



New Approach for Improvement of the First Insemination Conception rate in Ovsynch Treated Holstein Dairy Cows by Use of Estradiol Benzoate and Antioxidants

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ABSTRACT

The aim of the current study was conducted to improve the first insemination conception rate in Holstein dairy cows by the use of estradiol benzoate and antioxidants in conjunction with ovsynch program (GPG). A total number of 160 cyclic dairy cows (55-67 day in milk, 4-8 years old, 2nd to 5th lactations) were divided into 4 groups: G1 (n=40) treated with ovsynch program (control group). G2 (n=40) injected with 1mg estradiol benzoate i.m at the time of PGF₂ α injection in ovsynch protocol, G3 (n=40) injected with 15ml of vitesel (vit.E-Se) i.m at the time of PGF₂α injection in ovsynch protocol, G4 (n=40) injected with 1mg of estradiol benzoate plus 15ml vitesel i.m at the time of PGF₂α injection in ovsynch protocol. The expression and intensity of estrus signs were studied using behavioral and physiological changes. The conception rate after first insemination examined using transrectal ultrasonography on the 30th day from insemination and pregnancy rate was confirmed rectally after 60 days from insemination. The obtained results showed that total cows expressed estrus in G2 (ovsynch plus estradiol benzoate) and G4 (ovsynch plus estradiol benzoate and E-selenium) had highly significantly incidence (87.5% and 95.0%) compared with the cows of G1 (ovsynch group) and G3 (ovsynch plus E-selenium) 52.5% and 60% respectively P < 0.0001. The G2 and G4 had significant incidence of intense estrus signs (82.9% and 84.2%) compared with cows of G1 and G3 (57.1% and 62.5%) p = 0.040.

Also, it was noted that the G2 and G4 had highly significant incidence of first insemination conception (75.5% and 80%) versus (32.5% and 40%) in cows of G1 and G3 P < 0.0001. the pregnancy rate in G2 and G4 had highly significant incidence (67.5% and 80%) versus (30% and 40%) in cows of G1 and G3 P < 0.0001. Moreover, all cows showed intense estrus signs had 100% first insemination conception rate compared with 6.7% in weak estrus signs. It can be concluded that the beneficial effects of injection of estradiol benzoate and antioxidant with ovsynch program improve the reproductive efficiency through intensified signs of estrus and increase secretary activity of the tubular tract. These treatments improve the first insemination conception, pregnancy rates and enhances fertility in postpartum Holstein dairy cows.

Key words:

ovsynch – Estradiol benzoate – antioxidants – estrus intensity – conception rate – dairy cows.

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1. INTRODUCTION:

Fertility in dairy cows has declined over the past several years as milk production per cow has increased. The high milk production in dairy cows, led to a decline in the reproductive performance, lower circulating steroids, decline in estrus expression behavior and fertility (Lopez et al. 2004). The decreased reproductive efficiency in high producing dairy cattle is related to failure of estrus expression and detection of estrus (Walsh et al.

2011). The failure of estrus detection is the main reason for increased calving interval, failure of AI programs in dairy herds (Rounsaville et al. 1997). The problems associated with estrus detection and increased AI submission rates, researches has developed several synchrony programs based on timed artificial insemination such as ovsynch protocol (Pursley et al. 1995). Although ovsynch program improved AI submission rates, pregnancy

per AI remain similar to that in cows inseminated during normal estrus (Pursley et al. 1997b). Lower percentage of high producing cows showed estrus and fertility after application of ovsynch may be due to the lower circulating estradiol concentration near AI (Souza et al. 2007).

Vitamin E + Se are micronutrients act as antioxidants that play an important role in general animal health, production and reproduction (Chew, 1995 and Celi, 2011). Antioxidants improves the immune status, enhances the humoral and cellular immune response and combat infection (Chew, 1995). Vitamin E +Se are powerful free radical scavenger and prevent oxidative stress which decrease viability and development of the embryo. Oxidative stress and maternal low blood progesterone level during early embryonic development are a major cause of decreased pregnancy rate in dairy cows (Agarwal et al. 2006 and Tamura et al. 2008).

