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## Long term effect of post-weaning rhythm on the body fat and performance of rabbit doe

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**Abstract** – Reproductive protocols based on standard (Control: 11 days post-partum) or extended rhythm (PW: post-weaning at 27 days post-partum) were compared. Two groups of fifty 19-week-old New Zealand White females were inseminated for seven consecutive cycles. The kits were weaned at 26 days. On the day of AI, all the does were submitted to ultrasound scanning of the perirenal regions to measure fat thickness. The fertility rate and several indexes of efficiency were calculated. Fat thickness, estimated perirenal fat and live weight were higher in PW does. The does submitted to post-weaning rhythm had a higher sexual receptivity ( $P < 0.01$ ), and fertility rate ( $P < 0.01$ ) whereas litter size and pre-weaning mortality were not affected. Primiparous Control does showed a particularly low fertility rate; the value increased successively but was always lower than in PW does. PW rhythm in comparison with the standard one seemed more adapted to doe reproductive physiology even if there was a lower production (35.0 vs. 38.8 rabbit sold/year) and risk of fatness (18% of multiparous does).

**rabbit doe / fertility / rhythm of insemination**

### 1. INTRODUCTION

The efficiency of a rabbit farm mainly depends on the fertility and prolificacy of the does, which in turn, is modulated by genetic, feeding and management aspects. Among the management aspects, the interval between successive inseminations plays a key role. In European commercial farms, the most common reproductive rhythm is based on artificial insemination (AI) of the does around 11 days after kindling and on weaning of the young rabbits at 28–30 days of age. This protocol is well adapted to cycled production, but it does not take into account the reproductive physiology of the doe [1].

Sexual receptivity of the female rabbit, except immediately after kindling and after weaning [2], does not show any predictable trend. Previous studies have shown that intensive reproductive rhythms increase the annual production of the doe [3, 4], but the litter size, fertility rate and length of reproductive activity decrease [5]. Semi-intensive rhythms have shown intermediate results [6], whereas the long-term effect of extensive rhythm is practically unknown.

The modification of reproductive rhythms also involves some welfare aspects; it is reported [7] that extensive reproductive rhythms improve the body condition of the does and the length of reproductive activity.

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The aim of this study was to investigate the long-term effect of an extensive reproductive rhythm (AI post-weaning at 27 d post-partum) in comparison with the standard one (AI 11 d post-partum).

## 2. MATERIAL AND METHODS

### 2.1. Methods

The trial was carried out at the experimental farm of the Department of Botany and Agri-environmental Biotechnology and Animal Science of the University of Perugia. The environmental temperature ranged from +15 to +28 °C and relative humidity from 60 to 75%.

Two groups of 50 New Zealand White (NZW) nulliparous rabbit does were submitted to the following reproductive rhythms: AI 11 days post-partum (Control) or post-weaning (PW) 27 days post partum.

The young does were first inseminated at 19 weeks of age for 7 consecutive AI cycles. A 3-week cycled production protocol was adopted and non pregnant does of both groups were re-inseminated after 21d to avoid pseudo-pregnancy.

Twenty-four hours after birth, the number of suckling kits was adjusted to 8 per litter, and the pups were weaned at 26 d. Pre-weaning mortality rate was calculated as the % weaned kits/litter size after adjustment.

After three consecutive AI, the does that were not pregnant were eliminated.

### 2.2. Measurement

On the day of AI, all the does were submitted to ultrasound scanning (ALOKA model SSD-500,) of the perirenal regions (3 cm ahead of the 2nd-3rd lumbar vertebrae) after shaving of these parts with a hair trimmer. Scapular fat thickness was measured directly: the average of two measurements (left and right side) was calculated. Perirenal fat depots were estimated using a regression curve reported in a previous paper [7].

