Accepted Manuscript

Evaluation of alternative strategies to treat anoestrous dairy cows and implications for reproductive performance in pasture-based seasonal calving herds: a pilot study

E. Rojas Canadas, M. Gobikrushanth, P. Fernandez, J. Kenneally, P. Lonergan, S.T. Butler

PII: S0093-691X(18)30943-9

DOI: 10.1016/j.theriogenology.2019.01.008

Reference: THE 14838

To appear in: *Theriogenology*

Received Date: 14 October 2018

Accepted Date: 11 January 2019

Please cite this article as: E. Rojas Canadas, M. Gobikrushanth, P. Fernandez, J. Kenneally, P. Lonergan, S.T. Butler, Evaluation of alternative strategies to treat anoestrous dairy cows and implications for reproductive performance in pasture-based seasonal calving herds: a pilot study, *Theriogenology* (2019), doi: 10.1016/j.theriogenology.2019.01.008

Evaluation of alternative strategies to treat anoestrous dairy cows and implications for reproductive performance in pasture-based seasonal calving herds: a pilot study

E. Rojas Canadas[1,2], M. Gobikrushanth[3], P. Fernandez[1], J. Kenneally[1], P. Lonergan[2] and S.T. Butler[1]

[1]*Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland*

[2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland*

[3]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.*

* Corresponding author.Tel.: + 353 25-42252 *mail address*: stephen.butler@teagasc.ie (S.T. Butler)

ABSTRACT

The objective of the present study was to assess the effects on ovulation and reproductive performance of a single injection of either GnRH or hCG applied 9 days before the start of the seasonal breeding period in anovulatory anoestrus cows compared with a 7-day progesterone-Ovsynch protocol. The study was conducted on four grass-based seasonal calving dairy herds in Ireland. The total number of cows in the herds was 2112, of which 488 were diagnosed as anoestrus based on absence of behavioural oestrus during a 30 day period. Ovarian structures and the uterus were examined by transrectal ultrasound on all 488 presumptive anestrus cows 9 days before mating start date (MSD). The number of corpora lutea (CL), number of large follicles (≥ 10 mm) and uterine reproductive tract score were recorded. Only cows that had no CL, ultrasound reproductive tract score ≤ 2 and were ≥ 30 days in milk (DIM) were enrolled in the study (n = 214). Cows were blocked by parity, DIM and body condition score and randomly assigned to one of four treatments: i.m. injection of gonadotropin releasing hormone analogue [GnRH; (n = 57)], i.m. injection of human chorionic gonadotropin [hCG; (n = 48)], 7-day Progesterone-Ovsynch protocol [P4OV; (n = 60)] and Control (no hormonal intervention, n = 49). A second ultrasound examination was performed 7 days after treatment to determine ovulatory response. There was a treatment effect on ovulation rate ($P < 0.0001$), whereby Control cows had a lesser ovulation rate compared with GnRH-, hCG- and P4OV-treated cows. Submission rate during the first 21 days of the breeding period [SR21; ($P = 0.74$)], pregnant to first service [P/AI1; ($P = 0.24$)], pregnant within 42 days after the onset of breeding [P42; ($P = 0.73$)], and pregnant within 84 days after the onset of breeding were not affected by treatment. A tendency was observed ($P = 0.07$) for greater likelihood of pregnancy within 21 days after the onset of breeding (P21) for P4OV and Control cows compared with GnRH- and hCG-treated cows. GnRH- and hCG-treated cows tended ($P = 0.10$) to have greater P/AI1 when first service events occurred after day 21 of the breeding period compared with Control cows. P4OV cows had shorter MSD to first service interval ($P = 0.0001$) and shorter MSD to conception interval ($P = 0.02$) compared with Control, GnRH- and hCG-treated cows. In conclusion, treatment of anestrous cows with GnRH or hCG resulted in an increase in ovulation rate compared with untreated Control cows, but did not improve reproductive performance during the first 21 days of the breeding season. The best reproductive performance results were obtained with the P4OV treatment, but this treatment has the greatest cost, and has the greatest number of interventions. The observation of good P/AI1 in hCG- and GnRH- treated cows when the first insemination occurred later than day 21 after MSD warrants further investigation, and suggests that these interventions should be applied earlier than 9 days before the farm MSD.

Keywords: dairy cattle, ovarian cyclicity, human chorionic gonadotropin, gonadotropin releasing hormone

1. Introduction

The resumption of oestrous cycles before the breeding season is an important pre-requisite to achieve high reproductive efficiency in seasonally calving herds [1]. Extended postpartum anovulation and anoestrous periods are common, with approximately 11 to 35% of dairy cows anoestrus at the start of the breeding period [2] or at 63 days after calving [3]. The date for the planned start of calving is set to maximize the use of pasture during lactation in seasonal calving pasture-based systems [4]. To achieve a concentrated calving pattern, high submission rates and pregnancy per AI (P/AI) are essential [5]. Delayed postpartum commencement of ovulatory oestrous cyclicity and poor expression of oestrus are associated with reduced conception rates and increased intervals from calving to conception [6].

Witlbank et al. [7] described three categories of anovulation: (1) Anovulation with follicle growth only to 'emergence' state of development (no ovarian follicles ≥ 8 mm in diameter) associated with a relative deficiency in FSH; (2) anovulation with follicle growth to deviation but not ovulatory size, associated with low frequency pulses of LH and increased sensitivity to the negative feedback effects of estradiol on gonadotropin release, and (3) anovulation with follicular growth to larger than ovulatory follicle size (follicular cysts), often associated with an increased release of LH with an insensitivity to estradiol positive feedback.

Human chorionic gonadotropin (hCG) and gonadotropin releasing hormone (GnRH) treatments have similar effects on the ovary [8], inducing ovulation [9] and the formation of accessory corpora lutea (CL) [10] with a significant increase in plasma progesterone concentrations achieved 7 days after treatment [11], [for review, see De Rensis et al.,2010

[12]]. Hence, these two hormones have been widely used in fixed time artificial insemination protocols in both dairy [13, 14] and beef cattle [15] to cause ovulation or luteinisation of a dominant follicle and initiate a new follicular wave [16], and to increase endogenous progesterone (P4) production [17].

Exposure of anestrous cows to P4 may stimulate development and maturation of a dominant follicle by enhancing LH release, stimulating development of LH receptors in theca cells and secretion of estradiol by the follicle [2]. Treatment of anestrous cows with P4 resulted in greater follicular fluid and circulating concentrations of oestradiol, increased pulsatile release of LH and increased numbers of receptors for LH in granulosa and theca cells in pre-ovulatory follicles [18, 19].

