Conception rates following an Ovsynch and fixed-time insemination protocol with progesterone inclusion in cyclic dairy cows during the warm and cold seasons

*Giorgio Morini, Padet Tummaruk and Fabio De Rensis**

Abstract

Progesterone plasma levels during the warm period of the year (summer) are more variable and this can induce a reduction in the fertility of dairy cows. Therefore, the aim of this study is to investigate the effect of the combined use of progesterone supplements with an Ovsynch and FTAI protocol on conception rates during the summer in cows. The data were compared with treatments carried out during the cold season (winter). In total, 120 cows underwent oestrus synchronization by using an Ovsynch and FTAI protocol. After this, one group of 60 cows received progesterone supplements and another group of 60 cows did not. In each group, 30 cows were treated during the summer and 30 cows were treated during the winter. All cows were pre-synchronized with $PGF_{2\alpha}$ 25 days and 11 days before starting the Ovsynch protocol. The conception rate at days 30 and 40 post-insemination was not changed by the Ovsynch protocol being supplemented with progesterone. No interaction between progesterone supplementation and the season (winter or summer) was observed (P=0.17). The results of the study indicate that in cyclic cows with a functional *corpus luteum* the supplementation of the Ovsynch-FTAI protocol with progesterone did not improve the conception rate during the warm or cold seasons.

Key words: *progesterone; Ovsynch; warm and cold season; pregnancy rate; dairy cows*

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Introduction

Over the past decade, several protocols have been developed to control ovulation and allow artificial insemination without the need for oestrus detection (for reviews, see Colazo et al., 2014; De Rensis et al., 2015). These protocols, generally named Ovsynch-FTAI protocols, have minimized the limiting effects of the reduced efficiency and accuracy of oestrous detection (Lojkić et al., 2018), and are routinely utilized in the management of reproduction on dairy farms.

However, fertility after the use of the Ovsynch-FTAI protocol is sometimes lower compared to insemination in spontaneous ovulation (Mawhinney et al., 1999; MacMillan et al., 2003; Galvao et al., 2010). One reason for the lack of response to the Ovsynch-TAI protocol is that the emergence of a new follicular wave is synchronized only when the first GnRH administration causes ovulation and the development of a new follicular wave (Martinez et al., 2002). If the first GnRH does not synchronize the follicular wave emergence, ovulation following the second GnRH is poorly synchronized (Martinez et al., 1999), resulting in disappointing pregnancy rates following FTAI (Martinez et al., 2002). To avoid this problem, the cow can be pre-synchronized with a double administration of $PGF_{2\alpha}$ before the Ovsynch protocol. Another problem is due to the reduced exposure of the follicles to the correct level of progesterone before ovulation (Špoljarić et al., 2017). Blood concentrations of progesterone during the luteal phase before insemination are associated positively with conception rates (Fonseca et al., 1983; Folman et al., 1990; Rosenberg et al., 1990 a,b). In the absence of physiological levels of progesterone, spontaneous or GnRH-induced ovulation results in a greater risk of incomplete development of the corpus luteum. It follows earlier luteolysis at around day 10 of the oestrous cycle and the embryo cannot produce sufficient IFN-τ to block the luteolytic cascade (Rhodes et al., 2003; Inskeep, 2004) and there is pregnancy is loss (Špoljarić et al., 2017).

Therefore, several attempts have been made to optimize progesterone plasma levels during the Ovsynch plus FTAI protocol. One of them consists of the supplementation of the synchronization protocol in cows with progesterone. The results of these studies indicate that supplementation with progesterone is able to prevent early ovulation and increase the proportion of functional corpus luteum (CL) at the moment of $PGF_{2\alpha}$ administration (Lamb et al., 2001; Martinez et al., 2002; El-Zarkouny et al., 2004; Melendez et al., 2006; Bisinotto et al., 2013; Colazo et al., 2013), including in buffalo cows (De Rensis et al., 2005). However, these effects are not always repeatable, and in some studies supplementation of the Ovsynch protocol with progesterone gives a pregnancy rate similar to that in non-treated animals (Moreira et al., 2001; El-Zarkouny et al., 2004; Galvão et al., 2004; Sterry et al., 2007).

