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Research Article

Effect of GnRH at Estrus and 12-Day Post Matting and Propylene Glycol on Sera Metabolites Concentrations and Ewe Conception Rate

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Abstract

In this study GnRH and propylene glycol respectively used as hormonal treatment and to supply precursor material of glucose. Animals (56 crossbred ewes, Arkhamerino-Ghezel (**AG**) and Arkhamerino-Moghani (**AM**), 2.5 years old) were classified to 1 control and 3 treatment groups randomly. After estrus detection and before mating; one milliliter of physiological saline (0.9% NaCl, placebo) was administered to each ewe in control group, group 1 were injected with 2.5 ml GnRH and after 2 hours they were mated by ram; group 2 were injected with 5 ml GnRH 12 days after mating; group 3 were received 30 ml propylene glycol for 5 days after mating. Blood samples were taken on 6 d after mating by using vacuum tube. Glucose, urea, phosphorus, total protein, β-hydroxybutyrate (BHBA) and progesterone concentrations in blood serum were measured. Results shows that glucose in AM was significantly higher than AG ($P<0.01$). Urea and BHBA in AM were significantly ($P<0.01$) lower than AG. In repeat breeder ewes, progesterone concentration in estrus 2 was significantly ($P<0.05$) higher than estrus 1, and urea and BHBA concentrations in estrus 2 were significantly ($P<0.05$) lower than estrus 1. Administration 5 ml of GnRH on day-12 after mating was effective on reproductive performance than others. In conclusion, results showed that only one sheep in GnRH-2.5 ml treatment at estrus and five sheep in GnRH-5 ml on day-12 after mating showed repeat estrus and suggested that using 2.5 ml of GnRH before mating had better effect to prevent of repeat breeder .

Keywords: Crossbred Ewe; GnRH; Propylene Glycol; Conception Rate

Introduction

There are a number of factors (including the method of insemination, type of estrus (neutral or controlled), age, season, genetic, stress, embryonic and fetal mortality) that can influence fertility in a breeding program [1]. The time of mating or artificial insemination is important point to increase the fertility in animals. Insemination too early or too late in relation to ovulation is determinately to fertility [2]. The reduction in fertility may reflect failure of conception or embryonic mortality. Pervious study showed that reproduction of crossbred sheep could be improved by using of hormonal treatments and GnRH or its analogues have a positive effect on fertility [3]. Improvement of conception rates following GnRH treatment during estrus has been attributed to the prevention of ovulation failure or to reduced variation in the interval to ovulation [4]. It has also been suggested that GnRH induced increase in progesterone concentrations during the subsequent estrous cycle may have an effect on conception.

Pre-implantation embryonic loss is the major factor limiting optimum reproductive performance in farm animals. In sheep, 30-40% of fertilized eggs were lost during the first 3 weeks of pregnancy in 1996, Ullah et al. [1996] administered GnRH at detection time of estrus, following two daily visual observations and results showed the increased in conception of dairy cows in the suAMer, when conception rate was very low. Administration of GnRH at the time of insemination may modify the function pre- or postovulatory ovarian follicles or characteristics and secretary capacity of the developing corpus luteum [5, 6].

Administration of GnRH on day 12 post mating has been shown to improve early embryo survival and pregnancy rate in sheep. The administration on day 12 post mating is critical period for maternal recognition of regression of the corpus luteum in the natural estrous cycle. Hormonal induction of progesterone production after or on day 12 may increase interferon-τ production and may in turn prevent autolysis by preventing PGF2α secretion [7]. It is reported that daily drenching of propylene glycol to cows in early lactation would increase plasma glucose and insulin concentration and improve fertility in postpartum cows. After drenching, propylene glycol increased plasma glucose and insulin and decreased NEFA [8, 9]. The main aim of this study survey of effects of GnRH and propylene glycolon reproductive performance of crossbred ewes in north east of Iran during breeding season.

Materials and Methods

Locations

This study was performed at Animal Reproduction Laboratory of Tabriz University's, located in Tabriz province; Iran (38° 07' N and 46º 29' E) from June to December 2012. Ambient temperature during the experiment ranged from 20 to 24°C with annual rainfall in this region ranges from 226 to 250 mm.

