

## Effect of Different Gonadotropin Preparation on Ovulatory Response and Embryo Yield in Karayaka Ewes <sup>[1]</sup>

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### Summary

In this study, the main goal was to investigate the effect of stimulation with either oFSH (Ovagen®) or pFSH (Folltropin®) on the superovulatory response and embryo quality in Karayaka ewes. Thirty-five ewes were used in this study. The ewes were randomly allocated to two groups. Estrous cycles in ewes were synchronized with the controlled integral drug release dispensers (CIDR®) for 14 days. Starting on day 12 and during 4 consecutive days ewes were treated with oFSH in Group I (GI, n=16) and pFSH in Group II (GII, n=19). Ovarian examination and embryo recoveries were carried out with the aid of laparotomy on day 6 following CIDR withdrawal. There was no significant differences in the number of corpora lutea (CL), unfertilized oocytes (UFOs), total number of embryos (EMBTot), total number of freezable embryos (FEMBTot), ovulation rate (OR), fertilization rate (FR), recovery rate (RR) and embryo recovery rate (ERR) between the groups. On the other hand, embryo quality was better in GI compared to GII (P<0.05). In conclusion, it has been thought that application of oFSH for superovulatory treatment in Karayaka ewes was more effective than pFSH for the quality of embryos.

**Keywords:** Ewe, pFSH, oFSH, Superovulation, Embryo

## Karayaka Koyunlarında Farklı Gonadotropin Uygulamalarının Embriyo Verimleri ve Ovulasyon Yanıtları Üzerine Etkileri

### Özet

Sunulan çalışmada, Karayaka koyunlarında; oFSH (Ovagen®) ve pFSH (Folltropin®) uygulamalarının, süperovulasyon ve embriyo kalitesi üzerine etkilerinin karşılaştırılması amaçlandı. Bu çalışmada toplam 35 baş Karayaka koyunu kullanıldı. Koyunlar rastgele 2 gruba ayrıldı. Koyunların seksüel siklusları, 14 gün süreyle intravaginal CIDR kullanılarak senkronize edildi. Uygulamanın 12. gününden itibaren, 4 gün süreyle Grup I'e oFSH (GI, n=16), Grup II'ye pFSH (GII, n=19) uygulandı. CIDR çıkartıldıktan 6 gün sonra laparotomik olarak embriyo toplama işlemi ve ovarium muayenesi yapıldı. Gruplar arasında, korpus luteum, fertilize olmamış oosit, embriyo, dondurulabilecek embriyo sayıları, ovulasyon, fertilizasyon, geri kazanım ve embriyo elde etme oranları arasında istatikselsel olarak önemli bir fark bulunmadı. Diğer taraftan; gruplar arasında embriyo kaliteleri karşılaştırıldığında GI'den elde edilen embriyoların kalitesi daha yüksek bulundu (P<0.05). Sonuç olarak, Karayaka koyunlarında süperovulasyon ile elde edilen embriyoların kalitesi üzerinde oFSH kullanımının, pFSH kullanımına göre daha etkili olduğu saptandı.

**Anahtar sözcükler:** Koyun, pFSH, oFSH, Süperovulasyon, Embriyo

### INTRODUCTION

Sheep have an important place in the economy of Turkey and in the nutrition of population. A total of 625291 tones

of meat were produced from cattle, goat, sheep and buffalo in Turkey in 2009. 262000 tones of these came from sheep <sup>1</sup>.



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The Karayaka is the native sheep breed in Turkey, and it is distributed along the Black Sea coast. It constitutes about 3.5% of the total sheep population in Anatolia<sup>2</sup>. Karayaka is one of the most important breed in Turkey because of its high meat quality and adaptation ability<sup>3</sup>. However, Karayaka population has been decreasing in recent years. In order to protect endangered breeds, in 2007 with the TURKHAYGEN-I (In Vitro Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources-I) project in vitro conservation of the genetic resources of Anatolia started. Embryo bank was founded in this context. Embryos, cryoprotected in this bank were collected from Karayaka ewes and many other native breeds<sup>4</sup>.

