Reproductive performance improvement following the implementation of a modified Double-Ovsynch protocol compared to G6G in lactating Holstein cows

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ABSTRACT

This study evaluated a new presynchronization method, the combination of Presynch and Ovsynch, prior to the implementation of the Ovsynch-fixed timed artificial insemination (OVS+FTAI) protocol, as modified by Double-Ovsynch (MDO) compared to the G6G protocol. Lactating Holstein cows in G6G (n=250) and MDO (n=270) treatment groups received the following treatments, respectively: PGF2α-2d-GnRH(pre-GnRH1)-6d-OVS+FTAI (GnRH(GnRH1)-7d-PGF2α(PG)-2d-GnRH(GnRH2)-18h-FTAI) and PGF2α-7d-GnRH-7d-PGF2α-3d-GnRH(pre-GnRH1)-7d-OVS+FTAI. On average, the days in milk (DIM) of cows at the time of GnRH1 injection was 75±0.08 (mean±SEM). Pregnancy was diagnosed 32-38 days after timed AI by ultrasonographic examination. Modified Double-Ovsynch increased the pregnancies per AI (P/AI) compared to the G6G protocol (P=0.01). For all cows (n=520), the pregnancy rate was higher in cows that had preovulatory follicles ≥16 mm in diameter at the time of GnRH2 injection, compared to the cows that had preovulatory follicles <16 mm in diameter at the same time (P=0.03). Modified Double-Ovsynch increased the mean (±SEM) serum concentration of progesterone (P4, ng/ml) at PG compared to the G6G protocol (P<0.01). Therefore, MDO increased the pregnancy rate of cows at the first postpartum insemination compared to the G6G protocol, due to the induction of ovulation in noncyclic cows, improvement of synchronization in ovarian events, improvement of the quality of the ovulatory follicles and oocytes, and provision of an appropriate intrauterine environment for embryonic development and survival.

Key words: G6G; modified Double-Ovsynch; pregnancy; Holstein cows

Introduction

The reproductive performance of high-yielding dairy cows is low due to reductions in fertility, and expression and detection of estrus (WALSH et al., 2011; CROWE et al., 2018; KGARI et al., 2020). Thus, protocols that allow for TAI, such as Presynch-Ovsynch (MOREIRA et al., 2001), G6G (BELLO et al., 2006), and Double-Ovsynch (SOUZA et al., 2008) have been developed in recent years. The Ovsynch protocol combines GnRH and PGF2α to synchronize the time of ovulation

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in cows (SOUZA et al., 2008). The success rate of the OVS+FTAI protocol is dependent on the stage of the reproductive cycle of the cow at the time of the implementation of the protocol. It has been demonstrated that when the Ovsynch protocol is started in the early stages of the reproductive cycle (days 5-12 of the estrous cycle) the highest pregnancy rate will be achieved (VASCONCELOS et al., 1999; ABDEL AZIZ and ABDEL-WAHAB, 2017; NOWICKI et al., 2017; BORCHARDT et al, 2020). On this basis, researchers have designed presynchronization programs to increase the proportion of cows at the appropriate time of the estrous cycle at the beginning of the OVS+FTAI protocol. On the other hand, injections of GnRH and PGF2α in the form of presynchronization protocols can accelerate ovarian rebound and improve uterine health status in postpartum cows (HOEDEMAKER et al., 1992; SOUZA et al., 2008; LUTTGENAU et al., 2014). To obtain the optimal pregnancy rate following the implementation of the OVS+FTAI in a herd, presynchronization protocols should be designed to stimulate ovarian activity in anovular cows and to generate high synchrony rates in ovarian events (BELLO et al., 2006; STEVENSON and BRITT, 2017).

Compared to the G6G, the Double-Ovsynch protocol is more effective for increasing the pregnancy rate in primiparous cows by increasing the proportion of cyclic cows in the early lactation period (ASTIZ and FARGAS, 2013). It has been demonstrated that the proportion of noncyclic cows in the early lactation period is higher in primiparous cows than in multiparous cows (ASTIZ and FARGAS, 2013).

