The effect of using GnRH or eCG, 6 days before the implementation of the Ovsynch protocol on the ovarian rebound and pregnancy rate of anestrus dairy cows in the early postpartum period

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SUMMARY

Fertility in the postpartum period in cows is negatively affected by the prevalence of inactive ovaries. The aim of the recent study was to evaluate the effects of administration of 250 μg GnRH or 750 IU eCG before the implementation of Ovsynch protocol on reproductive parameters and pregnancy rate of dairy anestrus cows. The studied cows with an average of 61.1 ± 0.08 days in milk (DIM) at the 1st postpartum insemination were randomly assigned to one of the following treatment protocols and received the following treatments: Ovsynch (GnRH-7d-PGF2α-56h-GnRH(GnRH2)-18h-Fixed-time artificial insemination(FTAI), n=87), GnRH-Ovsynch (250 μg GnRH-6d-Ovsynch, n=85), and eCG-Ovsynch (750 IU eCG-6d-Ovsynch, n=88). The pregnancy diagnosis tests were done 30-32 days after AIs by ultrasonography. Non-pregnant cows were repeatedly treated with the same protocol in their groups. The period of the study lasted until day 168 after the beginning of the experiment. The eCG-Ovsynch protocol significantly increased the ovulation rate at GnRH2 compared to the Ovsynch protocol (p=0.03). The GnRH-Ovsynch and eCG-Ovsynch protocols significantly reduced the interval between treatment and pregnancy compared to the Ovsynch protocol (p=0.04 and p=0.02, respectively). The eCG-Ovsynch protocol increased the pregnancy rate of cows at the 1st postpartum insemination compared to the Ovsynch protocol (p=0.02). For cows that were inseminated ≤60 days postpartum, there was a statistically significant difference between the pregnancy rates of cows receiving the eCG-Ovsynch compared to the cows receiving the Ovsynch protocol (p=0.03). A similar result was achieved for cows that were inseminated >60 days postpartum between eCG-Ovsynch and Ovsynch protocols (p=0.02). For the GnRH-Ovsynch protocol, there was a statistically significant difference between the pregnancy rate of cows with DIM<60 compared to the cows with DIM>60 at the 1st postpartum AI (p=0.03). A similar result was achieved in the eCG-Ovsynch protocol (p=0.05). Implementation of the eCG-Ovsynch and GnRH-Ovsynch protocols significantly reduced the interval between treatment and pregnancy compared to the Ovsynch protocol. Implementation of the eCG-Ovsynch protocol increased the pregnancy rate of cows at the 1st postpartum insemination compared to the Ovsynch protocol.

KEY WORDS

Anestrus, eCG, GnRH, Holstein cows, pregnancy rate.

INTRODUCTION

During the postpartum period, the uterus experiences involution process and the hypothalamus-pituitary-ovarian axis resumes the cyclic secretion of gonadotrophic/gonadal hormones, then the 1st ovulation occurs and the estrous cycles are repeated. In the normal postpartum period, these events are completed within 6 weeks, and 90% of postpartum cows ovulate during this period[1]. Evaluation of ovarian follicles by rectal ultrasonography allows the detection of three critical follicular diameters during follicular growth: emergence (around 4 mm), deviation (around 9 mm), and ovulation (10-20 mm). Classification of anovulatory conditions on the basis of these three critical points aids in diagnosis and treatment of the condition.

Fertility in the postpartum period in cows is negatively affected by the prevalence of anestrus[2]. The anestrus is divided into physiological and pathological types[3]. Inactive ovaries syndrome is one of the pathological types of anestrus, which is characterized by minimal follicular growth, anovulation, absence of a corpus luteum (CL), and follicular or luteal cysts on the ovaries on two consecutive examinations 7-10 days apart[3,4]. Inactive ovaries occur in 10-20% of the dairy cows in the postpartum period in a normal herd[4,5]. In cows suffering inactive ovaries, the ovarian follicles grow to the emergence stage[6]. Inactive ovaries increase the interval between calving and pregnancy and reduce calf production during the economic life of cows[7].

To reduce the incidence of inactive ovaries in a herd the puerperal diseases and negative energy balance (NEB) in the early postpartum period should be minimized[6]. Negative energy balance reduces the frequency of LH pulses, which has negative effects on the development of ovarian follicles[8]. If energy needs for production are met, hormone therapies help induce cyclicity in cows suffering inactive ovaries[5]. Different hormone therapies have been used to induce cyclicity in anestrus cows in the postpartum period, such as exogenous progestogens[9,10], eCG[11-13], estradiol[14], GnRH, and PGF2α[2,15].

