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**CONTROL OF OVARIAN ACTIVITY AFTER ESTROUS
SYNCHRONIZATION WITH PRID, CRESTAR
AND PROSTAGLANDINS AND AS MONITORED
BY ULTRASONOGRAPHY IN DAIRY CATTLE
IN WEST OF ALGERIA**
(With 3 Tables and One Figure)

By

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(Received at 4/3/2007)

**مراقبة نشاط المبيض خلال عملية السيطرة على الشبق بواسطة
البروجسترون والبروستاجلاندين ف ٢ وترصدها بواسطة التصوير
بالموجات فوق الصوتية في الأبقار الحلوب بغرب الجزائر**

أبو عبدالله بن علو ، سامية ملياني ، جمال سعدي ، عبد اللطيف نيار

تمت هذه الدراسة على مجموعة من البقر الحلوب من فصيلة الهولشتاين ذي البقع السوداء وعددها ٩٢ بقرة، وذلك لتتبع الإباضة لديها عن طريق السيطرة على توقيت الشبق باستعمال الهرمونات الأكثر تداولاً في الجزائر، ولقد تم استعمال اللولب المهبلي (PRID)، الزرع تحت الجلد (CRESTAR) والبروستاجلاندين ($PGF2\alpha$). البقر المستعمل في هذه الدراسة وفي بداية العمل التجريبي كان يتواجد فسلجيا بين اليوم ٦٠ - ١٢٠ من فترة ما بعد الولادة وقد تحصل على نقطة تقييمية لحالة البدن بين ٢.٥ و ٣.٥. الإختلاف في قدر المحيطات للجريبات قبل الإباضة لم تكن معنوية، غير أن الإباضات بدأت تحدث ابتداء من معدل محيط يقارب ١٥ مم. لقد لوحظ أيضاً أن الإختلاف قد تجلى في معدلات ووقت ظهور أعراض الشبق لدى البقر بين البروتوكولات المستعملة، وخاصة لدى استعمال البروجيستيرونات وبالتحديد اللولب المهبلي (PRID)، الذي تحصلنا من خلاله على أحسن المعدلات لظهور أعراض الشبق ٧٢.٧٣% و ٨٧.٥% ومعدلات إباضة عالية تتراوح بين ٨١.٨٢% و ٧٥% وهذا ٩٦ ساعة بعد انتهاء المدة المخصصة للعلاج. غير أن ظهور أعراض الشبق لدى البقر المعالج بالبروستاجلاندين كانت ضئيلة جداً بمعدلات تتراوح بين ٢٠ و ٢٥% ولقد لاحظنا أن الأجسام الصفراء على مستوى المبايض لم تزل باقية حتى الساعة ٧٢ بعد العلاج ولم تعرف تغيراً معتبراً إلا عند الساعة ٩٦. النتائج المتحصل عليها باستعمال الكبسولة تحت الجلد كانت بين هذه وتلك، بمعدلات ظهور أعراض الشبق تتراوح بين ٤٠% و ٦٦.٦٧% ومعدلات إباضة ٥٧.١٤% و ٨٠%. بالإضافة لقد لاحظنا بعض الأبقار التي تحدث لديها الإباضة دون ظهور

أعراض الشبق لديها بمعدلات مختلفة بلغ أقصاها ٦٠% و ٧٥% باستعمال البروستاجلاندين وأدناها ٠% عن طريق اللولب المهبلي والحالة المشيمية الخيلية (eCG).

