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Article

Effect of follicular replacement (GnRH) and bovine somatotropin (bST) on the fertility of dairy cows exposed to heat stress

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Abstract:

Three reproductive protocols were evaluated: 1) PG: injection of PGF2α on d-50 postpartum and insemination (AI) based on estrus detection. 2) OVS (Ovsynch: d 0, GnRH; d 7, PGF2α; d 9, GnRH; d 10, AI); 3) ROV (GnRH + Ovsynch: d- 7, GnRH; d 0, GnRH; d 7 PGF2α; d 9, GnRB; d 10, AI). In addition, the effect of somatotropin (bST) to AI, on fertility at first postpartum service (FERT), and pregnancy rate at 99 d postpartum (PP) FERT was similar in ROV and OVS (36.2 *vs* 36.6 %) (P>0.05); and higher than PG (27.3 %) (P<0.05). Likewise, FERT was similar with and without bST (36.2 *vs* 30.6 %, P>0.05). PG and without bST (22.5 %) was lower than OVS with (38.5 %) and without bST (33.7 %), as well as than ROV with (37.0 %) and without bST (36.1 %), and PG with bST (32.9 %). The pregnancy rate at 99 d was: OVS (60.6 %); ROV (54.3 %), higher than PG group (46.8 %) (P>0.05). OVS with (64.7 %) and without bST (56.5 %) and ROV without bST were higher than PG without bST (41.1 %, P<0.05). In conclusion, GnRH before Ovsynch (ROV) and bST at AI did not increase fertility at the first service in Holstein cows under heat stress. OVS and ROV increased fertility of first service postpartum and pregnancy rate to 99 d postpartum. Somatotropin increased fertility of first postpartum service only in PG treated cows.

Key words: Dairy cow, Ovsynch, Somatotropin, heat stress, GnRH.

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Introduction

Heat stress (HS) compromises the estrous non-return rates[1,2] and conception rates of dairy cows[1-6]. Ovsynch protocols have increased the ovulation rate[7,8], the diameter of the ovulatory follicle[8], the fertility at first service[8-13] and the accumulated pregnancy rate at 120 d post-partum in high production dairy cows[9-11,14]. Injection of a luteolytic (i.e. prostaglandin F2α or PG) prior to Ovsynch[7,12] increases the ovulation rate of a follicle suitable for fertilization by more than 40 %[7], and the percentage of cows with higher levels of circulating progesterone 3 d after beginning Ovsynch[12]. The intravaginal insertion of a progesterone releasing device (PRD) increased the conception rate compared to cows that only receive Ovsynch[12,13]. However, under environmental conditions of HS, reproductive programs may be less efficient than under thermal comfort[8,12,14,15]. Double Ovsynch treatment has increased fertility by 10 %[16]. Several studies have determined that even though the fertility of *in vitro* fertilized oocytes are similar in winter and in the summer(17), the percentage of embryos that reached the blastocyst stage is compromised when using oocytes collected during the summer(17,18), especially in repeating cows(17). Holstein cows in full lactation(19) and non-lactating cows(20) exposed to heat stress during the summer(19), or during a follicular cycle(20), show a decrease in the number of healthy follicles(20), in the quality of the ovarian cluster(19), and in embryonic development(18-20). Follicle replacement is important for eliminating developed and affected follicles and is promoted with repeated GnRH treatments or by frequent aspiration of follicles 3 to 7 mm(19) or larger than 5 mm(20), and for generating the development of better-quality follicles and a higher percentage of embryos developed *in vitro* to the blastocyst stage. Follicle exchange prior to Ovsynch(21,22) did not improve fertility at the first service(21,22), but it did improve fertility in cows with uterine problems and low body condition(21).

On the other hand, bovine somatotropin (bST) has been used in Holstein cows for its beneficial effect in increasing milk production(23-26). It was considered that this increase in milk production could have a detrimental effect on the reproduction of the dairy cows. Treatment with 500 mg bST from 61 to 63 ds in milk and with repeated applications of this hormone every 10 ds(25) or 14 d(24,26) does not compromise fertility, the pregnancy rate(24-26) or the elimination of cows from the herd; the number of ds open; the number of mastitis cases, the incidence of follicular cysts and abortions(26), or animal welfare or health(27). Several authors have established that the use of bST at the onset of the estrus(28) and 10 ds after AI in dairy cows(29) has a positive effect on the pregnancy rate, improves the development of the corpus luteum and increases the production of progesterone(28,29) in both repeating cows(30,31) and embryo receptor cows(32). This favorable effect of bST has also caused a higher percentage of transferable embryos and fewer unfertilized oocytes in superovulated cows(29,32,33), and seems to be associated with insulin-like growth factor (IGF-I), and with final oocyte maturation, follicular development, and steroidogenesis(30,34,35). Together, beginning Ovsynch and bST at d 69 PP increased fertility at first insemination(28,36) and the accumulated pregnancy percentage at 120 and 365 d postpartum(32), but decreased the detection of estrus in cows treated with bST(37), and failed to increase the fertility of cows under heat stress(24).

