Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net

Document heading doi: 10.1016/S2305-0500(14)60051-8

Evaluation of norgestomet Crestar® on oestrus synchronization and reproductive performance of dairy cows in Algeria

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ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form 14 December 2014
Accepted 18 December 2014
Available online 20 March 2015

Keywords:
Norgestomet Crestar®
Timing of AI
Dairy cattle
Pregnancy

ABSTRACT

Objective: To evaluate the effectiveness of norgestomet Crestar® for synchronization of oestrus and its effect on pregnancy rate in cows. **Methods:** after a waiting period (~ 90 days postpartum), Holstein-Frisian females kept in different dairy farms were inseminated for the detection of estrous behavour (n=133). Females were allocated randomly to treated (n=69) or control (n=64) groups. The treated animals received the Crestar® treatment during 9-10 days. After implant removal, the treated animals were artificially inseminated (AI) for the detection of standing estrous behaviour. Blood samples were collected on Days 0, 21 and 30 after service. Plasma progesterone (P4) and pregnancy-associated glycoprotein (PAG) levels were determinate by radioimmunoassay. **Results:** the study showed a higher rate of heat synchronization in the treated group (94%) compared to the control group (86%). Out of 133 cows inseminated, thirteen (4 treated and 9 control) had a plasma progesterone concentrations higher than 1.0 ng/mL at service. The plasma PAG levels at Day 30 after AI were significantly lower in the treated group (7.13 ± 2.29) compared to the control group (8.37 ± 2.30). Pregnancy rate was 28% (16/57) and 42% (23/54) for treated and untreated group, respectively. There were no significant differences in the pregnancy rates of different parity in both groups. **Conclusion:** The norgestomet Crestar® treatment carried out during 9-10 days did not improve reproductive performance in dairy cattle. However, these data demonstrate that this hormonal protocol can be used to obtain high oestrus cow rates to initiate correct timing of insemination.

1. Introduction

Infertility is one of the pathological conditions qualified as disease of production. It is wide-spread in modern farming. Infertility affects the herd reproductive performance thereby negatively influencing productivity and return on investment of the farming business. The effectiveness of reproduction constitutes a crucial factor of productivity and profitability in the exploitation[1]. According to international standards, the interval calving-calving (IVV) of 12-13 month is considered economically optimal. The level of oestrus detection and the conception rate are essential components affecting the IVV. Hormone treatments to synchronize oestrus can reduce the calving to conception interval[2] and facilitate the use of the appropriate time insemination[3,4].

Management of reproduction is an important economic component in the success of a dairy operation. Good detection of oestrus is critically important in dairy husbandry. Incorrect detection of oestrus is related to loss of profit due to extended calving intervals, milk loss,

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veterinary costs, etc[5]. Synchronization of oestrus has been developed to help farmers manage reproduction more efficiently.

The hormones used pharmacologically to control the estrous cycle are identical to (or analogous to) the reproductive endogenous hormones secreted by the hypothalamus (GnRH), the pituitary (LH, FSH), the ovary (estradiol and progesterone), and the uterus (PGF2α) of female mammals. The biological activity of exogenous hormones is similar to the biological activity of endogenous hormones in the normal cow. In the review paper, the earliest oestrus synchronization method (developed in the 1960s) succeeded to block ovulation by administering exogenous progestogens[6].

Oestrus synchronization systems that use progestagens have three primary advantages over systems using prostaglandins alone. First, maintaining the blood progesterone concentration at a level greater than 1 ng/mL suppresses the LH surge and oestrus behavior. Thus, progesterone alone is a highly effective synchronization agent in cyclic cows that have regressed their corpora lutea or functional corpus luteum. Treatment with progesterone for 14 or 21 days resulted in a high oestrus response within 3 days of progesterone removal[7]. Hormonal treatments involving the use progesterone or progestagens (PRID® or Crestar®) to synchronize oestrus and ovulation in buffalo cow are interesting. Ovulation occurs 40–117 h following progestagen withdrawal[8], oestrus has been observed in 80%–93% of treated animals[9] and pregnancy rate following treatment varies from 20%[10] to 50%[11]. In the same way, eCG at norgestomet implant removal increased the rate of ovulation and pregnancy rates in cattle[12]. Crestar is a silicon implant containing 3 mg norgestomet. Treatment protocol with ear implant include an injection of 5 mg estradiol valerate and 3 mg norgestomet at the time of implant insertion to stimulate uterine–induced luteolysis[13], with implant removal after 9 day followed by oestrus detection and AI[14].

