The impacts of eCG administration, 3 days before OVSYNCH on the treatment of inactive ovary of dairy cows

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SUMMARY

Anestrus due to inactive ovaries decreases the probability of breeding and pregnancy rates, and increases days open in dairy cows. The objective of this study was to study the effect of equine chorionic gonadotrophin (eCG) administered three days before ovum synchronization (OVSYNCH) protocol in anestrous dairy cows. Therefore, one hundred sixty non-pregnant cows were included in the research as a clinical trial study with days in milk 60±5 days, (BCS 3±0.25, milk yield 46±8 kg, parity 3±1, during the last September to April) with no signs of estrus and suffering from inactive ovary. Transrectal palpations of ovaries were conducted at the time of animal selection, and were repeated 10 days later with an ultrasound probe. The anestrous cows without ovarian corpus luteum (CL), persisted follicle or persisted CL, ovarian cysts and large follicles (>10 mm) on that two examinations were assigned to the study. Selected cows were treated with OVSYNVH (n=40), controlled intra-vaginal device releasing (CIDR) +OVSYNCH (n = 40), eCG + OVCSYNCH (n = 40) or placebo (n=40). Pregnancy examination was carried out on day 33± 3 after the insemination with a transrectal ultrasound probe, and non-pregnant animals were treated repeatedly with the same protocol in their groups. Results showed that the rate of large follicles (>15mm) at AI was significantly more (P<0.05), and the treatment to conception interval was lesser in eCG treated animals (P<0.05). It was concluded that the injection of eCG three days before OVSYNCH protocol decreased treatment to conception intervals and increased proportions of cows with a large follicle at AI in anestrous dairy cows.

Keywords: Anestrus, CIDR-OVSYNCH, Dairy cow, eCG, Inactive Ovary

RÉSUMÉ

Impacts de l'administration d'eCG, 3 jours avant un protocole OVSYNCH dans le traitement de l'anoestrus des vaches laitières

L'anoestrus dû à l'inactivité des ovaires diminue la probabilité de reproduction chez les vaches laitières. L'objectif de cette étude visait à mesurer l'effet de la gonadotrophine chorionique équine (eCG) administrée trois jours avant la synchronisation des ovaires (OVSYNCH) chez les vaches laitières anesthésiées. Cent soixante vaches non gestantes ont été incluses dans l'étude à titre d'essai clinique avec des vaches au jour de lactation moyen de 60±5 jours, (BCS 3±0.25, rendement laitier 46±8 kg, parité 3±1, entre septembre et avril) sans signe d'œstrus et de souffrant d'ovaire inactif. Des palpations transrectales des ovaires ont été effectuées à l'admission des animaux, et ont été répétés 10 jours plus tard après une échographie. Les vaches en anoestrus sans corps jaune ovarien (CL), avec follicule ou CL persistant, avec kystes ovariens et gros follicules (>10 mm) sur les deux examens ont été incluses à l'étude. Les vaches sélectionnées ont été réparties en 4 groupes et traitées avec OVSYNVH (n=40), éponges vaginales CIDR + OVSYNCH (n = 40), eCG + OVCSYNCH (n = 40) ou placebo (n=40). Le diagnostic de gestation a été effectué 33± 3 jours après l'insémination artificielle (IA) à l'aide d'une sonde transrectale à ultrasons. Les animaux non gravides ont été traités à plusieurs reprises avec le même protocole. Les résultats ont montré que le taux de gros follicules (>15 mm) lors de l'IA était significativement plus élevé et que l'intervalle entre le traitement et la gestation était moindre chez les animaux traités par eCG (P<0,05).

