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FULL PAPER (ORIGINAL ARTICLE)

Evolution of the peripheral blood lymphocyte populations in multiparous rabbit does with two reproductive management rhythms

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ABSTRACT

The emergence of epizootic rabbit enteropathy is leading to changes in weaning protocols in commercial rabbitries. Traditional weaning protocols are being replaced with late weaning, beyond 35 days postpartum (dpp). The main objectives of this study were to compare the peripheral blood lymphocyte populations of multiparous rabbit does under two reproductive rhythms (insemination at 11 dpp and weaning at 28 dpp, insemination at 25 dpp and weaning at 42 dpp), and to assess the influence on those of kits. Samples of peripheral blood were taken in 22 adult females and 44 of their kits at different critical times, and several lymphocytic populations were evaluated by flow cytometry. Additionally, the perirenal fat thickness of does was also measured at partum and weaning to observe if body condition correlates with lymphocyte populations. During whole lactation, counts of total, T, $CD4^+$ and $CD8^+$ lymphocytes of females were generally lower with weaning at 42 dpp compared to 28 dpp. Moreover, counts of total, B and T lymphocytes in rabbit does weaned at 42 dpp correlated to their body condition (+0.60 to 0.82; $P<0.05$), contrary to that observed in rabbit does weaned at 28 dpp. Some correlations between lymphocyte counts in both does and weaning rabbits were observed. At weaning, those young rabbits weaned at 42 dpp had a significantly lower number of $CD4^+$ lymphocytes than those weaned at 28 dpp ($P<0.01$). In conclusion, longer lactation periods and major accumulated wear under prolonged reproduction rhythms could be interpreted as a minor immunological level for adult rabbit does.

KEYWORDS: rabbit; lymphocytic populations; peripheral blood; weaning age; body condition; flow cytometry.

INTRODUCTION

Digestive diseases are the main cause of morbidity and mortality in growing rabbits and are responsible for important economic losses in commercial rabbitries (Marlier et al., 2003), especially since 1997 when a gastro-intestinal syndrome called epizootic rabbit enteropathy appeared in Europe. This disease mainly affects young rabbits, usually after weaning (Licois et al., 2005). For this reason, some farmers are changing their rabbitries management. The usual weaning protocols at 28-32 days postpartum (dpp) (with inseminations at 11-13 dpp) are being replaced with late weaning, beyond 35 dpp (with inseminations at 18-25 dpp). Actually, this late weaning is an empirical practice that reduces mortality during fattening, probably because of the protective role of milk (Fortun-Lamothe and Boullier, 2007; Gallois et al., 2007) and young rabbits' greater maturity. However, a prolonged lactation period could affect the rabbit does' body condition, although knowledge about its possible effect on rabbit does is scarce (Pascual et al., 2006). Moreover, despite rabbits being one of the animal species most often used as experimental models in human and veterinary research, information on changes in lymphocyte subpopulations in peripheral blood is also scarce (Jeklova et al., 2007).

The main objective of the present study was to know the effect of weaning age (28 vs. 42 dpp) on the peripheral blood lymphocyte populations of multiparous rabbit does and their litters.

MATERIAL AND METHODS

Animals

The study sample included 22 adult rabbit females (*Oryctolagus cuniculus*) crossbred from two maternal lines selected by litter size (A and V lines, Universidad Politécnica de Valencia, Spain), aged between 11 and 15 months. Animals were housed in flat-deck cages of 700 x 500 x 320 mm, with a light cycle of 16 light hours and 8 dark hours under controlled environmental conditions.

Since their first parturition, at 5-months old, females were randomly allocated and maintained under one of the two possible reproductive management rhythms: insemination at 11 dpp and weaning at 28 dpp (11 animals, named 28D) or insemination at 25 dpp and weaning at 42 dpp (11 animals, named 42D).

After their fifth parturition, peripheral blood samples were taken at different times. For the 28D rabbit does, blood samples were taken at 16 dpp (around the maximum daily milk yield), 28 dpp (weaning), 35 dpp (during recovery of energy reserves) and 42 dpp (sixth parturition). With the 42D rabbit does, blood samples were taken at 16 dpp, 28 dpp, 42 dpp (weaning) and 49 dpp (during recovery of energy reserves). In both groups, blood samples from two young rabbits of each female were also taken at weaning.

