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A comparative study of HCG instead of replacing the second GnRH in Presynch protocol on the size of follicles and Estradiol concentration in the blood of Holstein dairy cow

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ABSTRACT

This research was done on cyclic Holstein Dairy Cows in Moganlivestock, industry farm where 40 cows were selected randomly 45 days after parturition;The Presynch method was used for 40 cows and they were divided to two groups. In control group in zero day,3 ml PGF2a was injected and in 14th day, the PGF2a was injected again. In 28th day the Gonadorelin was injected and in 35th day the third injection of PGF2a was done. In 37th day the second injection of Gonadorelin was done, and 16 to 22 hours later, the cows were inseminated artificially. In treatment groupthe protocol was repeated and instead of second GNRH, adose ofhumanchorionicgonadotropin was injected about 5000 unites. Both groups from 30th day of protocol were examined by ultrasonography, and the follicular progress was studied and recorded. Also from 7th to 9th days of protocol, the cows were sampled. there was significant differences between control and treatment groups in the rate of estradiol in 36th, 37th, 38th and 39th days and between the great and small diameters of follicular and through the level of greatest diameter of follicular ($p<0.05$). The rate of recorded pregnant in day 30 after insemination, 16 cows of 20 cows were pregnant and 4 cows were not pregnant while, in control group, 11 cows of 20 cows were pregnant and 9 of them were not pregnant; so 55% of the control group were pregnant while in treatment group 88% of cows were pregnant.

Keywords: Presynch, PGF2a, Gonadorelin, GNRH, HCG

INTRODUCTION

The reason for reduction of fertility in the dairy cows is not related only to increase of milk production. The epidemiologic studies show that other factors like reproduction disorders or parturition season have more influence than milk production and reproduction performance. It is obvious that milking as a physiologic process accompanies by low fertility(1). The cows with high production are healthy since they have nutritional and reproductive management. Due to high production, the reproductive cycle is delayed after parturition in these cows. Challenges in order to improve dairy cows reproductive performance involve perception of biochemical and physiologic principles of reproductive and milking and then integrated nutrition management and reproductive management systems in order to optimization of the cows fertility. Synchronization of ovulation and artificial insemination is reproductive tools management in daisy cows (2). Synchronization of different estrous phases shortens parturition season, increase calving and milking and facilitation of artificial insemination. Synchronization hormone protocols as a management tool reduces vulnerably expectation timeafter parturition in the dairy cows. Hormone protocol for ovulation in the cow by using GNRH is an effective method (3). But, it is seen that level of fertility resulted from

this protocol does not exceed 40%. One of the reasons is delay in removing ovule from follicle. Some researchers consider corpus luteum, inefficacy after fertilization as a reason for reduction of productivity. In addition to that human chorionic gonadotropin has similar properties (GNRH) but it acts like LH hormone. This hormone by attaching on LH receptors in small corpus luteum, cells increases progesterone production(4).Injection of this hormone during corpus luteum, phase causes to ovulation in the first follicular activity and form secondary corpus luteum. So it is possible that injection of progesterone hormone by HCG in different estrous phase increases plasma progesterone and as a result survival of the fetus and fertility in dairy cows. In addition treatment with CHG increases follicular three waves cycle. It is possible that such change in follicular dynamic due to number of three waves cows relative to two waves increase pregnancy rate. This hormone causes to ovulation inanition to growth of corpus luteum. The aim of this research is to compare fertility and estradiol hormone concentration in the Presynch method by replacement of HCG instead of injection of the second GnRH in the above method (5).

MATERIALS AND METHODS

Materials
1-Gonadoroline
2-HCG
3-Ultrsonogrphy system: 7.5 MH and rectal probe

This research was done on 40 cyclic Holstein Dairy Cows in the second station in Mogan livestock and industry farm. The average daily milk production was 30 kg and the cows were selected randomly 45 days after parturition. They were divided into two groups which every group included 20 cows. After control in zero day (the day after parturition) three 3 ml PGF2α was injected in every cow and in 14th day, the PGF2αwas injected again. In 28th day the Gonadorelin was injected and in 35th day the third injection of PGF2αwas done. In 37th day the second injection of Gonadorelin was done, and 16 to 22 hours later, the cows were inseminated artificially. In treatment group, this protocol was repeated and just instead of second injection of GNRH, adose of human chorionicgonadotropin by the brand of Choriomon was injected about 5000 unites. All cows of both groups (control and treatment groups) from 30th day of protocol were examined by ultrasonography Honda HS1500 with frequency of 7.5 megahertz, and the follicular progress was studied and recorded. Also from 7th to 9th days of protocol, the cows were sampled and the rate of blood's estradiol was studied, and for measuring the follicular progress (in first day of protocol and through the artificial insemination) and for conforming the pregnancy diagnosis (30days after the artificial insemination) were investigated by ultrasonography.The results are discussed in result section.

