Reprod Nutr Dev (1997) 37, 129-140

Original article

Absence of response to oestrus induction and synchronization treatment is related to lipid mobilization in suckled beef cows

B Grimard [1,2*], P Humblot [2], JP Mialot [1], N Jeanguyot [2], D Sauvant [3], M Thibier [2]

[1] *Laboratoire d'épidémiologie et de gestion de la santé animale, Ecole vétérinaire d'Alfort, 7, avenue du Général-de-Gaulle, 94704 Maisons-Alfort cedex ;*
[2] *Union nationale des coopératives d'élevage et d'insémination artificielle, Services techniques, 13, rue Jouet, BP 65, 94703 Maisons-Alfort cedex ;*
[3] *Station de nutrition, Institut national agronomique Paris-Grignon, 16, rue Claude-Bernard, 75231 Paris cedex, France*

(Received 3 October 1995; accepted 5 November 1996)

Summary — Energy status, follicular growth, oestradiol and LH secretion were investigated in 17 suckled Charolais cows synchronised 59.0 ± 3.6 days after calving with a 10 day ear implant containing 3 mg of Norgestomet. The cows received 3 mg of Norgestomet and 5 mg of oestradiol valerate by IM injection at implant insertion (day 0) and 600 IU PMSG at implant removal (day 10). They were artificially inseminated (AI) 48 and 72 h after implant removal. Energy status was assessed by measuring weekly plasma concentrations of non-esterified fatty acids (NEFA), β hydroxy-butyrate (BHB), glucose and insulin 7 weeks before AI. Progesterone plasma concentrations were measured during the same period to assess the presence of a functional corpus luteum. Follicular growth was followed daily by ultrasonography from day –3 to day 13. Oestradiol secretion was measured on day –3, day 6 and day 10 from five hourly samples. Oestradiol and LH plasma concentrations were measured hourly from 29 to 48 h after implant removal for seven cows. Cows were checked for pregnancy by ultrasonography 45 days after AI. Pregnant cows (P) were compared with non-pregnant cows (NP) for energy status, follicular growth, and oestradiol secretion by split-plot ANOVA. Two cows (11.8%) were cyclic before treatment, seven ovulated after treatment (41.2%) and five were found pregnant 45 days after AI (29.4%). There was no difference in body condition score and body weight between P and NP cows on day 0 (2.5 ± 0.2 and 685 ± 24 kg vs 2.5 ± 0.1 and 670 ± 13 kg; $P > 0.05$). Mean plasma NEFA concentrations before treatment were significantly lower in P than in NP cows (218 ± 29 μeq/L vs 279 ± 18 μeq/L; $P < 0.05$). No significant differences between P and NP cows were found for BHB, glucose and insulin concentrations. P cows presented more medium sized follicles

* Correspondence and reprints.
Tel: (33) 01 43 96 71 48; fax: (33) 01 43 96 71 50; e-mail: grimard@vet-alfort.fr

(5 mm ≤ diameter < 10 mm) than NP females during the period of observation (2.65 ± 0.19 vs 2.50 ± 0.12; $P = 0.05$). Plasma oestradiol concentrations were not different between P and NP cows on day −3 (8.4 ± 0.7 pg/mL vs 7.7 ± 0.4 pg/mL, $P > 0.05$), day 6 (10.4 ± 0.6 pg/mL vs 9.8 ± 0.4 pg/mL, $P > 0.05$) but were higher in P than in NP cows on day 10 (10.9 ± 0,6 pg/mL vs 7.8 ± 0.4 pg/mL; $P < 0.05$). After implant removal, oestradiol secretion only increased in P cows and a LH peak occurred whereas no increases in oestradiol (11.0 ± 0.4 pg/mL vs 6.3 ± 0.3 pg/mL, $P < 0.05$) and LH (6.0 ± 0.5 ng/mL vs 1.2 ± 0.5 ng/mL, $P < 0.05$) secretion were observed in NP cows. The conclusion was that follicular growth, oestradiol secretion, ovulation and pregnancy rate after oestrus synchronisation treatment are related to mobilization of energy stores before treatment in suckled beef cows in the same body condition score.

beef cow / anoestrus / oestrus synchronization treatment / lipid mobilization / non-esterified fatty acids

