

## Effects of body composition variations on the duration of the *postpartum* anovulatory period in milked ewes submitted to two different photoperiods

F Bocquier<sup>1</sup>, G Kann<sup>2</sup>, J Thimonier<sup>3</sup>

<sup>1</sup> INRA-Theix, Laboratoire Sous-Nutrition des Ruminants, F63122 Saint-Genès-Champagnelle;

<sup>2</sup> INRA UR Endocrinologie du Placenta et Périnatalité, F78352 Jouy-en-Josas Cedex;

<sup>3</sup> ENSA-INRA, Chaire de Zootechnie, Place Viala, F34060 Montpellier Cedex 1, France

(Received 3 March 1993; accepted 6 July 1993)

**Summary** — Forty-four dairy ewes were placed at lambing under long ( $n = 22$ ) or short ( $n = 22$ ) artificial photoperiods. In each photoperiodic treatment 2 diets (high or low) were offered to 11 grouped ewes. The duration of the anovulatory period (DAP, in d) was estimated by bi-weekly blood progesterone measurements. Within 2 months, ewes exposed to the long photoperiod had no resumption of ovulation, while most of the ewes (18/22) exposed to the short photoperiod ovulated. *In vivo* measurements of body composition ( $D_2O$ ) showed that the shortest DAP (ie 36 d) was observed for ewes which gained 1 kg body lipids during the first month *postpartum*. Above and below this threshold, DAP increased. Other relationships between DAP and ewe body composition variations (dynamic) and/or total chemical body mass (static) were determined. In dairy ewes, it seems that the dynamic aspects are more closely related to DAP than the static aspects. Results were compared to the available literature on anestrus in dairy cows.

***postpartum* / lactation / body composition / dairy sheep**

**Résumé** — Effets des variations de composition corporelle sur la durée de la période anovulatoire *post-partum* de brebis laitières placées en photopériodes artificielles. Quarante-quatre brebis laitières ont été soumises à 2 traitements photopériodiques constants dès la mise bas : jours longs (15 h 30L : 8 h 30D) ou courts (8 h 30L : 15 h 30D). Dans chaque traitement photopériodique, les brebis ont été divisées en 2 lots de 11 brebis recevant un régime alimentaire soit Haut, soit Bas. La durée de la période anovulatoire (DAP, jours) a été estimée par dosages bi-hebdomadaires de progestérone plasmatique. Les brebis exposées à la photopériode longue n'ont pas eu d'ovulation, alors que celles qui étaient en jours courts ont pratiquement toutes (18/22) ovulé dans les 2 mois *post-partum*. Les estimations *in vivo* de la composition corporelle des brebis ( $D_2O$ ) ont permis de montrer que la plus courte durée anovulatoire (DAP = 36 jours) est observée chez des brebis qui ont gagné 1 kg de lipides corporels au cours du premier mois. De part et d'autre de ce seuil de 1 kg de lipides, les DAP sont accrues. D'autres relations (tableaux II et III) ont été établies entre les DAP et

*les variations de composition corporelle (composantes dynamiques) et/ou les quantités de constituants chimiques corporels (composantes statiques). Il semble que, contrairement à la brebis tarie, la reprise de l'activité ovulatoire chez la brebis laitière soit davantage sous la dépendance de la composante dynamique que de la composante statique. Ces résultats sont comparés à ceux obtenus chez la vache laitière.*

## **post-partum / lactation / composition corporelle / brebis laitière**

### **INTRODUCTION**

The duration of the anovulatory *postpartum* period (ppp) in the lactating ewe is related to a number of factors including milking or suckling (Mauléon and Dauzier, 1965; Kann and Martinet, 1975; Kann *et al*, 1977), season (Hafez, 1952; Mauléon and Dauzier, 1965) or photoperiod (Orta-vant *et al*, 1988) and body condition (Ducker and Boyd, 1977; Wright *et al*, 1990). Coop (1962) introduced the terms "static" and "dynamic" to distinguish between effects of absolute difference in body weight and rapid increase in body condition in the dry ewe. Body reserve assessment was often based on body condition scores (Jefferies, 1961) or body weight but was rarely assessed by more accurate methods such as the water-labelled dilution technique. The aim of the present study was to analyze the respective influence of absolute quantities of chemical body components (static) and their changes (dynamic) in lactating dairy ewes under controlled photoperiods.

