Effects of postpartum energy intake on pregnancy rates in beef cattle subjected to GnRH- or CIDR-based timed artificial insemination protocols

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Wichtel, J. J., Charmley, E., Richardson, G. F., Duynisveld, J. L. and Lofstedt, R. 2008. **Effects of postpartum energy intake on pregnancy rates in beef cattle subjected to GnRH- or CIDR-based timed artificial insemination protocols**. Can. J. Anim. Sci. **88**: 439–447. The objectives were to determine the effects of three levels of postpartum metabolisable energy (ME) intake on pregnancy rates in beef cattle subjected to either GnRH-based (OVS) or progestin-based (CIDR) protocols for fixed time artificial insemination (TAI). Hereford cross cows were assigned to ME and TAI treatments (within ME) on the basis of parity and predicted calving date. The postpartum grass-silage-based diet was formulated to provide either Low (93 MJ d^{-1}), Medium (103 MJ d^{-1}) or High (120 MJ d^{-1})] ME from calving (January to February) to turnout (May 25). Lactating cows [$n = 175$, 5.7 ± 1.1 mean ($\pm$ SD) body condition score at calving] were subjected to their assigned TAI protocol; OVS [i.m. treatments of GnRH (100 μg) on day 0, $PGF_{2\alpha}$ (25 mg) on day 7, a second GnRH on day 9 and TAI 16 to 18 h later], or CIDR [i.m. treatment with 1 mg estradiol benzoate and 100 mg progesterone concurrent with CIDR (1.9 g progesterone) insertion on day 0, $PGF_{2\alpha}$ treatment at CIDR removal on day 7, a second estradiol treatment on day 8 and TAI 28 to 30 h later). Cows were 60 ± 13 d post-partum at the time of insemination. Lower ME intakes reduced ($P < 0.05$) maternal body weight and calf weight gain, but ME intake did not affect ($P > 0.05$) the proportion cycling ($113/175 = 65\%$, based on serum progesterone concentrations on days -5 and -14), ovulation following TAI, or the TAI pregnancy rates (based on ultrasonography). Timed insemination pregnancy rates were greater for CIDR- than OVS-treatment (63 vs. 45%, respectively, $P < 0.05$), regardless of ME intake.

Key words: Beef cow, estrus synchronization, Ovsynch, CIDR, energy intake, reproduction, calf gains

Wichtel, J. J., Charmley, E., Richardson, G. F., Duynisveld, J. L. et Lofstedt, R. 2008. **Incidence de l'apport énergétique postpartum sur le taux de conception des vaches de boucherie assujetties à un protocole d'insémination artificielle à temps prédéterminé par GnRH ou CIDR**. Can. J. Anim. Sci. **88**: 439–447. Les auteurs voulaient préciser les effets de trois apports postpartum d'énergie métabolisable (EM) sur le taux de conception des vaches de boucherie inséminées artificiellement à un moment fixe grâce à l'administration de GnRH (OVS) ou de progestine (CIDR). Des vaches Hereford hybrides ont été affectées à un traitement EM ou d'insémination artificielle en fonction de leur rang de mise bas et de la date de vêlage prévue. La ration à base d'ensilage d'herbe a été préparée de manière à fournir une faible (93 MJ par jours), une moyenne (103 MJ par jour) ou une forte (120 MJ par jour) quantité de EM, de la date de vêlage (janvier à février) à celle de la mise à l'herbe (25 mai). Les vaches en lactation [$n = 175$, note d'état corporel moyenne de $5{,}7 \pm 1{,}1$ ($\pm$ É.-T.) au vêlage] ont été affectées à leur protocole d'insémination artificielle : OVS [injection intramusculaire de GnRH (100 μg) le jour 0, de $PGF_{2\alpha}$ (25 mg) le jour 7, une deuxième injection de GnRH le jour 9 et insémination 16 à 18 h plus tard] ou CIDR [injection intramusculaire de 1 mg de benzoate d'œstradiol et de 100 mg de progestérone avec insertion du dispositif intravaginal CIDR (1,9 g de progestérone) le jour 0, injection de $PGF_{2\alpha}$ au retrait du dispositif le jour 7, nouvelle injection d'œstradiol le jour 8 et insémination 28 à 30 h plus tard). Soixante jours (± 13) s'étaient écoulés depuis le vêlage au moment de l'insémination. Un apport de EM plus faible réduit ($P < 0{,}05$) le poids corporel de la mère et le gain de poids du veau, mais l'apport de EM ne modifie pas ($P > 0{,}05$) la proportion d'animaux qui ovulent ($113/175 = 65$ %, selon la concentration de progestérone dans le sang entre les jours 5 et 14), l'ovulation après l'insémination artificielle ou le taux de conception après insémination (déterminé aux ultrasons). Le taux de conception après insémination à temps prédéterminé est plus élevé avec le CIDR qu'avec le traitement OVS (63 c. 45 %, respectivement, $P < 0{,}05$), peu importe l'apport de EM.

Mots clés: Vaches de boucherie, synchronisation de l'œstrus, Ovsynch, CIDR, apport énergétique, reproduction, gain de poids du veau

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Abbreviations: AI, artificial insemination; **BCS,** body condition score; **BW,** body weight; **CIDR,** controlled internal drug release; **CII,** calving-to-insemination interval; **DM,** dry matter; **TAI,** time artificial insemination; **GnRH,** gonadotropin releasing hormone; **ME,** metabolisable energy; **OVS,** Ovsynch®; **$PGF_{2\alpha}$**, prostaglandin $F_{2\alpha}$

Timed artificial insemination (TAI) refers to the administration of exogenous hormones to a group of cattle to induce synchronous ovulation, with or without behavioural estrus, making it possible to achieve an acceptable rate of conception to a single insemination at an appointed time. Such controlled breeding programs are intended to facilitate the introduction of artificial insemination in herds where day-to-day estrous detection is not possible or practical.

