TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2018, vol. 3, No 1(4): 70–76

COMPARISON OF HCG VS GNRH EFFECTS IN DOUBLE OVSYNCH ON FIRST-SERVICE CONCEPTION RATES IN ANESTRUS DAIRY COWS

Gundars Naglis[1], Ivan Fasulkov[1], Manol Karadaev[1], Radina Vasileva[2], Georgios Petrovas[3], Nasko Vasilev[1]

[1]Trakia University, Faculty of Veterinary Medicine, Stara Zagora, Bulgaria
[2]Five-year veterinary medicine student, Trakia University, Faculty of Veterinary Medicine, Stara Zagora, Bulgaria,
[3]Istanbul University, Faculty of Veterinary Medicine , Istanbul, Turkey

ABSTRACT

The aim of the present study was to compare first-service conception rates after inclusion of either hCG or GnRH in Double Ovsynch to anestrous dairy cows. The animals with follicle size ≥10 mm and no corpus luteum in ovaries were allotted into 2 groups. At the start of the programme, follicle sizes were 13.7±1.5 mm and 13.9±1.2 mm respectively (p=0.5). Higher percentage of ovulations (72.7%) were established in cows treated with hCG vs those treated with GnRH (64.4%, p<0.2094). Follicle sizes at the time of AI were 15.9±0.5 mm and 16.2±0.4 mm (p=0.5). Pregnancy was detected in 38.6% of cows treated with hCG and 42.2% of cows treated with GnRH (p<0.3501).

Key words: anestrus, dairy cows, double Ovsynch.

Introduction

The synchronisation of estrus and ovulation is an essential tool for reproduction management in dairy cows and improvement of reproductive performance in the herd. The application of estrus synchronisation programmes increase conception rates, reduce the average number of days in lactation, calving-to-first-service interval and intercalving intervals (Wiltbank and Pursley, 2014).

Numerous protocols for synchronisation of the estrus and ovulation are developed. In fact, there are so many that sometimes, the decision-making is the most difficult part of the entire process (Pursley et al., 1995).

Ovsynch protocol is the first synchronisation programme developed for timed artificial insemination (TAI). The protocol has two main advantages: does not require detection of the estrus and guarantees insemination of all cows. It saves costs for detection of cows in estrus and increases the overall number of animals, that would probably be pregnant (Pursley et al., 1997).

It has been established that cows respond better to programmes for estrus synchronisation, when they are preliminarily synchronised (Presynch). Most commonly, two applications of prostaglandin from the F2α group (PGF2α) at 14-day intervals, followed by the Ovsynch protocol 12 days later have been used to this end, resulting in increased conception rates in cycling but not in non-cycling cows (Moreira et al., 2001; El-Zarkouny et al., 2004; Navanukraw et al., 2004; Chebel and Santos, 2010). The second presynchronisation protocol is known as GnRH-PGF-GnRH. It includes treatment with gonadotropins (GnRH) at day 0, prostaglandin (PGF2α) at day 7 and gonadotropin (GnRH) on day 10, followed by Ovsynch protocol after 7 days (double Ovsynch). The first protocol is termed synchronising, and the second – breeding.

Presynchronisation is used only for the first artificial insemination after calving and it guarantees that all cows would be inseminated thus reducing the days in lactation to first-service (Bisinotto and Santos, 2012; Ribeiro et al., 2012b; Souza et al., 2008).

The aim of the present research was to compare first-service conception rates after application of either human choriongonadotropin hCG or GnRH in the synchronisation part of a double Ovsynch protocol to anestrus dairy cows.