**Table I.** Formulation, chemical composition and nutritional value of diet.

Ingredients (%)	
Barley meal	22.0
Wheat bran	13.5
Dehydrated alfalfa meal	37.0
Soybean meal 44% crude protein	14.0
Soybean extruded	7.0
Fat	1.0
Beet molasses	3.0
Calcium diphosphate	0.5
Vitamin -mineral premix*	0.85
Limestone	0.50
Salt	0.50
DL-Methionine	0.05
HCl Lysine	0.10
Chemical composition (%)	
Crude protein	18.7
Ether extract	4.8
Crude fibre	14.7
Ash	9.2
NDF	29.2
ADF	18.5
ADL	3.3
Cellulose	14.5
Hemicelluloses	10.6
Estimated digestible energy (MJ·kg <sup>-1</sup> )**	10.9

\* Added per kg: vit. A U.I. 11.000; vit. D<sub>3</sub> U.I. 2.000; vit. B<sub>1</sub> mg 2.5; vit. B<sub>2</sub> mg 4; vit. B<sub>6</sub> mg 1.25; vit. B<sub>12</sub> mg 0.01; vit. E mg 25; biotine mg 0.06; vit. K mg 2.5; niacine mg 15; folic ac. mg 0.30; D-panthothenic ac. mg 10; coline mg 600; Mn mg 60; Cu mg 3; Fe mg 50; Zn mg 15; I mg 0.5; Co mg 0.5.

\*\* Estimated according to Maertens et al. [9].

During the lactation, the feed consumption of the does was recorded daily. Diet formulation and analytical data are presented in Table I. Chemical analysis was done according to AOAC procedures [8] and Digestible Energy (DE) was estimated according to Maertens et al. [9]. The DE requirement was estimated according to the equations developed by Parigi-Bini and Xiccato [10]

and the DE deficit was estimated as the difference between requirement and ingestion.

The kits were nursed once a day and milk output was determined by weighting the doe immediately before and after suckling. Sexual receptivity was estimated by analysing the vulva colour and its turgescency and two classes were established: receptive (red or red-violet and turgescent) and non-receptive (whitish with non-turgescent vulva) following the IRRG recommendations [11].

AI was performed in the morning immediately after milking by inseminating 0.3 mL of diluted fresh semen, containing about 10 million sperms [12]. No hormonal synchronisation was done. Ovulation was induced by inoculating 10 µg of GnRH (Lutal-Hoechst).

### 2.3. Calculations

The fertility rate was estimated as the percentage of the number of kindlings/number of AI. Within the same kindling order, the results were divided according to the order of AI (1st and  $\geq$  2nd). Litter size was recorded at kindling and at 45 and 70 days of age.

The following indexes of efficiency were calculated: the overall productivity, as the number and the weight of rabbits sold/year/doe, and the production losses (%) of the system as the difference between the actual production and the theoretical one (theoretical production considering fertility rate = 100 and mortality of the young rabbits = 0).

The kindling order was ranked on the basis of preliminary analysis into three classes (0, 1  $\geq$  2 kindlings), whereas the perirenal body fat was ranked into 2 classes (20 g  $\leq$  medium  $\leq$  45 g and extreme < 20 g or > 45 g).

Statistical analysis was done with mixed models [13], (Anova and xtmixed procedures for continuous variables and xtprobit for binomial variables) adapted to repeated measures. The basic model evaluated the

fixed effect of the rhythm of insemination, kindling order and their interactions.

Since the fertility rate is the most important trait for the reproductive results, a further statistical model for the evaluation of the relative importance of some physiological variables on the fertility rate was tested. For this aim, the effect of lactation stage (dry, lactation), sexual receptivity (R+ or R-) and body fat classes (medium, extreme) at the moment of AI were evaluated; the rhythm of insemination, the kindling order and their interactions, being not significant, were omitted.