Therefore, the aim of the present pilot study was to assess the effects on ovulation and reproductive performance of a single injection of either GnRH or hCG applied 9 days before the start of the seasonal breeding period in anovulatory anoestrus cows to simplify herd management and to reduce costs compared with a 7-day progesterone-Ovsynch protocol. Specifically, we hypothesized that 1) treatment of non-cycling cows would result in a greater ovulation rate than no treatment; 2) treatment of non-cycling cows would shorten the interval from mating start date (MSD) to both insemination and conception.

2. Material and methods

2.1 Cattle, location and experimental design

All experimental procedures involving animals on this study were approved by the Teagasc Animals Ethics Committee. The study treatment and data collection schedule are illustrated in Figure 1. The study was conducted on 4 grass-based seasonal calving dairy herds located in the province of Munster, Ireland (n = 2112 Holstein-Friesian and Holstein-

Friesian x Jersey cross cows in total), and all enrolled cows calved during the spring (February to April) of 2017.Within each herd, all cows (n = 2112) were marked with tail paint (Tell Tail markers GEA © Farm Technologies, Mt Maunganui, New Zealand) 39 days before MSD in order to capture data on estrous cyclicity. Any cows with intact tail paint at Day -9 relative to MSD (n = 488) were assumed to be anoestrus and were subjected to ultrasound scanning.

2.2 Ultrasound examination

Ovarian structures and ultrasound reproductive tract score (URTS) of the uterus were evaluated by transrectal ultrasound 9 days before the farm MSD (8.5 MHz transrectal transducer Ibex Pro, Ibex®, Colorado, USA). Information recorded included number of corpora lutea, number of large follicles (≥ 10 mm), number of small follicles (≥ 5 mm and < 10 mm) and URTS, measured on a 4-point scale as previously described by Mee et al. (2009) [20]; G1: a typical spoke wheel-shaped lumen; G2: a spoke wheel-shaped lumen with an enlarged centre filled with a small volume (>2 mm, ≤5 mm) of fluid of mixed echogenicity; G3: a stellate-shaped lumen filled with a moderate volume (>5 mm, ≤10 mm) of fluid of mixed echogenicity; G4: a circular shaped lumen filled with a large volume (>10 mm) of fluid of mixed echogenicity. Only non-ovulatory cows with a UTRS ≤ 2 and ≥ 30 DIM were enrolled in the study.

The percentage of cows not detected in estrus by tail paint was 23.1% (488/2112). The percentage of cows with intact tail paint that presented with a CL or were classified as having a URTS > 2 was 39.9% (195 out 488) and 16.1% (79 out 488), respectively. These cows were not enrolled, resulting in 214 cows being available for inclusion in the study.

2.3 Treatment and AI

Anestrous cows were blocked by parity [primiparous (n = 32), second parity (n = 46), third parity or greater (n = 136)], DIM [early-calving (≥ 70 DIM, n = 60), mid-calving (43 to 69 DIM, n = 106), and late-calving (30 to 42 DIM, n = 48) at MSD] and body condition score [BCS, 1 to 5 scale in 0.25 increments [21]] and randomly assigned to one of four treatments 9 days before MSD. GnRH-treated cows (GnRH; n = 57) were administered an i.m injection of 2 mL of GnRH analogue containing 100 µg gonadorelin diacetate (Ovarelin ® 50 µg/mL, Ceva Santé Animale, Libourne, France). Human chorionic gonadotropin-treated cows (n = 48) were administered an i.m injection of 1500 IU hCG (Chorulon ®, MSD Ireland Ltd., Dublin, Ireland). Progesterone-Ovsynch-treated cows (P4OV; n = 60) had a progesterone–releasing intravaginal device (PRID E®; containing 1.55 g of progesterone; Ceva Santé Animale) inserted for 7 days with i.m. administration of prostaglandin F2α (Enzaprost®, 5 mL equivalent to 25 mg dinoprost, Ceva Santé Animale) at device removal, and gonadotropin-releasing hormone containing 100 µg gonadorelin diacetate (Ovarelin ® 50 µg/mL, Ceva Santé Animale) was administrated at PRID insertion and 56 h after PRID removal. P4OV cows were inseminated 72 h after PRID removal (16 h after the second GnRH) coinciding with MSD (Day 0**).** Animals in the Control group (n = 49) did not receive any treatment.

A second ultrasound examination was performed 7 days after treatment (Day -2) on all cows to determine ovulatory response by visualizing the presence or absence of a CL on either ovary. Cows were deemed to have ovulated if a CL was observed, or deemed to have not had an ovulation if a CL was not observed on either ovary.

All animals were served during the breeding season by AI after detection of estrus or tail paint removal, with the exception of the first service in the P4OV-treated cows, which was fixed time AI on the first day of the breeding season (Day 0). Between 30 to 42 days after first service an ultrasound examination was conducted to determine pregnancy status and viability of the embryo.

2.4 Reproductive parameters

Reproductive records including MSD, service dates and pregnancy status at the end of the breeding season for each enrolled herd were obtained from the Irish Cattle Breeding Federation (ICBF) website.

The 21-day submission rate **(SR21)** was constructed by coding cows with an insemination date within the first 21 days after MSD as 1, and cows without insemination date within the first 21 days after MSD were coded as 0.

Pregnant to first service **(P/AI1)** was coded as 1 if a cow was diagnosed pregnant by ultrasound on Day 38 ± 6 after the first service. Cows with more than one service, or where the cow was diagnosed as nonpregnant, were allocated a P/AI1 of zero. A similar description was used when the first service occurred during the first 21 days (**P/AI1 ≤ 21)** or later than the first 21 days **(P/AI1 > 21)** after MSD.

Pregnant within 21 days of MSD **(P21)** was coded as 1 if cows with at least one service did not receive a service following 21 days of breeding and was subsequently confirmed as pregnant. A cow received a P21 record of zero if a service was obtained sometime after 21 days of breeding, or if the animal was diagnosed as non-pregnant. Similar criteria were used for pregnant within either 42 **(P42)** or 84 days **(P84)** after MSD.

MSD to first service interval **(MSD-FS)** is the interval in days from MSD until the day of the first service. MSD to conception interval **(MSD-CI)** is the interval in days from

MSD until the day of the service that subsequently resulted in successful pregnancy diagnosis.

