ELSEVIER

Livestock Production Science 60 (1999) 229–241

LIVESTOCK
PRODUCTION
SCIENCE

Physiological mechanisms involved in the effects of concurrent pregnancy and lactation on foetal growth and mortality in the rabbit

L. Fortun-Lamothe[a,]*, A. Prunier[b], G. Bolet[c], F. Lebas[a]

[a]*Station de Recherches Cunicoles, INRA, BP 27, 31326 Castanet Tolosan, France*
[b]*Station de Recherches Porcines, INRA, 35590 Saint Gilles, France*
[c]*Station d'Amélioration Génétique des Animaux, INRA, BP 27, 31326 Castanet Tolosan, France*

Abstract

Rabbit does can be mated shortly after parturition and sustain concurrent pregnancy and lactation. Six experiments were performed in order to assess the effects of lactation on foetal growth and survival in primiparous rabbits, and to establish the origin of these effects. Comparison of reproductive performance of pregnant does, concurrently lactating or not, showed that foetal survival (−9.6%) and weight (−16%) at day 28 of pregnancy, were lower in females lactating 10 young. The harmful effects of lactation on foetal survival and growth were related to the size of the suckled litter (−5.2% and −9% in females suckling four young). Simultaneously pregnant and lactating does increased their feed intake (+56%). However, this increase was not sufficient to meet energy requirements for both milk production and foetal development. Therefore, the energy balance was negative (−11.8 MJ in does lactating 10 young) and these females had to mobilise both protein and lipid body reserves. The results suggest that the nutritional deficit occurring in lactating does induces a competition between the mammary glands and the pregnant uterus for the nutrient supply which was detrimental to foetal growth. The hyperprolactinemic status associated with lactation seems to be responsible, at least in part, for the lower foetal survival and the reduced concentration of progesterone (−21%) in lactating does. However, we could not establish a clear influence of this decrease in progesterone on foetal mortality.

Résumé

La lapine peut être fécondée aussitôt après la parturition et conduire une gestation et une lactation simultanées. Six expériences ont été réalisées afin de déterminer les effets de la lactation sur la mortalité et la croissance foetales chez la lapine primipare, et d'établir l'origine de ces effets. La comparaison des performances de reproduction de lapines gravides, allaitantes ou non, ont montré que la survie (−9.6%) et la croissance foetales (−16%) au 28ème jour de gestation, sont plus faibles chez les femelles allaitant 10 lapereaux. Cet effet négatif de la lactation sur la mortalité et la croissance foetales sont proportionnels à la taille de la portée allaitée (−5.2% et −9% chez les femelles allaitant quatre lapereaux). Les femelles simultanément gravides et allaitantes augmentent leur ingestion d'aliment (+56%). Cependant, cette augmentation est insuffisante pour satisfaire à la fois les besoins énergétiques pour la production laitière et le développement foetal. Par conséquent, le bilan énergétique de ces femelles est négatif (−11.8 MJ chez les femelles allaitant 10 lapereaux) et elles

---

*Corresponding author. Tel.: +33-5-6128-5318; fax: +33-5-6128-5319.
E-mail address: lamothe@toulouse.inra.fr (L. Fortun-Lamothe)

0301-6226/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0301-6226(99)00096-2

doivent mobiliser leurs réserves protéiques et lipidiques. Nos résultats suggèrent que le déficit nutritionnel qui survient chez les femelles allaitantes entraîne une compétition entre les glandes mammaires et l'utérus gravide pour la répartition des nutriments qui se réalise au détriment de la croissance foetale. L'hyperprolactinémie associée à la lactation semble, au moins partiellement, responsable de la diminution de la survie foetale et de la progestéronémie (−21%) chez les femelles allaitantes. Toutefois, nous n'avons pas pu établir de relations claires entre la diminution des niveaux de progestérone et la mortalité foetale chez ces animaux. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Lactation; Foetal growth; Foetal survival; Rabbits

---

1. Introduction

In rabbit does, ovulation is induced by coïtus and the high receptivity immediately after parturition allows concurrent pregnancy and lactation. When does are mated on the day of parturition, concurrent pregnancy and lactation does not affect milk production or milk composition during the first three weeks of lactation. During the fourth week post partum, milk production is slowly lowered in non-pregnant does. In contrast, milk production decreases sharply in pregnant does, and milk secretion stops spontaneously at day 28 of pregnancy, three days before the next parturition (Lebas, 1972).

As a general rule, receptivity is lower in lactating than in non-lactating females (review: Theau-Clément and Roustan, 1992). Previous workers have shown that occurrence of ovulation (Foxcroft and Hasnain, 1973), ovulation rate (Harned and Casida, 1969; Lamb et al., 1991) and fertilisation of oocytes (Torrès et al., 1977) are lower in lactating than in non-lactating does. Effects of lactation on embryonic mortality are controversial. A higher mortality was observed in some experiments (Garcia et al., 1983; Garcia and Perez, 1989), whereas no effect was observed in others (Harned and Casida, 1969; Torrès et al., 1977; Partridge et al., 1984; Lamb et al., 1988). The effects of concurrent pregnancy and lactation on foetal growth and survival have been scarcely investigated and the underlying mechanisms are not known.

The aims of the present experiments were to quantify the effects of concurrent pregnancy and lactation on foetal growth and mortality in rabbits (experiments 1 and 2) and to determine which mechanisms were involved (experiments 3 to 6). Two main non-exclusive hypotheses were tested: (1) the nutrient availability for foetal development is not adequate in lactating does due to the competition with milk production and (2) the hormonal milieu of lactation creates an unfavourable environment for the establishment and maintenance of pregnancy.

2. Material and methods

2.1. Animals

Females used in all the experiments were crossbred does from INRA lines (New Zealand × Californian). They were mated within 24 h after their first parturition (day 0, 22±0.2 weeks of age) and were assigned to one of the experimental groups according to their weight and litter size. Non-lactating females were weaned the day of parturition, while lactating females were allowed to suckle four (group FL in experiment 1) or 10 young (all the other groups of lactating females). Litters were equalised at birth by crossfostering or culling. Females were caged individually with a controlled light/dark cycle (16 h/8 h) and had free access to water. Does and their young (if present) were weighed weekly, and food intake was determined at that time.

