

## PATTERNS OF BODY CONDITION USE AND ITS IMPACT ON FERTILITY

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**Abstract:** A total of 60 nulliparous crossbred rabbit females were, *a posteriori*, divided into 2 experimental groups according to their natural “decision” to conceive or not at day 11 post first parturition (L: solely-lactating or LP: lactating-pregnant). This design allowed us to study the evolution of body reserves around first parturition and its influence on the future reproductive success of rabbit females. Primiparous rabbit females that failed to conceive when inseminated at day 11 post-partum (L) seemed to have a higher perirenal fat thickness (PFT) 12 d pre-partum (+0.25 mm;  $P=0.079$ ) than females that conceived (LP). In the subsequent days, L females showed a significantly higher mobilisation rate than LP females (on av. -0.12 and -0.07 mm of PFT per day, respectively;  $P=0.007$ ), reaching a lower PFT at partum (5.17 mm vs. 5.62 mm, respectively;  $P=0.002$ ). However, due to a greater recovery rate observed in L females (+0.15 vs. +0.08 mm of PFT per day for L and LP females, respectively;  $P=0.007$ ), PFT at 10 d post-partum was similar (on av. 5.86 mm). These different patterns showed that PFT level at 12 d pre-partum and PFT mobilisation rate onwards seems to drive the start of a new pregnancy. It also seems to modify the energy metabolism of rabbit females, allowing does to recover perirenal fat tissue without compromising the production of milk necessary for the current litter.

**Key Words:** rabbit females, reproductive rhythm, body condition changes, resources allocation.

## INTRODUCTION

Reproduction is a complex function that involves different systems and tissues such as the reproductive system and adipose tissue. While the reproductive system is responsible for producing gametes and sustaining gestation, adipose tissue is responsible for storing energy (as body fat) and informs the organism’s nutritional status (Chehab, 2000; Zieba *et al.*, 2005). As the nutritional status (i.e. amount of fat reserves) depends on the capacity of individuals to obtain the resources available from their living environment, nutritional status can be regarded as a signal used by organisms to read their environments. Therefore, if the organism perceives the environment as being good enough to sustain reproduction, it may reproduce.

However, in an environment where food is not limited (i.e. no constraints on resources), as in the case of commercial farms, other factors affects the nutritional status of an individual. In the case of rabbit females, their genetic potential (i.e. selection criteria), the quality of their food (i.e. nutritional composition), and their reproductive effort (i.e. size of the litter being raised and the interval between consecutive reproductive cycles) are recognised factors that affect their nutritional status (Fortun-Lamothe, 2006; Pascual *et al.*, 2013; Savietto *et al.*, 2015).

In commercial meat rabbit production, the genetic potential and food quality are normally set as constants while the real reproductive effort of females varies, affecting the female reproductive performance (Cervera *et al.*, 1993). A good example is the higher foetal mortality (+7.1%) and the lower body condition observed in rabbit females

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fully overlapping lactation and pregnancy when compared to females solely lactating (Fortun-Lamothe *et al.*, 1999). Another example is the higher sexual receptivity and pregnancy rate found in females following an extensive reproductive rhythm (Feugier and Fortun-Lamothe, 2006). Additionally, the previous reproductive effort affects the subsequent litter size (10.2 vs. 5.3 kits born alive, for females inseminated on post-weaning and post-partum, respectively), independently of the genetic potential of females (Theilgaard *et al.*, 2009).

Nowadays, adipose tissue is no longer simply considered a body reservoir, as it participates in different physiological contexts (e.g. energy homeostasis, immune and even neurological functions: Kershaw and Flier, 2004; Ahima, 2006), so the body reserve tissue (measured as body condition) could play an important role in the definition of reproduction and survival.

In this context, the use of *in vivo* methods to assess the evolution of fat tissue, such as the perirenal fat thickness method (Pascual *et al.*, 2000, 2004), allowed the description of how females manage their body reserves during reproduction. Although some works have studied the relation of body reserves around parturition and fertility (Rebollar *et al.*, 2011; Romero *et al.*, 2011), no works have described the direct effect of body-reserves mobilisation around partum on the fertility of rabbit females in their subsequent mating. Therefore, the main objective of this work was to describe the dynamic of fat reserves use and its effects on the fertility of primiparous rabbit females.