Résumé — L'absence de réponse au traitement d'induction de l'oestrus est associée à la mobilisation des réserves corporelles chez la vache allaitante. Le statut énergétique, la croissance folliculaire, la sécrétion de LH et d'oestradiol ont été suivis chez 17 vaches allaitantes charolaises soumises à un traitement de maîtrise des cycles 59.0 ± 3.6 jours après vêlage combinant un implant de 3 mg de Norgestomet laissé en place 10 jours, 3 mg de Norgestomet et 5 mg de valérate d'oestradiol à la pose (j0) et 600 UI de PMSG au retrait (j10). Les vaches ont été inséminées (IA) 48 et 72 heures après la fin du traitement. Le statut énergétique a été évalué par les concentrations hebdomadaires d'acides gras non estérifiés (AGNE), de β hydroxy-butyrate (BHB) de glucose et d'insuline durant les 7 semaines précédant l'IA. La progestéronémie a été mesurée dans ces échantillons afin de mettre en évidence la présence d'un corps jaune fonctionnel. La croissance folliculaire a été mesurée quotidiennement par échographie de j–3 à j13. Les concentrations plasmatiques d'oestradiol ont été mesurées à j–3, j6 et j10 dans cinq prélèvements réalisés à 1 heure d'intervalle. Les concentrations plasmatiques de LH et d'oestradiol ont été mesurées toutes les heures entre 29 et 48 heures après le retrait de l'implant chez sept vaches. La gestation a été contrôlée par échographie 45 jours après IA. Le statut énergétique, la croissance folliculaire, la sécrétion d'oestradiol et de LH des vaches gestantes après traitement ont été comparés aux mêmes paramètres chez les vaches non gestantes après traitement par split-plot ANOVA. Deux vaches étaient cyclées avant traitement (11,8 %), sept ont ovulé après traitement (41,2 %) et cinq ont été gestantes après première insémination (29,4 %). La note d'état corporel et le poids vif des vaches gestantes n'étaient pas différents de ceux des vaches non gestantes à j0 (2,5 ± 0,2 et 685 ± 24 kg vs 2,5 ± 0,1 et 670 ± 13 kg ; $p > 0,05$). Les concentrations plasmatiques d'AGNE ont été plus faibles avant traitement chez les vaches gestantes que chez les vaches non gestantes (218 ± 29 µeq/L vs 279 ± 18 µeq/L ; $p < 0,05$). Aucune différence significative entre les deux lots n'a été observée pour les concentrations de BHB, de glucose et d'insuline. Les vaches gestantes ont présenté plus de follicules de taille moyenne (5 mm ≤ diamètre < 10 mm) que les vaches non gestantes durant la période d'observation (2,65 ± 0,19 vs 2,50 ± 0,12 ; $p = 0,05$). Les concentrations plasmatiques d'oestradiol ont été identiques dans les deux groupes d'animaux à j–3 (8,4 ± 0,7 pg/mL vs 7,7 ± 0,4 pg/mL ; $p > 0,05$), j6 (10,4 ± 0,6 pg/mL vs 9,8 ± 0,4 pg/mL ; $p > 0,05$) mais ont été plus élevées chez les vaches gestantes que chez les vaches non gestantes à j10 (10,9 ± 0,6 pg/mL vs 7,8 ± 0,4 pg/mL ; $p < 0,05$). Après le retrait de l'implant, une augmentation de la sécrétion d'oestradiol et d'un pic de LH n'ont été observés que chez les vaches gestantes (respectivement 11,0 ± 0,4 pg/mL vs 6,3 ± 0,3 pg/mL, $p < 0,05$; 6,0 ± 0,5 ng/mL vs 1,2 ± 0,5 ng/mL, $p < 0,05$). En conclusion, la croissance folliculaire, la sécrétion d'oestradiol, le taux d'ovulation et le taux de gestation après traitement de maîtrise des cycles sont associés à la mobilisation des réserves corporelles avant traitement chez la vache allaitante.

vache allaitante / anoestrus / traitement de maîtrise des cycles / lipomobilisation / acides gras non estérifiés

INTRODUCTION

Postpartum anoestrus corresponds to the absence of ovarian cycles after parturition. Recovery of ovarian function after calving is preceded by restoration of pituitary sensitivity to GnRH (Webb et al, 1977; Fernandes et al, 1978; Schallenberger et al, 1978), an increase in the frequency and amplitude of LH pulses (Terqui et al, 1982; Nett, 1987; Weesner et al, 1987), re-establishment of the positive feedback of oestrogens (Schallenberger and Prokopp, 1985) and resumption of the growth of dominant follicles (Savio et al, 1990; Murphy et al, 1990).

Suckling delays resumption of oestrus after calving (Williams, 1990) by depressing basal LH concentrations in the peripheral circulation (Webb et al, 1980) as well as the frequency and the amplitude of LH episodic release (Carruthers and Hafs, 1980). Moreover, suckling reduces the pituitary response to GnRH (Foster et al, 1980) and the positive feedback of oestrogens (Radford et al, 1978).

In suckled beef cows, progestogen implant or intra-vaginal devices associated with PMSG are widely used to induce and synchronise oestrus after calving (Chupin, 1977; Aguer, 1981; Odde, 1990). Nevertheless, pregnancy rates (animals pregnant / animals treated) are highly variable and range from 30 to 70% (Grimard and Mialot, 1990; Odde, 1990). Parity, calving conditions, interval from parturition to treatment, body condition score, body weight at calving and at the time of treatment have been shown to influence pregnancy rate at induced oestrus (Chupin et al, 1977; Pelot et al, 1977; Aguer et al, 1981; Deletang, 1983; Walters et al, 1984; Fogwell et al, 1986; Odde, 1990; Grimard et al, 1992a, b; Humblot et al, 1996). Nevertheless the way these factors influence fertility rate after oestrus synchronisation treatment remains unclear. Moreover, high within-body condition score and body weight class variations were found (Grimard et al, 1992b). Dynamic aspects of lipid mobilization might be as important as body weight and body condition score at the time of treatment.

The present study was designed to determine if energy status, follicular growth, oestradiol secretion during treatment and oestradiol and LH secretion after implant removal are related to pregnancy rate at induced oestrus in synchronized suckled beef cows.

MATERIAL AND METHODS

Animals, diets and treatments

Charolais cows (eight primiparous and nine multiparous) which calved between 27th November and 19th January in an experimental herd of Saône-et-Loire were studied. Animals were kept in tethered housing and calves were allowed to suckle their dams twice daily. Diets were composed of grass silage, hay, barley, soya bean meal, minerals and vitamins to provide 100% of the Inra requirements (Petit, 1988). Cow body weight and body condition score (scale from 0 = thin to 5 = fat, Agabriel et al, 1986) were respectively 667 ± 11 kg (mean ± sem) and 2.6 ± 0.1 at calving and 674 ± 11 kg and 2.5 ± 0.2 at implant insertion. Mean calf daily weight gain was 958 ± 28 g/day.

Oestrus synchronisation treatment was applied 59.0 ± 3.6 days after calving. This treatment consisted of an ear implant lasting 10 days containing 3 mg of Norgestomet (Crestar ND, Intervet, France) with an intramuscular injection of 3 mg Norgestomet and 5 mg oestradiol valerate at the time of implant insertion (day 0), followed by an injection of 600 IU pregnant mare serum gonadotropin (Chronogest PMSG ND, Intervet, France) at implant removal (day 10). Cows were artificially inseminated 48 and 72 h after implant removal. Pregnancy was diagnosed by ultrasonography (Pierson and Ginther, 1984b; Mialot et al, 1991) 45 days after insemination (AI).

