PARTNERS IN REPRODUCTION

Volume 3, Issue 1 October 2007

In the Spotlight

Control of oestrus in nursing beef cows - European perspective

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Breeders of beef cattle aim in general to obtain one calf per cow per year. To realize this objective, a rapid restoration of cyclicity after calving is required. However, in lactating bovine females, anoestrus is often prolonged, due in particular to the sucking by the calf. Thereby, fertility constitutes the main limiting factor for the productivity of beef cows herds. In this context, the use of methods for better control of oestrus in lactating beef cows is of particular importance to reduce the economic losses associated with a delayed resumption of reproductive activity.

Particulars of post-partum in female beef cattle

The cows of beef cattle breeds who nurse their calves, display a prolonged anoestrus after calving. The principal factors responsible for post-partum anoestrus and influencing its duration, are well known:

- the presence of a suckling calf prolongs post-partum anoestrus,
- the nutritional status: the negative energy balance (generated by the limited appetite of the early post partum cows and the low quality of the available food) delays the restoration of cyclicity after calving,
- parity: primiparous animals who have not completed their growth phase and have a strong maternal behavior are characterized by a prolonged (by 1 to 4 weeks) anoestrus,
- the season: the hypothalamus-hypophysis axis in beef cattle is sen sitive to the length of daylight. Thus, cows that calve in the autumn season, having been exposed to the long days in the summer, return to cyclicity faster than do females that calve in spring,
- difficult or twin calving and placental retention are associated with prolonged post-partum anoestrus.

As in dairy cows, the production of FSH and the development of dominant follicles resume rapidly after parturition in suckling beef cows (Yavas & Walton 2000). Follicles of 5 - 10 mm in diameter can be observed from 5 - 10 days post-partum onwards, and dominant follicles between 10 and 21 days (Yavas & Walton 2000). The final maturation of the dominant follicle however does not take place, mostly due to the absence of LH pulses; no ovulation occurs and the follicle undergoes atresia. Thus, numerous waves of follicle growth develop in nursing females post-partum prior to first ovulation of a dominant follicle. In the first days post-partum, the scarcity of LH pulses is initially caused by depleted hypophyseal LH stocks at the end of gestation and unrelated to suckling. Thereafter, between 15 and 30 days p.p., the LH stocks are replenished but sucking then inhibits the hypothalamic release of GnRH and consequently also pulsatile release of LH.

Pulsatile LH secretion generally resumes around 30 days p.p. and cyclicity re-appears between 35 and 70 days p.p.. Further extension of the time to first estrus and ovulation is usually associated with nutritional and managerial deficiencies.

Restoration of cyclicity can be accelerated without recourse to pharmaceutical treatments in 2 ways:

- by separating the calves from their-mothers: complete temporary weaning (complete separation for 2 - 4 days) or partial weaning (only one sucking session of limited duration per day) is efficacious in reducing the anoestrus period. This method induces increased pulsatile release of GnRH and LH, leading to ovulation of a dominant follicle within a few days. However, weaning of the calves is not without drawbacks. It interferes with classical rearing practice in beef herds (rearing with the mother) increasing the work load and production cost, and potentially can lead to a decrease in growth rate of weaned calves due to stress and disturbed feeding pattern;
- by placing the mothers in contact with bulls: olfactory stimulation through the pheromones secreted by the bulls induces an increased pulstaility of LH secretion, sometimes triggering an LH peak and ovulation of a dominant follicle. This may shorten the post partum anoestrus period. The result is however variable as it depends on many other parameters (parity, body condition, period of animals' mix-up etc.). This technique, although it can accelerate the recovery of cyclicity, cannot be generalized owing to the unpredictability of its results.

Hormonal methods used for control of oestrus

The various methods theoretically applicable in the field, are based on the control of follicular growth and/or control of the life span of the corpus luteum (or the duration of the phase of treatment with exogenous progestagens in noncyclic females).

A. Prostaglandins: a strategy not well suited for suckling cows

Treatments for control of oestrus by means of prostaglandins were developed 30 years ago. A double injection of prostaglandin, 11 - 14 days apart, generates oestrus synchronization in treated females provided that they are cyclic, as the effectiveness of this protocol is based on the luteolytic effect of prostaglandins. Since the anoestrus rate in a suckling herd is generally high at the beginning of the breeding season (30 – 70 % of non-cyclic females around 70 days post calving), oestrus management with prostaglandins is not recommended, except in herds with a high rate of cyclicity.

