

PRELIMINARY RESULTS REGARDING ESTRUS SYNCHRONIZATION IN POSTPARTUM DAIRY COWS WITH GnRH ANALOGUE, PRID – INTRAVAGINAL DEVICE AND PGF2 α , FOLLOWED BY ARTIFICIAL INSEMINATION AT ESTRUS DETECTION

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Abstract

This research was carried out within a dairy farm from North Eastern of Romania, on an experimental group (group E, n = 43) and control group (group C, n = 50 cows) from Romanian Black Spotted Breed (Friesian origin).

The aim of study was to establish the efficiency of hormonal treatments applied to dairy cattle (group E) for estrus synchronization, consisting in: GnRH analogue (2.5ml Receptal) on day 0 (day of beginning treatment) and the insertion of an intravaginal device with progesterone (PRID Delta) for 7 days, prostaglandin F2 α (2 ml Estrumate) on day 6 and artificial insemination at estrus detected. The treatments applied to the experimental group were performed between November 2017 and July 2018, the mean calving to treatment interval for experimental group being 49 days.

The obtained results were represented by 90.7% inseminated cows from group E (n = 39); the treatment to first service interval (mean \pm standard deviation) was 22.77 \pm 3.95 days; treatment to conception interval (mean \pm standard deviation) was 44 \pm 7.99 days. We found in group E compared with group C the mean calving to first service interval with 18.53 days lower (79.6 \pm 4.2 days vs. 98.13 \pm 7.12 days); mean value of calving to conception interval with 43.03 day lower (98.9 \pm 8.2 days vs. 141.16 \pm 9.72 days) and the conception rate at first service with 8.8% higher (30.8% vs. 22.0%).

In conclusion, the hormonal treatment protocol in dairy cattle estrus synchronization consisting a GnRH analogue (Receptal 2.5ml) in day 0, intravaginal progesterone device (PRID) for 7 days and PGF2 α on day 6, can improve calving to first service interval, calving to conception interval and conception rate at first service being recommend it like a good alternative for improving reproduction.

Key words: dairy cattle, estrus synchronization, GnRH, PRID, Prostaglandin F2 α

INTRODUCTION

Reproductive efficiency is the most important factor which influences the economy of a dairy or meat farm. Therefore, minimizing the reproductive losses must be a major priority for cattle breeders.

The scientific progress made in the last 40-50 years in this direction has enabled a fast development of used technologies in order to increase the reproductive efficiency and genetic potential in cattle herds. Among reproductive biotechnologies, estrus

synchronization and artificial insemination are the most used and effective technologies for genetic improvement in cattle herds. Development of new and improved methods to synchronize estrus and ovulation occurred with the understanding of physiological and hormonal mechanisms that control estrous cycle. Research conducted in this field of study has shown that the basic physiological principles which underline how these hormonal products work are controlling follicular maturation and inducing corpus luteum regression.

Generally, estrus synchronization protocols development involves the following approaches: 1. temporary blocking of estrus

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cycle by ovulation inhibition, followed by spontaneous regression of corpus luteum (long-term progestin treatment); 2. induction of corpus luteum regression (PGF 2α treatment); 3. a combination of approaches 1 and 2. Most of the protocols used today can be classified in the third approach. The first approach requires long-term progesterone treatment (14 days) and is effective for estrus synchronization, but fertility in synchronized estrus is frequently reduced due to persistent follicles. The second approach results in good fertility, but animals that are in the first 5-6 days of the estrus cycle will not respond to PGF 2α injection, resulting in a low sync response. The third approach allows efficient estrus synchronization regardless the estrus cycle stage, without compromising the fertility. This is suitable when a GnRH injection is administered at the beginning of progestin treatment in order to ovulate a dominant follicle and to synchronize a new follicular wave. The success of estrus synchronization protocols depends on both mechanisms control, both the dominant follicle development, as well as luteal regression. During the estrus cycle, when a corpus luteum is present and the circulating concentrations of progesterone are high, estrus and ovulation are inhibited; however, when corpus luteum regresses and progesterone concentrations decrease, the circulating concentrations of estradiol increase and the animal returns in estrus. The progestins simulate the effects of progesterone produced by corpus luteum and inhibit ovulation and oestrus manifestation. After progestine elimination, progesterone concentrations decrease and estrus and ovulation will occur. Synthetic progesterone treatment towards the end of luteal phase results in a prolongation of follicle domination, with a significant decrease in pregnancy rate in relation to dominance duration from 4 to 8 days, further reducing if the dominance length exceeds 10 days (Mihm M. A. et al., 1994, Smith M. F. et al., 2006).