Energy status

In order to quantify energy status, blood samples were collected weekly for 7 weeks before

AI into heparinized tubes by caudal venipuncture before the morning feed. Plasma was collected after centrifugation (2 500 g for 20 min) and stored at –18 °C until measurement of non-esterified fatty acids (NEFA), β hydroxy-butyrate (BHB), glucose and insulin (Russel and Wright, 1983; Easdon et al, 1985).

Reproductive traits

Progesterone plasma concentrations were measured weekly in the previous samples to verify cyclicity prior to treatment. A plasma progesterone concentration higher than 1.5 ng/mL was considered to indicate the presence of a functional corpus luteum.

Ovaries were examined daily between day –3 and day 13 by ultrasonography using a PIE DATA linear scanner with a 5 mhz transducer (Pierson and Ginther, 1984a; Mialot et al, 1991). Follicles were assigned to three diameter classes according to Murphy et al (1990), ie, small (diameter < 5 mm), medium (5 mm ≤ diameter < 10 mm) and large follicles (diameter ≥ 10 mm). When a large follicle disappeared between two measurements after implant removal, ovulation was considered to have occurred.

Additionally, blood samples were collected hourly for 5 h, 3 days before implant insertion (day – 3), 6 days after implant insertion (day 6), and on the day of implant removal (day 10) to measure 17 β oestradiol concentrations.

Finally, seven cows were fitted with a jugular catheter on day 10 and sampled hourly from 29 to 48 h after implant removal to determine oestradiol and luteinizing hormone (LH) concentrations.

Assays

Plasma concentrations of insulin were measured by radioimmunoassay (RIA Kit INSI-PR, CIS bio international, France). Inter- and intra-assay coefficients of variation were respectively 8.7 and 9% and the sensitivity was 3 μIU/mL. Plasma concentrations of glucose and NEFA were measured by photometric methods (Kits PM 7576860 Dart glucose, Coulter diagnostics, USA, inter-assay CV = 0.6%, intra-assay CV = 0.8% between 0.2 and 1.2 g/L; NEFA C 46551, Wako Chemicals, Germany, inter-assay CV = 4.5%, intra-assay CV = 0.3% between 20 and 1000 μeq/L). Plasma BHB concentrations were measured by a method adapted from Barnouin et al (1986), (inter-assay CV = 3.7%, intra-assay CV = 1% between 20 and 600 mg/L).

Progesterone was measured by an assay procedure modified from Thibier and Saumande (1975) and previously described (Humblot et al, 1990). Inter- and intra-assay coefficients of variation were 8 and 2% respectively. The sensitivity of the assay was 50 pg/mL.

Blood concentrations of 17 β oestradiol were measured from single aliquots of plasma (1 mL) after extraction with 4 mL ether (Prolabo, Paris, France). The radioimmunoassay was subsequently performed with a polyclonal antibody (Ref 2054-S4, FRH, Fresnes, France) displaying less than 5% cross-reactivity with major oestrogens susceptible to interfere during the assay (oestrone 4%, oestriol 2.5%). This antibody was used at a final dilution of 1:30 000. Blank ether was found most often equal to 0 and was always lower than 2 pg. It was substracted systematically from individual plasmatic concentrations. Inter- and intra-assay coefficients of variations were respectively 8.8 and 6.7%. Sensitivity of the assay was 2 pg/tube.

LH was measured by a double antibody radioimmunoassay previously described by Thibier (1975) and Abdel Malak and Thibier (1982). Briefly, a highly purified ovine LH fraction (LER-106-C2) iodinated with ^{125}I (Amersham, France) was used as the labelled antigen. The bLH antiserum (Schams and Karg, 1969) was used at a final dilution of 1:200 000 and displayed low cross-reactivity with αTSH (< 4%), βFSH (0.3%) and αFSH (0.8%). The average of duplicate determination in each sample was expressed in term of bovine standard EHC-bL-1 concentrations (Loeber et al, 1987). Sensitivity of the assay was 50 pg/tube. Inter-and intra-assay coefficients of variation were 17 and 7% respectively.

Statistical analysis

After the end of experiment, cows were allocated to either the pregnant or the non-pregnant group and these two groups were compared for zootechnical parameters (calving to treatment interval, body weight, body condition score and calf daily weight gain) by *t*-test (Sas, 1988). Results are presented as mean ± standard error of the mean.

The sources of variation of plasmatic concentrations of NEFA, BHB, glucose and insulin were analysed using the GLM procedure of Sas (Sas, 1988) with the following model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha_i * \beta_j + \beta_j * \gamma_k + \alpha_i * \gamma_k + \theta_l * \alpha_i + \varepsilon$$

where:

- y_{ijkl} is the concentration of blood metabolite of the cow l, the week k
- α_i is the fixed group effect (pregnant vs non-pregnant)
- β_j is the fixed effect of the interval between calving and treatment
- γ_k is the fixed effect of the week of sampling
- $\theta_l * \alpha_i$ is the random effect of cow within group (pregnant vs non-pregnant)

All the tests were carried out using appropriate error terms determined by the expected mean squares (Sas, 1988).

Similar models were used to test 1) the effects of interval between calving and treatment, pregnancy status after treatment, day of observation and interactions on the number of follicles by class and on the size of the largest follicle; 2) the effects of interval between calving and treatment, pregnancy status, time of blood sampling and interactions on oestradiol plasmatic concentrations 3 days before treatment; 3) the effect of pregnancy status, period of observation (day 6 or day 10), time of blood sampling and interactions on oestradiol plasmatic concentration during and after treatment; 4) the effects of pregnancy status, time of blood sampling on oestradiol and LH plasmatic concentrations between 29 and 48 h after implant removal.