It has been shown in several studies that high serum concentrations of P4 in the preinsemination diestrous phase increase the quality of the ovulatory follicles and oocytes, and the chance of a successful pregnancy is increased following the ovulation of a qualified follicle (FONSECA et al., 1983; XU et al., 1997; RIVERA et al., 2011; BISINOTTO et al., 2015; SAAD et al., 2019).

Several parameters are proposed as potential indicators of preovulatory follicle quality, namely: follicle size (VASCONCELOS et al., 1999; VASCONCELOS et al., 2001; BELLO et al., 2006; KESKIN et al., 2016), follicle lifespan, duration of dominance (BLEACH et al., 2004; BURNS et al., 2005; CERRI et al., 2009; MONTEIRO et al., 2015), and serum concentrations of P4 during preovulatory follicle development (XU et al., 1997; NASSER et al, 2011; FAIR and LONERGAN, 2012; SAAD et al., 2019).

The main purpose of the recent study was to compare the pregnancy rates of dairy cows at the first postpartum insemination between the G6G and MDO protocols. The hypothesis of the current study was that the presynchronization program of the MDO compared to the G6G protocol induces cyclicity in a greater number of anovular cows, and produces higher synchrony and in turn pregnancy rates at the first postpartum insemination in dairy cows.

Materials and methods

Cows, housing, and feeding. This study was conducted on 520 milking Holstein cows on a commercial dairy farm near Mashhad from November 2019 to June 2020,. The cows had free access to fresh water, and twice daily were fed a total mixed ration consisting primarily of corn and alfalfa silage as forage, with a corn and soybean meal-based concentrate, balanced to meet the requirements for lactating dairy cows (NRC, 2001). The cows were milked three times daily. The farm had feedline head lockups and free stalls, bedded with a mattress/sawdust. All procedures, including hormonal injections, blood sampling, TAI, and ultrasonographic examination of the ovaries and uterus were approved by the Research Ethics Committee of Kermanshah University of Medical Sciences (Approval ID: IR.KUMS.REC.1399.447) and conducted while the cows were locked up in the feedline. At the time of the onset of the OVS+FTAI protocol, the cows had their body condition scored using five-point systems: 1 = thin to 5 = fat (EDMONSON et al., 1989). The average BCS for the cows was 2.6. Of the 250 cows that were assigned to the G6G protocol, 75 cows were primiparous, and 175 cows were multiparous. Of the 270 cows that were assigned to the MDO protocol, 86 cows were primiparous, and 184 cows were multiparous. The mean (±SEM) milk production of the cows was 35.2±0.22 kg/day on the day of the onset of the OVS+FTAI program. On average, the DIM of cows at the time of the onset of the OVS+FTAI program was 75±0.08 (mean±SEM).

Treatments and AI. Weekly, a cohort of cows at 51 ± 12 DIM were stratified by parity, milk yield, and DIM, and randomly assigned to one of the treatment groups: G6G or MDO. The timing of the hormonal injections in the G6G and MDO protocols is shown in Fig. 1. The PGF2α used in the recent study was 500µg *per injection* of cloprostenol sodium, estroPLAN, Parnell, Alexandria NSW 2015, Australia. The GnRH used in the recent study was 100µg per injection of *gonadorelin acetate*, GONAbreed, Parnell, Alexandria NSW 2015, Australia.

Two technicians performed the AIs of the cows and used two types of conventional semen.

