RESEARCH ARTICLE

The Effects of GnRH and hCG Used During and After Artificial Insemination on Blood Serum Progesterone Levels and Pregnancy Rate in Cows [1]

Zahid PAKSOY * ✎   Cahit KALKAN **

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 * General Directorate of Nature Conservation and National Parks, Ministry of Environment and Forestry, 06560 Ankara - TÜRKİYE
** Department of Obstetrics and Gynecology, Faculty of Veterinary, University of Firat, 23100 Elazığ - TÜRKİYE

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Summary

The aim of this study was to evaluate the effects of single and combined administrations of GnRH and hCG with and on the 12th day after artificial insemination (AI). Total 75 cows ranging from 2 to 3 years old were used as materials. All animals were divided into five groups randomly and inseminated in the first estrous period between days 40 and 80 after parturition. The first group received 10 µg buserelin acetate both during AI and on the 12th day of AI. The second group was treated with 10 µg buserelin acetate during AI and 1.500 IU hCG on the 12th day after AI. In the third group, 1.500 IU hCG was injected immediately and on the 12th day after AI. Cows in the fourth group was administered 1.500 IU hCG immediately and 10 µg buserelin acetate on the 12th day after AI. The last group was left as control. The pregnancy was diagnosed with rectal examination between days 45 and 60 following AI. The proportion of cows diagnosed as pregnant was 40.0% (6/15) for group 1, 4 and 5, 46.7% (7/15) for group 2 and 3. As a result of current study, it is identified that GnRH and hCG administration during and at the 12th day after AI failed in increasing pregnancy rate and caused no statistically significant alteration in progesterone levels.

Keywords: *Cow, Artificial insemination, GnRH, hCG, Pregnancy rates*

İneklerde Tohumlama Sırası ve Sonrası GnRH veya hCG Kullanımının Kan Serumu Progesteron Düzeyleri ve Gebe Kalma Oranları Üzerine Etkisi

Özet

Bu çalışmada, ineklerde GnRH veya hCG'nin tek başına veya birlikte suni tohumlama sırasında ve takip eden 12. günde kullanımının, gebelik oranları ve kan serumu progesteron düzeyleri üzerine etkisi araştırıldı. Materyal olarak, yaşları 2-3 arasında olan toplam 75 inek kullanıldı. Tüm hayvanlar, postpartum 40-80. günler arasında gösterdikleri ilk östrüste rastgele beş gruba ayrılarak tohumlandı. Birinci gruba hem tohumlamayı takiben hem de 12. günde 10 µg buserelin asetat uygulandı. İkinci gruba tohumlama anında 10 µg buserelin asetat ve tohumlama sonrası 12. günde 1500 IU hCG verildi. Üçüncü gruba; hem tohumlama anında hem de tohumlamadan sonraki 12. günde 1.500 IU hCG yapıldı. Dördüncü gruba tohumlamayla birlikte 1.500 IU hCG ve 12. günde 10 µg buserelin asetat uygulandı. Beşinci grup, kontrol grubu olarak bırakıldı. İneklerin gebelikleri, tohumlama sonrası 45-60. günlerde, rektal muayene ile teşhis edildi. Gebe olduğu teşhis edilen ineklerin oranı birinci, dördüncü ve beşinci grupta %40.0 (6/15), ikinci ve üçüncü grupta ise %46.7 (7/15) olarak bulundu Sonuç olarak, tohumlama sırası ve sonrası 12. günde GnRH ve hCG uygulamalarının gebe kalma oranlarını artırmada başarılı olmadığı ve progesteron düzeylerinde istatistiksel anlamda önemli bir değişikliğe neden olmadığı tespit edildi.

Anahtar sözcükler: *İnek, Suni tohumlama, GnRH, hCG, Gebelik oranları*

✎ İletişim (Correspondence)

☏ +90 312 2076068

✉ paksoyland@yahoo.com

INTRODUCTION

In dairy cattle farming, it is necessary to have a calf once a year and to ensure that the postpartum cows are pregnant at the first service. For this purpose, there should not be any problem in the herd regarding reproduction. Many diseases and environmental factors can decrease fertility in postpartum cows. For cows not detected in estrous; subestrous, anestrous, delayed ovulation, early embryonic death, high milk yield, negative energy balance, high environmental heat and various diseases are present as the reasons of decrease in fertility. Although a lot of studies have been done to decrease the infertility caused by these factors, this problem has not been eliminated until now [1-3].