All the blood samples were drawn from the median artery of the ear using vacuum tubes with EDTA. Diurnal variations in haematological parameters were minimised by collecting blood at approximately the same time (9:00-11:00h).

The Committee of Ethics and Animal Welfare of the Universidad Politécnica de Valencia approved this study. All the animals were handled according to the principles of animal care published by Spanish Royal Decree 1201/2005 (BOE, 2005; BOE = Official Spanish State Gazette).

Flow cytometry analysis

Blood samples were processed 1 hour after sampling. Before performing the flow cytometry studies, a white blood cells (WBC) count and the percentage of lymphocytes were determined using a haematology analyzer (MEK-6410, Nihon Kohden, Japan).

After mixing by inverting the tube, 50 μL of whole blood were pipetted into flow cytometry tubes and primary monoclonal antibodies (Table 1) were added, following the manufacturer's recommendations, and incubated for 15 minutes at room temperature in the dark. WBC were isolated by lysing erythrocytes by adding 1 mL of ammonium chloride lysing solution (8.02 g

100 NH_4Cl, 0.84 g $NaHCO_3$ and 0.37 g EDTA per litre of Millipore water) at 4ºC. After a 5-minute
101 incubation in the dark, samples were centrifuged at 400 x g for 5 minutes at room temperature,
102 the supernatant carefully eliminated and the pellet washed with 1 mL of phosphate-buffered
103 saline (PBS). After another wash, secondary antibodies (Rat anti-mouse IgG2a+b
104 Phycoerythrin and Goat anti-mouse IgM: R-Phycoerythrin -human adsorbed-) were added.
105 These were incubated for 20 minutes at room temperature in the dark. Finally, 1 mL of PBS
106 was added before running the flow cytometer. The resulting WBC suspensions were analysed
107 in a Cytomics FC500 flow cytometer (Beckman Coulter, Brea, CA). Specific data acquisition
108 protocols for rabbit WBC were designed using the CXP software (Beckman Coulter, Brea,
109 CA). Gates of each leukocyte type were adjusted with isotype negative control. All the samples
110 were processed in duplicate.
111 The total lymphocyte count was calculated as the product of the WBC count and the
112 lymphocyte percentage, and of the lymphocyte subset counts and percentages, as described by
113 Hulstaert et al. (1994).
114
115 **Ultrasound measurements**
116 The perirenal fat thickness (PFT) of does was measured at parturition and weaning by
117 ultrasound to evaluate body condition, as described by Pascual et al. (2000 and 2004). Fur was
118 removed from the thoracic and lumbar vertebrae areas by shearing to improve image retrieval.
119 Animals were placed in an immobilising box (150 x 370 x 150 mm) during the ultrasound
120 measurement and ultrasound gel was applied to the scanning area. The probe was always
121 placed in the same position to obtain a repeatable transversal section of perirenal fat at 3 cm
122 ahead of the 2^{nd} lumbar intervertebral space. Images were obtained with an ultrasound unit
123 (JustVision 200, Toshiba, Japan) equipped with an image analyzer software to determine
124 distances.
125

126 **Statistical analysis**

127 To analyse the evolution of the lymphocyte populations in the blood of multiparous rabbit
128 does, a mixed model (PROC MIXED; Statistical Analysis System, 2002) was used in
129 accordance with a repeated measures design that takes into account the variation between
130 animals and the covariation within them. Covariance structures were objectively compared
131 using the most severe criteria (Schwarz Bayesian criterion), as suggested by Littell et al.
132 (1998). The model [1] included weaning age (28 and 42 dpp), days from partum (16, 28, 35, 42
133 and 49 dpp), and their interaction as fixed effects. Random terms in the model included a
134 permanent effect of each animal (p) and the error term (e).

135 $$y_{ijk} = weaning_i + day_j + weaning_i*day_j + p_k + e_{ijk} \quad [1]$$

136 The model used to analyse the data of the lymphocytes populations in the blood of young
137 rabbits at weaning was a split plot design (PROC GLM; Statistical Analysis System, 2002) that
138 included weaning age (28 and 42 dpp) as a fixed effect. Random terms in the model [2]
139 included a permanent effect of mother (p) and the error term (e).