### **MATERIALS AND METHODS**

Two groups of 22 Préalpes ewes, previously exposed to a natural photoperiod of 13 h light (L): 11 h dark (D), were progressively changed over to a 2-wk period of either a constant long photoperiod (long days: 15 h 30L: 8 h 30D) or a short photoperiod (short days: 8 h 30L: 15 h 30D) so that light treatments (500 lux) were established

at lambing and kept constant during lactation. After lambing, ewes were allocated to either high or low diets within photoperiodic treatments (11 ewes per subgroup). Ewes were group-fed and diets were given in limited amounts to both groups. Diets differed both in mean energy and mean protein supply; respectively 19.04 and 10.48 MJ metabolizable energy and 370 and 237 g crude protein/ewe.

Ewes were milked twice daily and weighed and scored weekly. *In vivo* body composition was estimated on d 3 and 28 after lambing using the deuterium oxide (D<sub>2</sub>O) dilution technique (Bocquier and Thériez, 1984). Body lipid (BLIP), body protein (BPROT) and body energy (BENR) were calculated using specific equations for lactating ewes.

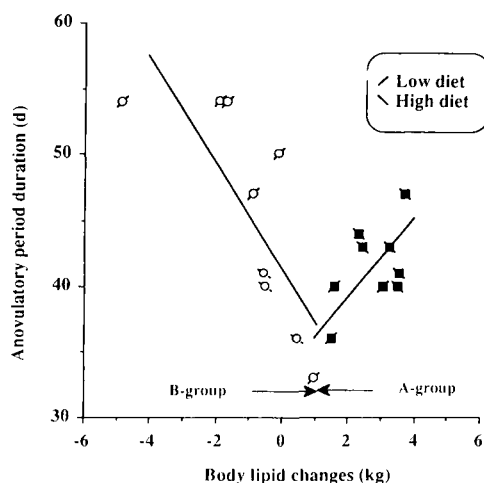
Blood samples collected twice weekly for 60 d after lambing were analyzed for progesterone (P4) by radioimmunoassay as described by Terqui and Thimonier (1974). Ewes were assumed to be cyclic when P4 reached a level of 2 ng/ml for 2 consecutive plasma samples (Thimonier, 1978). Short ovulatory cycles are generally not detected by this procedure. Pre-feeding blood samples were collected weekly and analyzed for non-esterified fatty acids by an automated method (NEFA-C test Wako) as described by Chilliard *et al* (1984). Data were analyzed by the GLM procedure (SAS, 1985). Non-reproductive sections of the results have already been published (Bocquier *et al*, 1990).

### **RESULTS**

None of the 22 ewes submitted to the long days treatment exhibited normal ovulatory activity during the first 60 d *post-partum*. Among the 22 ewes under the short days treatment, in 18 ewes there was a resump-

tion of ovarian activity: 8 in the high-fed and 10 in the low-fed group. In these females the anovulatory period ranged from 33 to 54 d *postpartum*. Because ewes were group-fed, it was not possible to calculate the individual energy balance (EB), although individual milk recordings and body composition (and variations) were available. Simple regression of lipid variation (BLIPc) to duration of anovulatory period (DAP) led to a regression coefficient ( $R^2$ ) of 0.38. Because of a curvilinear response, there was a better fit to the data ( $R^2 = 0.71$ ) with a polynomial adjustment. However, as the biological significance of the polynomial terms was difficult to assess, we divided the data set into 2 groups, one above (group A) and one below (group B) the minimum value for this function (+ 1.07 kg BLIPc). It appeared that independently of the experimental diet, with simple regressions there were good relationships between the duration of anovulatory period and body lipid changes if ewes above or below the threshold of + 1 kg BLIP changes were considered separately (fig 1). Ewes from these 2 groups had a similar initial body weight (57 vs 59 kg) with no difference in mean duration of *postpartum* anovulation: 45 vs 41 d (table I). Ewes above

(A,  $n = 9$ ) the threshold of + 1 kg lipid change had a mean lipid gain of 2.76 kg, while ewes below (B) had a mean loss of 1.02 kg body lipids. In fact, in the B group some ewes had a moderate body lipid gain ( $n = 2$ , fig 1). Body weight, body protein and energy variations showed similar patterns in the 2 groups (table I). The body



**Fig 1.** Effect of body lipid changes between d 3 and 28 *postpartum* on anovulatory period duration in dairy ewes under a constant short photoperiod (8.30 L : 15.30 D).

