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Replacing eCG with hCG in ewe laparoscopic artificial insemination protocols using hCG and a slow-release compound (Alhydrogel)

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Abstract The aim of this study was to investigate the effect of replacing equine chorionic gonadotropin (eCG) with human chorionic gonadotropin (hCG) and also improving the efficiency of hCG using a slow-release compound (alhydrogel) on the reproductive performance of Afshari ewes after laparoscopic artificial insemination. For this purpose, 48 Afshari ewes (2.5-4 years old with the mean weight of 68±2.5kg and an body condition score of 3.04±0.3 were treated with intravaginal controlled internal drug release (CIDR) for 14 days. The ewes were then divided into 4 groups: the first group: injection of eCG (400IU), 48 hours before CIDR removal (eCG_S), the second group: injection of eCG (400IU) combined with alhydrogel, 48 hours before CIDR removal (eCG_{SR}), the third group: injection of hCG (400IU), 48 hours before CIDR removal (hCG_S) and the fourth group: injection of hCG (400IU) combined with alhydrogel, 48 hours before CIDR removal (hCG_{SR}). The results showed that there was no significant difference between eCG_{SR} and eCG_S treatments in Measured parameters (P>0.05). The lowest pregnancy rate (25%) and fecundity (33%) were recorded in related to the hCG_S treatment. However, in the hCG_{SR} treatment, these parameters were increased to the values recorded in eCG_{SR} and eCG_S treatments. The results of this study showed that hCG in the presence of slow release compound can be a suitable substitute for eCG in the estrus synchronization protocol based on progesterone and eCG.

Keywords: alhydrogel, artificial insemination, estrus synchronization, equine chorionic gonadotropin, human chorionic gonadotropin

Introduction

To date, exogenous progesterone in combination with gonadotropin is the most accepted protocol for inducing and synchronizing the estrous cycle in ewes during the breeding and non-breeding seasons (Ali, 2007). Exogenous progesterone regimen is applied using intravaginal devices, such as sponges and controlled internal drug release (CIDR), for short (7 days) or long time (14 days) (Dias et al., 2015). eCG is the most common hormone that is used as the gonadotropin part of the above technique (Ali, 2007). In addition to improving estrous responses, eCG can also increase the twinning rate (Gordon, 1997). One of the major challenges for livestock farmers in using the estrus synchronization technique is the relatively high price of eCG. Therefore, finding a suitable substitute for eCG could plays an important role in increasing the acceptance of the estrus synchronization technique among livestock farmers. In addition, due to the ethical issues related to the production of eCG from pregnant mares, efforts are being made to find a suitable substitute for it (Gonzalez-Bulnes et al., 2020). Current studies are focused on compounds that can either stimulate LH secretion, such as gonadotropin-releasing hormone (GnRH), or exhibit LH-like activity, like hCG).

Due to a high degree of structural similarity with LH, hCG has the ability to bind to LH receptors and exert a stimulatory effect with LH at the level of mature follicles. In addition, some studies have shown that hCG has eCG-like activity and can increase the survival of antral follicles and subsequently causing an increase in the ovulation rate. Khan et al (2003) showed that the use of hCG (150IU) at the time of mating, significantly increased lambing and growth of fetal membranes. In another study, Cam and Kuran (2004) showed that the use of hCG (150IU), 12 days after mating, was associated with an increase in twinning and lambing rates. In the study of Zamiri and Hosseini (1998) on fat-tailed Ghezel ewes, the use of 500IU hCG was increased prolificacy, but fertility and fecundity rates decreased.

On the other hand, many parameters can influence the efficiency of eCG and hCG in synchronization protocols, including the dose (Quintero-Elisea et al., 2011), route of administration (Boshoff and Burger, 1973), season (Langford et al., 1983). Repeated injection (Driancourt et al., 1991) and the timing of administration relative to the CIDR removal (Ali, 2007). In hormone therapy, the duration of access to the effective concentration of hormone is one of the important key factors which can affect both the hormone efficiency and the hormone dosage. Indeed, in addition to the dosage, the pattern of hormone release is also very important. In this regard, Driancourt and Fry (1992) showed that at low dosages, the effectiveness of eCG is was only limited to 3 mm follicles and with increasing eCG dosage, its effective range increased and included almost all follicular phases. Their results showed that the duration of access to the effective eCG concentration is an important factor for increasing the efficiency of estrous synchronization. In other words, in addition to the dosage, the quality of eCG release also affects its performance (Driancourt and Fry, 1992). Various sustained release formulations of proteins, such as liposomes, cross linked hydrogels and implants have been evaluated for possible improvement in the pattern and quality of proteins release (Yahyaei et al., 2017, 2018). Simple mixing of proteins with various types of adsorbents is a simple and inexpensive method that results in sustained release of proteins (Bó and Mapletoft, 2014). One of these adsorbents is aluminum hydroxide gel that can adsorb proteins and release them gradually (Bó and Mapletoft, 2014; Kimura et al., 2007). Although a large number of studies have been carried out to evaluate different estrous synchronization protocols based on eCG, the numbers of studies addressing the replacement of eCG with hCG and eCG or hCG exposure time and efficiency of estrous synchronization protocols are limited. Therefore, the aim of this study was to investigate the effect of replacing eCG with hCG and also improving the efficiency of eCG and hCG using a slow-release compound (alhydrogel) on the reproductive performance of Afshari ewes after laparoscopic artificial insemination.