There are several reasons for these differences, but one of them can be the season in which the treatment takes place. During the summer, because of heat stress, progesterone plasma levels may not be optimal for follicular development, and fertility can be compromised (for reviews, see De Rensis at al., 2003; De Rensis et al., 2015). Since the inclusion of progesterone in the Ovsynch protocol increases the pregnancy rate in cows with low circulating progesterone concentrations (Stevenson et al., 2006), the aim of this study is to investigate if the supplementation of the Ovsynch protocol with progesterone can improve the conception rate during the summer season, a period of the year during which progesterone plasma levels are usually low.

Materials and methods

This study was carried out on dairy cow farms between December 2015 and August 2016. The animals were housed indoors, milked twice a day, and fed with a total mixed ration ad libitum to meet the nutritional requirements of lactating dairy cows. The mean milk yield at the start of the treatment was 43.2±1.1 kg/day. Only cows characterized by good reproductive health and without a history of reproductive illness were included in the study. Only cows with a parity of >1 or < 5 were included in the study. In addition, a body condition scoring system from 1 = very thin to 5 = very fat was used for each animal at the time of the first GnRH administration, and only cows with scores between 3 and 3.5 were included in the study. The voluntary waiting period for breeding was 60 days postpartum. All cows were examined between 20 and 30 days after calving by ultrasound to evaluate the recovery of postpartum cyclic ovarian activity through the detection of a corpus luteum.

In total, 120 cows were assigned randomly to two groups: Ovsynch-FTAI supplemented with progesterone (n=60) or Ovsynch-FTAI not supplemented with progesterone (n=60). In each group, 30 cows were treated during the warm season (May to August) and 30 cows during the cold season (December to March).

The experimental model is presented in Figure 1. All cows receiving the progesterone supplement were fitted with a progesterone-releasing intravaginal device (PRID-Delta, containing 1.55 g of P4; CEVA Salute Animale, Agrate Brianza (MB), Italy) and treated with GnRH (100 µg gonadorelin acetate; Cystoreline®, CEVA Salute Animale, Agrate Brianza (MB), Italy) on the day of the progesterone device insertion. The PRID was left in place for 7 days, and the animals were given $PGF_{2\alpha}$ (25 mg dinoprost i.m.; Enzaprost, CEVA Salud Animal) on its removal. Forty-eight hours later, the cows received a second GnRH dose. Cows in the non-progesterone-supplemented group were treated in the same way as the previous group but without progesterone administration. Cows in both groups were FTAI 16-22 hours after the second GnRH administration with frozen-thawed semen from a bull of known high fertility. Artificial insemination was performed by the veterinary surgeon supervising the herd. All cows were pre-synchronized with an administration of $PGF_{2\alpha}$ 25 days and 11 days before the start of the Ovsynch protocol. A pregnancy diagnosis was performed by ultrasound between days 30 and 40 post-insemination.

Statistical analysis

In the geographical area in the present study, there are only two clearly distinguishable meteorological seasons, which are summer and winter, i.e. warm (May-August) and cool (December-March) periods, respectively. During the warm period, the pregnancy rate is usually reduced (De Rensis et al., 2002). Treatment dates were thus used to analyze the effect of the treatment period (warm *vs.* cool seasons) on the conception rate. The conception rate was defined as the percentage of cows that became pregnant with fixed-timed insemination out of the total number of cows in each group. The differences in the conception rate of the first AI were analyzed using Fisher's exact test. P<0.05 was considered statistically significant.