2.2. Foetal growth and mortality

Does were slaughtered on day 28 post partum in order to study reproductive performance. The genital tract was removed and dissected immediately after slaughter. Ovulation rate was determined by counting the number of corpora lutea. Foetuses were divided into live (L) and dead foetuses (D). Foetal mortality (FM) was defined as $FM = (100 \times D)/(L + D)$, and foetal survival as $100 - FM$. Live foetuses and their placentas were individually weighed. Additionally, does were dissected in order to weigh carcass and dissectable adipose tissues. In some experiments, the litters (experiments 2, 3 and 4) and the carcasses of the does (animal without skin, digestive and genital tracts; experiments 3 and 4) were frozen immediately after slaughter until analyses. Representative samples of ground matter were freeze dried and analysed for dry matter (24 h at 103°C), proteins ($N \times 6.25$), ash (incineration for 6 h at 550°C) and energy (adiabatic calorimeter). Lipid percentage was estimated by difference.

2.3. Characterisation of the effects of concurrent lactation on foetal growth and mortality

2.3.1. Experiment 1 (Fortun et al., 1993; Fortun and Lebas, 1994a)

One hundred and twenty nine females (divided in two replicates) were assigned to one of three experimental groups. Females were weaned at parturition (W group, $n = 52$) or allowed to lactate ten (TL group, $n = 50$) or four young (FL group, $n = 27$) and were slaughtered on day 28 post partum. Females had free access to a commercial diet containing 17.5% of crude proteins and 10.9 MJ of digestible energy/kg of dry matter. Blood samples were taken on days 7 and 17 to assay plasmatic progesterone levels (Thibier and Saumande, 1975). Energy balance of the does during the experiment (0–28 days post partum) was estimated by difference between energy supply (from feed intake) and energy requirements (Fortun and Lebas, 1994b) for maintenance ($= 420$ and 468 kJ/day/kg$^{0.75}$ for pregnant and simultaneously pregnant and lactating does, respectively; Parigi-Bini et al., 1990a and 1992), for milk production (energy content of the milk $= 8.29$ KJ/g and efficiency of utilisation of digestible energy for milk production $= 0.63$; Parigi-Bini et al., 1990b and 1991) and for foetal growth (efficiency of utilisation of digestible energy for foetal growth $= 0.27$; Parigi-Bini et al., 1991).

2.3.2. Experiment 2 (Fortun-Lamothe and Lebas, 1998)

Sixty-two females were assigned to one of two experimental groups. Females were weaned at birth (W group, $n = 30$) or allowed to lactate 10 young (TL group, $n = 32$). At the second parturition, newborn rabbits were individually weighed and slaughtered. After slaughter, the milk was removed from the stomach and weighed. The weight at birth was calculated as live weight-milk weight in the stomach. Litters were then frozen in order to determine their chemical composition. Females had free access to a commercial diet containing 17.5% crude proteins and 10.9 MJ of digestible energy/kg of dry matter.

2.4. Effects of the energy balance of the mother on foetal growth and mortality

2.4.1. Experiment 3 (Fortun et al., 1994b)

Fifty nine females were weaned at birth and were fed 190 g/day (control group close to ad libitum feeding, CW group, $n = 20$), 120 g/d (to meet maintenance energy requirements, MW group, $n = 20$) or 120 g/day during the first week of gestation and 80 g/day thereafter (severely restricted females, RW group, $n = 19$). The feed was a commercial diet containing 17.5% crude proteins and 10.9 MJ of digestible energy/kg of dry matter. Females were slaughtered on day 28 post partum. Energy balance of the does (0–28 days post partum) was estimated as in experiment 1.

2.4.2. Experiment 4 (Fortun-Lamothe and Lebas, 1996)

Sixty eight females were allowed to suckle ten young and were given ad libitum access to a moderate-energy diet (9.88 MJ of digestible energy/kg of dry matter, group MTL, $n = 23$) or to one of two high-energy diets (12.2 MJ of digestible energy/kg of dry matter). In the high-energy diets, energy came from starch (HSTL group, $n = 23$) or starch + fat (3% of vegetable fat added, HFTL group, $n = 22$). Females were slaughtered on day 28 post partum.

2.5. Effects of hormonal (prolactin and progesterone) levels on foetal growth and mortality

2.5.1. Experiment 5 (Fortun et al., 1994a)

Sixty five females were weaned the day of parturition and had free access to a commercial diet containing 17.5% crude proteins and 10.9 MJ of digestible energy/kg of dry matter. Females received subcutaneously, 1 mg of ovine prolactin (Sigma; PrlW group, $n = 31$) or vehicle [1 ml of phosphate-buffered saline (PBS), pH 7.4; VehW group, $n = 34$] once a day from day 7 to day 21 post partum. Blood samples were collected on days 5 and 16, 2 h after the injection in order to assay plasmatic levels of prolactin (ovine and rabbit prolactin separately, oPrl and rPrl) and progesterone. A specific prolactin assay was developed using reagents supplied by the National Hormone and Pituitary Program (Dr. Parlow, Harbour-UCLA Medical Center, USA). Progesterone levels were measured according to a previously described method (Thibier and Saumande, 1975). Females were slaughtered on day 28 post partum.

2.5.2. Experiment 6 (Fortun, 1994)

Fifty-eight females were allowed to suckle 10 young and were given ad libitum access to a commercial diet containing 17.5% crude proteins and 10.9 MJ of digestible energy/kg of dry matter. On day 7, females received under general anaesthesia (xylazine, 5 mg/kg and ketamine, 20 mg/kg) two subcutaneous implants which were either empty (EmTL group, $n = 29$) or filled with crystalline progesterone (Sigma, PgTL group, $n = 29$). Each implant was a 5 cm segment of silastic medical tubing (Dow Corning 601-325) sealed by medical adhesive silicone. Implants were incubated in 0.9% NaCl overnight prior to use. Blood samples were collected on days 7, 16 and 25 in order to assay plasmatic levels of progesterone (Thibier and Saumande, 1975). Females were slaughtered on day 28 post partum.

2.6. Statistical analyses

All data were analysed by analysis of variance using the SAS (1990) package. When treatments differed significantly ($P < 0.05$), differences between means were tested using the Ryan–Einot–Gabriel–Welsch multiple F test.

3. Results

3.1. Experiment 1

Live weight of does was similar in non-lactating and both groups of lactating females from mating until day 14. During the second half of pregnancy, lactating does lost weight (−5.5% and −2.5% in TL and FL groups, respectively) while weaned does gained weight (+5.3%, $P < 0.001$). At slaughter on day 28, live weight as well as carcass and adipose tissues weights were the highest in W does and the lowest in TL does. Feed intake was higher in lactating (+42% in the FL group, +56% in the TL group) than in non-lactating females ($P < 0.001$). Energy balance over the period 0–28 days post partum was negative for lactating females (−8.9 and −11.8 MJ in FL and TL does, respectively) and positive for non-lactating females (+12.5 MJ). The number of live foetuses at day 28 of gestation was similar in the three groups, but foetal mortality was higher in the TL (14.1%) than in the W group (4.9%), and intermediate in the FL group (9.9%). Foetuses (−16%) and placentas (−9%) were lighter in TL than in W does ($P < 0.01$), and intermediate in FL does (Table 1). Progesterone levels were 21% lower in the TL and FL groups than in the W group on days 7 and 17 of pregnancy ($P < 0.001$).