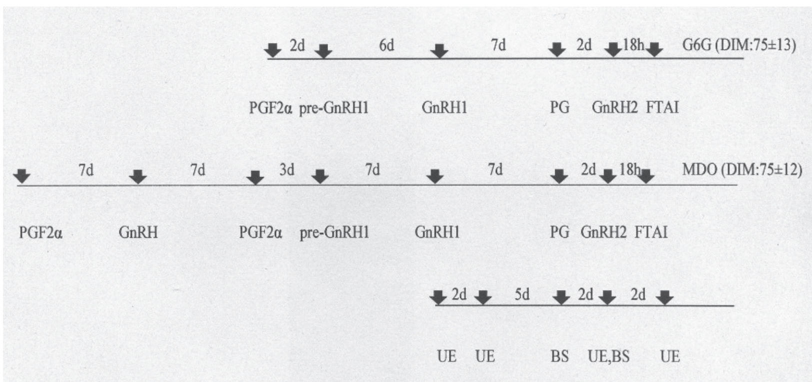

Fig. 1. Schematic image of hormonal injections, blood samplings, ultrasonographic examinations, and FTAI for the cows in the G6G and MDO protocols: DIM - The range of lactation days of cows at the time of the onset of the OVS+FTAI protocol, OVS+FTAI - Ovsynch+Fixed-time artificial insemination, MDO - Modified Double-Ovsynch, pre-GnRH1 - The latest GnRH in the presynchronization program, GnRH1 – The first GnRH in the OVS+FTAI protocol, PG – PGF2α in the OVS+FTAI protocol, GnRH2 – The last GnRH in the OVS+FTAI protocol, UE - Ultrasonographic examination, BS - Blood sampling.

Ovarian ultrasonography, ovulatory responses, and pregnancy diagnosis. Ultrasonographic examinations of the ovaries were done for all cows (n=520) with a 7.5 MHz linear array probe (Aloka SSD-900V, Aloka Co. Ltd., Wallingford, CT) at the time of GnRH1 injection, 2 days later, the time of the GnRH2 injection, and 2 days after that. Perpendicular cross-sections of the antrum of each follicle >4 mm in diameter were measured using a built-in caliper. Follicular measurements were recorded and their mean follicular diameter was obtained by averaging both cross-section measurements for each follicle. Before GnRH1 injection, the presence of a preovulatory follicle on the ovaries was evaluated and the diameter of the preovulatory follicle was also evaluated. Ovulatory response to GnRH1 injection was assessed 2 days later. The ovulatory response was characterized by the absence of the preovulatory follicle(s) on the ovaries 2 days after the GnRH1 injection. Before the GnRH2 injection, the presence of a preovulatory follicle on the ovaries was evaluated and the diameter of the preovulatory follicle was also evaluated. Two days after the GnRH2 injection, the ovulatory response of the preovulatory follicle to GnRH2 injection was evaluated (Fig. 1). Pregnancy was diagnosed 32–38 days after timed AI using ultrasonography examination.

Hormonal assays. Blood sampling was performed for all the cows (n=520) to measure the serum concentration of P4 before the PG and GnRH2 injections. All blood samples were taken from the coccygeal vein using tubes without an anticoagulant agent. Refrigerated samples were centrifuged (3000×g for 20 min) within 1 h after collection, and then kept at -20°C until the P4 concentrations were measured. Serum concentrations of P4 (ng/ml) were determined using a commercially available ELISA kit (Competitive EIA-LS-F10072, LifeSpan Biosciences, Inc, Seattle, WA). The intra and inter-assay coefficients of variation were 3.2% and 4.7%, respectively. The sensitivity of the assay was 0.15 ng/ml. A serum concentration of P4 ≥0.5 ng/ml indicated an active corpus luteum (CL) on the ovaries (WIJMA et al., 2017). Complete luteolysis was characterized by a decrease in the serum concentration of P4 from ≥0.5 ng/ml at the time of the PG injection to <0.5 ng/ml at the time of the GnRH2 injection.

Synchrony was defined as follows: a high serum concentration of P4 before the PG injection, a low serum concentration of P4 two days later, and ovulation following the GnRH2 injection.