**Table I.** Mean characteristics of dairy ewes under short photoperiod: duration of the anovulatory period (DAP, d) and body component changes (kg or MJ).

	Below T (n = 9) (B group)			Above T (n = 9) (B group)		
	Mean	Min	Max	Mean	Min	Max
DAP (d)	45.33	33	54	41.33	36	47
Body weight (d 3)	56.72	53	66	59.0	54	65
BW changes (28-3)	-4.16	-8.00	-1.00	+1.44	-3.0	+5.0
BLIP changes (28-3)	-1.02	-4.88	+0.99	+2.76	+1.49	+3.67
BPROT changes (28-3)	-0.38	-0.72	-0.07	-0.05	-0.56	+0.29
BENR changes (28-3)	-13.1	-50.1	+6.2	+24.3	+11.3	+33.4

Ewes were treated in 2 groups (A or B) according to a +1 kg body lipid change threshold (T).

composition (and variations) in the 4 ewes that did not exhibit ovulation were within the range of observed values in ewes that ovulated (18/22): BLIP (3 d) = 9.1 kg, BPROT = 6.6 kg and BLIPc = 1.6 kg, BPROTc = -0.2 kg.

Within these 2 groups, subsets of independent variables that could be correlated to the duration of anovulatory period (DAP, in d) were tested. Body weight (or body constituents) at d 3 and 28 pp were considered to be representative of static aspects, while dynamic aspects were expressed by their variations (table II; upper and lower parts). The best simple correlation with DAP was obtained with body energy changes (BENRc) in both B and A groups. This was particularly true for body lipid changes (BLIPc), but better predicted by body weight changes (BWc) than by body protein (BPROTc). Among static variables, the best correlations were always

found with initial values (d 3) rather than with final values (d 28). It is of interest to note that rank order of correlation coefficients was practically the same between the 2 groups (A and B), even though the differences were not always significant due to the small number of animals.

Mean standard milk yield for the first month of lactation (SMY, l: Bocquier *et al*, 1991), significantly increased DAP in overfed ewes ( $r = +0.73$ ,  $P < 0.02$ ) but not in ewes below +1 kg change in lipid content ( $r = -0.40$ , NS).

When using static and dynamic variables of body composition and standard milk yield simultaneously, the level of milk production delayed the recovery of ovulatory activity, especially when taking into account variations in body lipid or energy levels (table III). The best equation to predict DAP without taking milk yield into account was found with BLIPc (dynamic) and

**Table II.** Simple correlations between duration of anovulatory period (dap, d) and absolute values of body components (static effect), or their variations (dynamic effect).

	Below T (n = 9) (B group)	Rank	Above T (n = 9) (A group)	Rank
<b>Static effect</b>				
Energy (d 3)	+0.507	3	-0.339	3
Energy (d 28)	-0.212	7	-0.187	5
Lipid (d 3)	+0.475	4	-0.326	4
Lipid (d 28)	-0.257	6	-0.187	5
Protein (d 3)	+0.526	2	-0.472	1
Protein (d 28)	+0.331	5	-0.148	8
Body weight (d 3)	+0.569	1	-0.435	2
Body weight (d 28)	+0.154	8	-0.168	6
<b>Dynamic effect</b>				
BENR changes (28-3)	-0.768	1	+0.529	1
BLIP changes (28-3)	0.759	2	+0.519	2
BW changes (28-3)	-0.704	3	+0.377	3
BPROT changes (28-3)	-0.517	4	+0.309	4

Rank order of *R* within groups of ewes below or above the threshold (T) or +1 kg lipid change between d 3 and d 28.

**Table III.** Multiple regressions between duration of anovulatory period (DAP, d) and changes in body components (BLIPc, kg), body protein (BPROT d 3, kg) or standard milk yield (SMY, l/d), within groups of ewes below or above the threshold (T) of + 1 kg lipid change between d 3 and d 28.