Different researchers have reported different results following the implementation of various protocols to induce cyclicity in

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anestrus cows and it seems that more studies are needed to achieve comprehensive results.
The recent study hypothesized that injection of 250 μg GnRH or 750 IU eCG 6 days before the implementation of the Ovsynch protocol in postpartum anestrus cows can increase the pregnancy rate at the 1st postpartum insemination and decrease the interval between treatment and pregnancy compared to the Ovsynch protocol.
The aim of this study was to investigate the effects of injection of 250 μg GnRH or 750 IU eCG, 6 days before the implementation of the Ovsynch protocol on the induction of follicular growth and reproductive parameters in anestrus dairy cows and to compare the results with the Ovsynch protocol as the control group.

MATERIALS AND METHODS

Animals, feeding, housing

The recent study was carried out on 260 anestrus lactating Holstein cows with an average DIM of 61.1±0.08 at AI in 7 industrial farms near Tehran province, Iran, from November 2019 to May 2020 to investigate the effects of treatments on the induction of cyclicity and pregnancy rates of cows. Ultrasonographic examinations of the ovaries were performed at the time of the selection of the cows and 7-10 days later by two expert veterinary practitioners. Cows without follicular and luteal cysts, CL, and large follicles (>10 mm) on the ovaries in both consecutive examinations were assigned to the study as anestrus cows suffering inactive ovaries[2]. The selected cows had no complications such as dystocia, retained placenta, lameness, clinical and puerperal metritis, clinical mastitis, respiratory, and digestive system diseases following the latter parturition. The cows had free access to freshwater and were fed twice a day with a total mixed ration consisting of corn and alfalfa silages, hay as forage, soybean meal-based concentrate, vitamins, and minerals balanced to meet requirements for lactating dairy cows. The cows were housed in free stall barns with self-catching head-locks. Free stalls were bedded with mattress and straw. The cows were milked twice daily. At the time of the onset of the treatments, the average of body condition score (BCS) for the cows was 2.75 ± 0.01 based on a 5-point scale[16]. The average of milk production for the studied cows was 35.8 ± 0.2 kg/day on the day of AI. The mean number of parities for the studied cows was 2.4 ± 0.09. Cows were divided into two subgroups based on BCS as follows: ≤2.75 and >2.75. In terms of milk yield (kg/day), on the day of AI, cows were divided into two subgroups: ≤35 and >35. In respect of the number of parities, cows were classified as primiparous and multiparous. From the viewpoint of DIM at AI, cows were divided into two subgroups: ≤60 and >60. In terms of the diameter of the largest follicle at GnRH2 (mm), cows were divided into two subgroups: ≤13 and >13. From the viewpoint of the interval between treatment and pregnancy (day), cows were divided into two subgroups: ≤80 and >80.
All treatments including hormonal injections, AIs, and ultrasonographic examinations were done on the constrained cows. The research was conducted in accordance with the local Bioethics Committee of Medical Faculty of Kermanshah University.

Treatments

Studied cows (n=260) were stratified by BCS, parity, milk yield, and DIM and randomly assigned to one of the treatment protocols. Treatment protocols were Ovsynch, GnRH-Ovsynch, and eCG-Ovsynch. The timing of hormonal injections, ultrasonographic examinations, and FTAI for the studied cows is shown in Figure 1.
The dosage and analog of GnRH used in the recent study, in the OVS+FTAI program, were 100 μg per injection of gonadorelin acetate, GONAbreed, Parnell, Alexandria NSW 2015, Australia. The dosage and analog of PGF2α used in the recent study were 500 μg per injection of cloprostenol sodium, estroPLAN, Parnell, Alexandria NSW 2015, Australia. The analog of eCG used in this study was equine chorionic gonadotropin (Pregnecol, Bioniche, Canada). Seven technicians performed the AIs of the cows and used two types of conventional semen.

