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Original article

Comparison of an oestrus synchronisation protocol with oestradiol benzoate and $PGF_{2\alpha}$ and insemination at detected oestrus to a timed insemination protocol (Ovsynch) on reproductive performance of lactating dairy cows

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Abstract – A total of 226 out of 245 postpartum lactating dairy cows in a commercial dairy farm were allocated to two groups of oestrous synchronisation protocols in order to evaluate reproductive performance. One group was treated with oestradiol benzoate (ODB) and $PGF_{2\alpha}$ on day 10 of the oestrous cycle with insemination at the detected oestrus, the second group underwent the Ovsynch (OVS) protocol (GnRH + $PGF_{2\alpha}$ + GnRH) with timed AI. Pregnancy was diagnosed by ultrasonography on day 28 after AI and confirmed by rectal palpation on day 45. A higher ($P < 0.001$) proportion of cows in OVS (100%) were inseminated within (19.2 ± 3.8 h) following the second GnRH injection than those of cows in EPE (ODB + $PGF_{2\alpha}$ + ODB) (70.6%) inseminated at the detected oestrus within (35.6 ± 5.2 h) following the second ODB injection. Pregnancy rates for the first AI at day 28 (64.0 ± 4.6, 62.4 ± 5.5%) and at day 45 post-insemination (40.4 ± 4.7, 40.0 ± 5.6%) for OVS and EPE cows respectively, did not differ between the two treatments, whereas, the overall pregnancy rates tended to be higher ($P < 0.08$) for the OVS (85.1 ± 3.8%) cows than the EPE cows (74.1 ± 4.5%). No differences were observed in pregnancy rates for first AI and overall up to fourth AI between primiparous (34.7 ± 5.8 and 85.3 ± 4.7%) and multiparous cows (43.5 ± 4.5 and 77.4 ± 3.6%). Days open for pregnant cows tended to be lower ($P < 0.08$) for OVS (76.2 ± 3) than for EPE cows (84.7 ± 4), while days open were higher ($P < 0.05$) in primiparous cows (85.3 ± 4) than in multiparous cows (75.6 ± 3). The results indicate that pregnancy rates for first AI were similar, but overall pregnancy rates up to the fourth AI tended to be higher for OVS than EPE cows, while days open was tended to be lower for OVS than EPE cows.

dairy cows / oestrus-synchronisation / GnRH / oestradiol / PG / reproductive performance

1. INTRODUCTION

Pregnancy rate is the product of oestrus detection and conception rates. Low rates and accuracy of oestrus detection are major factors limiting in reproductive efficiency of dairy cows [1]. Cattle that fail to conceive following detection are expected to return to oestrus 18 to 24 days after insemination, the interval of a normal oestrous cycle. However, detection of oestrus is increasingly challenging for breeding management of

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dairy herds, particularly in larger herds, among cows in confinement, among cows housed entirely on concrete, and with increasing levels of milk production [2]. Delays in conception due to failure to detect oestrus increase intervals from calving to conception and lead to economic loss in dairy herds [3]. Therefore, pregnancy rates decline as a result of decreased conception rates and lowered rates of oestrus detection [1]. Moreover, oestrus detection and the interval to first postpartum service are the most important factors affecting the calving interval [4]. Under current production systems, the visual oestrus detection rate in high producing cows is approximately 50%, with 80 to 95% accuracy at best [5]. Therefore, development of treatment programs that can effectively induce oestrus and ovulation in cows can have a major effect on herd reproductive performance. The Ovsynch program was developed allowing cows to be inseminated without the need for oestrus detection and improving reproductive performance during the early postparum period (around 50 d) with no labour investment for detecting oestrous [6–9]. Such a protocol consists of an injection of GnRH agonist given at random stages of the oestrous cycle followed, 7 d later by an injection of $PGF_{2\alpha}$. Then another injection of GnRH is given 48 h following the $PGF_{2\alpha}$ injection. Treated cows are time inseminated at approximately 16 h after the last injection of GnRH without the need for oestrus detection. The ability of GnRH-$PGF_{2\alpha}$ based protocols to effectively synchronise oestrus and (or) ovulation is dependent upon the stage of follicular development at the time of initial GnRH injection; cows that started Ovsynch on d 5 to 13 had a higher probability of pregnancy and ended to have a lower rate of pregnancy loss than cows started at other stages [10].