When a significant effect of interval between calving and blood sample or calving and treatment was found, a linear regression was calculated correcting the effect of postpartum interval for cow effect (random effect). Post split-plot ANOVA multiple comparisons were made using Scheffe's test. Results are presented as lsmeans ± standard error of the lsmean.

RESULTS

Two cows presented a functional corpus luteum before oestrus synchronization treatment (11.8%; 0/8 primiparous, 2/9 multiparous; $P > 0.05$), seven ovulated (41.2%, 3/8 primiparous, 4/9 multiparous; $P > 0.05$) and five were found to be pregnant 45 days after AI (29.4%, 1/8 primiparous, 4/9 multiparous; $P > 0.05$). The two cows in their cycle period before treatment ovulated after treatment and were pregnant 45 days after AI.

Zootechnical parameters

There were no significant differences between pregnant and non-pregnant cows for interval between calving and treatment, body weight, body condition score and calf daily weight gain (table I).

Table I. Zootechnical characteristics of beef cows submitted to oestrus synchronization treatment and subsequently found pregnant or non-pregnant.

Characteristics	*Pregnant cows* $n = 5$		*Non-pregnant cows* $n = 12$		*P*
	m	*sem*	*m*	*sem*	
Calving to treatment interval (days)	67.8	3.1	55.3	4.6	NS*
Body condition score at calving	2.6	0.2	2.6	0.1	NS
Body weight at calving (kg)	685	23	660	13	NS
Body condition score at implant insertion	2.5	0.2	2.5	0.1	NS
Body weight at implant insertion (kg)	685	24	670	13	NS
Calf daily gain of weight (g/day)	1000	51	943	33	NS

* $P = 0.11$

Energy status

Plasma NEFA concentrations decreased with time postpartum (PP, interval effect; $P < 0.05$; regression equation: NEFA = –7 PP + 575).

When adjusted for postpartum interval, plasma NEFA concentrations were lower in pregnant cows than in non-pregnant cows during the 7 weeks before AI (218 ± 29 µeq/L vs 279 ± 18 µeq/L; $P < 0.05$; fig 1). No significant differences were found between pregnant and non-pregnant cows for BHB, glucose and insulin concentrations (table II) and no interaction was found between time of sampling and pregnancy status for any variable studied.

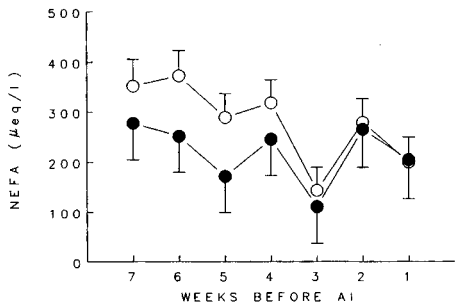

Fig 1. Non-esterified fatty acids (NEFA) plasma concentrations before insemination in oestrus synchronized postpartum beef cows which became subsequently pregnant (●; $n = 5$) or non-pregnant (○; $n = 12$) at fixed time AI's after treatment.

Follicular growth

A significant day effect was found on the number of small follicles, the number of large follicles and the size of the largest follicle (fig 2) but not on the number of medium sized follicles (2.57 ± 0.41). After implant insertion, the number of small follicles increased while the number of large follicles decreased. After implant removal, the number of large follicles increased. The size of the largest follicle was higher after implant removal than before or during treatment.

Pregnant cows presented more medium sized follicles than non-pregnant cows during the period of observation (2.65 ± 0.19 vs 2.50 ± 0.12; $P < 0.05$). Pregnant cows had less large follicles than non-pregnant cows 3 days before implant insertion (0.19 ± 0.37 vs 0.98 ± 0.25; $P < 0.05$) and 3 days after beginning of treatment (0.57 ± 0.37 vs 1.99 ± 0.25; $P < 0.05$; interaction day of observation * pregnancy status; $P < 0,05$).

Oestradiol secretion on day –3, day 6 and day 10

Oestradiol plasmatic concentrations before treatment (day –3) were not affected by interval between calving and blood sample nor by pregnancy status (8.1 ± 0.7 pg/mL).

Plasma oestradiol concentrations showed no difference between pregnant and non-

Table II. Plasma concentrations of energy metabolites and insulin during 7 weeks before insemination in oestrus synchronized postpartum beef cows subsequently found pregnant or non-pregnant.

	Pregnant cows n = *5*		*Non-pregnant cows* n = *12*		P
	lsmean	*sem*	*lsmean*	*sem*	
Non-esterified fatty acides (µeq/L)	218	29	279	18	0.04
β hydroxy-butyrate (mg/L)	22.0	1.0	21.5	0.6	NS
Glucose (g/L)	0.63	0.01	0.63	0.01	NS
Insulin (µIU/mL)	4.39	0.42	4.99	0.26	NS

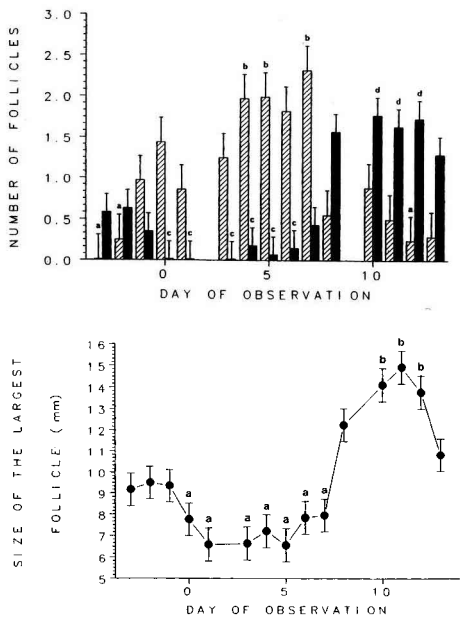

Fig 2. Number of small (diameter < 5 mm, ▨), large (diameter ≥ 10 mm; ■) follicles and size of the largest follicle (●) during oestrus induction and synchronization treatment (implant insertion at day 0; implant removal at day 10; AI on day 12 and 13) on the ovaries of 17 postpartum beef cows.

a vs b, $P < 0.05$
c vs d, $P < 0.05$

pregnant cows on day 6 (10.4 ± 0.6 pg/mL vs 9.8 ± 0.4 pg/mL; $P > 0.05$) but were higher in pregnant cows than in non-pregnant cows on day 10 (10.9 ± 0.6 pg/mL vs 7.8 ± 0.4 pg/mL; $P < 0.05$).

