# Chapter 4: FACTORS AFFECTING RESPONSES TO OESTRUS SYNCHRONISATION IN POST-PARTUM BEEF COWS

(EXPERIMENTAL)

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## 4.1 INTRODUCTION

In seasonally calving beef herds post-partum anoestrus and oestrus detection for precise timing of artificial insemination (AI) remain as major problems limiting reproductive efficiency. The effect of these on the interval from calving to conception influences the quantity and quality of calves weaned, since it determines both the reproductive rate and the spread of calving time. A wide range of treatment regimes have been developed to induce and synchronise oestrus and ovulation for the benefit and convenience of the cattle breeder. However, despite many years of research, results are still inconsistent. In Northern Australia in 1990/1991, for example, almost half (48%) of beef producers in a survey reported troubles with heat detection or cows not responding to synchronising treatments, and, consequently, 37% of artificial breeding programs did not use synchronising drugs and only 6% of programs used set time inseminations (Boothby *et al.,* 1992). This calls for further research into more effective oestrous synchronisation.

The present chapter reports three oestrous synchronisation experiments carried out on two commercial properties typical of the pasture-based seasonally calving beef enterprises in New England, Australia. The objectives were to examine the efficacy of several drug regimes, the effect of protected lipid supplementation and the influence of some important factors such as the nutritional status and reproductive state of animals prior to treatment on reproductive responses to oestrous synchronisation.

# 4.2 MATERIALS AND METHODS

## 4.2.1 ANIMALS AND TREATMENTS

### 4.2.1.1 Experiment 1

The first experiment was carried out on a post-partum Angus breeding beef herd on a commercial property ("Kilburnie", Walcha, NSW). In the first year (1994), the experiment aimed to compare the effectiveness of the CIDR-B and the Crestar (different sources of progesterone/progestogen) in treatment of oestrous synchronisation. Cows (n = 104) with an average age (Mean ± SE) of 6.5 ± 0.2 (4-11) years and a post-partum interval (PPI) from calving to treatment (Mean ± SE) of 73.1 ± 1.1 (50-93) days were allocated at random to two treatment groups, A and B. On day 0, at the start of treatment (1 November), all cows were weighed, assessed for body condition score (BCS) and ultrasonographically examined for ovarian activity. Cows in group A received a controlled intravaginal drug releasing device, of which there were limited numbers, (CIDR-B, Carter Holt Harvey Plastic Products, Haminton, NZ) containing 1.9 mg natural progesterone and 10 mg oestradiol benzoate (OB). Cows in group B received one Crestar implant (Intervet Pty. Ltd., NSW, Aust.) containing 3 mg Norgestomet (a synthetic progesterone) inserted subcutaneously in the ear. At the same time, a 2 ml Crestar injection containing 3 mg Norgestomet and 5 mg oestradiol valerate (OV) was given intramuscularly. Nine days later the CIDR-B device and the Crestar implant were removed. At this time, 500 i.u. of PMSG (Folligon, Intervet Pty. Ltd., NSW, Aust.) was injected intramuscularly into all cows.

Cows were detected for oestrus for three days after progesterone withdrawal and those showing heat were artificially inseminated around 12 hours after observed oestrus. The remainder of the herd were "blanket" inseminated 54-56 hours after PMSG injection. Calves were removed from the dams during the period up until artificial insemination (AI). Two weeks later "back-up" fertile bulls were placed with the herd for 8 weeks. Pregnancy diagnosis was undertaken 90 days following treatment.

The following year (1995), the experiment was repeated on the herd but only using the Crestar treatment. Pasture conditions were much better in 1995 compare with 1994. Cows (n = 166) had an average age (Mean ± SE) of 7.3 ± 0.2 (5-12) years and a post-partum interval (PPI) from calving to treatment (Mean ± SE) of 68.6 ± 1.0 (55-107) days. The Crestar treatment began on either 30 October or 13 November after the cows were weighed, assessed for body condition score (BCS) and ultrasonographically examined for ovarian activity. Nine days later the Crestar implant and CIDR-B device were removed and 500 i.u. of PMSG/head was injected intramuscularly into all cows. Other procedures were also the same as in 1994.

#### 4.2.1.2 Experiment 2

The second experiment tested the effects of PMSG dose on oestrous response and pregnancy rates and the effect of additional progesterone given post AI on conception following a short term CIDR-B synchronisation treatment. It was conducted in 1994 on 227 Hereford breeding cows on another commercial property ("Pine Hill", Mallanganee, NSW). The cows had an average age (Mean ± SE) of 5.1 ± 0.1 (3-9) years and the post-partum interval from calving to treatment (Mean ± SE) was 51.8 ± 0.7 (31-76) days. Cows were allocated randomly to treatment groups 1 and 2 with group 3 representing a later cycle calving group. At the start of treatment cows were weighed and assessed for BCS. All three groups were given a controlled internal drug release device - type B (CIDR-B, Carter Holt Harvey Plastic Products, Haminton, NZ) containing 1.9 mg natural progesterone (without OB). The CIDR-B device was removed nine days later from group 1 and 7 days later from groups 2 and 3. At CIDR-B removal each cow in groups 1 and 2 was given an injection of either 450 or 550 i.u. of PMSG (Pregnecol, Horizon Animal Reproduction Pty. Ltd., NSW, Aust.); all cows in group 3 received 550 i.u. of PMSG. Calves were removed from the dams after PMSG injection until after AI.

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<tr><th>Date</th><th>9/9</th><th>11/9</th><th>16/9</th><th>17/9</th><th>18/9</th><th>20-21/9</th><th>28/9</th><th>4/10</th><th>10/10</th><th>11/10</th><th>13-14/10</th><th>21/10</th><th>28/10</th><th>20/12</th></tr>
<tr><td>Gp 1</td><td>CIDR in</td><td></td><td>Scan</td><td>PG in</td><td rowspan="2">CIDR out and PMSG in</td><td rowspan="2">AI</td><td rowspan="2">Scan</td><td rowspan="3">CIDR in</td><td rowspan="2"></td><td rowspan="2">CIDR out</td><td rowspan="3">AI</td><td rowspan="2">Scan for Preg.</td><td rowspan="3">Bulls in</td><td rowspan="3">Scan for Preg.</td></tr>
<tr><td>Gp 2</td><td></td><td>CIDR in</td><td>Scan /PG in</td><td></td></tr>
<tr><td>Gp 3</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>PG in</td><td>CIDR out/ PMSG in</td><td></td></tr>
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Table 4.1: *Oestrus manipulation protocol of experiment 2*

Two days before CIDR-B removal all cows in groups 1 and 2 were monitored for ovarian activity. Those having a *corpus luteum* (CL) were injected with 0.5 mg of cloprostenol, a synthetic prostaglandin (Estrumate, Pitman Moore Co.). Oestrus was detected for 3 days following CIDR-B removal. Cows observed in oestrus in the morning were artificially inseminated in the afternoon and cows observed in oestrus in the afternoon were artificially inseminated the following morning. Those not showing oestrus were inseminated 54-56 hours after PMSG injection.

All cows in groups 1 and 2 were examined for ovulatory response (existence of a CL) ten days after the first CIDR-B removal. A second CIDR-B device was inserted into these cows two weeks after first AI in an attempt to increase fertility to first AI and synchrony of returns. The second CIDR-B remained in the cows for 7 days and those cows showing oestrus after removal of this CIDR-B were again artificially inseminated.

Cows in group 3 received only a single CIDR-B treatment, but all were given 2 ml of Estrumate (Pitman Moore Co.) one day before CIDR-B removal without being monitored for ovarian status. As for groups 1 and 2, oestrus was also detected for 3 days following CIDR-B removal. Cows observed in oestrus in the morning were artificially inseminated in the afternoon and cows observed in oestrus in the afternoon were artificially inseminated the following morning. Cows not showing oestrus were inseminated 54-56 hours after PMSG injection.

Two weeks following the second AI in groups 1 and 2 and the single AI in group 3, back-up fertile bulls were run with the herd for 8 weeks. Pregnancy was diagnosed 30 and 90 days following the first AI in groups 1 and 2, and 70 days following the single AI in group 3.

#### 4.2.1.3 Experiment 3

This experiment was undertaken in 1995 on 144 cows from the same herd as experiment 2. It was designed primarily to evaluate the potential of a protected lipid supplement called Rumentek (Protected Lipid, Rumentek Industries Pty. Ltd., NSW, Aust.), fed for a short period prior to AI, as a cost effective means to stimulate ovarian activity and therefore improve responses to oestrus synchronisation treatment in post-partum beef cows. Cows had an average age (Mean ± SE) of 5.9 ± 0.2 (4-10) years and the post-partum interval from calving to treatment (Mean ± SE) was 69.1 ± 1.1 (52-86) days. All cows, run at pasture, were initially fed a base supplement of 1 kg/h/d of cottonseed meal pellets (37% crude protein, <3% fat) for 2 weeks. On 1/9/1995, the cows were assigned (with approximate balance on ovarian cyclicity) to two feeding groups either to remain on the cottonseed meal supplement, as a control, or to be fed 0.5 kg/h/d of Rumentek (37.5% crude protein, 35% fat). The supplements were fed twice weekly. The different supplements were fed for 4 weeks prior to first AI and continued until the second AI (total 8 weeks). It has been proposed that the linoleic acid component of the total LCFA's is the most important determinant of responses. A feeding level of supplement supplying around 60-70g/h/d of linoleic acid was considered to be appropriate for this study and this was met by 0.5 kg/h/d of the Rumentek supplement, on the basis of 40% total LCFA, of which 50% is linoleic acid, with 70% protection.