3.2. Experiment 2

Litter size at the 2nd parturition was not significantly different between non-lactating and lactating females. However, the percentage of small litters (≤7 young) tended to be higher in the TL than in the W group (11/32 vs. 5/30, $P = 0.11$). The empty weight of new-born rabbits was 2.1% lower in the TL than in the W group ($P = 0.019$, Table 1). The lipid (−33.1%), protein (−2.3%) and energy contents (−4.8%) were lower, while the water (+2.8%) and the ash contents (+2.8%) of the new-born rabbits were higher in the TL than in the W group (Table 2).

3.3. Experiment 3

Feed intake, variations in the live weight throughout gestation, as well as weights of the carcass and of adipose tissues in control non-lactating females were similar in the present experiment and in non-lactating females fed ad libitum in experiment 1. Therefore, feed intake of the control females from the present experiment was close to ad libitum, as anticipated. Live weight as well as carcass and adipose tissues weights were lower in restricted (MW and RW) than in control females ($P < 0.001$). Lipid percentage and energy content per 100 g of

Table 1
Effects of lactation during pregnancy and size of the suckled litter on body composition and reproductive performance of primiparous rabbit does on day 28 post partum (adapted from Fortun et al., 1993; Fortun and Lebas, 1994a)

Experiment 1	Group			S.E.M.	Prob $> f$
	W	FL	TL		
Number of does	52	27	50		
Live weight of does (g)	4299 a	3943 b	3834 b	34.5	< 0.001
Carcass weight of does (g)	2392 a	2117 b	2061 b	23.6	< 0.001
Adipose tissue weight of does (g)	124 a	53 b	41 b	4.7	< 0.001
Number of corpora lutea	11.2	11.1	11.0	0.16	NS
Number of live foetuses	9.2	8.4	8.3	0.22	0.12
Number of dead foetuses	0.48 b	0.92 ab	1.36 a	0.10	< 0.001
Foetal mortality (%)	4.9 b	9.9 ab	14.1 a	1.1	< 0.001
Foetal weight (g)	40.0 a	37.6 b	33.6 c	0.45	< 0.001
Placenta weight (g)	7.7 a	7.1 b	7.0 b	0.1	< 0.001

Treatment groups: W: non-lactating females; FL: females suckling four young; TL: females suckling 10 young. For calculation of foetal mortality, see Section 2.2.

Table 2
Effects of lactation during pregnancy on weight and composition of newborn rabbits (adapted from Fortun-Lamothe and Lebas, 1998)

Experiment 2	Group[a]		S.E.M.	Prob $> f$
	W	TL		
Number of litters	30	32		
New born rabbits	9.3	8.5	0.3	NS
Weight at birth (g)	52.9	51.8	0.4	0.019
Water (%)	79.6	81.8	0.2	< 0.001
Proteins (%)	11.9	11.6	0.07	0.052
Lipids (%)	6.1	4.1	0.2	< 0.001
Ash (%)	2.48	2.55	0.02	0.055
Energy (MJ/kg)	5.60	5.32	0.3	< 0.001

[a] Treatment groups: W: non-lactating females, TL: females suckling 10 young.

maternal carcasses increased with feed intake, whereas percentage of water decreased ($P < 0.001$). The energy balance of the does was positive in the CW group (+11.8 MJ) and negative in the other groups (−6.4 MJ and −14.4 MJ in MW and RW females, respectively). Numbers of live and dead foetuses, and foetal mortality were not different between treatments. However, the weights of placentas and foetuses increased significantly with the feed intake of the does ($P < 0.001$, Table 3). Percentages of proteins ($P < 0.001$) and lipids in the foetuses increased with the feed intake of the does, whereas that of water decreased ($P < 0.001$).

3.4. Experiment 4

From days 0 to 21, digestible energy intake of lactating does increased with the energy content of the diet and with the introduction of fat (2.88, 3.09 and 3.34 MJ/day, in the MTL, HSTL and HFTL groups, respectively, $P < 0.05$). On day 28 of pregnancy, weights of carcasses were similar in the three groups, but the weight of adipose tissues was higher in the HSTL than in the two other groups ($P < 0.005$). Lipid and energy contents of does were lower, whereas water content was higher in the MTL group than in the two other groups ($P < 0.001$). Total milk production between birth and 21 days, estimated by litter growth (Lebas, 1969), was higher in HFTL (4.18 kg) than in HSTL does (3.58 kg, $P < 0.05$), the MTL group being intermediate (3.8 kg). There was no significant difference between treatments either in the numbers of live and dead foetuses, or in the weights of foetuses and placentas (Table 3). Water, protein and ash contents of foetuses were not altered by the diet, whereas lipid content tended to be higher in the HFTL group than in the two other groups ($P < 0.1$).

Table 3
Effects of the energy balance of pregnant does (adapted from Fortun et al., 1994b) or simultaneously pregnant and lactating does (adapted from Fortun-Lamothe and Lebas, 1996) on body composition, reproductive performance and chemical composition of the litters on day 28 post partum

