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Original Article

The effect of equine chorionic gonadotrophin (eCG) injection combined with prostaglandin F2α (PGF2α) and gonadotrophin releasing hormone(GnRH) treatment on reproductive performance of Zandi ewes during non-breeding season

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ABSTRACT

In this study, we aimed to investigate reproductive performance in estrus-induced Zandi ewes treated with equine chorionic gonadotrophin (eCG) injection in combination with prostaglandin $F_2\alpha$ ($PGF_2\alpha$)and gonadotrophin releasing hormone (GnRH) during non-breeding season. The estrous cycle was synchronized using controlled internal drug release (CIDR) for 12 days. The ewes were randomly assigned to five groups (n=100 in each group). The first group (control) did not receive hormone injection, the second group (eCG) received 400 IU of eCG at the time of CIDR removal, the third group (eCG+PGF2α) received 400 IU of eCG and 1 ml of PGF2α at the time of CIDR removal, the fourth group (eCG+GnRH) received 400 IU of eCG at the time of CIDR removal and 1 ml of GnRH injection on the day of insemination, and the fifth group (eCG+PGF2α+GnRH) received 400 IU of eCG, 1 ml of PGF2α at the time of CIDR removal, and 1 ml of GnRH on the day of insemination. The results showed that the heat response rate was higher in all the groups receiving hormonal injections compared with the control group ($P<0.05$). In addition, the lambing and parturition rateswere significantly ($P<0.05$) higher in the groups receiving GnRH compared with other treatments.In fact, in groups receiving eCG and PGF2α, lambing and parturition rateswere higher than the control group ($P>0.05$). In conclusion, use of eCG at the time of CIDR removal and GnRH on the day of insemination increased reproductive performance. Therefore, it can be applied as a strategy for increasing lambing rate in ewes.

Keywords: Lambing rate, eCG, GnRH, PGF2α, Zandi ewes

L'effet de l'injection de gonadotrophine chorionique équine combinée à un traitement de libération de prostaglandine F2α et gonadotrophine sur les performances reproductives des brebis Zandien dehors de la saison de reproduction

Résumé: L'objectif de cette étude était d'étudier les performances reproductives chez les brebis Zandien dehors de la saison de reproduction après un œstrus induit par le biais d'une injection de gonadotrophine chorionique équine (eCG) combinée à un traitement de libération de prostaglandine $F2\alpha(PGF_2\alpha)$et gonadotrophine (GnRH). Le cycle de l'œstrus a été synchronisé par la libération interne contrôlée de

médicament (controlledinternaldrug release, CIDR). Les brebis ont été réparties au hasard dans 5 groupes (n=100 dans chaque groupe). Le premier groupe (témoin) n'a reçu aucun traitement, le second (eCG) a été traité avec 400 IU d'eCG lors de la suppression de la CIDR, le troisième (eCG+PGF2α) a reçu 400 IU d'eCG et 1 ml de PGF2α lors de la suppression de la CIDR, le quatrième (eCG+GnRH) a été traité avec 400 IU d'eCG lors de la suppression de la CIDR et une injection d'1 ml de GnRH le jour de l'insémination. Les résultats montrent que le taux de réponse à la chaleur était plus fort dans tous les groupes ayant reçudes injections hormonales comparés au groupe témoin (P<0,05). De plus, les taux d'agnelage et de natalité étaient sensiblement plus élevés (P<0,05) chez les groupes traités avec de la GnRH comparés aux autres traitements. Cependant, les groupes ayant reçu de l'eCG et de la PGF2α montraient des taux d'agnelage et de natalité plus importants comparés au groupe témoin(P>0,05). En conclusion, l'utilisation de l'eCG lors de la suppression de la CIDR et de GnRH le jour de l'insémination améliore les performances reproductives. Par conséquent, ces traitements peuvent être utilisés comme une stratégie afin d'augmenter l'agnelage chez les brebis.
Mots clés: Taux d'agnelage,eCG, GnRH, PGF2α, Brebis Zandi