Material and methods

The study was conducted between May 2015 – May 2016 in a cattle farm in southeastern Bulgaria with capacity 320 Holstein-Friesian dairy cows. All animals were reared in free stalls, and machine milked three times per day, with Heat-time system for estrus detection implemented, permanent access to water and total mixed ration. The average 305-day lactation milk yield of the flock was 9180 L. The experiment included 96 cows without signs of estrus until the 45th day at postpartum, from which 7 animals were excluded due to diseases. The cows were divided into 2 groups. The first group of cows (n=44) was treated with standard double Ovsynch, e.g. 0.01 mg GnRH (Veterelin, Laboratorios Calier, Spain) at day 0, prostaglandin (Indupart, Vetpharma Animal Health, Spain) at day 7; 0.01 mg GnRH (Veterelin, Laboratorios Calier, Spain) at day 10 and 7 days apart, Ovsynch (GnRH-PGF-GnRH). In the second group (n=45) the programme started with 1 500 UI hCG (Chorulon, Intervet, Holland), prostaglandin (Indupart) at day 7, 0.01 mg GnRH (Veterilin) at day 10 and 7 days apart, Ovsynch (GnRH-PGF-GnRH).

Ultrasonographic studies were done with SonoScape A5 Vet (SonoScape, China) ultrasound equipped with linear multifrequency probe (5.0–12.0 MHz) for detection of ovarian findings and the cause of anestrus. The cows with follicle ≥10 mm and lack of corpus luteum in ovaries were included in the study (Fig. 1). Ultrasound investigations of ovaries were performed at the time of the first treatment with GnRH or hCG for measurement of available follicles and by the 7th day for detection of ovulation. The presence of ovulation after the first treatment with GnRH or hCG was assessed by the presence of corpus luteum in ovaries (Fig. 2). By the 17th day of the breeding protocol beginning of the double Ovsynch, echography was performed for detection of ovarian findings after the second GnRH application at the 10th day. On the 24th day, echography was done to document ovarian findings after the third treatment with GnRH. The presence of ovulation after GnRH treatments was determined by the presence of corpus luteum in ovaries. The measurement of the maximum follicle diameter and timed artificial insemination were conducted on the 27th day (Fig. 3). Seven days after TAI, ultrasonography was done to detect ovulation by the presence of a new corpus luteum. Pregnancy was diagnosed through ultrasound by the 32nd and the 60th day after TAI. Conception rates were calculated as ratio of pregnant cows and cows submitted to TAI. Blood samples for analysis of progesterone concentrations were collected on the same days. Progesterone determination was done by ELISA progesterone kit (Accu-Bind, Monobind Inc., USA).

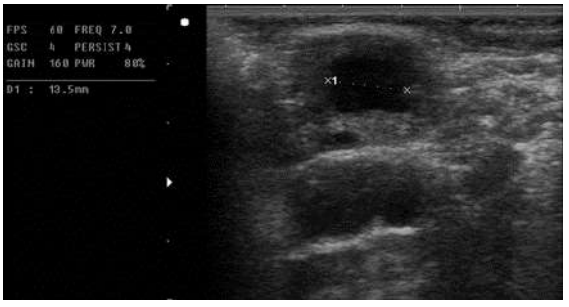

Figure 1: Ultrasonogram of a follicle over 10 mm in size.

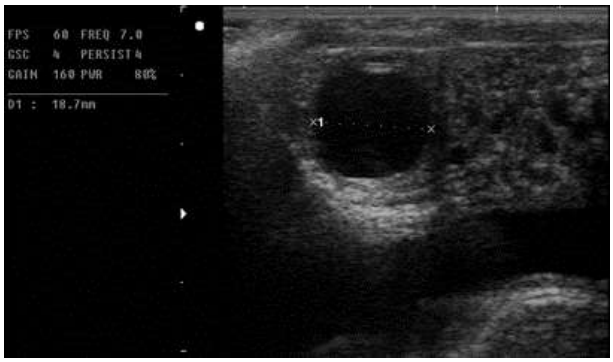

Figure 2: Ultrasonogram of a dominant follicle at the day of insemination.