The rate of detection of returning oestrus would be substantially improved if returning oestrus could be synchronized on a period of 20 to 25 days after AI[15]. To our knowledge, the impact of incorrect timing of insemination on conception rate has not been evaluated using plasma progesterone concentration and plasma pregnancy associated glycoprotein in the field following norgestomet Crestar® treatment during 9 or 10 days. The objective of the present investigation was to evaluate the effect of norgestomet Crestar® on oestrus detection and reproductive performance in Holstein–Frisian dairy cows using the implant device during 9–10 days. P4 radioimmunoassay was used to retrospectively assess the stage of the cycle (day of heat, day 0) and to determine the number of cows returning in heat (day 21). Rectal palpation and glycoprotein associated pregnancy were used to calculate the conception rate. In addition, we aimed at determining the effect of parity on pregnancy rates in females following the use of norgestomet Crestar® synchronization protocol.

2. Materials and methods

2.1. Animals and treatment

The experiment was conducted during the period from February to April 2004 in Bass Kabylie, Algeria (36°43′N, 5°04′W). A total of 133 lactating Holstein Frisian cows from different dairy herds were used in this study. After a waiting period (~90 days postpartum), whole females (2–10 years old, 0–8 parity) were inseminated after detecting oestrus. Cows were allocated randomly to treated (n=69) or control (n=64) groups. Body condition scores (BCS) of cows were recorded before the initiation of the study. Scores were given by the same researcher based on a 1 (thin) to 5 (obese) scales using a quarter–point system[16]. Females with BCS score between 2.5 and 3.5 were included in the experiment. Note that the thin females (under 2.5) have not been considered.

The treated animals received an intramuscular administration of 3 mg norgestomet (17α–acetoxy–11β–methyl–19–norpreg–4–en–3, 20–dione) and 3.8 mg of estradiol–valerate followed by the subcutaneous insertion in the ear of an implant (Crestar®, Intervet S.A., 49071 Beaucouzé, France) containing 3 mg norgestomet. The implants were placed and kept during 9–10 days using implant device in the middle section behind the ear. The artificial inseminations (AI) were performed once at 56–60 hours post withdrawal of implant. A late pregnancy diagnosis was determined by rectal palpation between 80–90 days after AI.

2.2. Collection of blood samples

Blood samples were withdrawn from the coccegeal vessels into a tube containing EDTA (Sarstedt®, Numbrecht, Germany) on days 0, 21 and 30 after service. Subsequently, the plasma was rapidly separated (1 500 x g for 15 min) and stored at −20 ℃ until assayed[17]. Note that day 0 was considered the day AI.

2.3. Hormone assays

Progesterone (Sigma Chemical Co., St. Louis, MO) was used as standard. The first antibody in rabbit immunized was raised against Progesterone-11-hemisuccinate-BSA injected in multiple sites according to the Vaitukaitis method[18]. Progesterone concentrations were determined in the plasma by using a direct solid-phase ^{125}I radioimmunoassay method (without extraction) performed in duplicate, as described and validated previously[19]. Plasma progesterone estimation was used to identify cases of insemination outside the non-luteal phase. Intra- and inter-assay coefficients of variation were 8.5% and 9.4%, respectively.

The measurement of plasma PAG concentrations was performed by two distinct double-antibody radioimmunoassay differing on their antisera as previously described and validated[20]. Antiserum R#497 was raised against a pure bovine PAG67kDa preparation[21]. A mixture of different (bovine, caprine and ovine) constituted the antiserum named pool[20]. A threshold of pregnancy diagnosis measured by RIA-497 and RIA-Pool was 0.8 ng/mL in all pregnant females[22]. Whereas, intra- and inter-assay coefficients of variation were 3.5% and 6.8% for RIA-497 and 4.5% and 17.3% for RIA-Pool. Assay progesterone and pregnancy associated glycoprotein concentrations were performed in laboratory of endocrinology and animal reproduction at University of Liege in Belgium.