The purpose of the present work was to evaluate the effect of follicular replacement (GnRH d-7) and the administration of bovine somatotropin (bST) at the time of insemination on fertility at the first postpartum service and the pregnancy rate in high production dairy cows exposed to heat stress.

Material and methods

The study was conducted on Holstein dairy cows (n= 553) from two intensive-production commercial herds in the central highlands of Mexico (Aguascalientes, Mexico), during the warm season, with shade only in the pens of the cows in production and dry cows. After calving, the cows were divided into batches by number of lactations with a whole feed, according to their milk production level. The estimated milk production at 305 d from the herd was 8 493 ± 349.6, and 9 116.3 ± 307.02 kg, respectively, in primiparous and multiparous cows.

Climate variables

During the study period from March to September, climate information regarding ambient temperature (°C) and relative humidity (RH) was recorded every 15 min at the INIFAP weather station in Aguascalientes, located at a distance of 5 km from the dairy herds where the study was conducted. The temperature-humidity index (THI) was calculated (Table 1) according to Ingraham *et al*[(37)], and the maximum temperature and average relative humidity were recorded using the following equation: THI= ° F – (0.55 – (((HR / 100) x 0.55)) * (° F – 58)).

Table 1: Temperature-humidity index (THI) during the study

Month	THI
March	73.4 ± 0.39^{a}
April	73.6 ± 0.39^{a}
May	76.6 ± 0.39^{b}
June	77.5 ± 0.39^{b}
July	77.1 ± 0.39^{b}
August	77.6 ± 0.41^{b}
September	76.5 ± 0.42^{b}

[abc] Different letters indicate significant difference (P<0.01).

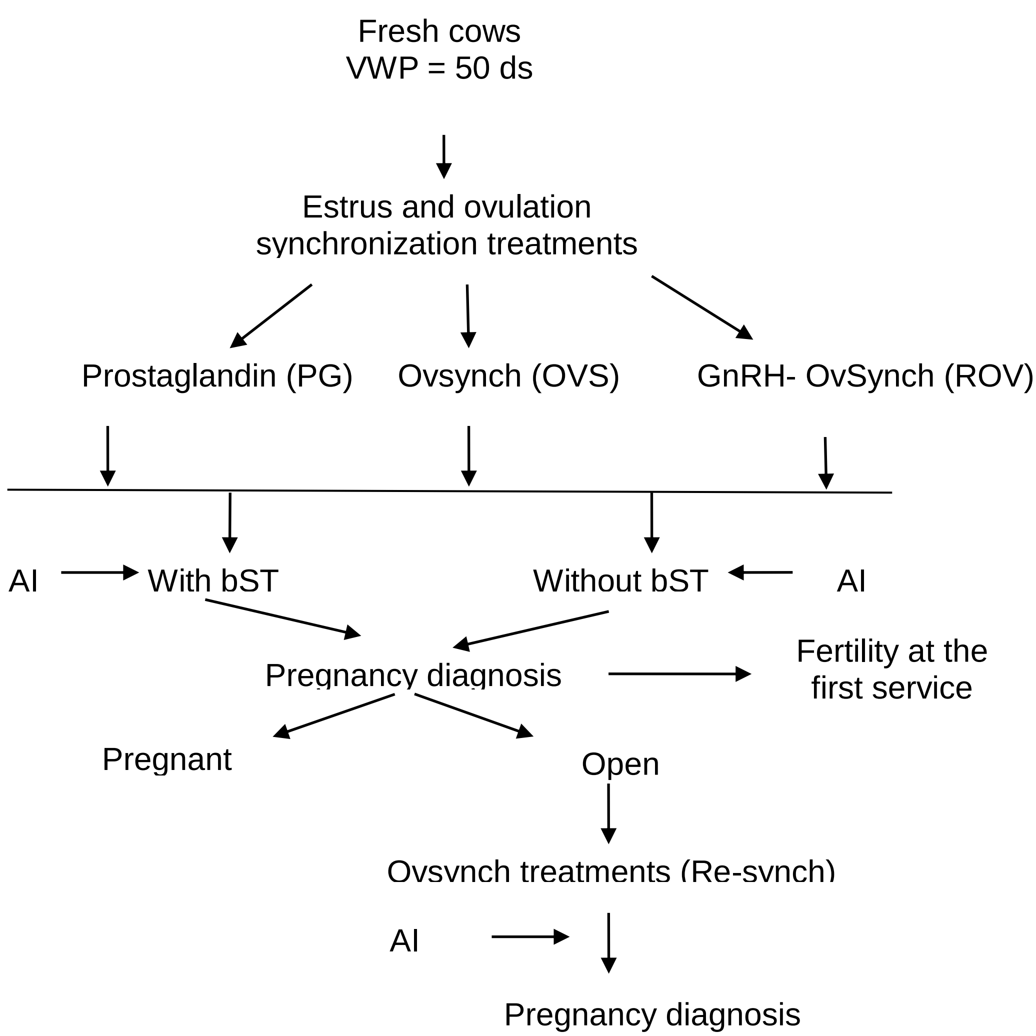
Reproductive management

The study included dairy cows that gave birth during the months of March and April. Reproductive management during early postpartum and the implementation of estrus synchronization programs took place during the warm months of the year, from May to