Animals and Treatments

The sixty Iranian crossbred ewes (Arkhar-Merino×Ghezel, 2-5 years old, weighing 40-50 kg). Each genetic group classified to 4 treated groups randomly. After estrus detection by ram and before mating, treated groups received the following treatments: group **A**) control group received only distilled water by intramuscular injection, **B**) animals injected with 2.5 ml GnRH (vetarolin, Aburaihan) 2 hours before mating; **C**) 5 ml GnRH injected, on day 12 after mating, **D**) 30 ml propylene glycol (Afarin Daro Company) for 5 days and 2 hours before mating orally were given.

Blood Sampling and Assay

Five ml of blood samples were collected on day 6 after mating using vacuum tube from jugular vein of each ewe. Sera were separated in the same day by centrifugation in 3500×g for 15 min. The serum was stored at -20ºc until starting to measure. Glucose, urea, phosphorus, total protein concentrations were measured using co-AMercial kits (Zist chimi, 10-505, 10-506, 10-516, 10-518). Respectively, by spectrophotometer (genus 20) and β-hydroxybutyrate (**BHBA**) concentrations were assayed by spectrophotometer method using Runbut kit (Randox, UK, RB-1007). Progesterone concentration was measured using Elisa co-AMercial kit (Monoband, ELA-1270). Standard commercial kits were used for analysis and the procedures were adopted as recommended by the manufacturer of these kits.

Statistical Analysis

Blood metabolites were analyzed by GLM procedure of SAS (V. 9.1) as least square means (LSM) ± standard errors (SE) according following model:

$$\mathbf{Y_{ijk} = \mu + A_i + B_j + T_k + AB_{ij} + AT_{ik} + BT_{jk} + e_{ijk}}$$

Y_{ijk}=Observation

μ= Average

A_i= Treatment effect (i=1, 2, 3, 4)

B_j= Genetic group effect (j=1, 2)

T_k=Ewe age effect (k=1, 2)

AB_{ij}= Interaction (genetic group ×treatment)

AT_{ik}= Interaction (ewe age×treatment)

BT_{jk}= Interaction (genetic group × ewe age)

e_{ijk}= residual effect

Discrete data (lambing, fertility, prolificaty and fecundity) were analyzed by LOGESTIC procedure of SAS (V.9.1) and expressed as mean ± SEM according this model:

$$\mathbf{Log\ (P/1\text{-}P) = \mu + A_i + Pr_j + BH_k}$$

P= Probability of each trait

μ= Average

A_i= ewe age

Pr_j= progesterone concentration

BH_k = β-hedroxy butyrate concentration

Results

Figure 1 shows that the effect of genetic group on serum concentration of glucose, urea and BHBA concentrations were significant (P<0.01). Glucose, Urea and BHBA in AM ewe was higher than AG ewe (P<0.01). Effect of breed × treatment were significant (P<0.05) and in AG glucose concentration in propylene glycol group were higher than control group (Figure 2). Propylene glycol group in AM genetic group had lower urea concentration than control group (Figure 3). Results about comparison between progesterone, urea and BHBA concentrations of first and second estrous cycles in repeat breeders have been showed in Figure 4. Progesterone concentration in estrus 2 was significantly (P<0.05) higher than estrus 1 and urea and BHBA concentration in estrus 2

were significantly (P<0.05) lower than estrus 1.

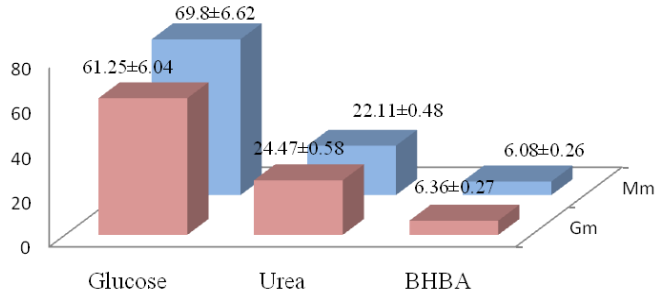

Figure 1. Genetic group effect on blood Glucose, Urea, and BHBA concentration (P<0.01).