### 3. RESULTS

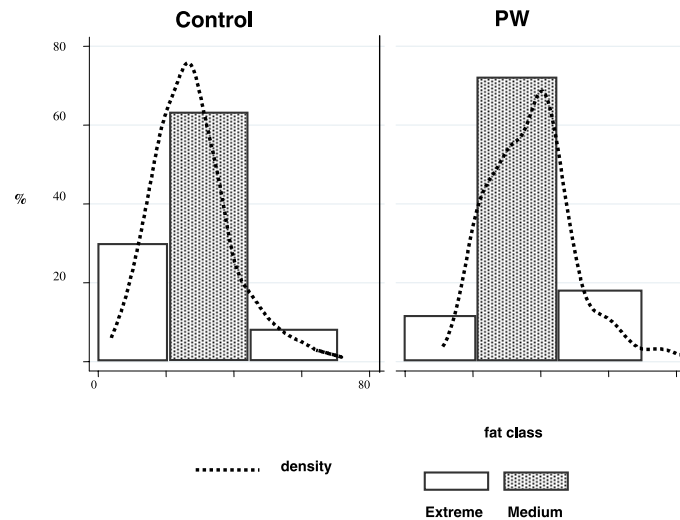
The distributions of the estimated perirenal fat and fat classes are reported in Figure 1. About 18% of the does inseminated PW had over 45 g of fat whereas 30% of the control does had less than 20 g; medium values were shown by 75 and 64% of the does, respectively.

The perirenal fat thickness at AI was always higher in PW than in the Control does (Tab. II). The estimated fat depot of PW does, compared to nulliparous does, increased in successive parities and was higher than the Control does which, throughout their productive career, were not able to restore the initial fat value.

Regardless of the group, with respect to nulliparous does, the primiparous with medium fat level decreased; successively multiparous PW does had a higher level (76.8 vs. 67.4%).

At kindling, weaning and AI, multiparous PW does were always heavier. Within the same parity, the fat depots at the first AI were lower than at the following AI mainly in PW does.

The DE intake, the DE requirement and the energy deficit during lactation were the same for the two reproductive rhythms. In both groups, the primiparous does showed a lower energy intake and milk production associated with a higher energy deficit.



**Figure 1.** Distribution of perirenal fat depots and fat classes.

Reproductive performance (Tab. III) was affected by kindling order as well as by reproductive rhythm. The nulliparous does showed the highest sexual receptivity and fertility rate whereas, regardless of the group, the primiparous does showed a lower litter size, weaned rabbits and pre-weaning mortality rate. Primiparous control does showed lower sexual receptivity and fertility rate (30.9% and 48.8%, respectively); the fertility increased for multiparous control does but was always lower than in PW does (60.2 vs. 79.0%).

The does of the PW group, compared to the control, showed higher sexual receptivity, fertility rate but the same litter size and pre-weaning mortality.

Within the same parity, the comparison of the 1st vs. following AI showed that PW does were more fertile at the 1st AI with a reduction in the following AI whereas the control had an opposite trend.

Regardless of group and parity, the fat (> 45 g) and lean (< 20 g) categories of animals (Tab. IV) showed the poorest fertility results (53.7%) while the best results (79.3%)

were obtained with a body fat depot ranging from 20 to 45 g per doe.

The does inseminated during lactation had lower fertility (52.1% vs. 80.8%) and the same reduction was shown by the non receptive does (52.8 vs. 90.1%).

When a doe was in the best conditions (dry, sexually receptive and with optimal fat depots), 93.2% fertility was reached. On the contrary, a non receptive doe inseminated during lactation with non optimal body condition had only 28.5% fertility.

These latter effects (sexual receptivity, lactation stage and fat rank) were significantly correlated with fertility rate ( $r=0.33$ ,  $0.30$ ,  $0.25$ , respectively); however if the doe was in the medium fat class, the amount of fat at AI did not exert a significant role ( $r=-0.04$ ; data not shown).

The performance of young rabbits was only affected by kindling order and the effect of rhythm was negligible (Tab. V). The early weaning did not depress growth rate and mortality rate. Primiparous does showed a lower number and lower weights of young rabbits, as well as lower post-weaning mortality.

**Table II.** Effect of reproductive rhythm on the perirenal fat thickness of does at AI and on some productive traits of rabbit does.

