

## Synchronization of the ovulation (OvSynch) in beef cattle

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### Abstract

Todorova, T., Todorova, Ts. & Todorov, I. (2021). Synchronization of the ovulation (OvSynch) in the beef cattle. *Bulg. J. Agric. Sci.*, 27 (5), 996–1001

The OvSynch protocol itself has not been successfully used to synchronize beef cows in postpartum anestrus. Recently, various modifications to the ovulation synchronization methods have been developed. One of these is the so-called PreSynch. Cows that are unresponsive to the first injection of GnRH may come to estrus early (36 to 48 hours before prostaglandin injection) or have follicles that are not “synchronized” at the time of prostaglandin injection. Pre-synchronization, as the name indicates, is a protocol that “pre-synchronizes” cows to the early stages of the cycle for optimal response to GnRH treatment, and thus improves ovulation synchronization results (OvSynch).

The experimental cows were divided into three groups of 12 animals each. One group was treated per week. The cows were artificially inseminated. After 35 days, if the cow did not come to spontaneous oestrus, a sonographic examination was performed to determine pregnancy. A total of 36 animals were treated. Out of 36 cows, 17 (47.2%) were pregnant after one treatment and the pregnancies are ongoing.

Nineteen cows, unfertilized after the first synchronization, were exposed 45 days later for another treatment called modified OvSynch. The modified OvSynch includes presynchronization before OvSynch (1 or 2 injections of PGF<sub>2α</sub> given 14 days apart, with the first injection of OvSynch given 12 to 14 days after the second injection of PGF<sub>2α</sub> or injections of PGF<sub>2α</sub> and GnRH every 7 days. Out of nineteen, 11 (57.9%) cows were pregnant 35 days after insemination.

*Keywords:* OvSynch; PreSynch; cattle; beef

### Introduction

The intensification of livestock breeding and productivity gains has been accompanied by increasing reproductive problems. That is leading us to the objectives for estrus management in a beef herd. The benefits are a compact breeding season followed by a short, planned calving season – induction of estrus and insemination of the maximum number of cows within an acceptable period postpartum, increased use of AI- to improve the genetic merit of herds and avoid the possibility of inbreeding. In practice, the terms “estrus synchronization” and “ovulation synchronization” are often confused. For estrus synchronization (e.g. with PGF<sub>2α</sub>), cows aim to reach estrus within a predetermined period of time

(e.g. 48 – 72 hours after the second injection of PGF<sub>2α</sub>). The purpose of ovulation synchronization is to induce ovulation over a defined period of time, which eliminates the need for monitoring estrus as opposed to estrus synchronization.

OvSynch and combinations of gestagens with OvSynch protocols are recommended for beef cows reared on pasture because of the high incidence of postpartum anestrus. On the other hand, prostaglandin regimens are cheaper and easier to administer on the field.

#### Fundamentals of the OvSynch system

For successful insemination without first detecting the estrus, Pursley et al. (1995; 1997a) develop a method for precise ovulation synchronization (OvSynch-Programm).

The original program involves three hormone injections at a given time.

The first injection of GnRH that can be done at any time of the cycle, according to Pursley et al. (1995) leads to ovulation or luteinization of the dominant follicle in most cows. A new wave of follicles is induced in all cows (Twagiramungu et al., 1995; Pursley et al., 1995), of which seven days later a follicle becomes dominant (Wolfenson et al., 1994).

The luteolysis of the cyclic yellow body, followed by ovulation, was induced 7 days later by the administration of PGF<sub>2α</sub>. Without further treatment, the interval between PGF<sub>2α</sub> injection and ovulation is highly variable in order to produce acceptable insemination results with single insemination (Stevenson et al., 1996).

According to Hegemann (1998) ovulation after GnRH-PGF<sub>2α</sub> treatment varies between two and seven days. To synchronize ovulation within a narrower range, 48 hours after PGF<sub>2α</sub> injection, a second injection of GnRH was administered.