As regards to the ethical aspects, the experimental protocol was approved by the ethical committee of the University, Abderahmane Mira (Bejaia, Algeria). Blood sampling of the Friesian Holstein females was carried out following the rules of good veterinary practice under farm conditions.

2.4. Data analysis

Statistical analyses were carried out in STATVIEW (Version 4.55). Statistical analysis was performed using t-test to compare treated and control females. The data were expressed as mean ± SD, and P<0.05 was considered significant.

3. Results

The pregnancy rates as determined by plasma PAG measurement on day 30 after AI in the treatment and control groups were 28% (16/57) and 42% (23/54), respectively (Table 1). There were not significant differences between both groups. The results of this experiment showed a higher rate of heat synchronization in the treated group (94%) in comparison to control group (86%).

Table 1

Effect of norgestomet Crestar® implant during day 9-10 post AI on reproductive performance of dairy cows.

Parameters	Treated group	Control group
AI (n)	69	64
AI at incorrect timing as per plasma P4 profile (n)[a]	4	9
Pregnancy on Day 30 as per plasma PAG estimations (n)[b]	16/57	23/54
Pregnancy rate (%)	28	42

[a]Progesterone>1.0 ng/mL at the time of AI, [b]a cut-off value of 0.8 ng/mL PAG for pregnancy diagnosis.

Out of 133 inseminated cows and heifers, thirteen were inseminated at a time when plasma progesterone concentration was much higher than 1.0 ng/mL (mean=4.6 ng/mL). In 4 out of 69 treated animals and in 9 out of 64 control animals the plasma progesterone was (4.53 ± 1.15) and (4.68 ± 2.82), respectively. As shown in Table 2, based on the progesterone profiles, the return rate on day 21 after AI was 60% in the treatment group and 42% in the control group.

Table 2

Response to norgestomet Crestar® based on progesterone profiles (mean ± SD) at day 0 and 21 after implant removal in dairy cows.

Groups		Percentage of animals % (P4 ng/mL)	
		Day 0	Day 21
Treated group	P4 level>1.0 ng/mL	6%[a](4.53±1.15)	40%[b](7.68±3.81)
	P4 level<1.0 ng/mL	94%[a](0.17±0.10)	60%[a](0.28±0.27)
Control group	P4 level>1.0 ng/mL	14%[a](4.68±2.82)	58%[b](7.72±2.97)
	P4 level<1.0 ng/mL	86%[a](0.18±0.11)	42%[a](0.39±0.33)

[a,b] Values with different superscripts in treated and control group differ statistically at different days (P<0.0001).

The mean pregnancy rates in different category of parity are illustrated in Figure 1. Note that nulliparous females from the implant and natural heat group exhibit rates of 25% (1/4) and 36% (4/11), respectively. No significant differences are depicted between the two groups (P=0.95). In contrast, the mean pregnancy rates of treated primiparous cows were lower than those of the control groups; 23% (8/37) compared to 40% (6/15), respectively. In the multiparous cows, mean pregnancy rates were somewhat lower in the treated (7/24) than in the control group (12/29) (29% and 41%, respectively). For this case also, no significant differences between the groups are recorded (P=0.95).

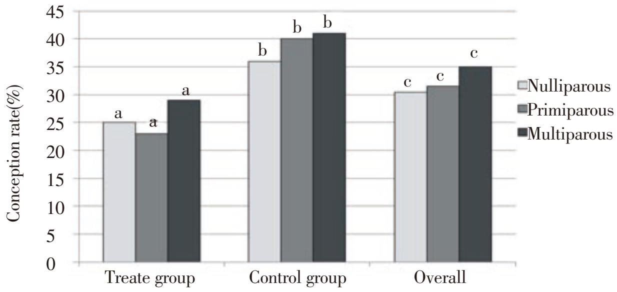

Figure 1. Mean pregnancy rate in nulliparous, primiparous and multiparous Holstein Friesian females after norgestomet Crestar® treatment during (%). [a,b,c]values with similar superscripts in the same group are not statistically different (P>0.05).