2.5 Data handling and Statistical analysis

All statistical analyses were performed using SAS software v 9.4 (SAS Institute, Cary, NC, USA). Five cows were culled after first service (Control = 1; GnRH = 2; hCG = 1, P4OV = 1) and were excluded from the analysis of reproductive performance outcomes except SR21. The GLIMMIX procedure was used to determine the effect of treatment on binary variables such as ovulation, SR21, P21, P42, P84, P/AI1, P/AI1≤21 and P/AI1>21. The model included as fixed effects treatment, parity, DIM, herd and their interactions, and cow was included as a random effect. The presence of a LF was included as a covariate in the analysis of ovulation rate. BCS was not included in the model as 88.7% (190/214) of the animals had a target BCS between 2.75 and 3.25. By design, cows in P4OV treatment received fixed time AI on the first day of the breeding period and consequently were removed from SR21 analysis.

The normality of both the raw data and residuals was tested for continuous variables, and the most appropriate Box-Cox transformation was used in MSD-FS. Mixed model procedures were used to determine the effect of treatment on MSD-FS and MSD-CI (in served and pregnant cows, respectively). Treatment, parity, DIM and herd were included as fixed effects and cow was included as a random effect.

Survival analysis was carried out using PROC LIFETEST procedure of SAS to examine the effect of treatment on MSD-FS and MSD-CI interval. For both MSD-FS and MSD-CI, cows that were not served or did not conceive during the study period were right-censored at 84 days (i.e., last day of the study breeding period).

Differences with $P < 0.05$ were considered significant and $0.05 \geqq P \leq 0.10$ were considered tendencies.

3. Results

3.1 Ovulation

The percentage of animals with no LF on the ovary on the day of enrolment and treatment initiation was 8.8% (19/214**.** There was a strong treatment effect on ovulation rate ($P < 0.0001$). Control animals had a lower ovulation rate compared with GnRH, hCG and P4OV treatment cows (Figure 2). There was no effect of DIM [early: 41/60 (68.3%), mid: 77/106 (72.6%) and late: 30/48 (62.5%); (P = 0.32)], parity [primiparous: 21/32 (65.6%) second parity: 29/46 (63.0%) and ≥ 3 parity 86/136 (63.2%); (P = 0.97)] and presence of LF [presence of LF: 122/195 (62.2%) absence of LF 14/19 (73.6%); (P = 0.28)] on ovulation rate.

3.2 Submission rate and P/AI1

The main reproductive performance outcomes are summarized in Table 1. The percentage of animals for which first service occurred during or after the first 21 days of breeding season was 77.5% (166/214) and 22.5% (48/214), respectively. SR21 was not affected by treatment (P = 0.74), ovulation rate after treatment (P = 0.63), parity (P = 0.69) or DIM (P = 0.19) (Table 1).

There was no effect of treatment (P = 0.24) or ovulation rate (P = 0.54) on P/AI1 (Table 1). A tendency (P = 0.10) was observed for greater P/AI1 in primiparous cows compared with cows in their second parity (+18.8 percentage points) and third or later parity (+21.7 percentage points). Similarly, there was a tendency (P = 0.10) for greater P/AI1 in early calving cows compared with mid (+14.7 percentage points) and late-calving cows (+16.6 percentage points). P/AI1 in cows that were served during the first 21 days of breeding season (P/AI1 ≤ 21) was not affected by treatment (*P* = 0.39) (Table 2). P/AI1 when insemination occurred after the first 21 days of the breeding period (P/AI > 21) tended to be affected by treatment (*P* = 0.10), with both GnRH and hCG treatment cows achieving greater P/AI1 > 21 than Control cows (see Table 2). Interestingly, there were 9 cows in the hCG treatment and 6 cows in the GnRH treatment that did ovulate before MSD and become pregnant at the first insemination between day 31 and day 35 of the breeding period (Figure 4).

3.3 P21, P42 and P84

The percentage of cows pregnant during the first 21 days of the breeding period tended to be greater (*P* = 0.07) in the Control and P4OV treatments compared with GnRH and hCG treatments (see Table 1). There was a tendency (*P* = 0.07) for P21 to be affected by DIM. Early-calving cows had greater P21 compared with mid and late-calving cows (+17.5 and +22.4 percentage points, respectively). No effect of treatment was observed on P42 (*P* = 0.73) or P84 (*P* = 0.88). During the first 42 days of the breeding period, primiparous cows tended (*P* = 0.09) to have greater pregnancy rate (+ 18 percentage points) compared with multiparous cows. Ovulation status on day -2 before the breeding season did not affect P21 (*P* = 0.41), P42 (*P* = 0.85) or P84 (*P* = 0.87) (Table 1).

3.4 MSD-First service interval and MSD-Conception interval

The MSD-FS interval for each treatment is illustrated in Figure 3. As expected, MSD-FS was affected by treatment (*P* = 0.0001), but it was not affected by ovulation rate after treatment (ovulation 16.2 d ±1.7; anovulation 16.5 d ±1.3; *P* = 0.89), parity (primiparous 18.2

d ±2.2; second parity 14.6 d ±2.1; third or greater parity 16.3 d ± 1.3; $P = 0.42$) or DIM (early-calving 15.0 d ±1.9; mid-calving 16.9 d ±1.4; late-calving 17.2 d ±2.1; $P = 0.68$).

The MSD-CI for each treatment is illustrated in Figure 4. P4OV had shorter MSD-CI in all cows ($P = 0.02$) compared with GnRH and hCG treatments, respectively (25.0 d ±3.2 vs 31.6 d ±2.6; and 35.0 d ±3.5) and tended to be shorter ($P= 0.09$) compared with Control cows (29.7 d ±3.6). Differences between P4OV and GnRH and hCG treatments in the number of days to become pregnant reflected the effect of treatment on pregnancy establishment during the first 3 weeks of the breeding season, with P4OV treatment cows achieving a greater percentage of cows pregnant (+14 points percentage) during first 21 days of the breeding period. MSD-CI was shorter in early-calving compared with late-calving animals [early 24.9 d ±3.0; mid 31.7 d ±2.5; late 35.4 d ±2.5; ($P = 0.03$)] and tended to be longer in multiparous compared with primiparous cows [primiparous 25.2 d ±3.6; second parity 29.0 d ±3.1; third or greater parity 37.2 d ±2.4; ($P = 0.07$)]. For cows that become pregnant during the breeding period, MSD-CI was not affected by ovulation rate after treatment [ovulation 20.3 d ±2.8; anovulation 22.7 d ±1.38; ($P = 0.38$)]

4. Discussion

The main finding in this study was that administration of GnRH or hCG 9 days before MSD increased ovulation rate in anovulatory anestrous cows but did not impact on reproductive performance during the first 21 days of the breeding period. As expected, P4OV-treated cows had a shorter MSD-FS and MSD-CI compared with Control, GnRH and hCG-treated cows, and had greater pregnancy rate in the first 21 days of the breeding season compared with GnRH and hCG-treated cows.