## MATERIAL AND METHODS

The experimental proceedings were approved by the Universitat Politècnica de València (UPV) ethic committee as set forth in Royal Decree 53/2013 on protection and use of animals for experiments and other scientific purposes, including teaching (BOE, 2013).

### *Animals and procedures*

A total of 60 crossbred rabbit females (UPV lines A×V; both selected for litter size at weaning) were used. Females were first artificially inseminated when they reached 4.2 kg of live weight. A pool of semen from males of a line selected for growth rate (UPV line R) was used to inseminate females. All females were inseminated at the same time, but only 33 females conceived at the first attempt. The remaining females were re-inseminated 22 d after the first attempt (the time required for pregnancy testing and re-insemination). Twenty six females conceived at this 2<sup>nd</sup> attempt, and only 1 female never conceived, so was excluded from the study. Another 12 females aborted or never gave birth, so they were excluded from the final analysis. All females were re-inseminated at 11 d post-partum. Throughout the experimental period, the lighting sequence was set to 16 h of light and 8 h of dark and animals had free access to a commercial diet [11.0 MJ of digestible energy per kg of dry matter (DM) and 180.0 g of crude protein per kg DM] and water.

Female live weight (LW), perirenal fat thickness (PFT) and dry matter intake (DMI) were measured at days: -30, -24, -18, -12, -06, -04, -02, zero, +04, +05, +10, +12, +17, +22, and +30, with respect to first parturition. Perirenal fat thickness was measured by ultrasound, placing the scanning probe between the 8<sup>th</sup> and 9<sup>th</sup> thoracic vertebrae on both sides, as described by Pascual *et al.* (2000 and 2004). Litter size and litter weight (offspring born in total, born alive and weaned) were controlled at birth and at weaning. To control the effect of different litter sizes on milk production, which consequently affects the evolution of LW and PFT during lactation, litters were standardised to nine offspring at birth. Dead offspring were not replaced. Milk yield was measured at days: +04, +05, +08, +10, +12, +17, +22 and +30 post-partum, by weighing females before and after suckling their litters. As a non-negligible amount of feed could be ingested by offspring after day 21 (Maertens and De Groote, 1990; Scapinello *et al.*, 1999), females and their litters were housed in separated cages to avoid overestimation of the female DMI. Once a day, females rejoined their litter for milking. Litters were weaned at 30 d post-partum.

### *Experimental Design*

Females were split into one of the 2 reproductive rhythm groups, according to the effectiveness of the insemination performed at 11 d post-partum. Pregnant females were assigned to the lactating-pregnant rhythm (LP: *n*=27) and empty females into the solely-lactating rhythm (L: *n*=20). Table 1 shows the number of the females at each management time thought the entire experimental period.

**Table 1:** Number of rabbit females at each management process through the experimental period.

Management process	Days respect to 1st partum	Reproductive Rhythm	
		Lactating-Pregnant (LP)	Solely-Lactating (L)
First effective insemination	-31		60
First pregnancy test	-19		59
First partum	0		47
Post-partum insemination	+11		47
Second pregnancy test	+23	27	20
Weaning	+28	27	19

### Statistical Analysis

Females body energy changes were estimated using the multiple regression equation described by Pascual *et al.* (2004) for reproductive rabbit does as follow:

$$EBE=2.51+(0.0012 \times LW)+(0.0018 \times PFT^3) \quad [1]$$

Where EBE is the estimate body energy content (MJ/kg of LW), LW is the live weight (g) and PFT is the perirenal fat thickness (mm). To avoid overestimation of EBE during the pregnancy, caused by the increment of female LW due to the foetus growth, a correction was performed. The weight of the foetus and its annexes was estimated using a Gompertz logistic growth function:

$$Y_{ij}=a_i \times \{\exp[-b_i \times \exp(-k_i \times t)]\} \quad [2]$$

where  $Y_{ij}$  was the observed weight of the litter  $i$  at time  $t_j$ , and  $a_i$ ,  $b_i$ ,  $k_i$  are the estimated parameters of this function. A total of 4 points (days 18, 25, 28, and 31 of pregnancy), obtained from literature (Argente *et al.* 2003, 2006 and 2008; Fortun-Lamothe *et al.* 1999; Quevedo *et al.* 2005; Theilgaard *et al.* 2009), and the current information on litter size at birth were used to estimate parameters  $a_i$ ,  $b_i$ , and  $k_i$ . These parameters were estimated using a nonlinear model (PROC NLIN from SAS, SAS Inst., Cary, NC); giving the following values:  $a_i=0.1000$ ,  $b_i=0.2824$  and  $k_i=1.6067$ .