For this reason; gonadotrophin releasing hormone (GnRH) and human chorionic gonadotrophin (hCG) have widely been used in the aim of synchronization of ovulation and estrous, especially; to stimulate luteal tissues and to control follicular waves in cows. Moreover, most of the studies are concentrated on obtaining higher pregnancy rates with fixed time insemination. In this way, some methods have been developed in order to avoid the estrous observation [4-8].

In several studies, it is emphasized that administration of GnRH and hCG before, during and post-insemination have increased the pregnancy rates and the decreased the early embryonic deaths [9-13]. Whereas; in some other studies it is claimed that administrations of GnRH and hCG reduce the pregnancy rate [14,15] or they are ineffective [16-19].

According to some studies, different results were achieved when GnRH was administered during artificial insemination (AI) and from 1st to 15th days post-AI. On the other hand, there are a few studies about the administration of hCG+hCG and GnRH+hCG during AI and between days 1 and 15 after AI [17,20].

In this study, it was aimed to investigate the effects of single administration of GnRH or hCG and their combined treatment during and on the 12th day of AI on pregnancy rates and serum progesterone levels in cows.

MATERIAL and METHODS

The present study was conducted on dairy cattle farming which belongs to Hünkar Limited Company located in Kahramanmaraş. It was started in July 2006 and finished in January 2007. Seventy-three Holstein and 2 Brown Swiss cows of 2-3 years old, between the 40th and 80th postpartum days were used, having no reproductive problem. Preceding the study, they were checked in terms of records, anamnesis, inspection and rectal examination. Cows were housed in free stall barns and they were fed and milked twice a day. During the lactation period animals were fed with corn silage, dry hay, concentrates and with the free access to water. Their body weights ranged from 300 to 400 kg. Cows detected in estrous were inseminated at the second half of estrous. These animals were divided into five groups as follows;

Group 1 (n= 15): Cows were inseminated after estrous was detected and they received 10 μg buserelin acetate (Receptal®, İntervet) intramuscularly both following AI and on the 12th day of AI.

Group 2 (n= 15): This group was given 10 μg buserelin acetate (Receptal®, İntervet) intramuscularly during AI and 1.500 IU hCG was given (Pregnyl®, Organon) on the 12th day after AI.

Group 3 (n= 15): This group was treated with 1.500 IU hCG (Pregnyl®, Organon) immediately after AI and on the 12th day of AI.

Group 4 (n= 15): Cows were administered 1.500 IU hCG (Pregnyl®, Organon) immediately after AI and 10 μg buserelin acetate was administered (Receptal®, İntervet) on the 12th day of AI.

Group 5 (n= 15): The last group was left as control. The control group was administered saline both following AI and on the 12th day after AI.

Blood was collected from the jugular vein of cows into blood collection tubes during AI and on the days 5, 10, 15, and 21 after AI. Blood samples were centrifuged at 3000 rpm for 10 min. to obtain serum. The serum was stored at - 20°C until it was assayed. Concentrations of progesterone were measured by commercial ELISA kits (Diametra, Foligno, Italy).

The pregnancy was diagnosed by rectal examination between days 45 and 60 after AI. Progesterone concentrations of the groups were compared using Kruskal-Wallis test and subgroup comparisons were performed by Mann-Whitney U test. Pregnancy rates were compared by Chi-square test [21].

