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Araştırma Makalesi – Research Article

The Effect of a GnRH Agonist Injection or Progesterone Implant at Diestrus in Cryopreserved Embryo Transferred Cows

Mesut KIRBAŞ[1] Bülent BÜLBÜL[1] Mehmet KÖSE[2] Şükrü DURSUN[1]
Mehmet ÇOLAK[3]

[1]Bahri Dağdaş International Agricultural Research Institute, Konya, Turkey
[2]Dicle University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Diyarbakır, Turkey
[3]Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Animal Science, Burdur, Turkey
mesutkirbas@gmail.com

Abstract

In this study, the effect of a single dose of GnRH on d 13 or progesterone implant for 7 days between d 13 and 20 on plasma progesterone levels and pregnancy rates on cryopreserved embryo transferred cows were investigated. Synchronized 48 Brown Swiss recipient cows were used as animal material. Seven days after estrus detection, cryopreserved cattle embryos were transferred into recipients and cows were assigned randomly into three groups. In GnRH group (n=16), cows were intramuscularly injected 10 μg buserelin acetate on d 13. In Implant group (n=16), ear implants containing 3 mg norgestomet were applied for 7 days between d 13 and 20. Control group (n=16) animals were intramuscularly injected 2 ml of saline on d 13. Progesterone levels in Implant group on d 16 and 20 were higher than that of in GnRH and Control groups ($p<0.05$). Pregnancy rates on d 28 were 43.75% (7/16), 50% (8/16) and 18.75% (3/16) in GnRH, Implant and Control groups, respectively, and it was higher in Implant group than that of in control group ($p=0.06$). In conclusion, progesterone supplementation between d 13 and 20 via ear implant increased the progesterone levels on d 16 and 20, and increased pregnancy rate compared to Control group.

Key Words: GnRH, ear implant, plasma progesterone, pregnancy, embryo transfer, cow

Dondurulmuş Embriyo Transfer Edilmiş İneklerde Diöstrüste GnRH Enjeksiyonu ya da Progesterone İmplantının Etkisi

Özet

Bu çalışmada, dondurulmuş-çözdürülmüş embriyo transferi yapılmış ineklerde 13. gün (östrüs=0) tek doz GnRH ya da 13-20. günler arasında progesteron uygulamasının kan plazması progesteron seviyesi ve gebelik oranları üzerine etkisi araştırıldı. Bu amaçla, 48 baş İsviçre Esmeri ırkı inek senkronize edilerek taşıyıcı olarak kullanıldı. Östrüs senkronizasyon protokolünü takiben tespit edilen östrüsten 7 gün sonra bütün ineklere ethilen glikolle dondurulmuş-çözdürülmüş embriyolar transfer edildi ve inekler rastgele üç gruba ayrıldı. GnRH grubunda (n=16), ineklere 13. gün 10 μg buserelin asetat i.m. yolla enjekte edildi. İmplant grubundaki (n=16) ineklere 3 mg norgestomet içeren implantlar kulak derisi altına yerleştirildi. Kontrol grubundaki (n=16) ineklere ise 13. gün i.m. olarak 2 ml fizyolojik tuzlu su solusyonu enjekte edildi. İmplant grubunda 16 ve 20. günlerde saptanan progesteron seviyeleri GnRH ve Kontrol gruplarından yüksek oldu ($p<0.05$). Yirmisekizinci gün gebelik oranları GnRH, İmplant ve Kontrol gruplarında sırasıyla %43.75 (7/16), %50 (8/16) ve %18.75 (3/16) olarak tespit edildi ve İmplant grubunda Kontrol grubundan yüksek olarak bulundu ($p=0.06$). Sonuç olarak, ineklerde östrüsten sonraki 13-20. günler arasında kulak implantı şeklinde progesteron uygulaması 16 ve 20. günlerde progesteron seviyesini yükseltti ve kontrol grubuna göre gebelik oranını artırdı.

