



## Effect of ovulation synchronization program and season on pregnancy to timed artificial insemination in suckled beef cows

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### ABSTRACT

This study was conducted to (i) evaluate the requirement for the administration of GnRH coincident with insertion of a progesterone-releasing intravaginal device (PRID) and (ii) the effect of supplementing with 400 IU eCG at PRID removal on pregnancy per AI (P/AI) in spring and autumn calving suckled beef cows, subjected to a 7-d CO-Synch + PRID timed artificial insemination (TAI) program. Suckled beef cows (n = 1408) on 62 commercial farms were enrolled and randomly assigned to either of three treatments: 1) cows received a PRID and 100 µg GnRH on Day –10, followed by 25 mg PGF<sub>2α</sub> at PRID removal (Day –3) and 100 µg GnRH 72 h later (Day 0) at TAI (Treatment 1; n: spring = 236, autumn = 248); 2) as Treatment 1, but without GnRH at PRID insertion on Day –10 (Treatment 2; n: spring = 232, autumn = 227); 3) as Treatment 1, but cows also received 400 IU eCG at PRID removal on Day –3 (Treatment 3; n: spring = 233, autumn = 232). At Day –10, ovaries were examined by ultrasonography to evaluate the presence or absence of a corpus luteum (CL) and follicle(s) ≥ 10 mm in diameter. Body condition score (BCS) was assessed on a scale of 1–5. Pregnancy diagnosis was carried out 30–35 d after TAI by transrectal ultrasonography. Data were analyzed using the GENMOD and LOGISTIC procedures of SAS. There was a treatment by season interaction for P/AI (P < 0.001). In spring, overall P/AI was 59.1% (414/701) and was affected by treatment (59.3 v 49.6 v 68.2%, for Treatments 1, 2 and 3, respectively P < 0.05). In contrast, in autumn, overall P/AI (51.5%, 364/707) was unaffected (P > 0.05) by treatment (50.1 v 53.7 v 48.7% for Treatments 1, 2 and 3, respectively). Overall, eCG had a positive effect on P/AI for cows lacking a CL at treatment initiation (P < 0.05). In addition, in cows with low BCS (≤2.25), eCG supplementation tended (P = 0.09) to improve P/AI. Seasonal differences in response to synchronization treatment may be reflective of different management regimens (grazing v confinement) and breed type and remain to be elucidated.

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DAK and FR conceived the study and recruited all participating herds and conducted the on farm measurements. PL, MP and MGD assisted with study design and execution. FL assisted with herd recruitment. AK and DAK conducted the statistical analysis. FR, PL and DAK drafted the manuscript. All authors reviewed and edited the manuscript.

### 1. Introduction

The two main drivers of reproductive efficiency in cattle operations are submission rate – the proportion of eligible cows

presented for breeding, and subsequent conception rate. Ovulation synchronization protocols which facilitate the use of timed AI (TAI), without recourse to estrus detection, provide the opportunity to dramatically increase the use of AI in beef cows because of the maximization of submission rate [1]. Such protocols, however, must be both labour and cost efficient as well as being effective. Using current protocols, it is now possible to expect herd pregnancy rates from AI in excess of 50% during the first week of the breeding season [2,3]. Several studies have demonstrated how the introduction of TAI as a management tool can positively impact the efficiency of beef operations [4,5]. In particular, the use of proven AI sires results in greater herd weaning weight and calf value, while facilitating lesser incidence of dystocia [2]. In addition, cows bred using estrous synchronization programs at the initiation of the breeding season are significantly more likely to wean a calf at the

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end of the subsequent year [4,6]. Although much work has been conducted internationally on the development of TAI protocols for beef cows (e.g. Refs. [1,7]), much of this work, particular that in South America, is reliant on the use of estradiol-based interventions which are not permitted in the EU, thus limiting their widespread adoption. Furthermore, in contrast to the US, use of equine chorionic gonadotropin (eCG) is currently permitted in the EU. For these reasons, it is of interest to evaluate the efficacy of eCG in combination with gonadotropin releasing hormone (GnRH) - progesterone (P4) - prostaglandin (PGF<sub>2α</sub>)-based programs, to establish robust, repeatable protocols requiring minimal animal assembly and interventions.