The decrease in fertility after persistent follicle formation and ovulation may result from changes in uterine environment due to increased secretion of estradiol (Butcher and Pope, 1979 cited by Smith M.F. et al., 2006) and (or) the early resumption of meiosis due

to prolonged exposure to high frequency of LH pulse (Mattheij et al., 1994, cited by Smith M.F. et al. 2006).

In addition to the right application of estrus synchronization protocol, various other environmental factors and the general farm management may influence the effectiveness of applied treatment, so the reported results by various authors were variable (1,2,3,6,7,9).

The aim of this study was to determine the effectiveness of an estrus synchronization protocol in dairy cattle consisting in administration of a GnRH analog (Receptal, 2.5ml, intramuscularly) on day 0, with the insertion of an intravaginal progesterone device (PRID Delta) for 7 days and prostaglandin PGF 2α on day 6, followed by artificial insemination on estrus detection.

MATERIAL AND METHOD

The study was conducted within a dairy cattle farm, form North Eastern of Romania, on an experimental group (Group E) consisting of 43 cows (Romanian Black Spotted Breed), compared to a control group (group C), consisting of 50 cows (Romanian Black Spotted Breed). Cows were maintained in loose housing system in shelters with a capacity of 200 animals (Fig. 1).



Fig. 1 Housing system in experimental group cows

Estrus synchronization treatments in cows from group E were performed between November 2017 and July 2018, within an average of 49 days from calving (variations between 40-64 days).

At the beginning of treatment, the cows were examined gynecologically by transrectal ultrasonography in order to identify ovarian structures and to establish the stage of estrus cycle.

The therapeutic protocol consisted in: administration of a 2.5 ml dose of GnRH (Receptal = 10 µg Buserelin, MSD Animal Health Netherlands, 4 µg/ml intramuscular) on

day 0 (regardless of estrus cycle) at the time of insertion intravaginal progesterone device (PRID Delta, 1.55g progesterone, Ceva Sante Animale France), a dose of PGF2α (2ml Estrumate = 500 mcg Cloprostenol, Intervet International BV, Netherlands) on day 6; intravaginal progesterone device removal (Delta Pride) on day 7 and artificial insemination on estrus detection (fig. 2).

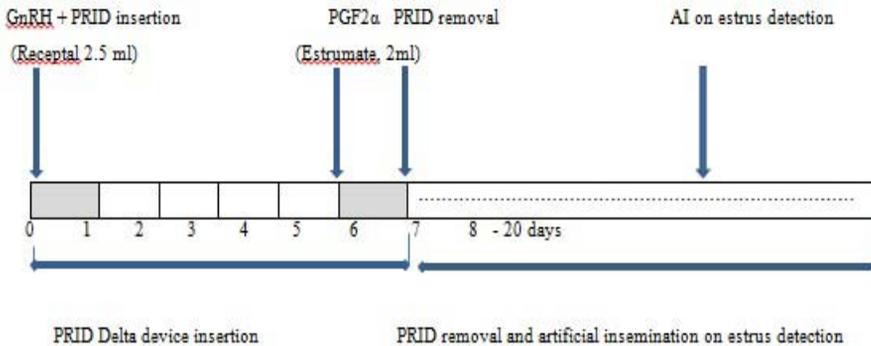


Fig. 2 Therapeutic protocol for estrus synchronization in cows based on GnRH on day 0+ PRID 7 days and PGF2 α on day 6 followed by insemination on estrus detection

Treatments effectiveness applied to cows within the experimental group was determined by assessing the following reproductive parameters: average intervals between treatment - first insemination, treatment - conception, calving - first insemination, calving - conception, pregnancy index (number of insemination / pregnancy), conception rates/ total inseminations number, as well as after 1st, 2nd, and 3rd artificial inseminations (AI).

RESULTS

After intravaginal progesterone device (PRID) removal, the cows were artificially inseminated after estrus detection in 90.7% of cases (39 out of 43 cows). Mean intervals from treatment to first insemination were 22.77 ± 3.95 days and from treatment to fecund artificial insemination (conception) registered a mean of 44 ± 7.99 days.

