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Evaluation of the Effects of GnRH and hCG Administrations during and after Insemination on Fertility Parameters of Dairy Cows

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ABSTRACT

Background: Getting pregnant and maintenance of the pregnancy in cows is crucial for the dairy farmers for sustainable production. Many methods are used for this purpose, one of them is the administration of gonadotropins at insemination and later in the early embryonic period. In this study, it was aimed to investigate the effects of different gonadotropin applications on fertility.

Methods: A total of 406 dairy cows were divided randomly into five treatment groups according to GnRH (10 µg buserelin acetate, im) and hCG (1500 IU, im) administrations in different time periods at AI and post AI as; GI (n=84, d7; hCG), GII (n=106, d7; GnRH), GIII (n=62, d0 and d7; hCG), GIV (n=80, d0 and d7; GnRH), GV (n=74, d0 and d7; placebo: 2.5 ml 0.9% NaCl). Pregnancy control was done on day 30 and 45 post AI. Fertility parameters (conception rate, first service pregnancy rate, pregnancy per AI, embryonic loss) were calculated with the obtained data.

Result: Our findings indicated that double dose hCG administration caused an improvement on the fertility parameters in cows with >150 DIM. Also, conception rates of the cows with higher parity, milk yield and lower BCS improved with the administration of hCG on d0 and d7. We concluded that double-dose hCG administrations on d0 and d7 can be used to improve fertility after 150 DIM in cows with higher parity and milk yield.

Key words: Conception rate, Dairy cow, GnRH, hCG.

INTRODUCTION

As the production volume is getting bigger day by day in dairy industry. Infertility of the cow is being one of the main problems of the producers. Farmers want their cows to conceive in the voluntary waiting period and get offspring every year. It is a well-known reality that the disruption of the pregnancy after insemination, which is one of the infertility problems in cows, is very important for dairy farmers. Declined pregnancy rates in cows are related to the failure in fertilization, embryonic mortality and foetal death (Lucy, 2001; Santos *et al*., 2004). High progesterone levels after inseminations are necessary for a successful pregnancy process (Lamming *et al*., 1989; Mann *et al*., 1995). Reduced progesterone levels are responsible for the embryonic and foetal loss during all periods of the pregnancy (Mann *et al*., 1999; Robinson *et al*., 2008; Spencer *et al*., 2008). Several methods have been tried by various authors for the maintenance of the pregnancy including gonadotropin administrations at the time of insemination or early luteal phase in cows (Nascimento *et al*., 2013; Shephard *et al*., 2014; Mendonca *et al*., 2017; Cunha *et al*., 2021).

Gonadotropins are administrated in several days of dioestrus of the cows for the embryo survival with an increase in progesterone levels (Nascimento *et al*., 2013; Shephard *et al*., 2014; Mendonca *et al*., 2017; Cunha *et al*., 2021) but it is reported administrations in the mid dioestrus (days 5-12) effect the fertility of lactating cows by increasing pregnancy per inseminations (Santos *et al*., 2001; Nascimento *et al*., 2013; Cunha *et al*., 2021). Besides that, these authors suggest that hCG treatment did not affect the fertility of the cows with higher parity (Nascimento *et al*., 2013; Cunha *et al*., 2021). On the other hand, this strategy could improve the fertility of the repeat breeder cows (Khoramian *et al*., 2011). All of these studies were based on the administration of GnRH or hCG on a certain day as at AI or 5 days after AI. The aim of this study firstly was to evaluate the effect of GnRH or hCG administration on day of AI and day 7 after AI or on both days on the fertility of dairy cows, then evaluating the fertility parameters of the cows relative to the starting day of treatments ≤150 days or >150 days in milk.

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MATERIALS AND METHODS

This study was carried out in 3 commercial dairy herds located in the central region of Türkiye. Cows with no reproductive disorders, within 60 to 350 days in milk were enrolled to the study. A total of 406 dairy cows with 133.03±68.08 DIM, 29.58±11.23 l/day milk yield, 2.72±0.21 BCS and 2.49±1.31 parity, were divided randomly into five treatment groups according to GnRH (10 µg buserelin acetate, im Receptal®, Intervet, Türkiye) and hCG (1500 IU, im, Chorulon®, Intervet) administrations on the day of AI (d0) and day 7 post AI or on both days; GI (n=84, d7; hCG), GII (n=106, d7; GnRH), GIII (n=62, d0 and d7; hCG), GIV (n=80, d0 and d7; GnRH), GV (n=74, d0 and d7; placebo: 2.5 ml 0.9% NaCl). Pregnancy control was done on day 30 and 45 post AI. The fertility parameters (conception rate, first service pregnancy rate, pregnancy per AI, embryonic loss) were calculated with the obtained data. Cows that were pregnant on Day 30 but not on Day 45 were considered to have undergone embryonic loss. Fertility parameters were calculated according to the following formulas.