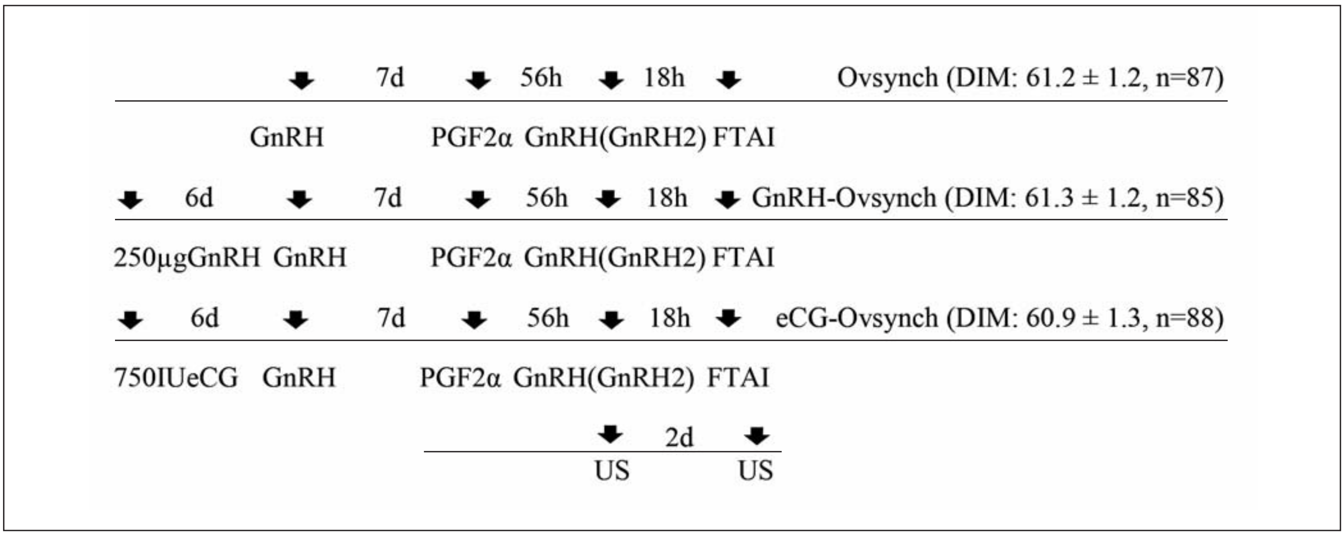

Figure 1 - Schematic image of hormonal injections, ultrasonographic examinations, and FTAI for the cows in the Ovsynch, GnRH-Ovsynch, and eCG-Ovsynch protocols. DIM=The mean(±SEM) of lactation days of cows at the time of the FTAI; FTAI=Fixed-time artificial insemination; GnRH2=The 2nd GnRH in Ovsynch+FTAI protocol; US=Ultrasonographic examination.

Measurement of follicle diameter, ovulatory response, and pregnancy diagnosis

Ultrasonographic examination of the ovaries was done with a 7.5 MHz linear probe (Aloka SSD-900V, Aloka Co. Ltd., Wallingford, CT) at GnRH2 and 2 days later to measure the diameter of the largest follicle present on the ovaries and to investigate the ovulatory response to GnRH2 injection, respectively. Perpendicular cross-sections of the antrum of the largest follicle >4 mm in diameter were measured using a built-in caliper. Follicular measurements were recorded and mean follicular diameter was obtained by averaging both cross-section measurements for each follicle. The ovulatory response was characterized by the absence of a pre-ovulatory follicle on the ovaries 2 days after GnRH2 injection. The pregnancy diagnosis tests were done 30-32 days after AIs. The cows that were diagnosed in standing heat in the AI to pregnancy diagnosis test interval were regarded non-pregnant and were inseminated at the proper time based on a.m.-p.m. rule. Non-pregnant cows were repeatedly treated with the same protocol in their groups. The period of the study lasted until day 168 after the beginning of the experiment.

Statistical Analyses

Pregnancies per AI as binomially-distributed data were analyzed by logistic regression, using the GLIMMIX procedure of SAS (SAS Institute, 2002-2003). Explanatory variables considered for inclusion in the model were treatment, BCS (categorized as ≤2.75 or >2.75), parity (primiparous vs multiparous), milk yield (categorized as ≤35 or >35 kg/day), DIM (categorized as ≤60 or >60 days), diameter of the largest follicle at GnRH2 (categorized as ≤13.1 or >13.1 mm), farm, technician, month of AI, semen type, and interactions. Classification of the studied variables (BCS, milk yield, DIM, diameter of the largest follicle at GnRH2) into two subgroups was done based on the median determination. The final logistic regression model removed variables by a 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, DIM, and interaction between treatment and BCS. A univariable analysis with PROC GLIMMIX was used for analyses of treatment effects on BCS, parity, milk yield, DIM, diameter of the largest follicle at GnRH2, ovulation at GnRH2, interval between treatment and pregnancy, and overall pregnancy rate (Table 1).

