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The effect of single epidural plus intramusculer injection of FSH on superovulatory response in Anatolian Black cow

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Summary: The present study was designed to determine whether a single epidural injection plus intramuscular (im) injection of FSH in order to reduce the number of injection treatments required to induce supurovulatory response, as well as to determine the effects of this treatment on Anatolian Black cow (*Bos Taurus*). The results will be compared to those obtained by administration of twice daily injections of FSH. Twenty-one Anatolian Black cows were used in this study. Anatolian Black cows received Cue-mate containing 1.56 g progesterone. The cows were divided into three groups (D1, D2 and E) based on the dose and administration route of FSH. In group D1 a total of 490 IU FSH and in group D2 a total of 700 IU FSH was given intramuscularly in decreasing doses for twice daily for 4 days. In both groups superovulatory treatments were initiated on day 7 of the cycle. In group E, FSH was given 175 IU epidural plus 175 IU im injection on day 7 of the cycle. On the 9th day of the cycle, the Cue-mate was removed and the cows received 500 µg prostaglandinF2α in all goups. The cows were artificially inseminated (AI) using semen obtained from native Anatolian Black bulls twice; in the evening of day 11 and in the morning of day 12 with 12 hours intervals. Superovulated cows were non-surgically flushed 7 days after AI. Embryos were morphologically evaluated and classified according to the criteria recommended by the IETS. No significant differences were observed in the mean number of Corpus luteum (CL) among groups which were 3.7 ± 0.56 in D1; 3.4 ± 0.55 in D2 and 6.0 ± 1.14 in E . There was no difference in transferable embryo yield among groups (10/42, 23.8% in D1; 14/32, 43.8% in D2 and 19/82, 23.2% in E). Untransferable embryo yield was not also significantly different among the groups (32/42, 76.2% in D1; 18/32, 56.2% in D2 and 63/82, 76.8% in E). The UFO rate was similar in all groups (17/77, 22.1% in D1; 13/71, 18.3% in D2 and 28/132, 21.2% in E). Recovery rate was found to be significant among groups (59/77, 76.6% in D1; 45/71, 63.4% in D2 and 110/132, 83.3% in E). To conclude, a single epidural injection plus an im injection of FSH application can be obtained acceptable results compared to twice daily injection of FSH for superstimulatory response. However transferable embryo rate of Anatolian Black cow was found to be low in both single epidural injection plus im injection and twice daily FSH applications. Further studies are required to obtain more concrete results on increasing transferable embryo yields in different breeds.

Key words: Cow, embryo, epidural injection, FSH, superovulation.

İntramuskuler uygulama ile birlikte epidural boşluğa uygulanan FSH'nın Yerli Kara inekte süperovulasyona olan etkisi

