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EFFECT OF UTILIZATION OF SINGLE OR DOUBLE PROSTAGLANDIN ADMINISTRATION WITHIN AN OVSYNCH FIXED-TIME ARTIFICIAL INSEMINATION PROTOCOL DURING SUMMER SEASON IN DAIRY COWS

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Abstract

This study investigated the fertility of dairy cows during summer season after treatment with a single or double $PGF_{2\alpha}$ in a progesterone (P4)-based Ovsynch protocol for fixed-time artificial insemination (FTAI). The data were compared to a treatment effectuated during winter season. Two groups of 60 dairy cows each were randomly assigned on day 60 post-partum to single $PGF_{2\alpha}$ or double $PGF_{2\alpha}$ group. At removal of the P4 treatment the single $PGF_{2\alpha}$ group received a single dose and the double $PGF_{2\alpha}$ group received two doses of $PGF_{2\alpha}$ at 24 hours apart. In each group, 30 cows were treated during the summer (June, July and August) and 30 cows during the winter (December, January and February) season. During summer season a double $PGF_{2\alpha}$ dose did not influence pregnancy rate at FTAI but improved cumulative pregnancy rate 60 days after FTAI (43% vs 69% for single $PGF_{2\alpha}$ and double $PGF_{2\alpha}$ administration, respectively; $P<0.05$). During winter season the single or double $PGF_{2\alpha}$ administration did not modify PR or cumulative PR. In conclusion, during summer the utilization of a double instead of single $PGF_{2\alpha}$ treatment into a P4-based Ovsynch-FTAI protocol did not increase pregnancy rate at FTAI but improved cumulative pregnancy rate.

Key words: FTAI, PGF2α, season, dairy cow

During the past two decades, an increase in the basic understanding of ovarian follicular dynamics allowed the development of treatment such as the synchronization protocol of estrus/ovulation generally named as Ovsynch (Pursley et al., 1997) followed by a fixed-time artificial insemination (FTAI). There are many variants of this protocol (for review see Nowicki et al., 2017; Colazo and Mapletoft, 2014) and

pregnancy rate is generally similar to animals inseminated after natural estrus and the calving-conception interval is reduced (Rajamahendran et al., 2001; Macmillan et al., 2003; De Rensis et al., 2015). Furthermore, in some circumstances, like in cows during the summer season, utilization of Ovsynch-FTAI has a positive effect on calving-conception interval (De Rensis et al., 2002; Dirandeh et al., 2014). However, a certain number of animals do not respond to the treatment and the percentage of successful pregnancy at FTAI is around the 30–40% (for review see Nowicki et al., 2017). This is due, among other factors, to a not optimal rate of luteal regression after a single $PGF_{2\alpha}$ treatment during the Ovsynch-FTAI protocol (Brusveen et al., 2009; Giordano et al., 2013; Martins et al., 2011; Colazo et al., 2013; Dirandeh et al., 2015, Souza et al., 2007; Behrouzi et al., 2016) with a reduction in LH pulse frequency and incomplete development of the dominant follicle which lead to a reduction in the pregnancy rate (Vasconcelos et al., 2001; Santos et al., 2010; Martins et al., 2011; Wiltbank et al., 2014). Therefore, studies have been developed to investigate if the luteolytic capacity of $PGF_{2\alpha}$ can be improved by a double instead of a single administration. When cows receive two $PGF_{2\alpha}$ administrations (Brusveen et al., 2009; Santos et al., 2010; Ribeira et al., 2012; Giordano et al., 2013; Carvalho et al., 2015) or the dose of $PGF_{2\alpha}$ is increased (Brusveen et al., 2009; Giordano et al., 2013) during an Ovsynch protocol, luteal regression is improved and pregnancy rate is increased compared with cows receiving only one $PGF_{2\alpha}$ administration (Wiltbank et al., 2015; Carvalho et al., 2015).

During summer season fertility is reduced because of heat stress that can alter luteal regression and then plasma progesterone becomes variable (Wolfenson et al., 2000; De Rensis and Scaramuzzi, 2003; De Rensis et al., 2017; Motavalli et al., 2017). Therefore, the objective of the present study has been to investigate if during summer the double, compared to a single, $PGF_{2\alpha}$ administration in a Ovsynch-FTAI protocol, can improve pregnancy rate. The results have been also compared to the winter season.