Introduction

Estrus synchronization or the induction of estrus is a valuable management tool for increasing the pregnancy rate in ewes. In addition, the use of estrus synchronization provides the opportunity for timed breeding and lambing. Several techniques have been developed to synchronize estrus in ewes during the past decades (Kermani Moakhar et al., 2012; Moradi Kor et al., 2012; Sirjani et al., 2012; Abdalla et al., 2014; Masoudi et al., 2016). The most widely applied protocol involves using controlled internal drug release (CIDR) inserted for 12 to 14 days followed by equine chorionic gonadotrophin (eCG) at CIDR removal (Gordon, 1975; Zarkawi, 2001; Husein and Hamit, 2005; Zonturlu et al., 2008). Despite the successful outcome of implementing such a protocol, reduced fertility rate is still reported in some studies (Kruip and Brand, 1975; Fitzgerald et al., 1985; Husein and Kridli, 2002; Kermani Moakhar et al., 2012). Numerous reasons have been stated for reduced fertility rate including heredity, different sheep breeds, seasonal variation, age, environment, nutritional levels, diseases, semen quality, and hormonal treatment (Cognié et al., 2003; Gonzalez-Bulnes et al., 2003; Ali, 2007; Mossa et al., 2007; Dadashpour Davachi et al., 2011; Dadashpour Davachi et al., 2014; Masoudi et al. 2012a,b). Gonadotropins have often been used to stimulate ovarian activity following progestin treatment in the sheep (Gordon, 1975; Menchaca et al., 2009). It was reported that gonadotropins are definitely required for ovarian follicles to reach a diameter of over 2.5 mm in ewes (McNatty et al., 1990). In addition, it was noted that using eCG and GnRH could improve ovulation rate in the ewe(Evans, 1988; Menchaca and Rubianes, 2004; Zeleke et al., 2010; Sirjani et al., 2012), and thereby, providing the potential for increasing pregnancy rate following artificial insemination (AI) in sheep. Titi et al. (2010)prpposed that combination of GnRH administration with progestogen sponge can be effective for synchronizing estrus and developing fecundity in the sheep and goats. Moreover, administration of GnRH 24 h after sponge removal enhanced ovulation rate in ewes treated with eCG during breeding season (Azawi and Al-Mola, 2011). Treatment with a combination of GnRH and PGF2α was used to control ovarian, follicular, and luteal functions and enhance the precision of estrus and ovulation synchronization in reproductive management programs. One to six days after PGF2a administration (during the period the sponges were still in place), several large antral follicles ovulated; these ovulations were not preceded by a preovulatory luteinizing hormone (LH)/follicle stimulating hormone(FSH) surge, and none of these ovulations were followed by the formation of corpus luteum (CL)(Bartlewski et al., 2003; Abdalla et al., 2014). According to the above-

mentioned studies, using these hormones during estrous cycle could affect reproductive performance and improve reproductive management. Therefore, the present study was conducted to compare the efficiency of combination of eCG, PGF2α, and GnRH treatment in synchronizing estrus in Zandi ewes before artificial insemination during non-breeding seasons.

MATERIALS AND METHODS

Treatments. The experiment was performed during March-June (non-breeding season) 2013. A total of 500 ewes with mean weight of 55±2.5 kg andage range of 3-4 years age were enrolled. The animals were provided with water and fed alfalfa hay, supplemented with grain pellets and ad libitum (CNCPS, 2003). The estrous cycles were synchronized using controlled intra-vaginal drug release (CIDR; EAZI-BREEDTM, CIDR®, New Zealand) for a period of 12 days. The day of CIDR insertion was considered as the onset of the experiment (day 0). The ewes were randomly assigned to five groups (n=100). The first group (control) did not receive hormone injection, the second group (eCG) received 400 IU of eCG (Folligon, Intervet, the Netherlands) as a single i.m. injection at the time of CIDR removal (day 12), the third group (eCG+PGF2α) was treated with 400 IU of eCG and 1 ml of PGF2α (1 ml of SynchroMate, each 1 ml solution containing 250 mg Cloprostenol, Coopers Co., Germany)at the time of CIDR removal (day 12), the fourth group (eCG/GnRH) was treated with 400 IU of eCG at the time of CIDR removal (day 12) and 1 ml of GnRH (Receptal, Intervet International, B.V. EU) on the day of insemination (day 14), and the fifth group (eCG+PGF2α+GnRH) received 400 IU of eCG, 1 ml of PGF2α at the time of CIDR removal (day 12), and GnRH was injected (1 mL) on the insemination day (five hours before insemination). Table 1 helps with understanding the experiment pattern;vasectomized rams were used to detect estrus in all the ewes from 24 h following CIDR removal.