RESULTS

3-2-Comparison of mean estradiol(control) in different days
According to the figure 1-3 and Kruskal-Wallis it is seen that mean estradiol is 56.40 pg/ml in 36th day, 59.26 pg/ml in 37th day,60.42 pg/ml in 38th day,62.41 pg/ml in 39th day that there is a significant difference in mean estradiol in four studied days with x^2=65.44with p=0.000 in confidence level of % 95(p<0.05).

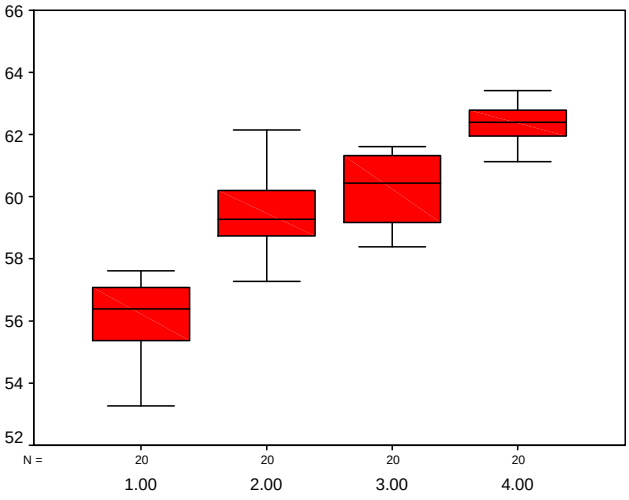

Figure 3-1:mean Estradiol in different days

3-2-Comparison of mean estradiol(treatment) in different days

According to the figure 3-2 and Kruskal- Wallis it is seen that mean estradiol is 58.63pg/ml in 36th day, 60.24pg/ml in 37th day, 57.25pg/ml in 38th day, 58.32pg/ml in 39th day that there is a significant difference in mean estradiol in four studied days with x^2=65.44with p=0.000 in confidence level of % 95(p<0.05).

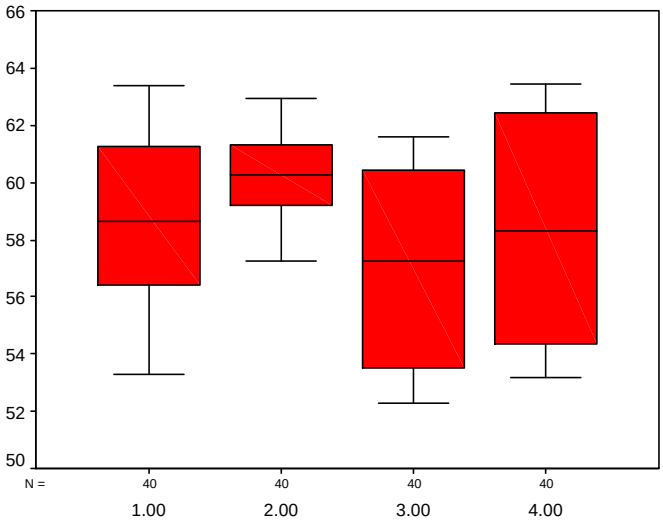

Figure 3-2: mean Estradiol (treatment) in different days

3-3-Comparison of mean estradiol(treatment and control) in day 36

According to the figure 3-3 and Mann- Whitney it is seen that mean day is 61.28 pg/ml in 36th day for estradiol(treatment) and 56.40 pg/ml in control group that there is a significant difference in mean day in two studied groups according to U=00 with p=0.000 in confidence level of % 95(p<0.05).