Anahtar Kelimeler: GnRH, kulak implantı, plazma progesteron, gebelik, embriyo transfer, inek

Introduction

Embryo transfer is a widely used technique for genetic improvement and to increasing the number of genetically superior cows. More than 50% of cattle embryos after recovery are frozen and later transferred to synchronized recipients (Hasler, 2001). However, the cost of pregnancy in cattle following embryo transfer is more expensive than artificial insemination (AI) and natural services because of the costs of drug for superovulation of donor cow and for synchronization of recipients, semen, registration, procurement and upkeep of recipient females etc. It is reported that if higher pregnancy rates are achieved by recipients, the cost of pregnancy would decrease (Looney et al., 2006).

It has been known that embryonic deaths are a major limiting problem to obtain reproductive efficiency in both dairy and beef cattle industry. Generally, preventing embryonic losses includes two main strategies. The first is the antiluteolytic strategies applied to prevent or delay the luteolytic response of the mother. The other is luteotropic application to increase level of progesterone. This strategy is based on the principle that progesterone is the responsible hormone for establishment and maintenance of pregnancy (Spencer et al., 2004). Moreover, Mann et al. (2006) showed that progesterone supplementation was the positive effect on early embryo development. It is also indicated that the decrease in peripheral plasma progesterone concentration due to inadequate luteal function deficiency in dams after AI or embryo transfer is the main cause of embryonic loss (Willard et al., 2003).

In previous studies, different hormonal treatments were applied to increase P4 concentrations and prevent embryonic loss, which is caused by luteal insufficiency, and improve pregnancy rates in recipients. For this aim, exogenous progesterone, gonadotropin releasing hormone (GnRH) and GnRH analogues or hCG were administered after or concomitant with embryo transfer and AI (Thatcher et al., 1994).

Exogenous progesterone supplementation can be done by progesterone releasing intravaginal devices (PRID), controlled internal drug release (CIDR) or Synchro-Mate ear implants during diestrus. With this method, progesterone concentration can be increased immediately through starting on the desirable time (Beltman et al., 2009). However, in previous studies, the effect of exogenous progesterone on pregnancy rates following embryo transfer is inconsistent. Tribulo et al. (1997) reported that the exogenous progesterone supplementation did not have any positive effect, while Smith et al. (1996) and Chagas e Silva et al. (2008) reported that it had a slightly positive effect on pregnancy rates.

Another method applied to increase progesterone concentrations in cattle is administration of GnRH on day 11 to 14 following estrus, which causes ovulation and/or luteinization of an antral follicle resulting in the formation of an accessory corpus luteum (CL). In a study, GnRH injection at diestrus promoted formation of an accessory CL by causing ovulation or luteinization (40% ovulation vs 60% luteinization) of the existing dominant follicle in the ovaries (Bülbül et al., 2009). However, the controversial results have also been obtained in the studies having used this method, like exogenous progesterone supplement method. While some studies have reported the positive effects of GnRH on day 11 (Block et al., 2003) or 13 (Tubino and Laraco, 1999), some other studies have failed to show similar effects on pregnancy rates on these days (Ellington et al., 1991; Galimberti et al., 2001; Franco et al., 2006).

Due to the inconsistent results of exogenous progesterone or GnRH treatments after transfer, further researches have to be done to explain the differentiation and to increase pregnancy rates in embryo transferred recipients (Smith and Grimmer, 2002; Block et al.,

2003). The aim of the present study was to evaluate the effect of GnRH treatment on d 13 or progesterone supplementation between d 13 and 20 after estrus on plasma progesterone concentrations and pregnancy rates in cryopreserved embryo transferred recipient cows.