Results

Regardless of progesterone supplementation, the overall conception rate was reduced during the warm season compared to the cold one (30% *vs.* 41% for the warm and cold seasons, respectively P<0.05; Table 1). Progesterone sup-

plementation in the Ovsynch-FTAI (Figure 2) did not improve the conception rate during the warm (P=0.13) or cold season (P=0.17).

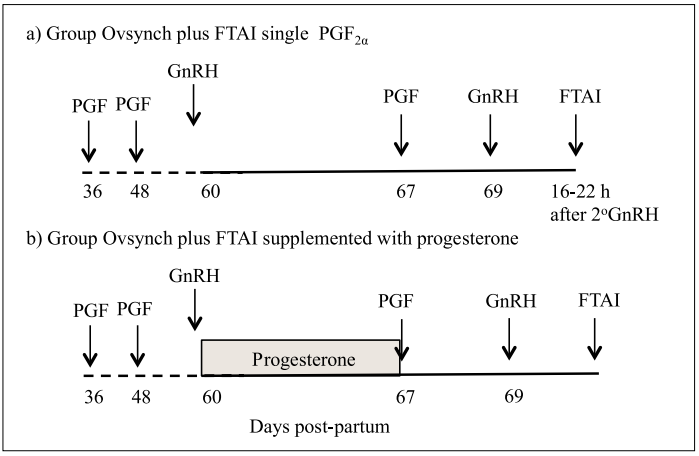

Figure 1. Experimental protocol

Table 1. Conception rate of cows after insemination at fixed times during the warm or cold season. [a] and [b] indicate the difference within a row (P=0.05).

Conception rate			
	Warm season	Cold season	Total
Ovsynch	15/60 (25%)	21/60 (35%)	36/120 (30%)
Ovsynch plus progesterone	20/60 (33%)	29/60 (48%)	47/120 (39%)
Total	35/120 (30%)[a]	50/120 (41%)[b]	

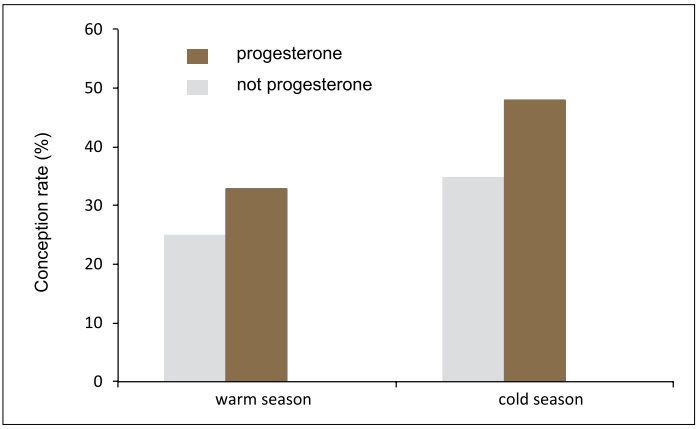

Figure 2. Conception rate of cows after insemination at fixed times (Ovsynch-FTAI) supplemented or not supplemented with progesterone during the warm or cold season.

Discussion

This study confirms previous data (for a review, see De Rensis et al., 2003) that during the warm season the overall conception rate is reduced compared to the cold season (30% *vs*. 41% for the warm and cold season, respectively; $P>0.05$). Concerning the efficacy of supplementation with progesterone on the conception rate, the results have not been consistent across studies. In dairy cows (El-Zarkouny et al., 2004; Mendelez et al., 2006; Galvao et al., 2010), non-cyclic cows (Lamb et al., 2001), anestrus cows (El-Zarkouny et al., 2004), beef heifers (Martinez et al., 2002a), Japanese black beef cows (Kawate et al., 2004), and anestrus buffalo cows (De Rensis et al., 2005) the likelihood of conception was greater using the Ovsynch protocol with progesterone supplementation compared to the standard Ovsynch protocol. However, other trials report that there is no improvement in response in either heifers or cows (Galvão et al., 2004; Mendonca et al., 2012). In the study of Stevenson et al., (2006), supplementation of Ovsynch plus FTAI with progesterone induced only a tendency towards a higher conception rate in treated animals (with a 9% difference in favor of treated animals), but this difference was not significant.