Therefore this study was conducted to improve the first insemination conception rate in Holstein dairy cows by the use of estradiol benzoate and antioxidants in conjunction with ovsynch program.

2. MATERIALS AND METHODS

This study was carried out on well managed Holstein dairy cows belongs the 2nd station of Al-biomey private dairy farm in Gamasa area at Dakhlia province during the period from October 2015 to May 2016. All cows free from infectious and contagious disease as proved by local veterinary authorities, they received periodical prophylactic treatment against internal and external parasites in addition to vaccination against brucellosis, lumpy skin disease, foot and mouth disease, ephemeral fever and respiratory complex virus.

160 cyclic dairy cows (55-67 day in milk (DIM), 4-8 years old, 2nd to 5th lactations) were selected for the present work. These cows were milked three times daily with an average daily milk yield 25-28 kg/head. The cows housed in open yard provided with shed according to milk yield as routine management in the farm.

All cows were fed total mixed ration (TMR) according to NRC recommendations (2009) based on corn silage, ground yellow corn, soya bean, wheat bran, cotton seed cake, barseem or hay and minerals-vitamins supplementations, all cows had free access of water.

All cows were in excellent health and the body condition score (BCS) ranged from 3.25 - 3.50 was

determined on a scale from (1-5) with a 0.25 unit increment according to (Edmonson et al. 1989).

The selected cows were clinically normal, free from dystocia, retained fetal membranes, endometritis and metritis. Moreover, cows free from subclinical and clinical mastitis, metabolic and digestive disorders and lameness. They had not any disorders in their reproductive tract.

The chosen cows were rectally examined before the start of the experiment showing normal size ovaries and the ovarian findings revealed that presence of growing follicles (10-15mm) in diameter plus corpus luteum (15-20mm) in diameter. Cows without ovarian structure were excluded from the experiment. Also, the tubular tract were checked rectally and vaginally revealed normal findings.

The cows were randomly grouped at 55-67 DIM into 4 groups:

Group 1 (40 cows):

Cows were synchronized with ovsynch protocol (GPG). Each cow was injected with 2.5 ml receptal i.m (GnRH analogue, 10 μ g Buserelin acetate, Intervet international GmbH-Germany) on day 0. On the 7th day cows were injected with 2ml Estrumate i.m (PGF₂ α , 500 μ cloprostenol, schering plough animal health, USA). followed after 56 hours by the same dose of receptal i.m (Pursley et al. 1995).

Group 2 (40 cows):

Each cow was injected with 1 mg estradiol benzoate i.m folone[®] (Misr company for pharmaceutical ind.- Cairo) at the time of PGF₂ α injection in ovsynch protocol.

Group 3 (40 cows):

Each cow was injected with 15 ml of vitesel i.m (each 1ml contains, vitamin E as alpha-tocopharyl acetate 68 mg and selenium as potassium selenate 1.5 mg , Norbrook laboratories, USA) at the time of PGF₂ α injection in ovsynch protocol .

Group 4 (40 cows):

Each cow was injected with 1 mg estradiol benzoate plus 15 ml vitesel i.m at the time of PGF₂ α injection in ovsynch protocol.

The cows in 4 groups were subjected to fixed time artificial insemination (FTAI) 18-20 hours after the second receptal dose. But that exhibit estrus during application of the program were inseminated according to (AM-PM breeding rule) to maximize conception.

The fertility in the experimental cows was assessed using the following measures:

- 1- **The expression and intensity of estrus signs.**

The expression and intensity of estrus signs were studied using behavioral, physiological changes and rectal examination. Cows in heat can be classified into intense and weak estrus signs according to (Van Eerdenburg *et al.* 1996). Intense estrus signs cows showed continuous bellowing, tail raising, restlessness, nervous, stand to be mounted and/or mounting other cows, high uterine tone, clear cervical mucous discharge straining from the vulva and relaxation of os-cervix, while weak estrus signs show some restlessness, mild uterine tone and slight mucus discharge.