Oestradiol and LH secretion after implant removal and response to oestrus synchronization treatment

Among the seven females frequently sampled after implant removal and PMSG injection, three were found subsequently to be pregnant vs four non-pregnant. Pregnant cows presented higher oestradiol plasmatic concentrations than non-pregnant cows (11.0 ± 0.4 pg/mL vs 6.30 ± 3 pg/mL; $P < 0.05$). A similar trend was found for LH plasmatic concentrations (6.0 ± 0.5 ng/mL vs 1.2 ± 0.5 ng/mL; $P < 0.05$). In the three pregnant cows, oestradiol plasmatic concentrations increased and reached a maximum of 17.1 pg/mL, but, on the contrary, no increase in oestradiol secretion was observed in the four other non-pregnant cows (interaction pregnancy status* time of sampling, $P < 0.05$, fig 3). In the three pregnant cows, LH plasmatic concentrations increased from 42 to 48 h after implant removal and a subsequent ovulation was observed by ultrasonography whereas there was no LH peak during the whole sampling period for the non-pregnant cows (interac-

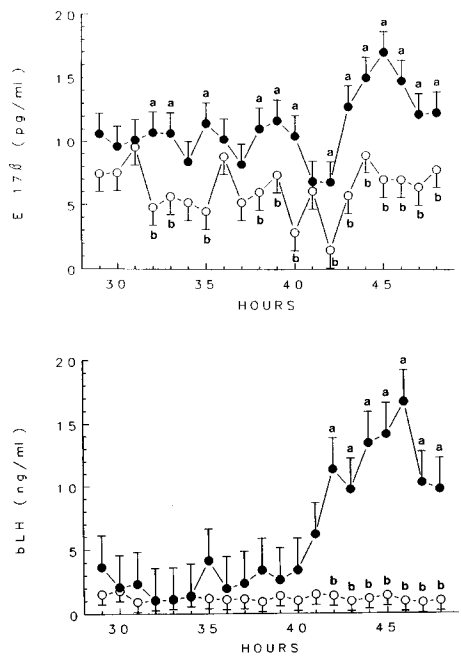

Fig 3. 17 β-Oestradiol and LH plasmatic concentrations 29 to 48 h after implant removal in oestrus synchronised postpartum beef cows subsequently found to be pregnant (●; $n = 3$) or non pregnant (○; $n = 4$).

a vs b, $P < 0.05$

tion pregnancy status* time of sampling, $P < 0.05$, fig 3) and no subsequent ovulation was observed.

In the ten remaining cows, only four ovulated within 3 days after implant removal and two were found to be pregnant 45 days after AI. The ovulation rate was significantly lower in the non-pregnant cows (2/12) than in the pregnant ones (5/5, Yates Chi-square, $P < 0.01$).

DISCUSSION

The low cyclicity rate before treatment observed here was similar to that reported previously in winter calving suckled Charolais cows in the same region (7 to 15% in primiparous cows, 25 to 30% in multiparous cows; Grimard et al, 1992a, b; Kabandana et al, 1993; Humblot et al, 1996) but lower than those reported by Ducrot et al (1994) and Pouilly et al (1994) in the same breed but in different regions. In such populations of cows, the treatment is designed to induced and to synchronise ovulation. The ovulation and pregnancy rate are lower than those cited in previous reports (Odde, 1990; Grimard and Mialot, 1990), but the females were younger (47% primiparous) and cyclicity rate was lower before treatment. These two factors are known to have a negative influence on ovulation and fertility rate after oestrus synchronisation treatment (Chupin et al, 1977; Pelot et al, 1977; Miksch et al, 1978; Aguer et al, 1981; Beal et al, 1984; Odde, 1990; Grimard et al, 1992a, b; Kabandana et al, 1993; Ponsart et al, 1996).

As shown by non-esterified fatty acids plasmatic concentrations, cows were mobilizing their energy stores after calving. In beef cows in good body condition score at the time of calving (BCS $\geq$ 2), the Inra's recommendations during the first month of lactation are calculated to be lower than the requirements (–0.72 Mcal net energy/day, Petit, 1988). As previously described by Easdon et al (1985) and Grimard et al (1995) in restricted beef cows, mobilization of energy stores decreased when postpartum interval increased (decrease of NEFA plasmatic concentrations) illustrating the adaptation of the cows to their diet. This fact has to be taken into account when studying relationship between nutrition and reproduction.

The decrease in the size of the largest follicles and the increase in the number of small follicles after implant insertion illustrate the effects of exogenous hormones on follicular growth. Oestradiol valerate is known to induce atresia of antral follicles (Rajamahendran and Walton, 1990; Bo et al, 1991). These observations do not match with the date of Lucy et al (1990), Rajamahendran and Taylor (1991) and Savio et al (1993) who described maintenance or an increase in follicular size after the beginning of oestrus synchronisation treatment. However, these authors administred prostaglandins before progestogen insertion. In this experiment, after atresia of the large follicle present on the ovary, a new wave of follicular growth was observed and, as reported by Lucy et al (1992), the number of large follicles increased. After implant removal and in response to PMSG injection, the size of the large follicles increased and some of them secreted oestradiol and ovulated.