A solution would exist in submitting to synchronization only those cows that have been diagnosed as cyclic, which in practice is complicated and runs counter to the basic purpose of triggering oestrus in all of the females of a given group.

Moreover the synchronization genera- ted by prostaglandins is far from optimal as these compounds do not synchronize follicular waves. Hence, expression of heat and ovulation is spread over a rather wide time-window. If such cows are inseminated, this is best performed at detected heat in order to obtain acceptable fertility results. Inseminations cannot be grouped into a single fixed-time session. Also, detection of heat is often suboptimal at beef farms.

For these reasons synchronization of heat with prostaglandins alone is not a method suited for management of beef herds.

B. The GPG/Ovsynch protocol: limited applicability in beef cattle

The GPG protocol for control of oestrus was elaborated in the United States by Pursley, and received the name of OVSYNCH (first results published by Pursley et al. in 1995). It represents a series of 3 injections that associate GnRH with prostaglandins (GnRH on Day 0, Prostaglandin on Day 7, GnRH on Day 9), followed by systematic blind artificial insemination (A.I.) 12 - 18 hours after the second GnRH. The association of a GnRH (or an analogue) with prostaglandin produces a combined control of follicular waves and of luteal activity, hence being more efficient than prostaglandins alone. As a consequence, the cows may be inseminated at a fixed time without estrus detection.

In detail:

- The first GnRH, at Day 0, elicits ovulation or luteinization of ovarian follicles larger than 10 mm diameter; this induces formation of a new corpus luteum and the emergence of a fresh follicular wave after about 48 hours,
- Prostaglandin administration on Day 7 causes luteolysis of the corpus luteum induced by GnRH administration on day 0 (as well as the corpus luteum that might have been present depending on the stage of the cycle at treatment initiation). Luteolysis removes the inhibitory effect of progesterone on LH secre tion, thus allowing final growth, maturation and ovulation of the dominant follicle.
- The second GnRH stimulates the LH surge that triggers ovulation after 20 – 24 hours.

In order to be fully effective, the protocol requires that the first injection of GnRH takes place in the presence of a dominant follicle. This condition statistically occurs in 65 – 70 % of females. In the remaining ones, follicles of insufficient size on Day 0 (below 10 mm in diameter) will not ovulate and a

fresh wave of follicular growth will not be recruited. In addition, around 30% of cows subjected to GPG may present high progesterone concentrations on Day 10, incompatible with a successful A.I., and up to 15 % of females may be seen in heat beyond Day 10 (Mialot et al., 1998). To limit this risk and ensure the presence of a suitable follicle on Day 0, a pre-synchronization treatment may be applied. This however means a cumbersome protocol with additional handling of the animals and increased costs, which reduces the attractiveness of its application in the European conditions.

In anoestrous cows, the GPG protocol has the ability to induce ovulation, but at lower rates than in cyclic ones (45 % of anoestrus cows vs. 85 % of cyclic cows, according to Mialot et al., 2003).

Generally saying, the GPG method gives best results in cyclic cows i.e. typically in dairy herds. GPG may give acceptable fertility results in suckling cows which display a high cyclicity rate at the time of treatment (pregnancy rate of 46 % was achieved in beef cows of which 80 % were cyclic, Mialot et al., 2003).

C. Progestagens: effective in females, both in cyclic and non-cyclic

i) Progestagens associated with oestrogens

Protocols for control of oestrus based on progestagens were set up 40 years ago; they involve:

- Initial injection of oestrogen (oestradiol ester) or its release from a vaginal device,
- Insertion of an auricular or vaginal device releasing a progestagen for 9 - 12 days,
- Injection of prostaglandin and eGC/PMSG, 24 - 48 hours prior and at the moment of withdrawal of the progestagen device, respectively is oftern recommended (see diagram 1 illustrating the CRESTAR® method).