June. The evaluation of uterine involution and clinical aspects of the reproductive system was performed around ds 20 and 40 postpartum (PP), and 500 µg of synthetic prostaglandin (Cloprostenol sodium, Virbac) were administered, approximately at d 50 PP. The voluntary waiting period and the target calving interval were 50 d and 13.8 mo, respectively.

The study included cows in full lactation (n= 553) that were clinically healthy, exhibiting no anatomical-pathological problems of the reproductive system, and which calved in the months of March and April, and the number of lactations of each cow was recorded. The experimental design is shown in Figure 1. At the beginning of the study (voluntary waiting period (VWP: approximately 50 d in milk), the days in milk (DIM) were recorded; and cows with an acceptable physical body condition (BCS) with an approximation of 0.25 points were used as described by Ferguson *et al*[38].

Figure 1: Experimental design



The cows were randomly assigned to the following treatments (T):

1) Prostaglandin (PG) (n= 247 cows) Induction of estrus synchronization with prostaglandin (500 μg of cloprostenol sodium, Virbac); the cows were given artificial insemination service 12 h after estrus detection through visual observation.
2) OVS (n= 161 cows). Ovsynch: d 0, GnRH; d 7, PG; d 9, GnRH; d 10, FTAI). Estrus synchronization and fixed time artificial insemination (FTAI) program, in which cows were administered 100 μg of gonadotropin releasing factor (GnRH) (gonadorelin acetate, SYVA) on day zero (treatment start); subsequently, on ds seven and nine, the cows were given 500 μg of PG and 100 μg of GnRH, respectively. The artificial insemination was performed between 12 and 16 h after the last administration of GnRH.
3) ROV (n= 145 cows). GnRH + Ovsynch: Seven ds prior to the Ovsynch treatment (OVS), 100 μg of GnRH (i.e., d-7) were administered. Artificial insemination was performed between 12 and 16 h after the last GnRH administration.

For the evaluation of the fertility of the first postpartum service with similar ds in milk of this service, only the cows that presented estrus and were inseminated in the PG treatments, and all those with fixed time service (OVS and ROV), were considered. The cows in each treatment exhibiting estrus were randomly assigned to two groups: a) with bovine somatotropin (*C*-bST) (n= 221), i.e. the cows that received 500 μg of bovine somatotropin (bST) (Lactotropine, Elanco) at the time of insemination, and b) without bovine somatotropin (*S*-bST) (n= 235), i.e., cows not treated with bST at the time of insemination.

For the evaluation of the fertility of the first postpartum service with similar ds in milk of this service only the cows that presented estrus were inseminated in the PG treatments were considered, and all those with fixed time service (OVS and ROV). The cows of each treatment which exhibited estrus were randomly assigned to two groups: a) bovine somatotropin (*C*-bST) (n=221); cows received at the time of insemination 500 mg of bovine somatotropin (bST) (Lactotropine, Elanco). B) without bovine somatotropin (*S*-bST) (n=235). Cows not treated with bST at the time of insemination.

Based on the interaction of the main treatment effects and the administrations of bST at the time of the first service, six 3experimental groups were formed to be evaluated:

1. Estrus synchronization with PG without bST (PG / *S*-bST) (n=80)
2. Synchronization of the strobe with PG plus bST at service (PG / *C*-bST) (n = 70).
3. Ovsynch without bST (OVS / *S*-bST) (n=83).
4. Ovsynch with bST at service (OVS / *C*-bST) (n=78).
5. GnRH – Ovsynch without bST (ROV / *S*-bST) (n = 72)
6. GnRH – Ovsynch with bST at service (ROV / *C*-bST) (n = 73).

The fertility of the first postpartum service (FERT) was the ratio of pregnant cows to the number of cows served. The date of the first service and conception was recorded. The calving at first service (FERT) and calving to conception intervals (CCI) were calculated. Regardless of the treatment received, all cows that were not pregnant at the first service were served again when a new natural estrus was observed; those that were empty at the time of the gestation diagnosis (approximately 30%) were resynchronized with a Re-synch protocol to provide a new artificial insemination service.