In this study, body condition score and body weight at calving or at implant insertion were not different between the pregnant and the non-pregnant cows. The body condition score reached the value of 2.5 recommended by Petit (1988) and Petit and Agabriel (1993). However, non-esterified fatty acids patterns shown that cows were in different energy status before AI's: non-pregnant cows were mobilizing their body reserves before AI while pregnant ones had reequilibrated their energy balance. This confirms earlier results of Grimard et al (1992b) who observed a lower fertility rate

after induction of ovulation in cows which lost more than 30 kg body weight after calving than in cows which lost less weight. Furthermore, in postpartum restricted beef cows, a rapid decrease in lipid mobilization is induced by flushing (Easdon et al, 1985) and this may be related to the positive effects on fertility rate usually reported after such treatment (Petit et al, 1977; Pelot et al, 1977; Aguer, 1981; Kabandana et al, 1993).

There is a trend for non-pregnant cows to have a smaller postpartum interval than pregnant cows (12.5 days) when synchronized. Moreover, a greater variation was observed in non-pregnant cows (more cows at earlier stages; larger range of stages postpartum). This may have contributed to the higher fatty acids concentrations and to the lack of conception. However, the effect of postpartum interval on the response to oestrus synchronization treatment observed from field studies (Chupin et al, 1977; Pelot et al, 1977; Aguer et al, 1981; Odde, 1990; Grimard et al, 1992b; Humblot et al, 1996) might be partly explained by the decrease in lipid mobilization with time (Grimard et al, 1995).

In this experiment, pregnant cows had less large sized follicles than non-pregnant females before implant insertion. Regulation of dominance seemed to be more effective in less negative energy balance beef cows as observed by Lucy et al (1992) in dairy cows. After implant removal, ovulation was observed in pregnant cows and the number of large follicles decreased on day 13 compared to non-pregnant cows which maintained a large follicle and did not ovulate within the 3 days following implant removal.

Ovulation rate was extremely low in non-pregnant cows when using oestrus synchronisation treatment and this confirms if necessary that, in anoestrus beef cows, the lack of induction of ovulation is the main limiting source of pregnancy rate (Chupin et al, 1977; Beal et al, 1984; Grimard et al, 1992a, b; Humblot et al, 1996). This was especially reported in restricted, light or thin cows treated soon after calving (Gauthier et al, 1981; Grimard et al, 1992b, Humblot et al, 1996; Ponsart et al, 1996). In this experiment, absence of ovulation was related essentially to mobilization of energy stores before AI and was preceded by a lack of (or delayed) increase in oestradiol secretion at the end of the treatment and after implant removal. In the absence of oestradiol secretion, no LH peak was induced and no ovulation was observed. On the contrary, in pregnant cows induced to ovulate, oestradiol secretion was effective and induced a LH surge at a time very similar to those reported in previous studies (36 to 51 h after implant or intra-vaginal device removal, Barnes et al, 1981; Peters et al, 1981; Roberson et al, 1989).

In conclusion, the low fertility rate after oestrus synchronization treatment observed in this study was due to low ovulation rate during the 3 days following implant removal in cows in negative energy balance. This was related to an absence of oestradiol secretion by the growing follicles and absence of a LH surge after treatment. Good body condition score at the time of treatment is important but this seems insufficient to achieve systematically good pregnancy rate when using treatments combining progestogen and PMSG. This study shows that cows need to reequilibrate their energy balance and that NEFA patterns are good indicators of individual energy status in beef cows. Additionally, energy status is influenced by the postpartum interval. Further studies are needed to investigate more precisely interaction between metabolic responses and fertility at different fixed stages postpartum.

ACKNOWLEDGMENTS

We are grateful to Y Durand (Chambre d'Agriculture de Saône-et-Loire), V Parez (laboratoire

Intervet, France), M Bessot and A Bonnet (Coopérative d'élevage et d'insémination, Verdun-sur-le-Doubs) for their financial and technical support. We also thank G Somme and M Magnin (Clonatec, France) for the gift of the monoclonal antibody against progesterone, Y Adeline (FRH, France) for the oestradiol antibody, D Schams (Institute for Physiology, Technische Universität, München, Germany) for the LH antibody, P Bas and F Ternois (Laboratoire de nutrition, Institut national agronomique, Paris, France) and A Ponter for English support.

REFERENCES

Abdel Malak G, Thibier M (1982) Plasma LH and testosterone responses to synthetic gondadotropin-releasing hormone (GnRH) or dexamethasone-GnRH combined treatment and their relationship to semen output in bulls. *J Reprod Fertil* 64, 107-113

Agabriel J, Giraud JM, Petit M (1986) Détermination et utilisation de la note d'état d'engraissement en élevage allaitant. *Bulletin Technique du Centre de recherches zootechniques et vétérinaires de Theix* 66, 43-50

Aguer D (1981) Les progestagènes dans la maîtrise des cycles sexuels chez les bovins. *Rec Med Vet* 157, 53-60

Aguer D, Pelot J, Chupin D (1981) Reproduction du troupeau à viande et synchronisation de l'oestrus. *Bulletin des Groupements techniques vétérinaires,* 1, 33-57

Barnes MA, Kazmer GW, Bierly ST (1981) Gonadotropic and ovarian hormone response in dairy cows treated with Norgestomet and oestradiol valerate. *Theriogenology* 16, 13-23

Barnouin J, El Idilbi N, Chilliard Y, Chacornac JP, Lefaivre R (1986) Micro-dosage automatisé sans déprotéinisation du 3-hydroxy-butyrate plasmatique chez les bovins. *Ann Rech Vét* 17, 129-139

Beal WE, Good GA, Peterson LA (1984) Estrus synchronization and pregnancy rates in cyclic and non-cyclic beef cows and heifers treated with synchromate B or Norgestomet and Alfaprostol. *Theriogenology* 22, 59-63