Material and methods

This study was performed on a commercial Holstein-Friesian dairy herd in Northern Italy (45°41’56”04 N 09°40’12”00 E). During the study period (October 2015 to November 2016), the mean number of lactating cows in the herd was 500 and mean annual milk production was 11,300 kg per cow. All animals were reared within the herd, housed indoors, milked twice per day and fed with a total mixed ration *ad libitum* to meet the nutritional requirements of lactating dairy cows. Mean milk yield at the start of the treatment was 42.2±1.1 kg/day. Only healthy cyclic cows with a history of no detectable reproductive disorders and free of clinical diseases were included. The genetics of the cows were very similar. In addition, body condition scoring system from 1 = very thin to 5 = very fat (Edmonson et al., 1989) was evaluated for each animal at the time of the first GnRH administration and only cows between score 3.0 and 3.5 were included in the study.

All the cows between 20 and 30 days after calving were examined by ultrasound machine equipped with a 6 MHz linear probe (Sonovet 180 plus Fujifilm Sonosite, Inc., USA) to evaluate the recovery of the post-partum cyclic ovarian activity by the detection of a corpus luteum. The voluntary waiting period for breeding ended at day 60 after calving and it was the moment at which the Ovsynch protocol was initiated (Figure 1). Before the Ovsynch protocol, all the animals were pre-synchronized with two $PGF_{2\alpha}$ treatments 12 days apart starting on Day 36 post-partum. Then, sixty cows during the summer (June, July and August) and 60 cows during the winter (December, January and February) season were fitted with a P4-releasing intravaginal device (PRID-DELTA, containing 1.55 g of P4; CEVA Salute Animale, Agrate Brianza, Italy) plus GnRH (100 µg i.m. Gonadoreline acetate; CystorelineÒ, CEVA Salute Animale, Agrate Brianza, Italy) administration upon PRID insertion. The PRID was left in place for 7 days. On PRID removal, the animals were randomly divided into two subgroups (30 animals each) and given one single administration of $PGF_{2\alpha}$ (8.0 mg Alfaprostol i.m.; Gabbrostim®, CEVA Salute Animale, Agrate Brianza, Italy) or two $PGF_{2\alpha}$ administrations 24 hours apart. Fifty-six hours after progesterone removal, all cows received a second GnRH dose and were artificially inseminated 16–22 hours later with frozen–thawed semen from a bull of known high fertility by the same practitioner.

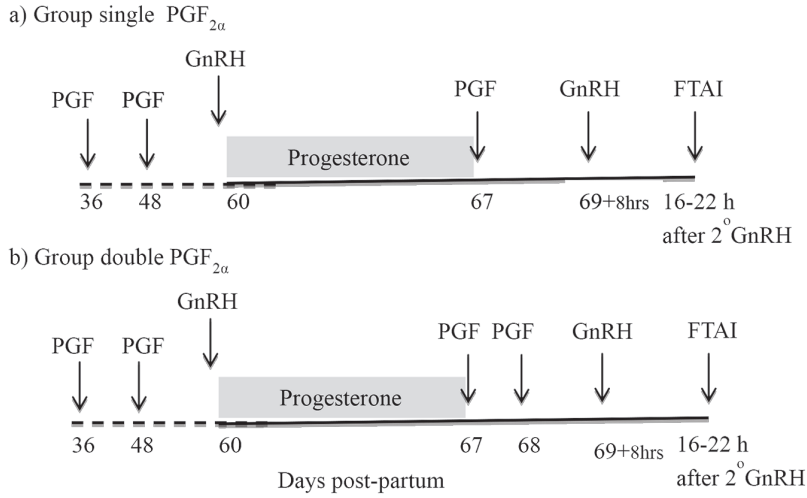

Figure 1. Experimental protocol

More than 80% of cows in summer groups were FTAI during the month of July and the cows in winter group during the month of January.

The cows that returned to estrus after FTAI were re-inseminated. Spontaneous estrus was detected by visual observation several times throughout the day and by pedometers. Pregnancy diagnosis was performed by ultrasound 30 days after FTAI to determine the pregnancy rate (PR) and 60 days after FTAI to determine the cumulative pregnancy rate (cumulative-PR).

During summer season a monthly mean of the maximum temperature >25ºC and/or of the maximum THI > 72 was observed. These temperatures are the thresholds to consider heat stress conditions in the herd (García-Ispierto et al., 2007; Zimbelman et al., 2009).