Parity order	Nulliparous		Primiparous		Multiparous		Significance			SE
	Control	PW	Control	PW	Control	PW	Parity	Rhythm	Parity × Rhythm	
Reproductive rhythm										
Weight of doe at kindling (g)	3982	4106	4027	4151	**	**	*	**	**	27
Weight of doe at weaning (g)	4144	4169	4148	4276	**	**	*	**	**	27
Weight of doe at AI (g)	4100	4160	4130	4265	**	**	*	**	**	27
Mean perirenal fat thickness (g)	0.65	0.72	0.66	0.85	**	**	**	**	**	0.02
Estimated perirenal fat <sup>1</sup> (g)	38.0	24.9	25.8	43.5	**	**	**	**	**	1.4
Fat at 1st AI (g)	39.1	24.2	25.5	42.3	**	**	**	**	**	1.0
Fat at ≥ 2 AI (g)	30.5	25.3	26.3	55.2	**	**	**	**	**	1.5
Does with medium fat level (%)	71.0	60.5	67.4	76.8	**	**	**	*	*	1.0
DE intake during lactation (kJ/d/kg. <sup>75</sup> )	1046	1042	1220	1219	**	**	ns.	ns.	ns.	15
Estimated DE requirement <sup>2</sup> (kJ/d/kg. <sup>75</sup> )	1246	1228	1306	1313	**	**	ns.	ns.	ns.	13
Energy deficit (kJ/d/kg. <sup>75</sup> )	162	149	86	94	**	**	ns.	ns.	ns.	4
Milk production (g·d <sup>-1</sup> )	172	170	185	190	**	**	ns.	ns.	ns.	2.5

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; ns.: not significant.

<sup>1</sup> According to the equation proposed by Dal Bosco et al. [7].

<sup>2</sup> According to Parigi-Bini and Xiccato [10].

PW: post weaning group. SE = standard error.

**Table III.** Reproductive performance of doe and pre-weaning mortality.

Parity order	Nulliparous Primiparous Multiparous				Significance		SE		
	Control	PW	Control	PW	Parity	Rhythm			
Reproductive rhythm					Parity × rhythm				
Sexual receptivity (%)	75.8	30.9	58.1	45.0	68.5	**	**	ns.	3.0
Fertility (%)	83.5	48.8	76.8	60.2	79.0	*	**	ns.	3.2
Fertility at 1st AI (%)	86.0	37.5	77.6	56.5	84.5	**	**	ns.	3.5
Fertility ≥ 2nd AI (%)	61.5	65.8	66.7	67.3	72.2	*	ns.	ns.	2.4
Live born (n)		7.0	7.0	8.3	8.5	**	ns.	ns.	0.2
Litter size at weaning (n)		6.2	6.0	6.9	7.1	**	ns.	ns.	0.1
Pre-weaning mortality* (%)		11.4	14.3	17.0	16.4	**	ns.	ns.	3.8

ns.: not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ . PW: post weaning group; SE = standard error.

**Table IV.** Effect of sexual receptivity, lactation state and fat classes on fertility rate.

Sexual receptivity		Lactation stage		Fat depot classes	
R+	R-	Dry	Lactation	Medium	Extreme
90.1	52.8	80.0	52.1	79.3	53.7
R+	93.2	81.1	Medium		
R-	52.3	28.5	Extreme		
R+	78.5	66.3	Extreme		
R-	64.8	45.7	Medium		

R+: sexual receptivity; R-: no sexual receptivity.

The indexes of overall productivity (Tab. VI) showed that the control group produced the highest number and weight of rabbits sold/year/doe.

The does submitted to the PW rhythm showed an 8-day longer kindling interval than the Control, however, the replacement doe and the non-pregnant does for three consecutive AI were higher in the Control group which also showed the highest production loss (44.2 vs. 33.2%).

#### 4. DISCUSSION

The live weight and the estimated perinatal fat depot of PW does at AI, compared to the control, showed higher values (Tab. II). Even starting with heavy nulliparous does (4 120 g at 19 weeks of age), the insemination of primiparous does 11 d post-partum was not successful, resulting in low sexual receptivity and fertility rate (Tab. III: 30.9% and 48.8% respectively).