Statistical analyses. Binomially-distributed data (i.e. ovulation following GnRH1, synchrony rate, and pregnancies per AI), concentration of P4 at PG, the preovulatory follicle diameter at GnRH1, and GnRH2 were analyzed by logistic regression, using the GLIMMIX procedure of SAS (SAS, 2002-2003). The explanatory variables considered for inclusion in the models were: treatment, ovulation at GnRH1, synchrony, serum concentration of P4 at PG (categorized as <6.2 or ≥6.2 ng/ml), preovulatory follicle diameter at GnRH1 (categorized as <14 or ≥14 mm), preovulatory follicle diameter at GnRH2 (categorized as <16 or ≥16 mm), BCS (categorized as <2.5 or ≥2.5), parity (primiparous vs multiparous), milk yield (categorized as <37.4 or ≥37.4 kg/day), DIM (categorized as <76 or ≥76 days), technician, month of AI, semen type, and interactions. Classification of the studied variables (serum concentration of P4 at PG, preovulatory follicle diameter at GnRH1 and GnRH2, BCS, milk yield, and DIM) into two subgroups was done on the basis of the median determination. The final logistic regression model removed variables by backward elimination. Probability values ≤0.05 were considered significant, whereas those between 0.051 and 0.1 were considered trends. The variables that were included in the final model for analysis of fertility were: treatment, synchrony, dominant follicle diameter at GnRH2, and parity. The final statistical model for analysis of synchrony included: treatment, DIM, ovulation at GnRH1, preovulatory follicle diameter at GnRH2, BCS, interaction between treatment and DIM, interaction between treatment and ovulation at GnRH1, interaction between treatment and BCS, and interaction between treatment and parity. The final statistical model for analysis of ovulation at GnRH1 included preovulatory follicle diameter at GnRH1, interaction between treatment and BCS, interaction between treatment and parity, and interaction between milk yield and parity. The serum concentration of P4 at PG and preovulatory follicle diameter at GnRH2 were analyzed using PROC MIXED. The final statistical model for analysis of serum concentration of P4 at PG included treatment and parity. The final statistical model for analysis of preovulatory follicle diameter at GnRH2 included parity, serum concentration of P4 at PG, ovulation at GnRH1, and interaction between treatment and ovulation at GnRH1. Univariable analysis with PROC GLIMMIX was used for analysis of the treatment effects on DIM, BCS, parity, and milk yield (Table 1). The correlation between the diameter of the preovulatory follicle at GnRH2 and ovulation following the GnRH2 injection was evaluated using PROC FREQ.

Results

The average DIM, BCS, parity, and milk yield did not differ between the treatment groups (Table 1). The variables of technician (P=0.53) and type of semen (P=0.4) had no effect on the P/AI. Also, similar percentages of pregnant cows after TAI were achieved in all months (P=0.27).

Cows receiving the MDO protocol had a greater P/AI than cows receiving the G6G protocol (P=0.01; Table 1). The differences in synchrony rates, ovulation following GnRH1, serum concentration of P4 at PG, and preovulatory follicle size at GnRH2 between the G6G and MDO protocols are shown in Table 2. Comparison of serum concentrations of P4 at PG (mean±SEM) showed a statistically significant difference between G6G and MDO treatment protocols (P<0.01; Table 2).

For all cows (n=520), synchronized cows had a greater P/AI compared to asynchronous cows (P<0.01; Table 3). Comparison of the P/AI of synchronized cows showed a statistically significant difference between the MDO and G6G protocols (P=0.05; Table 3).

Table 1. Mean (±SEM) effects of synchronization protocols on pregnancies per AI (P/AI), days in milk, body condition score (BCS), number of lactations, and milk yield in lactating dairy cows

	G6G	MDO	P-value
Pregnancies per AI (P/AI)[#]	37.2% (93/250)	48.8% 132/270)	0.01
Days in milk (range)	75.7±0.1 (64-88)	75.6±0.1 (63-85)	0.72
BCS (range)	2.6±0.01 (2.25-3.25)	2.5±0.01 (2.25-3)	0.45
Lactation number (range)	2.4±0.07 (1-6)	2.5±0.08 (1-6)	0.16
Milk yield (range)	34.7±0.29 (25-40.2)	35.8±0.32 (23-44)	0.16

[#]This analysis was done with GLIMMIX and accounted for synchrony, parity, and preovulatory follicle size at the last GnRH in OVS+FTAI in the model, OVS+FTAI - Ovsynch+Fixed-time artificial insemination, MDO - Modified Double-Ovsynch

Table 2. Mean (±SEM) effects of synchronization protocol various reproductive endpoints in lactating dairy cows

	G6G	MDO	P-value
Synchrony	82.8% (207/250)	86.2% (233/270)	0.14
OFGnRH1[a]	77.6% (194/250)	83.3% (225/270)	0.92
P4CPG[b] (ng/ml)	5.46±0.07 (2-8.6)	6.27±0.07 (2.6-9.6)	<0.01
FDGnRH2[c] (mm)	15.62±0.13 (8-20)	15.25±0.12 (10-20)	0.37