This study specifically examined the effect of treatment 9 days before the start of the breeding season on ovulation rate and reproductive performance in anovulatory anestrous seasonal-calving, pasture-based dairy cows. Cows were selected on the basis that they were not detected in oestrus and did not have a CL 9 days before MSD. This differs from previous studies where both cows that were anovular and cows that had ovulated but had not been detected in oestrus were enrolled [22]. In seasonal calving systems, there is not a defined voluntary waiting period for all cows. Thus, cows were on average 65 days after calving at time of treatment but ranged from 30 to 81 days. In the present study, a single injection of GnRH or a single injection of hCG were examined as potential strategies to simplify herd management and to reduce costs of treating anovulatory anoestrus cows compared with a P4OV protocol. Previous studies have focussed on examining the use of P4 alone or the P4OV protocol as treatments for anoestrous cows [19, 22, 23]. Hence, the results of the current study are an extension of the previous studies evaluating treatments in anoestrous cows.

4.1 Ovulation

The percentage of animals that were not detected in oestrus by tail-painting in the present study was 23.1%, which was less than previously reported by Rhodes et al. [18] [35%]. The percentage of animals that had not been detected in oestrus but did have a CL at examination, however, was 39.9% (195/488), a finding that was greater than previously reported by McDougall (2010) [22][between 20% and 30% of anoestrous cows had a CL at the veterinary examination].

Treatment had an effect on ovulation in the present study. Control cows had reduced ovulation rate compared with the other three treatments. GnRH triggers ovulation by stimulating a surge release of LH and FSH from the anterior pituitary [24]. Similarly, hCG exerts potent LH-like effects on the ovary [see review De Rensis et al., 2010 [12]]. Stevenson et al. [19] reported that 61.3% of anoestrous cows ovulated in response to GnRH injection, which is slightly less than in the current study, albeit with different cow genetics and milk production system. In suckling anoestrous beef cows (21 to 35 DIM), Sheffel et al [25] reported that 88.0% of cows formed a corpus luteum in response to an injection of 1000 iu of hCG. Interestingly, we did not find an interaction between treatment and large follicle status on ovulation rate, in agreement with [25] who stated that follicles smaller than 10 mm in diameter were able to form luteal tissue in response to hCG. Nevertheless, to the best of our knowledge, there are no reports on ovulation rate of small follicles (< 10 mm) using GnRH in anovulatory anestrus dairy cows.

4.2 SR21 and MSD-FS

The first postpartum ovulation is frequently associated with an absence of oestrous behaviour and is often followed by a luteal phase of short duration [26]. Horan et al. [27] reported average first postpartum luteal phase duration of 13.1 ± 0.8 d. Hence, we expected an increase in the number of services during the first week of the breeding season in GnRH and hCG treated cows due to greater ovulation rate in these two treatments during the week before MSD compared with the Control treatment. There were no differences between these three treatments in MSD-FS interval (Figure 3) or SR21 (Table1). This finding suggests one or a combination of more than one of the following events occurred: (i) a large proportion of Control cows had behavioural oestrus expression at the first spontaneous estrus event; (ii) the duration of the first luteal phase was prolonged in a large proportion of GnRH and hCG treatment cows that responded to the experimental treatments; or (iii) an increased incidence of silent oestrus events in GnRH and hCG treatment cows during the first 21 days of the breeding season.

4.3 Reproductive performance

Thatcher and Wilcox [28] and Darwash et al. [29] previously reported that a greater number of oestrous cycles before first insemination results in improved fertility at first service. In the current study, treatment with GnRH or hCG did not affect P/AI1 and P/AI1 ≤ 21. A tendency for greater P21 was observed in P4OV and Control cows compared with GnRH- or hCG-treated cows. Conversely, there was a tendency for greater P/AI1 in cows that received their first service event after day 21 of the breeding season (P/AI > 21) in GnRH- and hCG-treated cows compared with Control treatment cows. These findings might be explained by differences in the type of ovulation (induced ovulation vs spontaneous ovulation). Ovulation of smaller follicles results in a smaller CL and lower circulating plasma P4 concentrations [30]. Hence, in the current study, ovulation induced by GnRH or hCG treatment might result in lower circulating plasma P4 concentration during the ensuing oestrous cycle, potentially resulting in oestrous expression failure. In support of this, 9 and 6 cows in the hCG and GnRH treatments group, respectively, did ovulate before MSD, and become pregnant following their first service between day 31 and day 35 of the breeding period.

Primiparous and early-calving cows tended to have greater reproductive performance and had shorter MSD-CI compared with the other categories in agreement with Herlihy et al. [13] and Rojas Canadas et al. [35]. This might be explained by more favourable energy balance in primiparous [36] and early-calving cows at the MSD, and shorter time available to complete uterine involution in mid and late-calving animals.

Cows in the P4OV treatment had shorter MSD-CI compared with cows in the GnRH and hCG treatments and tended to have shorter MSD-CI interval compared with Control treatment cows. These differences reflected differences in MSD-FS (all cows in P4OV were served on the first day of breeding period) and similar P/AI1. MSD-CI in the Control treatment was shorter than reported by McDougall [22] [29.7 ±3.6 d vs 43.5 d ±2.6]. These differences can be explained by differences in the pregnancy rate at first service between studies (60.0% vs 34.3%).

In conclusion, treatment of anoestrous cows resulted in an increase in ovulation rate compared with untreated Control cows. While treatment did not improve overall reproductive performance in GnRH and hCG treated cows compared with Control cows, P/AI1 > 21 tended to be improved. A tendency for greater pregnancy during the first 21 days of the breeding period was observed in Control and P4OV treatment cows. It is apparent from the findings in the present study that stimulating ovulation in anoestrous cows with a single injection of either GnRH or hCG on day 9 before MSD was not a useful strategy to improve herd reproductive performance. The observed tendency for greater P/AI in GnRH and hCG treated cows when that first service event occurred after the first 21 days of the breeding period suggests that earlier administration of these treatments could have had a beneficial effect on reproductive performance and warrants further investigation.

Acknowledgements

The authors thank the owners and farm staff at the four farms that participated in this study for their collaboration. The authors acknowledge the assistance of undergraduate students. Also, the authors thank Ceva Santé Animale for supplying the pharmaceuticals used in the study. This work was founded by the Department of Agriculture, Food and Marine (Dublin, Ireland; Research Stimulus Fund grant 13S528).