Özet: Bu çalışma süperovulasyon oluşturmak için tekrarlanan dozlarda yapılan FSH uygulama sayısını azaltmak, FSH'nın tek uygulama şeklinde epidural-im olarak uygulanmasının süperovulasyon oluşturma yönünde etkisini belirlemek ve bu uygulamanın Yerli Kara inekte etkisini ortaya koymak amacıyla tasarlandı. Elde edilen sonuçlar günde iki kez azalan dozlarda yapılan FSH uygulama sonuçları ile karşılaştırıldı. Bu çalışmada 21 baş Yerli Kara inek kullanıldı. Yerli Kara ineklere 1.56 g progesteron içeren Cue-mate uygulandı. İnekler FSH uygulama şekli ve dozuna bağlı olarak 3 gruba (D1, D2 ve E) ayrıldı. Grup D1'e toplam 490 IU FSH ve grup D2'ye toplam 700 IU FSH süperovulasyon prokolünün 7. gününde başlamak üzere 4 gün süre ile azalan dozlarda im olarak uygulandı. Grup E'ye ise FSH süperovulasyon prokolünün 7. gününde 175 IU epidural, 175 IU im olmak üzere tek uygulama biçiminde yapıldı. Süperovulasyon protokolünün 9. gününde Cue-mate çıkarıldı ve gruplardaki tüm ineklere 500 µg prostaglandinF2α yapıldı. 11. gün akşam ve 12. gün sabah olmak üzere 12 saat ara ile tüm ineklere Yerli Kara boğalardan alınan spermalar ile suni tohumlama yapıldı. Süperovulasyon uygulanan inekler suni tohumlama uygulamasından 7 gün sonra cerrahi olmayan yöntemle embriyo elde etme çalışmasına alındı. Elde edilen embriyolar Uluslararası Embriyo Transferi Birliğinin bildirdiği kıstaslara göre sınıflandırıldı. Gruplar arasında ortalama CL sayıları yönünden istatistikî bir farklılık gözlenmedi. Ortalama CL sayıları grup D1'de 3.7 ± 0.56; grup D2'de 3.4 ± 0.55 ve grup E'de 6.0 ± 1.14 olarak belirlendi. Transferedilebilir embriyo oranları gruplar arasında farklı değildi (10/42, %23.8 grup D1; 14/32, %43.8 grup D2 ve 19/82, %23.2 grup E). Transferedilemez embriyo oranları da gruplar arasında farklı bulunmadı (32/42, %76.2 grup D1; 18/32, %56.2 grup D2 ve 63/82, %76.8 grup E). Tüm gruplar da UFO oranı benzerdi (17/77, %22.1 grup D1; 13/71, %18.3 grup D2 ve 28/132, %21.2 grup E). Embriyo geri kazanım oranı gruplar arasında farklı bulundu (59/77, %76.6 grup D1; 45/71, %63.4 grup D2 ve 110/132, %83.3 grup E). Sonuç olarak,

süperovulasyon oluşturma yönünden FSH'nın tek uygulama şeklinde yapılan epidural-im uygulamasının azalan dozlarda yapılan FSH uygulaması ile karşılaştırıldığında sonuçların azalan dozlarda yapılan uygulamaya yakın olduğu belirlendi. Ancak transferedilebilir embriyo oranı hem epidural-im uygulamada hem de azalan dozlarda yapılan uygulamalarda düşük bulundu. Transferedilebilir embriyo oranını artırmak için farklı ırklarda daha ileri çalışmaların yapılması gerektiği benimsendi.

Anahtar sözcükler**:** Embriyo, epidural enjeksiyon, FSH, inek, süperovulasyon.

Introduction

The Anatolian Black cow is Bos Taurus breed of Turkey which has ability of adaptation to harsh conditions. The population of pure Anatolian Black cow has been dramatically decreased, particularly in the last decade. This breed is one of the endangered genetic resources of Turkey. Information which indicates population number, distribution and risk status of this breed is not sufficient yet. For this reason a comprehensive inventory study should be initiated to alleviate the information gap and prepare a national action plan (14). Bovine embryo transfer has been applied widely around the world. This technology increases the number of offspring obtained from donors with high genetic value and is used to disseminate desirable genetics around the world (8). Superovulation is one of the main methods used to provide embryos for the embryo transfer technology (13). The objective of superovulatory treatments in cows is to obtain the maximum number of transferable embryos (11, 13). Many factors influence the variable yield and quality of embryos in superovulated cows (4). FSH treatments have been more commonly used for superovulation (36, 40). However, the biological half-life of FSH in cows has been reported relatively short (5 h or less) (15), thus it needs to be given frequently, in order to achieve high ovulation rates (32). It has been shown that twice daily injections of FSH with decreasing doses induce a greater superovulatory response than single injection daily treatments (32, 44). However, use of twice daily im injections cost high and is technically difficult. Furthermore excessive handling associated with frequent treatments might be stressful for animals and may result in reduced superovulatory response (15). On the other hand, the development of practical approaches to achieve enhanced responses to superovulation protocols still require further research (1). Simplified protocols of superstimulation are expected to reduce donor handling, costs and improve response for animals (1, 17). Although there are many reports on the efficiency of FSH treatment for ovarian superstimulatory response in the cow, there is no information about on epidural injection of FSH for superstimulatory response.

Thus, the present study was designed to determine whether a single epidural plus im injection of FSH reduces the number of injection treatments required to induce satisfactory superovulatory response, as well as to determine the effects of this treatment on Anatolian Black cow. Finally, The results were compared to those obtained by twice daily injections of FSH.