Table 1 comparatively emphasizes the reproductive indices obtained for cows from the experimental group E (with estrus synchronization treatment) and for cows from control group C (without treatments).

Thus, this results highlight that cows from group E have registered lower values of mean interval from calving to first insemination by 18.53 days (79.6 ± 4.2 days vs. 98.13 ± 7.12 days) and values of mean interval from calving to fecund artificial insemination (conception) by 43.03 days (98.9 ± 8.2 days versus 141.16 ± 9.72 days).

Table 2 shows conception rates obtained for cows from the experimental group E (with estrus synchronization treatment) and for cows from the control group C (without treatments).

Analyzing the results, we found that cows from experimental group presented conception rates at the first insemination higher by 8.8% compared to group C (30.8% vs. 22.0%), conception rates values after the first two inseminations higher by 4.6% (46.6% vs. 38%) and similar pregnancy index values (insemination number/pregnancy) of 2.0 ± 0.28 vs. 2.24 ± 0.28

These are preliminary results, registering thus, recent inseminated cows (RI) and undiagnosed cows between 23.25% (group E) and 24% (group C).

Table 1 Preliminary results regarding insemination and conception intervals after estrus synchronization treatments with GnRH on day 0 + PRID Delta for 7 days and PGF2 α on day 6 (group E, n = 43 cows) compared to cows from control group without treatments (group C, n = 50 cows)

| Specification | Statistical parameters | Animals | | Differences \pm GroupE/ Group C |
|---|------------------------|------------------|-------------------|-----------------------------------|
| | | Grup E | Grup C | |
| Calving - Treatment Interval | Mean \pm | 49.02 \pm 1.36 | | |
| | Min | 34 | | |
| | Max | 64 | | |
| | V% | 18.16 | | |
| Treatment – First artificial insemination (AI) Interval | Mean \pm | 22.77 \pm 3.95 | | |
| | Min | 1 | | |
| | Max | 81 | | |
| | V% | 102.7 | | |
| Treatment - Conception Interval | Mean \pm | 44 \pm 7.99 | | |
| | Min | 1 | | |
| | Max | 126 | | |
| | V% | 85.2 | | |
| Calving - First artificial insemination (AI) Interval | Mean \pm | 79.6 \pm 4.2 | 98.13 \pm 7.12 | -18.53 |
| | Min | 43 | 34 | |
| | Max | 142 | 258 | |
| | V% | 31.2 | 59.44 | |
| Calving - Conception Interval | Mean \pm | 98.9 \pm 8.2 | 141.16 \pm 9.72 | -42.26 |
| | Min | 43 | 35 | |
| | Max | 168 | 334 | |
| | V% | 38.8 | 48,18 | |

 Table 2 Preliminary results regarding conception rates in cows after estrus synchronization treatments with GnRH on day 0 + PRID Delta for 7 days and PGF2 α on day 6 (group E, n = 43 cows) compared to cows from control group without treatments (group C, n = 50 cows)

| Specification | UM | Animals | | Differences \pm GroupE/ Group C |
|--|------------|-----------------|-----------------|-----------------------------------|
| | | Group E | Group C | |
| Cows/Group | No. | 43 | 50 | |
| Cows inseminated after treatments | No. | 39 | | |
| | % | 90.7 | | |
| Total pregnant cows, of which | No. | 24 | 29 | |
| | % | 61.54 | 58.00 | + 3.54 |
| 1 st AI | No. | 12 | 11 | |
| | % | 30.80 | 22.00 | + 8.80 |
| 2 nd AI | No. | 6 | 10 | |
| | % | 15.39 | 20.00 | - 4.61 |
| 1 st and 2 nd AI | No. | 18 | 21 | |
| | % | 46.60 | 42.00 | + 4.6 |
| 3 rd AI or > | No. | 6 | 8 | |
| | % | 15.39 | 16.00 | - 0.61 |
| No. of AI/P | Mean \pm | 2.00 \pm 0.28 | 2.24 \pm 0.28 | -0.24 |
| | Min. | 1 | 1 | |
| | Max. | 6 | 7 | |
| Cows recent inseminated | No. | 10 | 12 | |
| | % | 23.25 | 24.00 | -0.75 |
| Anoestrus cows after insemination | No. | 5 | 9 | |
| | % | 11.63 | 18.00 | -6.37 |

At the beginning of estrus synchronization protocol cows from group E were examined by transrectal ultrasound in order to establish ovarian structures and the sexual cycle stage.