**Table V.** Performance of litters.

Parity order	Primiparous		Multiparous		Significance		SE
	Control	PW	Control	PW	Parity	Rhythm	
Milk/kit (g·d <sup>-1</sup> )	26.2	26.0	24.7	24.8	*	ns.	0.8
Kit weight at 19d (g)	325	330	357	360	**	ns.	39
Kit weight at weaning (g)	610	608	599	610	ns.	ns.	11.5
Litter at 70 d (g)	5.9	5.9	6.3	6.5	**	ns.	1.5
Individual weight at 70 d (g)	2 250	2 220	2 300	2 310	ns.	ns.	56
Mortality from weaning to 70 d (%)	4.9	3.5	8.4	9.8	**	ns.	2.1

ns.: not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ . PW: post weaning group; SE = standard error.

**Table VI.** Indexes of overall productivity.

Reproductive rhythm	Control	PW	SE	X <sup>2</sup>
Rabbits sold/year/doe (n)	38.8a	35.0b	1.2	
Live weight sold/year/doe (kg)	89.2b	79.8a	2.9	
Production losses (theoretical production-real %) (kg)	44.2b	33.2a	6.2	
Kindling interval (d)	58.4a	66.8b	4.7	
Kindling/year/doe (n)	6.2b	5.4a	1.3	
Annual replacement of doe (%)	80.0b	60.0a	–	7.6
Kindling doe (n)	7.8a	9.1b	1.9	
Doe with 3 consecutive negative AI (%)	35.3b	27.1a	–	5.8

Values in the same row with differing superscripts differ ( $P < 0.05$ ). SE: standard error.

Non pregnant primiparous control does inseminated during the dry period, showed about a 28% higher fertility rate; according to Theau-Clement et al. [14], such an increase seemed more correlated with the absence of lactation than with the gain of perirenal fat (+ 1.1 g per doe).

Although it is widely known that primiparous lactating does have a significant energy deficit (28% energetic losses, [10]) due to the high requirements for lactation, body growth and the stress of the first kindling, farmers often inseminate these does 11 d post-partum.

According to the present findings, this reproductive protocol does not seem sound and it is probably the reason why the Control does never reached the weight of PW does and were not able to maintain sufficient fat depots to counterbalance the high energy expenditure during lactation [15].

Control does showed quite the same weight and fat depots throughout the entire productive career with no significant increase in the following parities. This substantial weight stability disagreed with previous results [6]; besides the effect of the remating interval, this discrepancy could also depend on the body weight reached by nulliparous does which quite reached the mature body weight.

On the contrary, the PW insemination resulted in a higher percentage of does with medium fat level (Fig. 1: 75% vs. 64%) and sufficient body weight and fat level were

maintained, confirming the results of previous experiments [6].

Furthermore, independent of the group and in agreement with Rommers et al. [16], the body weight in heavier or smaller does remained sufficiently stable throughout the reproductive period.

Naturally, these results are affected by genetic strain which modulates feed ingestion and milk production; however, considering that the prolificacy of such does is lower than in other genotypes [17, 18], the body fat of more prolific strains should be even less.

The comparison of the fat estimation should be done considering that it occurred in different physiological phases: mainly during lactation in control does and during the dry period in PW.

The body fat depots, mainly with numerous suckling pups, decrease during lactation [14] thus, at the end of lactation, these depots could be lower than at 11 d of lactation. Since the feed intake and the estimated energy deficit during lactation were the same in PW and Control does, even in this trial, the trend should be the same.

Although the present results are not tailored to scrutinise this aspect, all the primiparous does at the first AI had the same change in perirenal fat level and all differences in estimated fat level are subsequent (PW does) and related to the non concurrence between gestation and lactation and the longer dry period. According to Feugier



et al. [19], 12 days after PW does from AI showed about 32% increase in fat depots, whereas those inseminated 11 days post-partum lost about 30% body fat.

However, due to the lower fertility rate of the Control group, about 60% of the control does were re-inseminated 3 weeks after non fertile AI during the dry period and this additional period determined an increase in fat depot and fertility rate.

Rabbit does are able to contemporarily sustain lactation and pregnancy; however, lactation negatively affects the reproductive functions mainly for hormonal and energetic reasons [20, 21]. Prolactin is mainly responsible for the hormonal antagonism [22], whereas the energy deficit is due to the relevant production of milk and to its high caloric value [10].