Materials and Methods

Study population: Twenty-one Anatolian Black cows (*Bos Taurus*) ages 3 to 3.5 years old were used with crossover design. The study was conducted at Lalahan Livestock Central Research Institute (39°58′ 23.56″ N, 33°06′ 28.51″ E) at an altitude of 1098m.

Average body weight of the cows ranged between 300 and 350 kg. The cows were housed outdoors in a sheltered pen and fed with concentrate daily together with good quality alfaalfa hay and barley straw. Water was supplied ad libitum. All cows were palpated per rectum prior to the study to determine possible reproductive tract abnormalities. The experimental procedures were approved by the Animal Care Committee of Istanbul University, Faculty of Veterinary Medicine.

Study design: Anatolian Black cows received a Cue-mate containing 1.56 g progesterone (Cue-mate, Bioniche Animal Health Ltd, New Zeland) on a random day of cycle (day 0). The cows were divided into three groups based on the dose and administration route of FSH (Folltropin, 700 IU, Bioniche Animal Health Ltd, Republic of Ireland). In group D1 a total of 490 IU FSH was given as intramuscular (im) injection in decreasing doses twice daily for 4 days. (87.5, 87.5, 70, 70, 52.5, 52.5. 35, 35 IU; n=21; Figure 1). In group D2 a total of 700 IU FSH was given as im injection in decreasing doses twice daily for 4 days (140, 140, 105, 105, 70, 70, 35, 35 IU; n=21; figure 2). In both groups superovulatory treatments were initiated on day 7 of the cycle. In group E, FSH was given 175 IU epidural plus 175 IU im injection on day 7 of the cycle (n: 21; figure 3). 9 days later begining of the cycle, the Cue-mate was removed and cows received 500 µg prostaglandinF2α (PGF2α; Cloprostenol, 250 µg, im, Estrumate®, Intervet, Turkey) in all goups. The cows were artificially inseminated using semen obtained from Anatolian Black bulls twice; in the evening of day 11 and in the morning of day 12 with 12 hours intervals. Ovaries of the cows were palpated at the begining of flushing to estimate the number of CL. Superovulated cows were non-surgically flushed 7 days after AI. Each cow was given epidural anaesthesia using 60 mg Adocain (Lidokain HCL, 20mg/ml, Sanovel, Turkey) prior to flushing. The vulva was scrubbed with disinfectant and Foley catheter (14-16

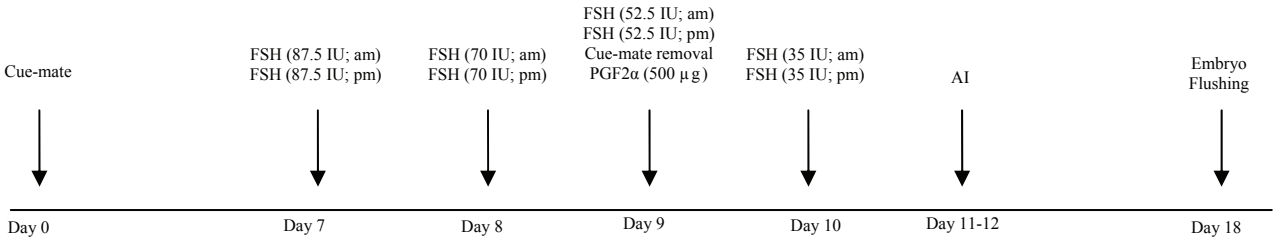

Figure 1. Schematic representation of group D1
Figür 1. Grup D1'in şematik sunumu

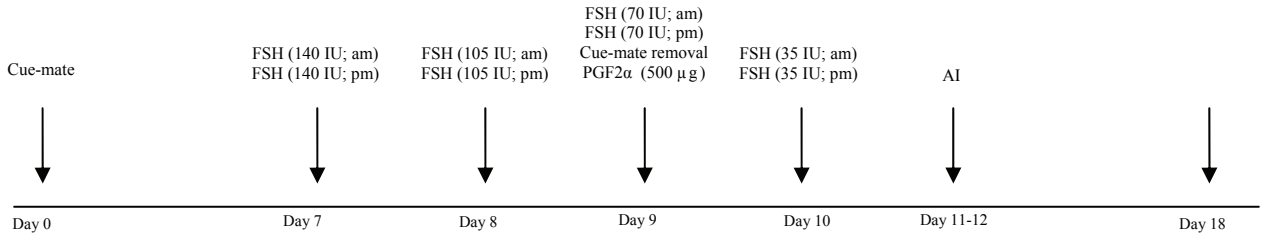

Figure 2. Schematic representation of group D2
Figür 2. Grup D2'nin şematik sunumu