These differences between studies could be related to the type of animal utilized (dairy or beef cows, heifers, primiparous or pluriparous cows), or the species (bovine or buffalo) or reproductive condition of the animal at the moment of treatment (anestrus, non-cyclic or cyclic cows). For example, progesterone supplementation only improved the pregnancy rate in acyclic cows (El-Zarkouny et al., 2004; Mendelez et al., 2006; Bisinotto et al., 2013). Bisinotto and Santos (2011) report that progesterone has a positive effect on pregnancy rates in cows in dioestrus, but this effect is not detectable in acyclic dairy cows. In our study, only cows that were cyclic and had a functional CL at the beginning of the treatment were utilized, and this could be the reason for the lack of an effect.

Another factor is that in our study the animals were pre-synchronized with $PGF_{2\alpha}$. Colazo et al. (2013) report that progesterone supplementation in the Ovsynch protocol increases pregnancies only in cows that have been not pre-synchronized with double $PGF_{2\alpha}$.

In conclusion, the effect of the inclusion of progesterone in a synchronization program can have a positive effect on fertility, even if this effect is not always repeatable, because several factors can affect the efficacy of the treatment, e.g. acyclic or cyclic animals, pre-synchronization before treatment, the presence or absence of a corpus luteum at the beginning of the treatment (for reviews, see Bisinotto et al., 2011; Colazo et al., 2014; Bisinotto et al., 2015). However, no information is available on the effect of the season (warm or cold) in which the treatment occurs. Our study indicates that during the warm and cold season the supplementation of the Ovsynch-FTAI protocol with progesterone does not improve the conception rate in cyclic cows with a functional *corpus luteum*.

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Postotak koncepcije nakon primjene Ovsynch protkola s točno određenim vremenom umjetnog osjemenjivanja s aplikacijom egzogenog progesterona u cikličnih mliječnih krava tijekom toplog i hladnog razdoblja godine

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Koncentracije progesterona u plazmi u mliječnih krava tijekom toplog razdoblja godine (ljeto) više variraju što može prouzročiti njihovu smanjenu plodnost. Stoga je cilj ovoga rada bio istražiti učinak aplikacije egzogenog progesterona u ovsynch protokolu u kombinaciji s točno određenim vremenom umjetnog osjemenjivanja (TOVO) na postotak koncepcije u mliječnih krava tijekom ljetnog razdoblja. Podatci su bili uspoređeni s istom kombinacijom protokola tijekom hladnog razdoblja godine (zima). Ukupno je u 120 krava sinkroniziran estrus klasičnim ovsynch i TOVO protokolom. Nakon toga, u 60 krava je apliciran egzogeni progesteron, dok u ostalih 60 krava nije apliciran spomenuti pripravak. U svakoj je skupini 30 krava tretirano tijekom ljeta i 30 krava tijekom zimskog razdoblja. Sve su krave bile podvrgnute postupku presinkronizacije s $PGF_{2\alpha}$ 25 dana i 11 dana prije početka ovsynch protokola. Aplikacija egzogenog progesterona u kombinaciji s ovsynch protokolom 30. i 40. dana nakon umjetnog osjemenjivanja nije utjecala na postotak koncepcije. Nije ustavljena značajna povezanost između dodatka egzogenog progesterona i godišnjeg doba (zima ili ljeto) (P=0.17). Rezultati ovog istraživanja pokazali su da aplikacijom egzogenog progesterona u kombinaciji s ovsynch-TOVUO protokolom nije poboljšao postotak koncepcije u krava s cikličnom aktivnosti jajnika (funkcionalno žuto tijelo) tijekom toplog ili hladnog dijela godine.

Ključne riječi: *progesteron, Ovsynch, toplo i hladno razdoblje godine, postotak gravidnosti, mliječne krave*