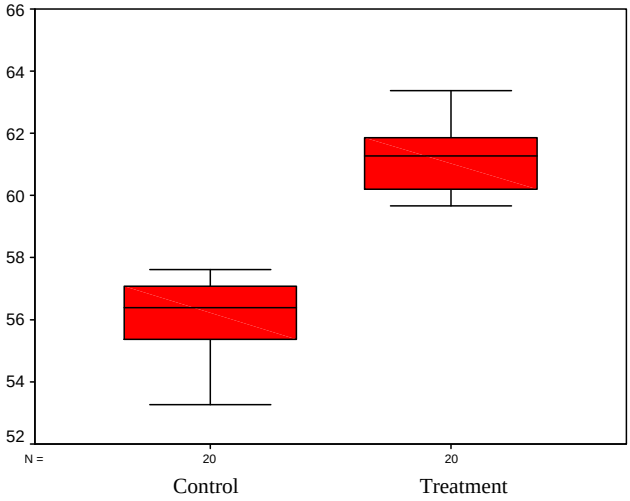

Figure 3-3: comparison of mean Estradiol (treatment and control) in day 36

3-4-Comparison of mean estradiol(treatment and control) in day 37

According to the figure 3-4 and Mann- Whitney it is seen that mean day is 61.29pg/ml in 37th day for estradiol(treatment) and 59.26pg/ml in control group that there is a significant difference in mean day in two studied groups according to U=00 with p=0.000 in confidence level of % 95(p<0.05).

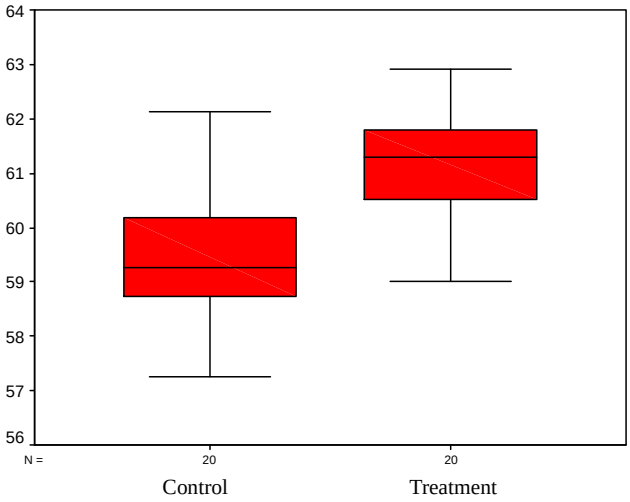

Figure 3-4: comparison of mean Estradiol (treatment and control) in day 37

3-5-Comparison of mean estradiol(treatment and control) in day 38

According to the figure 3-5 and Mann- Whitney it is seen that mean day is 53.50 pg/ml in 38th day for estradiol(treatment) and 60.42 pg/ml in control group that there is a significant difference in mean day in two studied groups according to U=00 with p=0.000 in confidence level of 95% (p<0.05).

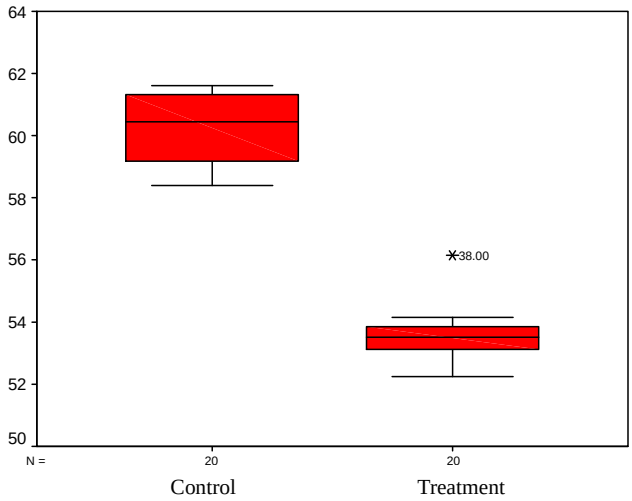

Figure 3-5: comparison of mean Estradiol (treatment and control) in day 38

3-6-Comparison of mean estradiol(treatment and control) in day 39

According to the figure 3-6 and Mann- Whitney it is seen that mean day is 54.36 pg/ml in 39th day for estradiol(treatment) and 62.41pg/ml in control group that there is a significant difference in mean day in two studied groups according to U=00 with p=0.000 in confidence level of 95% (p<0.05).