There are a number of TAI protocols in use at the present time in beef herds. The most commonly used protocols involve administering, singly or in various combinations, injectable prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), gonadotropin releasing hormone (GnRH), estradiol and oral or intra-vaginal progestin. Timed artificial insemination protocols induce synchronous follicular wave emergence and ovulation of a fertile oocyte at a defined time. To achieve this, the duration of the interovulatory period is controlled either by administration of $PGF_{2\alpha}$, which causes luteolysis of a natural or GnRH-induced corpus luteum, or by administration of an exogenous progestin for a defined period which mimics the luteal phase and suppresses ovulation (Thatcher et al. 1996). Emergence of a synchronous follicular wave during the period of progestin influence is induced by either GnRH or estradiol administered at the start of the protocol. Synchronous ovulation following removal of progestin influence is further facilitated by administration of GnRH prior to or at the time of insemination, or alternatively estradiol, which causes release of endogenous GnRH when administered during the preovulatory phase (Wenkoff 1987; Twagiramungu et al. 1995; Thatcher et al. 1996; Martinez et al. 2002).

Controlled breeding programs are not widely utilized by Canadian beef producers (Small et al. 1999). The reasons for this could include a lack of information on the protocols available, and the cost of certain protocols. Producers and veterinarians may be uncertain of the results that might be obtained if they were to invest in such programs, or may not know which program would be most appropriate for their herd. For example, herds with many non-cyclic cows at the time of treatment may obtain higher conception rates to TAI using protocols that include the administration of progestin, because such protocols would be expected to induce estrus and ovulation in anestrus cows more effectively than those that do not include an exogenous progestin (Roche et al. 1992; Lamb et al. 2001; Stevenson et al. 2003). The level of postpartum anestrus and overall reproductive performance are closely related to prepartum maternal body condition and postpartum nutrition (Wiltbank et al. 1964; Richards et al. 1986; Laflamme et al. 1992; Wettemann et al. 2003). This relationship is mediated in part through endocrine and metabolic signals which influence LH secretion. Early postpartum and underfed cattle have reduced pituitary secretion of LH leading to failure of ovulation and prolongation of the postpartum anestrus period (Schillo 1992; Wettemann et al. 2003; Hess et al. 2005). However, the effects of interaction between the estrus synchronization protocol and postpartum nutrient intake have not been specifically tested.

The objectives of this study were to compare the effectiveness of two estrus synchronization protocols for TAI programs in suckled beef cows, and to examine the effect of interaction between synchronization protocol and three levels of postpartum silage intake. It was hypothesized that administration of progesterone, estradiol and $PGF_{2\alpha}$ (a modified CIDR treatment) would result in higher TAI pregnancy rates in underfed cows, when compared with administration of GnRH and $PGF_{2\alpha}$ [Ovsynch (OVS) treatment]. The effects of diet on maternal body weight (BW), body condition score (BCS), milk production and calf weight gain were also determined.

MATERIALS AND METHODS

Design of the Trial

The cows used in this study were winter-calving Hereford-Beef Shorthorn cross cattle housed at the Agriculture and Agri-Food Canada Research Farm, Nappan NS. The experiment was replicated over 3 yr with the experimental population being 53, 64 and 58 cows for years 1, 2 and 3, respectively. Means and standard deviations for the ages of the cows were 3.5 ± 1.5 yr, 4.2 ± 2.0 yr and 3.9 ± 1.8 yr for years 1, 2 and 3, respectively, with approximately 15% of the experimental herd being replaced each year. The experimental period for each cow commenced each year on the cow's individual calving date, with all cows calving during January or February, and continued until cows were turned out to pasture on about 25 May each year. Mean and standard deviation for body condition score (BCS) at calving was 5.7 ± 1.1. Prior to calving each year, cows were randomly assigned to one of six treatments arranged in a 3×2 factorial design: three levels of postpartum ME intake (low, medium or high) and two of estrus synchronization protocols (OVS or CIDR) stratified by parity and predicted calving date, and irrespective of any previous experimental assignment. Assignment to pen was then stratified by treatment group to reduce the chance of a pen effect, and cows remained in their pens with calf at foot during the entire experimental period. Cows with significant post-partum complications following calving were not included in the experiment. All animals enrolled in this trial were cared for in accordance with the guidelines suggested by the Canadian Council on Animal Care (1993).

Diet Formulation and Feeding

The bunker silage used in this study (Table 1) was grown at Nappan, NS (lat. 45°N, log. 64°W) and harvested from a naturalized permanent sward containing predominantly timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.), Kentucky bluegrass

Table 1. Composition of silages in each year[z]

	2001	2002	2003
Dry matter (g kg^{-1})	347±9.1	321±26.8	307±42.7
Acid detergent fibre (g kg^{-1} DM)	411±7.4	352±9.6	392±12.5
Neutral detergent fibre (g kg^{-1} DM)	609±12.2	559±16.1	548±20.5
In vivo apparent DM digestibility (g kg^{-1})	635±14.6	–	–
ME (g kg^{-1} DM)[y]	9.24	10.1	9.5
Crude protein (g kg^{-1} DM)	129±1.4	159±8.4	136±12.4
pH	4.71±0.11	5.25±0.84	–

[z]Sampled daily, frozen and combined for analysis every 2 wk during the experimental period.
[y]ME calculated from ADF according to the equation ME (MJ kg^{-1} DM) = 15.0–0.013ADF.

(*Poa pratensis* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium multiflorum* L.). In all years, silage was taken at the first cutting from the same area and wilted to between 300 and 350 g kg^{-1}. Silages were similar in chemical composition, although the neutral detergent fibre (NDF) concentration was marginally higher in year 1 (Table 1), likely due to the slightly later harvest date.