[a] Ovulation at the beginning of OVS+FTAI, [b] Serum concentration of P4 at PGF2α in OVS+FTAI, [c] Preovulatory follicle size at the last GnRH in OVS+FTAI, OVS+FTAI - Ovsynch+Fixed-time artificial insemination, MDO - Modified Double-Ovsynch

Table 3. Effects of synchrony on pregnancies per AI (P/AI) following synchronization of lactating dairy cows with G6G or MDO protocols

	Synchrony (yes)	Synchrony (no)	P-value *, #
G6G	44.4% (92/207)	2.3% (1/43)	0.02
MDO	55.7% (130/233)	5.4% (2/37)	0.01
P-value **,#	0.05	0.52	-----
Overall	50.4% (222/440)	3.7% (3/80)	<0.01

[#]These analyses were done with GLIMMIX and accounted for parity and preovulatory follicle size at the last GnRH in OVS+FTAI in the model, *P-values for comparisons of synchrony (yes) vs synchrony (no), **P-values for comparisons of G6G vs MDO, OVS+FTAI - Ovsynch+Fixed-time artificial insemination, MDO - Modified Double-Ovsynch

The pregnancy rate of primiparous cows was significantly greater than that of multiparous cows in the MDO protocol (P=0.04; Table 4).

For MDO cows, the P/AI of cows that had preovulatory follicles ≥16 mm in diameter at GnRH2 was greater than the cows that had preovulatory follicles <16 mm in diameter at the same time (P=0.04; Table 5). Comparison of P/AI between G6G and MDO protocols showed a statistically significant difference for the cows that had preovulatory follicles ≥16 mm in diameter at GnRH2 (P=0.04; Table 5).

Table 4. Effects of the number of lactations on pregnancies per AI (P/AI) in lactating dairy cows following synchronization with G6G or MDO protocols

	Primiparous	Multiparous	P-value *,#
G6G	44% (33/75)	34.2% (60/175)	0.35
MDO	62.7% (54/86)	42.3% (78/184)	0.04
P-value **,#	0.07	0.17	-----
Overall	54% (87/161)	38.4% (138/359)	0.01

#These analyses were done with GLIMMIX and accounted for synchrony and preovulatory follicle size at the last GnRH in OVS+FTAI in the model, *P-values for comparisons of primiparous vs multiparous, **P-values for comparisons of G6G vs MDO, OVS+FTAI - Ovsynch+Fixed-time artificial insemination, MDO - Modified Double-Ovsynch

Table 5. Effects of FDGnRH2[a] (mm) on pregnancies per AI (P/AI) following synchronization of lactating dairy cows with G6G or MDO protocols

	High (≥16)	Low (<16)	P-value *,#
G6G	41.1% (67/163)	29.8% (26/87)	0.47
MDO	58.2% (85/146)	37.9% (47/124)	0.04
P-value **,#	0.04	0.53	-----
Overall	49.1% (152/309)	34.5% (73/211)	0.03

#These analyses were done with GLIMMIX and accounted for synchrony and parity in the model, *P-values for comparisons of high vs low FDGnRH2 cows, **P-values for comparisons of G6G vs MDO, [a] Preovulatory follicle size at the last GnRH in OVS+FTAI, OVS+FTAI - Ovsynch+Fixed-time artificial insemination, MDO - Modified Double-Ovsynch

Discussion

Economic benefits can occur in dairy herds with greater reproductive efficiency due to reductions in reproductive costs, such as the costs of semen, reproductive hormones, and veterinary services, such as pregnancy diagnoses (GIORDANO et al., 2011). The current study assessed a more complex presynchronization protocol than previous studies, in an effort to achieve a more synchronous phase of the cycle at the beginning of the OVS+FTAI protocol. The presynchronization program of the MDO protocol may seem excessive, but the ultimate goal of the study is to increase the pregnancy rate by increasing the quality of the ovulatory follicle and the hormonal environment in which the follicle grows. This study enhances our understanding of fertility-limiting factors in dairy cows, and could pave the way for the design of new practical protocols to increase reproductive performance in dairy herds. This study showed that the integration of Ovsynch and Presynch protocols as a presynchronization program before OVS+FTAI can increase the pregnancy rate of cows at the first postpartum insemination compared to the G6G protocol (P=0.01).