A frequency distribution of the calving to conception interval of cows that responded to synchronization with prostaglandins and were inseminated, and of those with fixed time insemination, was carried out to determine the number of classes and the amplitude of this interval[39]. The cumulative percentage of pregnant cows (CPPC) for each defined class was estimated thus: 1. Less than 100 d in milk 2. 101 to 150 d in milk. 3. 151 to 201 d of milk. 4. 2020 to 253 d in milk. 5. More than 253 d in milk.

Likewise, the percentage of pregnant cows at the first, second, third or fourth or more services was determined by treatment, bST administration, and their interaction. The calving to conception interval and the number of services per conception were calculated for all cows in the study, including those that did not respond to estrus synchronization with prostaglandin treatment without administration of bovine somatotropin at the time of service.

Variables to be evaluated

The variables evaluated were the number of lactations; days in milk (DIM) and body condition (BC) at the beginning of the study; interval from calving to first service postpartum (CFSI); fertility of first postpartum service (FERT); cumulative percentage of pregnant cows in different postpartum periods (CPPC); distribution of calving-to-conception interval of cows with response to synchronization up to 150 d in milk; as well as, the number of services per conception (NSC) and calving-to-conception interval (CCI), including in these last two parameters the cows with response to synchronization with prostaglandin and that were not treated with bST at the time of insemination.

Statistical analysis

The variables number of lactations (NL), ds in milk (DIM), body condition (BPC), calving to first service interval (CFSI), calving to conception interval (CCI) and number of services per conception (NSC) were analyzed by means of a randomized block analysis of variance. The cumulative percentage of pregnant cows in different postpartum periods and by number of services were analyzed by Chi-square. The expected value of the percentage of fertility of the first postpartum service was analyzed with a first-order multiple logistic regression model. The model was adjusted by the maximum likelihood method considering the effects: Treatment (T); the administration of bST (S); the interaction between treatment and bovine somatotropin (T x S); and the dairy herd was taken as a block[39].

Results

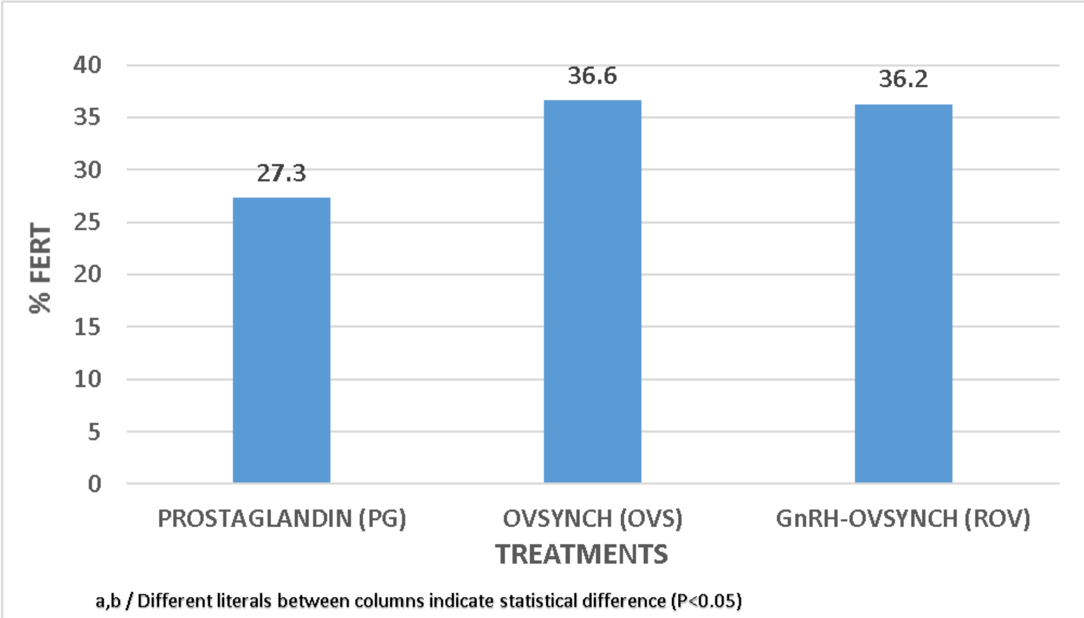
The ds in milk at the beginning of the treatment (BT= 56.6 ± 0.3), the body condition (BCS=3.1 ± 0.4), the number of lactations (NL= 2.7 ± 0.1), and the calving to first service interval (CFSI= 59.7 ± 0.3) were similar between treatment groups (*P*>0.05) (Table 2). The fertility at the first postpartum service of the Ovsynch (OVS, 36.6) and GnRH-Ovsynch (ROV, 36.2) treatments was higher than that observed in the Prostaglandin treatment (27.3 %) (*P*<0.05) (Figure 2).

