

# The Effects on Follicular Dynamics Caused by Changing the Application Time of PGF2 $\alpha$ and GnRh in the Cosynch Protocol Administered in Montofon Cows with Estrus Stimulated by Presynchronization <sup>[1][2]</sup>

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## Abstract

The objective of this study was to investigate how follicular dynamics are affected by changing the initial injection times of PGF2 $\alpha$  and GnRH in the Cosynch protocol in Montofon cattle that have been administered presynchronization. Initially, all cattle whose corpus luteum was identified were administered an injection of PGF2 $\alpha$ . Six days after estrus was identified, the Cosynch protocol was started. In keeping with this, Group I (GI; n=9) was administered GnRH on day 0 and PGF2 $\alpha$  7 days later. Fifty-six h later, artificial insemination was performed and GnRH was injected. In the second group of cows (GII; n=10), PGF2 $\alpha$  was administered in the Cosynch protocol 8 days after the first GnRH injection. Fifty-six h later, artificial insemination was performed and GnRH was injected. Development of the follicle and the corpus luteum was monitored each day with ultrasonography. On days 6, 7 and 8 of the procedure, a statistically significant difference in the follicle diameters was identified between the groups (P<0.05). No significant difference between the groups was identified in the dominant follicle diameter during artificial insemination (P>0.05). In conclusion, we determined that for cattle administered the Cosynch protocol on the 6th day of the sexual cycle, extending the application time of PGF2 $\alpha$  by one day in did not affect the size of the Graafian follicle.

**Keywords:** Cow, Corpus luteum, Cosynch, Follicular dynamics

## Presenkronizasyon İle Östrusu Uyarılan Montofon Irkı İneklerde Seksüel Siklusun Erken Luteal Döneminde Uygulanan Cosynch Protokolünde PGF2 $\alpha$ ve GnRH Uygulama Zamanının Değiştirilmesinin Folliküler Dinamik Üzerine Etkisi

### Özet

Bu çalışma ile presenkronizasyon oluşturulan Montofon ırkı ineklerde Cosynch protokolündeki ilk GnRH ile PGF2 $\alpha$  uygulanma arası zamanının değiştirilmesinin folliküler dinamikleri üzerine etkisini araştırmak amaçlanmıştır. İlk olarak corpus luteum belirlenen tüm ineklere tek doz PGF2 $\alpha$  enjekte edildi. Östrus tespitinden sonraki 6. gününde Cosynch protokolüne başlandı. Bu doğrultuda Grup 1'de (GI; n=9) 0. gün GnRH, 7 gün sonra PGF2 $\alpha$  enjekte edildi. Bu uygulamadan 56 saat sonra suni tohumlama yapıldı ve GnRH enjekte edildi. İkinci gruptaki ineklerde (GII; n=10) ise Cosynch protokolündeki PGF2 $\alpha$ , ilk GnRH enjeksiyonundan 8 gün sonra uygulandı. Bu uygulamadan 56 saat sonra suni tohumlama yapıldı ve GnRH enjekte edildi. Follikül ve corpus luteum gelişimi ultrasonografi ile günlük takip edildi. Uygulamanın 6, 7 ve 8. günlerinde gruplar arasında follikül çaplarında istatistiksel olarak önemli bir fark belirlendi (P<0.05). Suni tohumlama sırasında dominant follikül çapı büyüklüğünde gruplar arasında fark belirlenmedi (P>0.05). Sonuç olarak, seksüel siklusun 6. gününde Cosynch protokolü uygulanan ineklerde PGF2 $\alpha$  uygulama zamanının bir gün uzatılmasının graaf follikülünün büyüklüğünü etkilemediği belirlendi.