Cows were loose-housed in six pens that were equipped with free stalls. Silage was fed once a day between 0930 and 1100. Prior to calving, all cows were fed silage to meet requirements according to body weight (BW) measured at the time they entered the experimental housing (December). From the day of calving, silage was fed according to pre-determined amounts based upon BW at calving and the assigned dietary treatments (Table 2). Silage allowance to individual animals was controlled using a Calan Gate (American Calan Inc., Northwood, NH) feeding system. Minerals were top dressed once daily and feed refusals were measured each morning. Calves had creep access to good quality timothy hay at all times but could not gain access to silage.

Estrus Synchronization and Breeding

Estrus was synchronized for TAI during the first week of April. Lactating cows ($n = 53$, 64 and 58 for years 1, 2 and 3, respectively) were subjected to their assigned TAI protocol. The OVS consisted of i.m. treatments of GnRH (100 μg Cystorelin, Merial Canada, Baie d'Urfe, QC) on day 0, $PGF_{2\alpha}$ (25 mg Lutalyse, Pfizer Pfizer Animal Health, Montreal, QC) on day 7, a second GnRH on day 9 and TAI 16 to 18 h later. The CIDR protocol was adapted from Lammoglia et al. (1998) and Martinez et al. (2000) and consisted of i.m. treatment with 1 mg estradiol benzoate and 100 mg progesterone (dissolved in ethanol) concurrent with insertion of an intravaginal progestin releasing device (CIDR, 1.9 g progesterone, Pfizer Animal Health, Montreal, QC) on day 0, $PGF_{2\alpha}$ treatment at CIDR removal on day 7, a second estradiol treatment on day 8 and TAI 28 to 30 h later). Cows inseminated based on observation of estrus, after $PGF_{2\alpha}$ and before the appointed time of TAI, were deemed not pregnant to TAI. Inseminations were done by six experienced technicians using semen from 1 of 13 sires with proven fertility. Technicians and semen were balanced as much as possible across the ME and TAI treatments.

Table 2. Dietary treatments and recorded intakes of DM, protein and ME

	Intake		
	Low	Medium	High
DM intake (g kg^{-1} BW)	15.9	17.8	19.9
Protein intake (kg d^{-1})	2.2	2.5	2.8
ME intake (MJ d^{-1})[z]	93	103.4	120

[z]ME calculated from ADF according to the equation ME (MJ kg^{-1} DM) = 15.0–0.013ADF.

Assessment of Reproductive Performance

Cyclicity (defined as the proportion of cows that had recommenced estrous cycles following calving) was determined by serum progesterone concentration. Serum was obtained by centrifugation from jugular blood samples collected into non-heparinized vacuum tubes, 14 d and 5 d before the first day of treatment. Progesterone concentrations were categorized as low (< 1.67 ng mL^{-1}) or high (≥ 1.67 ng mL^{-1}) this value being the upper limit of the 95% confidence interval for serum progesterone concentrations of experimental cows known to be in estrus. Any cow with a high progesterone concentration at either of the sampling periods was classified as cyclic.

The proportion of cows showing behavioural signs of estrus after treatment was determined for year 1 only, and was based on activation of heat mount detectors (Kamar®, Kamar Inc., Steamboat Springs, CO). The proportion of cows ovulating in response to treatment at 0, 24 and 48 h post-insemination was determined each year by transrectal ultrasound examination using a 5 MHz rectal transducer (Aloka SSD 500, Image Solutions Médicales Inc., Vandreuil-Dorion, QC). The presence of a hyper-echoic ovulation site or corpus haemorrhagicum on either ovary was taken to indicate ovulation had occurred.

Returns to estrus following the TAI were recorded and estrous cows were artificially inseminated at first return. The proportion of cows pregnant to TAI was determined by ultrasound at 40–65 d post-insemination, using fetal development to discriminate between first and second service conception. Eight cows were excluded from reproductive analysis because they were detected in estrus and inseminated 24 h earlier than their appointed time of insemination. It could not be determined if these cows would have conceived to the TAI.

The excluded cows were distributed over the three years of the study.

Determination of Gain, Intake and Milk Production

Maternal and calf BW were recorded at calving and again at the end of the trial (at turn-out), and weight gains were calculated by obtaining the difference between these two measurements and dividing by the number of days on test for each cow-calf pair. Maternal BW at calving were estimated by taking the last pre-calving BW and subtracting twice the calf birth weight to account for the fetal fluids and placenta (Agricultural Research Council 1980). This avoided errors that might have arisen due to atypical feed and water intake, or fluid retention or loss, around the time of calving. In all cases, cows were weighed immediately prior to the daily feeding. At the time of weighing, cow BCS was also recorded, using a 1 to 9 scale (Lowman et al. 1976). Daily DM intake was determined from the amounts of feed offered and refused each day. The silage that was fed was sampled daily, frozen and combined to be submitted for analysis every 2 wk. For the same 2-wk period, uneaten feed was sampled once each week and the two samples were combined for analysis.

Cows in years 1 and 2 were milked for determination of milk yield. For each cow, milk yield was determined once between weeks 5 and 7 after calving. Cows were separated from calves at 0800 and milked after administration of oxytocin (Oxytocin® 60 IU i.m., MTC Pharmaceuticals, Cambridge, ON). Cows and calves remained separated until cows were again milked the following morning. Milk weight was corrected to a theoretical 24-h milk yield.