Table 3 shows results regarding insemination and pregnancy rates at first insemination depending on ovarian profile at the beginning of estrus synchronization treatments. Thus, after the protocol treatment finalization, 39 cows (90.7%) showed estrus signs and were artificially inseminated, being subsequently diagnosed as being pregnant at first insemination in 30.78% of cases.

Analyzing the pregnancy rate at first insemination after treatment according to

ovarian profile, some variations between 8.33% (small follicle, 4-6 mm in size one an ovary and medium follicle, size 8-10 or large follicle of 15 mm in size on the collateral ovary) and 25% (cows with small or large follicles on one ovary and corpus luteum on the collateral ovary.) Cows with small follicles (4-6 mm) on ovaries, atrophic ovaries or ovarian cysts have not been diagnosed.

After analyzing the results, it can be concluded that conception rate at first insemination after treatment depends on ovaries trophic state, sexual cycle stage in which is applied the treatment and according to ovarian follicle size.

Table 3 Ovarian profile at initiation of estrus synchronization protocol in Group E- treated cows with GnRH at day 0 + PRID for 7 days, PGF2 α at day 6 and insemination on estrus detection in relation to conception rate at first insemination after treatments

| Ovarian structures at the beginning of treatment Right ovary / Left ovary | Inseminated cows after treatment | | Pregnant cows at first insemination after treatment | |
|--|-------------------------------------|-------------|---|--------------|
| | No. | % | No. | % |
| Small follicle (4-6 mm) / Corpus luteum | 8 | 20.51 | 3 | 25.0 |
| Small follicle (4-6 mm) / Small follicle (4-6 mm) | 4 | 10.25 | 0 | 0 |
| Small follicle (4-6 mm) / Medium follicle (8-10 mm) | 2 | 5.13 | 1 | 8.33 |
| Medium follicles (10 mm) on both ovaries | 1 | 2.56 | 1 | 8.33 |
| Small follicle (4-6 mm) / Hypofunction | 7 | 17.95 | 2 | 16.70 |
| Big follicle (15 mm) / Small follicle (4-6 mm) | 2 | 5.13 | 1 | 8.33 |
| Big follicle (15 mm) / Big follicle (15 mm) / | 1 | 2.56 | 1 | 8.33 |
| Big follicle (15 mm) / Corpus luteum | 5 | 12.82 | 3 | 25.00 |
| Big follicle (15 mm) / Hypofunction | 2 | 5.13 | - | 0 |
| Ovarian cysts on both ovaries | 1 | 2.56 | - | 0 |
| Ovarian cyst/ Small follicle (4-6 mm) | 4 | 10.25 | - | 0 |
| Ovarian cyst/ Corpus luteum | 1 | 2.56 | - | 0 |
| Corpus luteum / Hypofunction | 1 | 2.56 | - | 0 |
| Total | 39 | 90,7 | 12 | 30.78 |

DISCUSSIONS

Similar results were also recorded by El-Zarkouny et al. 2004, which claimed that the addition of intravenous gestagens (PRID) to Ovsynch protocol resulted in increased conception rates (59%) compared to Ovsynch monotherapy (36%).

Good effects of estrus synchronization and significantly improved pregnancy rates were also indicated by other authors following the protocol application in meat and dairy cows, progestin-based as an intravaginal device for a 7 day period, followed by a dose of PGF2 α

injection (one day before device removal of) (Lucy MC et al., 2001), (4).

Some authors have found in dairy cows that using the intravaginal device protocol based on Gn-RH prevents premature estrus occurrence, with fertility-increasing effects by 11% (59% vs. 48%) (Lamb GC et al., 2001), (3).

In other studies, authors claim that in meat heifers intravaginal devices have limited effects (Lamb G.C., 2004). Gestagen administration in feed in meat heifers as Medroxyprogesterone acetate (MPA) for 14 days, followed by a PGF2 α dose on day 17, was a more efficient method to control estrus

cycle (Brown L.N. et al. 1988; Patterson D.G. et al. 1995;Thompson K.E. et al. 1999).