Gonadotropins are mainly used for ovarian stimulation in sheep MOET (multiple ovulation and embryo transfer) programs<sup>5,6</sup>. MOET is an inevitable method for the genetic conservation programs of endangered livestock. Superovulation and embryo collection are limited by the variation observed in ovarian response, embryo quality and fertilization rates of hormonally stimulated ewes<sup>7</sup>. The success of superovulatory treatment depends on many different factors such as different individual response, season and breed. In addition, the use of different commercial FSH preparations can affect the ovarian response and embryo yields. These differences are originated by FSH/LH ratio of the preparations<sup>8</sup>.

The combination of superovulation with cryopreservation is an important component in the preservation of endangered livestock<sup>9,10</sup>. The success of these conservation programs depends on the number of transferable embryos in response to superovulation stimulation. The procedure of embryo recovery is affected by various factors including the mating system (A.I. or natural)<sup>11</sup>, embryo flushing method<sup>12</sup>, progesterone devices<sup>13</sup>, season<sup>14</sup>, gonadotropins derived from different species<sup>15</sup>, dissolving agent for gonadotropins<sup>16</sup>. High degree of variation in the superovulatory response is the greatest problem in the MOET programs<sup>17</sup>. The ratio of FSH to LH in the commercial gonadotropin preparations affect the quality of embryos recovered from superovulated ewes<sup>18-22</sup>. High FSH:LH ratios cause lower embryo yield but increase embryo quality<sup>23</sup>.

The purpose of the present study was to investigate the effects of two different gonadotropin preparations on the ovulation response and embryo quality in Karayaka ewes.

## MATERIAL and METHODS

The experiments were conducted during the breeding season (September to December) in Central Anatolia (40°06'08.50" N, 32°37'18.65" E) at 850 m above the sea level. Thirty-five ewes were used. The ewes and rams were housed in a straw bedded semi-open sheepfold. They were fed with concentrate daily together with alfalfa hay and barley straw; water was *ad libitum*. All ewes were between 2 and 3 years

of age and the mean body weight was 48±7.6 kg. These ewes had not been used in any MOET program before. All surgical procedures were conducted by the same surgical team and all embryos were evaluated by the same person according to the criteria recommended by the IETS (International Embryo Transfer Society)<sup>24</sup>.

Oestruses were detected by rams which were aproned tied around body. After five days of oestrus, two different superovulatory treatments were performed in two different groups of ewes. In the first (GI; n=16) and second (GII; n=19) group of ewes, the oestrus cycles were synchronized with the controlled integral drug release dispensers containing 0.3 g progesterone (CIDR®; Eazi-Breed™; Pharmacia&Upjohn, Australia) for 14 days. Starting on day 12 of CIDR (48 h prior to CIDR removal) treatment and for 4 consecutive days first group of ewes were treated with (i.m.) oFSH (Ovagen®, 10 ml, 8.8 mg NIADDK oFSH-17, ICP Bio Reproduction, USA) and second group of ewes were treated with (i.m.) pFSH (Folltropin®, 10 ml, 200 mg NIH-FSH-P1, Bioniche Animal Health, Ireland) in eight doses of 1.5, 1.5, 1.5, 1.25, 1.25, 1, 1, 1 ml twice a day (06:00 and 18:00). At the time of second FSH injection in both groups, D-Cloprostenol (Dalmazin®, Fatro, Turkey) was administered (i.m.) at a dose of 150 mcg to cause luteolysis. The rams were used to detect oestrus in all ewes starting from 24 h after CIDR removal. All ewes in oestrus were natural mated twice a day over three days.