REFERENCES

[1] Butler S. Nutritional management to optimize fertility of dairy cows in pasture-based systems. animal. 2014;8:15-26.
[2] Rhodes F, McDougall S, Burke C, Verkerk G, Macmillan K. Invited review: Treatment of cows with an extended postpartum anestrous interval. Journal of dairy science. 2003;86:1876-94.
[3] Santos J, Bisinotto R, Ribeiro E. Mechanisms underlying reduced fertility in anovular dairy cows. Theriogenology. 2016;86:254-62.

[4] Shalloo L, Cromie A, McHugh N. Effect of fertility on the economics of pasture-based dairy systems. Animal. 2014;8:222-31.
[5] McDougall S. Reproduction performance and management of dairy cattle. Journal of Reproduction and Development. 2006;52:185-94.
[6] Morton J. Interrelationships between herd-level reproductive performance measures based on intervals from initiation of the breeding program in year-round and seasonal calving dairy herds. Journal of dairy science. 2010;93:901-10.
[7] Wiltbank M, Gümen A, Sartori R. Physiological classification of anovulatory conditions in cattle. Theriogenology. 2002;57:21-52.
[8] Rajamahendran R, Sianangama P. Effect of human chorionic gonadotrophin on dominant follicles in cows: formation of accessory corpora lutea, progesterone production and pregnancy rates. Journal of Reproduction and Fertility. 1992;95:577-84.
[9] Burns M, Buttrey B, Dobbins C, Martel C, Olson K, Lamb G, et al. Evaluation of human chorionic gonadotropin as a replacement for gonadotropin-releasing hormone in ovulation-synchronization protocols before fixed timed artificial insemination in beef cattle 1. Journal of animal science. 2008;86:2539-48.
[10] Schmitt E, Diaz T, Drost M, Thatcher W. Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. Journal of animal science. 1996;74:1084-91.
[11] Hanlon D, Jarratt G, Davidson P, Millar A, Douglas V. The effect of hCG administration five days after insemination on the first service conception rate of anestrous dairy cows. Theriogenology. 2005;63:1938-45.
[12] De Rensis F, Lopez-Gatius F, Garcia-Ispierto I, Techakumpu M. Clinical use of human chorionic gonadotropin in dairy cows: an update. Theriogenology. 2010;73:1001-8.
[13] Herlihy M, Crowe M, Berry D, Diskin M, Butler S. Factors associated with fertility outcomes in cows treated with protocols to synchronize estrus and ovulation in seasonal-calving, pasture-based dairy production systems. Journal of dairy science. 2013;96:1485-98.
[14] Sánchez J, Randi F, Passaro C, Mathew D, Butler S, Lonergan P. Effect of human chorionic gonadotrophin administration 2 days after insemination on progesterone concentration and pregnancy per artificial insemination in lactating dairy cows. Journal of dairy science. 2018.
[15] Sá Filho MF, Penteado L, Reis EL, Reis TA, Galvão KN, Baruselli PS. Timed artificial insemination early in the breeding season improves the reproductive performance of suckled beef cows. Theriogenology. 2013;79:625-32.
[16] Yu GM, Wu Y, Wang XL, Zhao S, Maeda T, Zeng SM. Evaluation of three hormonal protocols for anovulatory lactating cows under regulations restricting the use of estrogenic compounds. Animal Science Journal. 2018;89:640-7.
[17] Lonergan P. Influence of progesterone on oocyte quality and embryo development in cows. Theriogenology. 2011;76:1594-601.
[18] Rhodes F, Burke C, Clark B, Day M, Macmillan K. Effect of treatment with progesterone and oestradiol benzoate on ovarian follicular turnover in postpartum anoestrous cows and cows which have resumed oestrous cycles. Animal reproduction science. 2002;69:139-50.
[19] Stevenson J, Pursley J, Garverick H, Fricke P, Kesler D, Ottobre J, et al. Treatment of Cycling and Noncycling Lactating Dairy Cows with Progesterone During Ovsynch1. Journal of dairy science. 2006;89:2567-78.
[20] Mee J, Buckley F, Ryan D, Dillon P. Pre-breeding Ovaro–Uterine Ultrasonography and its Relationship with First Service Pregnancy Rate in Seasonal-Calving Dairy Herds. Reproduction in domestic animals. 2009;44:331-7.
[21] Edmonson A, Lean I, Weaver L, Farver T, Webster G. A body condition scoring chart for Holstein dairy cows. Journal of dairy science. 1989;72:68-78.
[22] McDougall S. Effects of treatment of anestrous dairy cows with gonadotropin-releasing hormone, prostaglandin, and progesterone. Journal of dairy science. 2010;93:1944-59.

[23] McDougall S, Compton C. Reproductive performance of anestrous dairy cows treated with progesterone and estradiol benzoate. Journal of dairy science. 2005;88:2388-400.
[24] D'Occhio M, Fordyce G, Whyte T, Aspden W, Trigg T. Reproductive responses of cattle to GnRH agonists. Animal Reproduction Science. 2000;60:433-42.
[25] Sheffel C, Pratt B, Ferrell W, Inskeep E. Induced Corpora Lutea in the Postpartum Beef Cow. II. Effects of Treatment with Progestogen and Gonadotropins 1, 2. Journal of animal science. 1982;54:830-6.
[26] McDougall S, Burke C, Macmillan K, Williamson N. Patterns of follicular development during periods of anovulation in pasture-fed dairy cows after calving. Research in veterinary science. 1995;58:212-6.
[27] Horan B, Mee J, O'connor P, Rath M, Dillon P. The effect of strain of Holstein-Friesian cow and feeding system on postpartum ovarian function, animal production and conception rate to first service. Theriogenology. 2005;63:950-71.
[28] Thatcher W, Wilcox C. Postpartum Estrus as an Indicator of Reproductive Status in the Dairy Cow1. Journal of Dairy Science. 1973;56:608-10.
[29] Darwash A, Lamming G, Wooliams J. The phenotypic association between the interval to post-partum ovulation and traditional measures of fertility in dairy cattle. Animal Science. 1997;65:9-16.
[30] Vasconcelos J, Sartori R, Oliveira H, Guenther J, Wiltbank M. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology. 2001;56:307-14.
[31] Rivera FA, Mendonça LG, Lopes G, Santos JE, Perez RV, Amstalden M, et al. Reduced progesterone concentration during growth of the first follicular wave affects embryo quality but has no effect on embryo survival post transfer in lactating dairy cows. Reproduction. 2011;141:333-42.
[32] Revah I, Butler W. Prolonged dominance of follicles and reduced viability of bovine oocytes. Journal of reproduction and fertility. 1996;106:39-47.
[33] Cerri R, Chebel R, Rivera F, Narciso C, Oliveira R, Amstalden M, et al. Concentration of progesterone during the development of the ovulatory follicle: II. Ovarian and uterine responses. Journal of dairy science. 2011;94:3352-65.
[34] Martins J, Wang D, Mun N, Rossi G, Martini A, Martins V, Purley J. Level of circulating concentrations of P4 during ovulatory follicle development affects timing of pregnancy loss in lactating cow. Journal of dairy science. 2018;In Press.
[35] Rojas Canadas Mh, J Keneally, J Grant, F Kearney, P Lonergan, ST Butler. Phenotypic association between ovarian cyclicity, uterine health, indicators of bioenergetic status, parity, genetics traits and reproductive performance in seasonally -calving, pasture-based lactating dairy cows. 2018.
[36] Wathes D, Cheng Z, Bourne N, Taylor V, Coffey M, Brotherstone S. Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. Domestic animal endocrinology. 2007;33:203-25.