The mean interval between treatment and pregnancy was calculated based on the mean days between the implementation of the OVS+FTAI protocol and conformation of pregnancy in the ultrasonographic examination for each treatment group. The overall pregnancy rate was determined based on the ratio of the number of pregnant cows to the total number of cows at the end of the experimental period in each treatment group.

RESULTS

There was no difference between the mean of BCS, parity, milk yield, DIM, diameter of the largest follicle at GnRH2, and overall pregnancy rate between treatment groups (Table 1).

Ovulation rate of cows at GnRH2 was increased significantly following the implementation of the eCG-Ovsynch protocol compared to the Ovsynch protocol (p=0.03, Table 1).

Table 1 - Mean (±SEM) effects of studied protocols on BCS, parity, milk yield, DIM, diameter of the largest follicle at GnRH2, ovulation at GnRH2, interval between treatment and pregnancy, pregnancy rate following the 1st AI, and overall pregnancy rate in anestrus lactating dairy cows.

	Studied parameters								
Studied groups	BCS	Parity	Milk yield (kg)	DIM	Diameter of the largest follicle at GnRH2 (mm)	Ovulation at GnRH2	Interval between treatment and pregnancy (day)	Pregnancy rate following the 1st FTAI$^{\#}$	Overall pregnancy rate
Ovsynch (n = 87)	2.78 ± 0.02	2.4 ± 0.09	35.9 ± 0.58	61.2 ± 1.2	11.7 ± 0.4	30/57 (34.4%)^{a}	93.5 ± 3.5^{a}	24/63 (27.5%)^{a}	72/15 (82.7%)
GnRH-Ovsynch (n = 85)	2.73 ± 0.02	2.38 ± 0.08	36.7 ± 0.53	61.3 ± 1.2	12.4 ± 0.3	40/45 (47%)^{ab}	84.8 ± 2.7^{b}	35/50 (41.1%)^{ab}	76/9 (89.4%)
eCG-Ovsynch (n = 88)	2.76 ± 0.02	2.43 ± 0.06	34.9 ± 0.62	60.9 ± 1.3	13.5 ± 0.3	51/37 (57.9%)^{b}	81.3 ± 2.6^{bc}	42/46 (47.7%)^{b}	80/8 (90.9%)
Total (n = 260)	2.75 ± 0.01	2.4 ± 0.09	35.8 ± 0.2	61.1 ± 0.08	12.5 ± 0.2	121/139 (46.5%)	86.5 ± 1.9	101/159 (38.8%)	228/32 (87.6%)
Significance	NS†	NS††	NS†††	NS††††	NS†††††	*	**	***	NS††††††

GnRH-Ovsynch=Injection of 250 µg GnRH 6 days before implementation of the Ovsynch and fixed-time artificial insemination program; eCG-Ovsynch=Injection of 750 IU eCG 6 days before implementation of the Ovsynch and fixed-time artificial insemination program; BCS=Body condition score; DIM=Days in milk; NS: Not significant; FTAI=Fixed-time artificial insemination. $^{\#}$This analysis was done with GLIMMIX and accounted for DIM. ^{abc}Different superscripts in each column show significant difference. †Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.43, p=0.67, and p=0.61, respectively; ††Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.52, p=0.43, and p=0.24, respectively; †††Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.55, p=0.59, and p=0.64, respectively; ††††Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.53, p=0.56, and p=0.65, respectively; †††††Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.18, p=0.08, and p=0.13, respectively; ††††††Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.32, p=0.11, and p=0.54, respectively; * Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.09, p=0.03, and p=0.23, respectively; ** Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.04, p=0.02, and p=0.52, respectively; ***Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.06, p=0.02, and p=0.24, respectively.

Implementation of the GnRH-Ovsynch and eCG-Ovsynch protocols significantly reduced the interval between treatment and pregnancy compared to the Ovsynch protocol (p=0.04 and p=0.02, respectively, Table 1).

Pregnancy rate of cows at the 1st postpartum insemination was significantly higher in the eCG-Ovsynch compared to the Ovsynch protocol (p=0.02, Table 1). There was a tendency to increase the pregnancy rate of cows at the 1st postpartum insemination following the implementation of the GnRH-Ovsynch compared to the Ovsynch protocol (p=0.06, Table 1).