Up to 60% of beef cows may still be anestrus at the initiation of the breeding season [8,9], which is an important concern in the design of effective TAI protocols. Synchronization programs that include P4 have the potential to significantly impact reproductive efficiency in suckled beef cows, as ovulation of a healthy oocyte can be induced in postpartum anestrus cows [for review, see 10, 11]. Furthermore, the importance of elevated P4 during the growth of the ovulatory follicle has become apparent and this knowledge is now incorporated into many synchronization protocols [12–14]. Although the effectiveness of administering eCG at the time of removal of the P4-releasing device has been variable in lactating dairy cows [15–17], improved pregnancy per AI (P/AI) has been reported for both beef heifers [18] and beef cows [18], particularly anestrus cows and those having a poor body condition score (BCS) [19,20]. Despite being the first country in the world to implement a genomic selection-assisted national breeding program for beef cattle, only approximately 20% of calves born in Irish beef herds are produced by AI [21], consistent with many other beef herds globally. This low usage of such a well-tested and effective technology is particularly worrying for the international beef industry and does not bode well for the rapid introduction of superior genetics, fundamental to sustained improvement in productive efficiency. Although a significant volume of literature has been produced on the topic of TAI in beef cattle [reviewed by 6,22], the adoption of this technology has been relatively limited, worldwide. The European beef industry is typified by very low average herd size and a multitude of different environmental conditions [23]. For this reason, we investigated the feasibility of using TAI programs under commercial conditions, where average herd size is relatively small and production systems include both pasture-based spring-calving as well as semi-confined autumn-calving herds.

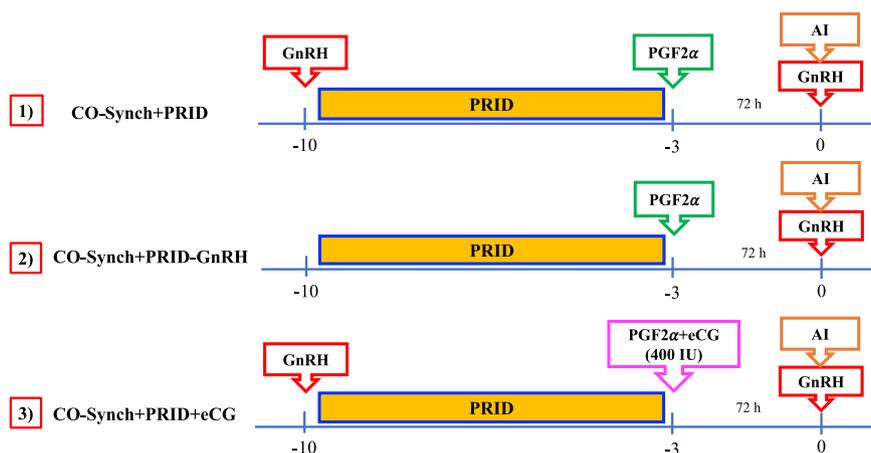
Thus, the overall objective of this study was to evaluate strategic manipulation of a P4-releasing intravaginal device (PRID)-based synchronization protocol for TAI in suckled beef cows to optimise efficacy, reliability and both labour and economic efficiency. Specifically, we examined the requirement for the administration of GnRH at the initiation of a standard 7-d CO-Synch + PRID treatment and the inclusion of 400 IU of eCG at PRID removal on P/AI, over two seasons (spring- and autumn-calving herds), under commercial conditions representative of many beef production systems throughout Europe. We hypothesized that GnRH at the start of the synchronization treatment and eCG at the end would enhance P/AI in suckled beef cows.

## 2. Materials and methods

All experimental procedures involving animals were sanctioned by the Teagasc Animal Ethics Committee and licensed by the Health Products Regulatory Authority ([www.hpra.ie](http://www.hpra.ie)) in accordance with Statutory Instrument No. 543 of 2012 (under Directive 2010/63/EU on the Protection of Animals used for Scientific Purposes).

This experiment was conducted on commercial beef farms throughout the island of Ireland between April 2014 and January 2015. A total of 1408 suckled beef cows consisting of approximately 16% primiparous and 84% multiparous cows located on 62 farms were enrolled in the study that was replicated in spring (n = 701 cows on 30 farms) and autumn (n = 707 cows on 32 farms) breeding seasons. The number of cows enrolled per herd ranged from 10 to 81, with an average of 23 cows per herd. Spring-calving cows grazed perennial ryegrass-based pastures from calving to pregnancy diagnosis, while in contrast, autumn-calving cows were typically fed a combination of grass silage and concentrates and were housed indoors for the duration of the trial.