Table 2: Body physical condition, days in milk at baseline, number of lactations and interval from birth to postpartum service per treatment

	Treatments		
Variables	**PG**	**OVS**	**ROV**
Number of observations	150	161	145
Initial fisical condition	3.1 ± 0.03	3.0 ± 0.03	3.1 ± 0.03
Lactation days	55.3 ± 0.3	57.6 ± 0.3	56.8 ± 0.3
Number of lactations	2.6 ± 0.1	2.8 ± 0.1	2.6 ± 0.1
Calving to first service interval	58.8 ± 0.3	60.5 ± 0.3	59.9 ± 0.3

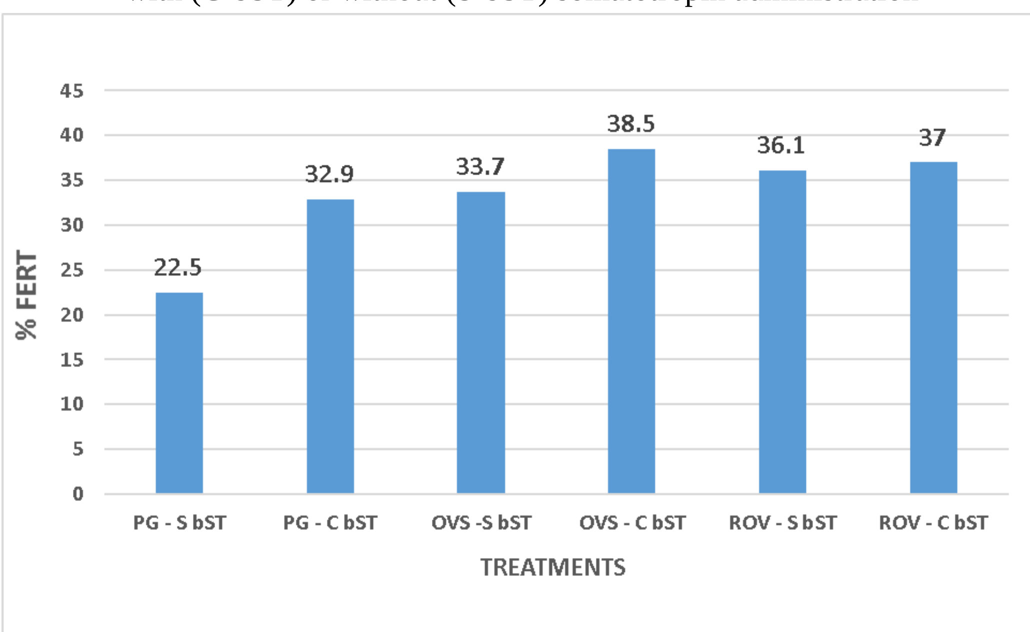
(*P*>*0*.05).

Figure 2: Fertility rate at first service postpartum by treatment effect



No single effect of bST (*P>0*.05) on fertility was detected at the first postpartum service of the dairy cows (36.2 *vs* 30.6 %, with and without bST, respectively). PG without bST presented a lower fertility rate at the first postpartum service (22.5 %) than the rest of the treatments (*P<0*.05) (Figure 3).

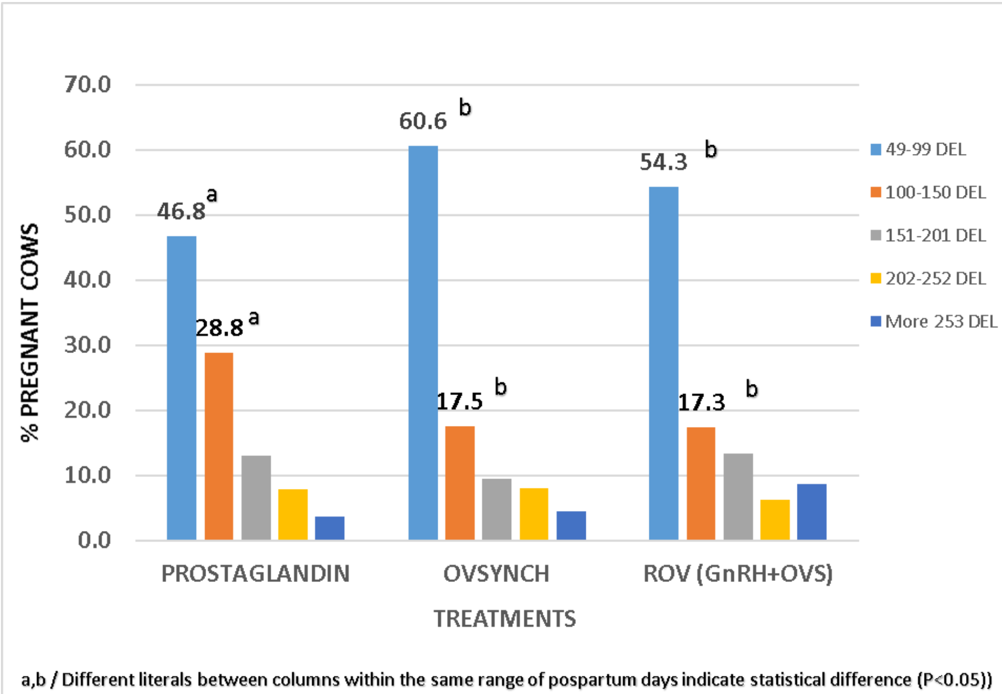
Figure 3: Fertility rate at first service postpartum (FERT) by treatment (PG – OVS – ROV) with (C-bST) or without (S-bST) somatotropin administration



a/b Different literals between columns indicate differences (*P*<0.05).