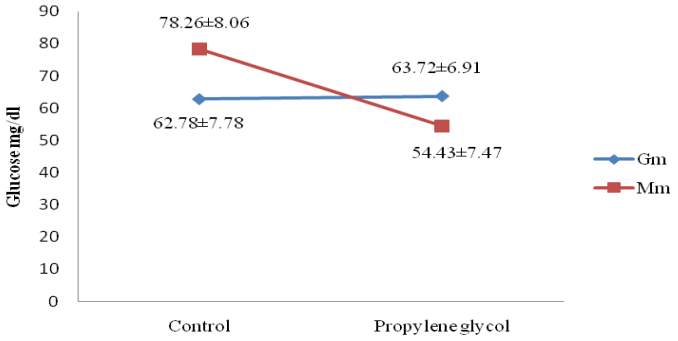

Figure 2. Effect of breed×treatment on Glucose concentration (P<0.05).

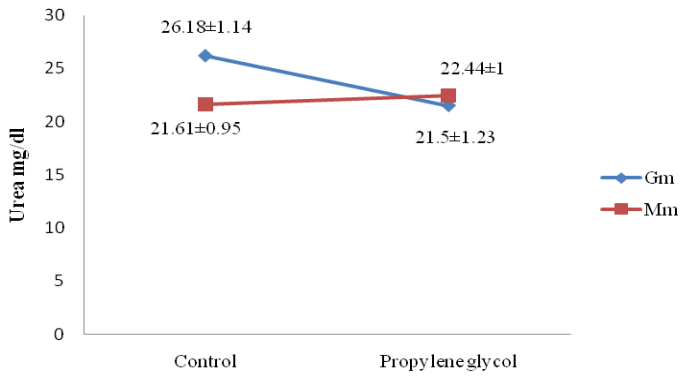

Figure 3. Effect of breed×treatment on urea concentration (P<0.05).

Reproductive performance by treatments has been showed in Table 1. In the AG genetic group 5 ml of GnRH treatment on day-12 after mating and in the AM genetic group, propylene glycol group had higher fertility, prolificacy and fecundity than others. Effect of treatments to prevent sheep of repeat breeder has been showed in Table 2. Comparison between control and treated groups clarified that only one sheep in GnRH-2.5 ml treatment at estrus and five sheep in GnRH-5 ml on day-12

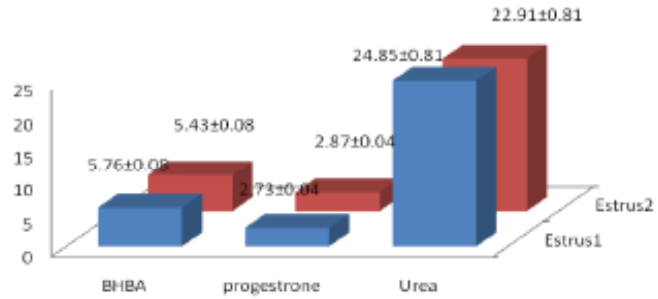

Figure 4. Comparison BHBA, progesterone and urea concentrations between estrus1 and estrus2 in repeat breeders (P<0.05).

Genetic group	treatments	n	Parturition n	Lamb n	Fertility %	Prolificacy %	Fecundity %
AG	Control	6	5	7	83.33	40	116.6
	GnRH-2.5 ml	6	5	5	83.33	0	83.33
	GnRH-5 ml	6	6	9	100	50	150
	Propylene glycol	6	5	6	83	20	100
AM	control	8	8	10	100	25	125
	GnRH-2.5 ml	8	8	9	100	12.5	112.5
	GnRH-5ml	8	6	7	75	16.66	87.5
	Propylene glycol	8	8	11	100	37.5	137.5
	Total	56	51	64	91.07	25.49	14.2

Table 1. Effect of GnRH treatment and propylene glycol on fertility, prolificacy and fecundity.

Genetic group	treatments	Ewe nom	Repeat breeder nom
AG	Control	6	1
	GnRH-2.5 ml	6	0
	GnRH-5ml	6	2
	Propylene glycol	6	1
AM	control	8	2
	GnRH-2.5 ml	8	1
	GnRH-5 ml	8	3
	Propylene glycol	8	2

Table 2. Effect of GnRH treatment and propylene glycol on decreasing of repeat breeder.

after mating showed repeat estrus and using 2.5 ml of GnRH before mating had better effect to prevent of repeat breeder. Results of logistic regression on probability of lambing numbers and lambing rate are shown in Table 3.

effect	DF	chi-square	pr<chi-square
Ewe age	1	4.01	0.04
BHBA	1	4.1	0.04
Progesterone	1	2.61	0.1

Table 3. Results of logistic regression on lambing rate.