**Anahtar sözcükler:** İnek, Corpus luteum, Cosynch, Folliküler dinamik



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## INTRODUCTION

For cattle to be able to calve every year, it is very important that signs of estrus be regularly monitored and to identify the best time for artificial insemination (AI) accordingly. Reproductive performance is limited on most farms because they fail to identify the signs of estrus more than 50% of the time. The rate of identifying estrus is low because the external signs of estrus are short and infrequent, as well as the fact that protocols for identifying estrus are limited [1]. Pursley et al. [2] developed the Ovsynch protocol to eliminate these problems in cattle. The Ovsynch protocol ensures follicular development and maturation of follicles during luteolysis prior to ovulation [2,3]. With the first GnRH injection, the existing dominant follicles start ovulation and approximately 2 days later a new wave of follicles begins [4]. At this time, one follicle gains dominance and the other large follicles (subordinate) undergo atresia. This separation occurs 6 days after the first GnRH injection [5,6]. In the Cosynch program, which is a modified form of the Ovsynch program, AI is performed at the same time as the second GnRH injection. A number of studies performed by researchers on cattle have reported pregnancy rates of 22.6%-58.5% with the Ovsynch and Cosynch synchronization programs [7-11]. It has been observed that changing the time of the second GnRH injection in the Ovsynch and Cosynch synchronization protocols has an effect on the pregnancy rates for lactating milk cows. In addition, it has been determined that pregnancy rates are higher in cows administered Ovsynch 56 (second GnRH injection administered 56 h after the PGF<sub>2α</sub> injection). Furthermore, it has been reported that cows administered Cosynch 48 (AI performed with the second GnRH injection 48 h after the PGF<sub>2α</sub> injection) had higher pregnancy rates than those administered Cosynch 72 (AI performed with the second GnRH injection 72 h after the PGF<sub>2α</sub> injection) [7]. Pursley et al. [2] achieved the highest pregnancy rate in the Ovsynch protocol with insemination performed 16 h after the second GnRH injection. Similarly, an increase in pregnancy rates was achieved by administering the Ovsynch protocol after 12-14 days in cows receiving PGF<sub>2α</sub> treatment at an interval of 14 days [7].

The objective of this study was to examine Montofon cows whose estrus was stimulated with presynchronization and investigate the effect on follicular dynamics starting the Cosynch synchronization program in the early luteal phase (the 6<sup>th</sup> day) of the sexual cycle and administering the PGF<sub>2α</sub> injection in the Cosynch protocol 8 days after the first GnRH injection.

## MATERIAL and METHODS

### Animals

This study was approved by the Kafkas University Local

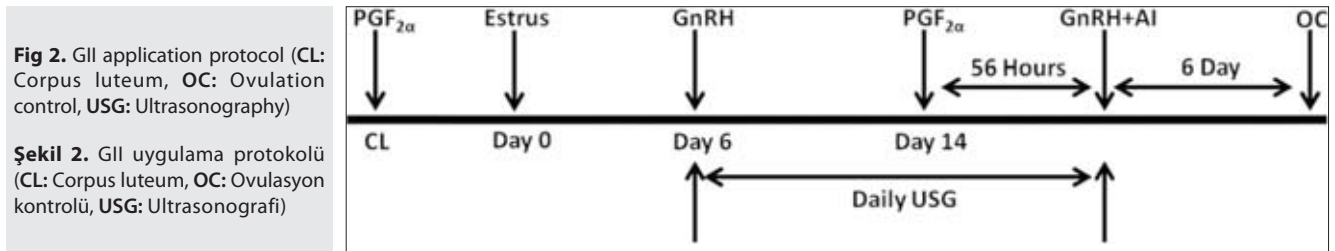
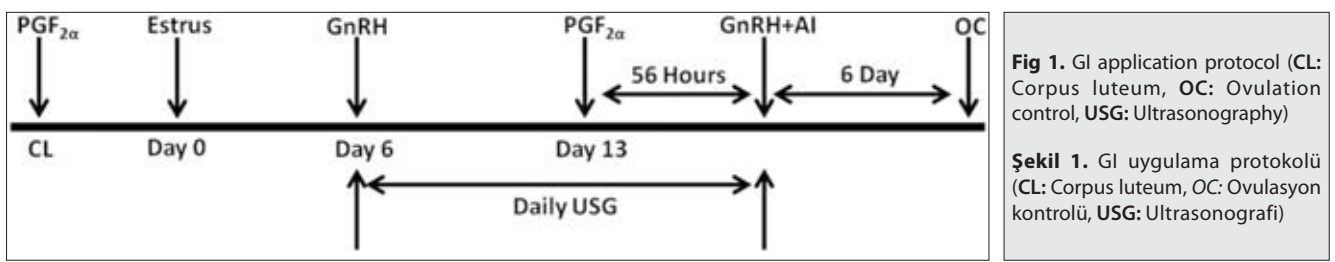
Ethics Committee for Animal Experiments, Kars, Turkey (KAÜ-HADYEK; 2010/04-09).