The effect of DIM on pregnancies per AI (P/AI) of cows following the implementation of Ovsynch, GnRH-Ovsynch, and eCG-Ovsynch protocols is shown in Table 2.

For the cows with DIM≤60 at the 1st AI the pregnancy rate of cows in the eCG-Ovsynch protocol was significantly higher than the Ovsynch protocol (p=0.03, Table 2).

For cows with DIM>60 at the 1st AI, the implementation of the eCG-Ovsynch protocol could significantly increase the pregnancy rate of cows compared to the Ovsynch protocol (p=0.02, Table 2).

For GnRH-Ovsynch protocol, there was a statistically significant difference between the pregnancy rate of cows with DIM<60 compared to the cows with DIM>60 at the 1st postpartum AI (p=0.03, Table 2). A similar result was achieved in the eCG-Ovsynch protocol (p=0.05, Table 2).

DISCUSSION

In the recent study, there was a tendency to increase the diameter of the largest follicle at GnRH2 following the implementation of the eCG-Ovsynch protocol compared to the Ovsynch protocol (p=0.08). Various studies have shown that eCG, with FSH-like effects, has beneficial effects on the growth and development of the ovarian follicles in cows[12,17,18]. It was demonstrated in the recent study that the ovulation rate of cows at GnRH2 was increased significantly following the implementation of the eCG-Ovsynch protocol compared to the Ovsynch protocol (p=0.03). Although the implementation of the GnRH-Ovsynch protocol increased the ovulation rate at GnRH2 compared to the Ovsynch protocol, this increase was not statistically significant (p=0.09). The growth rate of the follicles was increased following eCG administration and presumably, the grown follicles had a higher quality and a greater ability to secrete enough estrogen to induce an LH ovulatory surge. Although the injection of 250 µg of GnRH stimulated the growth of the ovarian follicles too, it seems that 750 IU eCG was more effective in that regard. It has been shown that the effect of GnRH on the growth of the ovarian follicles is exerted through the effect of GnRH on the anterior pituitary gland and the release of FSH and LH hormones[19,] while eCG binds directly to follicle receptors and exerts its FSH and LH like effects[20]. The half-life of GnRH is about 5 min[21], FSH is about 5 hrs[22], LH is about 40 hrs[23], and eCG is about 3 days[24] in cows. Due to the fact that the eCG has effects similar to both FSH and LH hormones and has a longer half-life than both of them, it can stimulate the growth of ovarian follicles for a longer period than GnRH. The longer half-life and greater effects of eCG on follicles compared to the GnRH make it possible for more follicles to grow and ovulate at GnRH2 following the implementation of the eCG-Ovsynch compared to the GnRH-Ovsynch protocol.

It was found in the recent study that the implementation of the GnRH-Ovsynch and eCG-Ovsynch protocols significantly reduced the interval between treatment and pregnancy compared to the Ovsynch protocol (p=0.04 and p=0.02, respectively). Postpartum pregnancy depends on the ovarian rebound, macroscopic and microscopic uterine involution, and the production of a qualified ovulatory follicle[2]. It seems that both the GnRH-Ovsynch and eCG-Ovsynch protocols had more success compared to the Ovsynch protocol in achieving the prerequisites for pregnancy at a shorter postpartum period. This result is consistent with the results of a study that showed the use of 400 IU eCG in the form of a CIDR-Ovsynch protocol in anestrus dairy cows reduced the interval between calving and pregnancy compared to the cows that did not receive eCG in the same protocol (p<0.01)[17]. Another study showed that injection of 1000 IU eCG 3 days before the implementation of the Ovsynch protocol significantly reduced the interval between calving and pregnancy in anestrus dairy cows compared to the Ovsynch protocol (p<0.05)[13]. The results of another study showed that injecting 250 µg of GnRH to anestrus dairy cows significantly reduced the interval between calving and pregnancy compared to cows in the control group which did not receive any treatment (p<0.001)[18]. The results of the recent study showed that the pregnancy rate of cows at the 1st postpartum insemination was significantly higher in the eCG-Ovsynch compared to the Ovsynch protocol (p=0.02). There was a tendency to increase the pregnancy rate of cows at the 1st postpartum insemination following the implementation of the GnRH-Ovsynch compared to the Ovsynch protocol in the recent study (p=0.06). These results indicate the positive effects of using 250 µg GnRH or 750 IU eCG 6 days before the implementation of the Ovsynch protocol to stimulate the growth of the ovarian follicles, ovarian rebound, and producing a qualified follicle that creates a qualified CL to support the pregnancy. Various studies have shown the beneficial effects of administering different doses of eCG on the growth and development of ovarian folli-

Table 2 - Effect of DIM on pregnancies per AI (P/AI) of cows following the implementation of Ovsynch, GnRH-Ovsynch, and eCG-Ovsynch protocols.