Materials and Methods

The experiment was conducted on 48 crossbred Brown Swiss cows housed in free stall barns and had water ad-libitum, in Bahri Dagdas International Agricultural Research Institute (latitude 37° 51' 16" N and longitude 32° 34' 45" E), Konya, Turkey. Cows, aged 3-5 years, were selected taking the criterions listed as followed: no dystochia and retained foetal membranes in previous calving; no purulent discharge during vaginal examination; 50 to 90 days postpartum; have shown estrus at least once; no AI or mating after previous calving. The cows were fed with a ration composed of corn silage, alfalfa hay and a concentrate-mineral mix. Recipients were synchronized with two injections of 0.500 mg cloprostenol (Iliren C, Intervet, Istanbul, Turkey) i.m. 11 d apart. A total of 48 lactating Brown Swiss cows showed estrus after estrus synchronization and had a CL at least 20 mm were used in this study as embryo recipients. The day of estrus was considered as day 0. On day 7, recipients were examined by transrectal ultrasonography (5-7.5 MHz linear array probe, Falko, Pie Medical, The Netherlands) for the presence and size of a CL.

Embryos were produced and frozen by a commercial American company (Sunshine Genetics Inc., Whitewater, Wisconsin, USA), following standard protocols for superovulation and uterus flushing (Herman et al., 1994) and embryo freezing (Hasler et al., 1997) with ethylene glycol for direct transfer previously reported (the embryos were loaded in the straws from which they would be directly transferred in ethylene glycol (1.5 M in PBS supplemented with 0.4% BSA) and allowed to equilibrate for 20 min. After holding in the freezing chamber at -6 °C for 15 min, seeding was applied at -6 °C and freezing was accomplished by cooling from -6 to 32 °C at 0.6 °C/ min and finally straws were plunged into liquid nitrogen (LN2) for storage). Only Grade-1 blastocysts were used in the experiment. Frozen embryos were stored in liquid nitrogen (-196 oC) until transfer.

Thawing was carried out in air at room temperature for 5 sec and then in a water bath at 25 °C for 25 s. All embryos were transferred (by the same technician) non-surgically on day 7 into the uterine horn ipsilateral to the CL under epidural anesthesia (3-5 ml lidokain HCl, Vilcain, Vilsan, Ankara, Turkey). Estrus synchrony between embryo and recipient was ±12 h. After the embryo transfer, recipients were divided randomly into three groups (16 cows per group). Cows in GnRH group received 10 μg buserelin acetate (Receptal, Intervet, Istanbul, Turkey) i.m. on d 13. Cows in Implant group received an ear implant containing 3 mg norgestomet subcutaneously (Crestar, Intervet, Istanbul, Turkey) from d 13 to 20. Control cows received 2 ml of saline solution i.m. on d 13. Pregnancy was determined by ultrasound on d 28.

Blood samples were collected by coccygeal venipuncture into heparinized tubes on d 7, 13, 16 and 20 for progesterone (P4) assay. The samples were centrifuged within 30 min of collection and plasma separated and immediately stored at -20 oC until analyzed. Plasma progesterone was measured using a commercial radioimmunoassay kit (progesterone RIA DSL-3900, Diagnostic system lab. Texas, USA) using a solid phase radioimmunoassay (Isocomp I, MGM-instruments inc., Hamden, USA).

Statistical analysis was performed using Chi-square test to compare pregnancy rates, and ANOVA to compare plasma progesterone levels among the groups (MINITAB, Release 12.0, Minitab Inc.).

Results

Mean CL diameters and P4 levels (±S.E.M.) on embryo transfer day in groups are presented in Table 1. There was no significant difference among groups for these data.

Table 1. Mean CL diameters and P4 levels on embryo transfer day in groups (±S.E.M.).

Group	n	CL (cm)	Progesterone (ng/ml)
GnRH	16	2.61±0.11	5.95±0.67
Implant	16	2.60±0.10	6.07±0.65
Control	16	2.58±0.10	5.66±0.65

There was no significant difference among groups on day 13 for P4 levels. However, recipients in the Implant group had higher progesterone level than that of in GnRH and control groups on d 16 and 20 ($p<0.05$). Mean progesterone levels in Implant and control groups on d 20 declined compared with that of on d 16 (Figure 1).