SUMMARY

Ninety two prim Holstein cows, from different dairy herds, were randomly assigned to progestin protocols (PRID or Norgestomet) or prostaglandin's, to synchronise estrous, according to the most used protocols in Algeria, in order to compare their efficiency on our dairy cattle. All cows were between day 60 and 120 post-partum at the beginning of the experiments, and at a score body condition between 2 and 3,5. The follicular diameters were not significantly different between treatments, whereas ovulations were observed around 15mm of diameter. Results of estrous synchronisation were very different between treatments; treatment with the Intra Vaginal Device (PRID) provided better rates of estrous expression (72,73% and 87,5%) and better ovulations rates (81,82% and 75%) about 96h post treatment. An intermediate rate was observed with Norgestomet protocols (66,67%; 40% and 42,86%) of animals in estrous, and (77,78%; 80% and 57,14%) of ovulations. Bet, a lesser rate was observed with PGF2 α protocol (20 and 25%) of estrous expression, rate of animals with retained corpus luteum, was very high and began to decline at 72h after the second injection. In this study, some animals ovulated without expression of heat; the highest rate (60% and 75%) was noted with prostaglandin's protocols, the intermediate results were observed with Norgestomet protocol (22,22%; 40% and 14,29%), and the lesser rate was noted with the PRID protocol (9,09% and 0%).

Key words: *Ovarian activity, estrous, prostaglandins, ultrasonography, dairy cattle*

INTRODUCTION

Fertility of dairy cows is of growing concern. Reproductive indices that are used to assess reproductive management show a negative trend during the past decade despite increased knowledge and professional attention (Van Eerdenburg *et al.*, 2002). Poor estrous detection is the major contributor to low fertility (Reimeirs *et al.*, 1985). Until recently, it was generally believed that poor estrous detection was caused by the lack of commitment of the farmer, due to other priorities (Alexander *et al.*, 1984).

However; standing behavior is not observed in over 50% of cows estrous, in a number of herds, and no data are available to relate the moment of ovulation to other estrous symptoms (Van Eerdenburg *et al.*, 2002).

Follicular size can be regarded as an indicator for functional capacity of the follicle. In these poor quality follicles, production of estradiol would be impaired resulting in lower expression of estrous behavior (Lyimo *et al.*, 2000). According to Britt (1992), current fertility problems in dairy industry might be due to low quality of follicles and oocytes as a result of postpartum negative energy balance.

The keys to successful estrous synchronization are closely synchronized, rapid declines in circulating progesterin concentrations and synchronous growth and ovulation of a viable follicle (Mapletoft *et al.*, 2003).

Although current techniques for estrous synchronization with PGF2 α are successful (Odde, 1990; Larson and Ball, 1992), and variation in ovarian follicular wave dynamics results in poor synchrony of estrous and ovulation because induction of luteolysis when a dominant follicle is mature will result in estrous and ovulation in 2 to 3 days, whereas the interval will be much longer if another follicle must be recruited from a new follicular wave (Kastelic and Ginther, 1991).

Although follicular dynamics during the normal estrous cycle have been extensively studied as an adjunct to develop effective estrous-synchronization strategies (Ireland *et al.*, 2000), less information on post-partum follicular dynamics is available (Rajamahendran and Taylor, 1990; Savio *et al.*, 1990). Moreover, the relationship between early follicular dynamics and subsequent fertility is not clear in modern dairy cows. Recently, it was documented that most cattle exhibit 2 or 3 follicular waves during the normal estrous cycle (Ireland *et al.*, 2000).

The objective of the present study was to compare the effect of different treatments protocols on largest follicles, daily follicle diameters evolution, corpus luteum, the time of estrous and ovulation in postpartum imported prim Holstein dairy cows in Algeria.

MATERIALS and METHODS

Animals: 92 Cyclic Postpartum multiparous (3 to 6 years old) Prim Holstein imported dairy cows, with a score body of 2 to 3,5 (1-to-5 scale, 1 = emaciated and 5 = obese), with average milk production of 5185 kg/cow/ year, from ten private farms in Tiaret area (North west of

Algeria), were assigned randomly to one of four treatments. Cows assigned to the prostaglandin (PG) protocol (n = 15) received a double injection of PGF 2α (ND Estrumate, 2ml) 11 days apart. Cows assigned to prostaglandin eCG protocol (PG + eCG) (n = 10) received the same treatment with an injection of eCG (500UI) at the time of the second injection of PGF 2α . Cows assigned to the first progesterone protocol, received a PRID® with the estradiol-17 β capsule (CEVA Laboratories, France; 1,55 g of progesterone) for 11 days; the (PRID) group (n=11) do not received other treatments, the (PRID+eCG) group (n=9) received an injection of eCG (500 UI) at the end of treatment (Fig. 1).