Groups	Variables			Constant term	CV (%)	R <sup>2</sup>
	X1	X2	X3			
Below T	-3.23 BLIPc*	+7.95 BPROT3 ns	-	-9.9	11.5	0.701*
Above T	+2.09 BLIPc <sup>a</sup>	-5.15 BPROT3 <sup>a</sup>	-	+70.3	5.8	0.541 <sup>a</sup>
Below T	-3.86 BLIPc**	-14.58 SMY*	-	+52.8	9.6	0.793**
Above T	+1.45 BLIPc ns	+4.97 SMY**	-	+33.3	4.8	0.689*
Below T	-0.40 BNRc <sup>a</sup>	-14.74 SMY**	-	+51.6	9.1	0.813**
Above T	+0.13 BNRc <sup>a</sup>	+4.80 SMY**	-	+33.3	5.0	0.654*
Below T	-3.43 BLIPc***	-14.71 SMY*	+8.07 BPROT3*	+0.6	6.4	0.922**
Above T	+1.63 BLIPc <sup>a</sup>	+3.96 SMY <sup>a</sup>	-2.65 BPROT3 ns	+51.5	4.8	0.743 <sup>a</sup>
Below T	-0.36 BNRc**	-14.81 SMY**	+7.38 BPROT3*	+4.0	6.6	0.919**
Above T	+0.16 BNRc ns	+3.50 SMY ns	-3.13 BPROT3 ns	+55.7	4.9	0.723 <sup>a</sup>

Statistical significance : <sup>a</sup> P < 0.1; \* P < 0.05; \*\* P < 0.01.

BPROT (3 d) (static) both for B ( $r = 0.839$ ) and A ( $r = 0.609$ ) groups, although body protein only slightly improved the fit of regressions (table III).

None of the weekly measured NEFA concentrations or mean or cumulative concentrations between d 3 and 28 pp were significantly correlated with DAP ( $\rho > 0.50$ ).

## DISCUSSION

The effect of long daylight on the absence of ovulation in the ewe is classic (Ortavant *et al*, 1988). The observed duration of the *postpartum* anovulatory period under the short photoperiod (33 to 54 d pp) in this experiment is in general agreement with previous measurements on well-fed ewes of the same breed: 31 d for non-suckling and 37 d for suckling ewes (Schirar *et al*, 1989).

In dry non-dairy ewes, spontaneous estrous activity has been described to be unrelated to the current plane of nutrition, but subjected to delayed (3–6 months) effects of previous nutrition (Oldham *et al*, 1990). In contrast, there has been an emphasis on the effect of plane of nutrition on ovulation or ovulation rate in the dry ewe. It is generally accepted that an increase in pre-mating liveweight is likely to be associated with an increase in lamb production (Gunn, 1983); this was partly related to the actual body mass (static) and to the extent of liveweight change (dynamic). Both Gunn and Maxwell (1989) and Smith and Stewart (1990) observed in the dry ewe that body weight (BW) better explained (resp  $R^2 = 0.45$  and  $R^2 = 0.42$ ) ovulation and lamb production (ovulation rate or ova loss) than body weight changes (BWc, resp  $R^2 = 0.13$  and  $R^2 = 0.18$ ). This clearly indicates that static effects of body weight are more effective on ovulation than dynamic or flushing effects which are less

significant and less repeatable (Smith and Stewart, 1990). The lack of repeatability of body weight change effects might be due to the fact that changes in food supply, often adopted in experimental designs, first affect gut content rather than body composition. Body fat mass is often supposed to be involved in reproduction without direct evidence, because body condition scoring is not an accurate method for fat determination (Dunn and Moss, 1992).

With *in vivo* measurement of body composition, a clear biphasic relationship was found between body lipid changes (BLIPc) between d 3 and 28 and DAP (fig 1); the minimum value of DAP (33 d) was observed when ewes gained +1 kg BLIPc. Any other variation in BLIPc resulted in a lengthening of DAP either above (A,  $n = 9$ ) or below (B,  $n = 9$ ) this threshold.

The rank order of correlations between DAP and chemical body components was the same for both groups of ewes (A and B). For dynamic variables the first was BENERc, and the second BLIPc followed by BWc and BPROTc (table II). For static variables the rank order of correlations was quite different from that observed with dynamic variables, *ie* first BW (3 d) or BPROT (3 d), followed by BENR (3 d) and BLIP (3 d) (table III). This seems to indicate that high body energy losses or large gains have a detrimental effect on reproduction, *ie* that DAP rises. In lactating dairy ewes, the resumption of ovulatory activity seems to be more dependent on dynamic aspects than on static aspects of body composition. The reverse has been observed for reproduction in dry ewes (Gunn and Maxwell, 1989; Smith and Stewart, 1990). Unfortunately there are no results in the literature for dairy ewes except those of Kassem *et al* (1989) on fat-tailed dairy ewes with induced estrus without control of food intake.