Cows were enrolled in the study after a minimum of 30 d postpartum. At initiation of the trial, BCS was recorded using a scale ranging from 1 to 5, where 1 corresponds to emaciated and 5 to obese [24]. Before the initiation of the synchronization program, all cows were evaluated by transrectal ultrasonography using a portable unit fitted with a 5–7.5 MHz probe (Easi-Scan, BCF Technology Ltd, Livingston, UK) in order to exclude any animals exhibiting evidence of uterine and/or ovarian pathologies and also to evaluate ovarian status to determine the presence or absence of follicle(s) ≥ 10 mm in diameter as well as the presence or absence of a corpus luteum (CL).



**Fig. 1.** Schematic diagram of the synchronization protocols used in the study. Suckled beef cows were ultrasound scanned (US) to record the presence or absence of a corpus luteum and a follicle(s) > 10 mm in diameter at treatment initiation and randomly assigned to receive a 7-day progesterone-releasing intravaginal device (PRID) with (Treatment 1, n = 484) or without (Treatment 2, n = 459) gonadotropin-releasing hormone (GnRH) at PRID insertion. A luteolytic dose of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) was administered at PRID removal and a third group of cows (Treatment 3, n = 467) received 400 IU equine chorionic gonadotropin (eCG) at PRID removal. GnRH was administered to all cows at timed AI 72 h after PRID removal.

The experimental protocol is illustrated in Fig. 1. Cows were randomly assigned to receive a 7-d P4-releasing intravaginal device (PRID® Delta, containing 1.55 g P4, CEVA Santé Animale, Libourne, France) with (Treatment 1, CO-Synch + PRID; spring n = 236, autumn n = 248) or without (Treatment 2, CO-Synch + PRID-no GnRH; spring n = 232, autumn n = 227) administration of a GnRH analogue (Ovarelin®, 100 µg of gonadorelin diacetate tetrahydrate, CEVA Santé Animale) at PRID insertion. All cows received a luteolytic dose of PGF<sub>2α</sub> (Enzaprost T®, 25 mg of dinoprost trometamol, CEVA Santé Animale) at PRID removal, whereas a third group of cows (Treatment 3, CO-Synch + PRID + eCG; spring n = 233, autumn n = 232) received 400 IU eCG (Syncrostim®, CEVA Santé Animale) at that time. GnRH was administered to all cows at TAI, 72 h after PRID removal. Treatments were balanced both within and across herds and across season.

The management of repeat breeding was in accordance with the practice prevailing on the farm. Where bulls were used, these were not introduced until Day 10 after TAI in order to remove any ambiguity between pregnancies achieved by AI and those resulting from natural mating. Pregnancy diagnosis was performed on all participating farms, by transrectal ultrasonography, 35–40 d after TAI.

Calving difficulty was retrospectively evaluated in a subsample of cows (n = 882) based on the score that farmers provided to the Irish Cattle Breeding Federation database following their guidelines for scoring calving ease. Calving assistance was scored on a scale of 1–4 as follows: (1) no assistance; (2) slight assistance (assistance by one person, without the need to use a calf puller); (3) considerable assistance (assistance by one person requiring the use of a calf puller or more than one person); (4) veterinary assistance (including Caesarean operations) [25].

### 2.1. Statistical analysis

Generalized linear mixed models (PROC GLIMMIX, SAS 9.4, SAS Institute, Cary, NC) with a binary distribution and the logit function specified were used to evaluate the effect of synchronization treatment on mean pregnancy rate. The model contained the fixed effects of synchronization treatment (Treatment 1, 2 and 3), season (Spring or Autumn) and the interaction of synchronization treatment x season. Random terms in the model included herd and herd x treatment interaction. The Co-Synch + PRID treatment group (1) was the control to which the Treatments 2 (- GnRH) and 3 (+eCG) were compared.

For cow level factors, days post-partum, BCS at protocol initiation and CL presence or absence, the interactive effects with synchronization treatments on pregnancy rate each cow factor were analyzed separately in a statistical model. Days post-partum were categorically coded <50 days, 51–65 days, 66–80 days and >80 days. Corpus luteum (presence or absence) and BCS (<2.25 (low), or

>2.5 (target)) at protocol initiation were retrospectively categorized. Each model contained individual cow level factors (days post-partum, BCS at protocol initiation and presence/absence of a CL) along with the fixed effects of synchronization treatment (Treatment 1, 2 and 3), season (Spring or Autumn) and their interactions. Interaction effects were removed sequentially from the model beginning with the effects with the largest P-value and continuing until only effects with P ≤ 0.1 remained in the model. Random terms in the model included herd and herd x treatment interaction. Parity (primiparous or multiparous) effects were examined but were non-significant (P > 0.10) in all analyses and thus excluded from the final models. Comparisons of least squares means (LSM) for pregnancy per AI between factors were performed using Tukey-Kramer adjustment for multiple comparisons. A statistically significant difference or effect was considered to exist when P < 0.05. Results are reported as statistic ± standard error of the statistic (e.g., LSM ± SEM).