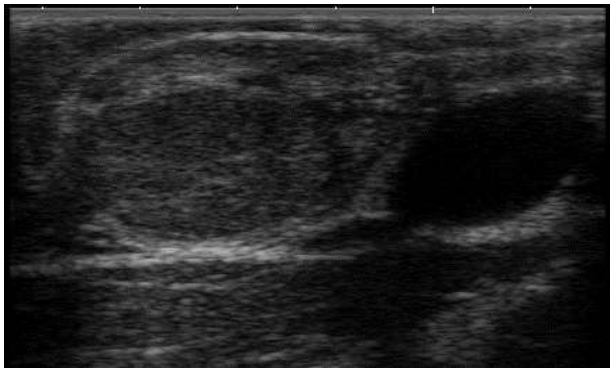

Figure 3: Ultrasonogram of a corpus luteum by the 7th post insemination day.

The results were statistically analysed by StatSoft (Statistica 7, Microsoft Corp. 1984–2000 Inc.) and presented as mean, SD, relative proportion (%) and levels of significant. Differences were interpreted as statistically significant at $p \leq 0.05$.

Results

The average number of lactations in both groups was 2.4 and 2.5 respectively, average daily milk yields: 38.6±2.3 kg and 39.2±2.7 kg, days in lactation: 56.8±3.4 and 57.6±4.7 without significant differences.

The follicle size at the start of the protocol, follicle size at TAI, ovulation percentage and conception rates are shown in Table 1. At the programme start, follicle size in both groups was almost equal – 13.7±1.5 mm and 13.9±1.2 mm ($p=0.5$). Higher percentage of ovulations (72.7%) was registered in the group treated initially with hCG vs the group treated with GnRH (64.4%) ($p<0.2094$). The average follicle size during the artificial insemination was 15.9±0.5 mm in the first and 16.2±0.4 mm in the second group without statistically significant difference ($p=0.5$). By the 32nd day, pregnancy was detected in 38.6% of animals treated initially with hCG and in 42.2% of cows treated with GnRH ($p<0.3501$).

Table 1: Follicle size at the start of the programme, ovulation percentage, follicle size at insemination and conception rates.

Parameter	hCG group (n=44)	GnRH group (n=45)
Follicle size at first treatment, mm	**13.7±1.5**	**13.9±1.2**
Blood progesterone concentrations, ng/ml	**0.36±0.12**	**0.39±0.14**
Ovulations, % (number)	**72.7 (**32/44)	**64.4 (**29/45)
Synchronisation, % (number)	**88.6 (**39/44)	**86.7 (**39/45)
Follicle size at the time of insemination, mm	**15.9±0.5**	**16.2±0.4**
Conception rate, % (pregnant/inseminated)	**38.6 (**17/44)	**42.2 (**19/45)

Discussion

Our study compared the ovulations percentage in response to treatment with either GnRH or hCG and investigated how conception rates were influenced, the ovulation response is an exceptionally important criterion for the successful synchronisation of ovulations in dairy cows. The Ovsynch protocol has several limitations as to the possibility for synchronisation of follicle growth, because the ovulations after the first GnRH is usually 45% - 60% because it was applied at several stages of the estrus (Gumen et al., 2003). After presynchronisation and subsequent Ovsynch application, ovulation rates attained up to 70% (Bisinotto and Santos, 2012; Ribeiro et al., 2012a). Presynchronisation of cows with two successive Ovsynch protocols (a.k.a. Double-Ovsynch) resulted in ovulation rates <72% after the first GnRH of the second Ovsynch (Souza et al, 2008; Giordano et al, 2013).

The main limitation of presynchronisation programmes with two-fold PgF2α application is the lack of possibility for improvement of conception rates in anestrus dairy cows, which comprise up to 41% of all cows until the end of the service period (Walsh et al., 2007; Santos et al., 2009). Better results in this group of cows could be obtained by inclusion of GnRH at the time of presynchronisation. The combination of GnRH and PgF2α has resulted in higher conception rates (Bisinotto and Santos, 2012). In fact, estrus presynchronisation with PgF2α and GnRH represents the so-called G6G protocol (Bello et al., 2006), that increases conception rates in cows submitted to TAI including with additional treatment with progesterone through CIDR (Ribeiro et al., 2012a). The double Ovsynch is a more efficient method for estrus presynchronisation in dairy cows and for induction of cycling in anovulatory cows (Ayres et al., 2013; Souza et al., 2008).

