

Various FSH Administration on Superovulatory Response and Embryo Yield in Anatolian Black Heifers

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ABSTRACT

The aim of the present study was to investigate effectiveness of superovulatory response and embryo yield in Anatolian Black heifers, induced with the administration of two different follicle stimulating hormone. Heifers received a progesterone releasing device (Cue-mate containing 1.56 g progesterone). The heifers were randomly assigned to four groups. Group Folltropin (F) were administered with Folltropin (400 mg) as a control, while those included in Groups Ovagen (O) were administered with Ovagen at doses of 8.8 mg (O1), 11.44 mg (O2) and 17.6 mg (O3) respectively. On the 9th day of the application, the Cue-mate was removed and the heifers received 500 µg prostaglandinF2α in all groups. The heifers were artificially inseminated (AI) using semen obtained from Anatolian Black bulls; in the evening of day 11 and in the morning of day 12 with 12 hours intervals. Embryos were collected by uterine flushing 7 days after AI. The mean number of CL determined as 6.33±0.718 in F, was found to be higher than the numbers obtained with the administration of the three different doses of O (3.82±0.502, 3.50±0.513, 3.58±0.448 respectively; p<0.001). The transferable embryo yield did not differ significantly among the treatment groups (p>0.05). In conclusion, findings show that although the administration of F was ascertained increase the number of CL and the total number of ova/embryos recovered, these increased numbers had no reflection on the number of transferable embryos in Anatolian Black heifers.

Key Words: Anatolian Black heifer, Folltropin, FSH, Ovagen, Superovulation

Yerli Kara Düvelerde Süperovulasyon ve Embriyo Verimi Üzerine Farklı FSH Uygulamalarının Etkileri

ÖZ

Bu çalışmanın amacı Yerli Kara düvelerde süperovulasyon oluşturma ve embriyo elde etme yönünde iki farklı follikül uyarıcı hormonunun etkinliğini gözlemlemektir. Düvelere 1.56 g progesteron içeren Cue-mate uygulandı. Düveler dört gruba ayrıldı. Folltropin (F) grubuna kontrol olarak 400 mg folltropin diğer taraftan Ovagen (O) grubuna ise sırasıyla 8.8 mg (O1), 11.4 (O2) ve 17.6 (O3) olmak üzere Ovagen uygulandı. 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. Suni tohumlama uygulamasından 7 gün sonra embriyo elde etme çalışması ile embriyolar toplandı. Ortalama CL sayıları F grubunda 6.33 ± 0.718 belirlenirken, O grubunda uygulanan üç farklı dozdan (sırasıyla 3.82 ± 0.502, 3.50 ± 0.513, 3.58 ± 0.448; p<0.001) elde edilen CL sayılarından daha yüksek bulundu. Transfer edilebilir embriyo oranları uygulama grupları arasında farklı değildi (p>0.05). Sonuç olarak, bulgular F grubunda yapılan uygulamanın CL, elde edilen embriyo ve ovum sayısını artırmasına rağmen transfer edilebilir embriyo sayısına olumlu bir etkisinin olmadığını gösterdi.

Anahtar Kelimeler: Yerli Kara Düve, Folltropin, FSH, Ovagen, Süperovulasyon.

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INTRODUCTION

Animal genetic resources are an integral element of biodiversity. Indigenous animal breeds have adaptation adverse environmental impact. The Anatolian Black, which is indigenous cattle breed characterised by delayed puberty, has well adapted to the severe climatic conditions of Anatolia and is commonly raised at high altitudes in Turkey (Compan and Cividini 2008). The genetic material of this particular breed needs to be preserved, by reason of the pure Anatolian Black cow population has dramatically decreased, particularly in the last decade (Taşdemir et al. 2012). Embryo transfer that has been applied all over the world is a suitable method for preservation of endangered animal species and breeds (Wildt et al. 1991, Solti et al. 2000). Besides, this method is used to increase the number of offspring obtained from donors with high genetic value (Baruselli et al. 2006) and it significantly reduces the time period required for genetic improvement and offers several advantages in comparison to natural breeding programme (Barati et al. 2006). There are various protocols applied for superovulation, which produce embryos (Silva et al. 2009). It is reported that, 5 to 6 transferable embryos have been obtained each superovulation treatment in the *Bos taurus* (Hasler 1992). It is well known the quality of the embryo obtained from donor cattle is influenced by both intrinsic and extrinsic factors. Intrinsic factors include genetics, individual sensitivity to gonadotropins, age, nutritional and health status (Silva et al. 2009). As for that extrinsic factors include the use of different commercial FSH preparations, dosage, season and administration route (Kafi and McGowan 1997, Taşdemir et al. 2012). There are many reports on the efficiency of FSH treatment for ovarian superstimulatory response in the cow. However, there are few studies that provide knowledge on superovulatory response and embryo yield in indigenous breed heifers. Therefore, the aim of present study was to investigate effectiveness of superovulatory response and embryo yield in Anatolian Black heifers, induced with the administration of various doses of two different follicle stimulating hormone.