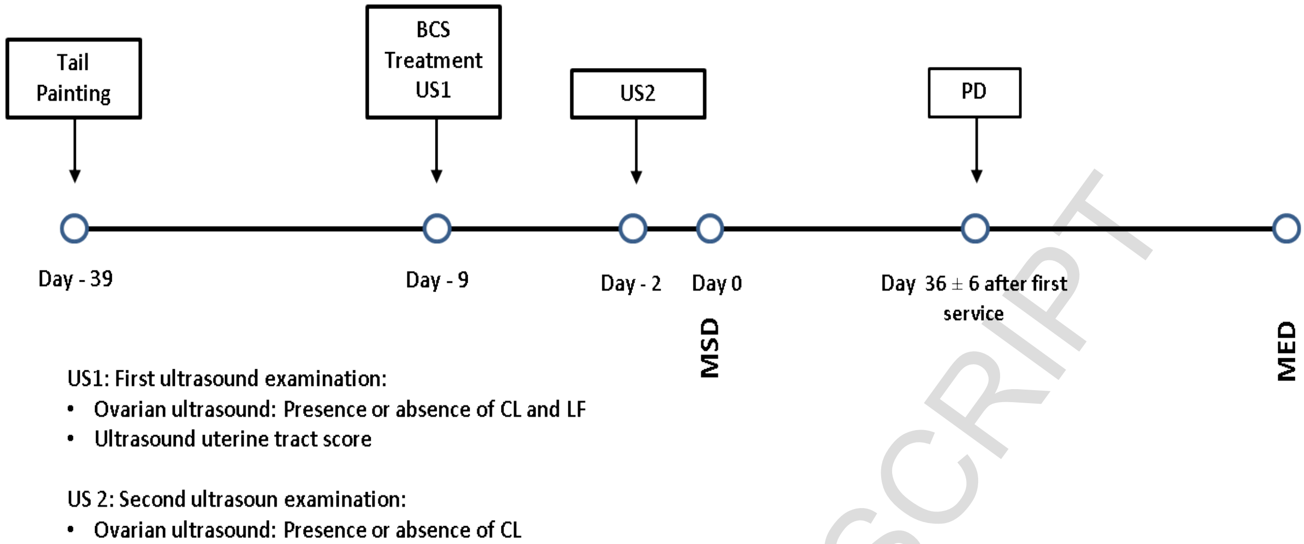


Figure 1. Schematic outline of activities. Tail painting: Whole herds were marked with tail paint 35 days before MSD to monitor oestrous behaviour. BCS = body condition score; 5-point scale. US1: First ultrasound examination to all cows with intact tail paint to identify cows as cycling or non-cycling and to assess uterine health. US2: Second ultrasound examination of all treated cows to assess the effect of treatment on ovulation. PD = Pregnancy diagnosis on day 36 ± 6 after first service by ultrasound. MSD = mating start date. MED = mating end date, end of breeding season. CL = corpus luteum. LF = large follicle

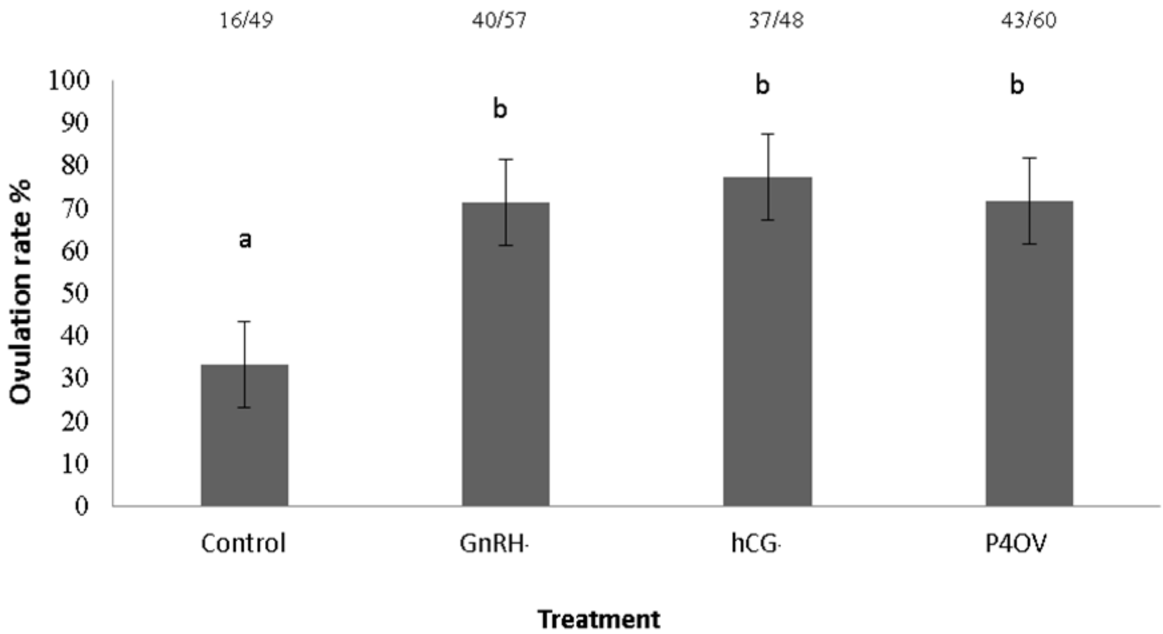


Figure 2. Effect of treatment on ovulation rate ($P < 0.0001$) measured 7 days after administration of experimental treatments. Control (no treatment); GnRH treatment (0.1 mg gonadorelin diacetate); hCG treatment (1500 IU of human chorionic gonadotropin); P4OV treatment (Progesterone-Ovsynch protocol).