The combination of oestrogen + progestagen in this synchronization treatment is very efficient as such protocol controls both luteal function and the dynamics of follicular waves:

- Luteal function: oestradiol administered at the beginning of the protocol exerts anti-luteotrophic effects on young corpora lutea as well as luteolytic effects on aging corpora lutea. As oestradiol has no action on mature corpora lutea, the protocols usually include administration of prostaglandin towards the end of treatment. Once the corpus luteum has regressed, synchronization of ovarian activity is generated by the exogenous progesterone/progestagen released from the progestagen-releasing device. The administration of exogenous progestagen creates an “artificial” corpus luteum. Removal of the progestagen containing device mimics synchronous luteolysis in a group of females.
- Follicular waves: Irrespective of their size, all follicles that are present on the ovaries at the start of the program (Day 0) will undergo atresia. Smaller follicles (3 - 10 mm), which require FSH for their growth and survival, degenerate under the effect of oestradiol that inhibits the FSH release (negative feed back). Larger follicles, which are LH-dependent, are “sterved” by the low LH concentration generated by the oestradiol + progestogen association introduced at the beginning of the protocol. This results in an emergence of a synchronized follicular wave in all treated females (after 4 days on the average, see Bó et al., 1995).

Continuous administration of exogenous progestagen does not prevent the growth of the new follicular wave, while blocking the ovulation of the dominant follicle of this wave (negative feedback of progesterone on LH).

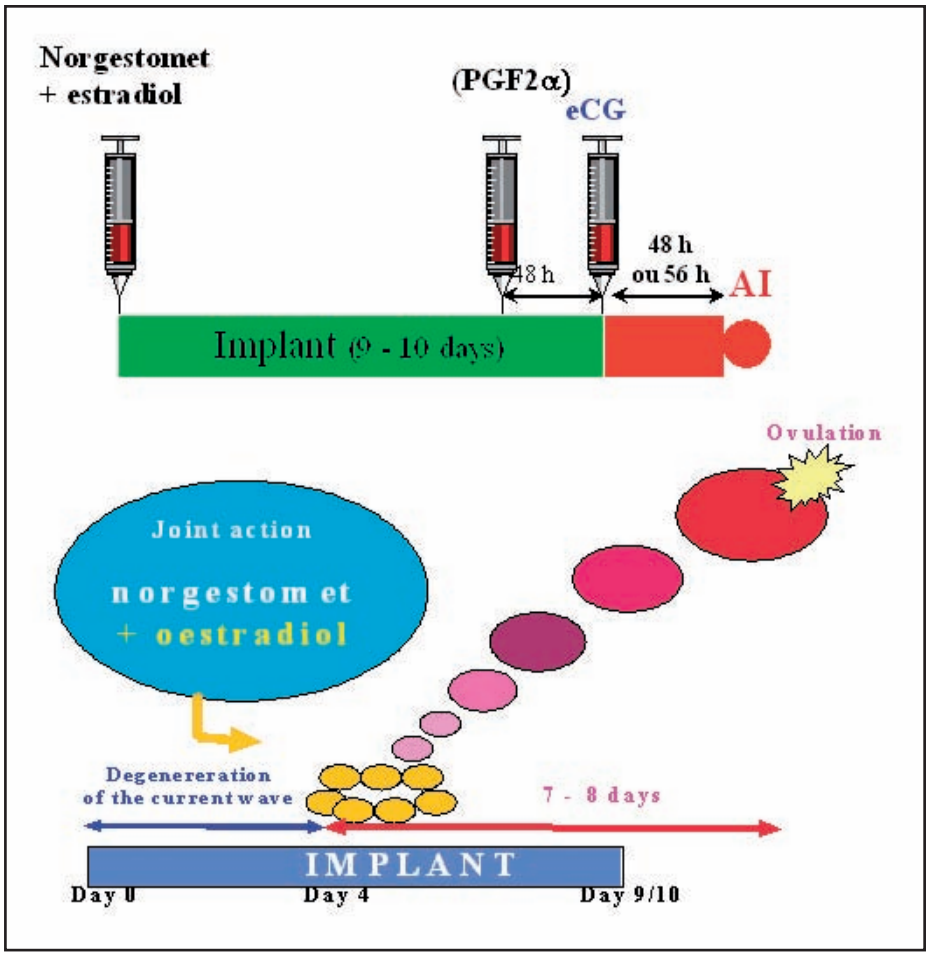
After withdrawal of the progestagen device, inhibition of the LH release stops and the dominant follicle undergoes final growth and maturation until ovulation, allowing A.I. at a preset time (fixed time).