In our study, GnRH was replaced with hCG in the beginning of the double Ovsynch. The result was statistically significantly increased conception rate in this group up to 72.2%, vs 64.4% in the group treated with GnRH ($p<0.2094$). In a previous study, Gumen et al. (2005) have utilised GnRH in a Presynch protocol (7 days before the last prostaglandin application) and registered 80% induction of ovulation in anestrus cows and 31% spontaneous ovulation in control animals.

According to our results, primiparous cows exhibited higher conception rates of 43.18%, vs 37.78% in multiparous ($p<0.2817$). Increased conception rates after application of Double-Ovsynch regardless of the parity number was probably due to the better results in primiparous heifers. It is generally acknowledged that these cows are more commonly anestrus than multiparous animals (El-Zarkouny et al., 2004; Chebel et al., 2006; Silva et al., 2007). Therefore, the differences in conception rates of the double Ovsynch depend on the parity, hence this protocol is more useful in primiparous cows.

In addition to the treatment of anestrus cows, it seems very probably that the start of the double Ovsynch was associated to better synchronisation of estrus stage in comparison to Presynch. The ovulation after the first administration of GnRH within the framework of Ovsynch and conception

rates depended on the stage of the sexual cycle at Ovsynch start (Vasconcelos et al., 1999). Further, cows ovulating after the first GnRH of the Ovsynch protocol exhibited higher conception rates than non-ovulated cows (Bello et al., 2006; Chebel et al., 2006).

Another factor influencing the Ovsynch performance, was the follicle size at the time of the second GnRH treatment or at the time of artificial insemination. In our research, cows in the group treated with hCG tended to produce follicles of relatively smaller size at the time of TAI than animals treated with GnRH (15.9±0.5 mm and 16.2±0.4 mm respectively). Lopes et al. (2007) reported that conception rates increased when follicle size was greater. On the other hand, Vasconcellos et al. (1999), Ozturk et al., (2010) established that smaller follicles resulted in higher conception rates in dairy cows. In our study, although the size of follicles was smaller in the hCG-PGF-GnRH (15.9±0.5 mm) than in the GnRH-PGF-GnRH group (16.2±0.4 mm), conception rate was lower in the former than in the latter group (38.6% vs 42.2%, $P<0.3501$). Other reports demonstrating higher conception rates after ovulation with follicles > 16 mm (Perry et al., 2005; Bello et al., 2007) in line with our data.

High blood circulating progesterone concentrations and higher proportion of cows with high progesterone (3 ng/ml) at the time of treatment corresponded to the better synchronisation of cows at the start of Ovsynch protocol and/or the higher percentage of ovulations due to the first GnRH application. Increased percentage of ovulations after the first gonadotropin treatment could produce a dominating follicle after the second treatment, as its size is less variable and closer to the ideal follicle size (Bello et al., 2006; Souza et al., 2007). The higher percentage of synchronisation at the time of Ovsynch could reduce the number of cows ovulating at an inappropriate moment (Vasconcelos et al., 1999), and thus, increase conception rates (Bello et al., 2006; Chebel et al., 2006).

The conception rates in this study (38.6% hCG vs 42.2% GnRH) was within the range reported by other researchers (Bello et al., 2006; Keskin et al., 2010). For example, Chebel et al., (2007) found out higher conception rate (~40% vs ~10%) in cows with corpus luteum at the day of treatment with prostaglandin during Ovsynch, as compared to cows without corpus luteum. Cows without corpus luteum during the first GnRH treatment of the protocol with TAI, anestrus or cycling, but treated during proestrus, estrus or metestrus, had lower progesterone concentrations than cows with corpus luteum at the start of the synchronisation protocol (0.5 ng/ml vs 3.4 ng/ml). This group comprises nearly 30% of all cows submitted to protocols with TAI at dairy farms. The lack of corpus luteum at the start of protocols was the main limitation for achievement of high conception rates (Bisinotto et al., 2010a).