This study was performed on 19 clinically and gynecologically healthy Montofon cows who were at least 45 days postpartum and were fed on dry grass and concentrate feed (minimum of 88% dry content, minimum of 16% crude protein, maximum of 14% crude cellulose, maximum of 9% crude ash, maximum of 1% HCl acid-insoluble ash, approximately 1.6% calcium, 0.4% phosphorus, 0.4% sodium, 1% NaCl and 2.500 kCal/kg of metabolic energy). Rectal and ultrasonography examinations (Titan®, Sonosite, USA, 5 MHz) were used on all cows to verify that uterine involution was complete in postpartum period. After the corpus luteum was identified in the cow during the ultrasonographic examination, PGF<sub>2α</sub> (2 ml, IM, 0.075 mg, D-Cloprostenol, Dalmazin®, Vetaş, Turkey) was administered and an electronic heat detection device (DEC®, IVM, France) able to identify the start time of estrus with an accuracy of 2 h was attached to the sacrum in order to identify signs of estrus. The day that estrus began was recorded as day 0 of the sexual cycle and the Cosynch protocol was started on day 6 (in the early luteal phase of the estrus cycle). After starting the Cosynch protocol, ultrasonographic measurements were made every day on both groups of cows to determine the sizes of the follicles and corpus luteum on the ovary. Measurements were made after identifying the follicle or CL that had the largest diameter on the screen. Daily ultrasonographic examinations were continued from the beginning of the Cosynch protocol until fertilization occurred. Furthermore, 6 days after AI, ultrasonography was used to investigate the presence of the corpus luteum to determine whether or not ovulation had occurred. In addition, body condition scoring (BCS) was measured for the groups from the beginning of the study using a 5-point system with quarter-point increments [12].

### Study Procedure

Group I (GI): This group of cows (n=9; average age of 5.0±0.5 years; BCS: 3.2±0.3; average milk production: 8.1±0.9 L) was administered a single dose of PGF<sub>2α</sub> for the purpose of presynchronization. The Cosynch protocol was started on the sixth day of the cycle after signs of estrus were identified in a given cow. According to this protocol, GnRH (2 mL, IM, 25 µg, Lecirelin acetate, Dalmarelin®, Vetaş, Turkey) was administered on day 0. Seven days later, PGF<sub>2α</sub> (2 mL, IM, 0.075 mg, D-Cloprostenol, Dalmazin®, Vetaş, Turkey) was administered, followed by AI and a GnRH injection 56 h after that (Fig. 1).

Group II (GII): This group of cows (n=10; average age of 5.0±0.5 years; BCS: 3.1±0.3; average milk production: 8.8±0.9 L) was administered a single dose of PGF<sub>2α</sub> for the purpose of presynchronization. As in GI, the Cosynch protocol was started on the sixth day of the cycle after estrus symptoms were identified in a given cow. In contrast



to the first group, however, this group was administered  $PGF_{2\alpha}$  8 days after the GnRH injection. Fifty-six hours after prostaglandin  $F_{2\alpha}$  was administered, AI was performed and GnRH was administered. In this group, it was not possible to perform AI on one animal because it developed severe diarrhea (Fig. 2).