Analysis

Silage offered and refused was analysed for DM by oven drying to constant weight at 50°C. Silage pH was determined on fresh 10-g samples macerated in 200 mL distilled water as described by (Charmley et al. 1997). All other feed analyses were performed on dried, ground samples. Ash-free NDF and ADF were analysed by methods described by (Van Soest et al. 1991). Ash was measured as the residual sample weight following 48 h combustion at 550°C in a muffle furnace. Nitrogen was determined using macro-kjeldahl procedures 7.033–7.037 (Association of Official Analytical Chemists 1990). Crude protein was assumed to be $N \times 6.25$. In year 1 only, apparent digestibility was measured in vivo using silage taken at the mid point of the feeding trial. Six steers (BW 350kg) were fed silage at 17 g kg-1 BW for 14 d. Apparent digestibility was measured by total faecal collection over the last 6 of the 14 d. Metabolisable energy (ME) was estimated as $DE \times 0.82$ (Agricultural Research Council 1980) in year 1. This direct measurement was compared with relationships from the literature in order to select the most suitable equation for estimating ME across the 3 yr of the study. The ME of silage for all years was estimated according to the following equation (Givens et al. 1989) where ADF is acid detergent fibre:

$$ME\ (MJ\ kg^{-1}DM) = 15.0 - 0.013ADF$$

Serum was stored frozen at −20°C until analysis for P_4 concentration, which was performed by sequential competitive chemiluminescent enzyme immunoassay (Immulite® Progesterone, Diagnostic Products Corporation, Los Angeles, CA).

Statistical Analysis

The dependent variable of primary interest was the proportion pregnant to TAI. The explanatory variables screened for inclusion in the multivariate logistic model were year, synchronization protocol, ME intake level, calving-to-insemination interval (CII), parity, pen, inseminate sire, AI technician, maternal BW at calving and maternal BCS at calving. These variables were tested for association with treatment using χ^2, converting continuous variables to trichotomous variables as necessary. Those variables associated with TAI pregnancy rates at $P \leq 0.25$ were retained. Main effects and interactions were then tested in mixed logistic models using the GLLAMM (Rabe-Hesketh et al. 2002) and LOGISTIC procedures of Stata (Stata Statistical Software, Release 7, Stata Corporation, College Station, TX). Terms were included to account for the effect of repeated measures for cows contributing data for more than 1 yr. The results are presented as proportions, along with associated odds ratios and standard errors derived from the logistic model. The contribution of each subset of parameters to the model was tested sequentially using the likelihood ratio test (Dohoo et al. 2003) and the probabilities are presented.

The effects of treatments on continuous dependent variables were tested using the MIXED procedure in SAS (SAS 8.2, SAS Institute, Cary, NC). The explanatory variables tested for inclusion in the multivariate linear model were the same as for the logistic model above, substituting days on test for CII, with the addition of BW at birth as a covariate where appropriate. Variables were first subjected to univariate analysis and those associated with TAI pregnancy rate at $P \leq 0.25$ were retained. Terms were included to account for any similarity between cows in the same pen, and the effect of repeated measures for cows contributing data for more than 1 yr. Results of the linear analysis are presented as least squares means with associated standard errors. All treatment effects are reported as statistically significant at $P < 0.05$.

RESULTS

A wide range in ME intake was achieved (Table 2), with intake of cows assigned to high and low treatments, expressed as a ratio of ME intake, being 1.3.

Reproductive Performance

The proportion of cows pregnant to TAI was greater for CIDR-treated cows than for OVS-treated cows (63% vs. 45% pregnant to first service, respectively; Table 3). The reproductive advantage associated with CIDR treatment was similar at all three levels of ME allowance, suggesting that, in this study, the effect of synchronization treatment did not depend on the level of ME intake (synchronization treatment × intake interaction $P = 0.84$). Parity, pen, inseminate sire, and technician were not associated with the proportion of cows pregnant to TAI.

In the final model, year was not significant as a main effect. The effect of ME intake on the proportion pregnant to TAI was also not significant as a main effect, even though lower intakes caused significant reduction in maternal BW and calf weight gain (Table 4).

Cyclicity, as evidenced by at least one high serum progesterone concentration prior to initiation of the TAI protocol, was not associated with an increased chance of pregnancy in this study. The proportions pregnant to TAI were 51 and 66% for those cows determined to be cyclic and non-cyclic, respectively ($P > 0.05$). Intake was not related to cyclicity, with 65, 69 and 70% of cows determined to be cyclic for low-, medium- and high-intake cows, respectively ($P > 0.05$). The significant predictors of cyclicity were BW at calving and parity, and these two predictors were correlated, i.e., 30% of primiparous cows were classified as cyclic prior to treatment as compared with 80% of multiparous cows ($P < 0.01$).

In the final model, parity, maternal BW at calving, maternal BCS at calving and CII were not statistically associated with the probability of pregnancy to TAI. However, the experimental cows were generally well-conditioned, with only 11% of cows having a BCS of < 5 at the time of calving. Furthermore, there was a very tight temporal distribution of calving in this herd, with only 3% of cows having a CII of less than 40 d.

No intravaginal inserts were lost during the experiment. Twelve of 85 OVS cows (14%) were detected in standing heat 24–72 h prior to the scheduled TAI, compared with none of 90 CIDR cows ($P < 0.01$). This occurred in each year of the study. The proportion of cows in estrus at the time of insemination (0 h), based on an activated or absent Kamar® patch, was greater for CIDR-treated cows than for OVS-treated cows (patches used in year 1 only). However, fewer CIDR- than OVS-cows had ovulated at 0 h (Table 3), with a significant synchronization treatment × intake interaction ($P = 0.03$) whereby CIDR treatment reduced the chance of ovulation at 0 h in the high-intake group, but not in the medium- and low-intake groups (3 vs. 25 and 28% of CIDR cows ovulated at 0 h, respectively). This interaction was responsible for most of the difference between synchronization groups in ovulation at 0 h. However, cows that had ovulated at the time of TAI (0 h) were no less likely to become pregnant that cows ovulating after TAI ($P = 0.43$). Overall, 93% of cows had ovulated by 24 h after insemination, with no effect of treatment (Table 3), and by 48 h this value was 99% (data not shown).

