semen evaluation. No sexual preparation was applied to bucks prior to collection. The rabbits were housed under a continuous photoperiod of 16 h light and 8 h darkness. They were fed *ad libitum* a commercial diet containing 175 g/kg protein and 145 g/kg fibre. Bucks were housed in individual cages arranged on flat decks, designed to facilitate semen collection. The room was heated and ventilated in winter so that the temperature did not fall below 18 °C. There was no cooling system and the maximum temperature recorded was 36 °C.

2.3. Semen traits

Immediately after semen collection, pH, volume and mass motility (coded from 0 to 9) were estimated, as described by Brun et al. (2002). Sperm cell motility was analysed from a 2 mL sample after dilution (1:40) in Galap (IMV Technologies, France) by a computer-assisted sperm analysis system (HTMA-IVOS, Version 10, Hamilton Thorne Research, Beverly, MA, USA) according to the set-up parameters described by Brun et al. (2006). The minimum cell number counted was 50. For each ejaculate, two drops of 10 μL each and three fields per drop were evaluated (Theau-Clément et al., 1996). The HTMA variables analysed (Boiti et al., 2005) were: percentage of motile sperms (Pmot), percentage of progressive sperms (speed > 40 μ/s and straightness > 90%, Ppro), track linearity (Lin, %), average path velocity of sperms (VAP, μm/s) and amplitude of lateral head displacement (Alh, μm). Concentration was estimated from 20 μL of pure semen diluted 1:200 in sodium citrate using a Thoma-Zeiss cell counter, and was only measured for the last four groups of males. Some additional variables were calculated: total number of sperm per ejaculate (TSE = volume × concentration), considered as a synthetic criterion of sperm production, and number of motile sperm per ejaculate (MSE = TSE × Pmot), combining both qualitative and quantitative aspects of sperm production.

2.4. Statistical analysis

Semen characteristics were analysed using a mixed linear model with the Proc Mixed procedure of SAS®. The buck was taken as a random effect. The fixed effects were the group of bucks (five levels), their age (three levels: growing males – from 23 to 27 weeks of age; young adults – from 29 to 35 weeks of age; and adults – from 37 to 43 weeks of age), the calendar season (four levels), the rank of ejaculate (three levels: 1, 2 and false 2, when semen was obtained only at the second collection), the technician in charge of collections (four collectors) (SAS, 2001), and the mother's parity at the buck's conception (three levels: 1, 2 and ≥3). All interactions, taken two-by-two, were first integrated into the model except for group × season because the intersection of these two factors had empty cells. Non-significant interactions on all semen parameters were eliminated. Only the group × ejaculate rank, season × ejaculate rank and age × season remained. For concentration, TSE and MSE, the factorial table age per season had empty cells and, consequently, the interaction between age and season was not estimable and was not included in the model.

3. Results and discussion

Out of 2243 ejaculates collected, 1958 ejaculates without urine were analysed.

The group of bucks influenced all semen characteristics except pH (Table 2). Indeed, bucks from Group 1 had higher semen performance (mass motility, semen volume, percentage of progressive sperm, linearity, average path velocity and amplitude of lateral head displacement), which sometimes did not significantly differ from Group 5. In contrast, bucks from Group 2 had lower performance (mass motility, percentage of motile or progressive cells, linearity and concentration). Between groups of bucks, sperm production per ejaculate ranged from simple to double: it was maximum for Group 5 (361 and 326 × 10^6, for TSE and MSE, respectively) and minimum for Group 2 (185 and 150 × 10^6, for TSE and MSE, respectively). This significant buck group effect that coincided with a time effect represented a decisive influence of uncontrolled environmental effects, not excluding differences in the genetic composition of groups due to sampling. Indeed, Groups 1 and 2 and Groups 3 and 4 had the same paternal origin.

The age of bucks significantly influenced volume, concentration, TSE and MSE, as reported by Luzi et al. (1996).