[a,b,c] values with different superscripts at different overall, treated and control in a same category of parity differ statistically (P<0.0001).

Table 3 illustrates the mean (± SD) pregnancy-associated glycoprotein (PAG-497 and PAG-Pool) and progesterone concentrations measured in the treated and the control group. The plasma pregnancy-associated glycoprotein levels measured by RIA-497 at day 30 after AI were practically similar in both groups. On the other hand, the mean PAG determined with RIA-Pool system were slightly higher in the treated group than in the control group, however this difference was not statistically significant. Concerning the P4 concentrations, the values obtained were significantly lower in the treated group than in the control group (7.13 ± 2.29 and 8.37 ± 2.30, respectively).

Table 3

Plasma PAG concentration (ng/mL) and P4 (ng/mL) at day 30 in treated and control pregnant females (Mean±SD).

Groups	PAG-497 concentration at Day 30*	PAG-Pool concentration at Day 30*	P4 concentration at Day 30
Treated group(n=16)	$1.83 \pm 0.61^{a,A}$	$5.00 \pm 2.51^{b,B}$	7.13 ± 2.29^{c}
Control group(n=23)	$1.85 \pm 1.41^{a,A}$	$4.29 \pm 2.86^{b,B}$	8.37 ± 2.30^{c}

[a,b] Values with different superscripts in same group between columns (P<0.0001). [A,B]Values with different superscripts in the same RIA-PAG concentration between rows (P<0.0001). [c]values with similar superscripts in the same P4 concentration are not statistically different (P>0.05). *A cut-off value of 0.8 ng/mL PAG for pregnancy diagnosis.

4. Discussion

Farms need to achieve good zootchnical performance and positive financial results to become profitable as quickly as possible[23]. Sá Filho and co-authors[4] reported that several factors, such as age, body condition score, stage of the oestrus at the beginning of the protocol, parity, breed, body condition score and semen quality, influence the timed of insemination artificial. Synchronization of oestrus has been developed to help farmers manage reproduction more efficiently[24]. Efficient and accurate detection of oestrus is vital to good reproductive performance in dairy herd where artificial insemination is used[25]. Sá Filho *et al.*[12] reported that cows that displayed oestrus were 3.3 times more likely to become pregnant than those without a display of oestrus. Numerous researchers have evaluated the potential of using a norgestomet-estradiol as a tool of synchronization oestrus in cyclic and anoestrus cattle[26-28]. However, incorrect timing of AI and the percentage of heat returning in females have not been well documented. Nevertheless, it is clearly known that these factors can significantly affect fertility. The present study was conducted to evaluate the effect of norgestomet Crestar® on pregnancy rate, taking into account the time of AI, and the ratio of oestrus returning at day 21.

A good oestrus response, with approximately 90% of animals showing oestrus soon after treatment has been achieved with PRID or Crestar®[29]. The results of this experiment showed a higher rate of heat synchronization in treated group (94%) in comparison with control group (86%). These results are in agreement with results reported in literatures which show that a rate of heat synchronization was high enough (98%) in Ndama living in different ecological zones[30]. Likewise, the work presented by Garcia and Salaheddine[31] indicates that different treatments used, including Crestar implant, were effective in inducing synchronous behavioral oestrus and ovulation.

In cattle, the detection of pregnancy-associated glycoprotein in maternal circulation is currently used as an accurate method for pregnancy diagnosis at approximately day 30 after breeding or artificial insemination[32,33]. The mean pregnancy-associated glycoprotein concentrations measured (day 30) by the two RIA systems in the treated and control groups were in agreement with those reported by Ayad and collaborators[34]. These values were not significantly different in both groups. However, PAG concentrations obtained by RIA-Pool were significantly higher than RIA-497, because Pool is a mixture of different antisera[19]. According to the PAG concentration at day 30 post-AI, our data showed differences in fertility between the implant-treated (16/57) and control group (23/54) when insemination was carried out after 90 days post calving. Note that the PAG concentrations decrease steadily in the postpartum period reaching undetectable levels only by day 100 postpartum[35]. These results confirmed the work performed by Williams *et al.*[36] in which norgestomet treatment revealed lower conception in cows compared with Syncho–Mate–B and Ovsynch.