Ovarian examination and embryo recovery were performed by laparotomy on day 6 after CIDR withdrawal. All ewes were fasted 24 hours before surgery. Animals were sedated with 0.1 - 1 mg/kg atropine s.c. (Atrol-F®, Sanovel, Turkey) and 0.5 mg/kg of diazepam i.v. (Diazem®, Deva, Turkey) and anesthetized (i.v.) with 2 mg/kg ketamine HCl (Alfamine®, EgeVet, Turkey). The abdominal area anterior the udder was shaved before the operation. For antisepsis 70% alcohol and 1% iodine solution were used. Mid lateral incision was performed after local anesthesia by infiltration of 2% lidocain HCl (Adokain®, Sanovel, Turkey) in the incision area. The number of CL (corpora lutea) was recorded. Each uterine horn was flushed with flushing media (20 ml mD-PBS+3 mg/ml BSA) using catheter (1.3 x 130 mm) inserted at near the utero-tubal junction. The embryos were recovered into a 90 mm petri dish using 2 way foley catheter (Rüsch®, no:10) inserted in the base of the uterine horns. Embryos were morphologically evaluated under a stereomicroscope (Leica, M 205C) and classified according to the criteria recommended by the IETS<sup>24</sup>. The embryos were classified as unfertilized oocyte (UFO), degenerated blastocyst (DgBl), compact morulae (CM, Grade I), early blastocyst (EBI, Grade II), blastocyst (Bl, Grade III), expanded blastocyst (ExBl, Grade IV). CM, EBI and ExBl were considered as freezable, UFO and DgBl were considered as unfreezable.

The mean ovulation rate (OR) was recorded by counting the number of CLs. The number of UFOs, DgBl, total embryos recovered (EMBTot), CMs, EBIs, Bls, ExBl, total structures recovered (TSR), total freezable embryos recovered (FEMBTot)

were recorded. Fertilization rates (FR), recovery rate (RR) and embryo recovery rate (ERR) were calculated using the following equations:  $TSR = CMs + EBIs + BIs + ExBIs + DgBIs + UFOs$ ;  $EMBTot = CMs + EBIs + BIs + ExBIs + DgBIs$ ;  $FEMBTot = CMs + EBIs + BIs + ExBIs$ ;  $FR = [EMBTot / (EMBTot + UFO)] \times 100$ ;  $RR = [(EMBTot + UFO + DgBI) / \text{no. of CL}] \times 100$ ;  $ERR = [EMBTot / \text{no. of CL}] \times 100$ . Differences in superovulation response and embryo yields were evaluated by the chi-square test. All data were analyzed using the statistical software package of SPSS (Version 15.0).

## RESULTS

All ewes exhibited the signs of oestrus 36 h after of CIDR removal in GI and GII. Seven ewes in GI and two ewes in GII showed no ovulatory response. Thus uterine flushing operation was not performed on them. When considering all ewes subjected to the different FSH treatment, the mean of OR on the right side ( $4.43 \pm 3.34$  and  $2.89 \pm 1.99$  for GI and II, respectively) and left side ( $4.62 \pm 4.16$  and  $3.47 \pm 3.07$  for GI and GII, respectively) and the total number of CLs per animal did not differ significantly between two groups. The means of TSR, EMBTot, FEMBTot were not significantly

different between GI and GII (Table 1). The rates of RR, FR and ERR did not differ significantly between groups (Table 2).

In GI, ExBI yield was found to be significantly higher than BI ( $P=0.05$ ). In GII, BI yield was significantly higher than ExBI ( $P<0.05$ ). Compared to GII, expanded blastocysts in GI were significantly higher ( $P<0.05$ ) (Table 3).

## DISCUSSION

In MOET programs, response to superovulatory treatments is higher in breeding season<sup>18</sup>. This feature has been taken into consideration in this study as well. Therefore seasonal effects were not observed. All ewes exhibited the signs of oestrus after 36 hours following CIDR removal. In terms of the number of ovulation, following the superovulatory treatment with different FSH preparations, there was no difference between the groups (GI and GII) and two sides (left ovary-right ovary). Type of FSH preparation had no effect on OR, EMBTot, FEMBTot, RR, ERR and FR. These results are similar to Bettencourt et al.<sup>18</sup>.