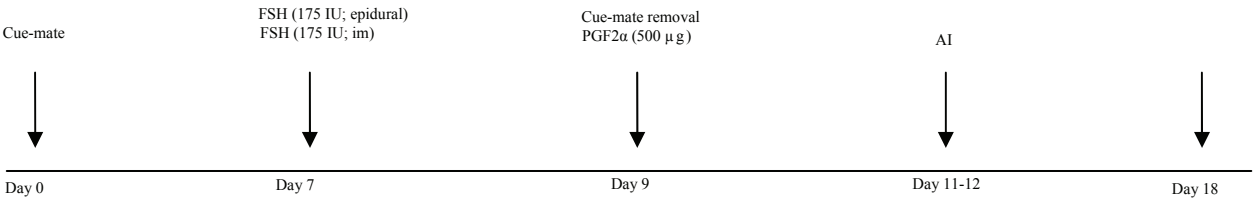

Figure 3. Schematic representation of group E
Figür 3. Grup E'in şematik sunumu

FR, Agtech, USA) with a 15 ml bulb, inserted through the vagina and the cervix. A stainless steel stylet was used to stiffen the catheter to allow passage through the cervix and both horns were flushed with flushing media (990 ml ringer lactate+10 ml calf serum) using folley cathater inserted next to the utero-tubal junction. The uterine horns were massaged and manipulated to facilitate medium return flow. The uterine exposed flushing media was collected into 1000 ml sterilized bottle. A stereomicroscope (Olympus, SZH10, Olympus, Japan) was used for searching the embryos. Embryos were morphologically evaluated and classified according to the criteria recommended by the IETS (45). Embryos classified as Grade 1 (excellent or good) and 2 (fair) were considered as transferable embryos, while embryos classified as Grade 3 (poor) and 4 (dead or degenerated) were considered as untransferable embryos.

Statistical analysis: The number of CL was submitted to variance analysis (Proc GLM). Embryo yields and recovery rate were evaluated by the chi-square test using MINITAB (MINITAB Release 13.0; Copyright 2000). Body condition, season of year, cows and calving intervals were considered and analysed as covariates The results are expressed as means or proportions (±S.D.). The differences were considered significant at $P<0.05$.

Results

As set out table 1, no significant differences were observed among treatment groups. The mean numbers for CL were determined as (3.7 ± 0.56 in D1, 3.4 ± 0.55 in D2 and 6.0 ± 1.14 in E groups; p=0.084). Transferable embryo yield also showed no significant difference among treatment groups (10/42, 23.8% in D1; 14/32, 43.8% in D2 and 19/82, 23.2% in E groups; P=0.071; table1). Untransferable embryo yield was also not significantly different among treatment groups (32/42, 76.2% in D1; 18/32, 56.2% in D2 and 63/82, 76.8% in E groups; p=0.071; table1). Unfertilized oocytes (UFO) rates were similar in all groups (17/77, 22.1% in D1; 13/71, 18.3% in D2 and 28/132, 21.2% in E groups; p=0.837; table1). Recovery rate was significantly

Table 1: Ovarian response and embryo yield different dose and administration of FSH
Tablo 1: Farklı dozlarda ve biçimde uygulanan FSH sonrasında elde edilen Corpus luteum (CL) sayıları ve embriyo oranları

	Group D1	Group D2	Group E	P value
CL number	3.7 ± 0.56	3.4 ± 0.55	6.0 ± 1.14	*P*=0.084
Transferable embryo yield	23.8% (10/42)	43.8% (14/32)	23.2% (19/82)	*P*=0.071
Untransferable embryo yield	76.2% (32/42)	56.2% (18/32)	76.8% (63/82)	*P*=0.071
Unfertilized oocyte	22.1% (17/77)	18.3% (13/71)	21.2% (28/132)	P=0.837
Recovery rate	76.6% (59/77) [a]	63.4% (45/71)[ab]	83.3% (110/132) [b]	P=0.006

a, b, ab: Different letters within the same line are significantly different (P < 0.05)

different among treatment groups (59/77, 76.6% in D1; 45/71, 63.4% in D2 and 110/132, 83.3% in E groups; p=0.006).