140 $$y_{ij} = weaning_i + p_j + e_{ij} \quad [2]$$

141 Finally, to test the relationship between the lymphocyte populations of multiparous rabbit does
142 and both the PTFs of females during lactation and the lymphocyte populations of young rabbits
143 at weaning, Pearson's correlation coefficients (ρ) were obtained using PROC CORR of the
144 Statistical Analysis System (2002).

145

146

147 **RESULTS**

148 Table 2 presents the means, standard deviations and coefficients of variation for peripheral
149 blood lymphocyte populations and ultrasound PFT measurements of rabbit does, both for all
150 animals and in terms of their weaning days. Lymphocyte populations were characterised by
151 higher T lymphocytes counts ($1191x10^6$/L) than B lymphocytes counts ($96x10^6$/L), which

152 became more variable later among individuals (CV=75%). Only 1.2% of T lymphocytes were
153 activated ($CD25^+$), and high variability was also observed (CV=152%). Very high variability
154 was noted in the change of PFT during lactation (CV=1250%), mainly in the 42D does
155 (CV=2589%).
156 Counts of total, T, $CD4^+$ and $CD8^+$ lymphocytes were significantly higher in the 28D than in
157 the 42D rabbit does (P=0.003, P=0.002, P=0.016 and P=0.042, respectively). Differences in
158 the evolution of total lymphocytes during the study period were detected depending on the
159 reproductive management rhythm (Figure 1). While there were no differences in the 42D does,
160 increased total lymphocytes at weaning (28 dpp) and next partum (42 dpp) were observed in
161 the 28D does. Compared with the 42D does, the 28D ones presented a higher T lymphocytes
162 count at 16 dpp and 28 dpp due to an increase in both the $CD4^+$ and $CD8^+$ populations (only
163 statistically significant at 28 dpp; P<0.05). At weaning, the 28D females also presented higher
164 counts of total, B, T, $CD4^+$ and $CD8^+$ lymphocytes than their 42D counterparts. These
165 differences were not significant one week later.
166 Table 3 shows the simple correlation coefficients between the body condition traits and the
167 lymphocyte population counts of the 42D rabbit does (no relevant correlation was found in the
168 28D rabbit does). The greater the PFT at partum, the higher the total and B lymphocytes counts
169 at 16 dpp. Furthermore, the greater the PFT at weaning or the lesser the PFT losses during
170 lactation, the higher the B and T lymphocyte counts one week later. In addition, the PFT
171 change during lactation negatively correlated with the $CD4^+/CD8^+$ ratio.
172 Regarding young rabbits, the only statistically significant difference observed was for the
173 $CD4^+$ lymphocyte count, which was higher at weaning for those weaned at 28 than at 42 days
174 (Table 4). Some significant correlations were detected between lymphocyte populations of
175 females and young rabbits (Table 5). In particular, the females' T lymphocyte count was
176 positively correlated with the young rabbits' T, $CD4^+$ and $CD8^+$ lymphocyte counts, but
177 negatively correlated with the young rabbits' $CD25^+$ lymphocyte count.

DISCUSSION

Rabbits have been used as experimental models for years, and haematological parameters and lymphocyte subsets in different types of animals can be found in the bibliography: conventional or SPF (free of common rabbit pathogens) rabbits, neonatal to pubescent rabbits, primiparous rabbit does and adult rabbits (Wells et al., 1999; Jeklova et al., 2007; Çetin et al., 2009; Jeklova et al., 2009). However to the authors' knowledge, there is no information about these parameters, mainly lymphocyte subpopulations, in adult rabbit does under field conditions. The main objective of the present work was to study the effect of two reproductive management rhythms on the lymphocyte subpopulations of multiparous rabbit does.

Several imbalances appear in the results obtained in the present study. The sum of the T and B lymphocyte percentages is lower than 100, averaging 52% in both rabbit does and weaning rabbits. Jeklova et al. (2007) referred to these non-detected cells as lymphocytes with pT^-, $CD4^-$, $CD8^-$, $CD79\alpha^-$ phenotypes that decreased with age, and constituted 45% and 16% of the total lymphocyte population in 1-day-old rabbits and 20-week-old rabbits, respectively. It is noteworthy that the sum of $CD4^+$ plus $CD8^+$ lymphocytes in the present study accounted for 65% and 76% of the T lymphocytes in rabbit does and weaning rabbits, respectively. This result may be partially explained because the $CD4^+CD8^+$ and $CD4^-CD8^-$ T lymphocytes were not assessed. Jeklova et al. (2007) reported the sum of $CD4^+$ plus $CD8^+$ lymphocytes as 93% and 96% of the T lymphocytes in 1-day-old and 20-week-old rabbits, respectively, while the rest were $CD4^+CD8^+$ T lymphocytes. These discrepancies in the non-detected cells (both lymphocytes and T lymphocytes) may relate with differences in age and the productive carrier of animals (present work: adult rabbit does at the end of their productive life; Jeklova et al. (2007): pubescent rabbits), but also with the panels of antibodies used between both studies.