Mots-clés : Anoestrus, Vaches laitières, OVSYNCH, eCG

Introduction

Pathologic anestrous due to the inactive ovary is the most important factor producing infertility in cows after uterine infection, repeat breeder, and mastitis [1,3]. The rate of inactive ovaries ranged approximately from 12 to 46 % by many authors [20]. About 20% of dairy cows in anestrous by the start of breeding programs [36] or by 63-d after calving [39] were suffering from the inactive ovary. It is reported that 28% of cows did not ovulate, on average, by 66-d after calving [43]. This syndrome is associated with reduced conception and pregnancy rates and increased calving to conception interval. [9,22,44]. Anestrous was correlated with an 18% decrease in first-service conception rate, a 41-d increase in calving-to-conception interval, and a relative risk of the overall conception of 0.68. These adverse effects increase calving intervals, culling rate and great economic losses [15].

Ovarian resumption may commence 30 to 70 days postpartum in normal cows. Factors affecting the Hypothalamus-Pituitary–Ovarian axis can delay the ovarian rebound [15,27,46]. Periparturient accidents, plane of nutrition [7] and metabolic workload, including; negative energy balance [18], non-reasonable body condition scoring (BCS) [25,34], an increase in total protein and globulin, hypophosphatemia [30], a decrease in tri-iodothyronine concentrations and body weight loss [12] may have some determinant effects on reproductive activities and increase parturition to ovarian activity interval. Many stressful conditions such as lameness [16], high milk yield [30], pneumonia, uterine infections, [40] and clinical [14] or subclinical mastitis [41,30] were considered as risk factors in anestrous cows. Sire breed of calf influences peripartum endocrine profiles and postpartum anestrous in Brahman cows [5]. In attempts to treat the inactive ovary, many different hormonal therapies are used in anestrous dairy cows [27]. After removing the risk factors, many different hormonal treatments are prescribed in anestrous dairy cows in attempts to cause a restoration of ovarian activity [27]. Gonadotropin-releasing hormone (GnRH) [8], controlled intravaginal drug release (CIDR) [27,23] and many other progesterone-like devices are used [2] to overcome inactive ovary and anestrus. GnRH treatment can induce ovulation or turnover of dominant follicles, induced a synchronized initiation of a new follicular wave, and increased the progesterone concentration from 4 d after treatment [48]. In order to induce ovarian activity in dairy cattle with prolonged postpartum anestrus, Hussein et al, (1992) injected repeated dosages of GnRH (two injections, 1hour apart) on the first day, and 12 repeated treatments twice weekly [20]. Finally, there was no significant difference among treatment groups in the number of days from calving to estrus or the number of days open. Hanlon et al, (2005) showed that supplementation of previous treated and inseminated anovulatory anestrous dairy cows with an intravaginal progesterone-releasing device for 7 days (commencing 4 or 5 days after insemination) did not significantly improve first-service conception rate [19]. Xu et al, (2000) demonstrated that noncyclic dairy cows treated with 10 µg GnRH and a progesterone-releasing CIDR insert on day 0, and 25 mg $PGF_{2\alpha}$ with CIDR removal on day 7, followed by 1 mg estradiol benzoate on day 9 (in cows that still had not shown estrus) had higher conception rate than cows treated only with CIDR and estradiol benzoate (47% vs. 29%)[48]. Anestrous cows were usually treated with intravaginal progesterone (P4) for 7 to 8 d in combination with 2 mg of estradiol benzoate (EB) at insert insertion and 1 mg of EB 1 d after insert removal until the European Union ban on the use of estradiol on food-producing animals [23]. Many cases of failure in amelioration of inactive ovaries, and the fact that vices are used [2] to overcome inactive ovary and anestrus indicate the need for further research in this field. Equine chorionic gonadotropin (eCG) has been used in small doses (400 -600 IU) alone or within ovum synchronization methods [4] in order to induce follicular growth and ovulation. Large doses of eCG (1000 IU) specially within the current ovum synchronization protocols can induce multiple ovulation [47]. Therefore, the effect of large doses of eCG (1000 IU), before the commencement of the ovum synchronization protocols has not been evaluated and may prevent of the multiple ovulation in spite of the more potency to induction of follicular growth and ovulation.

The aim of this study was to evaluate the effect of eCG treatment in anestrous dairy cows, three days before ovum synchronization (OVSYNCH) protocol.