Expanded blastocysts in GI were significantly higher than

**Table 1.** Ovulation rate (OR), total structures recovered (TSR), degenerated blastocysts (DgBI), unfertilized oocyte (UFO), total embryos recovered (EMBTot), total freezable embryos recovered (FEMBTot) in ewes treated with two different FSH preparations

**Tablo 1.** İki farklı FSH preparatı uygulanan koyunlarda; ovulasyon oranı (OO), geri kazanılan tüm yapılar (GKTY), dejenere blastosist (DjBI), fertilize olmamış oosit (FOO), geri kazanılan toplam embriyo sayısı (GKTES), geri kazanılan toplam dondurulabilir embriyo sayısı (GKTDES)

Parameters		Group I (n=16) (mean±S.D.)	Group II (n=19) (mean±S.D.)
OR	Right Ovary	4.43±3.34	2.89±1.99
	Left Ovary	4.62±4.16	3.47±3.07
	Total	9.62±6.97	6.36±4.66
TSR		5.18±5.57	3.26±3.61
DgBI		0.13±0.45	0.00
UFO		2.25±4.61	0.84±1.97
EMBTot		2.93±3.31	2.42±2.98
FEMBTot		2.75±3.21	2.42±2.98

**Table 2.** Recovery rate (RR), embryo recovery rate (ERR), fertilization rate (FR), in ewes treated with two different FSH preparations

**Tablo 2.** İki farklı FSH preparatı uygulanan koyunlarda; geri kazanım oranı (GKO), embriyo geri kazanım oranı (EGKO), fertilizasyon oranı (FO)

Groups	RR Median → (min-max)	ERR Median (min-max)	FR Median (min-max)
GI (n=16)	66.66 <sup>a</sup> (0-100)	20.77 <sup>b</sup> (0-100)	47.83 <sup>c</sup> (0-100)
GII (n=19)	33.33 <sup>a</sup> (0-100)	25.00 <sup>b</sup> (0-100)	100.00 <sup>c</sup> (0-100)

**Table 3.** Grades of embryos in Karayaka ewes applied two different superovulatory treatment

**Tablo 3.** İki farklı süperovulasyon uygulaması yapılan Karayaka koyunlarından elde edilen embriyoların sınıflandırılması

Parameters		Group I (n=16) (mean±S.D.)	Group II (n=19) (mean±S.D.)
FEMBTot	CM (Grade I)	0.00±0.00	0.15±0.5
	EBI (Grade II)	0.00±0.00	0.26±1.14
	BI (Grade III)	0.56±1.31 <sup>a*</sup>	1.21±1.61 <sup>b*</sup>
	ExBI (Grade IV)	2.18±2.31 <sup>a**</sup>	0.78±1.27 <sup>b**</sup>

CM: Compact morula, EBI: Early blastocyst, BI: Blastocyst, ExBI: Expanded blastocyst,

Means in the same row with different superscript letters and same column with different superscript asteriks differ significantly ( $P < 0.05$ )

GII. These differences have been attributed to the features of different commercial FSH preparations, especially LH-like features of these preparations are different<sup>8,18</sup>.

The overall success of MOET programs depends not only on ovulation rate, but also on the fertilization and embryo recovery rates. Controlling follicular development in ewes is not fully understood at the ovarian level<sup>8</sup>. Decreasing FSH/LH ratio causes luteal regression in the preovulatory peak<sup>25</sup>. Starting from this issue, some protocols of hormonal treatments were developed<sup>20</sup>. In this study, although there was no difference between the total numbers of embryos, a difference was observed in the quality of embryos. Effects of different sources of FSH, could cause this difference. oFSH (Ovagen®) and pFSH (Folltropin®) have different LH/FSH ratios. These ratios can affect both estradiol and progesterone levels, oocyte catch by infundibulum, oocyte transportation and quality of embryos<sup>8</sup>.