The first AI was performed following an 8-day CIDR-B synchronisation program. The CIDR-B contained 1.9 mg natural progesterone (without OB). Cows with *corpora lutea* based on ovarian examination on the day before CIDR-B removal were given 0.5 mg of cloprostenol (estroPLAN, Parnell Laboratories Pty. Ltd., NSW, Aust.). All cows were given 500 i.u. of PMSG (Pregnecol, Horizon Animal Reproduction Pty. Ltd., NSW, Aust.) at the time of CIDR-B removal. All cows were given a second 7-day CIDR-B plus PMSG treatment beginning two weeks after the first AI. The two cycles of AI were conducted based on detected oestrus. Calves were removed from their dams following CIDR-B removal until AI. Ovarian examination, assessment of BCS and live weight records were made at the initiation of treatment allocation and just prior to CIDR-B removal. Fertile bulls were introduced for paddock backup matings for 8 weeks beginning 2 weeks after the second AI. Pregnancy was diagnosed 62 days after the first AI.

### 4.2.2 OVARIAN ACTIVITY EXAMINATION, PREGNANCY DIAGNOSIS, BODY CONDITION SCORE ESTIMATION, AND OESTRUS DETECTION

Ovarian activity was assessed via ultrasonography using a real time, B-mode ultrasonic scanner equipped with a 7.5 MHz linear array transrectal transducer (*Aloka* 210, DXII, Aloka Co., Ltd., Tokyo, Japan). The cows were examined to detect the existence of the *corpus luteum* (CL), and the number and the size of follicles ≥5 mm in diameter. Pregnancy was also diagnosed using this equipment. Pregnancy was confirmed on the basis of the presence of a fluid sac or imaging of foetal head.

Body condition score (BCS) was assessed by palpation of spinous processes near the tail at the time of treatment and scored according to a 5-point scale (1 = emaciated to 5 = obese) at the start of treatment.

Oestrus detection was done by means of Kamar heat detector (Kamar Inc.) in experiment 1 for 3 days following PMSG injection. The Kamar was put on the rump of each cow at the time of Crestar and CIDR-B removal. If a cow was in behavioural oestrus the Kamar was broken and turned red due to the mounting of other cows or a teaser bull running with the herd. In experiments 2 oestrus was detected by means of tail paint, a paint-on product (Heat Paint, Tasman Chemicals), and direct observations for 3 days following termination of treatment. In experiment 3 oestrus was also detected by means of tail paint with an aerosol spray-on product (Heat Paint, Tasman Chemicals) and direct observations for 3 days following termination of treatment.

### 4.2.3 STATISTICAL ANALYSES

The effects of the various factors on binomial responses (yes or no) to oestrus control were assessed statistically by means of **iterative weighed least squares** for **binomial** data with the **logit** link function using the **Generalised Linear Models** procedure of the statistical analysis package **REG** (1992) with the following model:

$$\text{Logit(P)} = \text{Ln (P/1-P)} = \alpha + \beta X + e$$

where,
- **P**: **Pr (Y=1/X),** response probability
- **Y:** response, **Y** = **1** with probability **P**; = **0** with probability **1-P**
- **X:** a set of fixed effects and co-variables
- **β:** associated vectors of coefficients
- **α:** intercept parameter
- **e:** error term

For responses of normal distribution the **General Linear Models** procedure for analysis of variance of the same package was used with the following model:

$$Y = \alpha + \beta X + e$$

where,
- **Y:** response
- **X:** a set of fixed effects and co-variables
- **β:** associated vectors of coefficients
- **α:** intercept parameter
- **e:** error term

The responses and effects of concern in the three experiments are as follows:

*Experiment 1*:

The main responses (dependent variables) included synchronised oestrous response (oestrus observed following treatment termination, referred to as *oestrus*), synchronised oestrus fertility (pregnancy to AI of those showing oestrus, referred to as *oestrus fertility*), AI pregnancy (pregnancy to AI of all treated cows following CIDR-B treatment, referred to as *AI pregnancy*), total pregnancy (pregnancy to both AI and back-up bulls taken together, referred to as *total pregnancy).*

The explanatory (independent) variables included both fixed effects (factor-level) and co-variables (continuous variables). Treatment, year, CL (no or yes), and grouping of the largest follicle were treated as fixed effects. Body condition score, body weight, post-partum interval, number of follicles ≥ 5 mm in diameter, the size of the largest follicle were treated as co-variables of both first and second (quadratic) degree.

*Experiment 2:*

The responses were synchronised oestrus, synchronised oestrus fertility, ovulatory response (the existence of a CL 10 days following treatment, referred to as *ovulation*), CL development (the size of the CL, referred to as *CL size*), first AI pregnancy (pregnancy to AI of all treated cows following the first CIDR-B treatment, referred to as *first pregnancy*), overall AI pregnancy (pregnancy of all treated cows to all cycles of AI in experiments 2 and 3, referred to as *AI pregnancy*), total pregnancy (pregnancy to all AIs and back-up bulls taken together, referred to as *total pregnancy*), and interval to AI time following treatment.

The independent variables consisted of fixed effects, which were treatments, PMSG dosage, CL (no or yes), grouping of the largest follicle, and co-variables, which were body condition score, body weight, post-partum interval, number of follicles ≥ 5 mm in diameter, and the size of the largest follicle of both first and second (quadratic) degree.

*Experiment 3:*

The responses were synchronised oestrus, synchronised oestrus fertility, first AI pregnancy, overall AI pregnancy, total pregnancy, which were similar to those in experiment 2, and the interval to observed oestrus following treatment.

The explanatory variables were both fixed effects including supplement, CL (no or yes), and grouping of the largest and co-variables including body condition score, body weight, post-partum interval, number of follicles ≥ 5 mm in diameter and the size of the largest follicle of both first and second (quadratic) degree.

In all three experiments, possible interactions between effects were considered. Oestrous response (no or yes) was also treated as a fixed effect variable when analysing the dependence of pregnancy to AI on detected oestrus.

# 4.3 RESULTS

## 4.3.1 EXPERIMENT 1

### 4.3.1.1 Effects of Treatments

Only 3 of the 104 cows in 1994 were cycling (had a CL) at the time of commencement of treatment; therefore, the herd was subsequently considered to be acyclic with the three animals having a CL excluded from for the analyses. The two treatment regimes (CIDR-B plus PMSG and Crestar plus PMSG) produced significantly different levels of synchronised oestrous response and pregnancy rate to AI (Table 4.2). Crestar induced behavioural oestrus in 52.6%, compared to only 17.4% among cows treated with CIDR-B ($P < 0.001$).

Table 4.2: *Efficacy of CIDR-B and Crestar treatments - 1994*

| Response | Treatment Group | | Probability of |
|---|---|---|---|
| | CIDR | Crestar | Difference (P) |
| *No. of cows* | *23* | *78* | |
| Oestrus (%) | **17.4** | **52.6** | **< 0.001** |
| Oestrus Fert. (%) | 50.0 | 54.8 | > 0.05 |
| AI Preg.(%) | **8.7** | **32.1** | **< 0.05** |
| T. Preg. (%) | 82.6 | 78.2 | > 0.05 |

In spite of no significant difference in oestrus fertility, pregnancy rate to AI of all cows treated with Crestar was significantly higher (P < 0.05) than that of cows treated with CIDR-B, being 32.1 vs 8.7%, respectively.

### 4.3.1.2 Effects of Ovarian Cyclicity

The effects of ovarian cyclicity (presence of CL) were analysed separately in two years. The results are present in Table 4.3. In 1994 cows having a CL at initiation of treatment had a higher synchronised oestrous response than those not having a CL (P < 0.05). Pregnancy rates were higher in cycling cows on the average although the differences were not significant with the proportion of cycling cows being too small (3 out of 104). In 1995 no responses were significantly influenced by the existence of a CL at the start of progesterone treatment.

Table 4.3: *Effects of ovarian cyclicity on responses to treatment in experiment 1*

| Response | Ovarian Cyclicity (CL) | | | | | |
|---|---|---|---|---|---|---|
| | 1994 | | | 1995 | | |
| | No | Yes | P | No | Yes | P |
| *No. of cows* | *101* | *3* | | *36* | *39* | |
| Oestrus (%) | **44.6** | **100.0** | **< 0.05** | 72.2 | 69.2 | > 0.05 |
| Oestrus Fert. (%) | 54.4 | 66.7 | > 0.05 | 56.0 | 59.3 | > 0.05 |
| AI Preg.(%) | 26.7 | 66.7 | > 0.05 | 42.9 | 51.3 | > 0.05 |
| T. Preg. (%) | 79.2 | 100.0 | > 0.05 | - | - | - |

### 4.3.1.3 Effects of Follicular Development

The differences in the status of follicular development were measured by the differences in the number of follicles ≥ 5 mm in diameter and the size of the largest follicle. In 1994, fertility of synchronised oestrus was dependent (P < 0.05) on the size of the largest follicle; the larger the follicle the lower the fertility became (Figure 4.1).