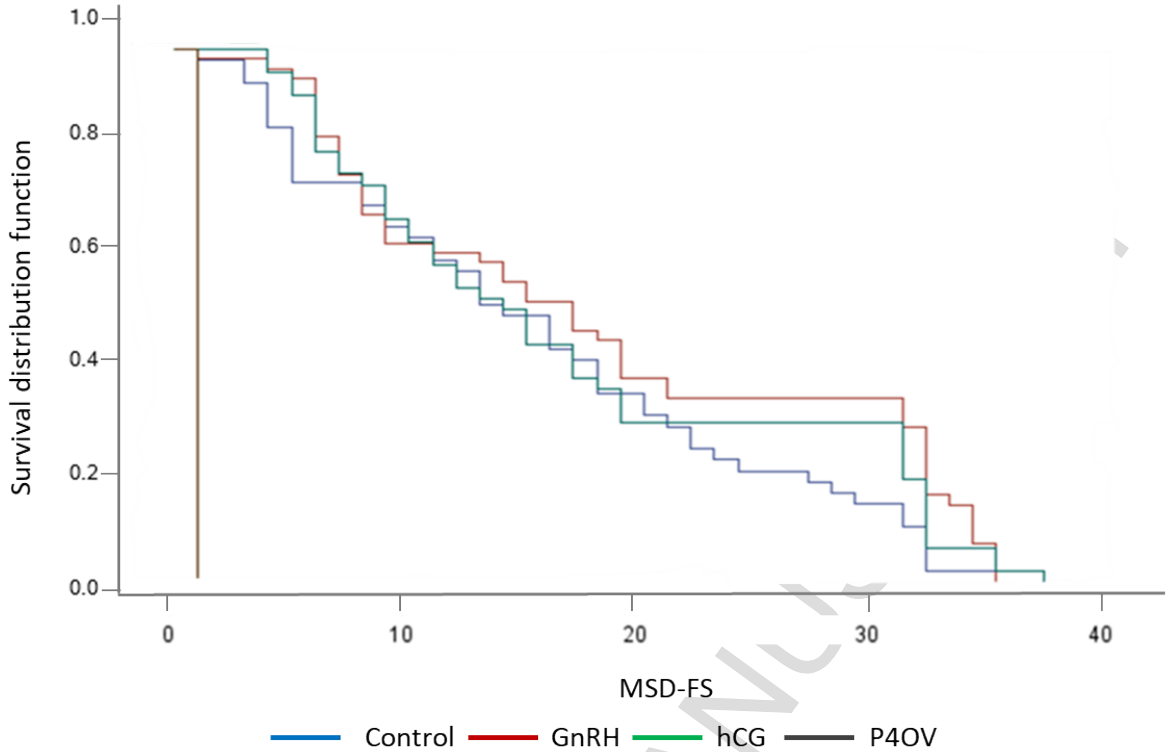
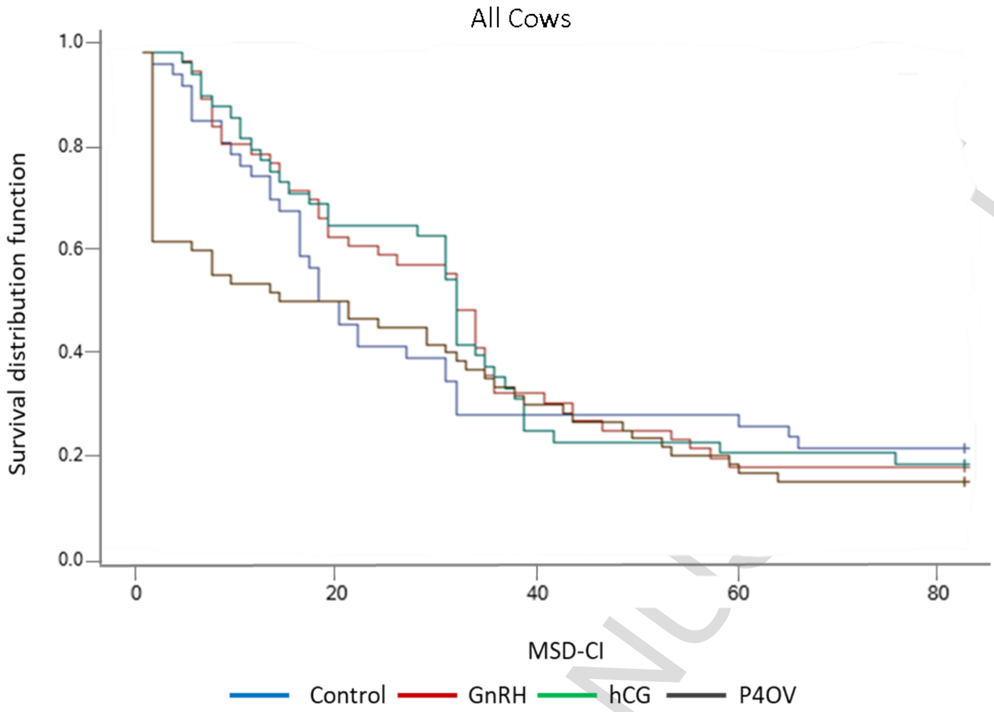


Figure 3. Kaplan–Meier survival curve for effect of treatment on mating start date to first service interval (MSD-FS). Control (no treatment); GnRH: 2mL of gonadotropin releasing hormone; hCG: 1500 IU of human chorionic gonadotropin; P4OV treatment (progesterone-Ovsynch protocol). There was a significant effect of treatment on MSD-FS (P = 0.04