Materials and methods

Animals and management

The experiment was carried out at the Animal Research Station, College of Agriculture, Arak University, Arak, Iran from November 2020 to April 2021. A total of 48 cyclic and non-lactating Afshari ewes (3-4 years, 70 ± 1.7kg body weight, body condition score 3.04±0.4 on scale 1 to 5) were housed in individual pens with free access to water and feed.

Experimental design

Estrous cycle was synchronized using CIDR (EAZI-BREEDTM, CIDR®, New Zealand) for a 14-day period during the breeding season. All ewes received a single dose of 2 mL of gonadorelin (GnRH) (Aborehan Pharmaceutical Company, Iran) (I.M.) at CIDR insertion and received a single dose of 1 mL of prostaglandin $F_{2\alpha}$ (Nasr Pharmaceutical Company, Iran) at the time of CIDR withdrawal. Then, based on the method and the type of gonadotropin injection, the ewes were divided into four groups (Figure 1). The first group was treated with 400IU eCG (n=12, eCG_{S}). The second group was treated with a simple mixture of 400IU eCG and alhydrogel (alhydrogel® adjuvant, Merck) (5 mL) (n=12, eCG_{SR}). The third group received 400IU hCG (n=12, hCG_{S}) and the fourth group was treated with a simple mixture of 400IU hCG and alhydrogel (alhydrogel® adjuvant, Merck) (5 mL) (n=12, hCG_{SR}). Then, all ewes were artificial through laparoscopic approach with frozen sperm of Rouge breed; 48h after CIDR withdrawal. Forty days after insemination, pregnancy was diagnosed via Ultrasonography (ultrasonography, Aloka, 5 MHz). The reproductive variables measured in experimental groups were the rate of return to estrus (%), pregnancy rate (%), parturition rate (%), fecundity and prolificacy.

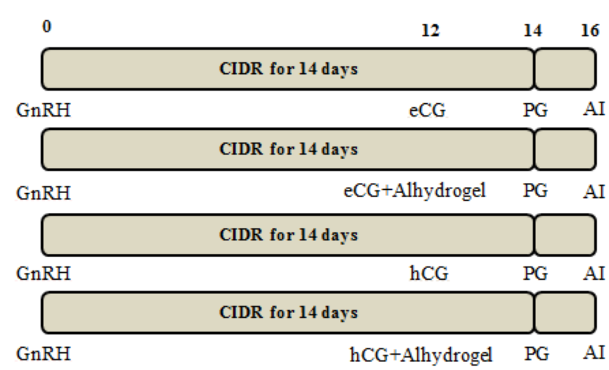

Figure 1. Schematic representation of the experimental design

Statistical analysis

The experiment was performed in a completely randomized design. The data were analyzed by PROC

GLM (SAS, 2003). Data on reproductive performance were analyzed using PROC GENMOD. Probability values of less than 0.05 (P<0.05) were considered significant.

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$

Y_{ij}: observed value of the dependent variable, μ: overall mean, T_i:fixed effect of the *i*th treatment and ε_{ij}: residual error.

Results and discussion

The reproductive performance data are given in Table 1. As it can be seen, there was no significant difference in return to estrus, pregnancy rate, parturition rate and lamb mortality parameters between the treatments. Numerically, the highest pregnancy rate was related to hCG_{SR} and eCG_{SR} treatments (58%). This shows that the simple mixing of hCG or eCG with alhydrogel was improved pregnancy rate. No abortion was observed during the pregnancy time and the parturition rate was 100% in all groups. The hormone type and their release pattern had no significant effect on the death of kids.

Table1. The effect of different hormonal treatments used for estrus synchronization on reproductive parameters in Afshari ewes

Variables	**Treatments**			
	hCG_{SR}	hCG_{S}	eCG_{SR}	eCG_{S}
Number of ewes	12	12	12	12
Rate of return to estrus (%)	5/12 (41%)	9/12 (75%)	5/12 (41%)	6/12 (50%)
Pregnancy rate (%)	7/12 (58%)	3/12 (25%)	7/12 (58%)	6/12 (50%)
Parturition rate (%)	7/7 (100%)	3/3 (100%)	7/7 (100%)	6/6 (100%)
Number of born lambs	9	4	8	7
Death of lambs (%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)

The highest and lowest fecundity were related to hCG_{SR} (75%) and hCG_{S} (33%) treatments, respectively. This shows that the use of alhydrogel, as a slow-release compound, had a positive effect on improving the efficiency of hCG. Unlike hCG, the presence of alhydrogel did not have a prominent effect on eCG function (Figure 2).

