

# Influence of metabolic status on oocyte quality and follicular characteristics at different postpartum periods in primiparous rabbit does

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## Abstract

Low reproductive performance of high-yield primiparous animals is closely associated with the metabolic stress caused by a simultaneous gestation and lactation. The aim of this work was (1) to analyze body composition and metabolic environment at three time points along lactation (at parturition time; in the lactation period [Day 11 postpartum]; and in the postweaning period [Day 32 postpartum]) of primiparous rabbit does (*Oryctolagus cuniculus*) and (2) to investigate the ovarian status at insemination time and the possible link with metabolic environment and with their reproductive performance. To this end, does were either submitted to a semi-intensive reproductive rhythm (Group S, inseminated on Day 11 postpartum) or an extensive rhythm (Group E, inseminated on Day 32 postpartum). Body energy ( $P < 0.05$ ) and protein content ( $P < 0.001$ ) as well as serum leptin ( $P < 0.05$ ) and protein concentrations ( $P < 0.001$ ) increased significantly along the postpartum period. At parturition, body lipid content was significantly lower and serum nonesterified fatty acids concentrations were significantly higher than that on Days 11 postpartum and 32 postpartum. Concerning ovarian status at insemination time, no significant differences were found in mean follicular stages, serum estradiol, progesterone, and prolactin (PRL) concentrations or in prolactin receptor (PRL-R) immunostaining. However, follicles in Group S showed a significantly higher apoptosis index than that of Group E ( $P < 0.001$ ). The nuclear and cytoplasmic oocyte maturation rates of Group S were also significantly lower than that in Group E. In addition, conception rate and prolificacy were improved in Group E ( $P < 0.001$  and  $P < 0.05$ , respectively). In conclusion, in the early postpartum period, metabolic status seems to impact negatively on ovarian follicle and oocyte quality leading to a poor reproductive outcome in primiparous rabbit does. © 2009 Elsevier Inc. All rights reserved.

**Keywords:** Follicular atresia; Metabolism; Oocyte maturation; Postpartum; Rabbit

## 1. Introduction

Rabbits show high sexual receptivity immediately after kindling. Then, it decreases in a nonpredictable trend and another peak appears only after weaning [1]. However, as occurs in cattle [2], insemination of female rabbits during lactation seems particularly negative due to nutritional competition, leading to a

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negative energy balance in these females [3,4]. This phenomenon is well known and particularly significant at the first parity in certain species [5,6]. Despite the rapid increase in doe feed intake during the early postpartum (postpartum) period, the doe is unable to cover its energetic needs for lactation of multiple litters, gestation, and its own growth [7]. Therefore, these animals show a poorer reproductive outcome than that of multiparous animals [2,8,9]. Artificial insemination (AI) around 11 days postpartum is the most common reproductive rhythm in commercial rabbit farms. It adapts well to cycled production, but this approach ignores the reproductive physiology of the females. This rhythm causes, especially in primiparous rabbit does, a pronounced reduction of conception rate mainly due to the low body condition and hormonal antagonism [10,11].

Body reserves status is reflected by changes in some metabolic parameters, such as serum protein, non-esterified fatty acids (NEFAs), and leptin concentrations [12]. They are present in follicles [13–15] and oviduct [16]; therefore, those parameters could affect the competence of the gametes before fertilization, which in turn influences oocyte maturation and presumably embryo survival [17–19]. In addition, during lactation, rabbit does show high prolactin (PRL) concentrations [20]. Prolactin is a hormone produced by the pituitary gland and also by extra-pituitary sites such as mammary gland, placenta, and uterus [21]. This hormone is the main responsible of the negative effect of lactation on the reproductive function [22]. The expression of PRL receptor in ovaries in several species [23,24] suggests a direct action of this hormone in follicular development and oocyte quality [25–27].

Most of the negative effects in the postpartum period due to metabolic stress and lactation status, which seem to affect follicle and gamete quality, have been extrapolated from studies in other species [5,28,29]. To our knowledge, no previous studies exist about the possible consequences on rabbit ovaries. In order to explain the observed decrease in reproductive performance, especially in primiparous does, the aim of this work was to study ovarian status at different insemination time points in terms of steroid concentrations and oocyte and follicle features. The evolution of metabolic parameters and body composition during the first postpartum period and the possible relationship of these parameters with the subsequent conception rate and prolificacy to establish the best physiology-based management for rabbit species were evaluated.

## 2. Materials and methods

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA). All the experimental procedures were approved by the Animal Ethics Committee of the Polytechnic University of Madrid (Spain) in compliance with the Spanish guidelines for the care and use of animals in research [30].

### 2.1. Animals and experimental design

Along the study, New Zealand  $\times$  California white rabbit does (*Oryctolagus cuniculus*) were held on the experimental farm at the Animal Production Department, Polytechnic University of Madrid (Spain). Animals were housed in individual flat-deck cages under a constant photoperiod of 16 h light per day, a temperature of 18 to 22 °C, and a relative humidity of 60% to 75% maintained by a forced ventilation system. They were fed ad libitum a commercial pelleted diet for lactating does (Cunimax; Cargill S.A., Barcelona, Spain).

To evaluate metabolic serum parameters, ovarian status, and reproductive performance related to different postpartum periods, a total of 90 primiparous does were used. Litter size of does was equalized to eight kits one day after parturition. Does were randomly allocated in two experimental groups: Group S ( $n = 45$ ), lactating does inseminated on Day 11 postpartum, following a semi-intensive reproductive rhythm; Group E ( $n = 45$ ), postweaned lactating does inseminated on Day 32 postpartum, following an extensive reproductive rhythm. In Group S, all animals were estrus-synchronized by transient doe-litter separation before artificial insemination (AI). Biostimulated does were separated from their litters by a metal screen for 24 h before AI, from Day 10 until Day 11 postpartum [9,31]. In Group E, animals were estrus-synchronized by weaning 4 days before AI. In both rhythms, weaning was performed at Day 28 of the lactation period. Artificial inseminations were carried out using a pool of fresh heterospermic semen with more than 20 million spermatozoa in 0.5 mL commercial diluent (Magapor S.L., Zaragoza, Spain). Ovulation was induced by intramuscular injection of 1  $\mu$ g buserelin (Suprafact, Hoechst Marion Roussel S.A., Madrid, Spain). Conception rate (number of parturitions/number of inseminations  $\times$  100) and prolificacy (total born and dead born per doe) were recorded for all animals inseminated.

At parturition time, at Day 11 postpartum and Day 32 postpartum, live body weight (LBW) and estimated

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