Experiments of Pursley et al. (1995) shows, that ovulation was observed in all cows within 32 hours. Animals can be inseminated at a specific time without the need for estrus detection (Small et al., 2009).

According to De Jarnette et al. (2001), in about 20% of cows treated with the OvSynch system, early estrus (up to 48 h after PGF<sub>2α</sub> injection) is observed and it is concluded that if estrus detection is applied during this period, insemination results are improved.

Currently, the timed AI protocols in beef and dairy cattle are well established, in which pregnancy rates between 30 and 65% are observed (Baruselli et al., 2017). However, the response to the timed AI protocol based on estrogen and P4 is approximately 80% in *Bos indicus* lactating beef cows (Sales et al., 2012) and approximately 85% in dairy cows in GnRH-based protocols and PGF<sub>2α</sub> (Silva et al., 2018).

The OvSynch protocol itself has not been successfully used to synchronize beef cows in postpartum anestrus, with pregnancy rates in anestrus cows significantly lower (14.9%) than in cycling cows (46.3%; Fernandes et al., 2001). Anestrus is a common condition in extensively managed suckled beef herds (Bó et al., 2007; Baruselli et al., 2004) and the use of OvSynch in beef herds has resulted in pregnancy rates as low as 15%, compared with 53% in cows treated with a progestin

device and oestradiol (Baruselli et al., 2004). Other trials have confirmed that OvSynch protocol yields a low conception rate in beef herds showing a high percentage of anestrus animals (Barros et al., 2000; Diskin et al., 2002). The effects of pre-synchronization with PGF before a CoSynch protocol on estrus synchrony, corpus luteum, preovulatory follicle diameters, and programmed AI has also been studied on beef heifers (Colazo et al., 2004). Pre-synchronization reduced the proportion of heifers in estrus before FTAI, suggesting that this may be useful in the successful application of GnRH based protocols in beef heifers.

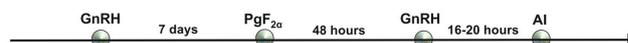
## Materials and Methods

In the period between July and December 2019, we applied the method to Limousin cows at the Experimental station “Zlatusha” of the Institute of Animal Science, Kostinbrod. Animals are kept free on pasture and without the presence of a bull. They are harvested in the barn only in the evening and this makes it difficult to detect oestrus promptly. That’s why we implemented the OvSynch scheme. We applied the scheme as described by Pursley et al. (1995; 1997a). We used the GnRH drug Fertagyl, (Intervet) and the prostaglandin Synchromate, (Bremerpharma). Frozen semen from one bull was used in all experiments to avoid the bull’s influence on the results.

## Results and Discussion

The cows were divided into three groups of 12 animals each. One group was treated per week. The cows were artificially inseminated. After 35 days, if the cow did not come to spontaneous oestrus, a sonographic examination was performed to determine pregnancy. Figure 1 shows the schedule for hormonal injection by the hour, and Figure 2 shows the chart for the days of the week.

The percentage of results is shown in Table 1.



**Fig. 1. Time points for hormone injection and artificial insemination in the OvSynch program (according to Pursley et al. 1995, modified by Wiltbank, 1998b)**

Monday 10.00 GnRH	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Monday 10.00 PGF <sub>2α</sub>	Tuesday	Wednesday 10.00 GnRH	Thursday 10.00 AI	Friday	Saturday	Sunday

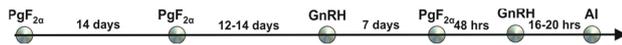
**Fig. 2. Exemplary diagram of the OvSynch system implementation on workdays of a week**

**Table 1. Results of the implementation of an OvSynch method for Limousin cows**

	I group	II group	III group	Total
Number	12	12	12	36
Pregnant	5 (41.6%)	8 (66.6%)	4 (33.3%)	17 (47.2%)

A total of 36 animals were treated. Out of the 36 cows, 17 (47.2%) were pregnant after one treatment and nine calves have been calved so far. Nineteen cows, unfertilized after the first synchronization, were subjected 60 days later to another treatment called modified OvSynch or PreSynch.