Studied groups	DIM		P-value
	≤ 60	> 60	
Ovsynch (n = 87)	7/33 (17.5%)[a]	16/31 (34%)[a]	0.08
GnRH-Ovsynch (n = 85)	10/29 (25.6%)[ab]	22/24 (47.8%)[ab]	0.03
eCG-Ovsynch (n = 88)	17/28 (37.7%)[b]	25/18 (58.1%)[b]	0.05
Total (n = 260)	34/90 (27.4%)	63/73 (46.3%)	<0.01
Significance	*	**	————

GnRH-Ovsynch=Injection of 250 µg GnRH 6 days before implementation of the Ovsynch and fixed-time artificial insemination program; eCG-Ovsynch=Injection of 750 IU eCG 6 days before implementation of the Ovsynch and fixed-time artificial insemination program; DIM=Days in milk; NS: Not significant; [abc]Different superscripts in each column show significant difference. *Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.14, p=0.03, and p=0.23, respectively; **Difference between Ovsynch & GnRH-Ovsynch, Ovsynch & eCG-Ovsynch, and GnRH-Ovsynch & eCG-Ovsynch at the levels of p=0.13, p=0.02, and p=0.24, respectively.

cles, the production of larger ovulatory follicles, and CL function that can produce more amounts of progesterone to stimulate embryonic growth[13,17,18].

For the cows with DIM≤60 at the 1st AI in the recent study the pregnancy rate of cows in the eCG-Ovsynch protocol was significantly higher than the Ovsynch protocol (p=0.03). Equine chorionic gonadotropin could effectively induce follicular growth and ovarian rebound in this group of cows. High-producing dairy cows experience a great NEB in the early stages (DIM<60) of lactation and the NEB is reduced as more days elapse from calving[25]. Negative energy balance suppresses follicular growth in cows and along with the disruption in the ovulation process, the 1st postpartum ovulation is delayed[2]. Recent results confirm that in cows that are inseminated in a shorter postpartum period (DIM≤60), administration of 750 IU eCG 6 days before implementation of the Ovsynch causes a greater success in follicular growth, induction of ovulation, and pregnancy compared to the Ovsynch protocol. It was demonstrated in the recent study for cows with DIM>60 at AI, the implementation of the eCG-Ovsynch protocol could significantly increase the pregnancy rate of cows compared to the Ovsynch protocol (p=0.02). It has been shown that as the DIM is increased and NEB is decreased, follicular growth and the number of hormone receptors in the cells of the follicle are increased in cows[26]. As a result, by increasing the number of days since parturition the ability of the cows to respond to hormonal stimuli to induce ovarian rebound is increased, and the injection of 750 IU eCG appears to be more effective than the injection of 250 µg of GnRH in this regard. It was demonstrated in the recent study that implementation of the GnRH-Ovsynch and eCG-Ovsynch protocols to improve the pregnancy rate of anestrus cows following the 1st postpartum insemination is more effective in cows with DIM>60 compared to the cows with DIM≤60 (Table 2). The results of the recent study showed that by increasing the interval between calving and insemination in anestrus cows the chance of pregnancy was significantly increased following the implementation of the GnRH-Ovsynch and eCG-Ovsynch protocols (Table 2).

CONCLUSIONS

Implementation of the eCG-Ovsynch and GnRH-Ovsynch protocols significantly reduced the interval between treatment and pregnancy compared to the Ovsynch protocol. Implementation of the eCG-Ovsynch protocol increased the pregnancy rate of cows at the 1st postpartum insemination compared to the Ovsynch protocol. Implementation of the GnRH-Ovsynch and eCG-Ovsynch protocols to improve the pregnancy rate of anestrus cows following the 1st postpartum insemination is more effective in cows with DIM>60 compared to the cows with DIM≤60.

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