The current difficulty for immunological research into rabbits lies in the still limited number of

204 (commercially available) monoclonal antibodies that recognise the various lymphocyte subsets
205 (Drouet-Viard and Fortun-Lamothe, 2002). In spite of this limitation, the comparison made
206 between the two reproductive rhythms of present study is not invalidated.
207 The number of total lymphocytes in young weaning rabbits is lower than in reproductive rabbit
208 does, which is in agreement with a gradual increase in lymphocytes during the previously
209 described young rabbits' maturation (Jeklova et al., 2007 and 2009). However, the mean values
210 of the total lymphocytes in both rabbit does and weaning rabbits are lower than previously
211 reported values (Wells et al., 1999; Kim et al., 2002; Jeklova et al., 2007 and 2009). These
212 discrepancies in adult animals may relate with the physiological characteristics of the studied
213 subjects. In the above-cited works, SPF young adult rabbits (20-weeks-old) or conventional
214 primipregnant rabbit does (16 to 24-week-old) were used. In order to know the accumulative
215 effect of successive gestation-lactation cycles in the immune system of high-performing
216 selected rabbit does, the animals studied in this work were at the fifth lactation and sixth
217 gestation stages and were older (11 to 15-months-old). In their study with Angora rabbits aged
218 2.0-2.5 years old, Çetin et al. (2009) found that the total lymphocyte count vastly differed
219 among males ($5700x10^6$/L), non-pregnant females ($4300x10^6$/L) and 22-25-day-old pregnant
220 females ($2800x10^6$/L), with the latest average being very similar to that obtained in this work
221 with younger, but more biologically stressed rabbit does (high-performing selected animals
222 that are either lactating or gestating, or both simultaneously). In humans, the absolute count
223 and the percentage of total lymphocytes have been described to decline with age (Hulstaert et
224 al., 1994). The lower lymphocytes count in the weaning rabbits of this study if compared with
225 previous works (Jeklova et al., 2007 and 2009) may relate with their mothers' immunological
226 status. Maternal stress in pregnant sows has been reported to possibly induce long-lasting
227 alterations in their offsprings' immunity (i.e., a decrease in lymphocytes) (Otten et al., 2010).
228 As indicated above, we may consider that the rabbit does of the present study were under

229 greater productive stress than the SPF primiparous rabbit does used as parents in the
230 aforementioned studies. Thus, it may be hypothesised that this fact affects their litters.
231 The T lymphocytes count is higher than that of the B lymphocytes, while the T/B lymphocyte
232 ratio is around 10 as an average of all the study animals, which is mainly due to very low B
233 lymphocyte counts. However, Jeklova et al. (2007) reported very similar lymphocytic
234 populations (with a T/B lymphocyte ratio of around 1) in both growing and young adult
235 rabbits. The discrepancies between both studies could be owing to the methodology used
236 (different antibodies) and/or to the physiological characteristics of the animals under study. A
237 lower amount of B cells in peripheral blood has been reported as being relatively common in
238 elderly persons (Franceschi et al., 1995) and older cows (Ohtsuka et al., 2009).
239 In general, the counts of total, T, $CD4^+$ and $CD8^+$ lymphocytes during lactation and at weaning
240 are lower in the 42D does than in the 28D ones. In addition, the 42D does are not apparently
241 capable of adapting to the physiological status since no differences in these counts were
242 detected throughout the experimental period. On the other hand, longer lactation periods and a
243 presumable major wear of this group likely implies that the body condition of these animals
244 becomes more relevant, to such an extent that the greater the PTF at partum, the higher the
245 total and B lymphocytes counts around the top milk yield (16 dpp); furthermore, the greater the
246 PTF at weaning or the lesser the PTF loss during lactation, the higher the T and B lymphocytes
247 counts at one week post-weaning. Milk yield requires great effort, even in its final period
248 (Pascual et al., 2003, 2006), and some studies have reported an improving of the body energy
249 balance of females by shortening lactation duration (Xiccato et al., 2004, 2005).
250 Conversely, the 28D does show an increased number of total lymphocytes at 28 dpp if
251 compared to 16 dpp, which is followed by a decrease at 35 dpp and a further increase at 42
252 dpp. The evolution of the total lymphocyte count during pregnancy in rabbit does reaches a
253 nadir on gestational days 22 to 24 (Wells et al., 1999; Kim et al., 2002), i.e., around 35 dpp in
254 our study.