	Experiment 3					Experiment 4				
	CW	MW	RW	S.E.M.	Prob > *f*	MTL	HSTL	HFTL	S.E.M.	Prob > *f*
Number of does	20	20	19			23	23	22		
Live weight of the does (g)	4371 a	3952 b	3838 b	42	< 0.001	3859	3923	3891	35.7	NS
Carcass weight of the does (g)	2369 a	2219 b	2172 b	23	< 0.001	1858	1960	1907	19.2	NS
Body composition of the does										
Adipose tissues (g)	121 a	85 b	71 b	5	< 0.001	28	52	38	2.5	< 0.01
Water (%)	59.1 c	62.2 b	64.6 a	0.5	< 0.001	66.8 b	63.0 a	64.3 a	4.0	NS
Proteins (%)	18.9	19.2	18.9	0.1	NS	21.1	21.1	21.0	0.3	NS
Lipids (%)	19.3 a	15.6 b	13.4 b	0.6	< 0.001	7.3 b	11.1 a	9.9 a	0.6	< 0.001
Ash (%)	3.6	3.7	3.8	0.1	NS	4.8	4.8	4.8	0.1	NS
Energy (MJ/kg)	11.8 a	10.5 b	9.6 c	0.2	< 0.001	0.78 b	0.92 a	0.88 a	0.02	< 0.001
Reproductive performance										
Number of corpora lutea	11.8	10.8	11.9	0.3	NS	11.4	11.0	11.1	0.2	NS
Number of live foetuses	9.5	8.2	8.6	0.3	NS	9.5	9.3	9.1	0.3	NS
Number of dead foetuses	0.85	0.45	1.00	0.1	NS	0.7	0.9	0.9	0.1	NS
Foetal mortality (%)	7.6	4.2	8.5	1.2	NS	5.8	8.7	6.7	1.1	NS
Foetal weight (g)	40.2 a	36.1 b	30.5 c	0.8	< 0.001	33.4	31.2	32.3	0.5	NS
Placenta weight (g)	7.1	6.6	5.5	0.2	< 0.001	7.0	6.9	6.7	0.1	NS
Body composition of the litters										
Water (%)	84.1 a	85.0 b	85.6 c	0.1	< 0.001	85.0	85.1	84.9	0.1	NS
Proteins (%)	10.3 a	9.6 b	9.1 c	0.1	< 0.001	9.7	9.6	9.6	0.1	NS
Lipids (%)	3.6	3.5	3.5	0.1	NS	3.4	3.4	3.6	0.1	NS
Ash (%)	1.9	1.8	1.8	0.01	NS	1.9	1.9	1.9	0.01	NS
Energy (MJ/kg)	3.55	3.53	3.36	0.01	NS	3.4	3.4	3.4	0.004	NS

Treatment groups: CW: non-lactating females fed close to ad libitum; MW: non-lactating females fed to the maintenance requirements; RW: non-lactating females severely feed-restricted; MTL: lactating females given a moderately energetic diet; HSTL: lactating females given a starch rich diet; HFTL: lactating females given a fat enriched diet. For calculation of foetal mortality, see Section 2.2.

3.5. Experiment 5

Plasma concentrations of prolactin and progesterone were similar in both groups before the period of treatment on day 5 of gestation. On the opposite, during the period of treatment on day 16 of gestation, prolactin was 4.5-fold higher in PrlW than in VehW does (112 vs. 24.4 ng/ml of oPrl + rPrl, $P < 0.001$). Simultaneously, progesterone was 21% lower in PrlW than in VehW does ($P < 0.01$). Prolactin supplementation decreased feed intake of non-lactating females during the period of treatment (146 and 196 g/day from days 7 to 21 of gestation in PrlW and VehW females, respectively; $P < 0.001$). However, feed intake was similar in both groups before and after this period. On day 28, the live weight ($P < 0.05$) and the weight of adipose tissues were lower ($P < 0.01$) in the PrlW than in the VehW group. The number of live foetuses was lower while foetal mortality was higher in the PrlW than in the VehW group ($P < 0.01$). Weights of foetuses and placentas were lower in the PrlW than in the VehW group ($P < 0.01$, Table 4). Nevertheless, when data were adjusted for feed intake during the period of prolactin supplementation (covariance analysis), there was no longer any difference between groups for the weight of foetuses.

3.6. Experiment 6

On day 7 (before inserting implants), the concentration of progesterone was similar in both

Table 4
Effects of prolactin (adapted from Fortun et al., 1994a) or progesterone (adapted from Fortun, 1994) treatment on reproductive performance of does slaughtered on day 28 post partum

	Experiment 5[a]				Experiment 6[a]			
	VehW	PrlW	S.E.M.	Prob > f	EmTL	PgTL	S.E.M.	Prob > f
Number of does	34	31			29	29		
Live weight of the does (g)	3920	3747	43	< 0.05	3703	3769	41	NS
Carcass weight of the does (g)	2089	2030	28	NS	1887	1925	23	NS
Adipose tissues of the does (g)	162	126	8	< 0.01	26	25	2	NS
Number of corpora lutea	10.7	11.2	0.2	NS	11.2	11.2	0.3	NS
Number of live foetuses	7.9	6.8	0.3	< 0.01	8.0	8.9	0.4	NS
Number of dead foetuses	0.9	2.3	0.2	< 0.01	0.7	0.9	0.2	NS
Foetal mortality (%)	10.6	24.5	2.4	< 0.01	10.8	9.5	3.2	NS
Foetal weight (g)	38.6	35.8	0.6	< 0.01	36.1	35.3	0.7	NS
Placenta weight (g)	7.3	6.7	0.2	< 0.01	7.5	7.2	0.2	NS

[a] Treatment groups: VehW: non-lactating females injected with vehicle; PrlW: non-lactating females injected with prolactin; EmTL: lactating females receiving empty implants; PgTL: lactating females receiving progesterone implants. For calculation of foetal mortality, see Sections 2.2.

groups. It was 29.4% higher in PgTL than in EmTL females on day 16 (15.4 and 11.9 ng/ml in PgTL and EmTL females, respectively; $P < 0.01$) and 41.4% higher on day 25 (14.0 and 9.9 ng/ml, $P < 0.01$). Progesterone treatment of lactating does had no effect on their feed intake, live weight or body composition at slaughter. Weights of foetuses and placentas, as well as the number of live foetuses and foetal mortality were not significantly different between the two groups (Table 4). For instance, there were 0.9 more live foetuses in PgTL than in EmTL females, but the difference was not significant.

4. Discussion

4.1. Effects of concurrent pregnancy and lactation on foetal growth and mortality, energy balance and hormonal levels in the does.

Our data show that the live weight of simultaneously pregnant and lactating does decreases during the second half of pregnancy. Such a decrease, which occurs despite development of the pregnant uterus, must be due to mobilization of body reserves. Indeed, comparison at the end of pregnancy of the weights of the carcasses and of adipose tissues, as well as comparison of the chemical composition between does which were lactating or not shows that this mobilization affects lipid and protein reserves. Similarly, Parigi-Bini et al. (1992) have shown that 6% and 49% of the initial body contents in proteins and lipids were respectively mobilized at the end of the second pregnancy-lactation. Additionally, present results show that the extent of body reserve mobilization increases with the size of the suckled litter.

Present data show that feed intake is higher for simultaneously pregnant and lactating does than for pregnant non-lactating does, and is influenced by litter size (thus milk production) as previously demonstrated (Lebas, 1987). Nevertheless, feed intake in pregnant-lactating does is not sufficient to meet nutrient requirements for both milk synthesis and foetal development and the energy balance is highly negative in pregnant-lactating does (−11.8 MJ for 10 young suckled) while it is positive in pregnant non-lactating does (+12.5 MJ). Energy deficit increases with the size of the suckled litter (−8.9 MJ for four young suckled). Body reserve mobilization allows to meet the energy and protein requirements of these pregnant lactating females. Inadequacy between energy intake and requirements during lactation have been previously shown in other farm species (reviews: Buttler and Smith, 1989; I'Anson et al., 1992; Quesnel and Prunier, 1995).