The injection of eCG/PMSG (on average 400 - 600 I.U.) at the end of treatment, brings considerable benefits in non cycling beef cows. Owing to its joint LH and FSH activity, eCG/PMSG stimulates follicular growth and oestrogen synthesis, therefore optimizing follicle and oocyte quality.

In summary, the features of this oestrogen + progestagen treament are:

- Efficient synchronization of follicular and luteal function, irrespective of the cyclicity status and stage of the cycle at treatment initiation.
- Presence of a “young” (7 - 8-day old) ovulatory follicle at the time of A.I. Ovulation of such “young” follicles is associated with optimum fertility. In contrast, persistent dominant follicles (older than 10-12 days) contain oocytes of reduced quality (Austin et al., 1999) in which meiosis has resumed prematurely and the somatic cells surrounding the oocyte (the cumulus) have started to dissociate.

Diagram 1. The CRESTAR protocol and its mode of action on follicular growth



The oestrogen + progestagen combination is a perfect approach for synchronization of oestrus in beef herds, where the rate of anoestrus is often high at the beginning of the breeding season.

The use of oestrogens in food producing animals has been banned in Europe as from October 2006 (as an application of Directive 2003/74/EC of September 22, 2003; in the various mamber countries). This may be problematic in beef cows, as it is well known that the use of progestagens alone is associated with a reduced fertility in noncyclic females (Ryan et al., 1995; Xu et al., 2000). The mechanisms responsible for this reduced fertility are well understood. In the absence of a corpus luteum, during the last days of treatment with exogenous progestagen suboptimal progestagen concentrations cannot provide a complete inhibition of the LH secretion. These deviant LH patterns fail to sucessfully induce the follicular turnover and emergence of a new follicular wave (Kojima et al., 1992; Kinder et al., 1996); As a consequence, persistent follicles containing aged oocytes emerge. With the CRESTAR® method and other progestagen-based programs, the inclusion of an oestradiol injection at the start of treatment reduced the risk of follicle persistency by inducing such turnover of responsive follicles in almost all cows (Kinder et al., 1996, Yelich et al., 1997).

ii) Progestagens in association with GnRH

As oestradiol esters can no longer be used in cattle in Europe, alternative methods were developped to generate oestrus synchronization, which include a GnRH injection instead of the oestradiol injection at the start of the treatment. Furthermore, a prostaglandin injection is needed 48h before the end of treatment to replace the anti-luteotrophic and luteolytic action provided by oestradiol at the start of treatment (Grimard et al., 2003).

This is the basis of the Crestar® SO method, which combines an injection of buserelin (a GnRH analogue), a norgestomet implant and prostaglandin administration.

At present, France is the only European country where this method has been registered and is marketed.

The synchronization of heat obtained with either CRESTAR® methods, the old one with oestradiol and the new one with buserelin, is based on two different mechanisms. While the norgestomet + oestradiol association (CRESTAR®) triggers regression (by atresia) of the large follicles, the CRESTAR® SO method synchronizes follicular waves by inducing ovulation of the LH-dependent follicles, owing to the LH releasing effects of the injection of buserelin. As follicular waves regularly occur in anoestrous females the LH peak generated by buserelin efficiently induces ovulation of a large proportion of non cycling beef cows.

Severalfeatures of the new CRESTAR® SO method need to be stressed:

- The follicle present on Day 0 is renewed by this new method in around 70% of the cows, as buserelin only triggers ovulation of follicles larger than 10 mm in diameter (Chastant-Maillard et al., 2005). The follicles that fail to ovulate following buserelin administration may continue their development until the ovulatory stage. This is why the duration of treatment has been set at 9-11 days to limit the occurrence of the old persistent follicles (fertility decreases dramatically when the duration of dominance exceeds 12 days).

- GnRH induced ovulation of a large LH sensitive follicle present at the beginning of treatment leads to the formation of an accessory corpus luteum. In females that are treated during luteal phase of their oestrous cycle, its progesterone production adds up to the progesterone produced by a naturally occuring corpus luteum. As a positive correlation has been demonstrated between progesterone concentrations in the days preceding the A.I. and fertility (Folman et al., 1990), this may help to maximize fertility. This additional production of progesterone is of particular interest in anoestrous beef cows that commonly have no endogenous progesterone when treated while in anoestrus.
- The insertion period cannot be shorter than 9 days. In fact, the corpus luteum generated by GnRH-induced ovulation on Day 0 becomes sensitive to prostaglandins around Day 7 (i.e. around 5 days of age). As prostaglandins have to be administered 48 hours before withdrawal of the implant, the implant cannot be removed before Day 9. In practice, the implant should be left in place for 9 – 11 days. While shorter treatement is better, this flexible duration ensures that no work is needed during weekends or public holidays.