2- Conception rate after first insemination.

Cows were examined using transrectal ultrasonography on the 30th day from insemination using an ultrasound scanner (Ultrascan 900, Allianco, Quebec, Canada) with a linear transrectal probe (6–8MHz).

3- Pregnancy rate after first insemination,

The pregnancy was confirmed rectally after 60 days from insemination.

Statistical analysis:

The obtained data were tabulated and statistically analyzed using statistical package for social sciences (SPSS) version 24.0. Chi-square (χ^2) test was used to compare the results between

groups. Results were presented as number, percent within brackets () and their corresponding 95% confidence interval [95% CI] results were considered statistically significant when $P \leq 0.05$.

4- RESULTS:

Total cows expressed estrus signs in G1 (ovsynch group), G2 (ovsynch plus estradiol benzoate), G3 (ovsynch plus E-selenium) and G4 (ovsynch plus estradiol benzoate and E-selenium) were 52.5% (21/40), 87.5% (35/40), 60% (24/40) and 95% (38/40) respectively (table 1).

Total cows expressed estrus signs in G2 and G4 had significantly higher incidence of expressed estrus signs (87.5% and 95.0% respectively). Compared with cows of G1 and G3 (52.5 % and 60.0% respectively) ($\chi^2 = 26.47$ and $P < 0.0001$).

The cows in G1, G2, G3 and G4 exhibited intense estrus signs in 57.1% (12/21), 82.9% (29/35), 62.5% (15/24) and 84.2% (32/38) respectively and weak estrus signs in 42.9% (9/21), 17.1% (6/35), 37.5% (9/24) and 15.8% (6/38).

The G2 and G4 had significantly higher incidence of intense estrus signs (82.9% and 84.2% respectively) compared with cows of G1 and G3 (57.1% and 62.5% respectively) ($\chi^2 = 8.34$ and $P = 0.040$).

Table (1): Comparison of expression and intensity of estrus signs during ovsynch protocol among 4 treated groups in Holstein dairy cows.

	Total cows expressed estrus signs %	Intensity of estrus signs %	
		Intense estrus signs	Weak estrus signs
G1 (n=40)	21 (52.5%) ^b [37.3% - 67.3%]	12 (57.1%) ^b [36.2% - 76.3%]	9 (42.9%) ^a [23.7% - 63.8%]
G2 (n=40)	35 (87.5%) ^a [74.8% - 95.1%]	29 (82.9%) ^a [68.0% - 92.5%]	6 (17.1%) ^b [7.5% - 32.0%]
G3 (n=40)	24 (60.0%) ^b [44.6% - 74.1%]	15 (62.5%) ^b [42.6% - 79.6%]	9 (37.5%) ^a [20.4% - 57.4%]
G4 (n=40)	38 (95.0%) ^a [84.9% - 98.9%]	32 (84.2%) ^a [70.3% - 93.1%]	6 (15.8%) ^b [6.9% - 29.7%]
Chi-square value (χ^2)	26.47	8.34	
P-value	<0.0001	0.040	

Percents within the same column not sharing the same subscripts are significantly differed at $P \leq 0.05$.

Table (2): Comparison of first insemination conception rate and pregnancy rate during ovsynch protocol among 4 treated groups in Holstein dairy cows .

	Conception rate % (30d)	Pregnancy rate % (60)
G1 (n=40)	13 (32.5%) ^b [19.6% - 47.8%]	12 (30.0%) ^b [17.6% - 45.2%]
G2 (n=40)	29 (75.5%) ^a [57.5% - 84.4%]	27 (67.5%) ^a [52.2% - 80.4%]
G3 (n=40)	16 (40.0%) ^b [25.9% - 55.4%]	16 (40.0%) ^b [25.9% - 55.4%]
G4 (n=40)	32 (80.0%) ^a [65.8% - 90.1%]	32 (80.0%) ^a [65.8% - 90.1%]
Chi-square value (χ^2)	26.92	26.28
P-value	<0.0001	<0.0001

Percents within the same column not sharing the same subscripts are significantly differed at $P \leq 0.05$.