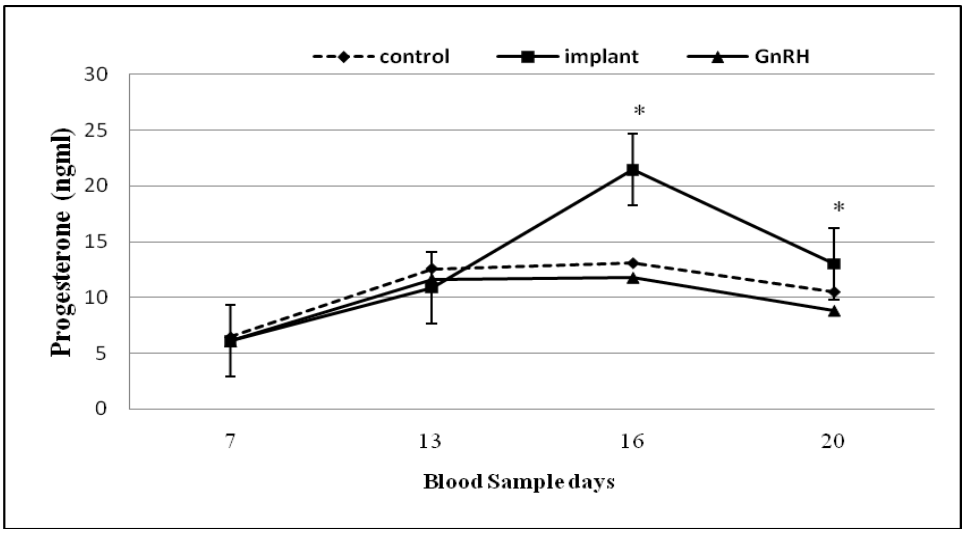

Figure 1. Mean progesterone levels in groups on d 7, 13, 16 and 20.

Pregnancy rates were 43.8% (7/16), 50% (8/16) and 18.8% (3/16) in GnRH, Implant and Control groups, respectively. There was a tendency for higher pregnancy rate in Group Implant compared to Control group (P= 0.06).

Discussion

The present study was carried out to evaluate the effect of GnRH injection on d 13 (estrus= 0) or progesterone application for 7 days between d 13 to 20 on pregnancy rates and P4 concentration in cryopreserved embryo transferred cows. Before GnRH and progesterone applications, mean plasma progesterone concentrations (on days of 7 and 13) and size of CL (at least 20 mm) on the ovary were similar ($p>0.05$).

One of the popular approaches to increase pregnancy rates after insemination or embryo transfer is GnRH injection to increase progesterone concentration during mid-luteal phase. At this phase, the aim of GnRH and analogues application arises from the functions of gonadotropins. In the present study, pregnancy rate obtained in GnRH group

was higher than that of in control group, but the difference was not significant. GnRH treatment on days 11-14 of estrus cycle following embryo transfer had positive effect in some reports (Tubino and Laraco, 1999; Block et al., 2003) while it had no effect in some others (Ellington et al., 1991; Galimberti et al., 2001). Moreover, results of many studies received GnRH after AI is inconsistent. Some reports displayed a positive effect of GnRH treatment 11-15 days after estrus (Rettmer et al., 1992a; Stevenson et al., 1993; Willard et al., 2003) while others (Rettmer et al., 1992b) indicated no effect like our study. Yildiz et al. (2009) reported that GnRH treatment on d 12 after insemination did not increase progesterone concentrations but improved pregnancy rate while Ataman et al. (2011) reported that the same application increased the plasma progesterone concentrations on d 18 and 21 but did not alter the pregnancy rates. Previous studies reported that GnRH treatment in mid-luteal period makes mid-cycle follicles to ovulate or luteinize, disrupts estradiol secretions (Bülbül et al., 2009; Retmer et al., 1992a). Increase of progesterone concentration was reported on 3rd (Stevenson et al., 1993) 6th day (Schmitt et al., 1996) after GnRH injection. In our study, the existence of seconder CL after GnRH injection was not controlled from d 20 until pregnancy examination. However, GnRH treatment showed statically no positive effect on progesterone concentration on day 16 and 20. Similar to our results, Schmitt et al. (1996) reported that luteotropic functions of luteal cells were not improved, and progesterone concentration was not affected by GnRH treatment. It is now well known that ovarian follicular growth is characterized by a follicle wave pattern, and an estrus cycle involves generally two or three waves in cattle. To achieve luteal support from GnRH applications in medium luteal phase depends on the phase of follicular wave. Previous results showed that effects of GnRH injection are atresiation, luteinization or ovulation of the follicle depending on its development stage (Doležel et al., 2002). A follicle may respond to the GnRH-induced LH surge when it has a diameter of 10 mm at the time of GnRH treatment. These varied responses may have been associated with different proportions of two and three-wave cycles in different populations of cows. The response of GnRH treatment may also be affected by dose of buserelin (Stevenson et al., 1993). Karimi et al. (2007) showed that administration of 5 or 10 ml Gonadorelin (a GnRH analogue) during mid-diestrus increased serum progesterone concentration; however, administration of 2,5 mL Gonadorelin did not increased serum progesterone concentration