An interesting experiment comparing fertility after synchronization with a progesterone releasing device alone and the same device together with an oestradiol or a buserelin injection at insertion is presented below. High fertility was observed in the two groups where a thight control of follicular waves was induced by GnRH or oestradiol (58-60%). In contrast, fertility after the use of the progestagen releasing device alone was clearly lower (belwo 50%) (Ryan et al., 1995) (Fig. 1).

Fig. 1. Comparison of fertility achieved in dairy cows using progestagen alone and in combination with oestradiol or buserelin (Ryan et al., 1995)

Pregnancy rate % (echography 25 - 45 days after A.I.)

Dairy cows 1st AI , vaginal device with 1.9 g of progesterone (CIDR) left in place for 8 days, PGF2-alpha on Day 7, A.I. when oestrus was detected

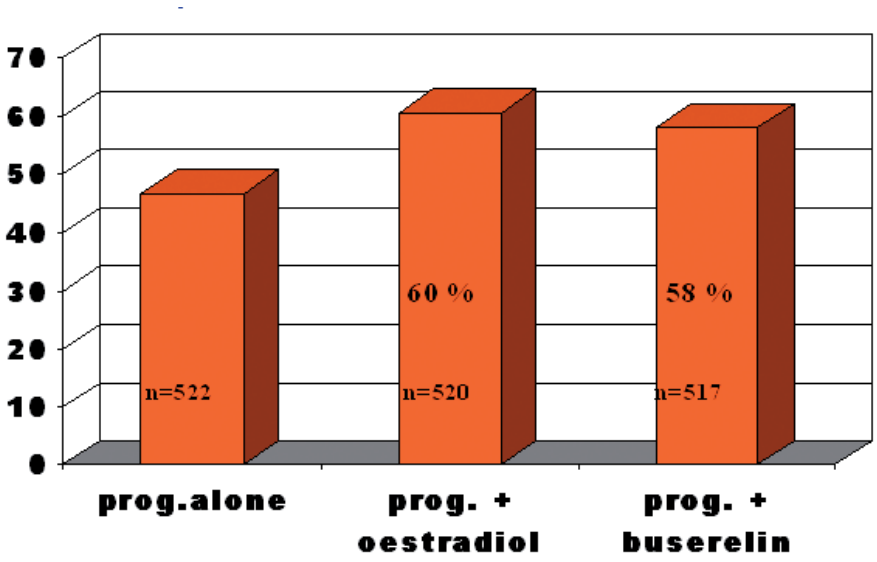
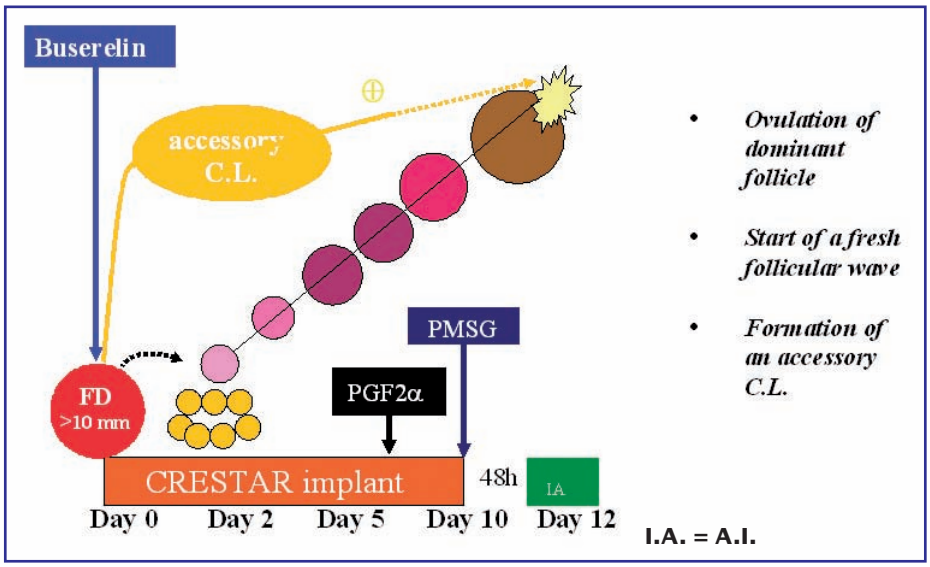