RESULTS

The proportion of cows diagnosed as pregnant was 40.0% (6/15) for group 1, 4 and 5, 46.7% (7/15) for group 2 and 3. When the pregnancy rates within the groups were compared, it was found out that the difference among the groups was not significant (P>0.05) *(Table 1).*

Groups	n	Pregnancy Rates		%
		(+)	(-)	
1. GnRH + GnRH	15	6	9	40.0
2. GnRH + hCG	15	7	8	46.7
3. hCG + hCG	15	7	8	46.7
4. hCG + GnRH	15	6	9	40.0
5. Plasebo+Plasebo	15	6	9	40.0
P		—	—	—

—: *P>0.05*

DISCUSSION

It has been reported that pregnancy rates have been increased by using GnRH, GnRH analogs and hCG during AI [5,9,13]. Conversely, it has been found out to be ineffective on pregnancy rates in some other studies [16,18,22]. Moreover, it has been shown that these hormones have negative effects on pregnancy rates [14,15].

There are a few studies using GnRH during AI and on 12th day around which have different results [9,18,23,24]. Ryan et al.[18], administered GnRH to lactating dairy cows at the time of AI or 12 days later and they noted this application did not improve pregnancy rate. In a similar study by Ryan et al.[24], GnRH was injected at the time of AI or both at the time of AI and on the 12th day after AI and it was reported that there was no change regarding pregnancy rate. In another study by Çınar [9], cows were synchronized by administrating prostaglandin F_2 alpha and after the synchronization, firstly; cows were injected GnRH during AI, secondly; they were injected GnRH on the 12th day, lastly; GnRH was injected both during AI

Among the groups a significant difference was detected only on the fifth day of the fourth group pregnant animals with respect to progesterone levels (P<0.05) *(Table 2).*

No significant difference was observed among the nonpregnant animals within the groups in terms of progesterone levels (P>0.05) *(Table 3).*

Table 2. *Progesterone levels (ng/ml±SEM) of the pregnant animals in the treatment and control groups*
Tablo 2. *Uygulama ve kontrol gruplarında gebe olan hayvanların progesteron düzeyleri*

Groups	Day 0 ($\bar{X}\pm S\bar{x}$)	Day 5 ($\bar{X}\pm S\bar{x}$)	Day 10 ($\bar{X}\pm S\bar{x}$)	Day 15 ($\bar{X}\pm S\bar{x}$)	Day 21 ($\bar{X}\pm S\bar{x}$)
1	0.50±0.10	3.44±1.19 [a]	12.32±4.64	13.94±5.86	16.30±5.78
2	0.73±0.14	9.08±3.08 [a]	16.98±6.04	16.05±3.27	12.47±5.82
3	0.55±0.12	5.56±0.88 [a]	7.27±1.70	9.76±2.40	10.53±4.52
4	0.28±0.97	0.70±0.26 [b]	5.18±2.52	5.18±1.20	7.18±3.13
5	0.42±0.16	6.68±2.72 [a]	11.50±3.27	15.12±4.05	22.48±4.94
P	—	—	—	—	—

a, b: *Means in the same columns with different letters are significantly different*
 : *P<0.05* —: *P>0.05*

Table 3. *Progesterone levels (ng/ml±SEM) of the nonpregnant animals in the treatment and control groups*
Tablo 3. *Uygulama ve kontrol gruplarında gebe olmayan hayvanların progesteron düzeyleri*

Groups	Day 0 ($\bar{X}\pm S\bar{x}$)	Day 5 ($\bar{X}\pm S\bar{x}$)	Day 10 ($\bar{X}\pm S\bar{x}$)	Day 15 ($\bar{X}\pm S\bar{x}$)	Day 21 ($\bar{X}\pm S\bar{x}$)
1	0.43±0.23	2.83±1.09	5.37±0.50	3.83±1.94	2.43±1.85
2	0.20±0.14	9.95±7.50	12.15±8.41	10.83±3.04	2.15±1.82
3	0.15±0.05	0.90±0.10	2.20±1.60	1.05±0.05	0.80±0.10
4	0.00±0.00	0.20±0.20	1.40±0.90	5.60±1.00	1.15±0.35
5	4.60±2.83	10.30±3.04	15.28±3.85	19.33±5.64	11.45±5.27
P	—	*	—	—	—

— : *P>0.05*

and on the 12th day after AI, then an increase in the pregnancy rates was reported. Similarly, Lopez-Gaitus et al.[23] reported that GnRH administration during AI and after 12 days increased the pregnancy rates.