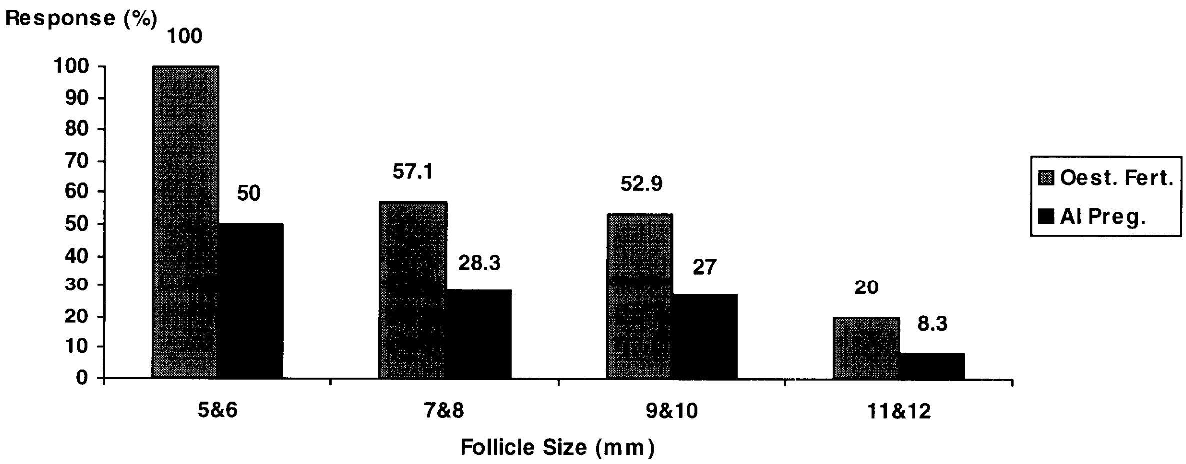


Figure 4.1: *Effects of size of the largest follicle on oestrus fertility and AI pregnancy, experiment 1, 1994.*

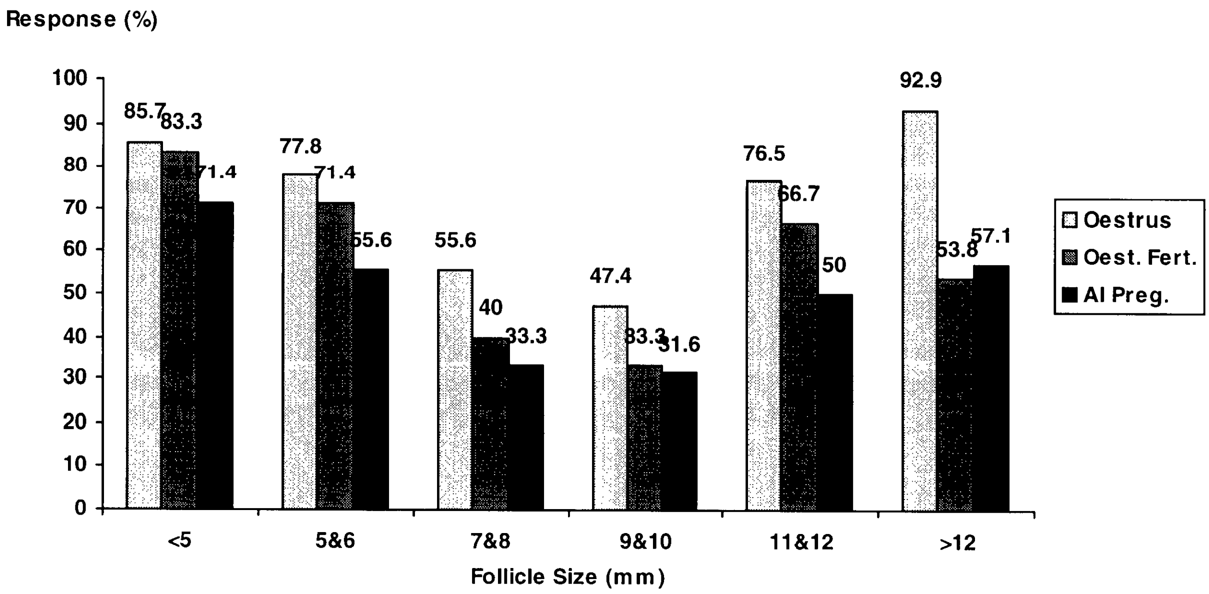


Figure 4.2: *Effects of size of the largest follicle on oestrous response and AI pregnancy, experiment 1, 1995.*

All cows showing oestrus with the largest follicle of 5-6 mm in diameter were pregnant to AI following observed oestrus, compared to only 20% of those having the largest follicle of 11-12 mm in diameter. Pregnancy to AI showed the same trend although it was not statistically significant. Only 8.3% of cows having the largest follicle of 11-12 mm were pregnant to AI following treatment, whereas 50% of those having the largest follicle of 5-6 mm were pregnant to AI.

In 1995, synchronised oestrous response was influenced by the size of the largest follicle in a non-linear manner ($P < 0.05$). Cows with the largest follicle of 9-10 mm in diameter had a lower response than those having smaller or bigger follicles (Figure 4.2). The effects of follicle size on pregnancy rate to AI and oestrus fertility showed a similar tendency although not significantly.

### 4.3.1.4 Effects of Years

As can be seen in Table 4.4, BCS and BW, indicators of the nutritional status of the herd at the start of synchronisation treatments, were significantly different between the

Table 4.4: Effects of years on reproductive responses to Crestar treatment. (Mean ± SE)

| Response | Year | | Probability of Difference (P) |
|---|---|---|---|
| | 1994 | 1995 | |
| *No. of Animals* | *68* | *166* | |
| BW1* (kg) | *468.3 ± 6.5* | *445.9 ± 5.2* | *<0.01* |
| BW2 **(kg) | *376.2 ± 4.7* | *532.7 ± 5.7* | *<0 .001* |
| BCS | *2.39 ± 0.07* | *4.50 ± 0.05* | *<0 .001* |
| Largest Fol. (mm) | *9.1 ± 0.2* | *10.7 ± 0.4* | *<0 .001* |
| Cycling (%) | *2.9* | *52.6* | *< 0.001* |
| Oestrus (%) | **53.2** | **80.3** | **< 0.001** |
| Oest. Fert. (%) | 54.8 | 61.4 | >0.05 |
| AI Preg. (%) | **31.6** | **50.6** | **<0.05** |

* in early March; ** in early November at start of treatment.

two years (P < 0.001), cows being in much better nutritional state in 1995. Also, in comparison to 1994, there were a greater proportion of cycling cows ( P < 0.001) and a larger mean follicle size (P < 0.001) in 1995. Reproductive responses to treatment were improved in 1995 for synchronised oestrus (P < 0.001), pregnancy rate to AI (P < 0.05). Oestrus fertility was also higher in 1995 although the difference was not statistically significant.

#### 4.3.1.5 Other Effects

There were no significant effects of PPI on synchronised oestrous response, oestrus fertility, pregnancy rate to AI and total pregnancy rate in this experiment.

Pregnancy to AI was found to be very strongly associated with behavioural oestrus observed (P < 0.001). Out of 29 AI-pregnant cows only 2 had not been observed in oestrus before insemination. Among 104 treated cows 56.2% of those having been observed in oestrus conceived to AI, while only 3.5% of cows not having showed oestrus conceived to AI.

### 4.3.2 EXPERIMENT 2

#### 4.3.2.1 Effects of Treatments

There were no significant differences in any responses between groups 1 and 2, which both had double CIDR-B treatments but differed in the duration of the first CIDR-B treatment (7 vs 9 days); therefore, the data set of groups 1 and 2 were pooled into one group as receiving double CIDR-B to compare with the other group which were given only a single CIDR-B. The results are shown in Table 4.5.

Oestrus fertility of the single CIDR-B treated group was higher (P < 0.05) than that following first CIDR-B treatment of the group of cows given double CIDR-B treatments (P < 0.05). Pregnancy rate to AI following the first CIDR-B treatment was also higher in single CIDR-B treated cows compared with that of double CIDR-B treated animals although the difference was not significant (P > 0.05). On the other hand, the overall pregnancy rate to AI of the single CIDR-B treated group, which received only one AI, was significantly lower (P < 0.05) than that of the double CIDR-B treated cows, which were given two cycles of AI; that is, the double CIDR treatment protocol resulted in an advantage of 11.4% in pregnancy rate to AI, although oestrous response and the total pregnancy rate to both AI and natural mating were not significantly different between the treatment groups (P > 0.05).

Table 4.5: *Effects of double vs single CIDR-B treatments.*

| Response | Treatment Group | | Probability of Difference (P) |
|---|---|---|---|
| | double CIDR | single CIDR | |
| *No. of cows* | *134* | *93* | |
| Oestrus* (%) | 83.6 | 78.5 | >0.05 |
| Oestrus Fert.* (%) | **62.5** | **75.3** | **<0.05** |
| First AI Preg.*(%) | 59.0 | 67.7 | >0.05 |
| Overall AI Preg.(%) | **79.1** | **67.7** | **<0.05** |
| T. Preg. (%) | 87.3 | 81.7 | >0.05 |

* after the first CIDR-B treatment.

### 4.3.2.2 Effects of PMSG Dosage

Table 4.6 shows the effects of the two doses of PMSG (550 vs 450 i.u.) on responses in double CIDR-B treated cows. Ovulatory response was significantly higher with 550 i.u. of PMSG (P < 0.05). Oestrous response was also much higher in this group (89.2 vs 78.3%), but the difference was not statistically significant. There were no significant differences in other responses.