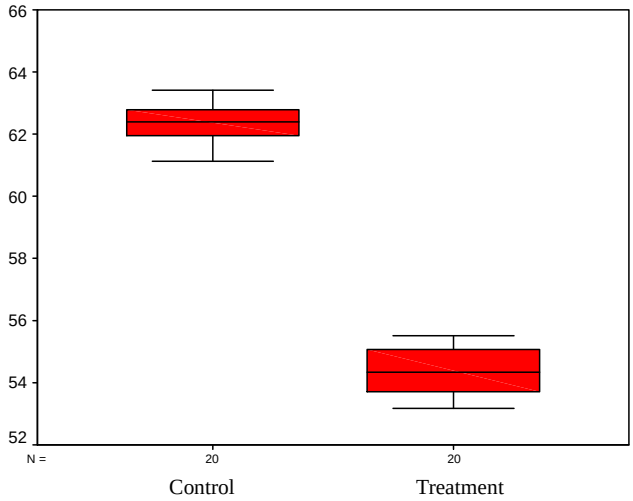

Figure 3-6: comparison of mean Estradiol (treatment and control) in day 39

3-7-Folliclelargediameter mean in control and treatment groups

According to figure 3-7 and T-test independent groups mean difference it was seen that in total follicle large diameter mean in a group received HCG was 17.13 and it was 16.16 in group that received GnRH2 that was calculated according to variance equality F=0.27 with confidence level of %95 and p=0.87 that shows the variance of both groups is equal. Thus t is used with unequal variance with t=-3.958 and p=0.00 in confidence level of %95. So,it can be said that the difference was significant in follicle largediameter mean in groups received HCG and GnRH2.

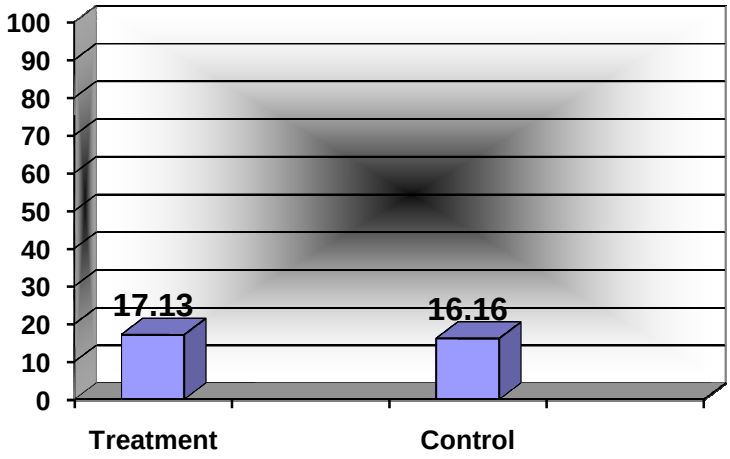

Figure 3-7: follicle large diameter mean in control and treatment groups

3-8-Follicle small diameter mean in control and treatment groups

According to figure 3-8 and T-test independent groups mean difference it was seen that in total follicle small diameter mean in a group received HCG was 23.15 and it was 22.51 in group that received GnRH2 that was calculated according to variance equality F=0.79 with confidence level of %95 and p=0.37 that shows the variance of both groups is equal. Thus t is used with unequal variance with t=-2.65 and p=0.00 in confidence level of 95%. So, it can be said that the difference was significant in follicle small diameter mean in groups received HCG and GnRH2 ($p<0.05$).