Materials and methods

SAMPLING PROCEDURES

One hundred sixty non-pregnant Holstein dairy cows (days in milk= 70±5) without any sign of estrus were selected from some dairy farms around Tehran (IRAN), through a clinical trial study during the last year (September 2017 to April 2018). Trans-rectal palpation and ultrasound (probe 5 MHz, iMAGO, ECM, Noveko International Inc, France) examination of ovarian follicles and corpora lutea were performed at the time of selection, and repeatedly 10 days later by an expert veterinary practitioner. Cows without ovarian cysts, persisted corpus luteum (CL) or follicle, normal CL and/or large follicles (>10 mm) in two subsequent examinations (10 days apart) were assigned to the study as anestrous animals, according to type I and II of inactive ovary, defined by Peter et al (2009) [33]. Involved cows were administered with the OVSYNCH (n=40) or the CIDR-OVSYNCH (n=40) protocol as positive control groups, the eCG-OVSYNCH (n=40) protocol as a test group, and the placebo (n=40) as a negative control group. The OVSYNCH protocol was treated with a GnRH (Cystorelin, synthetic gonadorelin, 5ml, im, Ceva Co. Malaysia) injection on day 0, a Prostaglandin $f_{2\alpha}$ ($PGf_{2\alpha}$) (Enzoprost, Dinoprost, 5ml, IM, Ceva Co. Malaysia) injection on day 7, the second GnRH injection on day 9 (56 hours after $PGf_{2\alpha}$), and a fixed time insemination at 16 hours later. In the CIDR-OVCYNCH protocol, a CIDR (Controlled Intravaginal Drug Release, Cattle Insert, Pfizer Animal Health Ltd., Auckland, New Zealand)) was inserted on day 0, simultaneous with the first GnRH injection, and removed from the vagina on day 7 at the same time with the $PGf_{2\alpha}$ injection. The second GnRH injection and the afterward-artificial insemination (AI) were the same to the OVSYNCH protocol. In the eCG-OVCYNCH protocol, an eCG (Pregnecol, 3.3 ml, 1000 IU, im, Bioniche, Canada) injection was injected intramuscularly on day 3 before the beginning of the OVSYNCH protocol. The other hormone injections and AIs were performed similarly to the OVSYNCH group. The animals in the control-placebo group were treated with 5 ml distilled water on day 0, 7, 9 of the study and inseminated only at estrus. The size of the follicles and the rates of cows with a large ovulatory follicle (>15mm) were evaluated at the time of insemination with the trans-rectal ultrasound probe. The proportions of animals with a CL were also evaluated on day 10±3 after AI with the trans-rectal ultrasound probe. Additional trans-rectal ultrasonography was conducted on day 3 after eCG injection only in the eCG + OVCYNCH group concurrent with the first GnRH injection. Pregnancy diagnosis was performed on day 33± 3 after insemination with the trans-rectal ultrasound probe, and non-pregnant animals were repeatedly treated with the same protocol in their groups. The period of the study lasted until day 160 after the beginning of the study.

INTERPRETIVE CRITERIA

Relevant reproductive indices such as the first service conception rate (Number of pregnant animals at the first AI×100÷total number of pregnant and non-pregnant inseminated animals), treatment to conception intervals, follicular size, the rates of cows with a large ovulatory follicle (>15 mm) at the time of AI, and the proportions of cows with an active CL on day 10±3 after AI (in order to determine the ovulation rates) were compared among the various groups as dependent variables. The different treatment protocols were considered as independent variables in this study. Parity, the levels of milk yield, clinical abnormalities and days in milk could affect not only on the involution of cervix and uterus, ovulation and estrous cycles, but also on the detection of estrus, conception rate, and days open, and so were considered as confounder or intervening variables in this study [14]. Therefore, in order to control and homogenize the various groups of the study, according to the confounder factors, the animals were selected and assigned to their group through a stratified sampling by the farm veterinary practitioner. Finally, body condition score (BCS) (3±0.25), the levels of milk yield (46±8 kg), parity (3±1), days in milk (70±5) and the month of the beginning of the study were homogenized among the various groups at the time of sampling.