Jeklova et al. (2007) reported $CD4^+/CD8^+$ ratios of around 2.8 in SPF 4-20-week-old rabbits. We obtained similar results in adult rabbit does (average 2.75), and lower values in 28-day-old rabbits (2.32±0.24,) especially, 42-day-old rabbits (1.91±0.22), mainly due to a drop in $CD4^+$ lymphocytes.

As regards the relationship between females and weaning rabbits, the T lymphocyte counts in females positively correlated with the T, $CD4^+$ and $CD8^+$ lymphocyte counts in young rabbits, but negatively correlated with the $CD25^+$ lymphocyte count. During human gestation, it has been reported that mothers acquire foetal lymphoid progenitors that develop into functional T cells (Khosrotehrani et al., 2008). Besides, human milk contains numerous lymphocytes (1.5-$3x10^5$ cells/mL), of which 80% are T cells (Lönnerdal, 2000). These cells contribute not only to locally protect the intestine, but also to general passive cell immunity as some can pass through the intestinal barrier and participate in the immunological reinforcement of young rabbits (Fortun-Lamothe and Drouet-Viard, 2002).

In conclusion, the 42 ddp rabbit does presented a lower number of total lymphocytes and lymphocytic subpopulations during lactation and at weaning, as well as lesser capacity of adjustment during the gestation-lactation cycle. This scenario might be related with longer lactation periods and major accumulated wear throughout their productive life under prolonged reproduction rhythms. This could imply a minor immunological level and them probably having a lower response capacity against infections. This fact apparently makes them depend on their body condition more than the 28 ddp rabbit does. In addition, their litters have a lower number of lymphocytes T $CD4^+$, which are a fundamental part of immune response coordination.

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372 **TABLES LEGENDS**

373 **Table 1.** Monoclonal antibodies used in this study.

374 **Table 2.** Means, standard deviations and coefficients of variation for the lymphocyte
375 population and ultrasound measurements of the perirenal fat thickness (PFT) of multiparous
376 rabbit does in terms of their weaning age.

377 **Table 3.** Simple correlation coefficients[1] between PFT thickness during lactation and the
378 lymphocyte populations in the peripheral blood of multiparous rabbit does weaned at 42 days[2]
379 (n=11).

380 **Table 4.** Effect of reproductive rhythm (weaning at 28 or 42 days) on the lymphocyte
381 populations in the peripheral blood of young rabbits at weaning (mean ± SE).

382 **Table 5.** Simple correlation coefficients[1] between the lymphocyte populations in the peripheral
383 blood of young rabbits at weaning and those of the multiparous rabbit does on different days
384 (n=22).

385

386 **FIGURE CAPTIONS**

387 **Figure 1.** Effect of reproductive rhythm (weaning at 28 □ or 42 ■ days) on the evolution of the
388 lymphocytes populations ($\times 10^6$/L) in the peripheral blood of multiparous rabbit does.

389 [a,b,c] Least square means not sharing the same superscript being significantly different at $P<0.05$.

390

391

Table 1. Monoclonal antibodies used in this study.

Monoclonal antibodies	Isotype	Specificity	Cell labeling	References	Company
Mouse anti-rabbit T lymphocytes: FITC	IgG1	T lymphocytes	T cell	Kotani et al., 1993a	Abd Serotec
Mouse anti-rabbit α-pan B	IgM	B lymphocytes	B cell	Davis and Hamilton, 2008	VMRD, Inc.
Mouse anti-rabbit CD4	IgG2a	CD4	T cell subset	Kotani et al., 1993a	Abd Serotec
Mouse anti-rabbit α- CD8	IgG2a	CD8	T cell subset	Davis and Hamilton, 2008	VMRD, Inc.
Mouse anti-rabbit CD25	IgG2b	CD25	Activated T cells	Kotani et al., 1993b	Abd Serotec
Mouse anti-human CD14: FITC	IgG2a	CD14	Monocytes and granulocytes	Jacobsen et al., 1993	Abd Serotec
Mouse anti-rabbit α- CD45	IgM	CD45	All leukocytes	Davis and Hamilton, 2008	VMRD, Inc.