Before synchronisation of ovulations for TAI, the percentage of cows in the beginning of diestrus at Ovsynch start, as well as the percentage of ovulating cows after the first GnRH treatment should be increased in order to attain higher blood progesterone concentrations during the Ovsynch. The Double-Ovsynch protocol offers a possibility for improvement of these variables at the time of Ovsynch in order to increased TAI conception rates.

In conclusion, according to the results of our study, the substitution of GnRH with hCG at the start of double Ovsynch protocol in anestrus dairy cows increased ovulation and synchronisation percentages, but did not improve conception rates.

Our sincere thanks to Todor Uzunov, agricultural producer, for kindly provided possibility to perform our experiments.

References

1. Ayres H., Ferreira R. M., Cunha A. P., Araújo R. R., Wiltbank M. C. (2013). *Double- Ovsynch in high-producing dairy cows: effects on progesterone concentrations and ovulation to GnRH treatments*. Theriogenology 2013: Vol. 79: 159–164.
2. Bello N. M., Steibel J. P., Pursley R. J. (2006). *Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows*. J Dairy Sci 2006: Vol. 89: 3413–24.
3. Bisinotto R. S., Chebel R. C., Santos JEP. (2010). *Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows*. Journal of Dairy Science 2010: Vol. 93: 3578–3587.
4. Bisinotto R. S., Santos JEP. (2012). *The use of endocrine treatments to improve pregnancy rates in cattle*. Reproduction Fertility and Development 2012: Vol. 24: 258–266.
5. Bisinotto R. S., Ribeiro E. S., Lima F. S., Martinez N., Greco L. F., Barbosa L. F., Bueno P. P., Scagion L. F., Thatcher W. W., Santos JEP. (2013). *Targeted progesterone supplementation improves fertility in lactating dairy cows without a corpus luteum at the initiation of the timed artificial insemination protocol*. Journal of Dairy Science 2013: Vol. 96: 2214–2225.
6. Chebel R. C., Santos JEP. (2010). *Effect of inseminating cows in estrus following a presynchronization protocol on reproductive and lactation performances*. Journal of Dairy Science 2010: Vol. 93: 4632–4643.
7. Chebel R. C., Santos J. E. P., Cerri R. L. A., Rutigliano H. M., Bruno R. G. S. (2006). *Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols*. J Dairy Sci 2006: Vol. 89: 4205–19.
8. 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 2004: Vol. 87: 1024–37.
9. Giordano J. O., Wiltbank M. C., Fricke P. M., Bas S., Pawlisch R., Guenther J., Nascimento A. B. (2013). *Effect of increasing GnRH and PGF2α dose during double- Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows*. Theriogenology 2013: Vol. 80: 773–783.
10. Gumen A., Guenther J. N., Wiltbank M. C. (2003). *Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cow*s. J Dairy Sci 2003: 86: 3184–94.
11. Gumen A., Souza A. H., Cunha A. P., Silva E., Guenther J. N., Wiltbank M. C. (2005). *Effect of GnRH between pre-synch injections and estradiol-17b during the Ovsynch protocol on conception rates in lactating dairy cows*. J Dairy Sci 2005: Vol. 88: 170 [Abstr.].
12. Keskin G., Yilmazbas-Mecitoglu, Gumen A., Karakaya E., Darici R., Okut H. (2010). *Effect of hCG vs. GnRH at the beginning of the Ovsynch on first ovulation and conception rates in cyclic lactating dairy cows*. Theriogenology 2010: Vol. 74: 602–607.
13. Lopes A. S., Butler S. T., Gilbert R. O., Butler W. R. (2007). *Relationship of pre-ovulatory follicle size, estradiol concentrations and season to pregnancy outcome in dairy cows*. Anim Reprod Sci 2007: Vol. 99: 34–43.
14. Mihn M., Baguisi A., Boland M. P., Roche J. F. (1994). *Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers*. J Reprod Fertil 1994: Vol. 102: 123–30.
15. Moreira F., Orlandi C., Risco C. A., Mattos R., Lopes F., Thatcher W. W. (2001). *Effects of presynchronization and bovine somatotropin on pregnancy rates to timed artificial insemination protocol in lactating dairy cows*. J Dairy Sci 2001: Vol. 84: 1646–59.
16. Navanukraw C., Redmer D. A., Reynolds L. P., Kirsch J. D., Grazul- Bilska A. T., Fricke P. M. (2004). *A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows*. J Dairy Sci 2004: Vol. 87: 1551–7.
17. Ozturk A., Cirit U., Baran A., Ak K. (2010). *Is Doublesynch protocol a new alternative for timed artificial insemination in anestrous dairy cows*. Theriogenology 2010: Vol. 73: 568–576.