Females LW, PFT, EBE, DMI, and milk yield (MY) were analysed using a mixed effect model (lme function of R-software; Pinheiro *et al.*, 2015), according to a repeated measures design that takes into account the variation between animals and co-variation within them. The co-variation structure was modelled considering an autoregressive correlation structure between repeated measures on the same individual. The model included the reproductive rhythm (LP and L), the measurement day as fixed effects, and the animal as random effect, as presented below:

$$Y_{ij}=\mu+rhythm_i+time_j+(rhythm_i \times time_j)+\epsilon_{ij} \quad [3]$$

Because animals were, more or less, evenly housed in 2 different rooms (Room 1 - LP=18, Room 1 - L=12, Room 2 - LP=9, Room 2 - L=8), the random effect of animal was nested to the room.

Litter size traits (number of offspring born in total, born alive and weaned) were analysed using a mixed model effect, taking into account the random effect of females nested to the room. The model included only the reproductive rhythm as a fixed effect:

$$Y_j=\mu+rhythm_j+\epsilon_j \quad [4]$$

Finally for the litter weight (weight of offspring born in total, born alive and standardised), two model were used by modifying model 4. Model 4.1, applied to analyse the weight of offspring born in total and born alive, included the number of offspring born in total as a covariate. Model 4.2, used to analyse the weight of standardised litter, included the number of offspring born alive as a covariate:

$$Y_j=\mu+rhythm_j+born\ total_j+\epsilon_j \quad [4.1]$$

$$Y_j=\mu+rhythm_j+born\ alive_j+\epsilon_j \quad [4.2]$$

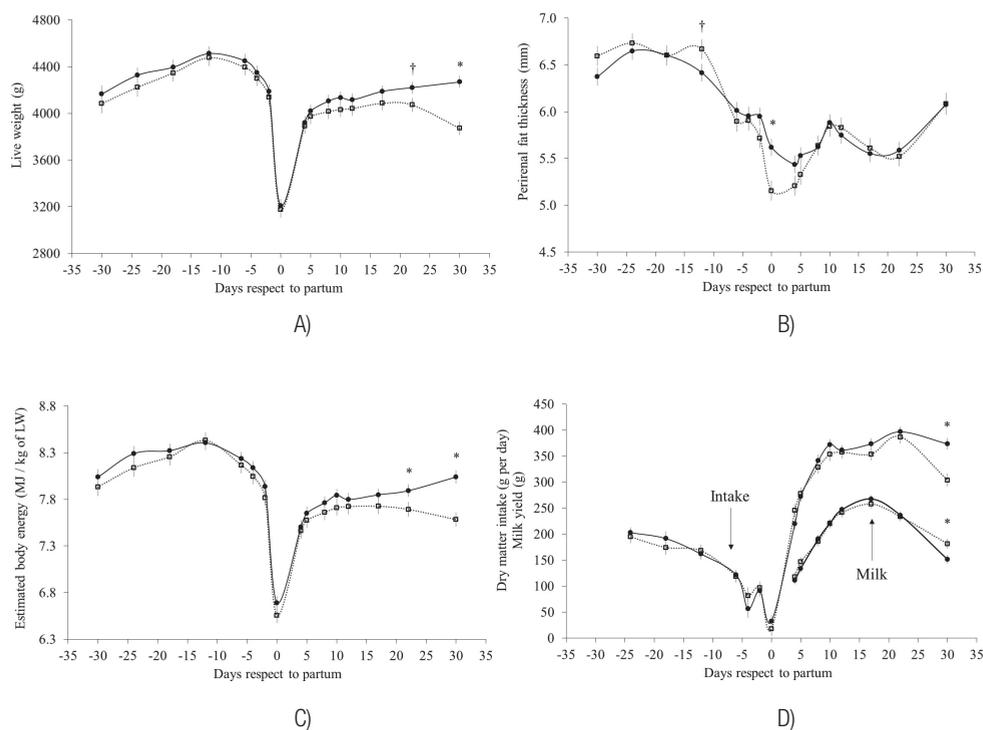
Weight of litter at weaning was analysed using model 4.