There was a significant interaction between PMSG dose and ovarian cycling state (Figure 4.3) on pregnancy rate to both cycles of AI (P < 0.01) and total pregnancy rate to both AI and backup bulls (P < 0.01). Overall AI pregnancy rate was higher in cycling cows treated with 450 i.u. of PMSG (95%) than in non-cycling cows treated

Table 4.6: *Effects of PMSG dose on responses to oestrous synchronisation.*

| Response | PMSG Dose (i.u.) | | Probability of |
|---|---|---|---|
| | 450 | 550 | Difference |
| *No. of cows* | *69* | *65* | |
| Oestrus (%) | *78.3* | *89.2* | >0.05 |
| Ovulation (%) | **85.5** | **95.4** | **<0.05** |
| CL Size (mm) | 13.5 | 15.1 | >0.05 |
| First AI Preg.(%) | 60.9 | 56.9 | >0.05 |
| Oestrus Fertility(%) | 66.7 | 58.6 | >0.05 |
| Overall AI Preg. (%) | 79.7 | 78.5 | >0.05 |
| T. Preg. (%) | 85.5 | 89.2 | >0.05 |

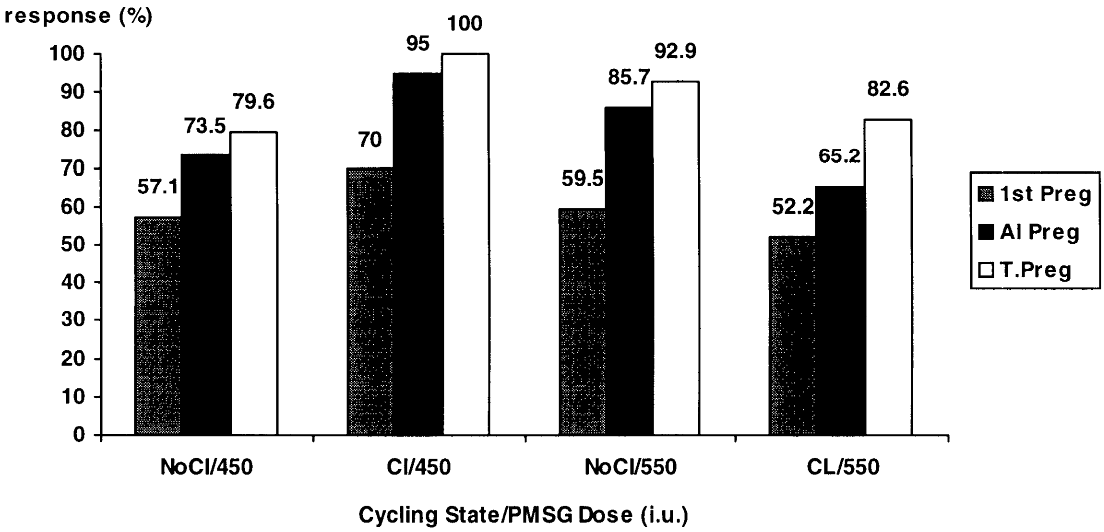


Figure 4.3: *Interaction between PMSG dose and ovarian cycling state on reproductive responses to oestrous synchronisation.*

with 550 i.u. of PMSG (85.7%), which was higher that in non-cycling animals treated with the lower dose (73.5%) or in cycling animals treated with the higher dose (65.2%). Similarly, total pregnancy was higher in cycling animals treated with 450 i.u. of PMSG (100%), compared with non-cycling animals treated with 550 i.u. of PMSG (92.9%) or with cycling cows treated with the higher dose (82.6%) and non-cycling cows treated with the lower dose (79.6%). Pregnancy rate to first AI showed the same tendency although the interaction was not statistically significant.

### 4.3.2.3 Effects of Ovarian Cyclicity

The effect of ovarian activity on responses was examined only in double CIDR-treated animals. Of 134 cows, 43 (32.1 %) had a CL and 91 (67.9%) had no CL two days before removal of the first device. On the average, as can be seen in Table 4.7, almost all the responses were greater in cows having a CL compared with those not found having a CL. Nevertheless, only the differences in oestrous response and in CL size were statistically significant (P < 0.01 and 0.05, resp.). All cows having a CL that ovulated showed oestrus; whereas up to 11% of cows not having a CL ovulated without being detected in oestrus.

Table 4.7: *Effects of ovarian cyclicity on reproductive responses in experiment 2.*

| Response | Ovarian Cyclicity (CL) | | Probability of Difference (P) |
|---|---|---|---|
| | No | Yes | |
| *No. of cows* | *91* | *43* | |
| Oestrus* (%) | **78.0** | **95.3** | **<0.01** |
| Ovulation* (%) | 89.0 | 93.0 | >0.05 |
| CL Size* (mm) | **13.6** | **15.7** | **<0.05** |
| Oestrus Fert.* (%) | 62.0 | 63.4 | >0.05 |
| First AI Preg.*(%) | 58.2 | 60.5 | >0.05 |
| Overall AI Preg.(%) | 79.1 | 79.1 | >0.05 |
| T. Preg. (%) | 85.7 | 90.7 | >0.05 |

* after the first CIDR-B treatment

### 4.3.2.4 Effects of Follicular Development

It was found that toward the end of the progesterone treatment there still existed a difference in the size of the largest follicle among treated animals and this strongly influenced the interval to oestrus expression as reflected by the time of AI which was based on detected oestrus (P < 0.001). The time from the termination of treatment to observed oestrus (AI) was non-linearly related to the size of the largest follicle, in which cows having the largest follicle of 9-10 mm diameter had the longest interval to AI as can be seen in Figure 4.4. However, the follicular status just before progestogen removal did not significantly affect oestrous response, oestrus fertility, ovulatory response, CL size or the different pregnancy rates (P > 0.05).

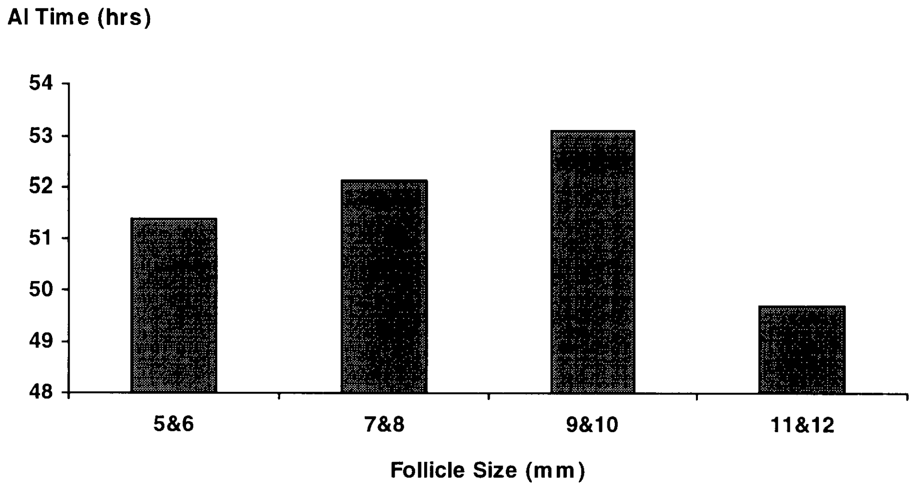


Figure 4.4: *Relation between the size of the largest follicle and the time of AI.*

### 4.3.2.5 Effects of Nutritional Status

Figure 4.5 shows the effects of BCS of the animal at the start of treatment on oestrous response and CL size. Cows with higher BCSs showed higher levels of these responses. The logit link function of oestrous response was linearly related to BCS (P < 0.05). An increase of 1 BCS resulted in an increase of 3.0 mm in diameter of the CL (P < 0.001). However, BW did not significantly influence any responses.

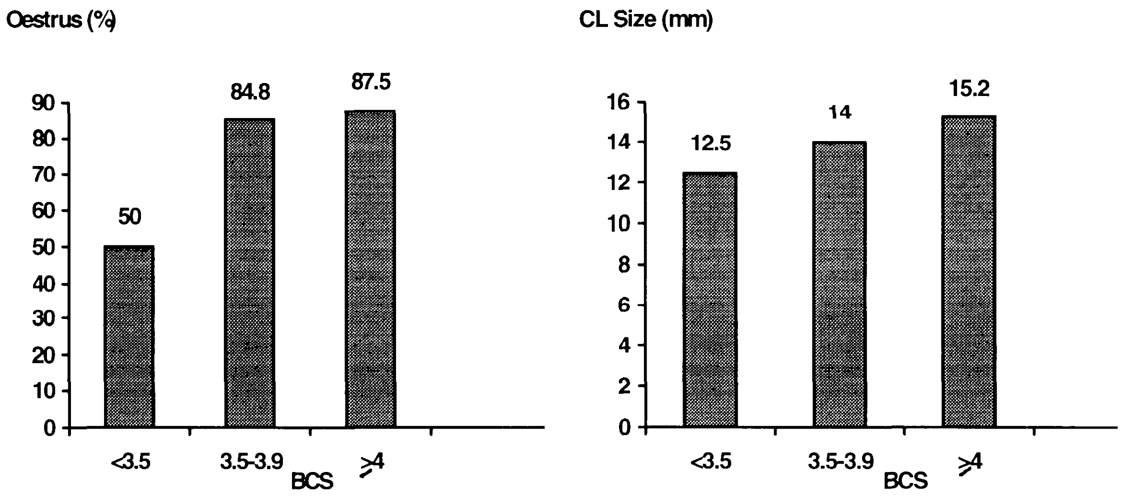


Figure 4.5: *Effects of BCS on oestrous response and CL size in experiment 2.*

#### 4.3.2.6 Other Effects

The effects of PPI on oestrous response, ovulatory response, CL size, pregnancy rates, and the interval to AI were not significant ($P > 0.05$).