CONCLUSIONS

1. Cows from group E showed estrus signs and were inseminated after the protocol application GnRH +PRID 7 days, PGF2 α on day 6 in 90.7% of all cases (39 from 43 cows).

2. The average interval from treatment to first artificial insemination within group E cows registered 22.77 \pm 3.95 days and from treatment to conception registered 44 \pm 7.99 days.

3. Estrus synchronization protocol application within group E cows compared to group C (without treatments), had effects of reducing the average interval from calving to first artificial insemination by 18.53 days (79.6 \pm 4.2 days compared to 98.13 \pm 7.12 days) and the average interval from calving to conception by 43.03 days (98.9 \pm 8.2 days compared to 141.16 \pm 9.72 days).

4. The experimental group cows (group E) registered conception rates values at first artificial insemination higher by 8.8% compared to control group cows (group C) (30.8% vs. 22.0%).

5. Depending on ovarian profile, pregnancy rate at first artificial insemination after treatment varied between 8.33% (small follicle of 4-6 mm size on one ovary, medium follicle of 10 mm size or 15 mm size on the collateral ovary) and 25% (4-6 mm small follicle or 15 mm large follicle on ovary and corpus luteum on collateral ovary).

6. None of the cows diagnosed with small follicles (4-6 mm) on both ovaries, ovarian hypofunction or ovarian cysts were diagnosed as being pregnant.

7. The GnRH + PRID for 7 days and PGF2 α on day 6 estrus synchronization protocol followed by artificial insemination at estrus detection can be recommended as an alternative to improve reproductive parameters in dairy cattle farms.

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REFERENCES

- [1] Brown LN, Odde KG, LeFever DG, King ME, Neubauer CJ. 1988: "Comparison of MGA- PGF2 α to Syncro-Mate B for estrus synchronization in beef heifers". *Theriogenology* 30:1-12.
- [2] El-Zarkouny, S.Z., Cartmill, J.A., Hensley, B.A., Stevenson, J.S., 2004: Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. *J. Dairy Sci.* John B. Hall, 2009-"GnRH Based Estrus Synchronization Systems for Beef Cows", Virginia Cooperative Extension, Virginia Tech, and Virginia State University. 87, 1024-1037.
- [3] Lamb GC, Stevenson JS, Kesler DJ, Garverick HA, Brown DR, Salfen BE., 2001: "Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2 α for ovulation control in postpartum suckled beef cows". *J Anim Sci* 79:2253-2259.
- [4] Lucy MC, Billings HJ, Butler WR, Ehnis LR, Fields MJ, Kesler DJ, Kinder JE, Mattos RC, Short RE, Thatcher WW, Wettemann RP, Yelich JV, Hafs HD., 2001: "Efficacy of an intravaginal progesterone insert and an injection of PGF2 α for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers". *J Anim Sci* 70:1904-1910.
- [5] Mihm, M., A. Baguisi, M. P. Boland, and J. F. Roche. 1994: Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. *J. Reprod.Fertil.* 102:123-130.
- [6] Mitchell W. Smith and Jeffrey S. Stevenson-1996: " Fate of the Dominant Follicle, Embryonal Survival, and Pregnancy Rates in Dairy Cattle Treated with Prostaglandin F2 α and Progestins in the Absence or Presence of a Functional Corpus Luteum", *Journal of Animal Science* 73:3743-3751.
- [7] Patterson DJ, Hall JB, Bradley NW, Schillo KK, Woods BL, Kearnan JM., 1995: "Improved synchrony, conception rate, and fecundity in postpartum suckled beef cows fed melengestrol acetate prior to prostaglandin F2 α ". *J Anim Sci* 73:954-959.
- [8] Smith M.F., Perry G.A., Atkins J.A., Busch D.C., Johnson C.L, and Patterson D.J., 2006: "Physiological principles underlying synchronization of estrus", *Proceedings, Applied Reproductive Strategies in Beef Cattle*, Rapid City, South Dakota 7.
- [9] Thompson KE, Stevenson JS, Lamb GC, Grieger DM, Loest CA.-1999: "Follicular, hormonal and pregnancy responses of early postpartum suckled beef cows to GnRH, norgestomet, and prostaglandin F2 α ". *J Anim Sci* 77:1823-1832.