The percentage of pregnant cows at 99 d postpartum was higher with Ovsynch (60.6 %) and ROV (54.3 %) than with Prostaglandin (46.8 %) (*P<0*.05). Later, between days 100 and 150 postpartum, 28.8 % of the cows treated with Prostaglandin calved; this percentage is superior to those observed with the Ovsynch (17.5 %) and ROV (17.3 %) (*P<0*.05) treatments (Figure 4). No simple effect of bST (*P>0*.05) was detected on the percentage of pregnant cows at 99 d postpartum (*P>0*.05).

Figure 4: Percentage distribution of pregnant cows during postpartum (DIM) by treatment effect



The percentage of pregnant cows at d 99 postpartum under treatment with Ovsynch with or without bST (64.7 and 56.5 %, respectively) and ROV without bST (61.7 %) was higher than that observed in PG treatment without bST (41.1 %) (*P<0*.01). On ds 100 to 150 postpartum, , 34.2 % of the cows treated with PG but without bST were pregnant; this value was higher than that observed with the OVS treatment with bST (13.2 %) and the ROV treatment without bST (11.7 %) (*P<0*.05) (Figure 5).

Figure 5: Distribution of pregnant cows during postpartum (DIM) by effect of treatment interaction with (*C*-bST) or without (*S*-bST) somatotropin administration

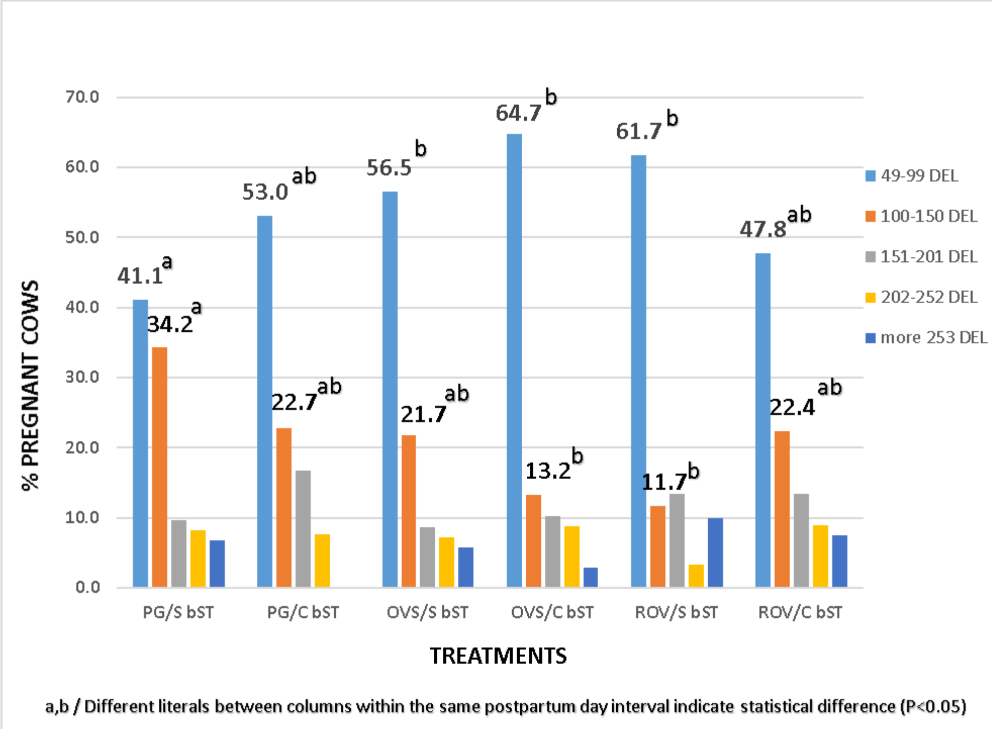


Figure 6 shows the percentage of pregnant cows accumulated in the first 150 d postpartum by effect of the synchronization treatment, in the OVS and ROV treatments from d 62 exhibited a cumulative percentage of pregnant cows (29.2 and 33.8 %) which is higher than that observed in the PG treatment (23.0 %) (*P*<0.05). This difference increased substantially towards d 65 in the OVS (38.7 %) and ROV (41.7 %) treatments, compared with the PG treatment (28.0 %) (*P*<0.05); the OVS and ROV treatments maintained this significant difference until the 109th and 145th ds in milk, respectively (*P*<0.05). After 150 d in milk, the cumulative pregnancy rate was similar for all three treatments (*P*>0.05).