### Statistical Analysis

Statistical analysis of the findings obtained in the study was performed using the SPSS 16.0 statistical program. One-way ANOVA was used in the statistical analysis of follicle sizes, corpus luteum diameters, BCS, age and milk production for the groups. Chi-square was used for statistical analysis of the ovulation rate and accessory corpus luteum development rate.  $P < 0.05$  was considered to be statistically significant.

## RESULTS

Dominant follicle sizes in the cows during the first application of GnRH in the Cosynch protocol were found to be  $12.3 \pm 3.3$  mm in GI and  $12.4 \pm 2.2$  mm in GII. At that same time, the size of the corpus luteum (CL) was observed to be  $20.4 \pm 3.2$  mm in GI and  $20.5 \pm 3.8$  mm in GII. No

statistically significant difference was found between the groups ( $P > 0.05$ ) with regard to the sizes of the dominant follicle and CL during the first administration of GnRH in the Cosynch protocol. For both GI and GII, development of an accessory CL was not identified in 2 cows. No statistically significant difference was found between the groups ( $P > 0.05$ ) with regard to the sizes of the dominant follicle and CL during administration of  $PGF_{2\alpha}$  in the Cosynch protocol (day 7 for GI and day 8 for GII). In addition, no statistically significant difference was found between the groups with regard to the follicle size in measurements performed 24 h after  $PGF_{2\alpha}$  was administered in GI and GII ( $14.2 \pm 1.0$  and  $13.0 \pm 2.0$ ;  $P > 0.05$ ; Table 1).

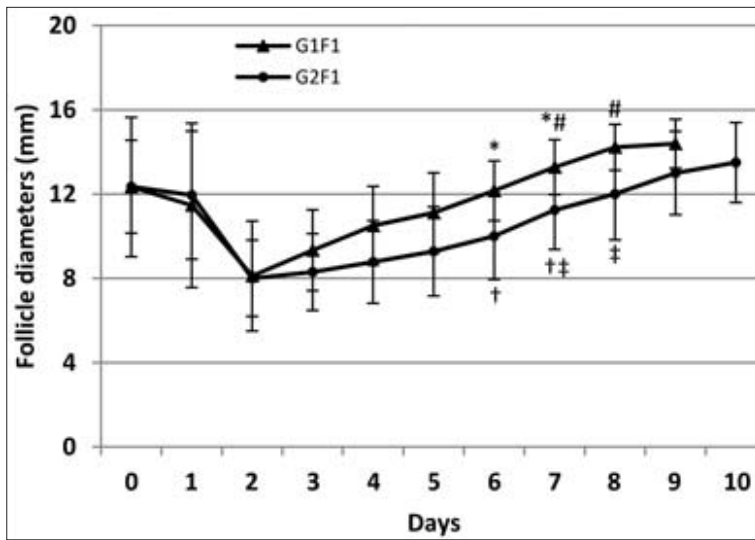
It was determined that there was no statistically significant difference in GI's follicle size as compared with GII as of day 4 of the study ( $P = 0.06$ ). However, follicle diameters were found to be statistically different between the groups on days 6, 7 and 8 (Fig. 3;  $P < 0.01$ ).

Fig. 4 shows the follicle sizes measured in the daily ultrasonographic examinations of the ovary after the Cosynch protocol was started (day 0) in GI and GII, while Fig. 5 shows the CL diameters. In our study, we determined that the subordinate follicles began to undergo atresia after