STATISTICAL ANALYSIS

The nonparametric data including the first service conception rates, the proportion of cows with a large ovulatory follicle (>15mm) at the time of AI, and the rates of cows with CLs on day 10±3 after insemination were compared among the groups with the Chi-square and the Log-linear test. The results of the treatment to conception intervals and the follicle sizes were given as averages plus or minus the standard deviation (M ± SD). These quantitative-parametric data were first analyzed with the Shapiro-Wilk and the Kolmogorov-Smirnov tests to prove the normality of the distribution and then were compared among the groups with the ANOVA Test. Finally, the post hoc Dunnett test was used to compare the treatment to conception intervals and the follicle sizes among the groups, because the variances of the means were not equal among the groups when the homogeneity of variances was analyzed with the Levene statistic test. Statistical criteria such as confidence interval=95% and confidence level α =5% were assumed. Therefore, the levels P<0.05 were considered as significant. PASW statistics (SPSS Version 22, IBM Co., New York 10504-1722, USA) was used to analyze the variables.

Results

The findings showed that the control-placebo group was always different from treated cows in all the variables measured with the exception of the follicle size for which no difference between groups was found (table 1). First service conception rates were not significantly different among the treatment groups (Table 1). Treatment to conception intervals were significantly lower in the eCG-OVSYNCH (96.9±7.3 D) than the OVSYNCH (116.4±8.7 D) or the CIDR-OVSYNCH (129.8±21.6 D) group (P<0.05). However, the difference of the variable was not significant between the OVSYNCH and the CIDR-OVSYNCH (Table I). The proportion of cows with a follicle larger than 15 mm at AI in the eCG-OVSYNCH (75%) were significantly higher than the OVSYNCH (52.5%) or the CIDR-OVSYNCH (60%) group (P<0.05). However, this variable was not significantly different between the OVSYNCH and the CIDR-OVSYNCH (Table I). The follicular size was not significantly different among the four groups (Table I). Transrectal ultrasonography on day 3 after the eCG injection in the eCG-OVSYNCH group, concurrent with the first GnRH injection, revealed that 40% of the treated cows (16/40) had one, and 25% (10/40) had 2-5 (average= 2.6) large follicles (>10 mm) on the ovaries. However, no case of multiple follicular growth at the time of AI and multiple CL formation on day 10 after the insemination was observed in any of the groups. The proportion of cows with a CL on day 10±3 after insemination was not significantly different among the treatment groups (Table I).

Treatment Groups	Reproductive Indexes: Pregnant cows after first AI	Treatment to conception interval	Cows with a large follicle (>15 mm[†]) at AI[*]	Follicular size at AI	Cows with an active CL on day 10 after AI
	n (%)	**Days (Means ±SD)**	**n (%)**	**mm (Means ±SD)**	**n (%)**
OVSYNCH (N=40)	12 [a] (30)	129.8±21.6 [a]	21 (52.5) [a]	11.5±7 [a]	15 [a] (37.5)
CIDR- OVSYNCH (N=40)	14 [a] (35)	116.4±8.7[a]	24 (60) [a]	13±6 [a]	18 [a] (45)
eCG- OVSYNCH (N=40)	18 [a] (45)	96.9±7.3 [b]	30 (75) [b]	13±5 [a]	22 [a] (55)
Control-placebo (N=40)	5 [b] (12.5)	141±15 [c]	10 [c] (25)	11.5±5 [a]	7 [b] (17.5)

Different lowercase letters [a-c] (in each column) = significantly different group (P <0.05)., *AI= Artificial Insemination, †mm=millimeter

Table I: Effects of different treatment protocols on reproductive indexes.