Figure 6: Cumulative pregnancy rate during the postpartum period by treatment effect

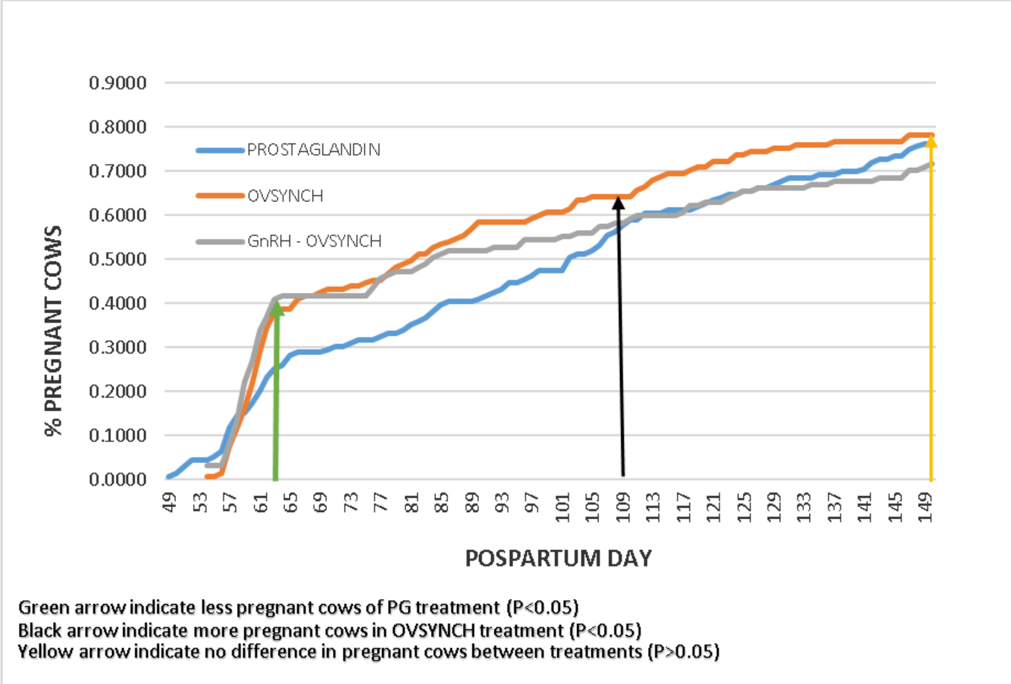


Table 3 shows a higher calving to conception interval in cows treated with PG without bST (144.6 d) than in those that were subjected to the rest of the treatments (*P*<0.05); fewer services per conception were observed in the groups of cows treated with Ovsynch and with PG plus bST at the time of service (2.2 and 2.3, respectively) compared to those observed in cows treated only with PG without bST at service (2.8), in those of the ROV group with bST (2.7) (*P*<0.05).

Table 3: Effect of treatment on calving-to-conception interval (CCI) and number of services per conception (NSC)

Treatment	n	IPC	NSC
PG witout bST	73	144.6 ± 6.7 a	2.8 ± 0.1 a
PG with bST	66	108.4 ± 9.4 b	2.3 ± 0.2 b
OVS without bST	69	115.4 ± 9.2 b	2.4 ± 0.2 ab
OVS with bST	68	106.4 ± 9.3 b	2.2 ± 0.2 b
ROV without bST	60	118.3 ± 9.9 b	2.4 ± 0.2 ab
ROV with bST	67	125.3 ± 9.4 b	2.7 ± 0.2 a

bST: Injection of somatotropin at the time of artificial insemination (AI)
OVS: [d 0, GnRH; d 7, PG; d 9, GnRH; d 10, AI]
ROV: [d -7, GnRH; plus Ovsynch].
[a,b] Different letters per column indicate significant statistical difference (P<0.05).

Discussion

Estrus synchronization and fixed-time artificial insemination (FTAI) programs improved first postpartum fertility in dairy cows under heat stress; thus, these programs show their goodness in overcoming the negative effect of dairy cow productivity on first postpartum fertility[40,41], and even as an additive negative effect to heat stress[1-6].