In cattle with luteal phase progesterone concentrations, oestradiol treatment reduced both FSH concentrations [11] and LH pulse frequency [12], resulting in follicle regression, followed by synchronous follicular wave within 3–5 d [13]. A strategically timed injection of 1 mg oestradiol benzoate (ODB) during the luteal phase of cycling cows has been demonstrated to be effective in synchronising the emergence of a wave of ovarian follicles [14]. The injection of 2 mg ODB at the time of progesterone (CIDR) treatment has also been found to result in greater synchrony of oestrus and normal fertility in postpartum cows, which have resumed the oestrous cycle [15]. Therefore, the first ODB injection on d 10 of the oestrous cycle initiates a new follicular wave and may provide a dominant follicle in a defined growth phase at the time of $PGF_{2\alpha}$ (present corpus luteum). In this manner, oestradiol is similar (albeit somewhat less efficient) than GnRH injection for follicular wave synchronisation, while the second ODB injection after luteolysis may induce an LH surge. A dose of 1 mg of ODB has been shown to be sufficient to elicit behavioural signs of oestrus in the anoestrus cow [16]. Substituting oestrogen for the GnRH injections is logical for numerous reasons, including cost availability and induction of normal oestrus characteristics such as mucus secretion, uterine tone and exhibition of sexual behaviour. The aim of this study was to compare the effect of the Ovsynch protocol on reproductive performance to the administration of oestradiol benzoate (ODB) on d 10 of the oestrous cycle plus $PGF_{2\alpha}$ 7 d later followed by another ODB injection 24 h later and insemination at detected oestrus (EPE).

2. MATERIALS AND METHOD

This study was conducted on a commercial dairy farm at Dulail area northeast of Jordan between October 2002 and April 2003 (autumn and winter). Lactating dairy cows were housed in free-stall barns provided with shade and were milked three times daily at approximately 8-h intervals. The rolling average milk yield for the herd was 8600 kg per lactation. The cows were fed according to NRC recommendations

[17] a total mixed ration (TMR) of 40% forage (corn silage and alfalfa hay) and 60% concentrate (whole cottonseed, barely, wheat bran, soybean meal, and commercial concentrate for lactation with trace minerals and vitamins) containing 1.70 Mcal of $NE_L \cdot kg^{-1}$ and 17.8% CP (percentage of DM). Cows had ad libitum access to fresh water. The experiment was implemented on a weekly basis according to the predetermined d postpartum.

A total of 245 lactating Friesian dairy cows (primiparous, *n* = 100) and (multiparous *n* = 145) were subjected to oestrus detection between 20–30 d postpartum by visual observation and the ALPRO™ system (Delaval International AB, Tumba, Sweden), which was utilised to detect the onset of oestrus and to record standing events associated with oestrus. An activity meter, which was hung around the same neckband as the ALPRO transponder, monitored the extra activity shown by a cow when she came into oestrus and transmitted this data every hour to the computer. If oestrus was not observed until d 30, each cow received an i.m. injection of 25 mg $PGF_{2\alpha}$ (Lutalyse, Phamacia & Upjohn S.A., Puurs, Belgium) after rectally palpating for the presence of a corpus luteum. Then the cow was observed for oestrus as in the previous procedure (visual observations and ALPRO™ system).