Cows assigned to the second progesterone protocol received an implant of Crestar® (Intervet, 3 mg of Norgestomet) for 11 days with 5mg estradiol valerate in IM at the day of implant, the (CR) group (n=15) do not received other treatment, the (CR+PG) group (n=16) received an injection of PGF 2α (ND Estrumate, 2ml) 48h before the end of treatment, the (CR + PG + eCG) group (n=16) received an injection of PGF 2α (ND Estrumate, 2ml) 48 h before and an injection of 500 IU of eCG at the end of treatment (Fig. 1).

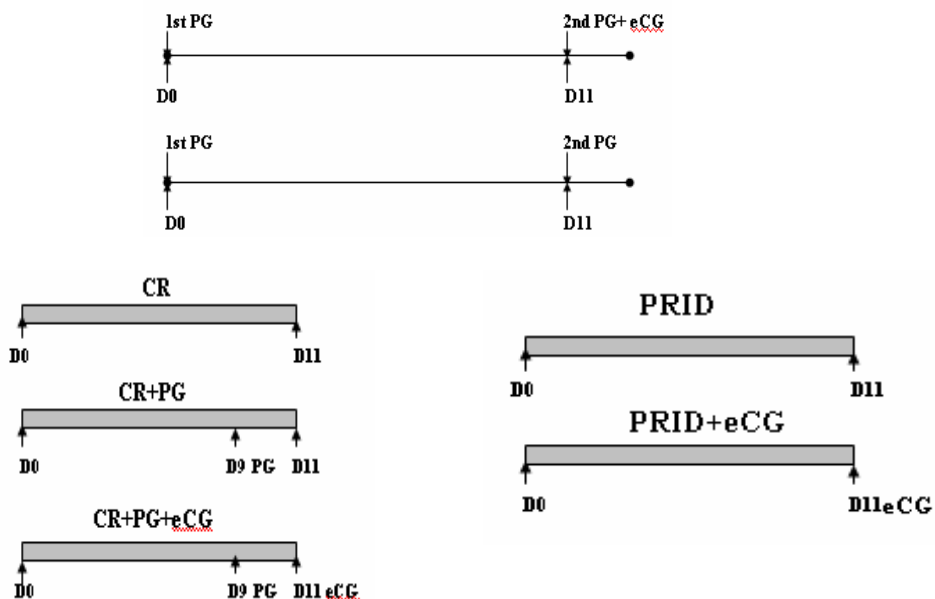


Fig. 1: Experimental design of treatment protocols, ultrasonography was performed every day for four days, and only diameters of dominant follicles were retained.

Ultrasonography, Estrous and Ovulation:

All cows were examined daily by transrectal ultrasonography to record follicular development using a Pie Medical Falcon 100 equipped with a 6-MHz linear-array transducer (Pie Medical Falcon 100, Netherlands) all follicles (≥ 10 mm) were recorded. Cows were examined daily from D0 to D4 after treatment until ovulation. A dominant follicle was defined as a large follicle, with other follicles.

Ovulation was determined by the disappearance of a large dominant follicle from an ovary. The presence of a corpus luteum on ovaries was also recorded.

Measurements were made on a single frozen image of the apparent maximal area of each follicle or the corpus luteum, using the average diameter in two directions at right angles. Only follicles ≥ 10 mm were retained. Cows were observed for signs of behavioral estrous beginning 1day before and continuing for 4 days following the final PG injection or Progestin insert withdrawal (for both PRID and CRESTAR protocols). Visual observations to detect signs of estrous were carried out during 4 days following synchronization treatment.

Statistical Analysis: Effects of treatment on follicle size at the end of each treatment were tested by Fisher's least significant difference test.

RESULTS

Table 1: Mean follicular diameters as daily observed after the end of treatments with PRID, CRESTAR, PG and eCG.