Previous studies concerning the effects of lactation on prolificacy were controversial (review: Fortun-Lamothe and Bolet, 1995). At day 28 of pregnancy, our experiments show that the number of dead foetuses is higher in simultaneously pregnant and lactating does than in pregnant non-lactating does. This negative effect of lactation on foetal viability seems to be proportional to the size of the suckled litter. In contrast, our data do not show any significant effect of the concurrent lactation on litter size at parturition, three days later. However, the percentage of small litters tends to be higher in lactating does compared to non-lactating does.

At day 28 of pregnancy, foetal (−16.0%) and placental (−9%) weights are lower in does suckling 10 young than in non-lactating does. This negative effect of lactation on foetal growth is proportional to the size of the suckled litter. At birth, the difference in weight of the rabbits born from lactating and from non-lactating does is of lower amplitude (−2.1%). Therefore, a compensatory growth probably occurs during the last three days of gestation after cessation of lactation. However, this compensatory growth is not sufficient to compensate for the retardation occurring before day 28 of gestation. Indeed, dry matter, protein, lipid and energy contents measured at birth in rabbits born from lactating does are reduced compared to rabbits from non-lactating does. This is in agreement with results of Parigi-Bini et al. (1992). As a general rule, it seems that physiological maturation of young from simultaneously pregnant and lactating does is delayed. Indeed, Gondret et al. (1997) demonstrated that maturation of the muscular fibres is delayed in such young rabbits. Moreover, the number of pre-ovulatory follicles at 70 days of age is lower in daughters born from lactating mothers compared to daughters from non-lactating mothers (Fortun-Lamothe et al., 1999).

Progesterone levels during pregnancy are lower in lactating than in non-lactating does. A difference in ovulation rate cannot be involved because it is similar in lactating and weaned does. The origin of lower progesterone levels remains unknown but several hypotheses can be evoked. Firstly, the action of oestrogens is regulated by a placental luteotrophic hormone (Gadsby and Keyes, 1984). Our results show that placentas are lighter in lactating than in non-lactating does. Consequently, compromised secretion of placental luteotrophin may contribute to decrease progesterone secretion. Secondly, high levels of prolactin inhibit steroidogenesis in the rabbit (Lin et al., 1987). Therefore, during lactation, hyperprolactinemia could inhibit progesterone secretion. Such a hypothesis is supported by the observation of lower concentrations of progesterone in does receiving prolactin injections (experiment 5). Thirdly, suckling stimulates oxytocin release from pituitary (Fuchs et al., 1984). Since this hormone seems to play a luteolytic role (Flint and Sheldrick, 1986; Sawyer et al., 1986), it could inhibit progesterone secretion in lactating does. Finally, it has been demonstrated in the pig, that increasing feed intake leads to a higher clearance rate of progesterone in the liver and hence to lower peripheral levels of this hormone (Symonds and Prime, 1989). Since feed intake is increased in lactating does, such phenomenon may contribute to decrease progesterone concentrations.

Taken together, these results indicate that, in pregnant-lactating does, spontaneous feed intake is not sufficient to meet the nutrient requirements for both foetal development and milk production, and that foetal growth and mortality are altered. Additionally, the endocrine status of pregnant does is modified by concurrent lactation: progesterone levels are lowered, while prolactin levels are greatly increased during lactation (McNeilly and Friesen, 1978). Therefore, the harmful effects of lactation on foetal growth and mortality may be related to the nutritional deficiency and/or to the hormonal milieu of lactation. Experiments have been performed in order to differentiate between the effects of the nutritional deficit due to the high metabolic demand for milk production and of the hormonal environment due to suckling (high prolactin levels, decreased progesterone levels).

4.2. Effects of energy balance of the doe on foetal growth and mortality

In order to assess, independently of suckling, the role of the nutritional deficit in the impairment of foetal survival and growth, non-lactating does were submitted to a feed restriction calculated to mimic the energy deficit occurring in lactating does (experiment 3). Feed restriction induced lower live weight, lipid percentage and energy content of the maternal body in agreement with previous results (Lebas, 1973; Sadurskis et al., 1991). These decreases were proportional to the level of feed restriction. Additionally, the estimated energy balance calculated in feed-restricted does was similar to that observed in simultaneously pregnant and lactating does. At day 28 of gestation, weights of foetuses and placentas, as well as dry matter and protein contents of the foetuses, were lower in feed-restricted females than in fully fed does, in accordance with previous results (Young and Widdowson, 1975; Lederman and Rosso, 1980; Rosso and Kava, 1980). Our results do not show any effect of the feed level on foetal mortality. However, a possible influence of feed restriction on foetal mortality could have been masked by the fact that foetal mortality was relatively high in the control group of experiment 3 compared to previous experiments (7.6% vs. 4.9%).

Our data show that increasing the dietary energy content allows to increase the daily digestible energy intake (experiment 5) in agreement with previous results (Partridge et al., 1986; Fraga et al., 1989; Xiccato et al., 1995). Since addition of fat to the diet increases milk production (Fraga et al., 1989; Cervera et al., 1993), it may remain without positive influence on the energy balance of lactating does as observed by Xiccato et al. (1995). Contrarily, our results indicate that lipid content of the maternal carcasses is higher and thus lipid mobilization is lower in the two groups of females receiving the high-energy diets regardless of the addition of fat. However, milk production and lipid mobilization are higher in the females given the fat-diet than in the females given the starch-diet. Our data do not show any significant effect of the dietary energy content nor of the fat addition on foetal growth or mortality. However, the lipid content of the foetuses tends to be increased in does receiving the fat-enriched diet. The permeability of rabbit placentas to fatty acids could explain this result (Elphick and Hull, 1977). Taken together these results suggest that, when females receive a high-energy diet, additional energy intake is used preferentially for milk synthesis (especially if energy comes from fat) or maternal storage (especially if energy come from starch) and only marginally for foetal growth.