In PW rhythm, these two main effects are virtually eliminated and the does partially compensated for the lower production intensity with a higher fertility rate (Tab. III, 79.0% vs. 60.2%). The higher fertility rate of the PW group reduced the differences in kindling interval (theoretical 16 d vs. real 8 d; Tab. VI) emphasising the importance of the high fertility rate in a cycled production system [23]. Cycled production implies that non-pregnant does follow the same insemination schedule as pregnant ones and when the fertility rate is low many does must wait until the next insemination cycle (generally at 3 weeks) and the actual kindling interval increases. As a result 18.8% difference in fertility rate reduces the kindling interval to 8 days.

However, even the PW group showed a lower productivity than expected; since the major causes of hypo-fertility are eliminated, fertility should be the same as nulliparous. One critical point was the excessive deposition of fat (Fig. 1: 18% of does > 45 g), confirming that non-pregnant does for one or more AI are too fat (Tab. II), a condition which reduces fertility (Tab. III: about -10 to -12%) as the opposite body situation [24]. In this former circumstance it could be interesting to study if the excessive fat is

only the effect of the dry period being too long or if there is an alteration of energetic and lipid metabolism.

One other cause of hypo-fertility could be the potential presence of infection/inflammation processes even at a sub-clinical level [25, 26], which restricts the transfer of spermatozoa to the oviduct [27, 28] thus reducing the fertility rate.

Independent of the group and parity, the success of AI was affected by lactation stage, sexual receptivity and fat depot class. Besides the confirmation of the relationship between fertility, sexual receptivity and lactation stage [20], even the fat class played a relevant role.

The ultrasound measurement of perirenal fat was correlated with the performance of the does; when it was particularly low or high, the fertility rate dropped (Tab. IV) [18]. To this respect it seems important that at AI the doe should not be too lean or too fat; in this condition, the specific perirenal level was not significant.

Assuming that the fat depot of the doe is an index of body condition, the present findings offer additional information on the complex relationships between reproductive performance and body conditions. In other species (milking cows, sows) these relationships are well described [29] and even in rabbit does some attempts have been made even if the fur renders such evaluation more difficult [30].

When all the physiological conditions are the best (dry does, sexually receptive, medium fat), about 93% fertility rate was obtained.

PW insemination represents a suitable compromise among all these factors (75% medium fat classes; 68.5% sexual receptivity) permitting good reproductive efficiency to be obtained without the use of any synchronising treatment (hormones or biostimulation).

It is also evident that excessive fatness should be controlled by performing a sudden AI of non-pregnant does or controlling feed ingestion during the dry period.

In agreement with previous results [6, 31], weaning at 26 days seemed sufficient for a correct weight gain of the litters.

The milk intake of kits and the performances of litters during the post weaning period were not affected by the reproductive rhythm.

Even if this study was not focussed on animal welfare, some points should be emphasised. The post-weaning rhythm, compared to insemination at 11 days, seemed more adapted to doe reproductive physiology (higher sexual receptivity and fertility), maintained a more sustainable equilibrium of body weight and fat depots and increased the length of the reproductive activity simultaneously reducing production losses (44.2 vs. 32.2%).

According to this assessment, some Italian rabbit farmers, although experiencing lower productivity, use post-weaning insemination due to the simplicity of management and better work organisation.

## 5. CONCLUSIONS

This study confirmed that the standard reproductive rhythm, without any oestrus synchronisation, determines a progressive reduction of perirenal fat depots and shows a low overall efficiency.

The insemination of primiparous does during lactation seems particularly negative and these findings strongly suggest that AI after weaning is the best at least for such a category of animal.

Nevertheless, even PW did not reach a very high reproductive efficiency due to excessive fatness. Thus, it could be interesting to find a welfare-oriented reproductive rhythm in which AI is conditioned to the body reserves of the doe. Such a rhythm, besides the improvement of animal welfare, must:

- show a high fertility rate;
- be simple and adapted to cycled production;
- be profitable.

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