Plasma progesterone concentration on day 0 is a measure of the accuracy of oestrus detection. Table 1 shows interesting results for progesterone concentrations classified as either low (<1.0 ng/mL) or high (>1.0 ng/mL). Incorrect timing of AI based on P4 concentrations at day 0 was practically half in the treated compared to untreated group (6% and 14%, respectively). In another study, the percentage of cows in the luteal phase on the day of insemination (i.e. false oestrus detection) was similar in the treated group[37,38]. It is well known that 90% of the cows standing to be mounted are in oestrus[39]. This is the most accurate indicator of oestrus but not the most sensitive[40,41]. In this study, the proportion of females inseminated in non opportune time could be explained by the overestimation of oestrus signs as pretended by farmers. Indeed, the sampling for progesterone assays on the day of artificial insemination might have a positive effect on reproductive performance of dairy cattle, in particular for the oestrus detection. Poor oestrus expression and a prolonged inter–calving interval compromise the reproductive efficiency of female buffaloes[42].

In 2011, Sá Filho and co–authors[43] concluded that administration of estradiol increased the occurrence of oestrus and pregnancy per AI in suckeld Bos indicus beef cow submitted to an estradio/progesterone–based synchronization protocol timed AI. Moreover, Mann and Lamming[44] detected a positive effect of estradiol production during proestrus on progesterone production in the subsequent luteal phase and on embryonic survival. In addition, recent study concludes that the beneficial effects of estradiol supplementation on conception rate were likely caused by post–fertilization processes[45]. It appeared that, on the basis of the variation in blood progesterone levels at day 21, the return rate to oestrus of females treated by implant were higher than in untreated group (60% compared to 42%, respectively). The higher return rate of females to oestrus could be justified by a fertilization failure or an early embryonic death[46,47]. But, it is very difficult to confirm it, because there is no availability of biological or immunological methods that could be used to measure pregnancy associated glycoprotein concentrations during the early stage.

Significant differences in the pregnancy rates in nulliparous, primiparous and multiparous cows were recorded in both groups. The results obtained with treated and untreated groups in primiparous and multiparous were similar to the findings of Cavalieri and Macmillan[48]; and Bülbül and Atman[48], respectively. Ponsart *et al.*[49] obtained higher pregnancy rate in multiparous cows than primiparous cows when synchronization protocols used progesterone implant. In Buffalo cattle, parity shows an effect on synchronization treatment efficacy with multiparous animals responding better than primiparous animals[51]. In our work, the pregnancy rates were similar to previous research. These differences suggest that parity could affect the efficiency of a synchronization protocol[49]. In contrast, nulliparous females exhibited higher pregnancy rates than primiparous cows (25% vs. 23%). These slight differences could be due to reproductive problems after calving. These divergence between nulliparous and primiparous may be also explained by the nutritional status, body score and time of initiation of the protocol[52]. In conditions sub–tropical, the pregnancy rate in heifers was very higher than in cows[53]. Additionally, other experiment has reported an effect of eCG on pregnancy outcome only in primiparous cows[54]. Ansari–Lari and collaborators[55] concluded that high milk production is risk factor for decreasing fertility. The reproductive tract of lactating dairy cows may provide a less favorable environment for very early embryo development than that of the heifers[56].

In conclusion, the norgestomet Crestar® treatment during 9–10 days did not improve reproductive performance in dairy cows. However, these data demonstrate that this hormonal protocol can be used to obtain satisfactory cyclic cow rates and less incidence of incorrect timing of insemination. Furthermore, there were no differences in overall rates pregnancy between nulliparous, primiparous and multiparous when using implant protocol.

Conflict of interest statement

The authors declare that they have no conflict of interests.

Acknowledgements

Dr Ayad A. gratefully acknowledges Professor Beckers J.F. (Laboratory of Physiology of Animal Reproduction, Faculty of Veterinary Medicine, ULg, Belgium) for furnishing of reagents, technical assistance and reception in his laboratory. The authors thank M. Harrats Ch. and Ouail O. for the English correction.

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