Discussion and Conclusion

In the present study, the mean number of CL was found greater in the group that received a single epidural injection FSH plus an im injection of FSH than the groups which received FSH twice daily for 4 days. Although the difference between the mean numbers of CL among the groups were not statistically significant, they were found lower than some previous studies ranging from 7.3-31.3 (2, 9, 10, 23, 34). However, findings are similar to Yu et al. (47) who superovulated Tianzhu white yaks with twice daily injection for 4 days and reported 4.8 CL. The changes in ovarian response have been found to be related with differences in superstimulatory treatments such as total dose, duration and timing of treatment, and the use of additional hormones in the treatment protocol (38, 39). Additional factors for the differences in ovarian response might be the genetic of the animal and its environment (3), dietary intake (46), breed (1, 9, 42), season (33), age (27, 29), ovarian status at the time of the treatment (5, 18) and the effects of repeated superstimulations (30). It has been reported that application of FSH in various forms and ways other than im injection can induce superovulatory response and subcunaneous fat amount is considered to be a determinant factor in cattle (10, 25). Similarly, the present findings show that epidural injection of FSH resulted in an acceptable superovulatory response to compare twice daily injection of FSH in Anatolian Black cows. Reasons for this result are unclear; however, it could be speculated that obtaining results probably were dependent on administration way of FSH by which it was absorbed slowly.

A series of studies were conducted in severel different donor breeds to determine the appropriate dose and administration way of FSH for eliminating stress due to multiple injection of FSH (12). It has been reported that a single im and sc injections of FSH has the same effects with twice daily FSH treatments (1, 21, 35, 43). In the present study, the proportion of transferable embryo yield also showed no significant difference among treatment groups. The current result was consistent with some reports in which the proportion of high-quality embryos is inversely associated with the responsiveness of cattle for superovulation (20, 22). When comparing with other studies conducted with different FSH protocols, transferable embryo proportions in present study were lower than in breeds of Sistani (49%), Angus (52%), Finnish Ayrhire (61%), Holstein (54%), Korean native (62%) and Japanese Black (53%), (6, 26, 31, 34, 37, 41). However, similar results were reported in Nelore cows (1, 7). Donaldson (16) reported that the number of low-quality embryos collected increased when an LH surge was induced extremely early, extremely late, or not induced at all. The relationship between the time interval to the induction of an LH surge and the number and/or quality of embryos is not completely understood. A lower number of transferable embryos in the present study might be related to the early regression of CL or poor reproductive performance of Anatolian Black cow.

It was reported that the lower rates of fertilization could be a consequence of decrease in the numbers of growing follicles (28). Unfertilized oocytes (UFO) rates were similar in all groups. In this study, UFO rate was found higher than some previous studies which ranged from 23.5% to 39% in different breeds (26, 31, 38, 41). This may due to asynchronous ovulation and fertilization failure.

Recovery rate was found significantly different among all groups and it was the highest in group E (175 IU epidural plus 175 IU intramusculer injection of FSH day 7 of the cycle). Studies on embryos/ova recovery rate (per ovulated follicle) using nonsurgical embryo recovery were varied widely from as high as 60–80% (23, 24) to as low as 20–25% (19). Recovery rate found in this study was consistent with the previously reported acceptable rate.

In conclusion, a single epidural injection plus an im injection of FSH application can be obtained acceptable results compared to twice daily injection of FSH for superstimulatory response. However, transferable embryo rate of Anatolian Black cow was found to be low in both single epidural injection plus im injection and twice daily FSH applications. Further studies are

required to obtain more concrete results on increasing transferable embryo yields in different breeds.

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