Pregnancy rate in Implant group was higher than that of in control group in this study. In cattle, establishment and maintenance of pregnancy is a complex process, which depends on interactions between ovary, embryo and uterus. In order to achieve this interaction, progesterone, which is produced from CL on ovary, is the indispensible hormone of pregnancy because it stimulates and maintains endometrial functions necessary for maternal support of embryos survival and development. In cattle, the period between 15 to 17 days after estrus is more critical because luteolysis generally begins in these days. Moreover, the conceptus undergoes elongation and it begins secretion of interferone-tau in this period (Spencer et al., 2004). Mann and Lamming (2001) showed that embryos developed in lower luteal phase plateau were in a smaller size and they produced little or no interferone-tau compared to that developed in a higher luteal phase plateau. According to the results of this study, during this critical period, size of conceptus is very important, and have to secret sufficient amounts of interferone-tau to prevent luteolysis. Apart from size of conceptus during this critical period, high progesterone concentration could be important for maintenance of bovine pregnancy. Bogacki et al. (2002) showed that high progesterone prevents the luteolysis via suppressing the ability of oxytocin to induce endometrial secretion of prostaglandin (PG) in vitro. Therefore, progesterone supplementation may be useful to block PG secretion from endometrium during this critical period. In our study, ear implant application increased P4 concentration on day 16

compared with other groups. Mehni et al. (2012) stated that CIDR application as a source of releasing continuous progesterone increased pregnancy rate probably through preventing early embryonic mortality compared with progesterone and GnRH injection. CIDR, PRID or ear implant applications have more advantages than progesterone, GnRH or hCG injections because they can be applied at the desired and critical time, and plasma P4 levels rise in a short time. There are a few reports, involving direct progesterone supplementation starting immediately after transfer or at mid-luteal phase after embryo transfer, and removing on d 19-21 post-estrus (Smith et al., 1996; Tribulo et al., 1997; Purcell et al., 2005). These reports indicated no positive effect of P4 supplementation on pregnancy rates. Following AI, however, some researchers reported enhanced pregnancy rates when the supplemental P4 was administered at mid-cycle (Mehni et al., 2012) while others reported no effect on pregnancy rate (Long et al., 2010). Moreover, Chenault et al. (2003) reported reduced pregnancy rates for CIDR treatment between d 14-21 after AI in lactating dairy cows. These reports show that there are inconsistent results of exogenous P4 supplements following both AI and embryo transfer on pregnancy rates.

In conclusion, direct progesterone supplementation between d 13 and 20 via ear implant increased the progesterone level on d 16 and 20 and increased the pregnancy rate compared to control group. Moreover, it can be suggested that both a single dose GnRH injection and progesterone implant can be used to prevent embryonic losses in cryopreserved embryo transferred cows.

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