Calving interval data were available for 130/175 cows. After controlling for the significant effects of year, parity and maternal BW at calving, calving interval was affected by neither synchronization treatment nor intake, the mean values being 361 d (SEM = 1.8) for both OVS and CIDR-treated cows, and 359, 362 and 363 (SEM = 2.2) for low-, medium- and high-intake cows, respectively.

Body Weight, Body Condition, Milk Production and Calf Weight Gain

Cows assigned to high intake were fed ad libitum, with DM consumption approaching 20 g kg^{-1} BW. At the lowest level of feeding, total DM intake was 79% of ad libitum intake. During the experimental period, cows on restricted silage feeding were in negative energy balance and lost BW ($P < 0.01$) while those fed ad libitum maintained weight (Table 4). The changes in maternal BCS mirrored those in BW, but differences between groups were not significant. Changes in rump fat and back fat thickness were measured for year 1 only. Between-group differences were not significant and did not mirror changes in BW (data not shown).

Table 3. Effect of synchronization treatment and postpartum ME intake on reproductive indices

	Synchronization treatment (S)[z]		Postpartum intake (I)[y]			Probability [x]	
	OVS	CIDR	Low	Med	High	S	I
Number of cows	76	90	57	53	56		
Ovulated at 0 h (%)[w]	39	19	31	35	18	0.98	0.10
Ovulated at 24 h (%)[w]	96	91	92	92	95	0.22	0.87
Pregnant to TAI (%)	45	63	53	55	57	**0.01**	0.49

[z]OVS, Ovsynch® protocol; CIDR, modified CIDR-B® protocol.
[y]Low, medium or high dry matter intake; refer to Table 2.
[x]The significance probability for the effect calculated using the likelihood ratio test.
[w]The proportion of cows having ovulated at the time of insemination (0 h) and 24 h after insemination (24 h). S × I significant $P = 0.03$; High intake reduced the chance of ovulation at 0 h in the CIDR group (1/29 cows) but not the OVS group (9/21 cows).

Table 4. Effect of postpartum ME intake on body weight change of the experimental cows, and gain of their calves

	Postpartum intake				
	Low	Med	High	SEM	Probability
Number of cows	57	53	56		
BW at calving	620	616	621	12	0.91
DM intake (g kg^{-1} BW)	15.7	17.4	19.8	0.2	>0.01
BW change of cows (kg d^{-1})	−0.21	−0.14	0.06	0.06	>0.01
BCS change of cows (units)[z]	−0.21	−0.06	0	0.12	0.37
BW of calves at birth (kg)	43.1	43.5	43.7	1.6	0.88
Calf body weight at turnout (kg)	115	123	121	2	0.03
BW gain of calves (kg d^{-1})	0.81	0.89	0.88	0.41	0.02

[z]Scale of 1–9, increasing as body condition increases.

Restricting maternal silage intake reduced rate of gain in calves ($P<0.02$) and resulted in a 6% reduction in calf weight at turnout at the lowest level of intake (Table 4). Milk yield was measured during years 1 and 2. Yield was 4.7, 5.6 and 5.34 kg d^{-1} (SEM = kg d^{-1}) for the low-, medium- and high-intake groups, respectively ($P=0.27$).

Calf weaning weights coming off pasture in the fall were available for year 1 only, because calves were enrolled in unrelated projects in subsequent years. There was a trend towards heavier weaning weights in the high feed intake group ($P=0.12$), the average values being 240, 241 and 263 kg (SEM = 5 kg) for the low-, medium- and high-intake groups, respectively.

DISCUSSION

Effect of Synchronization Protocol on the Proportion Pregnant

In agreement with other studies using similar protocols in suckled beef cows (Geary et al. 1998; Martinez et al. 2000; Small et al. 2001) both the OVS and CIDR protocols resulted in a high degree of ovulation synchrony, with acceptable TAI pregnancy rates. This appeared to hold true over a range of postpartum ME intakes. Overall, the CIDR protocol resulted in higher pregnancy rates than the OVS protocol under the conditions of this study. No other studies have directly compared the traditional OVS protocol to a modified CIDR protocol incorporating estradiol in suckled beef cows.

Cyclicity prior to treatment was not associated with an increased chance of pregnancy in this study, regardless of treatment used. The lack of effect of cyclicity in time insemination studies has been observed previously (Bridges et al. 1999). In agreement with most previous work in post-partum beef cattle (Derouen et al. 1994; Marston et al. 1995; Lalman et al. 1997) the most important predictors of cyclicity were BW at calving and parity. Extreme changes in energy intake, both before and after calving, can affect reproductive performance (Wiltbank et al. 1964), but the reproductive performance of cows appears to be quite unresponsive to moderate under-feeding during the postpartum period, providing that they calve in adequate body condition (Marston et al. 1995).

Adequate progestin stimulation is an important prerequisite for inducing a fertile ovulation in postpartum cows (Munro 1989). The mechanism of action of progesterone in the postpartum cow is complex. Progesterone appears to act through changes in estradiol receptor number in the hypothalamus, reduced negative feedback on GnRH release, enhanced LH release, stimulation of LH receptors in the follicle, and increases in follicular estradiol production, as recently reviewed (Rhodes et al. 2003). In the case of CIDR protocols, this progesterone requirement is satisfied by the use of an exogenous source of progestin delivered via an intravaginal insert (Macmillan et al. 1993). In OVS protocols, progesterone stimulation follows the GnRH-induced ovulation or luteinization of a dominant follicle (Pursley et al. 1995; Martinez et al. 1999); however, typically, a proportion of OVS-treated cows will exhibit estrus and/or ovulate before the appointed time of AI (Pursley et al. 1997; Martinez et al. 2000). This was a feature of our study, and explained in part why OVS performance was inferior to CIDR: 14% of OVS cows came into heat 24–72 h before the appointed time of insemination in this study, whereas early heats were not noted in CIDR cows. Cows ovulating more than 24 h early fail to conceive in AI programs where insemination is restricted to an appointed day, thus reducing the apparent effectiveness of the program (Martinez et al. 2000).