In well-fed ewes (group A), higher milk yield significantly delayed ( $P < 0.024$ ) the

occurrence of ovulatory activity ( $r = +0.734$ ). In underfed ewes (group B), no significant correlation was found between DAP and milk yield. This is in agreement with previous observations by Butler *et al* (1981) who did not observe a significant correlation between milk yield and interval to first ovulation in dairy cows ( $r = 0.30$ ). They also found that body weight changes were poorly related to DAP (Butler and Smith, 1989). Calculated EB was the most sensitive variable in predicting impact of both milk yield and energy intake on reproductive recovery ( $r = -0.60$ ; Butler *et al*, 1981). In our experiment, *in vivo* measurement of body energy changes revealed a strong relationship with DAP (in the B group;  $r = -0.77$ , table I). These results are consistent with the observations of Canfield and Butler (1991), with a significant correlation between cumulative (or average) EB, but not with daily EB near ovulation and anestrus duration (Butler *et al*, 1981). This agrees with the fact that the NEFA concentration (instant energy balance index) was not significantly correlated with the interval to first ovulation, either in dairy cattle (Butler *et al*, 1981) or in dairy ewes (present study). In our experiment, most ewes ovulated while still probably in negative energy balance (group B).

In dairy ewes, the dynamic effect of body component changes is far closer to the EB calculated with dairy cows. The better fit of the relationships observed in this experiment may be due to the direct measurement of body energy (Bocquier et Th  riez, 1984; Vermorel *et al*, 1985) or body composition changes rather than calculated balances. It is of interest to note that body lipids, which are clearly energy reserves, constitute the best dynamic variable and that body proteins, which are not truly mobilizable, are the best static variable.

The adverse effect of rapid body reserve recovery on resumption of ovulation has not been described in cows, probably because it is an infrequent situation in both dairy and beef cattle. It has, however, been shown in the dry ewe that excess body condition score may impair reproduction (Gunn, 1983; Gunn and Maxwell, 1989).

## CONCLUSION

This experiment confirmed that in milked ewes the first limiting factor to resumption of normal ovulation is photoperiod. Under favourable daylength (short photoperiod), variations in chemical body components (*ie* lipids, protein) or body energy better explained anovulatory duration than body weight changes. The optimum lipid variation, which led to the earliest recovery of ovarian activity, seemed to be close to + 1 kg body lipids associated with a high body protein mass at the beginning of the first month of lactation.

It should in fact be pointed out that with the experimental design used in the present experiment, relationships between body composition and anovulatory *postpartum* duration are only correlational; causal relationships still have to be confirmed, particularly because 4 ewes did not exhibit ovulation during the experimental period. It is possible that some of the circulating metabolites (glucose) or metabolic hormones (insulin, growth hormone, prolactin) may also be involved in the regulation of resumption of ovarian activity. However, it seems that for future research on *postpartum* anovulation in sheep, knowledge of body composition variation should greatly help the understanding of this phenomenon.

## ACKNOWLEDGMENTS

Appreciation is expressed to P Lebecque and M Tourret for technical assistance and to B Mirman and staff for animal care.