Statistical analysis

Two cows from $PGF_{2\alpha}$ and one cow from $PGF_{2\alpha}$ group were not included in the analysis on the cumulative-PR because they were removed from the farm for reason not correlated to the study.

Pregnancy rate (PR) was defined as the percentage of cows that became pregnant at FTAI out of the total number of cows in the corresponding group. The cumulative-PR was defined as the percentage of cows that were pregnant within 60 days after FTAI out of the total number of cows in the corresponding group.

Because of the small sample number, possible significant differences of season (summer or winter) between groups were analyzed by the Fisher's exact test. The overall differences in pregnancy rate between treatments (single or double $PGF_{2\alpha}$) were calculated by the chi-square test. $P<0.05$ was considered statistically significant.

Results

Overall pregnancy rate at FTAI was lower during summer compared with winter season (30% *vs* 41.7%; $P<0.05$).

During summer the percentage of pregnant cows that became pregnant at FTAI after treatment with double or single $PGF_{2\alpha}$ was not different (Table 1) while cumulative-PR at day 60 after calving was significantly ($P<0.05$) improved in the group treated with double compared to single $PGF_{2\alpha}$ treatment (42.9% and 69% for $PGF_{2\alpha}$ and $2PGF_{2\alpha}$ cows, respectively) (Table 1). During the winter season no differences were observed in PR and cumulative-PR after single or double $PGF_{2\alpha}$ administration (Table 1).

Table 1. Pregnancy rate (at FTAI) and cumulative pregnancy rate (60 days post-FTAI) during the summer or winter season of cows treated with a single or a double $PGF_{2\alpha}$ administration within a P4-based Ovsynch protocol plus FTAI. Different letters indicate a significant difference ($P<0.05$) within a column (x,y)

	Summer season	Winter season
Pregnancy rate		
$PGF_{2\alpha}$	8/30 (26.7%)	11/30 (36.7%)
$2PGF_{2\alpha}$	10/30 (33.3%)	14/30 (46.7%)
Cumulative pregnancy rate		
$PGF_{2\alpha}$	12/28 (42.9%)x	21/30 (70%)
$2PGF_{2\alpha}$	20/29 (69%)y	23/30 (76.7%)

Discussion

It has been reported that increasing the dose of $PGF_{2\alpha}$ as a single or a split administration enhanced luteolytic response and the odds of pregnancy (Brusveen et al., 2009; Santos et al., 2010; Ribeira et al., 2012; Giordano et al., 2013; Carvalho et al., 2015). In our study pregnancy rate at FTAI during summer and winter season did not differ between cows receiving one versus two $PGF_{2\alpha}$ administrations. During summer an improvement in pregnancy rate was detectable in cumulative pregnancy rate 60 days after TAI in cows receiving two $PGF_{2\alpha}$. These differences between our and previous studies in pregnancy rate at FTAI could be related to several factors. For example, the fact that all cows were cyclic and the Ovsynch protocol started at day 60 after calving, could have masked a possible positive effect of a double $PGF_{2\alpha}$ dose. Furthermore, there are some differences in the FTAI protocols utilized between our and the remaining studies that may have influenced the final results. Finally, we consider only the summer and winter season. Indeed, in a contemporary study to ours (Heidari et al., 2017) the results are very similar to ours with a pregnancy rate at 32 days after TAI that does not differ between cows receiving one versus two $PGF_{2\alpha}$ administrations but an improvement in fertility was detectable at 60 days after TAI in cows receiving two $PGF_{2\alpha}$.

Our observation of a positive effect of double $PGF_{2\alpha}$ administration detected on cumulative-PR could be related to the fact that during summer season heat stress can affect $PGF_{2\alpha}$ release from uterus and/or the luteolytic action of $PGF_{2\alpha}$ (Wolfenson et al., 2000; De Rensis et al., 2017; Motavalli et al., 2017) with the development of luteinized follicles or of persistent dominant follicle (Sirois and Fortune, 1990; Wolfenson et al., 2002) and the full recovery of reproductive activity may be delayed. Therefore, our study supports the previous observations of Dirandeh et al. (2015) in which the administration of a double $PGF_{2\alpha}$ dose favors the full recovery of the hypothalamus-pituitary-ovarian axis during the following estrous cycle.

Conclusion

In this study, the use of a double instead of a single $PGF_{2\alpha}$ dose into a P4-based Ovsynch protocol did not increase the pregnancy rate at FTAI during the summer and winter seasons but during the summer season it improved cumulative pregnancy rate on day 60 after FTAI.

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