To attain acceptable TAI pregnancy rates, ovulation must be highly synchronous and should occur on or around the day of insemination (Small et al. 2001). To achieve this, truncation of the randomly staged follicular waves, and the induction of new, synchronous follicular waves must occur approximately 1 wk before the synchronous ovulation is required, so dominant follicles in all cows reach maturity at approximately the same time. This is achieved in the OVS protocol by the first GnRH injection, while in the CIDR protocol, it is the combined administration of progesterone and estradiol that causes truncation of the follicular wave. In the presence of low progesterone concentrations and a

developing follicle, precise timing of ovulation is further enhanced by the second injection of GnRH, or estradiol, in the OVS and CIDR protocols, respectively (Bo et al. 2002). That a proportion of OVS-treated cows exhibit estrus and/or ovulate prematurely (i.e., before the appointed time of AI) appears to result from a failure of ovulation and/or luteinization following the first GnRH injection of the OVS protocol and progression to a non-synchronous ovulation during the interval between the $PGF_{2\alpha}$ and second GnRH injection (Pursley et al. 1997; Vasconcelos et al. 1999). Conception rates of cows displaying premature estrus are lower if inseminated strictly at the time of TAI. Ovulation in response to GnRH is dependent on stage of follicular development (Silcox et al. 1993; Moriera et al. 2000). Small follicles (with an antral diameters of less than 10 mm) may not respond due to a lack of LH receptors (Xu et al. 1995). Combining OVS with an oral progestin such melengestrol acetate, or with intra-vaginal progesterone, provides an exogenous source of progestin, which effectively suppresses premature estrus (Dejarnette et al. 2001). Similarly, pre-synchronization of randomly cycling dairy cows with two injections of $PGF_{2\alpha}$ 14 d apart improves the degree of synchrony when OVS is employed (El-Zarkouny et al. 2004). Further, OVS protocols that include a progestin (such as a CIDR) appear to be more effective in causing a synchronous ovulation when compared with those that do not include a progestin for the treatment of post-partum anestrus (Lamb et al. 2001). A significantly larger proportion of OVS-treated cows had ovulated at the time of TAI when compared with CIDR-treated cows, with a significant synchronization treatment $\times$ intake interaction whereby CIDR use reduced the chance of ovulation at 0 h in the high-intake group, but not in the medium- and low-intake groups. This relationship between postpartum ME intake and time to ovulation in TAI programs deserves further study; however, its impact on the results of this study was not great: cows that had ovulated at the time of TAI (0 h) were no less likely to become pregnant when compared with cows ovulating after TAI.

Effect of ME Intake on the Proportion of Cows Pregnant

We examined the effect of interaction between synchronization protocol and postpartum ME intake on TAI pregnancy rates. To our knowledge, no other studies have specifically addressed this subject. There is evidence that exogenous progesterone is an effective inducer of estrus and ovulation in anoestrus cows (Roche et al. 1992; Lamb et al. 2001; Stevenson et al. 2003). Therefore, we hypothesized that the benefit associated with progesterone (CIDR) treatment would be more pronounced in the underfed cows in our study, because this group would be most at risk of anestrus at the time of treatment. However, an interaction between synchronization protocol and ME intake was not identified in this study. This indicated that the effect of synchronization treatment was similar at all levels of ME intake. Despite the disparate between-group intakes, the underfed cows in this study were no more likely to be anestrous than cows fed ad libitum. It is possible that the ME intake for the Low-intake cows was sufficient to support cyclicity, even though reduced maternal BW and calf gains were noted. Previous studies have noted that the effect of postpartum intake is not great in cows that calve in moderate to good condition (Marston et al. 1995; Lalman et al. 1997).

Parity, maternal condition at calving, days between calving and insemination, and the presence of suckled calves are other potent drivers of cyclicity (Wiltbank et al. 1964; Bellows et al. 1982; Stagg et al. 1998; Hess et al. 2005). In this study, however, parity, maternal BW at calving, maternal BCS at calving and CII were not statistically associated with the probability of pregnancy to TAI. This could be explained by the fact that the experimental cows were well-conditioned, with only 5% of cows having a BCS of <4.5 at the time of calving. Furthermore, there was a very tight temporal distribution of calving in this herd, with only 3% of cows having a CII of less than 40 d. Collectively, these factors may have promoted postpartum cyclicity across all levels of postpartum ME intake, thereby decreasing the likelihood of observing an interaction between progesterone treatment and ME intake. It is possible that in many commercial herds, cows may calve in much poorer body condition and have a greater degree of negative energy balance prior to breeding than was noted in the present study.

Effect of Intake on Body Weight, Milk Production and Calf Weight Gain

We have included data on maternal body weight, milk production and calf weight gain to confirm that the experimental levels of postpartum ME intake achieved in this study were sufficiently disparate to reasonably test the effect of plane of nutrition on reproductive performance. We observed a decrease in maternal BW as a result of restricted ME intake, which was similar to that observed in previous work (Charmley et al. 1999). In that study, calf gain was not affected by maternal ME intake in the presence of supplemental protein (Charmley et al. 1999). In the present study, however, maternal ME intake did influence calf gain up to turn-out. The effect was apparent in all 3 yr, but the major effect was noted when comparing the low and medium intake levels, with no further response above an intake of approximately 100 MJ d^{-1} ME. Levels of milk production were typical for silage-fed beef cows (Charmley et al. 2004). Milk yield responded numerically to increased ME intake, and this response was consistent with the modest increase in rate of gain by calves. We conclude from these data that reproductive performance is less sensitive to post-partum energy intake than other

performance indicators such as change in maternal bodyweight and calf gain.