Pregnancy rate to AI was much higher ($P < 0.01$) in cows showing oestrus (67.2%) compared to those not showing oestrus (40.9%) when animals were inseminated at a fixed time. There was also an obvious difference in ovulatory response between cows exhibiting and not exhibiting oestrus ($P < 0.001$) with 98.2% of the cows showing oestrus ovulating, compared to only 54.5% of the cows which did not exhibit oestrus.

### 4.3.3 EXPERIMENT 3

#### 4.3.3.1 Effects of Supplementation of Protected Lipids

Table 4.8 shows the effects of supplementation with Rumentek in comparison with "control" cottonseed meal on reproductive responses to the double CIDR-B treatment.

cows. Rumentek treatment resulted in significantly higher synchronised oestrous response ($P < 0.05$) and total pregnancy rate after two cycles of AI ($P < 0.05$). There was a significant 16% advantage in pregnancy rate to 2 cycles of AI in favour of Rumentek compared to cottonseed meal treatment. Oestrus fertility and pregnancy rate to first AI were also higher in the Rumentek fed group although the differences were not statistically significant. In addition, there were more Rumentek treated cows pregnant among those not returning ($P < 0.05$). The mean follicle size was greater ($P < 0.05$) in the Rumentek supplemented group. Cows supplemented with Rumentek had a significant shorter ($P < 0.001$) and less variable interval from the termination of synchronisation treatment to observed oestrus with the mean and standard derivation being 40.8 ± 6.0 vs 45.0 ± 7.3 hrs.

Table 4.8: *Effects of protected lipids supplementation on reproductive responses (mean± SE) to oestrous synchronisation.*

| Response | Treatment Group | | Probability of |
|---|---|---|---|
| | Cottonseed meal | Rumentek | Difference (P) |
| *No. of cows* | *70* | *74* | |
| *PPI (days)* | *67.8 ± 1.4* | *68.6 ± 1.4* | *>0.05* |
| *BW (kg)* | *433.2 ± 5.4* | *435.5 ± 5.1* | *>0.05* |
| *BCS* | *4.38 ± 0.04* | *4.39 ± 0.05* | *>0.05* |
| Largest Fol. (mm) | **7.5 ± 0.3** | **8.4 ± 0.4** | **<0.05** |
| Oestrus* (%) | **80.0** | **91.9** | **<0.05** |
| Oestrus Fert.* (%) | 58.2 | 61.8 | > 0.05 |
| Oestrus Time (hrs) | **45.0 ± 1.0** | **40.8 ± 0.7** | **<0.001** |
| First AI Preg.*(%) | 46.4 | 60.8 | = 0.08 |
| Overall AI Preg. (%) | **60.9** | **77.0** | **<0.05** |
| Preg./not return (%) | **63.0** | **79.0** | **<0.05** |

* after the first CIDR-B treatment

#### 4.3.3.2 Effects of Ovarian Cyclicity

Synchronised oestrus response was higher in those cows having a CL compared to cows not having a CL; however, the difference was not statistically significant (Table 4.8). As in experiments 1 and 2, there were no significant differences in oestrus fertility and AI pregnancy rates between cows with and without a CL at commencement of the experiment. The only significant difference between cycling and non-cycling cows was in the interval to observed oestrus following treatment; cows with a CL showed oestrus later than those without a CL (P < 0.05).

Table 4.9: *Effects of ovarian cyclicity on reproductive responses in experiment 3.*

| Response | Ovarian Cyclicity (CL) | | Probability of |
|---|---|---|---|
| | No | Yes | Difference (P) |
| *No. of cows* | *48* | *16* | |
| Oestrus* (%) | 77.1 | 93.8 | >0.05 |
| Oestrus Time* (hrs) | **42.4 ± 1.2** | **47.6 ± 2.0** | **<0.05** |
| Oestrus Fert.* (%) | 56.8 | 53.3 | >0.05 |
| First AI Preg.* (%) | 47.9 | 50.0 | >0.05 |
| Overall AI Preg. (%) | 66.7 | 62.5 | >0.05 |

* after the first CIDR-B treatment

#### 4.3.3.3 Effects of Follicular Development

The size of the largest follicle prior to first implant withdrawal was positively related to pregnancy rate to AI (P < 0.05) and to oestrus fertility (P < 0.05) after first CIDR-B treatment (Figure 4.6). Total pregnancy rate to both cycles of AI showed the same tendency in relation to the size of the largest follicle although the difference was not significant. The effect of the stage of follicular development on oestrous responses was not linear (P > 0.05).

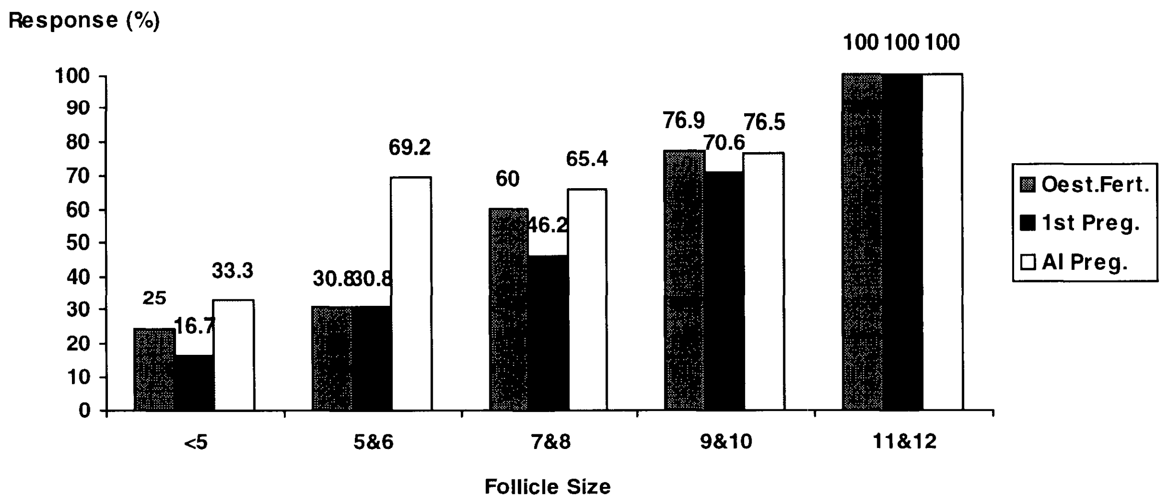


Figure 4.6: *Effects of size of the largest follicle at the end of progesterone treatment on responses in experiment 3.*

### 4.3.3.4 Effects of Nutritional Status

BCS positively influenced synchronised oestrous response (P < 0.05), pregnancy rate to first AI (P < 0.05), and pregnancy rate to both cycles of AI (P < 0.01). BCS was linearly related to the logit link function of these responses. In absolute terms, cows of higher BCSs showed higher levels of these responses (Figure 4.7). However, the effects of BW on reproductive responses were not significant.

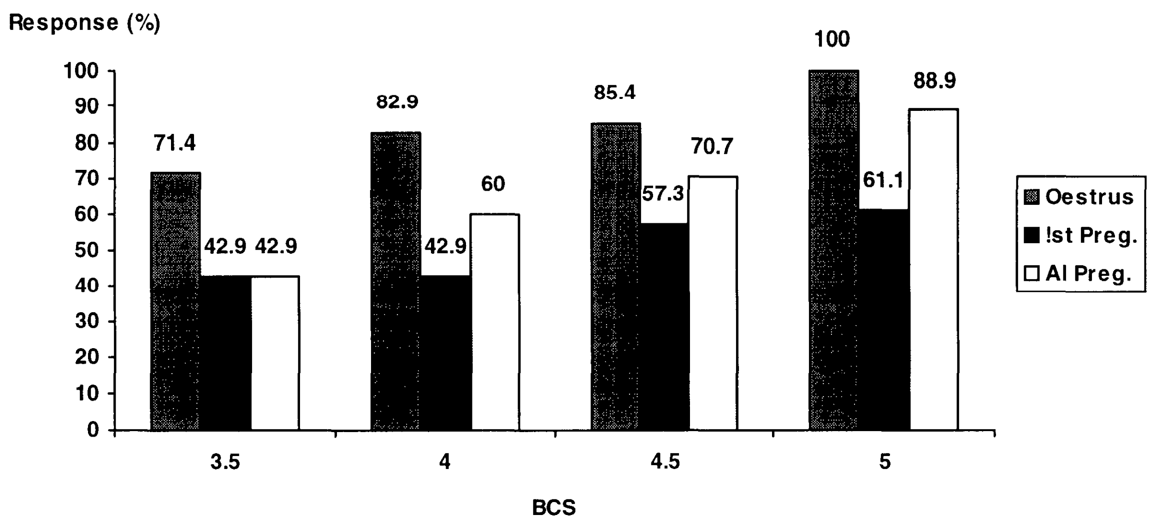


Figure 4.7 : Effects of BCS on reproductive response in experiment 3.