Nineteen cows did not respond to $PGF_{2\alpha}$ injection on d 30 and were excluded from the study. On d 10 (mid oestrous cycle) after the observed oestrus (Fig. 1), the cows were randomly assigned into two treatments [OVS (GnRH + $PGF_{2\alpha}$ + GnRH and TAI), *n* = 124] and [EPE (ODB + $PGF_{2\alpha}$ + ODB and AI at detected oestrus), *n* = 102]. The cows in the OVS were injected with 10 µg GnRH agonist (Buserelin, Receptal®, Hoechst Roussel Vet GmbH), followed 7 d later by an i.m. injection of 25 mg $PGF_{2\alpha}$ (Lutalyse, Phamacia & Upjohn S.A., Puurs, Belgium) then another injection of 10 µg GnRH 48-h later and time inseminated from 16 to 24 h after the second GnRH injection. Cows in the EPE were injected with 2 mg ODB in oil (Intervet International B.V., Boxmeer, Holland), followed by an i.m. injection of 25 mg $PGF_{2\alpha}$ 7 d later, then another injection of 1 mg ODB 24-h later. They were then inseminated at the detected oestrus. Because this study was performed on a private farm, the cows continued under the oestrus detection program during the hormonal treatment and were inseminated at any detected oestrus in order to maximise conception. The expected breeding period (EBP) started by 53 d and 50 d postpartum in the OVS and EPE groups, respectively (NS difference). Pregnancy was based on visualisation of an embryonic heartbeat by transrectal ultrasonography (scanner 100 Vet, equipped with a 5.0 MHz transducer; Pie Medical, Maastricht, The Netherlands) at d 28 and it was reconfirmed by rectal palpation at d 45 post insemination. The cows, which were diagnosed pregnant at d 28 and later diagnosed nonpregnant were considered as having experienced late embryonic mortality (LEM).

The cows in the OVS and EPE, which returned to oestrus, were re-inseminated at detected oestrus and those cows which were diagnosed not pregnant either on d 28 or 45 received another injection of 25 mg $PGF_{2\alpha}$ and were re-inseminated at the detected oestrus to maximise pregnancy rate. For cows in EPE, oestrus detection rate or AI submission rate was defined as the proportion of cows that cycled within 96 h after the second ODB injection. The pregnancy rate was defined as the proportion of all treated cows that were pregnant at 28 and at 45 d post insemination, and overall pregnancy rate (up to fourth AI). Days open were defined as the number of days from calving to conception for pregnant cows till the fourth insemination.

Logistic regression models (Proc logistic) of SAS [18] were used to analyse pregnancy rates, late embryonic mortality between d 28 and d 45 post AI and oestrus detection rate or AI submission rate using a stepwise selection procedure to determine independent