Discussion

Many researchers prefer the timed AI Protocols such as OVSYNCH or CIDR-OVSYNCH instead of simple injections of GnRH, eCG, etcetera. McDougall (2010) studied the effects of treatment of anestrous dairy cows with GnRH, $PGf_{2\alpha}$, and CIDR (OVSYNCH-56+CIDR, simple OVSYNCH, COSYNCH-72+CIDR), and found that treatment of anestrous cows before the start of breeding resulted in earlier conception than no treatment, but had no effect on the final pregnancy rate [24]. The addition of CIDR to the OVSYNCH program resulted in earlier conception and in more cows with normal subsequent luteal phase lengths [24]. However, in our study, CIDR insertion in OVSYNCH protocol did not show any beneficial effect on the treatment to conception intervals and the proportions of cows with an active CL on day 10 after AI. El-Zarkouny et al, (2011) compared OVSYNCH with SELECT-SYNCH in order to overcome inactive ovaries of lactating dairy cows and found that OVSYNCH cows had greater cumulative pregnancy rates and fewer days open than control (161 ±20 vs. 258 ± 29 d), but did not differ from SELECT SYNCH (233 ± 19 d) [12]. Timed AI produced comparable fertility and superior cumulative pregnancy rates, fewer days to the first service, and fewer days open than AI at observed estrus in cows inseminated ≤100 DIM [12]. In our study control-placebo group with an AI at observed estrus showed a lesser conception rate at first service and a more treatment to conception intervals. Unlike the beneficial effects of CIDR or CIDR-OVSYNCH reported in some mentioned studies, OVCYNCH and CIDR-OVSYNCH protocol induced lesser reproductive performance than eCG-OVSYNCH in anestrous cows enrolled in our study. Jubb et al, (1989) evaluated a regimen using a CIDR and eCG as a treatment for postpartum anestrus in dairy cattle [21]. The CIDR was inserted for 7 days and the animals were treated with 400 IU of eCG (im) at the CIDR removal. There was no clinical useful difference among cows receiving the CIDR-eCG, a placebo at the CIDR removal and untreated cows in the intervals of treatment to either the first estrus or conception, the conception rates to first service or the percent of pregnant by the end of mating. However, contrary to the findings of that study, eCG had a significant effect on the first service conception rates compared only with the control-placebo group, and treatment to conception intervals compared with all other groups in our study. Administration of eCG in GnRH/PG/P4 breeding protocols improved reproductive efficiency in seasonally calving, anestrous dairy cattle [6]. ECG injection (400 IU, IM) at progesterone device (0.5 g, during 8 days from 60-70 postpartum,) removal improved ovulation rate, serum progesterone concentrations and CL area in anestrous cows [28]. In our study, eCG injection had a beneficial effect on the ovulation rats measured by the number of active CL on day 10 after AI only compared with control-placebo group. ECG (500 IU) administration on day 6 postpartum could assist the early resumption of ovarian activity by enhancing ovarian follicle growth and early ovulation in postpartum cows. However, subsequent human chorionic gonadotrophin (hCG, 500 IU) injection did not provide any more beneficial effect [37]. The injection of eCG (500 IU) on Day 6 postpartum in Holstein dairy cows reduced days to first service and calving to conception interval [45]. In a recent study, the injection of 300-IU of eCG intramuscularly on day 8 of an estrus synchronization protocol followed by timed artificial insemination resulted in an increased final follicular growth, ovulation rate, and fertility in Bos indicus (especially in primiparous) cows [38]. However, in our study, eCG injection 3 days before the OVSYNCH protocol resulted only in an increased proportion of cows with a large follicle (>15mm) at AI and a decreased treatment to conception intervals.

ECG has long-lasting luteinizing hormone (LH) and follicle stimulating hormone (FSH)-like effects that stimulate the follicular growth, and estradiol and progesterone secretion [11]. Thus, eCG administration in dairy cattle results in the recruitment of more small follicles showing an elevated growth rate, the sustained growth of medium and large follicles and improved development of the dominant and pre-ovulatory follicle. Despite the fact that doses of more than 1000 IU of eCG could induce multiple ovulations [10], no multiple ovulation cases were observed in our study. The implementation of the OVSYNCH protocol three days after the eCG injection may prevent multiple ovulation in eCG treated animals.