#### 4.3.3.5 Other Effects

The post-partum interval from calving to synchronisation treatment did not significantly influence synchronised oestrous response, oestrus fertility, pregnancy rates, and the interval to observed oestrus ($P > 0.05$).

Observed oestrus was strongly associated with pregnancy to AI; cows showing oestrus had a subsequent pregnancy rate to AI of 62.7%, compared to only 13.2% in those not exhibiting oestrus ($P < 0.001$). In addition, the interval from termination of treatment to observed oestrus was non-linearly related to the incidence of subsequent pregnancy ($P < 0.001$), the cows with highest pregnancy rate to AI having an interval of 41.5 hrs.

## 4.4 DISCUSSION

The purpose of the three experiments was primarily to examine the effectiveness of different drug regimes and the influence of long term nutritional status, the reproductive state of animals and the effects of supplemented protected lipids on reproductive responses to oestrous synchronisation. This discussion integrates the three experiments to look at these aspects.

### 4.4.1 EFFECTS OF SYNCHRONISATION TREATMENTS

In experiment 1, the CIDR-B treatment produced much poorer reproductive responses in comparison not only with the Crestar treatment. The responses to CIDR-B in this experiment were also lower in comparison to responses to CIDR-B treatment used for post-partum cows in previous reports (see reviews by Odde, 1990, Larson *et al.*, 1992, Macmillan *et al.,* 1993) and also with the CIDR-B treatment in experiment 2. However, the number of cows treated with CIDR-B in this experiment was small and the treatment was examined in only one year when very poor nutritional conditions existed as reflected in BW and BCS. Therefore, further comparative investigation is necessary to see if CIDR-B actually produces poor results in nutritionally deprived beef cows.

Better responses to Crestar compared to CIDR-B treatment in this study suggests that the Crestar treatment protocol can be applied to regulate oestrus and ovulation in post-partum beef suckled cows which are in poor condition. This may be due to the action of Norgestomet as a progestogen in comparison with natural progesterone in CIDR-B. Moffatt *et al.* (1993) found that Norgestomet bound to the bovine endometrial progesterone receptor with an affinity for the receptor that was somewhat greater than that of progesterone. Such a difference may produce a longer progestogen exposure in the Crestar treated animals. However, more comparative studies should be undertaken to investigate if there are any differences in the patterns of release and dynamics of progesterone levels in the blood during treatment, particularly in cattle in poor condition.

In experiment 2, the differences between the three treatment groups lie in the differences in the duration of the first CIDR-B treatment (9 days vs 7 days), whether there was a second (post-insemination) CIDR-B treatment or not, and in prostaglandin treatment (to only cows having a CL or to all). No significant differences between groups in oestrous response, ovulation rate, CL size and pregnancy rate to AI after the first CIDR-B treatment indicates that 9 day CIDR-B and 7 day CIDR-B treatments in these combinations may result in similar results. The present three treatment protocols resulted in high oestrous response and ovulation rate with reasonably good fertility compared with other oestrus synchronisation protocols as reported by Odde (1990), Larson *et al.* (1992) and Macmillan *et al.* (1993).

The better fertility of the single CIDR-B treated group compared to double CIDR-B treated cows may result from the effect of PG. All single CIDR-B treated cows (100%) received Estrumate regardless of whether they had a CL or not (without monitoring), whereas in the double CIDR-B treated groups only cows with a CL (32.1%) were given Estrumate in order to cause luteolysis. Prostaglandins not only have luteolytic effects in cows having a CL but can also hasten uterine involution (Lindell *et al.*, 1983), be effective in the treatment of metritis (Korenic, 1984), increase GnRH-induced LH release (Harrison *et al.*, 1984), and are considered an important modulator in the initiation of oestrous cycles (Madej *et al.*, 1984, Velez *et al.*, 1993) in post-partum cows. Therefore, this group may have benefited from the administration of PG to all cows. Beal *et al.* (1986), McClary *et al.* (1989), Patterson *et al.* (1995) and Jaeger *et al.* (1995) have demonstrated that administration of prostaglandins improved reproductive performance of post-partum cows. However, Morton *et al.* (1992) reported that a single post-partum prostaglandin treatment failed to improve the reproductive performance of dairy cows. Additionally, Johnson *et al.*

(1992) demonstrated that changes in PGF2α during follicular growth were not related to the formation of corpora lutea of normal life span in post-partum beef cows. These conflicting results call for further detailed studies on the effects of prostaglandin in post-partum cows.

Equivocal results have been reported on the use of progesterone treatment after insemination in order to increase fertility owing to high levels of progesterone maintained following AI (Van Cleeff *et al.* 1989, Robinson *et al.*, 1989, Munro and Bertram, 1990, Favero *et al.,* 1993, Macmillan *et al.*, 1993). In the present experiment, pregnancy rate to first AI was not increased as a result of insertion of a second CIDR-B device 2 weeks later. Double CIDR-B treated groups had a higher overall pregnancy rate to AI than the single CIDR-B treated group merely as a result of 2 successive CIDR-B treatments and 2 subsequent AIs. Since the single CIDR-B treated group had more cows conceiving to backup bulls, the total pregnancy rate was not different among groups. Here, a higher overall pregnancy rate to AI was obtained owing to added synchronised returns to service (the second cycle) at the expense of added costs associated with more drug treatments and AIs of the two cycles. There was no advantage in fertility of a second CIDR-B treatment post-insemination in the present experiment. However, this effect was confounded with the effect of PG and the difference between groups in the time of previous calving.

### 4.4.2 EFFECTS OF PMSG DOSE

PMSG has been used as a single injection at the end of progesterone treatment to stimulate follicular development in post-partum cows (see chapter 3). In the present study, a PMSG dose of 550 i.u. produced better ovulatory response than 450 i.u, but the differences were not significant for other responses. This confirms the results of Macmillan *et al.* (1987) and Macmillan *et al.* (1993) who indicated that a higher dose of PMSG (600 vs 400 i.u.) increased the percentage of animals which ovulated, but did not increase the percentage detected in oestrus and inseminated. They also found no advantage of increased PMSG dose on fertility. However, significantly higher ovulatory responses in cows receiving higher doses of PMSG in the present experiment as well as in previous reports indicate that a dose of around 550 i.u. of PMSG is necessary to stimulate follicular development to a point where a follicle can ovulate following progesterone treatment.

Munro (1987) found increased calving rates in post-partum anoestrous Hereford cows when increasing PMSG dose from 0 to 375 and then 750 i.u. injected at removal of PRID treatment (similar to CIDR). In another study, Munro (1989) found that a high dose of PMSG (750 i.u.) tended to produce higher calving rates in anoestrous cows while a lower dose (375 i.u.) seemed more favourable on cyclic cows following progesterone treatment. The significant interaction between PMSG dose and ovarian cyclicity in present experiment confirmed this finding. Cycling cows responded better to a dose of 450 i.u. of PMSG, whereas non-cycling cows responded better to 550 i.u. of PMSG. Therefore, on one hand, the difference between the two doses in the present study may not be great enough to give statistically significant differences in most responses, on the other hand, the significant higher ovulatory response with no significant difference in AI pregnancy rates in the present study suggests a lower fertilisation rate or higher early mortality with high doses of PMSG. This deserves further detailed investigation.

### 4.4.3 EFFECTS OF OVARIAN CYCLICITY

The differences in reproductive response of cows with or without a CL at the start of treatment of experiments 1 and 3 essentially represent the differences between cycling and anoestrous cows, although there may be up to 25% of cycling cows which had no CL as they were in the follicular phase at the time of scanning. Likewise in experiment 2, those cows having a CL 2 days before CIDR removal can be considered to represent cycling cows as only a small proportion of cycling cows either were in the follicular phase at initiation of treatment or had a CL which had naturally regressed during the early treatment period before monitoring.

Cows having a CL tended to show a better synchronised oestrus response in most experiments and a larger CL size as seen in experiment 2. This is presumably because cows with a CL had commenced normal ovarian cycles, so that treatment was only to synchronise oestrus and ovulation in these cows, whereas treatment must have first stimulated ovarian activity to induce oestrus in acyclic cows. Endocrine priming, particularly progesterone, would have been optimal for CL formation in cycling animals compared with that of non-cycling animals. Characteristically cows in post-partum anoestrus have small ovaries (Fielden *et al.*, 1973) and first ovulation may occur without oestrus (Peters *et al.*, 1987a), demonstrating a lack of progesterone priming. Ovulation without oestrus also occurred in the present experiment. On the other hand, the interval from implant removal to observed oestrus in experiment 3 was shorter ($P < 0.05$) for cows having no CL. The longer interval in cycling cows may result from a deeper negative feedback of progesterone priming from the CL in these animals.

Pregnancy rates were not significantly different between cows having and not having a CL in all three experiments. Similar results have been reported by Richards *et al.* (1990) who indicated that the presence of a functional CL prior to synchronisation contributed to variability in the onset of oestrus (earlier in cows without a CL), but not to pregnancy rate. The influence of the cycling state may be less important for cows in a good nutritional status as can be seen in the Angus herd where no significant effect of the CL on reproductive responses was found in 1995 when the cows had high body condition scores. Similarly, in the Hereford herd the effect of cycling state was more apparent in 1994 when the cows were in poorer condition. Based on these findings, it can be expected that pregnancy rates following a synchronisation treatment which is initiated after one month post-partum are not significantly affected by the cycling state in animals of good body condition. However, Mares *et al.* (1977) indicated that pregnancy rate following SMB treatment was lower in herds in which less than 50% of the cows were cycling prior to treatment compared with herds in which greater than 50% of the cows were cycling. Experiments using large numbers are needed to look at the interactions between cycling state and different treatments so that an ideal treatment protocol may be arrived at for commercial post-partum beef suckled cows without a need for ovarian monitoring.