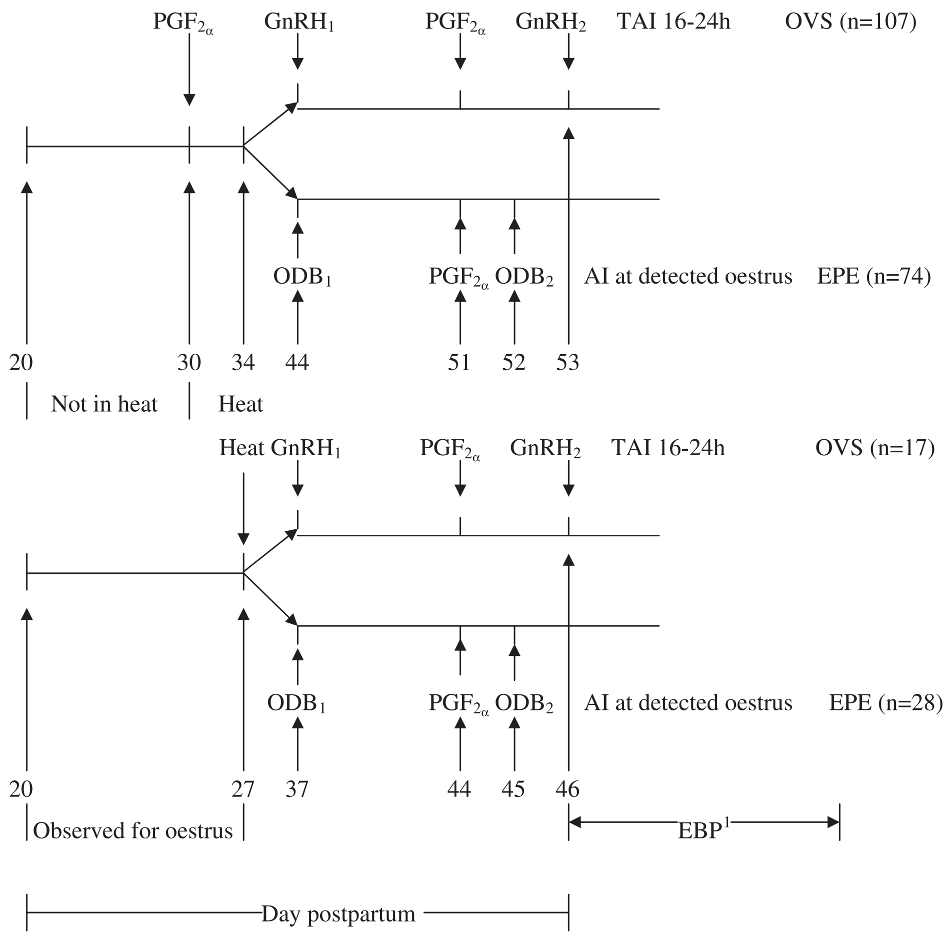

Figure 1. Schematic diagram of treatment protocols used in the experiment. [1] Expected breeding period for OVS group: timed AI between 16–24 h from $GnRH_2$, for the EPE group: AI at detected oestrus within 96 h from ODB_2.

variables at predetermined significance levels (a variable entered with $P \leq 0.30$ and stayed with $P \leq 0.20$ in the model). Mathematical models for reproductive responses included effects of treatments (OVS and EPE), parity (primiparous and multiparous), season or month (week pp when cows enter the experiment), observed oestrus, 120-day total milk yield and milk production between 40 and 60 d postpartum. The general linear model (GLM) of SAS was then utilised using the reduced model of the significant variables determined by the logistic regression. For all cows, days from calving to EBP, days from calving to first AI, hours from second injection of GnRH and ODB to first AI, services per conception, days open for pregnant cows were analysed using two-way analysis of variance with treatments, parity, season and their interactions.

Table I. Oestrus and pregnancy rates for OVS and EPE cows.

Criteria	Treatment[1]	
	OVS (n = 114)	EPE (n = 85)
AI submission rate	(n) %	(n) %
During EBP[2]	(114/114) 100^{a}	(60/85) 70.6^{b}
After EBP[3]	(11/114) 9.7^{a}	(25/85) 29.4^{b}
Pregnancy rates[4]		
During EBP	(41/114) 36.0	(24/60) 40.0
After EBP	(5/11) 45.5	(10/25) 40.0
All	(46/114) 40.4	(34/85) 40.0^{NS}

[1] OVS: (GnRH + $PGF_{2\alpha}$ + GnRH and TAI), EPE: (ODB + $PGF_{2\alpha}$ + ODB and AI at detected oestrus), 10 and 17 cows from OVS and EPE groups were exhibited oestrus and inseminated prior to the last hormonal injection and were excluded from the analysis.
[2] Expected breeding period for OVS: cows AI between 16–24 h from the second GnRH injection, for EPE: cows AI at detected oestrus within 96 h from the second ODB injection.
[3] Expected breeding period for OVS: cows in heat and AI after 24 h from the second GnRH injection, for EPE: cows in heat and AI after 96 h from the second ODB injection.
[4] At day 45 post AI.
^{a,b} Percentages within the same row with different superscripts differ ($P < 0.001$).
^{NS} Not significant.

3. RESULTS

Amongst the 226 cows enrolled in the study, 45 were detected in oestrus between 20 and 30 d postpartum. No difference was detected between OVS (27.1 ± 0.6 d, n = 17) and EPE (26.7 ± 0.5 d, n = 28). Ninety percent (181 out of 200) of the cows came in oestrus 4.4 ± 0.2 d after $PGF_{2\alpha}$ administration on d 30 (this high detection rate was due to the use of the ALPRO system). The mean days from calving to first oestrus did not differ significantly between OVS and EPE cows.