In the present study, the proportion of cows with large ovulatory follicles (>15 mm) at AI was significantly more in the eCG treated (75%) than the OVSYNCH (52.5%), the CYDR-OVSYNCH (60%) or the control-placebo group (25%). However, the size of follicles at AI showed no difference among eCG treated (13± 5 mm), OVSYNCH (11.5± 7 mm), CIDR-OVSYNCH (13± 6 mm) and the control-placebo (11.5 ± 3 mm) group. In a recent study, suckled Bos Taurus beef cows treated with 400 IU eCG had significantly larger follicles (15.1 ± 0.3 mm) than 300 IU eCG (14.0 ± 0.2 mm) and no treated-control animals (13.5 ± 0.3) [32]. In our study, ECG, as like as many other studies had a significant impact on some reproductive indexes. However, the lack of eCG positive effects in our study that observed in some other studies may be due to some more effective factors such as metabolic defects, and strong stresses (heat stress, lameness, mastitis). Effects of age, milk yield and clinical abnormalities [14] on the detection of estrus, conception rate, and days open were studied by many researchers and should be considered as a confounder and conflicting factors. In the present study, the levels of milk yield, BCS, parity and the month of the beginning of the study were the conflicting factors and so were controlled and homogenized via stratified sampling. In a recent study, additional 400 or 600 IU eCG in timed breeding protocols (D0–P4 device insertion, with estradiol benzoate, D8–P4 device removal, $PGF_{2\alpha}$ and 400 or 600 IU eCG) was inefficient to alter follicular and luteal dynamics and increase pregnancy per AI in high-producing dairy cows [13]. The author discussed that the lack of positive effects of eCG in that study could be explained by the small percentage of cows with poor body condition score and lesser incidence of anestrus. ECG has usually not improved fertility except in cows with inactive ovaries, suffering from anestrus, or cows with low BCS [35,42]. Some researchers reported that in situations with greater negative energy balance or other physiological situations that would reduce LH pulses, a follicular growth stimulus with eCG could improve outcomes with OVSYNCH [47]. In a recent study, heat-stressed and high-producing dairy cows on a single commercial farm were given an eCG (500 UI, n = 214) on days 11-17 after calving did not show improved ovarian activity, ovulation rate, and fertility as long as 60 days after AI [31]. On the other hand, Freick et al., (2017) showed a lack of effects of an eCG administration between days 9 and 15 postpartum on the reproductive performance in a Holstein dairy herd [17]. In our study, the lack of a treated group with the common doses of 300 to 600 IU was a shortcoming of the administered treatment method. The cause of this lack was the insufficient number of assigned animals. Pessoa et al (2016) showed that the different doses of 300 or 400 IU of eCG injected at progesterone (P4) device removal in suckled Bos Taurus beef cows were equally efficient to improve pregnancy to artificial insemination [32]. As the same as many other studies, the present study was not performed based on the power of the test (ß statistics criterion) and sample size; and so had a restricted importance. In the present study, the first service conception rates and the rates of cows with an active CL on day 10 after AI in the eCG-OVSYNCH group were non-significantly higher than OVSYNCH or CIDR-OVCYNCH treated cows. However, the proportions of a large follicle (>15 mm) at AI were significantly higher in the eCG-OVSYNCH compared with OVSYNCH or CIDR-OVSYNCH group. In addition, treatment to conception intervals were significantly lower in eCG-OVSYNCH treated compared with OVSYNCH or CIDR-OVSYNCH administered cows. Probably, the positive effect of eCG on the follicular growth, ovulation and CL function reduced treatment to conception intervals. Luteotrophic [29] and microvascularization effects of eCG on bovine LCs have been reported in some studies [26], and so it may improve maintenance of pregnancy in treated animals.

Conclusion

Based on the findings of our study, it was concluded that the administration of eCG three days before OVSYNCH in anestrous dairy cows due to inactive ovaries decreased treatment to conception intervals and increased proportions of cows with a large follicle at AI. This protocol has not had a beneficial effect on the follicl size at AI, first service conception rates and proportions of cows with an active CL on day 10 after AI.

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Conflicts of interest

The author has no conflicts of interest.

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