A multivariate logistic regression prediction model was constructed using LOGISTIC procedure in SAS to examine biological drivers pregnancy rate across synchronization regimens. Days postpartum, BCS, presence of a CL and/or follicle(s) ≥ 10 mm at treatment initiation, previous calving difficulty and parity. Logistic binomial regression analysis using the Wald statistic was used to investigate the associations with the predictor variables. Multivariable model selection proceeded from a maximum model containing all variables that were associated (P < 0.15) and variables were removed from the model in a stepwise process until only those reaching P < 0.05, remained. Regression coefficient, prediction model R-square, along with prediction variables odds ratios with the associated 95% confidence intervals (CI) were derived.

### 3. Results

Summary data are presented in Table 1. A total of 111 cows were excluded from the trial at initial enrolment due to the presence of uterine pathological conditions diagnosed at ultrasonographic examination. This consisted of 19/722 (2.6%) and 92/799 (11.5%) for spring and autumn replicates, respectively (P < 0.001). As indicated in Table 1, more cows were cyclic based on the presence of a CL at PRID insertion in the autumn (69.8%) than in the spring (49.7%).

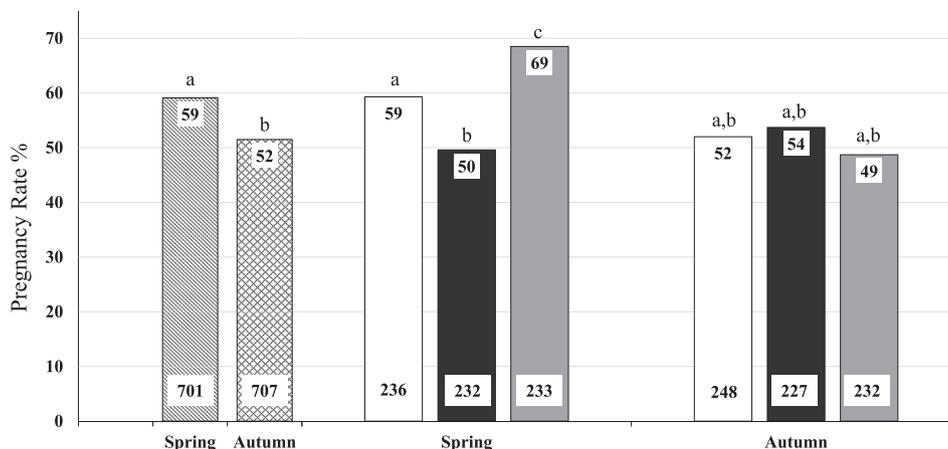
A treatment x season interaction (P < 0.001) was detected for P/AI (Fig. 2). For example, in the spring replicate, GnRH administration at PRID insertion improved the P/AI (59.3% vs 49.6%; P < 0.05) and eCG supplementation at PRID removal further improved the P/AI (59.3% vs 68.2%; P < 0.05). In contrast, in the autumn replicate, no differences were observed in pregnancy/AI among the three treatments, with P/AI across treatments ranging from 49 to 54%. In spring, overall P/AI was 59.1% (414/701) while in autumn, overall P/AI was 51.5% (364/707).