Sperm collection and dilution. Semen sampleswere collected (two ejaculations from each ram) using artificial vagina from 20 mature Zandi rams with mean weight of 75±2.5 kg and age range of 3-4 years. For eliminating individual differences, the samples were pooled and then diluted 1:1 (v:v) with skim milk, and finally, loaded in straws (0.25 ml) and stored for about 2 hours at 4ºC until insemination. Each straw contained about 500 million sperm cells. Samples with at least 60% progressive motility were selected for insemination. Motility was evaluated via an optical microscope.

Table1. Management of treatments and time of injections among the groups

Time (day) Groups	CIDR[1] insertion	CIDR removal	eCG[2] injection	PGF2α[3] injection	GnRH[4] injection	AI[5]
Control	0	12	-	-	-	14
eCG	0	12	12	-	-	14
eCG+ PGF2α	0	12	12	12	-	14
eCG+GnRH	0	12	12	-	14	14
eCG+ PGF2α+GnRH	0	12	12	14	14	14

1- controlled internal drug release, 2- equine chorionic gonadotrophin, 3- prostaglandin F2α, 4- gonadotrophin releasing hormone, 5- artificial insemination

Artificial insemination and pregnancy detection. Artificial insemination was performed cervically 54 hours after CIDR removal in the morning. In this method, semen was deposited in cervical entrance by the gun of artificial insemination after opening the vagina via a speculum. Moreover, pregnancy diagnosis was performed by an ultrasound unit equipped with a 3.5 MHz sector probe in day 50 after insemination.

The measured traits. Estrus response is the number of ewes showing signs of estrus/total ewes treated x 100. Pregnancy rate is the number of pregnant ewes/number of ewes inseminated x100. Lambing rate is the number of lambs born/number of ewes inseminated. Parturition rate is the number of ewes lambed/number of ewes inseminated.

Table 2. Reproductive parameters in the ewes receiving different hormonal treatments during non-breeding season

	Experimental groups				
	Control	eCG	eCG+PG	eCG+GnRH	eCG+PG+GnRH
Heat rate(%)	22[b]	48[a]	45[a]	50[a]	47[a]
Pregnancy rate(%)	13[c]	28[b]	30[b]	45[a]	43[a]
Parturition rate(%)	10[c]	26[b]	23[b]	44[a]	43[a]
Lambing rate(%)	10[c]	26[b]	23[b]	44[a]	43[a]

Different superscripts (a, b, c) indicate a significant difference between the groups (P<0.05)
equine chorionic gonadotrophin, gonadotrophin releasing hormone

Statistical analysis: Heat, pregnancy, parturition, and lambing rates were analyzed using GENMOD Procedure of SAS 9.1 software through performing Chi-squared test.

RESULTS

The rates of estrous synchronization in the control, eCG, eCG+PGF2α, eCG+GnRH, and eCG+PGF2α+GnRH groups (P<0.05) were 22.0%, 48.0%, 45.0%, 50.0%, and 47.0%, respectively (Table 2). The pregnancy rate was higher in the ewes receiving hormonal treatment compared with the control group (Table 2). The highest pregnancy rate was observed in groups of ewes injected with GnRH. Pregnancy rate in the control, eCG, eCG+PGF2α, eCG+GnRH, and eCG+PGF2α+GnRH groups were 13.0%, 28.0%, 30.0%, 45.0% and 43.0%, respectively. Parturition and lambing rates were higher in ewes receiving hormonal treatments, compared to the control group (P<0.05), and among the hormonal groups, higher parturition and lambing rates were observed in groups of ewes treated with GnRH (P<0.05; Table 2). Parturition and lambing rates in the control, eCG, eCG+PGF2α, eCG+GnRH, and eCG+PGF2α+GnRH groups were 10.0%, 26.0%, 23.0%, 44.0%, and 43.0%, respectively. No twins were born and twinning rate was 0% in all the groups.