Diagram 2. The CRESTAR® SO protocol and its mode of action



3. Practical recommendations and conclusion

A) Selection of a method for synchronization of oestrus in beef cows and expected results

In suckling cows, the methods for control of oestrus may help to induce a fertile heat in non-cyclic females that are introduced into the breeding herd as single animals or in groups. Once cycling, these cows may be inseminated or presented to a bull. More often however, the control of the oestrous cycle is a zootechnical or organizational approach to synchronize heat in a group of females, whether cyclic or not, and use fixed time AI.

Synchronization generated by progestagen containing devices is best suited for beef cows. This technique offers the double advantage of reducing the workload (both for the cattle breeder and the inseminator) and of precise planning of the calving dates. It also allows for an improvement in overall fertility of a herd by inseminating all females at an earlier stage of the breeding season (in particular those that have not started to display heat prior to treatment).

The substitution of oestradiol by buserelin in the new CRESTAR® SO method generates high fertility results, similar to those achieved with insemination at detected heat.

Several trials conducted in France have confirmed the efficacy of CRESTAR® SO in synchronizing ovulation of beef heifers and cows of various breeds and in herds displaying various ratios of cycling versus non cycling females (Table 1).

Two new experiments have recently been conducted with CRESTAR® SO in Charolais females in France (Grimard et al., 2007). They generated the following additional conclusions

- In heifers, fertility following AI was similar whether AI was performed 36 or 48 hours after removal of the implant. Hence, in practice there is a 12-h time range over which the heifers may be inseminated, making the planning of work more flexible.
- In cows, a single systematic A.I. 48 hours after removal of the implant is adequate to maximize fertility. Adding a second insemination tends to decrease fertility, in particular in primiparous animals.

B) Factors determining successful synchronization

In beef cows, fertility following natural service or A.I. is related to a range of parameters that by now have been well identified. Among the main factors, some are animal-related (parity, conditions of the previous calving, etc.) while others are associated with the their management (nutrition, season of gestation, suckling, etc.).

i) Nutritional status and its consequences for the animal; optimal body condition scores.

As a rule, females should not be too lean at the beginning of the breeding season. Differences of 10 - 25 points in pregnancy rates have been demonstrated between cows that were lean versus well nourished at the beginning of a progestagen treatment (Grimard et al., 2003). A score of 2.5 (on a 1-5 scale) should be targetted for multiparous cows, while 3 appears optimal in primiparous animals. Moreover, the cows should not lose weight throughout the synchonization period. Nutrition during winter shoud therefore be carefully planned, especially in cows that return lean from pasture in autumn. Making sure that primiparous cows are not undernourished during the winter stabling period will allow to avoid a delay in the resumption of their cyclicity at the beginning of the breeding season.

Flushing (2 supplementary UFs during the synchronization treatment and continued until 3 weeks after A.I.) gives favourable results in animals in poor body condition (score ≤ 2) and may improve pregnancy rates by 10 - 25 points (Grimard et al., 2005b). It should be kept in mind that flushing is contraindicated in animals in good condition (Kabananda et al., 1993). Additionally overconditioning of cows should also be

Table 1. Fertility results obtained in France with the CRESTAR® SO method in various beef breeds (Fournier et al., 2004; Barreteau et al., 2005; Grimard et al., 2005a).

	No. of females	Breed	Cyclic	Pregnancy diagnosis	AI CRESTAR®SO	Fertility 1st AI CRESTAR®	Fertility 1st AI CRESTAR SO®
Exp. 1	379 heifers and cows	Charolaise	51 %	Echography 40 - 50 days after A.I.	Single service, at 48 hours	46 %	51 %
Exp. 3	292 cows	Charolaise	49 %	Echography 30 - 50 days after A.I.	Two servicess, at 48 and 72 hours	59 %	60 %
Exp. 2	139 cows	Blonde d'Aquitaine, Limousine	72 %	PSPB determination 40 days after A.I.	Single service, at 48 hours	73 %	75 %

avoided. Besides the extra cost of the surplus feeding, overfed females calve with greater difficulty, may exhibit a delayed resumption of ovarian activity post partum and a lower fertility later during the breeding season.