For cows in which a CL was not detected at PRID insertion, those

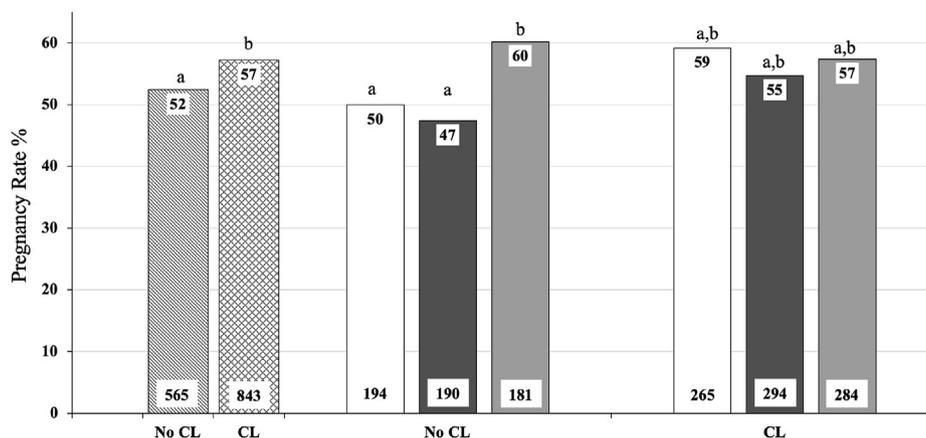
**Table 1**  
Summary statistics of the suckled beef cows employed prior to initiation of the ovulation synchronization protocols.

Variable	N	Mean	Std Dev	Median	Min	Max	Spring	Autumn
BCS	1408	2.77	0.47	2.75	1	4.75	2.75	2.78
Days postpartum	1281	69.67	41.4	63	23	474**	72.53	67.19
Calving difficulty score*	822	1.16	0.531	1	1	4	1.14	1.18
Parity	1139	3.98	2.59	3	1	15	4.76	3.44
CL %	1408	59.8	—	—	—	—	49.7	69.8
Follicle ≥10 mm %	1408	51.9	—	—	—	—	35.3	46.9

BCS: body condition score; CL %: presence of a corpus luteum at treatment initiation; Follicle ≥10 mm %: presence of a follicle ≥10 mm at treatment initiation. \*Scored on a 4-point scale 1 = unassisted calving, 2 = assisted by only 1 person with no aids, 3 = assisted by more than one person and/or calving aids 4 = veterinary intervention. \*\*In total, only 3/1408 total cows were >400 days postpartum. Indeed, as little as 5% of the total cows enrolled were >105 days postpartum.



**Fig. 2.** Effect of treatment and season on mean pregnancy per AI following timed AI in suckled beef cows. Cows received a 7-day progesterone-releasing intravaginal device (PRID) with (Treatment 1, white bar) or without (Treatment 2, dark grey bar) gonadotropin-releasing hormone (GnRH) at PRID insertion. A luteolytic dose of prostaglandin F<sub>2α</sub> was administered at PRID removal and a third group of cows (Treatment 3, light grey bar) received 400 IU equine chorionic gonadotropin at PRID removal. GnRH was administered to all cows at timed AI 72 h after PRID removal. Values at the base of the bars indicate the number of cows involved. Values at the top of the bars indicate the pregnancy per AI (%). A treatment × season interaction was detected ( $P < 0.001$ ). Within season, superscripts indicate treatment differences ( $P < 0.05$ ).



**Fig. 3.** Effect of treatment and corpus luteum (CL) presence or absence at protocol initiation on mean pregnancy per AI following timed AI in suckled beef cows. Cows were ultrasound scanned to determine the presence or absence of a CL and randomly assigned to receive a 7-day progesterone-releasing intravaginal device (PRID) with (Treatment 1, white bar) or without (Treatment 2, dark grey bar) gonadotropin-releasing hormone (GnRH) at PRID insertion. A luteolytic dose of prostaglandin F<sub>2α</sub> was administered at PRID removal and a third group of cows (Treatment 3, light grey bar) received 400 IU equine chorionic gonadotropin at PRID removal. GnRH was administered to all cows at timed AI 72 h after PRID removal. Values at the base of the bars indicate the number of cows involved. Values at the top of the bars indicate the pregnancy per AI (%). A treatment × CL presence or absence interaction was detected ( $P = 0.05$ ).

on the CO-Synch + PRID + eCG treatment exhibited improved P/AI by 10–13% points ( $P < 0.05$ ) compared to those enrolled in the other two treatments (Fig. 3). In contrast, for cows in which a CL was present at PRID insertion, pregnancy/AI was not affected by synchrony treatment. Irrespective of treatment, the presence or absence of a follicle(s)  $\geq 10$  mm at protocol initiation did not affect pregnancy/AI ( $P > 0.05$ ).

An overall effect of BCS ( $P = 0.01$ ) was detected with cows in low BCS having a lesser pregnancy/AI (48% versus 57%) compared with those in moderate to high BCS (Fig. 4).