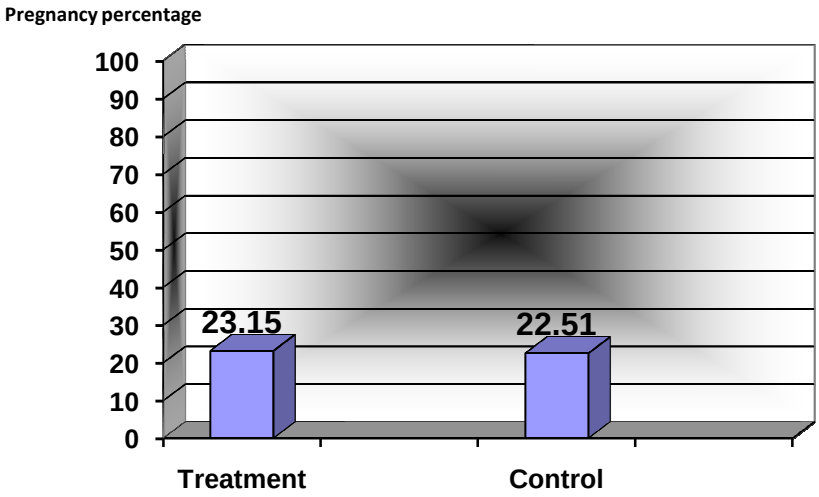

Figure 3-8: Pregnancy Percentage

3-9-Folliclelarge section level mean in control and treatment groups

According to figure 3-9 and T-test independent groups mean difference it was seen that in total follicle large and small diameter mean in a group received HCG was 310.41 and it was 289.99 in group that received GnRH2 that was calculated according to variance equality F=0.897 with confidence level of %95 and p=0.349 that shows the variance of both groups is equal. Thus t is used with equal variance with t=-2.82 and p=0.007 in confidence level of %95. So, it can be said that the difference was significant in follicle large and small diameter mean in groups received HCG and GnRH2 and this value was high in treatment group relative to control group (p<0.05).

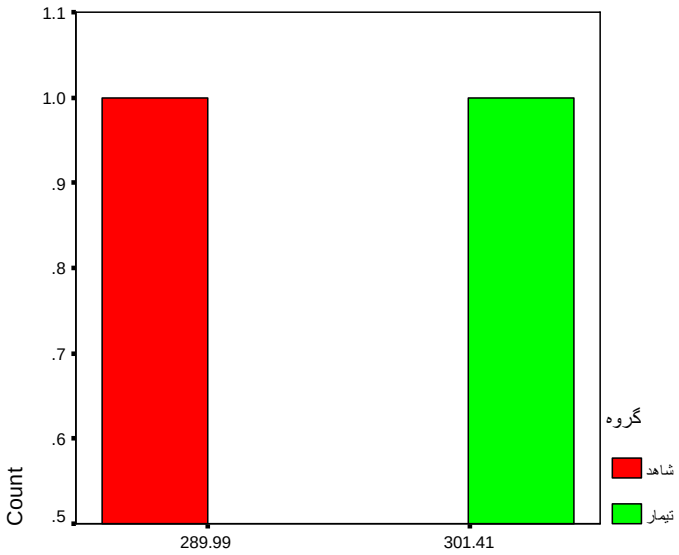

Figure 3-9-follicle large section level mean in control and treatment groups (red: control, green: treatment)

3-10-Pregnancy percentage

In this research after sonography of the cows in control and treatment groups in day 30th after insemination of 20 treatment cows 16 cows were pregnant and our were healthy. In control group of 20 cows 11 cows were pregnant as 55% and 9 cows were healthy that pregnancy percentage for control and treatment groups and pregnancy diagnosis was 80% for treatments and it was 55% for control group.

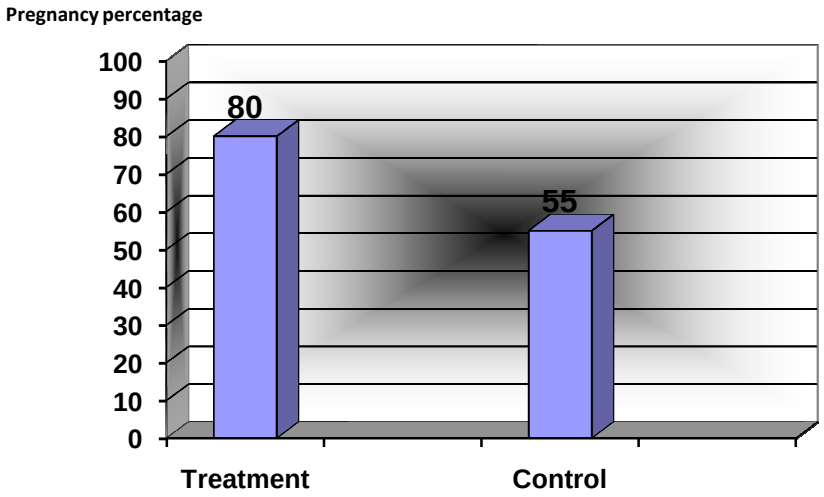

Figure 3-10 Pregnancy Percentages

DISCUSSION AND CONCLUSION

Several studies conducted on the use of HCG on dairy cows that have reported different results. In another study by Niasari et al, varying HCG doses were administered in heifer of Holstein in 10 days cycle with progesterone.The results showed no difference between the concentrations of progesterone and features ofthe LH and the dynamics of the follicle. In this study, the same protocol of ovulation in dairy cows showed that the ovulation was high in cows treated with HCG. These results are consistent to our results.