In the recent study, Presynch was combined with the Ovsynch protocol as a presynchronization program, so the cows benefited from both protocols before implementing the OVS+FTAI program.

The higher pregnancy rate of synchronized cows in the MDO compared to the G6G protocol (P=0.05; Table 3) is presumably due to the increased quality of the oocyte, ovulatory follicle, and provision of an appropriate intrauterine environment for embryo acceptance, which are achieved following the implementation of the MDO compared to the G6G protocol.

The mean (±SEM) serum concentration of P4 at PG was higher in cows receiving MDO than in cows receiving the G6G protocol (P<0.01; Table 2). Various studies have shown that high serum concentrations of P4 in the preinsemination diestrous phase increase the quality of the oocyte, ovulatory follicle and embryo, the survival rate of the embryo, and decrease the development of co-dominant follicles and multiple ovulation (FONSECA et al., 1983; XU et al., 1997; BELLO et al., 2006; NASSER et al, 2011; RIVERA et al., 2011; WILTBANK et al., 2011; FAIR and LONERGAN, 2012; SAAD et al., 2019; CONSENTINI et al., 2021).

In the recent study, the MDO protocol was able to establish a higher pregnancy rate in primiparous cows compared to the multiparous cows (P=0.04; Table 4). It has been demonstrated that injections of GnRH stimulate the onset of ovarian cycles in anestrous cows before the implementation of the OVS+FTAI protocol (SOUZA et al., 2008; LUTTGENAU et al, 2014). Since two doses of GnRH have a greater effect on stimulating the onset of ovarian cycles in anestrous cows, the number of primiparous cows that were cyclic at the time of the onset of the OVS+FTAI protocol was probably higher in the MDO compared to the G6G protocol.

As the number of previous parturitions increases in cows, the prevalence of reproductive and metabolic disorders also increases (DE KRUIF, 1978; KHAN et al., 2016; GETAHUN et al., 2021). For this reason, in relation to all the cows in the present study (n=520), the pregnancy rate of the primiparous cows was higher than that of the multiparous cows (P=0.01; Table 4).

It has been demonstrated that by increasing the number of injections of GnRH and PGF2α before the implementation of the OVS+FTAI protocol, the health status of the uterus will be increased in cows (LE BLANC et al., 2002; LUTTGENAU et al., 2014). An alternative approach for the treatment of clinical and subclinical endometritis is the use of the Presynch or Ovsynch protocol (MOREIRA et al., 2000; ADNANE et al., 2017; WAGENER et al., 2017). The beneficial effects of GnRH and PGF2α injections on improving uterine health status can be seen in the increase in the pregnancy rate of multiparous cows in the MDO compared to the G6G protocol, although this increase was not statistically significant (P=0.17; Table 4).

In the present study, in the MDO protocol the ovulation and pregnancy rates for cows that had a preovulatory follicle ≥16 mm in diameter at GnRH2 were significantly higher than in cows that had a preovulatory follicle <16 mm in diameter at the same time (P<0.01 and P=0.04, respectively). The results of the study by BELLO et al. (2006) showed that the highest pregnancy rate, based on the diameter of the preovulatory follicle at the time of the last GnRH injection in the OVS+FTAI protocol, was obtained in cows with a preovulatory follicle size of 16 mm in diameter (P<0.05). It was demonstrated in another study that the pregnancy rate was higher in cows that ovulated follicles of intermediary size (15–19 mm, 47%) as compared to those ovulating smaller (<14 mm, 36%) or larger (>20 mm, 38%) follicles (SOUZA et al., 2007). Although in relation to the G6G protocol in the present study, the pregnancy rate of the cows with a preovulatory follicle ≥16 mm in diameter at GnRH2 was higher than that of the cows with a preovulatory follicle <16 mm in diameter at the same time, this difference was not statistically significant (P=0.47; Table 5). Although the size and function of the preovulatory follicle at the time of the last GnRH injection in the Ovsynch protocol seem to play important roles in pregnancy success, many other known and unknown factors, including the intrauterine environment and the postovulatory hormonal environment, contribute to pregnancy success (IRELAND and ROCHE, 1982; BELLO et al., 2006; WALKER et al., 2012; RAMOS et al., 2015). The results of the current study suggest that the MDO protocol is likely to be more effective than the G6G protocol in enhancing the quality of the ovulatory follicles, oocytes, and the provision of an appropriate uterine environment for pregnancy survival.