In this table the effects of ewe age, BHBA and progesterone on lambing rate were significant. The results of regression showed that with increasing 1 unit of ewe age and progesterone concentration, the probability of lambing/no and lambing rate is increasing 4.01 and 4.1 unit, respectively. Also with decreasing 1 unit on BHBA concentration this probability is increasing 2.61 units.

Discussion

In the present study in attempt to reduce embryonic morality and hence to improve reproductive performance crossbred ewes, have been employed single administration of GnRH treatment during the breeding season. The results of propylene glycol effect on serum glucose, urea and BHBA concentrations observed in this study has been supported in [8] that reported daily drenching of propylene glycol to cows in early lactation would increase plasma glucose and insulin concentrations and improve fertility in postpartum cows. After drenching, propylene glycol increased plasma glucose and insulin and decreased NEFA (P<0.01). In 2006, Abu El-ELLA was provided evidence that luminal administration of propylene glycol produced a greater molar percentage of luminal propionate and increased concentrations of glucose and insulin and decreased concentration of NEFA in plasma. [10] was found that increasing amount of propylene glycol linearly increased glucose and insulin and decreased BHBA and NEFA in blood. A certain amount of propylene glycol is metabolized to propionate, which stimulates insulin secretion, or intermediates of propylene glycol metabolism may stimulate insulin secretion directly [8]. Glucose is the primary metabolic fuel used by the central nervous system, so that inadequate availability of utilizable glucose reduces hypothalamic release of GnRH. Glucose is a major source of energy for the ovary. Low blood glucose may be detected by the hypothalamus in a threshold-depend manner such that GnRH secretion will be impaired if glucose availability is inadequate [11, 12] was reported that effect of feeding with propylene glycol in pregnant ewes on glucose, urea and cortisol concentration was significant. Glucose, urea, BHBA and cortisol were significantly different among control and treated groups (P<0.01). The results of the present study showed that administration of GnRH on day-12 post mating increased lambing rate than other treatments and the ewes in GnRH group had more twins and consequently they had a higher total number of lambs born than others. The difference between GnRH group and others reflected the effect of hormonal treatment on reducing the early embryonic mortality that accounted for 70-80% of the total embryonic loss. These results indicated that GnRH administration before the time of maternal recognition of pregnancy improved pregnancy rate, twinning rate and the number of lambs born by improving embryo survival. [4] reported that GnRH administration before the time of maternal recognition of pregnancy improved reproductive performance in sheep during the breeding season. The results of the present study are in agreement with these findings. The effect of GnRH on embryo survival may occur through GnRH-stimulated LH surge [13] stimulating production of progesterone by CL and/or causing ovulation and the formation of accessory CLs (Present study showed that the treatment of ewes with GnRH at estrus and before mating improved their conception rate. In a study, GnRH administered at detection of estrus increased conception rate in dairy cows in suAMer, when conception rate was very low (Ullah et al, 1996).In another study, GnRH administered at estrus increased both the LH peak height and the area under the curve. These findings could by related to the fact that repeat breeder heifers have a smaller pre-ovulatory LH surge than virgin heifers and therefore, an increase in the spontaneous surge that results from the administration of GnRH at estrus affects the conception rate favorably. In the present study, significant difference (P<0.05) in plasma progesterone concentrations between control and GnRH groups were observed and in control group was higher than others. In various studies, progesterone concentration has either decreased during the luteal phase, following GnRH administration [14].recently, possible relationships between low LH surge and low in vitro secretion of progesterone by luteinized granulose cells, and between a low GnRH-induced LH surge and low plasma progesterone concentrations at mid-luteal phase were reported [15, 16].

Conclusion

Propylene glycol decreased blood urea and BHBA level near to optimum range. Administration of 5 ml GnRH on day-12 after mating had significant effect on reproductive performance than others and had better effect on conception rate. Administration of 2.5 ml GnRH before mating had better effect to prevent of repeat breeder animals. In conclusion it can be suggested that for improvement of reproductive performance in sheep, GnRH and propylene glycol be used in mating period.

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