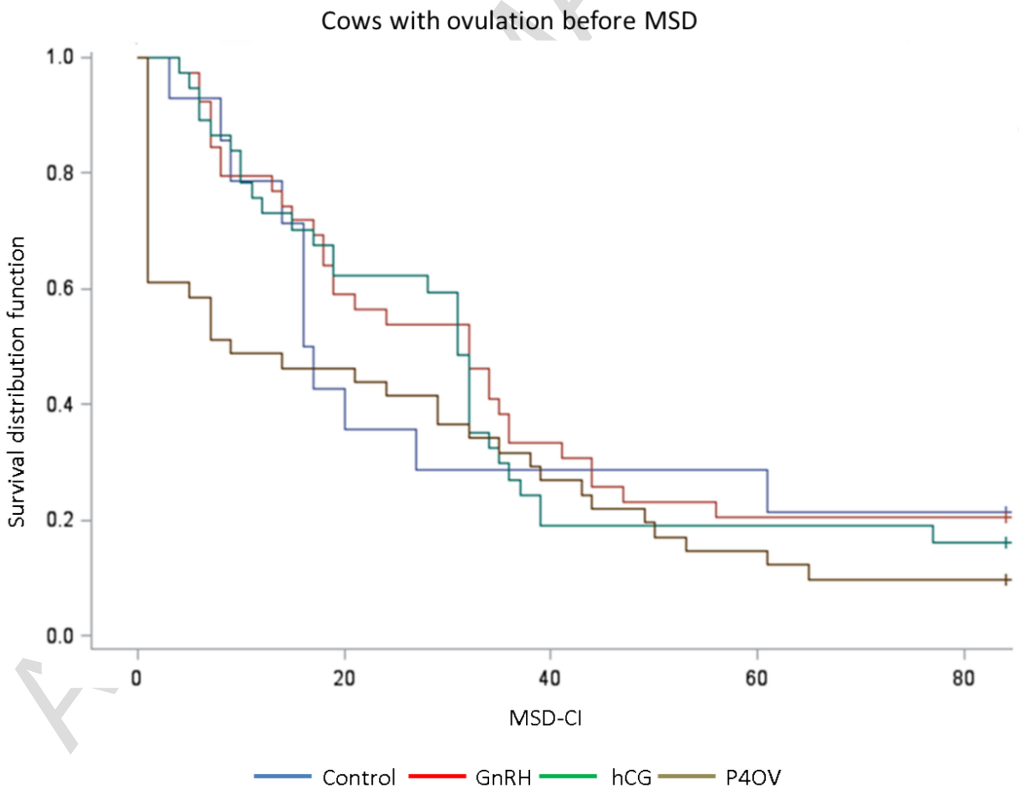


Figure 4. Kaplan–Meier survival curve for effect of treatment on mating start date to conception interval (MSD-CI). Top: All cows. Bottom: Only cows that did ovulate before MSD. Control (no treatment); GnRH treatment (0.1 mg gonadorelin diacetate); hCG treatment (1500 IU of human chorionic gonadotropin); P4OV treatment (progesterone-Ovsynch protocol); There was a significant effect on MSD-CI (*P* = 0.02). Cows that failed to conceive during the breeding season were right-censored at 84-days. MSD = Mating Start Date.

Table 1. Effect of ovulation after treatment, treatment (control, GnRH, hCG or Progesterone Ovsynch protocol), parity and days in milk on reproductive performance

	SR21	*P* value	P/AI1	*P* value	P21	*P* value	P42	*P* value	P84	*P* value
Ovulation after treatment										
Yes	79.5% (109/137)	0.63	57.0% (77/135)	0.54	45.9%[a] (62/135)	0.41	71.1% (96/135)	0.85	80.7% (109/135)	0.87
No	72.7% (56/77)		51.3% (38/74)		40.5% (30/74)		67.5% (50/74)		78.3% (58/74)	
Treatment										
Control	69.3% (34/49)	0.74	56.2% (27/48)	0.24	50.0%[a] (24/48)	0.07	66.6% (32/48)	0.73	72.9% (35/48)	0.88
GnRH	66.6% (38/57)		61.8% (34/55)		38.1%b (21/55)		69.0% (38/55)		81.8% (45/55)	
hCG	70.8% (34/48)		59.5% (28/47)		34.0%b (16/47)		76.6% (36/47)		80.8% (38/47)	
P4OV	100%* (60/60)		45.7% (27/59)		52.5%[a] (31/59)		69.4 (41/59)		84.7% (50/59)	
Parity										
1	71.88% (23/32)	0.69	70.9%[a] (22/31)	0.10	48.3% (15/31)	0.54	83.7%[a] (26/31)	0.09	83.7% (26/31)	0.42
2	78.2% (36/46)		52.1%[b] (24/46)		50.0% (23/46)		76.0%[a,b] (35/46)		93.4% (43/46)	
≥ 3	78.6% (107/136)		49.2%[b] (65/132)		40.9% (54/132)		65.1%[b] (86/132)		75.0% (99/132)	
DIM										
Early	85.0% (51/60)	0.19	66.6%[a] (38/57)	0.10	57.8%[a] (33/57)	0.07	77.1% (44/57)	0.48	84.2% (48/57)	0.29
Mid	76.4% (81/106)		51.9%[b] (54/104)		40.3%[b] (42/104)		69.2% (72/104)		81.7% (85/104)	
Late	70.8% (34/48)		50.0%[b] (24/48)		35.4%[b] (17/48)		64.5% (31/48)		72.9% (35.48)	

Control: No treatment; GnRH: Gonadotropin releasing hormone; hCG: Human chorionic gonadotropin; P4OV: 7 days-Progesterone-Ovsynch protocol; Significant differences when ($P < 0.05$). Tendency when ($0.05 = P \leq 0.10$). [a] [b]Different superscripts within category indicate tends to differ. *Not included in the statistical analysis, as all cows submitted by design.

Table 2. Effect of treatment (control, gonadotropin releasing hormone, human chorionic gonadotropin or 7 days-Progesterone Ovsynch protocol) on pregnancy rate at first service during and after 21 days of mating start date

	Control	**GnRH**	**hCG**	**P4OV**	***P*-value**
P/AI1≤21	58.8% (20/34)	57.1% (20/35)	48.4% (16/33)	45.7% (27/59)	0.39
P/AI1>21	50.0%^{a} (7/14)	72.7%^{b} (14/19)	85.7%^{b} (12/14)	-	0.10

P/AI≤21: Pregnancy rate at first service during first 21 days of breeding period. P/AI≥21: Pregnancy rate at first service after 21 days of mating start date. Control: No treatment; GnRH: Gonadotropin releasing hormone; hCG: Human chorionic gonadotropin; 7 days-P4OV: Progesterone-Ovsynch; Significant differences when (P < 0.05). Tendency when (0.05 = P ≤ 0.10). $^{a\,b}$Different superscripts within same row indicate tends to differ.

Highlights

A single injection of GnRH or hCG increased ovulation rate compared with untreated Control cows.

GnRh or hCG treatment did not improve overall reproductive performance.