The data from this study show that embryo quality is affected by the type of FSH preparation which is used for superovulatory stimulation on Karayaka ewes. Folltropin and Ovagen have different levels of bioactive FSH and LH. Ovagen has more bioactive FSH than Folltropin<sup>26</sup>. High FSH:LH ratios may lower overall embryo yield but will increase embryo quality<sup>23</sup>. Similarly in this study, oFSH preparation has a significant positive effect on the embryo quality. In addition, MOET programs can be applied successfully in Karayaka ewes. The superovulatory response in Karayaka ewes was found similar to other sheep breeds<sup>10,27</sup>. On the contrary, yields of Karayaka ewe such as number of corpora lutea and embryo yields showed difference compared to some other breeds<sup>18,28</sup>. The different results using the same treatment shows that genetic differences may have potential importance in the response of superovulatory treatment in sheep. The strategy of studies on this subject should be planned on the basis of this issue.

## REFERENCES

- 1. FAOSTAT:** Total meat produced in Turkey. Food and Agricultural Organization Statistics, 2009. <http://faostat.fao.org/site/569/default.aspx#ancor>, Accessed: 06.10.2011.
- 2. Kaymakçı MI, Oğuz C, Un G, Bigen T:** Basic characteristics of some Turkish indigenous sheep breeds. *Pakistan J Biol Sci* 4, 916-919, 2001.
- 3. Olfaz M, Ocak N, Erener G, Cam MA, Garipoglu AV:** Growth, carcass and meat characteristics of Karayaka growing rams fed sugar beet pulp, partially substituting for grass hay as forage. *Meat Sci*, 70, 7-14, 2005.
- 4. TURKHAYGEN-I Consortium:** *In Vitro* Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources-I, 2011, [http://www.turkhaygen.gov.tr/doc/turkhaygenproject\\_pr\\_en.pdf](http://www.turkhaygen.gov.tr/doc/turkhaygenproject_pr_en.pdf), Accessed: 06.10.2011.
- 5. Gordon I:** Embryo Transfer and Associated Techniques in Sheep. In, Gordon I (Ed): *Controlled Reproduction in Sheep and Goats*. Vol. 2, pp. 280-317, CAB International, New York, NY, 1997.
- 6. Gonzalez-Bulnes A, Baird DT, Campbell BK, Cocero MJ, Garcia-Garcia RM, Inskoop EK, Lopez-Sebastian A, McNeilly AS, Santiago-Moreno J, Souza CJH, Veiga-Lopez A:** Multiple factors affecting the efficiency of multiple ovulation and embryo transfer in sheep and goats. *Reprod Fertil Dev*, 16, 421-425, 2004.
- 7. Cognie Y, Baril G:** State of the art in sheep-goat embryo transfer, *INRA Prod Anim*, 15, 199-207, 2002.
- 8. Gonzalez-Bulnes A, Santiago-Moreno J, Cocero M J, Lopez-Sebastian A:** Effects of FSH commercial preparation and follicular status on follicular growth and superovulatory response in Spanish Merino ewes, *Theriogenology*, 54, 1055-1064, 2000.
- 9. Taşdemir U, Agaoglu A R, Kaymaz M, Karakaş K:** Ovarian response and embryo yield of Angora and Kilis goats given the day 0 protocol for superovulation in the non-breeding season. *Trop Anim Health Prod*, 43 (5): 1035-1038, 2011.
- 10. Mayorga I, Mara L, Sanna D, Stelletta C, Morgante M, Casu S, Dattena M:** Good quality sheep embryos produced by superovulation treatment without the use of progesterone devices, *Theriogenology*, 75, 1661-1668, 2011.
- 11. Azawi OI, Al-Mola MKMA:** Effect of season and mating system in Awassi ewes superovulated with FSH on fertilization rate and embryo recovery, *Iraqi J Vet Sci*, 24 (2): 75-79, 2010.