Table 2

Table 2. Means, standard deviations and coefficients of variation for the lymphocyte population and ultrasound measurements of the perirenal fat thickness (PFT) of multiparous rabbit does in terms of their weaning age.

Variable	Description	No.[1]	Mean	SD[2]	Minimum	Maximum	CV(×100)[3]
All the females							
|Lym|	Lymphocytes (10^6/L)	88	2554	819	943	4566	32.08
|LymB|	B Lymphocytes (10^6/L)	88	96	72	0	339	74.88
|LymT|	T Lymphocytes (10^6/L)	88	1191	380	473	2262	31.94
|$CD4^+$|	$CD4^+$ (10^6/L)	88	543	283	85	1268	52.22
|$CD8^+$|	$CD8^+$ (10^6/L)	87	216	112	38	580	52.00
|$CD25^+$|	$CD25^+$ (10^6/L)	86	14	19	0	91	136.99
|$CD4^+$|/|$CD8^+$|		87	2.75	1.46	0.56	7.15	53.09
LymB	B Lymphocytes (%)	88	3.75	2.39	0	10.74	63.68
LymT	T Lymphocytes (%)	88	48.48	13.39	21.42	83.24	27.61
$CD4^+$	$CD4^+$ (%)	88	47.86	17.23	10.55	78.71	36.00
$CD8^+$	$CD8^+$ (%)	87	17.57	5.49	5.15	30.85	31.23
$CD25^+$	$CD25^+$ (%)	86	1.20	1.82	0	10.95	152.46
$CD4^+/CD8^+$		87	2.96	1.49	0.61	8.32	50.22
UP	PFT at partum (mm)	22	4.92	0.37	4.10	5.60	7.60
ΔUPW	Partum to weaning PFT change (mm)	22	–0.04	0.51	–1.10	1.10	–1250.21
Weaning at 28 days							
|Lym|	Lymphocytes (10^6/L)	44	2843	842	1535	4566	46.66
|LymB|	B Lymphocytes (10^6/L)	44	100	80	0	339	80.47
|LymT|	T Lymphocytes (10^6/L)	44	1335	400	632	2262	29.94
|$CD4^+$|	$CD4^+$ (10^6/L)	44	639	296	196	1268	46.46
|$CD8^+$|	$CD8^+$ (10^6/L)	44	240	123	78	580	51.48
|$CD25^+$|	$CD25^+$ (10^6/L)	43	14	19	0	85	130.19
|$CD4^+$|/|$CD8^+$|		44	3.09	1.58	0.56	7.15	51.14
LymB	B Lymphocytes (%)	44	3.51	2.61	0	10.74	74.34
LymT	T Lymphocytes (%)	44	49.08	15.39	21.42	83.24	31.36
$CD4^+$	$CD4^+$ (%)	44	50.24	13.90	12.79	77.75	27.68
$CD8^+$	$CD8^+$ (%)	44	17.09	5.55	5.15	30.85	32.46
$CD25^+$	$CD25^+$ (%)	43	0.91	1.07	0	5.33	117.64
$CD4^+/CD8^+$		44	3.32	1.55	0.61	8.32	46.67
UP	PFT at partum (mm)	11	4.80	0.37	4.10	5.30	7.71
ΔUP28d	Partum to 28 days PFT change (mm)	11	–0.06	0.57	–1.1	1.10	–897.98
Weaning at 42 days							
|Lym|	Lymphocytes (10^6/L)	44	2265	691	943	3627	30.53
|LymB|	B Lymphocytes (10^6/L)	44	91.43	62.34	0	307	68.18
|LymT|	T Lymphocytes (10^6/L)	44	1046	300	473	1734	28.70
|$CD4^+$|	$CD4^+$ (10^6/L)	44	448	237	85	983	52.90
|$CD8^+$|	$CD8^+$ (10^6/L)	43	192	95	38	439	49.64
|$CD25^+$|	$CD25^+$ (10^6/L)	43	14	20	0	91	145.15
|$CD4^+$|/|$CD8^+$|		43	2.40	1.25	0.77	5.95	52.02
LymB	B Lymphocytes (%)	44	3.99	2.15	0	9.27	53.81
LymT	T Lymphocytes (%)	44	47.87	11.17	27.29	73.14	23.34
$CD4^+$	$CD4^+$ (%)	44	45.48	19.90	10.55	78.71	43.74
$CD8^+$	$CD8^+$ (%)	43	18.05	5.44	5.15	30.53	30.15
$CD25^+$	$CD25^+$ (%)	43	1.48	2.33	0	10.95	156.99
$CD4^+/CD8^+$		43	2.60	1.35	0.87	6.89	51.85
UP	PFT at partum (mm)	11	5.05	0.35	4.60	5.60	6.89
ΔUP42d	Partum to 42 days PFT change (mm)	11	–0.02	0.47	–0.70	0.70	–2589.30