It can be said that the MDO was more effective in comparison to the G6G protocol for increasing the pregnancy rate because, presumably, it had more beneficial effects on the cows that had inactive ovaries or experienced postpartum uterine infections.

Conclusions

Integration of the Ovsynch and Presynch protocols as a presynchronization program before the implementation of the Ovsynch and timed artificial insemination program could increase the pregnancy rate in lactating dairy cows at the first postpartum insemination compared to the G6G protocol, due to the improvement of synchronization in ovarian events, presumably, induction of ovulation in anovular cows, improvement of the quality of the ovulatory follicles and oocytes, and provision of an appropriate intrauterine environment for embryonic survival and development.

Ethics approval

All procedures, including hormonal injections, blood sampling, TAI, ultrasonographic examination of the ovaries and uterus were approved by the Research Ethics Committee of Kermanshah University of Medical Sciences (Approval ID: IR.KUMS.REC.1399.447).

Declaration of competing interest

All authors declare that they have no conflicts of interest

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KOHSARI, H., K. BERENJIAN, S. HEYDARIAN: Poboljšanje reproduktivne sposobnosti nakon primjene modificiranog protokola Double-Ovsynch u usporedbi s protokolom G6G u holštajnskih krava u laktaciji. Vet. arhiv 94, 353-364, 2024.

SAŽETAK

U radu je istražena nova metoda predsinkronizacije, kombinacija protokola *Presynch* i *Ovsynch*, prije primjene protokola *Ovsynch* s ciljanim vremenom umjetne oplodnje (OVS + FTAI) kao modificiranog protokola *Double-Ovsynch* (MDO). Nova metoda uspoređena je s protokolom G6G. Holštajnske krave u laktaciji u pokusnim skupinama G6G (n = 250) i MDO (n = 270) bile su pod sljedećim terapijskim protokolima: PGF2α-2d-GnRH(pre-GnRH1)-6d-OVS+FTAI (GnRH(GnRH1)-7d-PGF2α(PG)-2d-GnRH(GnRH2)-18h-FTAI) i PGF2α-7d-GnRH-7d-PGF2α-3d-GnRH(pre-GnRH1)-7d-OVS+FTAI. Prosječna je vrijednost dana laktacije (DIM) u vrijeme injekcije GnRH1 iznosila 75±0,08 (prosječna vrijednost ± SEM). Gravidnost je ustanovljena ultrazvučno, 32 - 38 dana nakon ciljanog vremena umjetnog osjemenjivanja (UO). Modificirani protokol *Double-Ovsynch* povećao je stopu gravidnosti u odnosu na broj umjetnih osjemenjivanja (P/AI) u odnosu na protokol G6G (P=0,01). Kod svih je krava (n=520) stopa gravidnosti bila veća kod onih čiji su predovulacijski folikuli bili promjera ≥16 mm u vrijeme injekcije GnRH2 u usporedbi s kravama koje su u isto vrijeme imale predovulacijske folikule manje od 16 mm u promjeru (P=0,03). Modificirani protokol *Double-Ovsynch* povećao je prosječnu vrijednost (±SEM) serumske koncentracije progesterona (P4, ng/mL) u usporedbi s protokolom G6G (P<0,01). Zaključno, MDO je povećao stopu gravidnosti u krava pri prvoj postpartalnoj oplodnji u usporedbi s protokolom G6G, zahvaljujući indukciji ovulacije u krava koje se ne tjeraju, poboljšanju sinkronizacije ovarijskih funkcija, poboljšanju kvalitete ovarijskih folikula i oocita i osiguravanju odgovarajućeg intrauterinog okruženja za razvoj i preživljavanje embrija.

Ključne riječi: G6G; modificirani protocol; *Double-Ovsynch*; gravidnost; krave holštajnske pasmine