CONCLUSIONS

This study specifically examined the interaction between synchronization protocol and postpartum energy intake on the reproductive performance of postpartum beef cattle in a TAI program. Reducing postpartum ME intake had little effect on reproductive performance irrespective of the TAI protocol used. Both the OVS and CIDR protocols achieved satisfactory TAI pregnancy rates in a beef herd at low risk of anestrus (based on body condition and serum progesterone) over the range of postpartum energy intakes. Although lower ME intakes reduced maternal BW and calf weight gain, good body condition at calving may have overcome the potential negative effect of reduced dietary intake on TAI pregnancy rate.

Overall, the CIDR protocol resulted in higher TAI pregnancy rate than the OVS protocol. However, TAI pregnancy rates in this trial were comparable to those in the literature using similar estrus synchronization techniques.

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Agricultural Research Council. **1980**. The nutrient requirements of livestock. CAB International, Wallingford, UK.

Association of Official Analytical Chemists. **1990**. Official methods of analysis. 15th ed. AOAC, Arlington, VA.

Bellows, R. A., Short, R. E. and Richardson, G. V. 1982. Effects of sire, age of dam and gestation feed level on dystocias and postpartum reproduction. J. Anim. Sci. **55**: 18–27.

Bo, G. A., Baruselli, P. S., Moreno, D., Cutaia, L., Caccia, M., Tribulo, H. E. and Mapletoft, R. J. 2002. The control of follicular wave development for self-appointed embryo transfer programs in cattle. Theriogenology **57**: 53–72.

Bridges, P. J., Lewis, P. E., Wagner, W. R. and Inskeep, E. K. 1999. Follicular growth, estrus and pregnancy after fixed-time insemination in beef cows treated with intravaginal progesterone inserts and estradiol benzoate. Theriogenology **52**: 573–583.

Canadian Council on Animal Care. **1993**. Guide to the care and use of experimental animals. 2nd ed. CCAC, Ottawa ON.

Charmley, E. and Duynisveld, J. L. 2004. The partial replacement of silage with straw, with or without barley or soybean meal, in rations for winter calving beef cows. Can. J. Anim. Sci. **84**: 245–253.

Charmley, E., Savoie, P. and McQueen, R. E. 1997. Influence of maceration at cutting on lactic acid and bacteria populations, silage fermentation and voluntary intake and digestibility of precision-chopped lucerne silage. Grass Forage Sci. **52**: 110–121.

Charmley, E., Small, J. A. and McRae, K. B. 1999. Influence of post-calving supplemental protein on calf performance and reproductive efficiency for beef cows fed silage. Can. J. Anim. Sci. **79**: 97–106.

Dejarnette, J. M., Wallace, R. W., House, R. B., Salverson, R. R. and Marshall, C. E. 2001. Attenuation of premature estrous behavior in postpartum beef cows synchronized to estrus using GnRH and PGF2a. Theriogenology **56**: 493–501.

Derouen, S. M., Franke, D. E., Morrison, D. G., Wyatt, W. E., Coombs, D. F., White, T. W., Humes, P. E. and Greene, B. B. 1994. Prepartum body condition and weight influences on reproductive performance of first-calf beef cows. J. Anim. Sci. **72**: 1119–1125.

Dohoo, I. R., Martin, W. and Stryhn, H. 2003. Veterinary epidemiologic research. AVC Inc. Charlottetown, PE.

El-Zarkouny, S. Z., Cartmill, J. A., Hensley, B. A. and Stevenson, J. S. 2004. Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. J. Dairy Sci. **87**: 1024–1037.

Geary, T. W., Whittier, J. C., Downing, E. R., LeFever, D. G., Silcox, R. W., Holland, M. D., Nett, T. M. and Niswender, G. D. 1998. Pregnancy rates of postpartum beef cows that were synchronized using Synchro-Mate-B or the Ovsynch protocol. J. Anim. Sci. **76**: 1523–1527.

Givens, D. I., Everington, J. M. and Adamson, A. H. 1989. The nutritive value of spring-grown herbage on farms throughout England and Wales over 4 years. III. The prediction of energy values from various laboratory measurements. Anim. Feed Sci. Technol. **36**: 215–218.

Hess, B. W., Lake, S. L., Scholljegerdes, E. J., Weston, T. R., Nayagihugu, V., Molle, J. D. C. and Moss, G. E. 2005. Nutritional controls of beef cow reproduction. J. Anim. Sci. **83**: E90–E106.

Laflamme, L. F. and Connor, M. L. 1992. Effect of postpartum nutrition and cow body condition at parturition on subsequent performance of beef cattle. Can. J. Anim. Sci. **72**: 843–851.

Lalman, D. L., Keisler, D. H., Williams, J. E., Scholljegerdes, E. J. and Mallett, D. M. 1997. Influence of postpartum weight and body condition change on duration of anestrus by undernourished suckled beef heifers. J. Anim. Sci. **75**: 2003–2008.

Lamb, G. C., Stevenson, J. S., Kesler, D. J., Garverick, H. A., Brown, D. R. and Salfen, B. E. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2a for ovulation control in postpartum suckled beef cows. J. Anim. Sci. **79**: 2253–2259.

Lammoglia, M. A., Short, R. E., Bellows, S. E., Bellows, R. A., MacNeil, M. D. and Hafs, H. D. 1998. Induced and synchronized estrus in cattle: dose titration of estradiol benzoate in peripubertal heifers and postpartum cows after treatment with an intravaginal progesterone-releasing insert and prostaglandin F2alpha. J. Anim. Sci. **76**: 1662–1670.

Lowman, B. G., Scott, N. A. and Sommerville, S. H. 1976. Condition scoring of cattle. Bulletin 6. Rev. ed. East of Scotland College of Agriculture, Penicuik, Scotland.

Macmillan, K. L. and Peterson, A. J. 1993. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrus. Anim. Reprod. Sci. **33**: 1–25.