Eight percent (10/124) and 16.7% (17/102) of the cows in the OVS and EPE groups exhibited oestrus and were inseminated prior to the last hormonal injection ($P > 0.05$). Only 20% (2/10) and 17.7% (3/17) of the cows in OVS and EPE respectively were pregnant ($P > 0.05$) at d 45 and were excluded from the analysis. Oestrus and pregnancy rates for cows in OVS and EPE are presented in Table I. Treatment differences ($P < 0.001$) were detected for AI submission rates for cows in OVS and EPE groups. These differences occurred by design, since all cows in OVS were inseminated at (19.2 ± 3.8 h) following the second GnRH injection, around 9.7% (11/114) of those returned to oestrus at (113.9 ± 13.6 h) from the second GnRH injection and were inseminated 12 h later, whereas 70.6% (60/85) and 29.4% (25/85) of the cows in EPE exhibited oestrus at (35.6 ± 5.2 and 151.9 ± 7.9 h, respectively) following the second ODB injection and inseminated at 12 h later. Only 40.4% (46/114) and 40.0% (34/85) of the cows in the OVS and EPE groups were pregnant (Tabs. I and II).

There was no effect of treatment, parity and season or interactions on pregnancy rate for first AI. Pregnancy rates at 28 and at 45 d post AI were similar for OVS (64.0 ± 4.6 and 40.4 ± 4.7%) and EPE cows (62.4 ± 5.5 and 40.0 ± 5.6%, respectively; Fig. 2), whereas, overall pregnancy rates for cows in OVS tended to be higher ($P < 0.08$) than for cows in EPE (85.1 ± 3.8 and 74.1 ± 4.5%, respectively; Tab. I). No difference

Table II. Reproductive performance for OVS and EPE cows.

Parameter	Treatment[1]	
	OVS (n = 114)	EPE (n = 85)
	(n) Mean ± LSM	(n) Mean ± LSM
Days from calving to first AI[2]	(114) 54.5 ± 0.5	(85) 54.4 ± 0.6
Hours from last hormonal injection to first AI[3]	(114) 19.2 ± 3.8[a]	(60) 35.6 ± 5.2[b]
Hours after EBP[4]	(11) 113.9 ± 13.6[c]	(25) 151.9 ± 7.9[d]
Overall pregnancy rate[5]	(97) 85.1 ± 3.8[e]	(63) 74.1 ± 4.5[f]
Days open for all pregnant cows[5]	(97) 76.2 ± 3[e]	(63) 84.7 ± 4[f]
Services per conception[5]	(97) 1.78 ± 0.09	(63) 1.78 ± 0.12[NS]

[1] OVS: (GnRH + $PGF_{2\alpha}$ + GnRH and TAI), EPE: (ODB + $PGF_{2\alpha}$ + ODB and AI at detected oestrus), 10 and 17 cows from OVS and EPE groups were exhibited oestrus and inseminated prior to the last hormonal injection and were excluded from the analysis.
[2] Artificial insemination.
[3] OVS: cows AI between 16–24 h from the second GnRH injection, EPE: cows AI at detected oestrus within 96 h from the second ODB injection.
[4] Expected breeding period for OVS: cows AI between 16–24 h from the second GnRH injection, for EPE: cows AI at detected oestrus within 96 h from the second ODB injection.
[5] Up to the fourth AI.
[a,b] Percentages within the same row with different superscripts differ ($P < 0.001$).
[c,d] Percentages within the same row with different superscripts differ ($P < 0.01$).
[e,f] Percentages within the same row with different superscripts differ ($P < 0.08$).
[NS] Not significant.

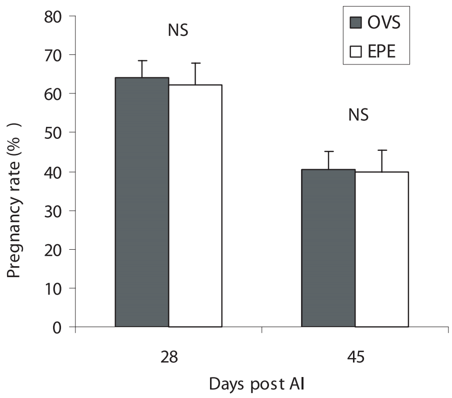

Figure 2. Least squares means and SE for pregnancy rates at d 28 and at 45 post first AI for cows in the OVS and EPE groups.

was observed on pregnancy rates at the first AI and overall up to the fourth AI between primiparous (34.7 ± 5.8 and 85.3 ± 4.7%) and multiparous cows (43.5 ± 4.5 and 77.4 ± 3.6%, respectively).