The fertility rates observed in the first postpartum service in the FTAIs coincide with those reported by other studies[7-16,20], which may be related to a higher ovulation rate of a follicle that is suitable for fertilization[7], and to a higher circulating concentration of the hormone progesterone[12,15], compared to schemes where cows were not pre-synchronized. However, other studies conducted in dairy cows under heat stress found a less efficient reproductive response than that observed in cows in thermal comfort[12,14,15]. An established effect of heat stress on dairy cows is that it reduces the quality of follicles and oocyte competence[16-20,42], the ovulation rate[7,8], and the diameter size of the ovulatory follicles[8]. Some studies determined that administering gonadotropin releasing factor (GnRH)[19] or replacing dominant follicles[20] in dairy cows during the summer season improved oocyte competence for fertilization and resulted in a higher percentage of embryos that would reach the blastocyst stage in *in vitro* studies, and concluded that oocyte competence has also been reported by other authors[17,18] under heat stress conditions, especially in the case of repeating cows[17,31], even though the fertility of *in vitro* fertilized oocyte was similar in winter and in the summer[17]. This emphasizes the importance of minimizing the effect of heat stress and shows that the FTAI schemes may have had a beneficial effect, improving the follicular quality and oocyte competence. The administration of gonadotropin release factor (GnRH: i.e., ROV treatment) prior to the Ovsynch program with the intention of generating follicular replacement did not improve the fertility rate of the first postpartum service, which was similar to the one observed in cows of the OVS group (Ovsynch). These results may indicate that the latter scheme of fixed-time artificial insemination was in itself sufficient to improve the follicular quality and oocyte competence, and they agree with those observed in other studies[21,22], and that alone the follicular exchange prior to the start of the Ovsynch program can improve fertility in cows that exhibited uterine problems in the early postpartum period and in those with a low body condition[21].

It has been determined that the loss of the cow’s physical body condition and the depth of the negative energy balance affect the fertility of the first postpartum service[43,44] by affecting oocyte competence[45,46]; and given the acceptable health and physical body condition of the cows in this study, it may be inferred that these negative effects were controlled. On the other hand, the fertility rate of the first postpartum service of over 36% obtained in the fixed-time artificial insemination programs observed by the present study under heat stress is excellent, compared to the fertility reported in cows with a high productive potential[40,41,47] and under heat stress[1-6]. On the other hand, the administration of bovine somatotropin (bST) at the moment of the fixed-time artificial insemination did not have a determining effect as a main variable to improve fertility in the first postpartum service, as reported by Jousan *et al.*[24]; therefore, it is possible that estrus synchronization and fixed-time artificial insemination programs were sufficient to eliminate the damaged follicle due to heat stress, induce the emergence of new follicles, and improve the quality of the ovulatory follicle and the competence of the oocyte as described in other studies[19,20]. However, the fact that the group of cows treated only with prostaglandin without administration of somatotropin at the time of service had a fertility rate 10.4 to 16 percentage points lower in the first postpartum service than that observed in cows treated with somatotropin significantly indicates a positive effect of somatotropin on fertility in the first postpartum service, as documented in other studies with bST treatments from 61 to 63 ds in milk with repeated applications of this hormone every 10[25,48,49] or 14 d[26,35,36], having improved the development of the corpus luteum and increased its production of progesterone[28,29], both in repeating cows[30,31], and in embryo receptor cows[32]. Furthermore, it has been inferred that this beneficial effect involves insulin-like growth factor type I (IGF-1), which appears to be associated with the process of final oocyte maturation, follicular development, and steroidogenesis[34,50,51] and which, in *in vitro* studies, increases the pregnancy rate of transferred embryos[52].

On the other hand, in programs in which bST is administered every 14 ds to dairy cows in order to increase their milk production[24-26], the expression of the estrus has been observed to be negatively affected[25,36]; therefore, it has been suggested that the use of bST should be accompanied by fixed-time insemination protocols in order to ensure insemination of 100% of the cows[36]. Thus, although the application of bST as the main variable does not have a relevant effect on the fertility rate of the first postpartum service under heat stress, at least with the FTAI the risk of not detecting cows in heat is eliminated.

Consequently, an increase of 15.4 to 23.6 % in the percentage of pregnant cows in the first third of lactation in fixed-time insemination programs compared to traditional management, as confirmed in other studies[9-11,14], ensures a new production cycle and reduces the risk of eliminating cows from the herd due to reproductive causes. On the other hand, pregnant cows using estrus synchronization treatments with prostaglandins without bST at service exhibited between 19.3 and 38.6 more open ds than with the other treatments; this implies the loss of at least one to two lost estrus cycles, which entails extra costs in the reproductive cycle of the dairy cows.

Conclusions and implications

The administration of gonadotropin releasing factor (GnRH) prior to the Ovsynch program (ROV) and the administration of bovine somatotropin (bST) at the time of insemination did not improve the fertility rate of the first postpartum service. Fixed-time artificial insemination schemes improved the fertility rate of the first postpartum service and increased the number of pregnant cows in the first 99 d postpartum. Under heat stress, bovine somatotropin increases the fertility rates at first service postpartum in cows treated with prostaglandin, but not in cows in fixed-time insemination programs.

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