18. Perry G. A., Smith M. F., Lucy M. C., Green J. A., Parks T. E., MacNeilv M. D., Roberts A. J., Geary T. W. (2005). *Relationship between follicle size at insemination and pregnancy succes*. PNAS USA 2005: Vol. 102: 5268–73.
19. Pursley R. J., Mee M. O., Wiltbank M. C. (1995). *Synchronization of ovulation in dairy cows using PGF2a and GnRH*. Theriogenology 1995: Vol. 44: 915–23.
20. Pursley R. J., Kosorok M. R., Wiltbank M. C. (1997). *Reproductive management of lactating dairy cows using synchronization of ovulation*. J Dairy Sci 1997: Vol. 80: 301–6.
21. Revah I., Butler W. R. (1996). *Prolonged dominance of follicles reduced viability of bovine oocytes*. J Reprod Fertil 1996: 106: 39–47.
22. Ribeiro E. S., Bisinotto R. S., Favoreto M. G., Martins L. T., Cerri RLA., Silvestre F. T., Greco L. F., Thatcher W. W., Santos JEP. (2012a). *Fertility in dairy cows following presynchronization and administering twice the luteolytic dose of prostaglandin F2α as one or two injections in the 5-day timed artificial insemination protocol*. Theriogenology 2012a: Vol. 78: 273–284.
23. Ribeiro E. S., Monteiro A. P., Lima F. S., Ayres H., Bisinotto R. S., Favoreto M., Greco L. F., Marsola R. S., Thatcher W. W., Santos JEP. (2012b). *Effects of presynchronization and length of proestrus on fertility of grazing dairy cows subjected to a 5-day timed artificial insemination protocol*. Journal of Dairy Science 2012b: Vol. 95: 2513–2522.
24. Santos JEP, Rutigliano H. M., Sá Filho M. F. (2009). *Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows*. Animal Reproduction Science 2009: Vol. 110: 207–221.
25. Silva E., Sterry R. A., Fricke P. M. (2007). *Assessment of a practical method for identifying anovular dairy cows synchronized for first postpartum timed artificial insemination*. J Dairy Sci 2007: Vol. 90: 3255–62.
26. Souza A. H., Gumen A., Silva EPB, Cunha A. P., Guenther J. N., Peto C. M. (2007). *Supplementation with estradiol-17b before the last gonadotropin-releasing hormone injection of the Ovsynch protocol in lactation dairy cows*. J Dairy Sci 2007: Vol. 90: 4623–34.
27. Souza, A. H., Ayres H., Ferreira R. M, Wiltbank M. C. (2008). *A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows*. Theriogenology 2008: Vol. 70: 208–215.
28. Vasconcelos J. L., Silcox R. W., Rosa G. J., Pursley R. J., Wiltbank M. C. (1999). *Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows*. Theriogenology 1999: Vol. 52: 1067–78.
29. Walsh R. B., Kelton D. F., Duffield T. F., Leslie K. E., Walton J. S., LeBlanc S. J. (2007). *Prevalence and risk factors for postpartum anovulatory condition in dairy cows*. Journal of Dairy Science 2007: Vol. 90: 315–324.
30. Wiltbank M. C., Pursley J. R. (2014). *The cow as an induced ovulator: timed AI after synchronization of ovulation*. Theriogenology 2014: Vol. 81: 170–185.