In some of the studies [11,12] GnRH administration 12 days after AI indicates an increase in pregnancy rate. In contrast, Szenci et al.[25] suggest that GnRH usage 12 days after AI does not show a difference in pregnancy rate in cows. In two different experiments [11,26] cows were divided into three groups. Buserelin (10 µg) was injected to the first group on the day of AI. Cows in the second group were injected on the 12th day after AI. For the last group cows were injected with buserelin either on the 8th day or the 10th day after AI. It was suggested that only the second group increased the pregnancy rates. In our study, although 40% of pregnancy rate was obtained in the group of GnRH administration during AI and after 12 days, no significant difference was achieved statistically when compared with the control group. This result is in parallel with the trial of Ryan et al.[24]. Although this study has higher pregnancy rates than the study of Çınar [9] and Lopez-Gaitus et al.[23] in which GnRH was administered during AI and after 12 days, our study is found to be unsuccessful in increasing pregnancy rates.

Only one study was found using GnRH during AI and hCG after AI in which [17], 836 cows and heifers were used and treated with GnRH at AI and hCG on the day 15th. In conclusion, it was reported that 65 cows out of 124 cows were pregnant (52.4%). There was no difference in the pregnancy rates between treatment and control groups. In the present study, 46.7% of pregnancy rate was obtained in the group of GnRH administration during AI and hCG after 12 days, a significant difference could not be achieved statistically when compared with the control group. Our findings were similar with the work of Lewis et al.[17] in terms of pregnancy rates.

Although hCG administration during AI and GnRH administration after 12 days to the fourth group, 40% of pregnancy rate was obtained, a significant difference could not be detected statistically compared with the control group. Also such a similar research has not been observed during the literature survey.

In some studies where both ovulation and luteal structures were stimulated in order to increase pregnancy rates, hCG was used during and after AI. In one of these studies [20], hCG was administered to four groups. In the first group, hCG was administered on the days 1, 7, and 14. In the second group, hCG was injected during and on the day 5 after AI. In the third and fourth group, hCG was used during AI and on the day 5, respectively. The highest pregnancy rate (35%) has been observed in the first group. Nevertheless a significant increase on pregnancy rates could not be observed.

In the present study, 46.7% of pregnancy rate was obtained in hCG administered group during AI and after 12 days, a statistically significant difference could not be achieved when compared with the control group. Even though our pregnancy rates are higher than that of Kharche and Srivastava [20] in which hCG was administered during AI and on the days 7 and 14 after AI, our results were similar with their findings (40 vs. 35%).

In many studies where GnRH analogs and hCG were used, it was aimed to increase the progesterone levels in serum, to decrease the early embryonic deaths and as a result to increase the pregnancy rates. In some of the studies, it was reported that in most of them progesterone levels raised whereas pregnancy rates did not change [19,27,28], it was also reported that pregnancy rates increased but progesterone levels did not rise [13,29], or pregnancy rates increased depending on the increase of progesterone levels [30,31]. In this study, no significant change was observed in the progesterone levels after the administration. However, it was found that the fifth day values of fourth group were less than the other groups. This difference can be interpreted as test error due to the pregnancy of animals. In the same way, the values of the nonpregnant animals in the control group did not show a difference but together with this, progesterone levels were above 1 ng/ml in the days 0 and 21. This can also be accepted as a test error, not coming into estrous on the day 21, or not showing any estrous signs. Moreover, in the nonpregnant animals of 1st, 2nd and 4th group progesterone levels were higher than 1 ng/ml on the day 21. The high levels of these values can be interpreted as a probable test error, not coming into estrous on the day 21, or occurrence of embryonic death in some animals.

In conclusion, it is suggested that GnRH+GnRH, GnRH+hCG, hCG+hCG and hCG+GnRH administrations during and 12 days after AI period has no effect on pregnancy rate and progesterone levels. Although it was aimed to enhance pregnancy rates depending on the increase in the concentration of blood serum progesterone and to stimulate the luteal structures and ovulation; yet desired fertility parameters were not achieved.

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