**Table 1.** Follicle, CL diameters and ovulation in the groups on different days

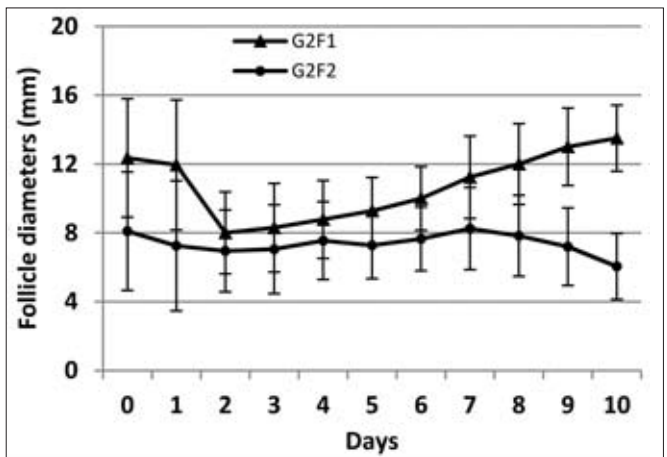
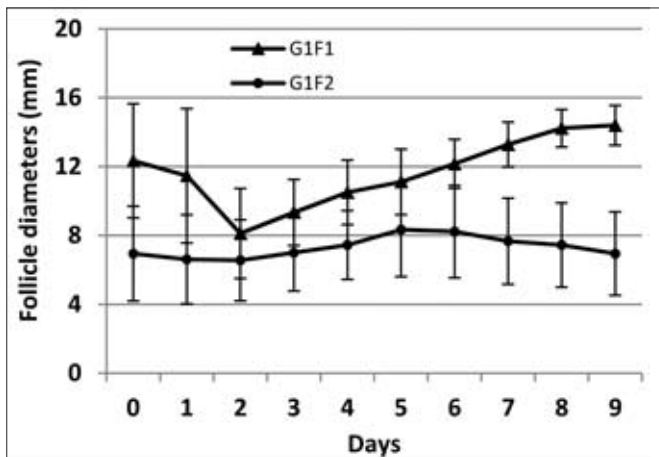
**Tablo 1.** Grupların farklı günlerdeki follükül ve CL çapları ve ovulasyon oranları

Parameters	Group I Mean $\pm$ SD	Group II Mean $\pm$ SD	P
Size of dominant follicle during the first administration of GnRH (mm)	12.3 $\pm$ 3.3	12.4 $\pm$ 2.2	$P > 0.05$
Size of CL during the first administration of GnRH (mm)	20.4 $\pm$ 3.2	20.5 $\pm$ 3.8	$P > 0.05$
Rate of developing an accessory CL (%)	77.7 (7/9)	88.8 (8/10)	$P > 0.05$
Size of dominant follicle during administration of $PGF_{2\alpha}$ in the Cosynch protocol (mm)	13.3 $\pm$ 1.3	12.0 $\pm$ 2.2	$P > 0.05$
Size of CL during the administration of $PGF_{2\alpha}$ in the Cosynch protocol (mm)	23.2 $\pm$ 5.1	22.4 $\pm$ 3.7	$P > 0.05$
Size of the Graafian follicle during artificial insemination (mm)	14.4 $\pm$ 1.2	13.5 $\pm$ 1.9	$P > 0.05$
Ovulation rate 6 days after artificial insemination (%)	75 (7/9)	100 (9/9)	$P > 0.05$



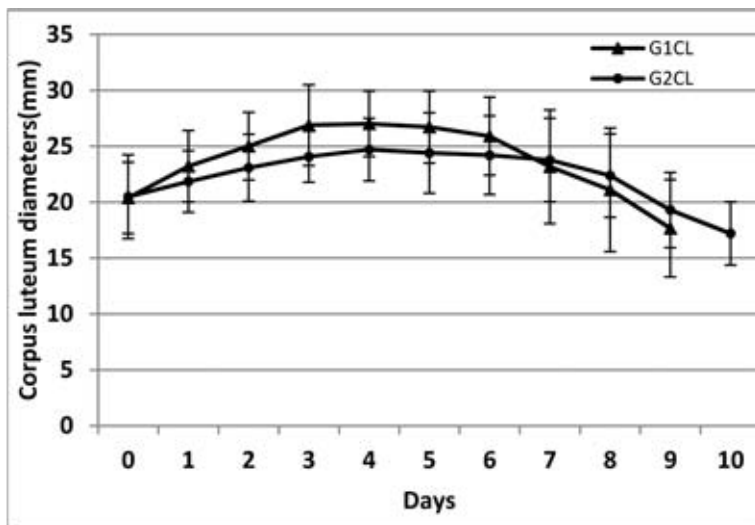
**Fig 3.** Dominant follicle diameters measured in G1 and GII during the period lasting from the beginning of the Cosynch protocol until the time artificial insemination was performed (F1: Dominant follicle); \* : #, † : ‡ = P<0.05; \* : †, \*# : †‡, # : ‡ = P<0.01