Overall, our data support that the energy deficit induced by milk production is involved in the impairment of foetal growth in concurrently pregnant and lactating rabbit does whereas it is probably not responsible for the increased foetal mortality. Nutritional competition between the mammary glands and the pregnant uterus could be a competition for blood flow or(and) a competition for nutrient uptake. Indeed, mammary glands and foetuses utilise the same substrates such as glucose, amino acids, long-chain fatty acids and free fatty acids (Elphick and Hull, 1977; Jones and Parker, 1981; Jones and Rolph, 1985; Fraga et al., 1989; Stephenson et al., 1990). Moreover, blood flow is preferentially directed toward the uterus during pregnancy, mainly due to oestrogens, whereas it is redirected toward the mammary glands during lactation, mainly due to prolactin (Bauman and Currie, 1980). The influence of blood flow on foetal growth has been pointed out by Johnson et al. (1985) who have shown that foetal weight is correlated with the placental blood flow ($r = 0.67$).

4.3. *Effects of the hormonal status of the does on foetal growth and mortality*

In most mammal species, and more especially in the rabbit, progesterone levels must be sufficient to ensure a uterine environment favourable to the establishment and maintenance of pregnancy (Niswander and Nett, 1988). Therefore, low concentrations of progesterone in lactating does during pregnancy (see above) may be detrimental to foetal growth and survival. Treatment of lactating does with progesterone implants allowed to maintain their progesterone concentrations (experiment 6) at levels similar to those observed in non-lactating does (experiment 1). However, it did not prevent the negative effects of lactation on foetal growth and survival as shown by similar foetal weight and mortality at day 28 of pregnancy in both treated and control lactating females.

Suckling occurs once a day in the rabbit species (Zarrow et al., 1965) and stimulates prolactin release within 15 min. Concentrations of prolactin after nursing are two- to 10-fold higher than the levels observed during pregnancy (McNeilly and Friesen, 1978). This hyperprolactinemia may be involved in the impairment of foetal growth and survival occurring in pregnant lactating does. In order to test this hypothesis, we have determined the effects of ovine prolactin supplementation injections in pregnant non-lactating does (experiment 5). The pattern of injection which was used (1 mg of ovine prolactin/day/doe) allowed to increase prolactin, as measured 2 h after the injection, to similar levels as those found in lactating rabbits after suckling (McNeilly and Friesen, 1978). Feed intake was reduced during the period of prolactin injection. In prolactin-treated females, foetal survival and weight were reduced. However, it seems that the effects of prolactin on foetal growth were essentially linked to the reduction in feed intake. Several mechanisms could explain the detrimental effect of hyperprolactinemia on foetal survival. High levels of prolactin could affect the relations between the uterus and the foetuses. Indeed, prolactin modifies endometrial cellular differentiation, quality of the uterine secretions (Daniel and Juneja, 1989; Chilton and Daniel, 1985; Young and Bazer, 1987) as well as water and ionic transport throughout the epithelium (Rabbee and McCoshen, 1986). In addition, prolactin increases the progesterone receptor endometrial concentration (Chilton and Daniel, 1987) and stimulates the secretion of prostaglandins, which may themselves modify the endometrial vascularization during pregnancy (Hodgen and Itskovitz, 1988).

Taken together our results suggest that hyperprolactinemia due to suckling could be involved in the lower foetal survival occurring in pregnant-lactating does whereas the influence of the decreased levels of progesterone is not clear.

5. Conclusion

Concurrent lactation during pregnancy reduces foetal growth and survival, and these harmful effects increase with the size of the suckled litter. At least

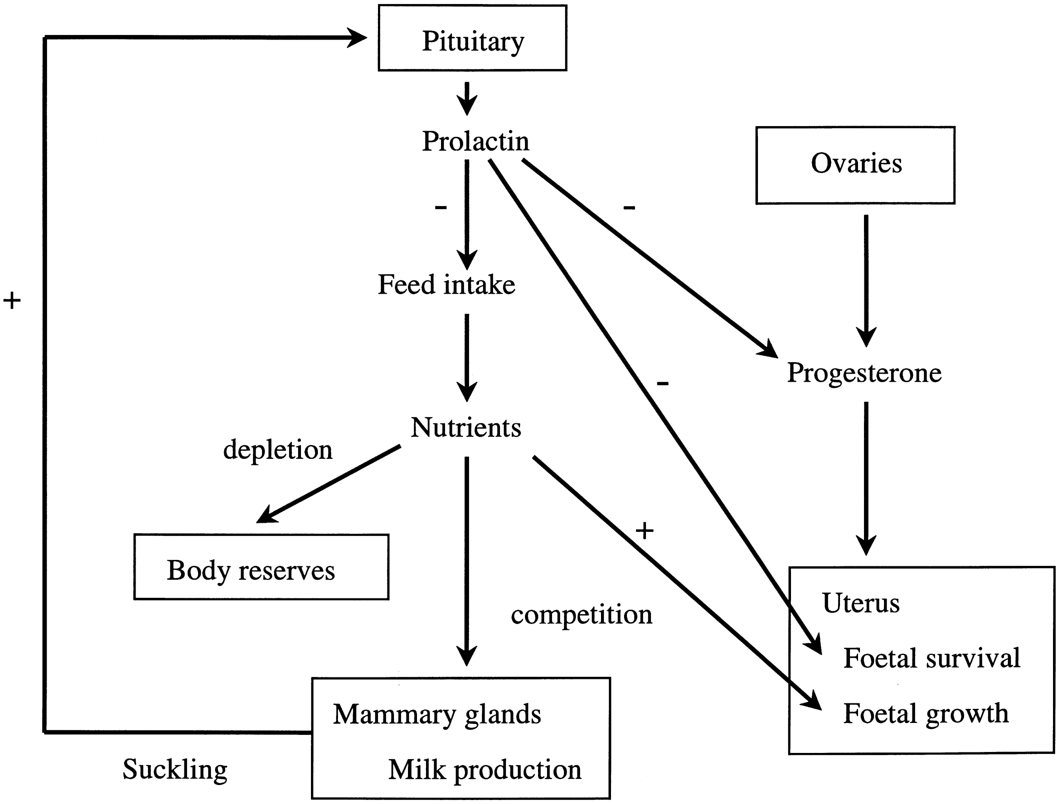

Fig. 1. Mechanisms involved in the detrimental effects of lactation on foetal growth and mortality.

two phenomena seem to be involved in these effects. Firstly, in primiparous rabbit does, the spontaneous energy intake is not sufficient to meet the nutrient requirements for both milk synthesis and foetal development. A nutritional competition occurs between the mammary glands and the pregnant uterus which is detrimental for foetal growth. However, additional energy intake when females are offered a high-energy diet is used preferentially for milk synthesis or maternal tissues without clear influence on foetal growth. Secondly, high levels of prolactin during pregnancy due to suckling can explain, at least partly, the higher foetal mortality and the lower progesterone levels observed in lactating does (Fig. 1).