## RESULTS

The evolution of LW, PFT, EBE, DMI, and MY of primiparous rabbit females conceiving (LP) or not (L) at 11 d post-partum is shown in Figure 1. Regarding the evolution of LW, PFT, EBE, and DMI, there were no significant differences between groups from the moment of the first effective insemination till the parturition time, except for PFT. The differences between LP and L females began at 12 d pre-partum, where PFT of L females seemed to be greater (+0.25 mm;  $P=0.079$ ) than PFT of LP females (6.42 mm). In the subsequently days, L females showed a significantly higher mobilisation rate than LP females (on av.:  $-0.12$  and  $-0.07$  mm of PFT/day, respectively;  $P=0.007$ ), reaching a lower PFT at partum (5.17 mm vs. 5.62 mm, respectively;  $P=0.002$ ). However, PFT at 10 d post-partum, was similar (on av.: 5.86 mm) due to a greater recovery rate observed in L females ( $+0.15$  vs.  $+0.08$  mm of PFT/d for L and LP, respectively;  $P=0.007$ ).

After parturition, females from both groups showed similar evolution for the DMI, MY, LW and EBE during the first 3 wk of lactation. However, LP females showed higher LW at 30 d post-partum (+399 g;  $P<0.001$ ) and higher EBE at 22 and 30 d post-partum than L females (+0.20 and +0.46 MJ/kg of LW, respectively;  $P<0.05$ ). DMI was also higher for LP females at 30 d post-partum (+69 g of DM/d;  $P<0.001$ ), but milk yield was lower ( $-30$  g/d;  $P=0.02$ ).

Main litter parameters of rabbit females according to their reproductive rhythm are shown in Table 2. No significant differences were observed for litter parameters at birth and weaning between LP and L females.



**Figure 1:** Evolution of live weight (A), perirenal fat thickness (B), estimated body energy (C), and daily feed intake and daily milk yield (D) of primiparous rabbit females effectively (LP: lactating-pregnant) and not effectively (L: solely-lactating) inseminated at 11 d post-partum († $P<0.10$ ; \* $P<0.01$ ). —●— LP; ...□... L.

**Table 2:** Litter parameters of rabbit females according to their reproductive rhythm. Means followed by the standard error of means.

Variables	Reproductive Rhythm		P-value
	Lactating-Pregnant (LP)	Solely-Lactating (L)	
Number of females (n)			
At partum	27	20	-
At weaning	27	19	-
Litter size (n)			
Born in total	11.2±0.62	11.7±0.71	0.662
Born alive	10.6±0.64	10.6±0.74	1.0
Standardised	9.0	9.0	-
Weaned	8.8±0.12	8.5±0.14	0.119
Litter weight (g)			
Born in total	502±17	517±19	0.550
Born alive	466±22	477±25	0.743
Standardised	421±14	428±17	0.746
Weaned	4832±114	4672±134	0.370

## DISCUSSION

The question we aim to tackle is how resources allocation (*i.e.* different uses of body reserves) may condition the success of reproduction after first parturition. A series of measures were performed in 2 groups of females naturally assigned to 2 experimental groups, based on whether these females conceived or not on day 11 of their first lactation.