Bo GA, Pierson RA, Mapletoft RJ (1991) The effect of oestradiol valerate on follicular dynamics and superovulatory response in cows with synchromate-B implants. *Theriogenology* 36, 169-183

Carruthers TD, Hafs HD (1980) Suckling and four-time-daily milking, influence on ovulation, estrus and serum luteinizing hormone glucocorticoïds and prolactin in post partum Holstein. *J Anim Sci* 50, 919-925

Chupin D (1977) Maîtrise de la reproduction chez les bovins. Principes, résultats, limites. *Ann Méd Vét* 121, 329-338

Chupin D, Pelot J, Petit M (1977) Induction et synchronisation de l'ovulation chez les femelles de race à viande. In : *Physiologie et pathologie de la reproduction. Journées ITEB-UNCEIA* ITEB ed, Paris, France, 45-49

Deletang F (1983) Objectifs et réussite de la synchronisation des chaleurs chez la vache laitière et allaitante. In : *Synchronisation de l'oestrus chez les femelles domestiques.* Association pour l'étude de la reproduction animale ed, Lyon, France C1-C3

Ducrot C, Grohn YT, Humblot P, Bugnard F, Sulpice P, Gilbert RO (1994) Postpartum anestrus in French beef cattle: an epidemiological study, *Theriogenology* 42, 753-764

Easdon MP, Chesworth JM, Aboul-Ela MBE, Henderson GD (1985) The effect of undernutrition in beef cows on blood hormone and metabolite concentrations post partum. *Reprod Nutr Dev* 25, 113-126

Fernandes LC, Thatcher WW, Wilcox CJ, Call EP (1978) LH release in response to GnRH during the postpartum period of dairy cows. *J Anim Sci* 46, 443-448

Fogwell RL, Bartlett BB, Reid WA (1986) Synchronized estrus and fertility of beef cows after weaning calves for short intervals. *J Anim Sci* 63, 369-376

Foster JP, Lamming GE, Peters AR (1980) Short-term relationships between plasma LH-FSH and progesterone concentrations in postpartum dairy cows and the effect of GnRH injection. *J Reprod Fertil* 59, 321-327

Gauthier D, Yaouanc A, Cochaud J, Mauleon P (1981) Influence d'une sous-alimentation de la vache allaitante sur l'induction de l'ovulation par l'hormone gonadotrope serique (PMSG) au cours du postpartum. *Reprod Nutr Dev* 21, 577-583

Grimard B, Mialot JP (1990) Avancer et regrouper les vêlages grâce à la maîtrise des cycles sexuels dans les systèmes allaitants traditionnels. *Elev Insémin* 240, 15-30

Grimard B, Humblot P, Thibier M (1992a) Synchronisation de l'oestrus chez la vache charolaise : effets de la parité et de la cyclicité prétraitement sur les taux d'induction d'ovulation et de gestation. *Elev Insémin* 247, 9-15

Grimard B, Humblot P, Parez V, Mialot JP, Thibier M (1992b) Synchronisation de l'oestrus chez la vache charolaise : facteurs de variation de la cyclicité prétraitement, du taux d'ovulation après traitement et du taux de fertilité à l'oestrus induit. *Elev Insémin* 250, 5-17

Grimard B, Humblot P, Ponter AA, Mialot JP, Sauvant D, Thibier M (1995) The influence of postpartum energy restriction on energy status, plasma LH and oestradiol secretion and follicular development in beef cows. *J Reprod Fertil* 104, 173-179

Humblot P, de Montigny G, Jeanguyot N, Tefedoie F, Payen B, Thibier M, Sasser RG (1990) Pregnancy specific protein B and progesterone concentrations in French Alpine goats throughout gestation. *J Reprod Fertil* 89, 205-212

Humblot P, Grimard B, Ribon O, Khireddine B, Dervishi V, Thibier M (1996) Source of variation of postpartum cyclicity, ovulation and pregnancy rates in primiparous Charolais cows treated with Norgestomet implants and PMSG. *Theriogenology* 46, 1085-1096

Kabandana F, Grimard B, Humblot P, Thibier M (1993) Effet d'une supplémentation alimentaire sur l'efficacité des traitements d'induction et de synchronisation de l'oestrus chez la vache allaitante : références particulières aux primipares non cyclées. *Elev Insémin* 258, 1-14

Loeber JG, Van de Wiel DFM, Gaines Das RE (1987) An international collaborative study on the First International Standard of bovine luteinizing hormone for immunoassay. *J Reprod Feril* 79, 145-152

Lucy MC, Thatcher WW, MacMillan KL (1990) Ultrasonic identification of follicular populations and return to oestrus in early postpartum dairy cows given intravaginal progesterone for 15 days. *Theriogenology* 34, 325-340

Lucy MC, Savio JD, Badinga L, De la Sota RL, Thatcher WW (1992) Factors than affect ovarian follicular dynamics in cattle. *J Anim Sci* 70, 3615-3626

Mialot JP, Levy I, Grimard B (1991) L'échographie dans la gestion de la reproduction des bovins. *Rec Med Vet* 167, 21-31

Miksch ED, Lefever DG, Mukembo JC, Spitzer JC, Wiltbank JN (1978) Synchronisation of oestrus in beef cattle. II. Effect of an injection of Norgestomet and oestrogen in conjonction with a Norgestomet implant in beef heifers and cows. *Theriogenology* 10, 201-218

Murphy MG, Boland MP, Roche JF (1990) Pattern of follicular growth and resumption of ovarian activity in postpartum beef suckler cows. *J Reprod Fertil* 90, 523-533

Nett TM (1987) Function of the hypothalamic-hypophysical axis during the postpartum period in ewes and cows. *J Reprod Fertil* 34, 201-213