ii) The parity status

Generally, primiparous cows are characterized by an anoestrus period that is about 3 weeks longer than in multiparous ones (Grimard & Mialot 1990) and their fertility is around 20 points lower. This is because primiparous cows are particularly predisposed to a delayed resumption of ovarian activity post calving through the combined effects of an intense maternal behaviour and a deeper negative energy balance (a significant proportion of the energy ingested is still used for growth in primiparous females). Moreover, difficult calvings occur more often in such females increasing the incidence of post partum anoestrus. Therefore, particular attention should be given to first parity females that should calve in adequate body condition and not below a certain minimum weight (2/3 of the adult weight). Additionally, as fresh primiparous cows they must not lose too much weight during the first 2 months post partum, otherwise they will find it very difficult to "make up" within an acceptable period of time.

iii) Conditions of calving and complications

Difficult calving (forced extraction, cesarean section), poor hygiene in the parturition box, as well as lesions of the reproductive tract (vaginal laceration etc.) are associated with delayed resumption of cyclicity and low fertility. This decrease in reproductive performance is partly linked to an increased frequency of uterine infections. A reduction in fertility (by up to 15 - 30 points) have been detected in females having undergone forced extraction or cesarean section versus those having calved without difficulty (Grimard et al., 2003; Humblot et al., 199¬6).
This is why selection of sires should be made cautiously in order to limit the risk of difficult calvings. In addition, interventions during calving should be kept to the strict minimum and be performed with the best possible hygiene (cleaning of vulva, wearing disposable gloves, etc.).

iv) The breeding season

Calving in autumn (September to mid-December) is associated with a more rapid resumption of ovarian ctivity than calving in winter (in particular in February and March).
Negative effects of the winter season on reproductive results are generated through several factors: shorter daylight, poor nutrition, dark stables with reduced physical exercise, dim lights, etc.
Whereas 70 % of beef cows as a rule are cycling at 70 days post calving in autumn, this proportion drops to 30 % in winter, and as low as 15 % in the case of primiparous animals (French data on Charolaise breed).

v) Suckling

The suckling calf constitutes the chief factor accounting for delayed restoration of cyclicity in beef cows. Suckking hampers the synthesis of GnRH and therefore also of pulstaile LH secretion. In European breeding conditions most calves are reared "with the mother" without the possibility of temporary weaning. In these conditions, the restoration of cyclicity is observed in general not sooner than 55 - 60 days post calving.
Risk factors limiting reproductive performance are known and the manager of a cow-calf operations should take all measures to to reduce their occurrence. In a recent study (Humblot et al.,1996) the fertility of primiparous Charolais cows synchronized by the CRESTAR® method, reached 72 % in females having calved without assistance, being in suitable body condition at the time of A.I. (score > 2.5) and having had an at least 70-day interval between calving and A.I.. In contrast, fertility was only 30 % in primiparous cows that needed assistance during calving, had a low body conndition score (< 2.5) and were inseminated early post calving (shorter than 70-day interval between calving and A.I.).

Pharmacological management of oestrus allows for synchronization of heat and ovulation and insemination performed simultaneously in a group of animals, without the need for heat detection. Among the applicable methods, the synchronizations based on the use of progestagens appears to be the most suitable for suckling beef cows, as they result in the highest reproductive performance especially in herds where a large proportion of females is not cycling, as commonly seen in beef breeds. Classically, the methods used include progestagens combined with oestrogens (oestradiol esters). Following the European ban on the use of oestrogens in cattle, investigations have shown that GnRH in combinaison with progestagens also result in high fertility, irrespective of the cyclicity, of the stage of the cycle of the cycling females and of their parity.

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In the next issue:

Although the next issue of the Intervet Reproduction Newsletter will appear in December we will discuss a very "hot" issue there, the influence of high ambient temperatures on reproduction function in dairy cows. So called heat stress has been an increasing problem in high producing dairy herds and not only in typically tropical zones. Last summer many practitioners from Europe reported dramatic decrease of reproductive performance in dairy herds during the summer months. As both managerial and pharmacological methods can be adopted to ameliorate the negative influence of high temperature on fertility in dairy cows, we would like to place the newsletter early enough to allow all of us, practitioners and breeders to prepare for the next "hot season".

R0234_07

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