Marston, T. T., Lusby, K. S., Wettemann, R. P. and Purvis, H. T. 1995. Effects of feeding energy or protein supplements before or after calving on performance of spring-calving cows grazing native pasture. J. Anim. Sci. **73**: 657–664.

Martinez, M., Kastelic, J. P., Adams, G. P., Cook, B., Olson, W. O. and Mapletoft, R. J. 2002. The use of progestins in regimens for fixed-time artificial insemination in beef cattle. Theriogenology **57**: 1049–1059.

Martinez, M. F., Adams, G. P., Bergfelt, D. R., Kastelic, J. P. and Mapletoft, R. J. 1999. Effect of LH or GnRH on the dominant follicle of the first follicular wave of beef heifers. Anim. Reprod. Sci. **57**: 23–33.

Martinez, M. F., Kastelic, J. P., Adams, G. P., Janzen, E., McCartney, D. H. and Mapletoft, R. J. 2000. Estrus synchronization and pregnancy rates in beef cattle given CIDR-B, prostaglandin and estradiol, or GnRH. Can. Vet. J. **41**: 786–790.

Moriera, F., de la Sota, R. L., Diaz, T. and Thatcher, W. W. 2000. Effect of day of the estrous cycle at initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J. Anim. Sci. **78**: 1576.

Munro, R. K. 1989. The effects of duration and concentration of plasma progesterone on the fertility of post-partum cows treated with pregnant mare serum gonadotrophin and intravaginal progesterone. Aust. Vet. J. **66**: 43–45.

Pursley, J. R., Mee, J. F. and Wiltbank, J. N. 1995. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology **44**: 915–923.

Pursley, J. R., Wiltbank, M. C., Stevenson, J. S., Ottobre, J. S., Gaverick, H. A. and Anderson, L. L. 1997. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J. Dairy Sci. **80**: 295–300.

Rabe-Hesketh, S., Skrondal, A. and Pickles, A. 2002. Reliable estimation of generalized mixed models using adaptive quadrature. The Stata Journal **2**: 1–21.

Randel, R. D. 1990. Nutrition and postpartum rebreeding in cattle. J. Anim. Sci. **68**: 853–862.

Rhodes, F. M., McDougall, S., Burke, C. R., Verkerk, G. A. and Macmillan, K. L. 2003. Treatment of cows with an extended postpartum anestrous interval. J. Dairy Sci. **86**: 1876–1894.

Richards, M. W., Spitzer, J. C. and Warner, M. B. 1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J. Anim. Sci. **62**: 300–306.

Roche, J. F., Crowe, M. A. and Boland, M. P. 1992. Postpartum anoestrus in dairy and beef cows. Anim. Reprod. Sci. **28**: 371–378.

Schillo, K. K. 1992. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. J. Anim. Sci. **70**: 1271–1282.

Silcox, R. W., Powell, K. L. and Kiser, T. E. 1993. Ability of dominant follicles to respond to exogenous GnRH administration is dependent on their stage of development. J. Anim. Sci. **71** (Supp 1): 513.

Small, J. A., Ambrose, J. D., McCaughey, W. P., Ward, D. R., Sutherland, W. D., Glover, N. D. and Rajamahendran, R. 2001. The effects of gonadotrophin releasing hormone in prostaglandin F2a-based timed insemination programs for beef cattle. Can. J. Anim. Sci. **81**: 335–343.

Small, J. A. and McCaughey, W. P. 1999. Beef cattle management in Manitoba. Can. J. Anim. Sci. **79**: 539–544.

Stagg, K., Spicer, L. J., Sreenan, J., Roche, J. F. and Diskin, M. G. 1998. Effect of calf isolation on follicular wave dynamics, gonadotropin and metabolic hormone changes, and interval to first ovulation in beef cows fed either of two energy levels postpartum. Biol. Reprod. **59**: 777–783.

Stevenson, J. S., Lamb, G. C., Johnson, S. K., Medina-Britos, M. A., Grieger, D. M., Harmoney, K. R., Cartmill, J. A., El-Zarkouny, S. Z., Dahlen, C. R. and Marple, T. J. 2003. Supplemental norgestomet, progesterone, or melengestrol acetate increases pregnancy rates in suckled beef cows after timed inseminations. J. Anim. Sci. **81**: 571–586.

Thatcher, W. W., de l. S. R., Schmitt, E. J., Diaz, T. C., Badinga, L., Simmen, F. A., Staples, C. R. and Drost, M. 1996. Control and management of ovarian follicles in cattle to optimize fertility. Reprod. Fertil. Dev. **8**: 203–217.

Twagiramungu, H., Guilbault, L. A. and Dufour, J. J. 1995. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: a review. J. Anim. Sci. **73**: 3141–3151.

Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. **74**: 3583–3597.

Vasconcelos, J. L. M., Silcox, R. W., Rosa, G. J. M., Pursley, J. R. and Wiltbank, M. C. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology **52**: 1067–1078.

Wenkoff, M. S. 1987. The management of drug-induced manipulation of the estrous cycle in normal cows and heifers. Can. Vet. J. **28**: 366–373.

Wettemann, R. P., Lents, C. A., Ciccioli, N. H., White, F. J. and Rubio, I. 2003. Nutritional- and suckling-mediated anovulation in beef cows. J. Anim. Sci. **81**: E48–E59.

Wiltbank, J. N., Rowden, W. W., Ingallis, J. E. and Zimmerman, D. R. 1964. Influence of post-partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. J. Anim. Sci. **23**: 1049.

Xu, Z., Gaverick, H. A., Smith, G. W., Smith, M. F., Hamilton, S. A. and Youngquist, R. S. 1995. Expression of follicle-stimulating hormone and luteinizing hormone receptor messenger ribonucleic acids in bovine follicles during and first follicular wave. Biol. Reprod. **53**: 951–957.