**Şekil 3.** GI ve GII'de Cosynch protokolü başlangıcından suni tohumlama yapılncaya kadar geçen sürede ölçülen dominant follikül çapları (F1: Dominant follikül) \* : #, † : ‡ = P<0.05; \* : †, \*# : †‡, # : ‡ = P<0.01



**Fig 4.** Follicle development in G1 and GII during the period lasting from the beginning of the Cosynch protocol until the time artificial insemination was performed (F1: Dominant follicle, F2: Subordinate follicle)

**Şekil 4.** GI ve GII'de Cosynch protokolü başlangıcından suni tohumlama yapılncaya kadar geçen sürede folliküllerin gelişimi (F1: Dominant follikül, F2: Subordinate follikül)



**Fig 5.** Corpus luteum development in G1 and GII during the period lasting from the beginning of the Cosynch protocol until the time artificial insemination was performed (CL: Corpus luteum)

**Şekil 5.** GI ve GII'de Cosynch protokolü başlangıcından suni tohumlama yapılncaya kadar geçen sürede corpus luteum gelişimi (CL: Corpus luteum)

day 6 of the protocol in G1 ( $8.2 \pm 2.7$  mm) and after day 7 in GII ( $8.3 \pm 2.4$  mm) (Fig. 4). Measurements taken on these

days did not reveal any statistically significant difference between the subordinate follicle diameters ( $P > 0.05$ ).

## DISCUSSION

The response to administering GnRH in cows varies depending on the development phase of the follicle. Ovulation occurs 100% of the time from a follicle that is developing with GnRH, 33% of the time from a follicle in the static phase and 1% of the time from a follicle that is in the regression phase [2,13]. It has been reported that the pregnancy rate is higher when

the Ovsynch protocol is administered between days 5-12 of the sexual cycle in dairy cows. Starting the Ovsynch protocol in the late luteal stage may cause estrus before the second GnRH injection as well as premature CL regression. As a result, an abnormal CL may develop from the ovulating follicle, producing a small amount of progesterone, which can cause a reduction in pregnancy rates [3]. In our study, we started the Cosynch protocol on day 6 of the cycle in order to achieve the best follicle for ovulation.

Follicular waves are optimally synchronized for some cows in the Ovsynch protocol, but they are not sufficiently synchronized for other cows. Insufficient development of follicular waves depends on the initial response to GnRH. Differences in the dominant follicle's development stage when the second GnRH is administered causes negative results [14]. Presynchronization with PGF<sub>2α</sub> prior to starting the Cosynch protocol has been observed to increase the pregnancy rate in multiparous cows [8]. Studies on cows that have been administered presynchronization have reported follicle diameters of 15.5 mm [15] and 14.4 mm [16] during the first GnRH injection administered in the Ovsynch protocol. Furthermore, the ovulation rate was found to be 69.5% after this procedure [15]. In our study, we found the dominant follicle to be 12.3 mm in GI during the first GnRH injection administered in the Cosynch protocol and 12.4 mm for GII in which PGF<sub>2α</sub> was administered at a different time. Furthermore, the ovulation rate after administering GnRH was found to be 77.7% and 88.8%, respectively ( $P>0.05$ ). In our study, measurements taken on days 6, 7 and 8 revealed that dominant follicle diameters were larger in Group I than Group II and we found that there was a statistically significant difference between these follicles. In one study performed on heifers, the PGF<sub>2α</sub> injection time was changed (7 or 8 days after the first GnRH injection) and progesterone was administered during this time period. In that study, follicle diameter was found to be 13.5 mm before ovulation and the ovulation rate was reported to be 100% in the heifers in all groups [17]. In addition, preovulatory follicle diameters of 14.0 mm [15] and 15.7 mm [16] have been reported during the second GnRH injection administered in the Ovsynch protocol with presynchronization. Furthermore, the ovulation rate was found to be 96.2% after the second GnRH injection [15]. In our study, we also found the preovulatory follicle diameter to be 14.4 mm and 13.5 mm, respectively, during