DISCUSSION

During non-breeding season, only 22% of the ewes in the control group exhibited estrus behavior and ovulation compared with the hormonal treatment groups. This result is not congruent with findings of some former studies (Boscos et al.; Leyva et al., 1998; Naqvi and Gulyani, 1998; Zonturlu et al., 2008),in which progesterone treatment without using exogenous gonadotropins predisposed cyclic ewes to show estrous behavior and ovulation during breeding season. The response to estrus synchronization obtained in the present studyfrom Zandi ewes was higher than the estimations of Kermani Moakhar et al. (2012) (Chall ewes, 83%), Abdel-Megeed (2006)(Rahmani ewes, 84%), and Abdullah et al. (2002)(Awassi ewes, 80.9%), while it was lower than the 100% rate reported in Karakul (Safdarian et al., 2006) and Akkaraman crossbred ewes (Ataman and Aköz, 2006). eCG treatment induced a synchrony of estrus and ovulation in almost all ewes (Cline et al., 2001; Zeleke et al., 2010). The absence of estrus may be due to inadequate estradiol secretion by the ovarian follicles, indicating incomplete follicular growth and development (Baird

and McNeilly, 1981). (Fuerst et al., 2009)exhibited an increase in estradiol concentration following eCG treatment. GnRH induced a preovulatory LH surge closely synchronized within 2 h after intra-muscular injection in sheep (Rubianes et al., 1998). Therefore, GnRH treatment 36 h after progestin removal could induce ovulation before the normal expression of estrus (Luther et al., 2007). The efficiency of GnRH+PGF2α+eCG treatment in inducing a high level of estrus (90%) in the present study was similar with the results of Beck et al. (1996)obtained from Welsh halbred ewes treated with a combination of buserelin and PGF2α or Akkaraman cross-bred ewes treated with GnRH–PGF2α–eCG (Ataman and Aköz, 2006). Our findingsdemonstrated that the use of CIDR in combination with eCG treatment promotes pregnancy and lambing rates in comparison with the use of CIDR alone that was similar with previous results in ewes (Luther et al., 2007; Moradi Kor et al., 2012; Sirjani et al., 2012). They also showed that eCG administration significantly increased the number of large ovarian follicles in estrus (Noël et al.; Rubianes et al., 1995; Kermani Moakhar et al., 2012). There are several possible mechanisms through which eCG can increase the number of large follicles. For instance, it may enhance the entry rate of small and medium follicles into larger sized follicles nad prevent the occurrence of natural follicular atresia (Bister et al.; Mandiki et al., 2000). eCG injection may lead to the growth of large anovulatory estrogenic follicles,which may in turn, adversely affect early embryonic development and oviductal transport (Bister et al.; Mandiki et al., 2000). The current studyrevealed that GnRH treatment 48 h after CIDR removal promoted the pregnancy, lambing, and parturition rates. In agreement with our results,Sirjani et al. (2012) reported GnRH injection at 48 h after CIDR removal increased multiple birth and lambing rates andlitter size in ewes. GnRH injection may enhance the number of ovulations, and consequently, lead to increased lambing and multiple birth rates and litter sizein synchronized ewes. It was found that a single dose of GnRH injection 24 h after CIDR removal could boost the number of embryos in multiple ovulation and embryo transfer protocols (Herbert and Trigg, 2005; Pawson and McNeilly, 2005; Turk et al., 2008; Menchaca et al., 2009). In cattle, GnRH injections promote LH release,thereby, inducing either ovulation or atresia of the dominant follicle and resulting in emergence of a new follicular wave in a synchronous way (Dirandeh et al., 2009) and a derived new dominant follicle with the best oocyte quality (Mhim et al., 1999).Treatment with a combination of GnRH and PGF2α was used to control ovarian follicular and luteal functions and increase the precision of estrus and ovulation synchronization in reproductive management programs;however, in the present study, adding PGF2a injection to eCG or eCG+GnRH treatment did not affect pregnancy, lambing, multiple birth, and parturition rates. Nonetheless, the fertility rate of GnRH+PGF2α+eCG treated ewes is comparable with the results obtained byMoradi Kor et al. (2012). They reported PGF2α–FGA–GnRH treatment resulted in the lowest fertility rate after natural service. The reasons for this might be ascribed to early injection of GnRH with a subsequent LH early surge, which blocked the ovarian steroidogenesis (Hay and Moor, 1975) and prevented the occurrence of estrus. Moreover, it wasproposed that GnRH administration might induce premature ovulation (Keisler and Keisler, 1989) with subsequent reduced fertility.

In conclusion, eCG treatment at the time of CIDR removal combined with GnRH treatment 48 h after CIDR removal increased pregnancy, lambing, and parturition rates during non-breeding season in ewes.In fact, adding PGF2α to the same treatments did not affect reproductive performance.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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