Odde KG (1990) A review of synchronization of estrus in postpartum cattle. *J Anim Sci* 68, 817-830

Pelot J, Chupin D, Petit M (1977) Influence de quelques facteurs sur la fertilité à l'oestrus induit. In: *Physiologie et pathologie de la reproduction. Journées ITEB-UNCEIA* ITEB ed, Paris, France, 49-52

Peters AR, Kingsley SR, Riley Gm (1981) Plasma gonadotrophin response in beef cows to progesterone plus prostaglandin treatment. *Theriogenology* 16, 259-265

Petit M (1988) Alimentation des vaches allaitantes. In: *Alimentation des bovins ovins et caprins.* INRA ed, Paris, France, 159-184

Petit M, Agabriel J (1993) Etat corporel des vaches allaitantes charolaises : signification, utilisation pratique et relations avec la reproduction. *Prod Anim* 6, 311-318

Petit M, Chupin D, Pelot J (1977) Analyse de l'activité ovarienne des femelles bovines. In: *Physiopathologie de la reproduction. Journées ITEB-UNCEIA* ITEB ed, Paris, France, 21-28

Pierson RA, Ginther OJ (1984a) Ultrasonography of the bovine ovary. *Theriogenology* 21, 495-504

Pierson RA, Ginther OJ (1984b) Ultrasonography of pregnancy and study of embryonic development in heifers. *Theriogenology* 22, 225-233

Ponsart C, Sanaa M, Humblot P, Grimard B, Jeanguyot N, Ponter AA, Viel JF, Mialot JP (1996) Variation factors of pregnancy rates after oestrus synchronization treatment in French Charolais beef cows. *Vet Res* (in press).

Pouilly F, Viel JF, Mialot JP, Sanaa M, Humblot P, Ducrot C, Grimard B (1994) Risk factors for postpartum anoestrus in Charolais beef cows in France. *Prev Vet Med* 18, 305-314

Radford HM, Nancarrow CD, Mattner PE (1978) Ovarian function in suckling and non-suckling beef cows post partum. *J Reprod Fertil* 54, 49-56

Rajamahendran R, Walton JS (1990) Effect of treatment with oestradiol valerate on endocrine changes and ovarian follicle populations in dairy cows. *Theriogenology* 33, 441-452

Rajamahendran R, Taylor C (1991) Follicular dynamics and temporal relationships among body temperature, oestrus, the surge of luteinizing hormone and ovulation in Holstein heifers treated with norgestomet. *J Reprod Fertil* 92, 461-467

Roberson MS, Wolfe MW, Stumpf TT, Kittok RJ, Kinder JE (1989) Luteinizing hormone secretion and corpus luteum function in cows receiving two levels of progesterone. *Biol Reprod* 41, 997-1003

Russel AJF, Wright IA (1983) The use of blood metabolites in the determination of energy status in beef cows. *Anim Prod* 37, 335-343

Sas (1988) STAT user's guide. SAS Inst Inc Ed 6.03, Cary, NC, USA, 1 028 p

Savio JD, Boland MP, Hynes N, Roche JF (1990) Resumption of follicular activity in the early postpartum period of dairy cow. *J Reprod Fertil* 88, 569- 579

Savio JD, Thatcher WW, Badinga L, De la Sota RL, Wolfenson D (1993) Regulation of dominant follicle turnover during the oestrus cycle in cows. *J Reprod Fertil* 97, 197-203

Schallenberger E, Prokopp S (1985) Gonadotrophins and ovarian steroids in cattle. IV: Reestablishement of the stimulatory feedback action of oestra-

diol-17 β on LH and *FSH. Acta Endocrinol* 109, 44-49

Schallenberger E, Schams D, Zottmeier K (1978) Response of lutropin (LH) and follitropin (FSH) to the administration of gonadoliberin (GnRH) in pregnant and postpartum cattle including experiments with prolactin suppression. *Theriogenology* 10, 35-53

Schams D, Karg H (1969) Radioimmunogishe LH-Bestimmung Im Blutserum Vom Rind unter besonderer Beücksichtigung des Brunstzyklus. *Acta endocrinol* (Kbh) 61, 96-103

Terqui M, Chupin D, Gauthier D, Perez N, Pelot J, Mauleon P (1982) Influence of management and nutrition on post-partum endocrine function and ovarian activity in cows. In: *Current topics in veterinary medecine and animal science. Factors influencing fertility in the post partum cow.* (Martinus Nijhoff ed), The Hague, the Netherlands, 384-408

Thibier M (1975) Variations circadiennes de la LH plasmatique chez le jeune taurillon. *Ann Endocrinol* 36, 205-210

Thibier M, Saumande J (1975) Oestradiol 17-β, progesterone and 17 α-hydroxyprogesterone concentrations in jugular venous plasma in cows prior to and during *oestrus*, *J Steroid Biochem* 6, 1433-1437

Walters DL, Bunel WC, Witbank JN (1984) Influence of exogenous steroids, nutrition and calf removal on reproductive performance of anestrus beef cows. *Theriogenology* 21, 395-406

Webb R, Lamming GE, Haynes NB, Hafs HD, Manns JG (1977) Response of cyclic and postpartum suckled cows to injections of synthetic LH-RH. *J Reprod Fertil* 50, 203-210

Webb R, Lamming GE, Haynes NB, Foxtrot JR (1980) Plasma progesterone and gonadotrophin concentrations and ovarian activity in postpartum dairy cows. *J Reprod Fertil* 59, 133-143

Weesner GD, Norris TA, Forrest DW, Harms PG (1987) Biological activity of luteinizing hormone in the peripartum cow: least activity at parturition with an increase throughout the postparum interval. *Biol Reprod* 37, 851-858

Williams Gl (1990) Suckling as a regulator of postpartum rebreeding in cattle: a review. *J Anim Sci* 68, 831-852