A compensatory growth occurs between weaning of the suckled litter and birth of the following one. However, this compensation is only partial and body composition of the rabbits from the second litter is greatly affected. Beyond birth, it seems that muscle differentiation and sexual maturation of these rabbits are also impaired.

Acknowledgements

The authors want to acknowledge C. Béranger, B. Lamboley, J. DeDapper, D. Guéméné, A. Lapanouse, M. Mills, A.M. Mounier, and J. Belleraud for expert technical assistance as well as A. Aumaitre, M. Etienne and J.Y. Dourmad for valuable comments and ideas.

References

Bauman, P.E., Currie, W.B., 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorrhesis. J. Dairy Sci. 63, 1514–1529.

Buttler, W.R., Smith, R.D., 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72, 767–783.

Cervera, C., Fernandez-Carmona, J., Viudes, P., Blas, E., 1993. Effect of remating interval and diet on the performance of female rabbits and their litters. Anim. Prod. 56, 399–405.

Chilton, B.S., Daniel, J.C., 1985. Influence of prolactin on DNA synthesis and glandular differentiation in rabbit uterine endometrium. Prolactin Basic Clin. Correlates 1, 351–359.

Chilton, B.S., Daniel, J.R., 1987. Difference in the rabbit uterine response to progesterone as influenced by growth hormone or prolactin. J. Reprod. Fertil. 79, 581–587.

Daniel, J.C., Juneja, S.C., 1989. Amplification of uterine secretion by altering prolactin-progesterone administration. J. Endocrinol. 122, R5–R6.

Elphick, M.C., Hull, D., 1977. The transfer of free fatty acids across the rabbit placenta. J. Physiol. 264, 751–766.

Flint, A.P.F., Sheldrick, E.L., 1986. Ovarian oxytocin and the maternal recognition of pregnancy. J. Reprod. Fertil. 76, 831–839.

Fortun, L., 1994. Influence of progesterone supplementation on foetal survival in concurrently pregnant and lactating rabbit does. Reprod. Nutr. Dev. 34, 125–131.

Fortun, L., Lebas, F., 1994. Influence of the number of suckling young and the feed level on foetal survival and growth in rabbit does. Ann. Zoot. 43, 163–171.

Fortun, L., Lebas, F., 1994. Estimation of the energy balance in concurrently pregnant and lactating rabbit does during their second pregnancy. Proc. Symposium on Animal and Human Nutrition, Comparative Physiology of Digestion and Metabolism, Tours, 13–14 January. Reprod. Nutr. Dev. 34, 632.

Fortun, L., Prunier, A., Lebas, F., 1993. Effects of lactation on fetal survival and development in rabbit does mated shortly after parturition. J. Anim. Sci. 71, 1882–1886.

Fortun, L., Bolet, G., Lebas, F., 1994. Influence of prolactin on foetal survival in rabbit does. Anim. Reprod. Sci. 37, 75–84.

Fortun, L., Prunier, A., Etienne, M., Lebas, F., 1994. Influence of the nutritional balance on foetal survival and growth and blood metabolites in rabbit does. Reprod. Nutr. Dev. 34, 201–211.

Fortun-Lamothe, L., Bolet, G., 1995. Les effets de la lactation sur les performances de reproduction chez la lapine. INRA, Prod. Anim. 8, 49–56.

Fortun-Lamothe, L., Lebas, F., 1996. Effects of dietary energy level and source on foetal development and energy balance in concurrently pregnant and lactating primiparous rabbit does. Anim. Sci. 62, 615–620.

Fortun-Lamothe, L., Lebas, F., 1998. Effects of simultaneous pregnancy and lactation in primiparous rabbit does on weight and composition of new born rabbits. 2nd International Conference on Rabbit Production in Hot Climates, Adana, 7–8 September.

Fortun-Lamothe, L., Powers, S.J., Collet, A., Read, K.L.Q., Mariana, J.C., 1999. Effects of concurrent pregnancy and lactation in rabbit does on the growth of follicles in daughter's ovaries. Reprod. Nutr. Dev., in press.

Foxcroft, G.R., Hasnain, H., 1973. Effect of suckling and time to mating after parturition on reproduction in domestic rabbit. J. Reprod. Fertil. 33, 367–377.

Fraga, M.J., Lorente, M., Carabano, R.M., DeBlas, J.C., 1989. Effect of diet and of remating interval on milk composition of the doe rabbit. Anim. Prod. 48, 459–466.

Fuchs, A.R., Cubile, L.M., Dawood, Y., Jorgensen, F.S., 1984. Release of oxytocin and prolactin by suckling in rabbits throughout lactation. Endocrinology 114, 462–569.

Gadsby, J.E., Keyes, P.L., 1984. Control of corpus luteum function in the pregnant rabbit: role of the placenta (''placental luteotropin'') in regulating responsiveness of corpora lutea to estrogen. Biol. Reprod. 31, 16–24.

Garcia, F., Perez, A., 1989. Effects of lactation and litter size on mating, ovulation and embryo viability evaluated by means of laparoscopy in multiparous rabbits. Inf. Tec. Econ. Agraria. 20 (80), 3–10.

Garcia, F., Baselga, M., Pla, M., 1983. Mortalidades embrionaria y fetal en las distintas etapas de la gestacion en el conejo de carne. An. Inst. Nac. Invest. Agrar. 18, 11–27.

Gondret, F., Fortun-Lamothe, L., Bonneau, M., 1997. Effects of simultaneous pregnancy and lactation in rabbit does on postnatal growth and muscular characteristics of the young. Reprod. Nutr. Dev. 37, 163–172.

Harned, M.A., Casida, L.E., 1969. Some post partum reproductive phenomena in the domestic rabbit. J. Anim. Sci. 28, 785–788.

Hodgen, G.D., Itskovitz, J., 1988. Recognition and maintenance of pregnancy. In: Knobil, E., Neill, J. et al. (Eds.), The Physiology of Reproduction, Raven Press, New York, Ch. 48.

I'Anson, H., Foster, D.L., Foxcroft, G.R., Booth, P.J., 1992. Nutrition and reproduction. Oxford Rev. Reprod. Biol. 8, 239–311.

Johnson, R.L., Gilbert, M., Meschia, G., Battaglia, F.C., 1985. Cardiac output distribution and uteroplacental blood flow in the pregnant rabbit: a comparative study. Am. J. Obstet. Gynecol. 151, 682–686.

Jones, C.S., Parker, D.S., 1981. The metabolism of glucose, acetate, and palmitate in the lactating rabbit. Comp. Biochem. Physiol. 69B, 837–842.