## REFERENCES

- Bocquier F, Thériez M (1984) Prediction of ewe body composition at different physiological states. *In: In vivo Measurements of Body Composition in Meat Animals* (Lister D, ed) Elsevier, London, 152-156
- Bocquier F, Kann G, Thériez M (1990) Relationships between secretory patterns of growth hormone, prolactin and body reserves and milk yield in dairy ewes under different photoperiod and feeding conditions. *Anim Prod* 51, 115-125
- Bocquier F, Barillet F, Guillouet P (1991) Prediction of gross energy content of ewe's milks from different chemical analysis: a proposal of an energy corrected milk for dairy ewes. *In: Proc 12th Symp Energy Metabolism in Farm Animals*. Kartause Ittingen, Switzerland, 1-7 Sept 1991, 345-348
- Butler WR, Everett RW, Coppock CE (1981) The relationships between energy balance, milk production and ovulation in *postpartum* Holstein cows. *J Anim Sci* 53, 742-748
- Butler WR, Smith WR (1989) Interrelationships between energy balance and *postpartum* reproductive function in dairy cattle. *J Dairy Sci* 72, 767-783
- Canfield RW, Butler WR (1991) Energy balance, first ovulation and the effects of naloxone on LH secretion in early *postpartum* dairy cows. *J Anim Sci* 69, 740-746
- Chilliard Y, Bauchart D, Barnouin J (1984) Determination of plasma non-esterified fatty acids in herbivores and man: a comparison of values obtained by manual or automatic chromatographic, titrimetric, colorimetric and enzymatic methods. *Reprod Nutr Dev* 24, 469-482
- Coop IE (1962) Liveweight-reproductivity relationships in sheep. *NZ J Agric Res* 5, 249-264
- Ducker MJ, Boyd JS (1977) The effect of body size and body condition on the ovulation rate of ewes. *Anim Prod* 24, 377-385
- Dunn TG, Moss GE (1992) Effects of nutrient deficiencies and excesses on reproductive efficiency of livestock. *J Anim Sci* 70, 1580-1593
- Gunn RG (1983) The influence of nutrition on the reproductive performances of ewes. *In: Sheep Production* (Haresign W, ed) Butterworths, London, 99-110
- Gunn RG, Maxwell TJ (1989) A note on the effect of direction of liveweight change about the time of mating on reproductive performances of Greyface ewes. *Anim Prod* 48, 471-474
- Hafez ESE (1952) Studies on the breeding season and reproduction of the ewe. I. The breeding season in different environments. *J Agric Sci (Camb)* 42, 189-265
- Jefferies BC (1961) Body condition scoring and its use in management. *Tasmanian J Agric* 32, 19-28
- Kann G, Martinet J (1975) Prolactin levels and duration of *postpartum* anestrus in lactating ewes. *Nature (Lond)* 357, 63-71
- Kann G, Martinet J, Schirar A (1977) Hypothalamo-pituitary control during lactation in sheep. *In: Control of Ovulation* (Crighton DB, Haynes NB, Foxcroft GR, Lamming GE, eds) Butterworths, London, 319-333
- Kassem R, Owen JB, Fadel I (1989) Rebreeding activity in milking Awassi ewes under semi-arid conditions. *Anim Prod* 49, 89-93
- Mauléon P, Dautzier L (1965) Variations de la durée d'anoestrus de lactation chez les brebis de race Ile-de-France. *Ann Biol Anim Biochim Biophys* 5, 131-143
- Oldham CM, Lindsay DR, Martin GB (1990) Effects of seasonal variation of liveweight on the breeding activity of Merino ewes. *In: Reproductive Physiology of Merino Sheep. Concepts and Consequences* (Oldham CM, Martin GB, Purvis IW, eds) School of Agriculture, Univ Western Australia, 41-58
- Ortavant R, Bocquier F, Pelletier J, Thimonier J, Volland-Nail P (1988) Seasonality of reproduction in sheep and its control by photoperiod. *Austr J Biol Sci* 41, 69-85
- SAS (1985) *User's Guide: Statistics*. SAS Institute Inc, Cary, NC
- Schirar A, Cognié Y, Louault F, Poulin N, Levasseur MC, Martinet J (1989) Resumption of oestrus behaviour and cyclic ovarian activity



- in suckling and non-suckling ewes. *J Reprod Fertil* 87, 789-794
- Smith JF, Stewart RD (1990) Effects of nutrition on the ovulation rate of ewes. In: *Reproductive Physiology of Merino Sheep. Concepts and Consequences* (Oldham CM, Martin GB, Purvis IW, eds) School of Agriculture, Univ Western Australia, 85-101
- Terqui M, Thimonier J (1974) Nouvelle méthode radioimmunologique rapide pour l'estimation du niveau de progestérone plasmatique. Application pour le diagnostic précoce de gestation chez la brebis et la chèvre. *CR Acad Sci Paris Ser D* 279, 1109-112
- Thimonier J (1978) L'activité ovarienne chez les bovins. Moyens d'étude et facteurs de variations. *Ann Méd Vét* 122, 81-92
- Vermorel M, Bocquier F, Vernet J, Brelurut A (1985) Mobilization and reconstitution of body reserves in dairy ewes studied by indirect calorimetry and D20 dilution technique. In: *Energy Metabolism in Farm Animals* (Moe PW, Tyrrell HF, Reynold PJ, eds) Rowman and Littlefield, Totowa, NJ, 314-317
- Wright PJ, Geytenbeek PE, Clarke IJ (1990) The influence of nutrient status of *post-partum* ewes on ovarian cyclicity and on oestrus and anovulatory responses to ram introduction. *Anim Reprod Sci* 23, 293-303