In a survey by Santsoe and et al (2001)on Holstein dairy cows the pregnancy rate was significantly high in cowsreceived HCG injection simultaneously with insemination (6). In the same study it was noted that the use of HCG before or after insemination was effective in induction of ovulation of follicles and the size of the luteum of ovulation of these follicles was large in terms of size and efficient. The results from our study also indicate that in the cyclic cows under the protocol Presynch they were injected HCG on day 37 and follicle size was largerand serum estradiol levels were also higher than the control group and also percent of pregnancy in the first pregnancy testimproved significantly (7). All livestock 18-22 hours after inoculation were forced to insemination and treatment group hadgood fertility. Ovulation induction in treatment group might be due to better occurrence of confluence Spermatozoa with ovulation in proper time.

In another study by Santvz, Webl and et al the HCG Protocol CIDR + Cosynch was used and pregnancy rate to insemination in the treatment group was significantly lower than the control group (8). In this study also it is stated that the size of the dominant follicle declined on day zero and 3 in the treatment group compared with the control group. These results are not consistent to our results although, protocol implemented in this study is different. In general, among methods of synchronization, using Co synch is not recommended due to dramatic declines in fertility rates. May be the reason for this is due to the low pregnancy rate in Co synch method. The results of HCG injection in the above research can be interpreted that increased progesterone concentrations resulted from secondary corpus luteum causes to reduced growth of the dominant follicle and reduced growth or even stop the growth of dominant follicles causing a deterioration or feedback negatively in the secretion of GnRH and subsequently LH because the authors reported the size of dominant follicles in the control group were significantly larger than those in the treatment group. The results of our study show that the fertility and pregnancy is high in the treatment group compared to control group that the fertility rate in the first insemination after HCG injection is considered as mentioned in the results. In a study by Yen et al conducted on the dairy heifers it was reported that follicle graph disappearance, the increase in corpus luteum tissue volume, increased concentrations of progesterone and injection of HCG is meaning and more effective than GnRH (9). The study also showed that follicle graph disappearance and sudden decline in estradiol levels which is the reason for ovulation in the daily scan ultrasonographic images before and after protocol and HCG injection assured us that the disappearance of follicle graph indicates ovulation that confirm the results of Yen et al. Differences in blood half-life of LH and HCG may be responsible for the disappearance of large follicles in the calf after using 1000 IU HCG compared with GnRH 100 micrograms.

Another study conducted by Diaz and Ferivascuele et al pregnancy rates was low in the cows received HCG before synchronization (10). They justified that HCG induced ovulation of ovulation in small follicles and this leads to poor fertility and consequently will be more likely to produce small corpus luteum that have less capacity on progesterone production. But in our research significant increase of pregnancy percentage in control and treatment groupsin the first pregnancy test results are not consistent with this approach has been shown to improve fertility. Perhaps it was due to an increase in fertility and increased mean follicle in cows with Presynch protocol that it can be justified that Presynch protocol initially causes to growth and distinctive selection of a follicle to grow and as follicle dominate and eventually follicle graph.

Injection of HCG in this research was done in a second injection of GnRH therefore; the aim of injecting HCG was act like LH to ovulation in the follicle graph.

Simply the objective of this study was induction of ovulation in follicle graph between the two treatmentsand control groups compared to the subsequent inoculation at TIA 18 - 22 hours after ovulation with a probability of more than 95 % in follicles graph.

The Size of the follicles had a significant difference than the control group in our study indicating that HCG in this way only through effects like LH causes to ovulation which passes through increased efficiency of oocytes so newly young ovulated has been fertilized by sperm released from the significant increase in fertility.

In another study conducted on the diameter of the largest follicle, Stevenson and et all found no significant differences in diameter of the largest follicle in the group who received compared to the control group that received no GnRH (11,12). In this study the mean size of the large and small follicle diameter was significantly different in the treatment group than the control group that does not confirm the above results. However, there is a major difference between this study and studies that investigate these heifers, but in our study the dairy cows tested by protocol. It can be justified that theresults of endocrinology and LH receptors ofthe calving dairy cows are different with milking heifers.

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