Days postpartum at treatment initiation significantly influenced P/AI ( $P < 0.05$ ); highest P/AI was achieved in those cows 66–80 d postpartum (Fig. 5).

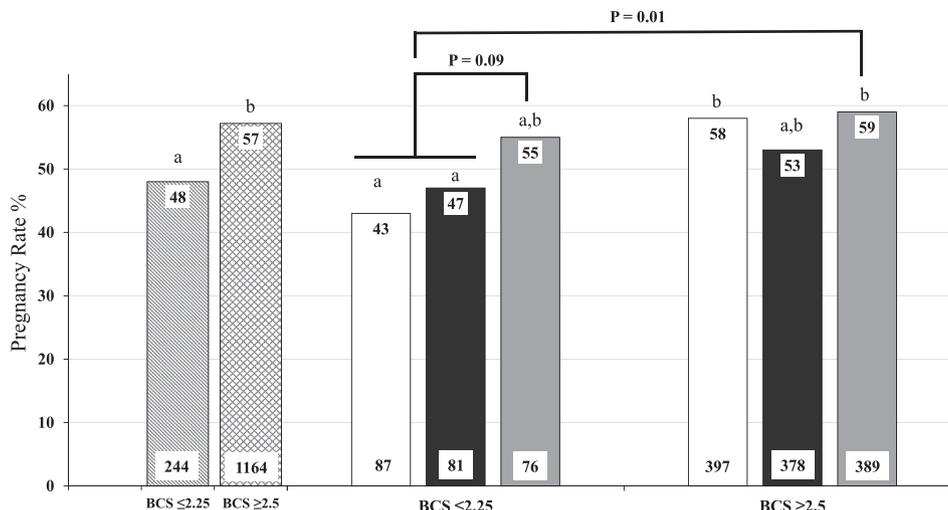
Across all the variables examined, multivariable logistical analysis identified BCS ( $P = 0.01$ ; odds ratio 1.35; 95% wald CI 1.06–1.73) and days postpartum ( $P = 0.06$ ; odds ratio 1; 95% Wald CI 0.99–1) as the two cow-related drivers of pregnancy success rate in a synchronization regimen. The pregnancy prediction model was as follows = intercept (−0.79) + BCS (0.31) + days postpartum (0.002) + synchronization treatment (Treatment 1 (−0.12);

Treatment 2 (0.006); Treatment 3 (0)) + Season (spring (0.16); autumn (0)).

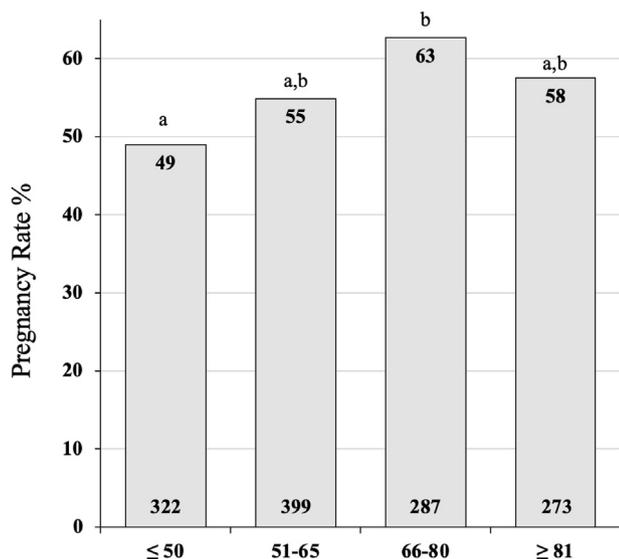
#### 4. Discussion

The main findings from this study were that (i) P/AI in excess of 65% are achievable in suckled beef cows using a minimal intervention TAI regimen without recourse to estrus detection and the associated labour requirement; (ii) GnRH administration at treatment initiation and eCG at PRID withdrawal both improved pregnancy per TAI in spring-calving but not in autumn-calving suckled cows.