MATERIALS and METHODS

Animals

Twenty-one heifers (*Bos Taurus*) aged 2-4 years and of a mean body weight of 250-300 kg, which were housed out doors in a sheltered pen, constituted the material of the study. The heifers were fed three times a day with concentrated feed together with high quality alfalfa hay and barley straw. Water was supplied ad libitum. The same heifers were used in

each group with crossover design. All heifers 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 heifers received a progesterone releasing device (Cuemate-1.56 g progesterone, Bioniche Animal Health Ltd, New Zealand) on a random day of cycle (day 0). The heifers were divided into four groups based on the dose of Foltropin (F) (400 mg NIH-FSH-P1, Bioniche Animal Health Inc., Ontario, Canada) and Ovagen (O) (17.6 mg NIADDK-oFSH- 17 standart, Immuno-Chemical Products Ltd. Auckland, New Zealand). In group F a total of 400 mg FSH was given as intramuscular (im) injection in decreasing doses twice daily for 4 days (80, 80, 60, 60, 40, 40, 20, 20 mg; n=21). In group O1, O2 and O3 a total of 8.8, 11.44 and 17.6 mg Ovagen were given as im injection in decreasing doses twice a daily for 4 days (1.76, 1.32, 1.32, 0.88, 0.88, 0.88, 0.88, 0.88 mg in group O1; 2.10, 1.66, 1.66, 1.32, 1.32, 1.32, 0.88, 0.88 in group O2; 3.52, 3.52, 2.64, 2.64, 1.76, 1.76, 0.88, 0.88 in group O3 respectively; n=21). In all groups superovulatory treatments were initiated on day 7 of the cycle. nine days later beginning of the cycle, the Cue-mate was removed and heifers received 500 µg prostaglandinF2α (PGF2α; Cloprostenol, 500 µg, im, Estrumate®, Intervet, Turkey). The heifers were artificially inseminated twice using semen obtained from Anatolian Black bulls; in the evening of day 11 and in the morning of day 12 with 12 hours intervals. Ovaries were checked at the beginning of flushing to determine number of CL by ultrasonographic examinations (Falco 100, Pie- Medical- USA). Superovulated heifers were flushed at day 7 after AI. Each heifer 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 (13-14 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+1 ml kanamycin sulphat) using foley catheter 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. Collected media was filtered using 0.22 micrometer filter. A stereomicroscope (Olympus, SZH10, Olympus, Japan) was used for searching the embryos. Embryos were morphologically evaluated and classified same person according to the criteria recommended by the IETS (Stringfellow and Seidel 1998). 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

Statistical analyses were made using the MINITAB (MINITAB Release 13.0; Copyright 2000) statistical software. This study provides informative statistical data on the parameters investigated. A square root transformation was performed before the groups were compared for the number of CL by analysis of variance (Proc GLM). Multiple comparisons were made using Tukey's test was performed for the comparison of the groups for embryo recovery rates (RR) and embryo yields.

RESULTS

As shown table 1, the mean number of CL determined as 6.33 ± 0.718 in group F, was found to be higher than the numbers obtained with the administration of the three different doses of O ($P < 0.001$). In the current study, the transferable embryo yield did not differ significantly among treatment groups ($P > 0.05$) and were determined as 33.3% (13/39) in the F, 40.0% (4/10) in the O1, 69.2% (9/13) in the O2 and 34.6% (9/26) in the O3. Ovum-embryo recovery rate was greater in F group than the rates obtained with the administration of the three different doses of O ($p < 0.001$).

Table 1: The effects of FSH administration on ovarian response and embryo yield

Tablo 1: Süperovulasyon ve embriyo verimi üzerine farklı FSH uygulamasının etkileri

	Group F	Group O1	Group O2	Group O3	P
CL number	6.33 ± 0.718 a	3.82 ± 0.5 02 ^b	3.50 ± 0.5 13 ^b	3.58 ± 0.4 48 ^b	0.001
Transferable embryo yield	33.3% (13/39)	40.0% (4/10)	69.2% (9/13)	34.6% (9/26)	0.129
Untransferable embryo yield	66.7% (26/39)	60.0% (6/10)	30.8% (4/13)	65.4% (17/26)	0.129
Recovery rate	83.5(111/133) ^a	41.5(27/65) ^b	47.6(30/63) ^b	51.5(35/68) ^b	0.000

a,b:Different letters within the same line are significantly different.