### 4.4.4 EFFECTS OF FOLLICULAR DEVELOPMENT

A wave-like pattern of follicular growth has been shown to occur not only in cycling but also in post-partum acyclic cows (chapter 3). It is desirable that a synchronisation treatment should stimulate and synchronise these waves of follicles and therefore a high degree of response synchrony. Combinations of progesterone and oestradiol have been reported to suppress follicle growth effectively and thereby can synchronise the next follicular wave at a consistent interval post treatment regardless of the phase at which treatment is initiated (Bo *et al.*, 1994b, Adams, 1994). The Crestar and the CIDR-B used in experiment 1 were both combinations of progesterone and oestradiol. They were randomly given to cows at different stages of follicular development. However, the dependence of synchronised oestrus response on the size of the largest follicle at initiation of treatment suggests that these combinations were not completely effective in synchronising follicular development.

The effects of progesterone treatment on follicle growth, regression and wave development have been examined in many studies (see Adams *et al.*, 1992b, Bo *et al.*, 1993, Savio *et al.*, 1993a, 1993b, Taylor *et al.*, 1994). Results showed that progesterone alone could only suppress the dominant follicle in a dose-dependent manner when given during the growing phase but had no effects on static or regressing phase follicles. If growth of the dominant follicle was terminated the period of dominance was shortened, followed by an early emergence of the next follicular wave. If the dominant follicle was maintained it ovulated following treatment termination and the emergence of the next wave was delayed. In experiment 2, monitoring the ovaries two days before termination of progesterone treatment, revealed that a difference in the status of follicular development still existed toward the end of exogenous progesterone treatment and that it influenced the interval to behavioural oestrus. This finding further supports the argument that progesterone alone is not completely effective in suppressing follicular development.

Also in experiment 2 the interval between treatment termination and oestrus increased when the size of the largest follicle rose to 9-10 mm in diameter but was shorter if the largest follicle was > 10 mm. This may be due to the fact that follicles of ≤ 10 mm should have been in the growing phase at initiation of treatment and thus were suppressed by exogenous progesterone and therefore it took time to develop an ovulatory follicle from the next wave; the larger the follicle the longer the time was needed. On the other hand, follicles larger than 10 mm were maintained and ovulated following treatment in a shorter time with increasing size.

However, the effects of follicular development on reproductive responses to oestrous synchronisation were not consistent between years and treatments in the present experiments. The trends of the relation between the follicle size and reproductive responses were also conflicting. For example, oestrus fertility may be related to the size of the largest follicle in a positively linear, negatively linear or non-linear manner, or not at all. These differences may be due to some confounding effects or interactions which were not possible to examine in the present studies.

### 4.4.5 EFFECTS OF LONG TERM NUTRITIONAL STATUS

The importance of long term nutrition to the response of post-partum cows to oestrus synchronisation treatment was demonstrated in the comparison of responses in 1994 and 1995 in experiment 1. The two years were quite different in terms of nutritional conditions, 1994 reflecting drought conditions for the previous 10 months and 1995 favourable pasture growth. This was reflected in the differences in BCS, BW and the change in the mean BW during a period before treatment in the two years. The average BW of the herd *declined* by 92.1 kg from early March till early November, 1994; while in 1995 it *increased* by 86.8 kg over the same period. BW (532.7 vs 376.2 kg, $P < 0.001$) and BCS (4.50 vs 2.39, $P < 0.001$) at the start of treatment were much higher in 1995 compared to 1994. As a consequence, a greater proportion of cattle were cycling (52.6 vs 2.9%, $P < 0.001$) and the mean size of the largest follicle was greater in 1995 (10.7 vs 9.1, $P < 0.001$). Therefore, it can be said that the herd was nutritionally deprived for a long period in 1994 and probably the improved condition status of the cows in 1995 had a positive effect on reproductive states and responses to the treatment. In addition, experiments 2 and 3 showed that BCS had significant effects on synchronised oestrous response, pregnancy rates, CL development and the interval to observed oestrus. This demonstrates how important the nutritional status of the animal is when a treatment is initiated and highlights the need for cows to be in "good" condition to maximise responses to oestrous synchronisation.

It is generally recognised that nutrition is a major determinant of duration of post-partum anoestrus. Randel (1990) indicated that inadequate nutrition lowered pregnancy rates as well as first service conception rate and extended the anoestrous period in suckled post-partum beef cows. Lucy *et al.* (1991a) saw a higher proportion ovulating associated with greater positive energy balance in post-partum dairy cows. Wright *et al.* (1992) demonstrated that body condition at calving was negatively correlated with the duration of the post-partum anoestrous period. Odde (1990) maintained that thin cattle had longer PPI to oestrus and therefore were more likely to be non-cycling at the time of treatment, resulting in reduced conception rates. These findings are in line with the results of the present experiments in which cows under poor nutritional circumstances/in poor body condition had poor reproductive responses to oestrous synchronisation.

The mechanisms whereby poor nutrition suppresses reproductive performance in post-partum cattle is not completely understood (see section 2.3.2.1.2). Undernutrition may affect reproduction by altering GnRH secretion and/or altering pituitary sensitivity to GnRH, which reduces gonadotropin secretion causing decreased follicular development, absence of oestrus and ovulation (Richards *et al.*, 1989, Randel, 1990). The importance of nutritional status to follicular and ovarian function has previously been identified in other studies (Lucy *et al.*, 1991a; 1992, Dominguez, 1995). Jolly *et al.* (1995) indicated that moderate levels of underfeeding, before or after calving, may interfere with the mechanism(s) of final follicle maturation, whereas more pronounced nutritional deficiencies may affect the mechanism(s) regulating dominant follicle size and the dynamics of dominant follicle growth and regression. The changes are consistent with likely effects of reduced LH or FSH secretion that have been associated with inhibition of both tonic and surge release of GnRH. These should have important impacts on reproductive responses to artificial oestrus synchronisation in nutritionally deprived post-partum cows, as seen especially in 1994 - experiment 1. Also, the significant effects of nutritional status (BCS) on reproductive responses in the present experiments may support the influence of nutrition via follicular development as the size of the largest follicle was found to be strongly dependent on BCS ($P < 0.001$) as well.

Body condition score and body weight are functional indicators of energy status after calving (Randel, 1990). Therefore, these variables were used to examine the effect of nutritional status of post-partum cows on responses to oestrous synchronisation. However, it should be noted that in the present studies BCS had significant effects on reproductive responses, whereas BW was not found to have any significant effects. This suggests that BCS, which may be somewhat imprecise or subjective, is a better functional indicator of the nutritional status of the cow than BW and therefore of greater use in predicting the response to oestrous synchronisation.

### 4.4.6 EFFECTS OF SUPPLEMENTATION OF PROTECTED LIPIDS

As discussed above, nutrition has long been known to be a major contributor to the variability in post-partum fertility and responses to oestrous manipulation programs. Identification of possible nutritional aspects affecting responses to artificial manipulation of oestrus and ovulation in a herd of post-partum beef cows is of importance. Nevertheless, manipulating the nutritional status of the cow pre and/or post calving can be an expensive exercise. Therefore, investigations of the potential for specific supplements, fed for short periods, to provide cost effective strategies to stimulate ovarian activity soon after calving may be beneficial for improved reproductive responses to oestrous synchronisation.

The main stimulus to examine supplements containing protected lipids has come from positive responses found in previous studies. For example, De Luna *et al.* (1982) reported that all of the group of cows supplemented with protected fat showed standing oestrus or oestrous activity by 45 days post-partum compared with less than 50% of the control cows. Ferguson *et al.* (1988) and Sklan *et al.* (1991) observed greater conception rates and fewer days open in dairy cows supplemented with bypass lipids. Furthermore, Lucy *et al.* (1991a, 1991b, 1992) demonstrated that supplements containing calcium salts of long chain fatty acids (LCFA) altered ovarian follicle development in post-partum dairy cows.

In the present experiment, all the responses were significantly improved by the Rumentek supplementation in comparison with the "control" cottonseed meal. This suggests that the improved reproductive responses were due to effects of protected

LCFA in the Rumentek supplement as this product has a much higher level of protected lipids than cottonseed meal (35 vs 3% fat rich in LCFA with 70% protection) while the levels of protein in the two supplements were almost the same (37.5 vs 37% crude protein), the cottonseed group recieving more protein. The question that arises is what are the possible mechanisms of action of LCFA to improve reproductive states in post-partum beef cows.

In post-partum dairy cows, Lucy *et al.* (1991a, 1991b, 1992) indicated that increased energy balance resulting from supplements containing LCFA altered ovarian follicle development. The role of energy balance (nutritional status) as a powerful factor affecting gonadotropin secretion and therefore ovarian development has previously been discussed in sections 2.4.2.1.2. and 4.4.5. However, the two diets in the present study were approximately isocaloric and there was no effect of the treatment on body condition just prior to AI. These facts suggest that the effect of the Rumentek supplement, fed for a short period, was independent of the effects of changes in nutritional status or energy balance.