Challenges associated with estrous detection have led to effort in the development of effective estrous synchronization protocols for beef and dairy cows [6,14]. Prohibition of the use of estradiol within the EU [26], however, has rendered many effective protocols used elsewhere obsolete [27]. Although alternative programs (i.e. using a combination of GnRH-P4-PGF<sub>2α</sub>) which facilitate the use of TAI for beef cows have been developed in some countries [2], such protocols have not been thoroughly tested under European



**Fig. 4.** Effect of treatment and body condition score (BCS) score at protocol initiation on mean pregnancy per AI following timed AI in suckled beef cows. Cows received a 7-day progesterone-releasing intravaginal device (PRID) with (Treatment 1, white bar) or without (Treatment 2, dark grey bar) gonadotropin-releasing hormone (GnRH) at PRID insertion. A luteolytic dose of prostaglandin F<sub>2α</sub> was administered at PRID removal and a third group of cows (Treatment 3, light grey bar) received 400 IU equine chorionic gonadotropin at PRID removal. GnRH was administered to all cows at timed AI 72 h after PRID removal. Values at the base of the bars indicate the number of cows involved. Values at the top of the bars indicate the pregnancy per AI (%). Superscripts indicate BCS treatment differences (P < 0.05).



**Fig. 5.** Effect of days postpartum at protocol initiation on mean pregnancy per AI following timed AI in suckled beef cows. Bars not sharing a superscript differ significantly (P < 0.05). Values at the base of the bars indicate the number of cows involved. Values at the top of the bars indicate the pregnancy per AI (%).

seasonal systems of production. Compact calving before turn-out to pasture in spring is an essential component of pasture-based suckled beef production systems to ensure maximum pasture utilization and, hence, profitability [28]. Notwithstanding this, some producers elect to maintain an autumn-calving component to their herd in order to either reduce requirements for labor and facilities and/or reduce the seasonality of animal sales. Regardless of season, achieving a highly concentrated period of calving requires both high submission and high conception rates within a short period following the planned start of mating. In beef cows, high submission rate in the first 6 weeks of the breeding period is highly dependent on cows having resumed estrous cycles by 50 d post-calving [28]. Compared with dairy cows, there is typically considerable variability in the duration of the postpartum anestrus period in suckled beef cows with mean duration often extending to

beyond 80 d, even in cows of moderate to good BCS [11] due to the failure of successive dominant follicles to ovulate [29]. Although management practices such as restricted suckling [30,31] and/or exposure to an intact male [32–34] can stimulate increased hypothyseal LH pulsatility and hasten the resumption of ovarian cyclicity in some cows, such approaches, particularly calf separation, may be impractical and/or labor intensive at farm level. Although AI and estrous synchronization programs for ovulation control remain the most important and widely applicable reproductive biotechnologies available for beef cattle operations, their use has found limited application mainly because of associated time, logistics, labor and cost constraints.

In the current study, a significant treatment by season interaction was observed for P/AI. In the spring replicate, GnRH administered at the start of the PRID treatment resulted in an increase in P/AI (49.6% vs 59.3%). In contrast, no effect of initial GnRH was observed in the autumn replicate. The size of the pre-ovulatory follicle has been positively correlated with pregnancy rate after induced ovulation in beef cows [35] and heifers [36]. The proportion of beef cows ovulating in response to GnRH typically ranges from 36% in noncyclic [37] to 50% in cows that have resumed cyclicity [37]. Anestrus cows that ovulate a dominant follicle after the first GnRH administration typically have larger dominant follicles at the time of the second GnRH administration and exhibit higher systemic concentrations of estradiol when synchronized with a CO-Synch + P4 program [37]. Furthermore, cyclic cows that ovulated after the first GnRH injection in the aforementioned study had similar follicular size at the second GnRH to those that did not respond to the first GnRH. This effect is apparently consistent, regardless of stage of the cycle and serum P4 concentrations at protocol initiation [37]. In contrast, another study reported that cows ovulating to the first GnRH injection had reduced P/AI after TAI when synchronized with a 7-d CO-Synch + P4 protocol with or without administration of 200 IU of eCG [30].

An overall positive effect of GnRH administration at the initiation of the synchronization program was evident in spring-calving cows, where a greater proportion of cows (50%) did not have a CL at the initiation of the treatment protocol. This is consistent with the results of Atkins et al. [38], who reported that cows in anestrus which ovulate to the first GnRH presented a larger follicle at the

time of AI and second GnRH, and that a smaller follicle diameter at the time of AI resulted in lesser P/AI [12]. In contrast, this was not observed in the autumn replicate where more than 70% of cows had a CL at PRID insertion. In cyclic cows, it has been reported that, regardless of ovulation response to the first GnRH, cows presented a similar follicle size at the time of AI [37]. In a recent large retrospective analysis, initiating a CO-Synch + P4 program with or without GnRH produced similar pregnancy outcomes [39] and is consistent with other studies [40].