Overall, the results showed that nulliparous rabbit females accumulate body reserves (*i.e.* LW and PFT) until the last 12 d of gestation and then mobilise the accumulated reserves, reaching the lowest body condition at parturition. This pattern is surprising, as it is the inverse to that observed in sows (Sigfridson, 1996) and cows (Nielsen *et al.*, 2003), where the maximum body condition score is achieved at parturition. However, this high mobilisation pattern of nulliparous rabbit females in late pregnancy has been already reported, when *in vivo* methods such as total body electrical conductivity (Bolet and Fortun-Lamothe, 2002) or bioelectrical impedance analyses (Rebollar *et al.*, 2011; Romero *et al.*, 2011) were used. Rabbit females are able to conceive a few hours after giving birth, so lactation and pregnancy fully overlap, which implies that rabbit females must be able to ensure the survival of the current litter (lactating litter) without compromising the development of the future one (litter being gestated). However, for an optimal change of priorities between the current and the future litter, rabbit females should ensure that the current litter is developing quickly enough and then change their priority to the future one. The survival ability of the current litter is, in part, guaranteed by using the accumulated pre-partum reserves to ensure an adequate body weight and composition of the newborn offspring. In fact, Fortun-Lamothe *et al.* (1999) observed a similar body composition of newborn offspring, independently of the reproductive rhythm of their dams, which may be achieved at the expense of their mother's body condition. Coureaud *et al.* (2007) also observed mortality rates below 3% in new born offspring weighing more than 48 g at birth. The present results shows that rabbit females, regardless of their future reproductive rhythm (L vs. LP), mobilise body reserves ensuring an adequate litter weight at first parturition (46 g on average). Based on our observations and the data available in the literature, we argue that the body reserves mobilisation observed in the pre-partum period is a natural reproductive strategy developed by rabbit females to ensure correct foetus development and thus offspring survival in the post-partum period.

One of the most interesting features observed in this study was the different pre-partum mobilisation rate of body reserves between L and LP females. Depending on the level of fat reached in late gestation (day -12 of Figure 1B), different mobilisation patterns took place. Females reaching day 18 of first gestation with a high amount of body reserves (L females) showed the greatest mobilisation rate in the pre-partum period and the lowest level of PFT at partum. This mobilisation pattern occurred irrespectively of the feed intake (it was similar between L and LP females). A similar mobilisation pattern was observed by Rebollar *et al.* (2011). However, L and LP females reached a similar

level of PFT 10 d after partum. This implies a steeper body condition accretion curve for L females than for LP females, with similar feed intake and milk production effort. The consequence of this strategy may be the “inability” of L females to conceive at 11 d post-partum, when all females were re-inseminated.

Friggens *et al.* (2004) hypothesised that body condition is genetically driven, because before starting a new reproductive cycle, animals (dairy cows in their case) must attain a target level of body reserves, otherwise they do not conceive. In their reasoning, Friggens *et al.* (2004) proposed that dairy cows recover body condition after a nutritional insult and defend the target level when they are overfed by diverting the energy surplus to produce milk. In the case of rabbits, Quevedo *et al.* (2006) and Theilgaard *et al.* (2009) observed that independently of the genetic type and feed management used in the pre-partum period and the reproductive rhythm applied, rabbit females recover body energy in the first 10 d post-partum. However, if both L and LP females achieved a similar level of body condition at insemination, why were the conception success rates so different? If we assume that, as in dairy cows, rabbit females also have a target level of body condition that must be attained in the post-partum period before being able to face a new reproductive cycle, L females try to recover the target level. However, this greater accretion effort was made without increased resource acquisition or milk yield reduction, revealing an alteration of nutrient partitioning which resulted in a negative attempt to conceive.

From day 11 post-partum to weaning, (Figures 1A to 1D) the differences between groups were perceived at the end of lactation, and seem to be related with the competition for nutrients between the mammary gland and gravid uterus (Fortun-Lamothe *et al.*, 1999). In this sense, LP females presented higher intake and reduced milk production compared to L females. We argue that LP females split the acquired resources between the current and the next litter, as described by Fortun-Lamothe *et al.* (1999) and cited by Pascual *et al.* (2013) and Savietto *et al.* (2015). At this point the priority is the future litter, as the current one is able to compensate the lower milk yield by eating, a strategy that does not compromise offspring survival. On the other hand, in comparison to non-pregnant females, LP females start to recover fat reserves to reach the maximum level again before next parturition to ensure the next litter's pre-partum growth, presenting a greater body weight and estimated body energy at the end of lactation.

## CONCLUSIONS

This paper provides new knowledge on the patterns of body condition in young reproductive rabbit females. The results show that rabbit females start to invest their resources in the current litter in late pregnancy, using the body reserves to promote foetal growth and the onset of milk production.

Fat tissue accretion in the early lactation could be due to the fact that rabbits have evolved a fitness strategy to conceive just after parturition. In addition, the degree of fat tissue mobilisation in late pregnancy and fat tissue recovery in early lactation seemed to condition the reproduction success during lactation.

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