No differences were observed in late embryonic mortality due to treatment effects, parity and season or interactions. The frequency of LEM from 28 to 45 d after insemination was 23.7% and 22.5% for cows in OVS and EPE, respectively. Least squares means for days from calving to first AI were similar for OVS and EPE cows (54.5 ± 0.5 and 54.4 ± 0.6, respectively), while the hours from the last hormonal treatment to the first AI was less ($P < 0.001$) for OVS than EPE cows (19.2 ± 3.8 vs. 35.6 ± 5.2, respectively; Tab. II). Services per conception for overall pregnant cows were not affected by treatment, parity and season or interactions. Services per conception were similar ($P > 0.05$) for OVS (1.78 ± 0.09) and EPE cows (1.78 ± 0.12). Similar observations were found in primiparous (1.89 ± 0.12; n = 64) and multiparous cows (1.68 ± 0.10; n = 96). Days open for pregnant cows in

OVS tended to be lower ($P < 0.08$) than those of the EPE groups (76.2 ± 3 and 84.7 ± 4, respectively, Tab. II), but days open for primiparous (85.3 ± 4) were higher ($P < 0.05$) than for multiparous cows (75.6 ± 3). Milk yield between days 40 to 60 postpartum and the total yield at 120 days did not affect pregnancy rates.

4. DISCUSSION

In this study, synchronisation in OVS and EPE cows was initiated on day 10 of the oestrous cycle. Eight percent of the cows in OVS were detected in oestrus (by the ALPRO system) and inseminated before the second GnRH injection. Stevenson et al. [9] reported that 8 to 16% of cows showed oestrus in the OVS protocol around the time of $PGF_{2\alpha}$ injection. According to Vasconcelos et al. [10] these cows failed to ovulate after the first GnRH injection; they were primarily in the second half of the oestrous cycle when they received the first GnRH injection and exhibited oestrus before the second GnRH injection because the corpus luteum regressed and the cows naturally came into oestrus. A greater percentage of cows were time inseminated in OVS between 16–24 h after the second GnRH injection, while 9.7% of those cows showed oestrus after that time and were re-inseminated to maximise the pregnancy rate. These cows likely initiated growth of a new follicular wave in response to ovulation of a dominant follicle; however, the follicle grows quickly and loses dominance during the nine-day interval between GnRH injections and failed to ovulate after the second GnRH injection [10]. Therefore, only 82.2% of the cows were synchronised, which agrees with the results of [10, 19]. Recently, Cartmill et al. [20, 21] reported that 83.8% of the cows were cycling in the OVS program, while 5 to 15% of the cows were not cycling at the time of insemination.

Around 16% of the cows in EPE were detected in oestrus and inseminated before the second ODB injection which means they had dominant follicles when the first ODB was injected and those ovulated before the second ODB injection as reported by [22]. It has been demonstrated that 1 mg of ODB injection during the luteal phase of the oestrous cycle generally promotes a synchronous timing of new emergence. The intervals to new wave emergence and oestrus are still variable [23]. This variability could be due to a number of factors such as breed type, age, lactation status and dose of oestradiol [24]. Moreover, ODB may also be used to induce oestrus with or without concurrent ovulation [25]. Furthermore, eight percent in the first group (OVS) and 16% in the second group (EPE) showed oestrus (due to CL regression before the second hormonal injection) and these cows were inseminated but had low pregnancy rates. In the present study, more cows were observed in oestrus and inseminated within 35.6 ± 5.2 h after the second ODB injection (70.6%). These results are in line with those of Bortman et al. [26] who reported that following treatment of lactating dairy cows with GnRH, 7 days later by $PGF_{2\alpha}$ and oestradiol cypionate (ECP), an esterified form of oestradiol-17β, after 24 h then AI at detected oestrus, 63% of the cows were observed in oestrus. In another study Stevenson et al. [27] detected only 40% of the cows in oestrus when using a similar synchronisation method. Administration of ODB one day after luteolysis may induce an LH surge and behavioural signs of oestrus in anoestrus cows [28]. Recent studies by Pancarci et al. [29] and Stevenson et al. [27] found that cows injected with 1 or 0.5 mg of ECP induced an LH surge in lactating dairy cows when given 24 h after a luteolytic dose of $PGF_{2\alpha}$. Moreover, Pancarci et al. [29] reported that oestrus in the Heatsynch protocol (ECP replaces the second GnRH injection in the OVS protocol) occurred at 29.0 ± 1.8 h after ECP injection with ovulation occurring at 55.4 ± 2.7 h after ECP injection of 66.6% of the cows with only one cow not ovulating until 83 h after ECP.