The first insemination conception rate in G1, G2, G3 and G4 were 32.5% (13/40), 75.5% (29/40), 40% (16/40) and 80% (32/40) respectively (table 2). Regarding the first insemination conception rate, it was noted that the G2 and G4 had highly significant incidence (75.5% and 80%) compared with cows of G1 and G3 (32.5% and 40% respectively) ($\chi^2 = 26.92$ and $P < 0.0001$). The pregnancy rate in G1, G2, G3 and G4 was 30% (12/40), 67.5% (27/40), 40% (16/40) and 80% (32/40) respectively (table 2).

Pregnancy rate it was noted that the G2 and G4 had highly significant incidence (67.5% and 80.0%) compared with cows of G1 and G3 (30.0% and 40.0% respectively) ($\chi^2 = 26.28$ and $P < 0.0001$).

All cows show intense estrus sings had 100% first insemination conception rate. Significantly higher first insemination conception rate ($P < 0.0001$) was observed in cows showing intense estrus signs. Lower first insemination conception rate was found in cows showing weak estrus signs (table 3, Fig. 1).

Table (3): Effect of estrus intensity signs on first insemination conception rate in Holstein dairy cows.

	Cows with Intense Estrus Signs (n=88)		Cows with Weak Estrus Signs (n=30)		P-value
	No.	%	No.	%	
Conceived	88	100.0%	2	6.7%	<0.0001
Non-conceived	0	0.0%	28	93.3%	

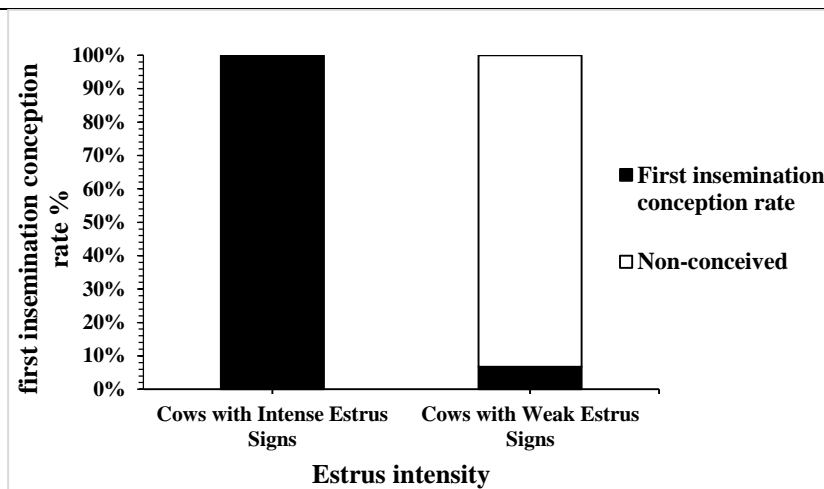


Fig. (1): Effect of estrus intensity signs on first insemination conception rate in Holstein dairy cows.

4-DISCUSSION:

Estrus synchronization is a management tool to enhance reproductive efficiency and genetic improvement in dairy cows through the use of AI. The major and dangerous problem in the reproductive management is a diminished behavioral and physiological estrus signs in lactating dairy cows (Kojima, 2003 and Jae et al., 2013). Restlessness, bellowing and mounting other cows are most frequently observed expression signs of estrus (Keown and Kononoff, 2007)

In the present study cows in G2 (ovsynch plus estradiol benzoate treated group) and G4 (ovsynch plus estradiol benzoate and E-selenium treated group) showed expressed estrus signs in 87.5% and 95% versus 52.5% in G1 (ovsynch treated group). Also, these cows in G2 and G4 showed intense estrus signs in 82.9% and 84.2% versus 57.1% in G1. Moreover, these cows in G1 and G3 showed weak estrus signs in 42.9% and 37.5% versus 17.1% and 15.8% in G2 and G4 respectively. In this respect incorporation of estradiol benzoate in ovsynch program appeared to be an effective tool to enhances estrus expression and intensity.