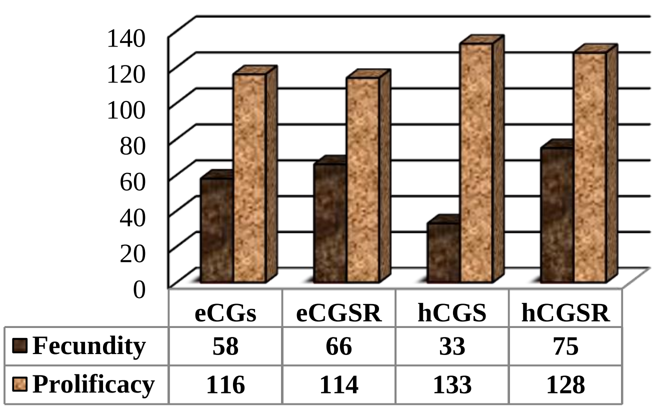

Figure 2. The effect of different hormone treatments during estrus 325 synchronization on fecundity and prolificacy rates in Afshari ewes (P>0.05)

The results of orthogonal comparisons showed that there was no significant difference in any of the reproductive parameters between the eCG and hCG groups. A similar result was observed between single and slow release groups. This shows that through the simple mixing of hCG with alhydrogel compound it is possible to obtain reproductive performance similar to eCG that is economically viable in comparison to eCG. The orthogonal comparisons results also showed alhydrogel compound does did not have a negative effect on hCG or eCG and it may be used as a safe compound for increasing the exposure time to hormone.

Most researches have studied the effect of hCG in the presence of eCG with the aim of reducing embryo mortality (by increasing progesterone concentration and reducing estrogen concentration) or increasing the ovulation rate during natural mating (Azari et al., 2020; Bruno-Galarraga et al., 2021; Shahir et al., 2017; Tohidi et al., 2019). Very few studies have investigated the effect of hCG on reproductive efficiency during artificial insemination. In the studies of Braden et al. (1960) and Radford et al. (1984) on Merino ewes, it was shown that the use of hCG was increased the ovulation rate. We also observed that hCG had a positive effect on increasing the ovulation rate. In another study, Zamiri and Hosseini (1998) investigated the effect of hCG on the reproductive performance of Ghezel ewes and showed that 500IU hCG significantly increased the prolificacy rate. The observed differences between the results of this study and others could be attributed to the breed of sheep, stage of breeding season, hCG dosage, injection time, type of protocol and pattern of hCG release. Radford et al, (1984) showed that 250 IU of hCG gave resulted in the most uniform increase in the ovulation rate when compared with 125 and 500 IU. This result showed hCG dosage is one of the effective factors on its performance. In the study of Zamiri and Hosseini (1998) on Ghezel ewes, reproductive performance was affected by hCG dosage, so that 125 and 250 IU of hCG as compared to 500 IU of hCG did not affect the prolificacy. They also reported that as compared with control group, fertility and lambing rate decreased in hCG-treated groups. These results show that there is an optimal hCG dosage to achieve the desired reproductive performance. In addition to the dosage, the time of hCG injection affects its performance. This possibility was investigated by Radford et al. (1984) and Zamiri and Hosseini (1998). They found that better mating and pregnancy performance was found when hCG was injected before the expected time of estrus. Based on the results of different studies. it seems that by modifying the pattern of hCG release, we can solve the problem of time and dosage of hCG injection. In our study, the hCG_{SR} treatment had a higher pregnancy rate compared to the hCG_S treatment (58% vs. 25%) and this shows that pattern of hCG release can have a positive effect on its performance. A proposed mechanism to improve the performance of hCG in the presence of alhydrogel compound can be as follows. Possibly, a combination of hCG with alhydrogel can impact on of the pattern of hCG release from the injection site. Circulating concentrations of hCG following injection depends on absorption from the site of administration and metabolic clearance from the circulation. According to metabolic clearance rates law it would be expected that the clearance rate of hCG from blood flow is irrespective of its method of treatment and concentration. As a result, rapid absorption at the injection site should result in rapid hCG clearance. Therefore, dosage and the length of time during which the effective concentration of hCG are available for follicles will be short and high, respectively. It is expected that the problem of high dosage and short access time of hCG will be solved by simple mixing of hCG with alhydrogel. It has been shown that a mixture of FSH and aluminum hydroxide gel prolonged the peak serum FSH level through slowing the rate of absorption (Demoustier et al., 1988). Scaramuzzi et al. (2011) presented the maintaining effective FSH concentration for different lengths of time as a strategy for increasing the number of ovulatory follicles in ewes. The findings of this study indicated that the exposure time to gonadotropins would have greater impacts on the number of ovulatory follicle rather than the gonadotropin dosage. In another study, Kimura et al. (2007) found that follicular growth and ovarian weight in rats were significantly increased when single treatment of pFSH in saline was replaced with single treatment of pFSH in alhydrogel.

Conclusions

In summary, administration of 400IU hCG in alhydrogel resulted in similar reproductive performance to 400IU eCG in Afshari ewes. The reason for this improvement is unclear but we assume that mixing of hCG with alhydrogel could improve the pattern of hCG release at injection site. However, further studies are needed to investigate this issue.

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