The effect of season on beef cow fertility, particularly the length of postpartum anestrus, has been known for some time [41]. Cows calved in late spring have shorter postpartum intervals compared with those calving earlier [42]. This may be due to differences in daylength but also feed availability. Moreover, increasing the photoperiod in winter-calving suckled cows with artificial exposure to 18 h of daylight in wintertime reduced the length of the postpartum period compared with the untreated cows and also in some cases reduced the interval from calving to conception [43–45].

Equine chorionic gonadotropin, a glycoprotein with gonadotrophin-like activity, has been employed in P4-based protocols for TAI and also for timed embryo transfer as it seems to improve synchrony of ovulation, and has shown beneficial effects on embryo development and survival [46]. Such effects are particularly evident in cows in which LH secretion and ovarian activity are reduced or compromised, for instance, during the early postpartum period, under seasonal heat stress, in anestrus cows or in cows with a low BCS [reviewed by [47,48]]. The use of eCG has been reported to increase P/AI following TAI, particularly in anestrus cows or those with low BCS at the time of the synchronization [reviewed in 3,6]. In some studies, eCG supplementation has been as efficient as, or even more efficacious than, temporary calf removal in inducing fertile ovulations in anestrus postpartum suckled cows [31,49], although this was not confirmed by others [30]. The improvement in P/AI may be partly explained by the increased diameter of the ovulatory follicle [30,31,50,51], and the associated increase in luteal volume [31,50,51] and P4 production [50–53]. Elevated P4 concentrations in the week post-ovulation have been shown to alter uterine endometrial gene expression [54] and to be associated with advanced conceptus growth [55] and increased likelihood of pregnancy [56,57].

The positive effects of eCG administration were particularly evident in the spring replicate, where overall P/AI across all animals enrolled increased from 59.3% with the standard CO-Synch + PRID to 68.5% in the CO-Synch + PRID + eCG-treated cow. The use of eCG in synchronization programs for beef cows has been comprehensively reviewed [58]. Administration enhanced fertility of suckled cows with low BCS at the time of treatment application, raising the ability of these cows to conceive to the level achieved by cows in moderate to high BCS  $\geq 3$ . These beneficial effects are achievable only for cows that were gaining body condition during the breeding season, compatible with the spring-calving group in this study, as pasture production quality progressively increases during the breeding season [59]. Cows that are not gaining body condition during the breeding period do not benefit from the effects of eCG [58]. During the winter season, suckled cows in the Irish system are housed and tend to lose weight and BCS [60]. Supplementation with eCG did not increase P/AI in the cows synchronized in the autumn, 70% of which presented a CL at the time of protocol initiation, in contrast to the increase in fertility in the spring where only approximately 50% of cows had a CL at PRID insertion. It has been previously reported that the supplementation with eCG enhances P/AI in cows lacking a CL at the time of protocol initiation, but does not increase the fertility performances of cows presenting a CL at the time of protocol initiation [58].

The larger incidence of uterine pathological conditions after the

imposed voluntary waiting period of 30 d observed in this study in autumn-calving cows may be related to the fact that autumn calving cows are typically bred to more muscular bulls and calve off a better plane of nutrition. These two facts could combine to potentially give rise to more dystocia and a higher incidence of uterine problems [61]. Although not available at the initiation of the study, we included recorded calving difficulty score in our analysis, retrospectively, and found that it was balanced across treatment groups for both seasons of the study. Previous reports have established that calving difficulty could impact on the duration of postpartum anestrus in suckled beef cows and calving interval [45] and that dystocia could account for as much as 10.5% of the variation observed in P/AI in beef cows [62]. In contrast, under Irish conditions a previous study did not find any impact of previous calving difficulty on the subsequent reproductive performance of suckled beef cows [42].

## 5. Conclusions

Results indicate that the main drivers of a successful synchronization regimen at farm level are BCS and days postpartum at treatment initiation. Use of GnRH at the start of the protocol and supplementation with eCG at PRID removal under certain circumstances (spring replicate) had a positive effect on pregnancy outcome. The use of artificial insemination is still the most cost-effective way to increase genetic progress in beef herds. Indeed, the uptake of synchronization programs for TAI has become widespread in many major beef producing countries (e.g., Brazil). Results demonstrate the effectiveness of this technology, within the context of a large-scale field trial conducted under pasture-based conditions, the results of which provide a basis for increased uptake of AI with the associated benefits in terms of genetic improvement.

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