Conception rate (%) =

$$\frac{\text{Number of pregnant cows after first insemination}}{\text{Number of cows with first insemination}} \times 100$$

First service pregnancy rate (%) =

$$\frac{\text{Number of pregnant cows}}{\text{Number of cows}} \times 100$$

Pregnancy per AI (P/AI) =

$$\frac{\text{Number of pregnant cows}}{\text{Number of inseminations for pregnant cows}}$$

Embryonic loss (%) =

$$\frac{\text{Number of embryonic losses}}{\text{Number of pregnant cows}} \times 100$$

While calculating the fertility parameters, the groups were evaluated by dividing them into two as the treatment started before and after 150 days in milk. Also, conception rates were evaluated in terms of parity, milk yield and BCS.

Data were analysed using IBM SPSS Statistics for Windows (Version 19.0. Armonk, NY: IBM Corp.) and differences between groups with a P-value 0.05 were considered signiûcant. Chi-square analysis were performed to compare fertility parameters and conception rates according to parity, milk yield and BCS.

RESULTS AND DISCUSSION

Although no differences were detected among DIM at the start of treatment, the highest DIM was obtained in GV cows with 140.10±6.38 days, while the lowest DIM was obtained 127.30±5.35 days in GI on the contrary. Besides that, parity number of the GII (2.27±0.09) was lower than GV (2.84±0.13). Group I animals had the highest milk yield (32.48±1.10 l/day) and lowest milk yield was obtained in GIII. The milk yield was in the G III lower than in GIV and GV but the BCS was higher in GIII than in both groups (P<0.05; Table 1).

When the fertility parameters of all cows included in the study were examined, regardless of DIM, highest CR was obtained in GIII (50.00%). Although no differences were found in FSPR, P/AI and EL between the groups (Table 2). Besides, it was observed that there was a statistically significant difference in FSPR, P/AI and EL parameters in GI (P<0.01 and P<0.001) when cows were evaluated among DIM (≤150 and >150). Significant differences were found in GII in terms of EL (P<0.001) and in GIII in terms of P/AI (P<0.001) (Table 3). Conception rate in the GIII group was found to be significantly higher (P<0.002 and P<0.05) than both GII and GV groups, with a rate of 57.69% in cows with ≥3 parity. Conception rates in both GII and GIII groups were significantly higher than GV group, with 50.00% and 66.67% in cows with a milk yield of >30 l/day (P<0.05). Furthermore, CR were found to be statistically significantly higher, again with the rates of 44.68% and 50.00%, in the GII and GIII

Table 1: Parity, DIM, Milk Yield and BCD under the groups.

	Parity	DIM (day)	Milk Yield (l/day)	BCS
GI (n=84)	2.49±0.11	127.30±5.35	32.48±1.10	2.72±0.02
GII (n=106)	2.27±0.09[a]	128.27±5.08	28.39±1.19	2.73±0.01
GIII (n=62)	2.43±0.12	135.73±7.46	26.41±2.13[a]	2.79±0.02[a]
GIV (n=80)	2.46±0.09	136.65±5.96	29.58±1.70[b]	2.67±0.01[b]
GV (n=74)	2.84±0.13[b]	140.10±6.38	29.14±1.70[b]	2.70±0.02[b]

The values are given as mean±standard errors. Different superscripts within the column indicate significant differences (P<0.05).

Table 2: Fertility parameters of the groups after treatment.

Parameter	GI	GII	GIII	GIV	GV
CR (%)	33.33	37.73	50.00	35.00	29.72
FSPR (%)	25.00	29.24	30.64	23.75	25.67
P/AI	0.67	0.81	0.56	0.72	0.67
EL (%)	0.00	2.50	9.67	0.00	4.54

There is no significant difference between the groups (P>0.05). CR: Conception rate, FSPR: First service pregnancy rate, P/AI: Pregnancy per artificial insemination, EL: Embryonic loss.

groups than in the GV group in dairy cows with <2.75 BCS (P<0.01; P<0.05). In addition, while a significant difference was found between the CR of dairy cows with <3 and ≥3 parity in GII with 44.44% and 23.53% (P<0.05), it was determined that CR was significantly lower in cows with <2.75 BCS than those with ≥2.75 in GV (Table 4).