[1] No.: Number of observations
[2] SD: Standard deviation
[3] CV: Coefficient of variation

Table 3. Simple correlation coefficients[1] between PFT thickness during lactation and the lymphocyte populations in the peripheral blood of multiparous rabbit does weaned at 42 days[2] (n=11).

Population[3]	Time	UP[3]	U42d[3]	ΔUP42d[3]
|Lym|	16d	+0.6477*		
|LymB|	16d	+0.8215***		
	49d		+0.6030*	+0.7030**
|LymT|	49d		+0.6875**	+0.7901**
$\|CD4^+\|/\|CD8^+\|$	42d			–0.6646*
	49d			–0.7217**

[1] Only relevant and significant correlations are presented: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.
[2] Any relevant simple correlation for the data from multiparous rabbit does weaned at 28 d were obtained.
[3] Abbreviations as in the Table 2.

Table 4. Effect of reproductive rhythm (weaning at 28 or 42 days) on the lymphocyte populations in peripheral blood of young rabbits at weaning (mean ± SE).

		Weaning age		
Population[1]	No.	28 d	42 d	*P*-value
|Lym| (10^6/L)	41	1713 ± 163	1635 ± 152	0.7317
|LymB| (10^6/L)	41	66.0 ± 14.5	97.7 ± 13.5	0.1172
|LymT| (10^6/L)	41	749 ± 100	735 ± 93	0.9197
|$CD4^+$| (10^6/L)	42	409 ± 41	255 ± 39	0.0095
|$CD8^+$| (10^6/L)	42	181 ± 24	159 ± 22	0.5039
|$CD25^+$| (10^6/L)	38	6.25 ± 3.19	8.68 ± 2.9	0.4794
|$CD4^+$|/|$CD8^+$|	40	2.32 ± 0.24	1.91 ± 0.22	0.2213
LymB (%)	41	4.39 ± 1.26	6.81 ± 1.12	0.1678
LymT (%)	41	48.30 ± 7.05	43.76 ± 6.25	0.6348
$CD4^+$ (%)	42	55.75 ± 5.02	45.89 ± 4.62	0.1642
$CD8^+$ (%)	42	24.79 ± 2.05	25.49 ± 1.89	0.8031
$CD25^+$ (%)	38	0.44 ± 0.48	1.48 ± 0.44	0.1265
$CD4^+/CD8^+$	40	2.26 ± 0.19	1.77 ± 0.16	0.0691

[1]Abbreviations as in the Table 2.
SE: Standard error.

Table 5. Simple correlation coefficients[1] between the lymphocyte populations in the peripheral blood of young rabbits at weaning and those of the multiparous rabbit does on different days (n=22).

	Lymphocyte populations of pups at weaning[2]				
Lymphocyte population of females[2]	|Lym|	|LymT|	|$CD4^+$|	|$CD8^+$|	|$CD25^+$|
|Lym|					-0.3343^{*}
|LymB|		$+0.3534^{*}$			
|LymT|		$+0.3740^{**}$	$+0.3505^{*}$	$+0.3418^{*}$	-0.3958^{**}
|$CD4^+$|/|$CD8^+$|	$+0.3196^{*}$		$+0.3233^{*}$		

[1] Only relevant and significant correlations are presented: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.
[2] Abbreviations as in the Table 2.