The modified OvSynch includes presynchronization of OvSynch (1 or 2 injections of PGF<sub>2α</sub> given 14 days apart, with the first injection of OvSynch given 12 to 14 days after the second injection of PGF<sub>2α</sub> or injections of PGF<sub>2α</sub> and GnRH every 7 days. It is assumed, that PreSynch improves first service conception rate compared to OvSynch. The PreSynch scheme is shown in Figure 3.



**Fig. 3. A daily injection schedule of a presynchronization protocol with 2 PGF injections (14 days apart) and initiation of OvSynch 12 days after the second PGF. Out of nineteen, 11 cows were pregnant 35 days after insemination**

After applying the method of presynchronization, out of 19 treated cows, 11 (57.9%) became pregnant. At the time of writing this article, the pregnancies were still ongoing. The results show that the tendency is to improve the results with the application of the modified OvSynch system. In our opinion, this is due to a better synchronization of the estrus following directly the previous treatment. Mild hormonal stimulation may also enhance the concept. Of course, the small number of animals treated does not allow us to draw any firm conclusions, but the tendency is obvious.

The application of programmed insemination requires accurate determination of the time of ovulation since no estrus detection is used. The second injection of GnRH stimulates ovulation by initiating a preovulatory peak of LH. In one study involving 675 animals (Peters & Pursley, 2003); the optimal time for the second injection of GnRH had to be determined.

In one group, the classical OvSynch program was administered, while in the other group, the second GnRH injection was administered concomitantly with PGF<sub>2α</sub>, 7 days after the start of treatment (modified OvSynch). In each case, insemination was performed 16 hours after the last injection of

GnRH. Fertility in the classic OvSynch system was significantly better than the modified system (31.3% vs. 14.7%).

In subsequent experiments, the authors treated 457 cows with increasing intervals between PGF<sub>2α</sub> injection and the second injection of GnRH, as follows: GnRH II 0, 12, 24, or 36 hours after PGF<sub>2α</sub> injection. The authors observed a linear increase in the degree of fertility as the interval between the injection of prostaglandin and the second injection of GnRH increased. The highest fertility rate was observed in the group in which the second GnRH injection was made 36 hours after PGF<sub>2α</sub> administration.

In conclusion, Peters & Pursley (2003) found that the second injection of GnRH should be given at least 24 hours after the administration of PGF<sub>2α</sub>.

Other authors, such as Mawhinney et al. (1999) administered a second injection of GnRH, 52-56 hours after prostaglandin. Stevenson et al. (1999) in experiments with 308 Holstein cows compared “OvSynch-33” and “OvSynch-48” with different prostaglandin programs. They found that the results were significantly better at the 48-hour interval between the injection of prostaglandin and the second injection of GnRH.

Recently, various modifications to the ovulation synchronization methods have been developed. One of these is the so-called PreSynch. Pre-synchronization, as the name indicates, is a protocol that “pre-synchronizes” cows to the early stages of the cycle for optimal response to GnRH treatment, and thus improves ovulation synchronization results (OvSynch) (Morera et al., 2000; El-Zarkouny et al., 2004; Chebel et al., 2013; Wiltbank & Pursley, 2014; Stangaferro et al., 2019).

Although the first injection of GnRH stimulates 90% of the follicles if the cow is between 5 and 12 days of the cycle, in cows that are between 13 and 17 days of the cycle only 50% of the follicles respond to treatment (Geary et al., 2000).

Cows that are unresponsive to the first injection of GnRH may come to oestrus early (36 to 48 hours before prostaglandin injection) or have follicles that are not “synchronized” at the time of prostaglandin injection.

The main protocols for presynchronization of cows before the implementation of the OvSynch protocol are based either on a PGF<sub>2α</sub> or the addition of another OvSynch protocol (i.e. Double OvSynch). Other presynchronization protocols have been developed based on the use of GnRH (Bisino et al., 2014; Fricke et al., 2014; Borchardt et al., 2017).