Item	PRID (n=11)	PRID+eCG (n=8)	CR (n=9)	CR+PG (n=10)	CR+PG+eCG (n=14)	PG (n=15)	PG+eCG (n=8)
Days							
D0	14±4,24	12	15,71±2,98	12±2,71	16,25±2,06	13±1,87	13±3
D1	14,91±3,33	13,57±2,76	17±3,07	12,6±2,51	15,8±3,7	13,92±1,44	15,67±1,53
D2	15,82±3,87	13,86±2,91	17,14±3,80	13,8±2,05	16±5,20	14,64±1,86	17±2,31
D3	14,2±1,48	14±1,73	15±4,24	14,33±2,89	15,75±3,5	15,42±1,62	16,5±0,71
D4	15		14±4	16		13,67±2,5	
Ovulation	15,33±2,16	15±2,97	17,5±3,59	14,2±2,49	17±2,94	14,9±1,79	17,75±1,5
Evolution mm /24h	0,87±0,06	0,21±0,10	1,24±1,28	1,10±0,47	1,15±0,21	0,96±0,45	2,00±0,94

Table 2: Percentage of cows observed in estrous and ovulated after the end of treatments with PRID, CRESTAR, PG and eCG.

Days		D0	D1	D2	D3	D4	ALL COWS
PRID (n=11)	Estrous %	0	0	27.27	45.45	0	72.73
	OV %				54.54	27.27	81.82
PRID+eCG (n=8)	Estrous %	0	0	37.5	37.5	12.5	87.5
	OV %			12.5	50.00	12.50	75.00
CR (n=9)	Estrous %	11.11	0	33.33	11.11	11.11	66.67
	OV %		11.11	0	55.55	11.11	77.78
CR +PG (n=10)	Estrous %				40.00	0	40.00
	OV %				40.00	40.00	80.00
CR+PG+ eCG n=14	Estrous %	14.29.	0	0	28.57	0	42.86
	OV %		14.29	0	28.57	14.28	57.14
PG (n = 15)	Estrous %	6.67	0	0	6.67	6.67	20.01
	OV %		6.67	6.67	13.33	46.67	73.34
PG + eCG (n= 8)	Estrous %				25.00	0	25.00
	OV %				25.00	50.00	75.00

Table 3: Percentage of cows with CL on ovaries after the end of treatments with PRID, CRESTAR, PG and eCG.

Item	PRID (n=11)	PRID+eCG (n=8)	CR (n=9)	CR+PG (n=10)	CR+PG+eCG (n=14)	PG (n=15)	PG+eCG (n=8)
days							
D0	9,09	25	55,56	20	42,86	93,33	50
D1	9,09	0	22,22	0	28,57	93,33	50
D2	0	0	11,11	0	14,29	86,67	50
D3	0	0	11,11	0	14,29	73,33	50
D4	0	0	11,11	0	0	20	0

DISCUSSION

Three cows (two from PG + eCG group and one from the PRID + eCG group) were excluded from this study, cows from the PG + eCG presented inactive ovaries, and the cow from the PRID + eCG group presented cystic follicles (>25 mm) persistent more than 1 week.

Six cows (two from the CR group, two from CR+PG group and two from CR + PG + eCG group) lost their implants. In total five cows (three from CR+PG group and two from CR) didn't respond to treatments and presented inactive ovaries. Three cows (two from CR group and one from CR+PG group) presented cystic follicles (>25 mm) persistent more than 1 week (Lucy, 2001). These cows were also excluded from this study.

Totally, seventeen cows were excluded from this study. So, seventy five cows were retained.

Follicular diameter

The mean of follicular diameter didn't differ between treatments ($P > 0.10$); however, follicular diameter varied from 12 to 17mm (Table1). Follicles ovulation with prostaglandins based protocols was observed at a mean diameter of $14,9 \pm 1,79$ mm with a mean evolution of $0,96 \pm 0,45$ mm/day, which is comparable with results of SARTORI *et al.* (2002) $13,7 \pm 0,3$ mm Vs $15,8 \pm 0,4$ mm, and an evolution diameter of $0,8 \pm 0,1$ mm/day Vs $1,0 \pm 0,1$ mm/d respectively; with SMITH and STEVENSON (1995), $16,4 \pm 0,7$ mm ; BRITO *et al.* (2002) 13,2 mm with a mean evolution of 1,02 mm/day. The injection of prostaglandins controls the lifespan of the corpus luteum but don't affect the follicular diameter so that the follicle continues to grow until estrous and ovulation and it can take from 2 to 4 days times necessary for requirement.