It is a well-known strategy to use GnRH and hCG for the improvement of pregnancy rates in cows at the time of AI or after AI. Most of studies are based on improving ovulation rates and luteinization of the follicles to increase the P4 levels for supporting the embryo for a successful pregnancy (Paksoy and Kalkan, 2010; Khoramian *et al*., 2011; Nascimento *et al*., 2013; Mendonca *et al*., 2017; Cunha *et al*., 2021).

For this purpose, the gonadotropins are used in the studies were administrated between the 5th and 7th days of oestrus or early pregnancy, during the period when the follicles are sensitive to gonadotropins (Vasconcelos *et al*., 1997; Santos *et al*., 2001; Beltran and Vasconcelos, 2008; Paksoy and Kalkan, 2010; Shephard *et al*., 2014; Agarwal *et al*., 2021). GnRH administrations are effective in the presence of endogenous LH release (De Rensis *et al*., 2010) but hCG has a longer half-life (Rizkallah *et al*., 1969) and can stimulate luteinization even in follicles smaller than 10 mm (Sheffel *et al*., 1982). It also stimulates P4 production by stimulating the transformation of theca and granulosa cells into small and large luteal cells and small luteal cells into large luteal cells (Farin *et al*., 1988; Sianangama and Rajamahendran, 1992).

In this study, it was aimed to investigate how the above-mentioned effects of GnRH and hCG would have an effect on fertility parameters in cows with different regimes. Some of the studies stated that GnRH and hCG administrations both on day of insemination and on day 7 after insemination did not affect the pregnancy rates (Vasconcelos *et al*., 1997; Buttery *et al*., 2007; Agarwal *et al*., 2021) similar to our findings. However, when the data were evaluated according to the DIM, parity, milk yield and BCS of the cows, there were statistical differences between the groups. Especially, when animals with a milk yield of ≥30 /day or ≥3 parity or >150 DIM or <2.75 BCS are evaluated separately, conception rates were significantly higher in GIII (hCG; d0 and d7) than GV (placebo; d0 and d7).

According to Mendonca *et al*. (2017) GnRH administration at the time of insemination did not affect the P/AI and the rates of embryonic losses. In our study, P/AI was found to be highest in the GI group given hCG on d0 in ≤150 DIM cows. Also, in the same group P/AI was significantly higher cows with ≤150 DIM than >150 DIM, 0.91 and 0.33 respectively Many researchers reported that hCG administrations after insemination stimulates the formation of primary and accessory corpus luteum and causes an increase in luteal diameter and luteal area, but not affected the pregnancy rates (Sianangama and Rajamahendran,

Table 3: Fertility parameters of the groups after treatment among DIM.

	DIM	GI	GII	GIII	GIV	GV	P
CR (%)	≤150	35.48	36.59	43.59	34.55	29.17	NS*
	>150	27.27	41.67	60.87	36.00	30.77	NS*
FSPR (%)	≤150	32.26[a]	30.49	30.77	25.45	18.75	NS*
	>150	4.50[b]	25.00	30.43	20.00	19.23	NS*
P/AI	≤150	0.91[x]	0.88	0.77[x]	0.76	0.67	NS*
	>150	0.33[y]	0.67	0.42[y]	0.64	0.67	NS*
EL (%)	≤150	0.00	0.00[x]	5.88	0.00	0.00	NS*
	>150	0.00	10.00[y]	14.28	0.00	7.14	NS*
	P	a:b = 0.01 x:y = 0.001	x:y = 0.001	x:y = 0.001	NS*	NS*	

Different letters indicate significances. NS*: non-Significant (P>0.05). CR: Conception rate, FSPR: First service pregnancy rate, P/AI: Pregnancy per artificial insemination, EL: Embryonic loss.

Table 4: Conception rates of the groups according to parity, milk yield and BCS.