Presynchronization with PGF<sub>2α</sub> consists of the administration of 2 injections of a luteolytic dose of PGF<sub>2α</sub> at 14 days interval. This treatment synchronizes estrus in the majority of cows between 2 to 4 days after the second injection of PGF<sub>2α</sub>. The OvSynch protocol is then initiated 10, 11 or

14 days after the second PGF<sub>2α</sub> injection, which would be the ideal stage of the cycle (i.e. 5 to 12 days post-ovulation). The choice of the interval between the second injection of PGF<sub>2α</sub> and initiation of the OvSynch protocol is generally dictated by the herd management (preferred day of the week of injections and timed AI). Despite the complicated nature of the program, the majority of field trials have shown an increase in pregnancy rate by 6 to 12 percentage points using the presynchronization – OvSynch compared to OvSynch alone (Chebel & Santos, 2010; Chebel et al., 2013; Wiltbank & Pursley, 2014; Stangaferro et al., 2019; Karakaya-Bilen et al., 2019). PreSynch-11 was found to be superior to the PreSynch-14 (Galvao et al., 2007).

In two studies with dairy cows (Sartori et al., 2000; Yu et al., 2018) pregnancy rate following timed AI was higher in cows treated with the “PreSynch-OvSynch” protocol than in those treated with OvSynch alone (49% vs 37%; 47% vs 38%; *P*, 0.01).

The effect of varying the interval between the second PGF of PreSynch and initiation of OvSynch on pregnancy rate in lactating dairy cows has been examined. Although an interval of 12 d between the second PGF and the first GnRH improved pregnancy rate by 10% to 12% (30.31), the dairy industry adopted an interval of 14 d so that all treatments are done on the same days of the week. Recently, Galvão et al. (2007) reported that a reduction in the interval between PreSynch and the first GnRH from 14 to 11 days increased the percentage of animals ovulating to the first GnRH (61% versus 45%; *P*, 0.01) and consequently pregnancy rate (41% versus 34%; *P*, 0.05). However, the increase in the proportion of animals ovulating to the first GnRH was observed only in those animals that were cycling.

Portaluppi and Stevenson (2005) determine the rate of fertilization after a change in injection time of the second GnRH dose, insemination time, or both in the combined PreSynch + OvSynch protocol. They conclude that insemination 48 or 72 hours after PGF<sub>2α</sub> when GnRH is injected 48 hours after prostaglandin results in lower results than the GnRH injection 72 hours after PGF<sub>2α</sub> in cows whose estrous cycles were synchronized before application of OvSynch protocol.

Silva et al. (2018) examined the effect of administration of PGF<sub>2α</sub> 12 days before initiation of treatment for presynchronization and preparation for OvSynch protocol. They found no effect on the ovulatory response of the first GnRH injection. The authors found a link between PGF<sub>2α</sub> injection 12 days apart before resynchronization and an increase in pregnancy rates 66 days after synchronized insemination in cows with a serum concentration of P4 > 1.0 ng / ml. They also reported decreased early embryonic mortality between 31 and 66 days after insemination (Mendonca et al., 2019).

## Conclusion

The majority of studies have been performed on dairy cows but based on the data by the authors cited and our results, we believe, that the method of presynchronization (PreSynch) and ovulation synchronization (OvSynch) may be recommended for use in industrial beef cattle breeding. We applied it to beef cows, raised on pasture, and artificially inseminated. The results are comparable to those of dairy cows. The problem is not biological but rather organizational, especially if it is combined with artificial insemination. The method can be applied year-round without much difference in results.

## Acknowledgments

The research that led to these results was funded under the National Scientific Program “Reproductive Biotechnology in Animal Breeding in Bulgaria (NNP REPROBIOTECH)” № 0406-105 of the Ministry of Education and Science of the Republic of Bulgaria.

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*Received: April 27, 2020; Accepted: October 21, 2020; Published: October, 2021*