the second GnRH injection in both groups. We found the ovulation rate to be 75% in Group I and 100% in Group II. Our findings are similar to the results of the aforementioned study. Assey et al. [18] found the average corpus luteum diameters to be  $20.8\pm 0.1$  mm at day 7 after PGF<sub>2α</sub> injection in cows. Similarly in mentioned study, we found the corpus luteum diameters to be  $23.2\pm 5.1$  mm in Group I and  $22.4\pm 3.7$  mm in Group II. Rastegarnia et al. [19] found the average corpus luteum diameters to be  $12.5\pm 0.29$  mm in river buffalo while administering PGF<sub>2α</sub> in the protocol. Based on this data, we found the corpus luteum diameters to be larger when PGF<sub>2α</sub> was administered. Similar to our findings, Bulbul et al. [20] found no effect on the diameter of CL following initiation of the Ovsynch protocol between on day 0 and 8 of sexual cycle in Montofon cows.

In a study performed on cows that were administered the Cosynch protocol, follicle size at ovulation when administering GnRH 56 h after the PGF<sub>2α</sub> injection was found to be smaller than Cosynch 72 and larger than Cosynch 48 [7]. Ovulation of the maturing dominant follicle is stimulated with the first GnRH injection and a new follicular wave is produced within approximately 2 days [4]. In both groups in our study, the existing dominant follicles ovulated with the first GnRH injection, followed by a new follicular wave 2 days later. Studies on follicle dynamics have observed a significant difference in growth of the dominant follicle and the subordinate follicle by 6 days after the GnRH injection. It has been reported that in the following days, the subordinate follicle will undergo atresia while the dominant follicle will develop even further for ovulation. It has been observed that the subordinate follicle grows approximately 8 mm and then becomes smaller, in contrast to the dominant follicle [5,6]. Similarly, our study also found that the subordinate follicles in both groups grew approximately 8 mm and then became smaller. Furthermore, these follicles began to undergo atresia on day 6 in Group I and on day 7 in Group II.

In conclusion, our study determined that changing the injection time of PGF<sub>2α</sub> after the first GnRH injection in the Cosynch protocol does not affect the follicle size at ovulation.

## REFERENCES

1. Fricke PM, Guenther JN, Wiltbank MC: Efficacy of decreasing the dose of GnRH used in a protocol for synchronization of ovulation and timed AI in lactating dairy cows. *Theriogenology*, 50, 1275-1284, 1998.
2. Pursley JR, Mee MO, Wiltbank MC: Synchronization of ovulation in dairy cows using PGF<sub>2</sub> alpha and GnRH. *Theriogenology*, 44, 915-923, 1995.
3. El-Zarkouny SZ, Cartmill JA, Hensley BA, Stevenson JS: Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. *J Dairy Sci*, 87, 1024-1037, 2004.
4. Colazo MG, Gordon MB, Rajamahendran R, Mapletoft RJ, Ambrose DJ: Pregnancy rates to timed artificial insemination in dairy cows treated with Gonadotropin-Releasing Hormone or Porcine Luteinizing Hormone. *Theriogenology*, 2, 262-270, 2009.