Conception rate	Parity		Milk Yield (l/day)		BCS		
	<3	≥3	<30	≥30	<2.75	≥2.75	P
GI (%)	32.65	34.29	32.14	35.71	35.56	30.77	NS*
GII (%)	44.44[x]	23.53[y,(a)]	35.56	50.00[(a)]	44.68[(a)]	32.20	x:y=0.05
GIII (%)	44.44	57.69[(b)]	46.00	66.67[(a)]	50.00[(a,b)]	50.00	NS*
GIV (%)	34.09	36.11	35.82	30.77	33.33	36.84	NS*
GV (%)	25.00	33.33[(a,c)]	33.33	17.65[(b)]	13.51[(c)]	45.95[y]	x:y = 0.01
P	NS*	a:b P<0.002 a:c P<0.05	NS*	a:b P<0.05	a:c P<0.01 b:c P<0.05	NS*	

x:y indicates differences within the group in terms of, parity, milk yield and BCS. a:b:c indicates differences between the groups. NS*: non-significant (P>0.05).

1992; Vasconcelos *et al*., 1997; Nascimento *et al*., 2013; Shephard *et al*., 2014). Similarly, in a study where hCG administrated at day 0 with insemination or on one of post insemination days; 0, 7, 14 or together, an increase in luteal diameter and area was detected, but no difference was reported in pregnancy rates (Agarwal *et al*., 2021). increasing the dose of hCG, causes an increase in the stimulation of ovulations, luteal volume and P4 levels (Cabrera *et al*., 2021). Considering the long half-life of hCG (Rizkallah *et al*., 1969), it is thought that the high conception rates obtained in GIII after may be depending on the higher doses of hCG due to repeated administrations.

Pryce and Løvendahl (1999) reported that relationship between milk yield and BCS is depending on the association between BCS and energy balance and tissue mobilization, furthermore genetically, there is a moderate to strong negative correlation between milk yield and BCS. Cows with <2.75 BCS had higher milk yield in this study. Conception rates obtained among BCS was found higher in animals with BCS <2.75 and in the group in which hCG was administered twice in agreement with Santos *et al*. (2001). Contrary to the findings of the same author, a higher rate was found in animals with high milk yield (≥30 l/day) in GIII compared to GnRH (GI) and placebo (GV) treatment. Rethmeier *et al*. (2019) reported that conception rates decreased compared to data from Germany between 1996 and 2002 and the USA in 2012 and the target for this rate was below 35%, regardless of milk yield or herd size. When the conception rates obtained in this study were evaluated according to DIM, parity, milk yield and BCS, they were found to be 43.59% and above in GIII and these data suggest that double-dose hCG administration increased CR in dairy cows with ≥3 parity or ≥30 l/day milk yield or <2.75 BCS. The findings of this study showed that hCG administrations in d0 and d7 caused an improvement in conception rates, considering the parity (≥3), milk yield (≥30 l/day) and BCS (<2.75) parameters.

Earlier pregnancy diagnosis after insemination is economically important for shortening service period (Abdullah *et al*., 2017). It has been reported that as the day of pregnancy diagnosis increases after insemination, embryonic losses gradually decrease (Vasconcelos *et al*., 1997; Santos *et al*., 2004, Mahajan *et al*., 2022). hCG is used for synchronisation of the estruses cycle, induction of ovulations or to prevent early embryonic losses (Buttery *et al*., 2007, Beltran and Vasconcelos, 2008, Rahman *et al*., 2017). It is thought that the pregnancy loss rates of 7.14% and above detected in animals with >150 DIM in GII, GIII and GIV in this study may be related to the fact that pregnancy controls were performed on the 30th and 45th days after insemination in this study. In this study, it was found that double-dose hCG administrated dairy cows with ≥3 parity in GIII had higher CR than placebo treatment in contrast with the studies in which the effects of gonadotropins, either at the time of insemination or after insemination, were used seasonally (Vasconcelos *et al*., 1997; Beltran and Vasconcelos, 2008; Mendonca *et al*., 2017) or in embryo transfer programmes (Vasconcelos *et al*., 1997) or only to increase pregnancy rates (Bartolome *et al*., 2005; Nascimento *et al*., 2013; Agarwal *et al*., 2021), presented that administrations helped to improve the luteal structure and increase P4 synthesis.

CONCLUSION

Results of this study indicated that administration of hCG or GnRH at AI or d7 post AI statistically had no effect on the fertility parameters of the study cows. However, when the 150th day in milk was taken as the limit, it was determined that double dose hCG administration caused a proportional improvement on the fertility parameters in cows with >150 DIM. Also, conception rates of the cows with higher parity, milk yield and lower BCS improved with the administration of hCG on d0 and d7. We concluded that double-dose hCG administrations on do and d7 can be used to improve fertility after 150 DIM in cows with higher parity and milk yield.

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