- 12. Ramon-Ugalde JP, Folch J, Cocero MJ, Piña-Aguilar RE, Alabart JL:** Embryo recovery from the oviduct in superovulated ewes: A method to improve MOET systems, *Czech J Anim Sci*, 53 (4): 145-151, 2008.
- 13. Thompson JGE, Simpson AC, James RW, Tervit HR:** The application of progesterone containing CIRDTM devices to superovulated ewes, *Theriogenology*, 33 (6): 1297-1304, 1990.
- 14. Torres S, Sevellec C:** Repeated superovulation and surgical recovery of embryos in the ewe. *Reprod Nutr Dev*, 27, 859-863, 1987.
- 15. Boland MP, Crosby TF, Gordon I:** Ovarian response in ewes following horse anterior pituitary extract and progestagen treatment, *Anim Reprod Sci*, 6, 119-127, 1983.
- 16. Dattena M, Vespignani S, Branca A, Gallus M, Ledda S, Naitana S, Cappai P:** Superovulatory response and quality of embryos recovered from anestrus ewes after a single injection of porcine FSH dissolved in polyvinylpyrrolidone. *Theriogenology*, 42, 235-239, 1994.
- 17. Leoni G, Bogliolo L, Pintus P, Ledda S, Naitana S:** Sheep embryos derived from FSH/eCG treatment have a lower in vitro viability after vitrification than those derived from FSH treatment, *Reprod Nut Develop*, 41, 239-246, 2001.
- 18. Bettencourt EM, Bettencourt CM, Chagas e Silva J, Ferreira P, Manito CI, Matos CM, Romao RJ, Rocha A:** Effect of season and gonadotropin preparation on superovulatory response and embryo quality in Portuguese Black Merinos. *Small Rumin Res*, 74, 134-139, 2008.
- 19. Cognie Y, Chupin Y, Saumande J:** The effect of modifying the FSH/LH ratio during the superovulatory treatment in ewes. *Theriogenology*, 25, 148, 1986.
- 20. D'Alessandro A, Martemucci G, Colonna MA, Cafueri C, Todeda F:** Some affects of adding pLH in defined amounts to purified pFSH to modify FSH/LH ratios during the superovulatory treatment of anoestrus ewes, *Anim Reprod Sci*, 47, 91-98, 1997.
- 21. Armstrong DT:** Recent advances in superovulation of cattle. *Theriogenology*, 39, 7-24, 1993.
- 22. Dixon IE, Hopkins GJ:** Superovulation of cattle using porcine pituitary gonadotropin preparation (Pluset Serono). In, *Pluset Scientific Literature Serono Veterinary*, Rome, Italy, 22-23, 1996.
- 23. Hemming PJ:** Hormone therapy and estrus cycle control. In: *Comparative Veterinary Reproduction and Obstetrics*, Lecture 4, 2007, <http://art-breedingcenter.com/Articles.php?a=Content/ReproHormones.htm>, Accessed: 10.11.2011.
- 24. Stringfellow DA, Seidel SM (Eds.):** Manual of the International Embryo Transfer Society, Savoy Il, International Embryo Transfer Society, Inc., USA, 1998.
- 25. Cahill LP, Saumande J, Ravault JP, Blanc M, Thimonier J, Mariana JC, Mauleon P:** Hormonal and follicular relationships in ewes of high and low ovulation rates. *J Reprod Fertil* 62, 141-150, 1981.
- 26. McNatty KP, Hudson NL, Ball K, Mason A, Simmons MH:** Superovulation and embryo recovery in goats treated with Ovagen and Folltropin. *N Z Vet J*, 37, 27-29, 1989.
- 27. Simonetti L, Forcada F, Rivera OE, Carou N, Alberio RH, Abecia JA, Palacin I:** Simplified superovulatory treatments in Corriedale ewes, *Anim Reprod Sci*, 104 (2-4): 227-237, 2008.
- 28. Cordeiro MF, Lima-Verde JB, Lopes-Júnior ES, Teixeira DIA, Farias LN, Salles HO, Simplício AA, Rondina D, Freitas VJF:** Embryo recovery rate in Santa Inês ewes subjected to successive superovulatory treatments with pFSH, *Small Rumin Res*, 49, 19-23, 2003.