Figure 1

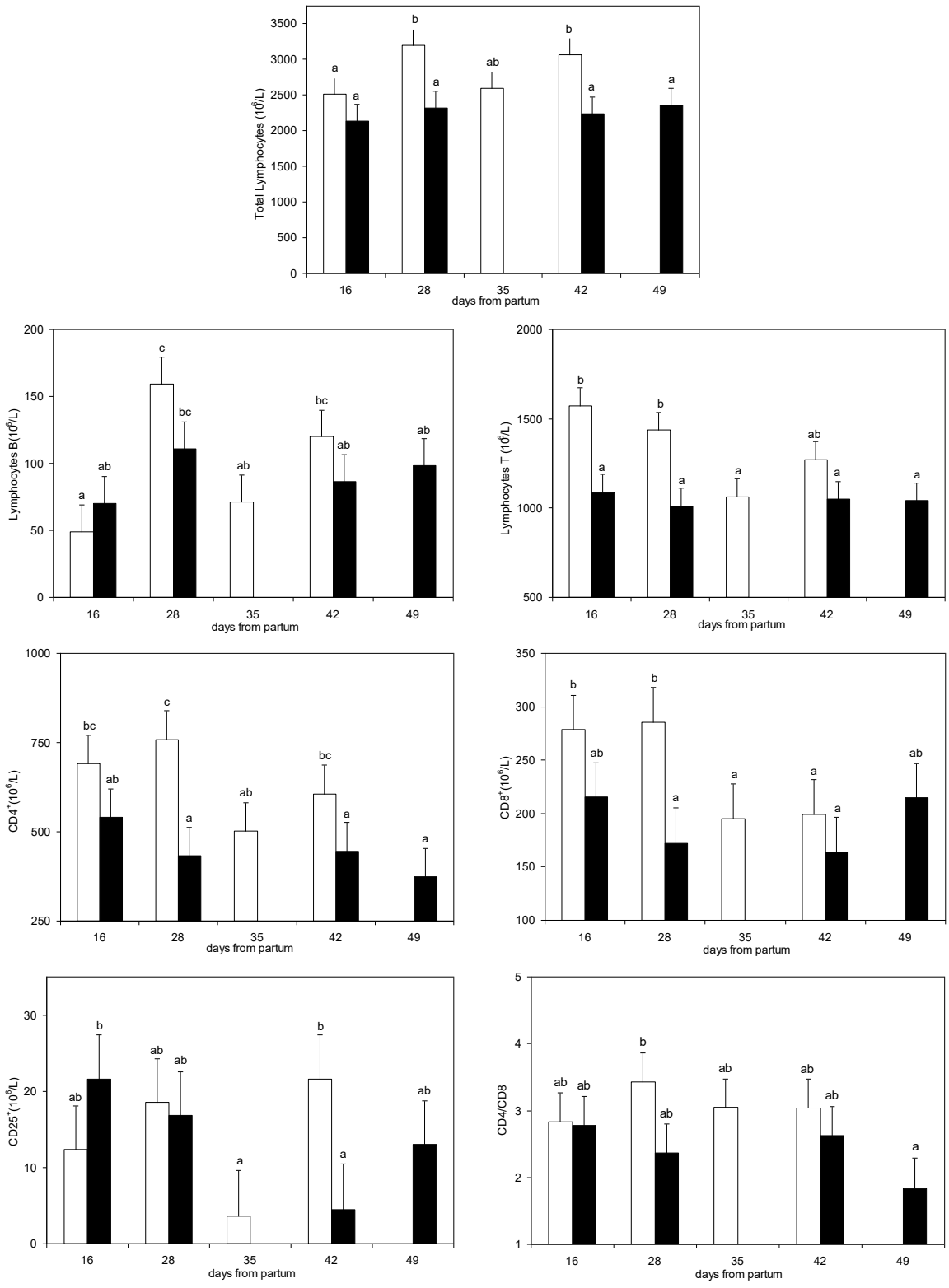

Figure 1. Effect of reproductive rhythm (weaning at 28 □ or 42 ■ days) on the evolution of the lymphocytes populations ($\times 10^6$/L) in peripheral blood of multiparous rabbit does.
[a,b,c] Least square means not sharing the same superscript being significantly different at $P<0.05$.