Jones, C.S., Rolph, T.P., 1985. Metabolism during fetal life: a functional assesment of metabolic development. Physiol. Rev. 65 (2), 357–430.

Lamb, I.C., Partridge, G.G., Fuller, M.F., Racey, P.A., 1988. Fertility on the early post partum, lactating rabbit. Theriogenology 30, 75–82.

Lamb, I.C., Strachan, W., Henderson, G., Atkinson, T., Lawson, W., Partridge, G.G., Fuller, M.F., Racey, P.A., 1991. Effect of reducing the remating interval after parturition on the fertility and plasma concentrations of luteinizing hormone, prolactin, oestradiol-17β and progesterone in lactating domestic rabbits. J. Reprod. Fertil. 92, 281–289.

Lebas, F., 1969. Alimentation lactée et croissance pondérale du lapin avant sevrage. Ann. Zootech. 18, 197–208.

Lebas, F., 1972. Effet de la simultanéité de la lactation et de la gestation sur les performances laitières chez la lapine. Ann. Zoot. 21, 129–131.

Lebas, F., 1987. Influence de la taille de la portée et de la production laitière sur la quantité d'aliment ingérée par la lapine allaitante. Reprod. Nutr. Dev. 27, 207–208.

Lebas, F., 1973. Variations des reserves corporelles de la lapine au cours d'un cycle de reproduction. J. Rech. Avicole Cunicole. Dijon, France. Comm. III, 3.

Lederman, S.A., Rosso, P., 1980. Effects of food restriction on fetal and placental growth and maternal body composition. Growth 44, 77–88.

Lin, K.C., Okamura, H., Mori, T., 1987. Inhibition of human chorionic gonadotropin. Induced ovulation and steroidogenesis by short term hyperprolactinemia in female rabbits. Endocrinol. Japon 34, 675–683.

McNeilly, A.S., Friesen, H.G., 1978. Prolactin during pregnancy and lactation in the rabbit. Endocrinology 102, 1548–1558.

Niswander, G.D., Nett, T.M., 1988. The corpus luteum and its control. In: Knobil, E., Neill, J. (Eds.), The Physiology of Reproduction, Raven Press, New York, Ch. 13.

Parigi-Bini, R., Xiccato, G., Cinetto, M., 1990. Energy and protein retention and partition in rabbit does during the first pregnancy. Cuni-Science 6, 19–30.

Parigi-Bini, R., Xiccato, G., Cinetto, M., 1990. Répartition de l'énergie alimentaire chex la lapine non gestante pendant la première lactation. 5th J. Rech. Cunicole France, Paris 12–13 December, Commun. 47.

Parigi-Bini, R., Xiccato, G., Cinetto, M., DalleZotte, A., 1991. Energy and protein utilization and partition in rabbit does concurrently pregnant and lactating. Anim. Prod. 55, 153–162.

Parigi-Bini, R., Xiccato, G., Cinetto, M., Dalle Zotte, A., 1992. Energy and protein utilization and partition in rabbit does concurrently pregnant and lactating. Anim. Prod. 55, 153–162.

Partridge, G.G., Allan, S.J., Findlay, M., Corrigall, W., 1984. The effects of reducing the remating interval after parturition on the reproductive performance of the commercial doe rabbit. Anim. Prod. 39, 465.

Partridge, G.G., Lobley, G.E., Fordyce, R.A., 1986. Energy and nitrogen metabolism of rabbits during pregnancy, lactation, and concurrent pregnancy and lactation. Br. J. Nutr. 56, 199–207.

Quesnel, H., Prunier, A., 1995. Endocrine bases of lactational anoestrus in the sow. Reprod. Nutr. Dev. 35, 395–414.

Rabbee, M.A., McCoshen, J.A., 1986. Epithelial regulation of prolactin effect on amniotic permeability. Am. J. Obstet. Gynecol. 154, 130–134.

Rosso, P., Kava, R., 1980. Effects of food restriction on cardiac output and blood flow to the uterus and placenta in the pregnant rat. J. Nutr. 110, 2350–2354.

Sadurskis, A., Sohlstrom, A., Kabir, N., Forsum, E., 1991. Energy restriction and the partitioning of energy among the costs of reproduction in rats in relation to growth of the progeny. J. Nutr. 121, 1798–1810.

SAS Institute, 1990. SAS Statistical Analysis System. SAS User's Guide: Statistics, SAS Institute, Cary, NC.

Sawyer, H.R., Moeller, C.L., Kowzlowski, G.P., 1986. Immunocytochemical localization of neurophysin and oxytocin in ovine corpora lutea. Biol. Reprod. 34, 543–548.

Stephenson, T.J., Stammers, J.P., Hull, D., 1990. Maternal to fetal transfer of free fatty acids in the in situ perfused rabbit placenta. J. Dev. Physiol. 13, 117–123.

Symonds, H.W., Prime, G., 1989. The influence of volume of food intake by gilts on blood flow in the portal vein and clearance of progesterone from plasma. Anim. Prod. 48, 620–621.

Theau-Clément, M., Roustan, A., 1992. A study on relationships between receptivity and lactation in the doe, and their influence on reproductive performance. In: Proceedings of the 5th World Rabbit Congress, WRSA. Corvallis, pp. 412–421.

Thibier, M., Saumande, J., 1975. Oestradiol-17β, progesterone and 17α-hydroxyprogesterone concentrations in jugular venous plasma in cows prior to and during oestrus. J. Steroid Biochem. 6, 1433–1438.

Torrès, S., Gerard, M., Thibault, C., 1977. Fertility factors in lactating rabbits mated 24 hours and 25 days after parturition. Ann. Biol. Anim. Bioch. Biophys. 17 (1), 63–69.

Young, K.H., Bazer, F.W., 1987. Development of a homologous radioreceptor assay (RRA) for porcine prolactin (pPRL). Biol. Reprod. 36, 74, (abstract).

Young, M., Widdowson, E.M., 1975. The influence of diets deficient in energy, or in protein, on conceptus weight, and the placental transfer of a non-metabolisable amino acid in the guinea pig. Biol. Neonate 27, 184–191.

Xiccato, G., Parigi-Bini, R., DalleZotte, A., Carazzolo, A., Cossu, M.E., 1995. Effect of dietary energy level, addition of fat and physiological state on performance and energy balance of lactating and pregnant rabbit does. Anim. Sci. 61, 387–398.

Zarrow, M.X., Denenberg, V.H., Anderson, C.O., 1965. Rabbit: frequency of suckling in the pup. Science NY 150, 1835–1836.