DISCUSSION

The superovulation process is affected by several factors in cattle. One of the main factors which limit embryo production is the effect of gonadotropins, used commercially for superovulation, on the ovaries and these hormones enabling the ovulation of an

adequate number of oocytes (Kelly et al. 1997). Granulosa cells possess a limited number of FSH receptors, which activate the growth and development of follicles. When used at favourable level for superovulation purposes, FSH binds to these receptors and produces a satisfactory induction (Gonzales et al. 1990, Hockley et al. 1992). Some authors reported the variability of the ovarian response to superovulation treatments being related to gonadotropin administration route, total dose, timing, LH residue on FSH, duration and the use of additional hormones (Mapletoft et al. 2002, Taşdemir et al. 2011). The current results related to CL number, in contrast to previous findings in which obtained similar superovulation results with the use of O and FSH-P in Nelore cattle (Silve et al. 2009). In a study (Nilchuen et al. 2012) in which 200 and 250 mg of FSH (NIH-FSH-P1) did not induce different superovulatory responses in cows and heifers too. Ağaoglu et al. (2012) suggested that O had a stronger effect on superovulation in Karayaka sheep, compared to that of F. Besides, present results are different to the report obtained from Anatolian Black cow study in which the administration of high and low doses of FSH elicit similar effects on superovulatory response (Taşdemir et al. 2012). On the other hand, the current results obtained in the present study are similar to those reported by some researcher (Carvalho et al. 2013). It was observed that, the administration of O, which contains ovine FSH, had less effect on the superovulatory response, in comparison to F, which contains porcine FSH. The results obtained generate that, porcine FSH was more effective than ovine FSH on the superovulatory response in Anatolian Black heifers. The differences observed in the present study, in comparison to those reported in previous research, were attributed to the genetic differences of Anatolian Black heifer and O containing a lower quantity of FSH, when compared to F.

Gonadotropins, which contain a high level of LH, adversely affect fertilization rate and embryo quality, gonadotropins containing low levels of LH are preferred to be used (Herrler et al. 1991, Hockley et al. 1992). F and O have different bioactive FSH and LH composition; the FSH content of O is more active compared to that of F (McNatty et al. 1989). In the present study, transferable embryo with the administration of both F and O in the Anatolian Black heifers, were lower than those previously reported to have been obtained in local cattle breeds (Baruselli et al. 2006, Silva et al. 2009). However, transferable and non-transferable embryos were found to be similar to those obtained in previous research (Barati et al. 2006, Nichuen et al. 2012). Silva et al. (2009) reported that the transferable embryo obtained with the use of different FSH

preparations in Nelore cattle did not differ from each other. Similar to our results, in a previous study which Bonsmara cows and heifers were administered with the respective doses of 320 mg and 200-260 mg of NIH-FSH-P1 Folltropin-V, the number of transferable embryos recovered was 5.1 ± 0.9 in the cows and 3.7 ± 0.8 in the heifers (Carballo et al. 2008). In a study on Mertolenga cattle, informed no difference was determined between Folltropin and FSH-P administration for embryo production with respect to the number of total ovum and embryos (Quaresma et al. 2003). On the contrary to the findings of this study, Ağaoğlu et al. (2012) reported that embryo quality was affected by the type of FSH preparation which had been used for superovulatory stimulation on Karayaka ewes. Based on the current results, difference between the findings may be related to species, genetic differences or poor reproductive performance of Anatolian Black heifer. The ovum-embryo recovery rate determined in the present study was lower than previously reported in the same breed cattle (Taşdemir et al. 2012). Studies on embryos/ova recovery rate (per ovulated follicle) using nonsurgical embryo recovery varied widely from as high as 60–80% (11, 23) to as low as 20–25% (Ireland et al. 2007). Recovery rate found in this study was consistent with the previously reported acceptable rate. However, in the present study, the ovum-embryo recovery rate achieved with the administration of F was higher than the rates obtained with the administration of the three different doses of O. It is thought that, as also highlighted by Kanagawa et al. (1995), this difference may be related to several factors, including the experience of the staff performing the treatment, and the timing of collection. The birth canal of Anatolian Black heifer being narrow, complicates the performance of both rectal palpation and embryo collection procedures, and may be responsible for lower embryo recovery rates.

In conclusion, findings show that although the administration of F was ascertained increase the number of CL and the total number of ova/embryos recovered, these increased numbers had no reflection on the number of transferable embryos in Anatolian Black heifers. It is suggested that O can be used instead of F since the required dose for superovulation is less without affecting the transferable embryo yield in indigenous breed.

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