The Rumentek fed cows had a larger mean size for the largest follicle ($P < 0.05$) coupled with much greater oestrous response and pregnancy rates. In addition, the higher proportion of Rumentek fed cows pregnant among those not returning to oestrus following the second CIDR-B treatment suggests lower embryo mortality in this group. These improved responses may result from an effect of LCFA on enhanced follicular development and normal luteal function in post-partum beef cows as a direct result of alteration of post-partum endocrine characteristic as demonstrated in previous studies. For example, Talavera *et al.* (1985) indicated that hyperlipidemic-hypercholesterolemic diets may enhance luteal progesterone biosynthesis, release or clearance in heifers. Hightshoe *et al.* (1991) reported that calcium soaps of fatty acids (CSFA) increased plasma cholesterol accompanied by decreased serum oestradiol, enhanced follicle growth, increased LH, and greater progesterone during the luteal phase of the first post-partum oestrous cycle.

Wehrman *et al.* (1991) also demonstrated that diet-induced hyperlipidemia in cattle modified the intrafollicular cholesterol environment, modulated ovarian follicular dynamics, and hastened the onset of post-partum luteal activity. In addition, Hawkins *et al.* (1995) reported increased concentrations of cholesterol and progesterone associated with increased lipid accumulation within the CL and a slower rate of disappearance of progesterone from serum when CSFA were added to diets of beef heifers. Espinoza *et al.* (1995) concluded that supplementation of fatty acids during pre- and post-partum periods in beef cows resulted in increased serum cholesterol and increased percentages of cycling and pregnant cows in the early breeding season, thus improving reproductive efficiency. Thus it seems that changes in lipid metabolic status may modify reproductive potential in cattle, independently of dietary energy intake (Wehrman *et al.*, 1991). A likely mechanism of action of protected lipid supplements is increased concentrations of lipids as precursors for biosynthesis and/or reduced rates of clearance of steroid hormones from the blood, coupled with enhanced follicular development, resulting in normal luteal function in post-partum cows.

In addition to their more obvious possible effects on energy balance and on production and metabolism of steroid hormones, supplemented LCFA may provide precursors for biosynthesis of uterine prostaglandin $F2\alpha$ (Espinoza *et al.*, 1995), which has been implicated as an important modulator of the post-partum period as discussed earlier in sections 1.3.1.2 and 4.4.1. However, Lucy *et al.* (1991b) did not see any influence of feeding CSFA on 15-keto-13,14-dihydro-prostaglandin $F2\alpha$. Therefore, examination of endocrine changes in comparative studies on post-partum beef cows in both poor and adequate nutrition is needed to verify the possible nutritional and/or direct endocrine effects of the Rumentek supplement.

### 4.4.7 OTHER EFFECTS

Besides the effects mentioned above, the present experiments were also concerned about the post-partum interval from calving to initiation of oestrous synchronisation treatment and the association between pregnancy rate to AI and observed oestrus. Cows were at different stages post-partum, from 31 to 107 days. The difference in PPI among these cows did not significantly influence reproductive responses in any experiment. This supports the report of Macmillan *et al.* (1993) showing that post-partum intervals at CIDR-B insertion (30-40, 41-60, >60 days) were not a significant factor contributing to treatment response patterns. The post-partum interval from the previous calving may be related to uterine involution and the resumption of ovarian activity, and thereby may affect rebreeding ability in early post-partum cows (Peters *et al.*, 1987a, Short *et al.*, 1990, Bazer *et al.*, 1993, Jainudeen *et al.*, 1993). However, in suckling cows uterine involution has usually been completed by about 30 days (Peters *et al.* 1987) and these cows would not have been affected by this factor thereafter. Graves *et al.* (1968) also found that fertilisation rates and pregnancy rates were very low when cows were bred < 20 d after calving, but fertility returned to normal between 20 to 40 days after calving. Therefore, beyond 30 days post-partum the timing of treatment is not of importance even when the animals are still anoestrous.

The strong association between pregnancy rate to AI and observed oestrus may be due to the following: 1) cows that were able to show oestrus following treatment should be in a better reproductive state, especially the uterine environment as a result of previous progesterone priming, 2) cows showing oestrus have a higher probability to ovulate as found in experiment 2 and only cows ovulating can conceive to AI, and 3) cows observed in oestrus are inseminated in a more optimal time frame to become pregnant as AI was performed according to detected oestrus.

## 4.5 CONCLUSION

From the three present experiments some main concluding points are:

1. Better reproductive responses were obtained from the use of the Crestar compared to the CIDR-B treatment for nutritionally deprived post-partum *Bos taurus* cattle.

2. Short term (7-9 day) CIDR-B treatment (without oestrogen) in combination with prostaglandin and PMSG can be applied to synchronise oestrus and ovulation in post-partum suckling beef cows.

3. Double CIDR-B regime resulted in a higher overall pregnancy rate to two cycles of AI; however, no positive effect of the second CIDR-B treatment on fertility following first AI was found.

4. Progesterone alone was unable to suppress and synchronise follicular wave development. Progesterone-oestrogen combination did not completely ablate the effects of follicular development on synchrony of responses.

5. A low PMSG dose (450 i.u.) was suitable for cycling cows while a higher dose (550 i.u.) was good for non-cycling cows in the present oestrous synchronisation treatments.

6. Nutrition was of significance in determining the response to treatment of oestrus and ovulation synchronisation in post-partum suckled beef cows. BCS was a better indicator of the nutritional status and thus reproductive responses of the cow than BW.

The different pasture conditions in two years 1994 and 1995 influenced the nutritional status of the cow and, consequently, significantly affected reproductive responses to oestrus synchronisation.

7. Short term Rumentek supplementation before AI can result in better reproductive responses following synchronisation treatment compared to cottonseed meal.

8. The interval from calving to oestrous synchronisation treatment was not significant in affecting reproductive responses when synchronisation treatments were initiated after one month post-partum.

9. Even in a late post-partum herd the majority of cows may be still anoestrous. However, cycling state in late post-partum cows seem to influence only the expression of behavioural oestrus but not to affect pregnancy rates following these oestrous synchronisation regimes.

10. Pregnancy rate to AI was strongly associated with oestrus observed within 3 days following oestrous synchronisation treatment.

# Chapter 5:

# GENERAL CONCLUSION AND IMPLICATIONS

In the present thesis, physiological mechanisms for controlling oestrus and ovulation and current methods for inducing and synchronising oestrus and ovulation in female cattle have been reviewed. Three experiments have been integratively reported looking at the efficacy of and important factors affecting some treatment regimes for the artificial manipulation of oestrus and ovulation in post-partum suckled beef cows on two pasture-based seasonally calving properties in Northern NSW, Australia in two years 1994 and 1995.

It was found in one experiment that the Crestar produced better reproductive responses than the CIDR-B treatment for the *Bos taurus* cattle in very poor condition. Further detailed comparative studies should be carried out to examine the efficacy of the CIDR-B and the Crestar under different nutritional circumstances. In addition, these progesterone-oestrogen combinations were not able to completely ablate the effects of follicular development on synchrony of responses. Therefore, the suppressive effect of progesterone-oestrogen combination on follicular wave development as previously reported should be questioned and also deserves more investigation.

Short term (7-9 day) CIDR-B treatments (without oestrogen) in combination with PMSG and prostaglandin in the other two experiments were able to stimulate and synchronise oestrus and ovulation in suckling beef cows. Double CIDR-B treatment protocol can be applied to increase the overall pregnancy rate to AI. However, the positive effect of the second CIDR-B on fertility to first AI should be further examined and the costs involved should be considered. The dosage of incorporated PMSG should be based on the cycling state of the herd, a higher dose given to a herd with the majority non-cycling and a lower dose given to a herd with the majority cycling. These experiments have also highlighted the need for further research to verify the effects of prostaglandin incorporated with steroid treatments of oestrous synchronisation for post-partum beef suckled cows. In addition, since the CIDR-B was not effective in suppressing follicular waves, causing variability in the time of oestrous expression, fixed time AI should not be applied following the CIDR-B (both with and without oestrogen) as well as Crestar treatments because pregnancy rate to AI was strongly associated with observed oestrus.

Nutrition was found to be of significance in determining the response to treatment of oestrus and ovulation in post-partum suckled beef cows. BCS was a better indicator of the nutritional status and thus responses of the cow than BW. The nutritional status, as reflected by BCS and BW, may very greatly from year to year and, as a consequence, responses to oestrous synchronisation may be affected by years in pasture-based seasonally calving beef enterprises. However, it would be difficult and expensive to manipulate the nutritional state of a herd in this situation. Specific supplements, fed for short periods, may provide cost effective strategies to stimulate ovarian activity soon after calving. Whatever the mechanisms of action may be, the encouraging results obtained from feeding Rumentek as a protected lipid supplement incorporated with an oestrous synchronisation treatment suggests a promising means for increased post-partum reproductive efficiency of commercial beef herds.

It has also been shown in these studies that cycling cows responded better to synchronisation treatments than anoestrous cows, especially in poor condition, and that even in a late post-partum herd the majority of cows may be still anoestrous. Therefore, a method of oestrus synchronisation for post-partum cows should be directed first to induce oestrus in anoestrous animals. Nevertheless, the insignificant effects of PPI on reproductive responses to synchronisation implies that artificial oestrus manipulation can be initiated as early as 30 days post-partum, if not earlier, with the same efficacy as carried out later in the post-partum period.

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