This occur due to injection of estradiol benzoate in ovsynch protocol sensitize the hypothalamus for induction of behavioral, physiological and intensity of estrus signs (Muhammad et al., 2015). Moreover, lactating dairy cows have an elevated estradiol metabolism that decrease the circulating level of estrogen (Sangsrivong et al. 2002 and Lopez et al. 2004). This findings cleared the incorporation of estradiol benzoate induce a preovulatory like surge of LH and ovulation (Lammoglia et al., 1998) and can exert luteolytic activity during luteal phase (Salfen et al., 1999). The positive effects of estradiol benzoate on LH secretion due to progressive increase the sensitivity of the anterior pituitary gland to gonadotropin hormone, this may be achieved by increasing GnRH receptor number (Beck and Convey, 1977; Adams and Spies 1981).

Other benefits from the addition of estradiol benzoate to the ovsynch program is the action of estradiol on the cervix and uterine muscles. In this regard (Das et al., 2009) reported that uterine tonicity and cervical canal relaxation are the two important monitors for measuring the intensity of heat. Moreover estrogen plays an important role in uterine functions, efficiency of sperm transportations, sperm capacitation and increase

fertilization (Hawk and Cooper 1978; Bathla et al. 1999).

Regarding to the conception and pregnancy rates in our experiment revealed that, 32.5% and 30.0% in G1, 75.5% and 67.5% in G2, 40.0% and 40.0% in G3 and 80.0% and 80.0% in G4 respectively. Results of the current study revealed the significantly increase in conception and pregnancy rates in G2 and G4 than in G1 and G3. This attributed to the addition of vitamin E-selenium to the estradiol benzoate in ovsynch protocol act as antioxidant, improves the general health, immune status, production and reproduction (Chew, 1995 and Celi, 2011). The oxidative stress and lower progesterone concentration in the maternal blood during the early embryonic development causing decreased conception and pregnancy rates. Moreover the oxidative process causes luteolysis of the corpus luteum (Yildiz, et al. 2015). Therefore addition of antioxidant drugs (Vit E-selenium) in our experiment improves the conception and pregnancy rates.

Concerning the relationship between the intensity of heat signs and first insemination conception rate. The present results revealed that a highly significant conception rate was obtained in cows with intense heat signs 100% versus 6.7% in cows with weak estrus signs as shown in (table 3, fig. 1). at $P < 0.0001$. higher conception rate was also obtained by Das et al., 2009 in cows showing intense heat signs compared to lower conception in cows with moderate to weak heat signs. In addition Antoli et al. 2012 reported better conception rate in cows evidenced intense heat signs and presence of cervical mucus at the time of AI. The same results were also obtained by Gunasekaran et al. 2008 recorded that O, 14.74% and 42.42% conception rates in cows showing weak, moderate and intense uterine tone respectively. They attributed their results the highest conception rates in cows due to the highest intense heat and highest uterine tone. Moreover lower conception and pregnancy rates were due to the association between weak expression of estrus signs and the incidence of delayed ovulation, reduced preovulatory estradiol concentration and poor oocyte quality (Cutullic et al. 2009).

5- CONCLUSION:

The present study indicated that the beneficial effects of injection of estradiol benzoate and antioxidants with $PGF_{2\alpha}$ in ovsynch protocol improve the reproductive efficiency through

intensified signs of estrus and increase secretory activity of the tubular tract. These treatments significantly improve the first insemination conception, pregnancy rates and enhances fertility in postpartum Holstein dairy cows.

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