Custer *et al.* (1994) reported that the average diameter at ovulation is about 14 ± 03 mm and 17 mm; with a growth rate of $1,3 \pm 0.1$ mm/day and 0.7 mm/day respectively for the PRID and CRESTAR; Same results have been reported by Smith and Stevensen (1995) for the PRID and CRESTAR (16.4 ± 0.8 mm).

Percentage of observed oestrous and ovulations

For cows synchronized by prostaglandins, several authors have reported variable rates; Beal *et al.* (1983) reported a rate of 28% spread out over 7 days which is similar to that noted in our study (20 and 25%). For Brito *et al.* (2002), the rate noted was of 36% (percentage of cows observed in heats at the end of 96h). On the other hand, a rate of 62 % has been reported by Smith and Stevenson (1995). This will be explained by the fact that at the time of luteolysis, the dominant follicle had not yet completed its full development, and this which involve a spreading out of oestrous after treatment (Mialot *et al.*, 1999; Driancourt, 2001). Otherwise, we have noted that 75% of ovulations have been concentrated between 48 and 96h for cows treated by prostaglandins (Table 2).

The most important problem found with $PGF2\alpha$ is the variability of the interval from injection to estrous (ODDE, 1990).

Luteal tissue and plasma progesterone concentration were reported to be highly correlated in heifers and cows (Spêecher *et al.*, 1989; Kastlic and Ginther, 1990). Causes of luteolysis failure (occurring

in $\geq 10\%$ of the cows treated with prostaglandins) are not clear but may be related to several factors (Peters and Ball, 1995).

The rate of cows observed in oestrous when synchronized by PRID was quit important compared with cows treated with prostaglandins, and varying between 72, 73 and 87, 5% with a better regrouping between 48 and 72h. We judged that the PRID ensures a good luteolysis effect and a decrease of circulating progesterone; witch gives a good rate of synchronization (Table 2). Also for the two processes, we have noted an ovulation rate varying between 75 to 87, 5%, and then concentrated between 48 and 96h. However there are a number of cows which ovulated without showing any sign of oestrous.

For Crestar protocols, ovulation diameters were compared with those observed by Smith and Stevenson (1995), of 15, $8 \pm 0,8$ mm. For the three groups treated with Crestar, heats were spread out over all the duration of this study, with rates of cows seen in heats varying from 40 to 66%. We have even noted a number of cows which expressed behavioural heats, the day of implant withdrawal. The rate of cows presenting a CL the day of Crestar withdrawal, also varied from 20, 42, 86 and 55, 6% for the 3 groups of Crestar treated cows (Table 2). On the other hand, we have noted for the two protocols (Crestar and prostaglandins) a rather significant rate of ovulation varying from 60 to 80%, and concentrated between 48 and 72h. However, we have also noted that around of 40% of cows ovulates without showing any sign of oestrous Table 2 and 3).

Causes of luteolytic failure (occurring in $\geq 10\%$ of prostaglandin treated cows) are not clear, but may be related to several factors (Peters and Ball, 1995).

The growth rate of largest follicles could also play an important role at the time of estrous and ovulation. The longer persisting progesterone concentration might influence the growth rate of the largest follicles and cause the failure of clinical sings of estrous and ovulation (Repasi *et al.*, 2005).

CONCLUSION

Problems of estrous detection have to be resolved in dairy cows. Even after oestrous synchronisation, we have a number of cows witch do not respond to the treatment. This problem should be taken seriously by inseminators. Ignorance of ovarian activity at the time of insemination involves a poor cow's fertility, and put the doubt in reproductive performance in dairy cows imported by our country.

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