Pregnancy rates in OVS and EPE cows were similar at 28 and 45 d post AI but numerically higher up to fourth AI in OVS than EPE cows. A similar observation, at first AI, was found in beef cows using a progesterone releasing device insertion between the first GnRH or ODB injections and $PGF_{2\alpha}$ injection and timed AI after the second GnRH or ODB injections [30]. Meanwhile, pregnancy rates did not differ between OVS and Heatsynch timed insemination in lactating dairy cows as reported by [27, 29]. The concept of OVS is to achieve high pregnancy rates by synchronisation of ovulation and timed AI [19]. If service rate is close to 100% the success of the OVS protocol depends on the conception rate achieved by timed AI. In the present study, amongst the 91.9% (114/124) cows that were time inseminated in OVS, 82.2% of the cows were, actually in oestrus while 9.7% of the cows were inseminated without showing oestrus. In the EPE, the cows which were inseminated at the detected oestrus gave a better conception rate. But, 5 cows exhibited oestrus around 20 d after EBP, which indicates that those cows were probably in oestrus at the first AI but they did not conceive. Probably, if cows in EPE were time inseminated around 30 h after the second ODB could have a better conception rate. Cows in the OVS group pregnant up to the fourth AI had a tendency to lower days open than cows in EPE. This is the first study, that involved the two protocols. Several studies have demonstrated that pregnancy rate to the OVS program was comparable to or greater than the pregnancy rates to various programs that involved insemination at detected oestrus following injections of $PGF_{2\alpha}$, GnRH followed by $PGF_{2\alpha}$ or spontaneous oestruses. However, the reproductive management advantage of the OVS program is that the insemination rate is higher. That reduces the mean days to first AI and days open and accelerated the rate of accumulated pregnancies [6, 8, 31]. In the current study, pregnancy rates either to the first or overall up to the fourth AI for primiparous and multiparous cows were similar. These results were in agreement with previous studies [29, 32].

The average frequency of LEM between d 28 and 45 for both treatments was 23% and not affected by month treatment, parity or interactions. Several studies on lactating dairy cows have indicated that LEM between 27 and 45 d after AI range from 8 to 21% [33–35]. In some cases, LEM have been reported to be higher than 21% [21, 36]. According to Starbuck et al. [37] some factors were associated to LEM like concentrations of progesterone at week 5 of gestation, twin ovulation, body condition, age and service sire.

The hypothesis of this study was that the OVS treatment would result in higher pregnancy rates and consequently improved overall reproductive performance since all cows would be inseminated without depending on the observed oestrus. Yet, the lack of a difference in reproductive performance obtained in the results could have been due to the high efficiency of detecting oestrus in the cows in the EPE group (since oestrogen is expected to induce oestrus characteristics) and the oestrus detection method used on the farm allowed a large number of cows to be detected in oestrus and consequently inseminated.

5. CONCLUSIONS

The results indicate that timed AI following the GnRH + $PGF_{2\alpha}$ + GnRH synchronisation protocol had similar pregnancy rates for first AI and numerically decreased days open as compared to insemination at the detected oestrus following the ODB + $PGF_{2\alpha}$ + ODB synchronisation protocol. Meanwhile, ODB can be used to induce follicular turnover and ovulation. Future investigations are needed to evaluate this protocol with timed AI around 30 h after the second ODB.

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