- 5. Lucy MC, Savio JD, Badinga L, De La Sota RL, Thatcher WW:** Factors that affect ovarian follicular dynamics in cattle. *J Anim Sci*, 70, 3615-3626, 1992.
- 6. Sartori R, Fricke PM, Ferreira JCP, Ginther OJ, Wiltbank MC:** Follicular deviation and acquisition of ovulatory capacity in bovine follicles. *Biol Reprod*, 65, 1403-1409, 2001.
- 7. Brusveen DJ, Cunha AP, Silva CD, Cunha PM, Sterry RA, Silva EPB, Guenther JN, Wiltbank MC:** Altering the time of the second gonadotropin-releasing hormone injection and artificial insemination (AI) during ovsynch affects pregnancies per AI in lactating dairy cows. *J Dairy Sci*, 91, 1044-1052, 2008.
- 8. DeJarnette JM, Marshall CE:** Effects of Pre-synchronization using combinations PGF<sub>2</sub> alfa and (or) GnRH on pregnancy rates of Ovsynch- and Cosynch-treated lactating holstein cows. *Anim Reprod Sci*, 77, 51-60, 2003.
- 9. Kacar C, Yildiz S, Pancarci SM, Kaya M, Oral H, Gurbulak K, Gungor O:** Administration of GnRH treatment prior to ovsynch protocol to stimulate cyclicity in cows with functional anoestrus. *Bull Vet Inst Pulawy*, 50, 497-501, 2006.
- 10. Kaçar C, Kamiloğlu NN, Uçar Ö, Arı UÇ, Pancarci ŞM, Güngör Ö:** Effect of Ovsynch and Cosynch synchronization programmes combined with  $\beta$ -carotene+Vitamin E administration upon the pregnancy rates in cows. *Kafkas Univ Vet Fak Derg*, 14, 45-50, 2008.
- 11. Yilmaz C, Yilmaz O, Ucar M:** Effect of PGF<sub>2a</sub> and GnRH injections applied before Ovsynch on pregnancy rates in cows and heifers. *Kafkas Univ Vet Fak Derg*, 17, 641-644, 2011.
- 12. Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G:** A body condition scoring chart for holstein dairy cows. *J Dairy Sci*, 72, 68-78, 1989.
- 13. Sanchez T, Wehrman ME, Kojima FN, Cupp AS, Bergfeld EG, Peters KE:** Dosage of the synthetic progestin, norgestomet, influences luteinizing hormone pluse frequency and endogenous secretion of 17- $\beta$  estradiol in heifers. *Biol Reprod*, 52, 464-469, 1995.
- 14. Vasconcelos JL, Sartori R, Oliveira HN, Guenther JG, Wiltbank MC:** Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology*, 56, 307-314, 2001.
- 15. Stevenson JS, Pulley SL, Mellieon HI:** Prostaglandin F<sub>2a</sub> and gonadotropin-releasing hormone administration improve progesterone status, luteal number, and proportion of ovular and anovular dairy cows with corpora lutea before a timed artificial insemination program. *J Dairy Sci*, 95, 1831-1844, 2012.
- 16. Bello NM, Steibel JP, Pursley JR:** Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. *J Dairy Sci*, 89, 3413-3424, 2006.
- 17. Hittinger MA, Ambrose JD, Kastelic JP:** Luteolysis, onset of estrus, and ovulation in Holstein heifers given prostaglandin F<sub>2a</sub> concurrent with, or 24 hours prior to removal of an intravaginal, progesterone-releasing device. *Can J Vet Res*, 68, 283-287, 2004.
- 18. Assey RJ, Purwantara B, Greve T, Hyttell P, Schmidt MH:** Corpus luteum size and plasma progesterone levels in cattle after cloprostenol-induced luteolysis. *Theriogenology*, 39, 1321-1330, 1993.
- 19. Rastegarnia A, Niasari-Naslaji A, Hovareshti P, Sarhaddi F, Safaei M:** The effect of different doses of gonadorelin on ovarian follicle dynamics in river buffalo (*Bubalus bubalis*). *Theriogenology*, 62, 1283-1291, 2004.
- 20. Bulbul B, Kirbas M, Kose M, Dursun S, Colak M:** The affect of Ovsynch started in different phase of oestrus cycle on oestrus synchronization in cows. *Istanbul Univ Vet Fak Derg*, 35, 7-17, 2009.