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COMPARISON OF THE EFFECTIVENESS OF HORMONAL TREATMENT (GnRH AND HCG) ON PLASMA PROTEINS AND UREA CONCENTRATIONS IN CYPRUS DOES DURING THE NON-BREEDING SEASON

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Abstract

The aim of this study was to investigate the effect of GnRH and HCG on plasma proteins and urea concentrations in Cyprus does for induction of estrus during the non-breeding season. A total 33 Cyprus does 2-5 years old and average 45 kg. The does were divided randomly into three groups (11 does per group). The first group (G1) regarded as a control group, intramuscular injection with normal saline. The GnRH intramuscular injection of the second group (G2) was 20 µg / animal and divided two doses, whereas, the third group (G3) HCG intramuscular injection 250 IU/ animal, divided two doses. Bucks were introduced after 24h of the treatment in all groups, ensuring that does exhibit estrus were mated for two consecutive estrus cycles. Study revealed that there were significant increased ($P< 0.05$) in plasma total protein, albumin and urea concentrations in G2 and G3 groups affected by GnRH and HCG after treatment compared with G1 group. Values of plasma globulin and urea concentrations at the time of estrus were significant ($P< 0.05$) in G2 and G3 groups compared with G1. Thus, it can be concluded that the hormonal treatment (GnRH and HCG) had pronounced effect on induction of estrus as revealed by changing in plasma proteins and urea concentrations in Cyprus does during the non-breeding season.

Keywords : Cyprus does , GnRH , HCG, plasma proteins , urea

Introduction

Reproductive seasonality in goat breeds which lived in subtropical, middle and high latitudes display seasonal changes in reproductive activity (Gómez-Brunet *et al*., 2012) was characterized by changes in behavioral, endocrine, and ovulatory patterns (Rosa and Bryant, 2003). Cyprus goats or Damascus goat or Shami goats were seasonal breeds that it considered the most important breeds of goats in the Middle East (Mavrogenis *et al*., 2006). Due to its reputation as a fertility, prolificacy and milk production (Khazaal, 2009). Therefore, Cyprus goats were introduced in Iraq. The breeding season of Cyprus goats start in late August and extends through mid-December (Mavrogenis *et al*., 2006). The attempt to mate at a frequency greater than once a year will require one breeding season during or anestrous. Consequently, one of the most common ways to invest the non-breeding season by manipulation the sexual activity of animals (Delgadillo, 2011) by using of exogenous hormonal treatments (Abecia *et al*., 2011; AL-Ameri,2019) for estrus synchronization provides the opportunity for timed breeding.

Researchers used Gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (HCG) for inducing estrus during non-breeding season in goats. In general, protocols based on the use of GnRH source were associated with of norgestomet ear implant to induce goats for synchronization estrus and ovulation during the non-breeding season and improve fertility rate (Medan *et al*., 2002; Acar *et al*., 2013), MAP (Melenagesterol acetate) with PMSG – GnRH induced ovulation and increased plasma progesterone (Cameron *et al*., 1988), FAG (Fluorogestone acetate) with GnRH led to LH release in response to GnRH and inducing ovulation (Knight *et al*., 1988).While the use of another of the protocol of P4-priming + E2 + GnRH, was actived in synchronizing estrus and ovulation (Carrillo *et al*., 2019).

The administration of human chorionic gonadotropin (HCG) has been used in different protocols to synchronize the reproductive activity during the non-breeding season (Rowe and East, 1996; Fonseca *et al*., 2005; Fonseca *et al*., 2009; Pietroski *et al*., 2013; González-Álvarez *et al*., 2016; Rodríguez-Martínez *et al*., 2018). The physiological basis for using HCG to induce sexual activity (González-Álvarez *et al*., 2016; Rodríguez-Martínez *et al*., 2018), ovulation (Rahman *et al*., 2017; Dias *et al*., 2018), supported CL formation, increased serum progesterone concentration (Ishida *et al*., 1999; Lankford *et al*., 2010; Kelidari *et al*., 2010; Shabankareh *et al*., 2012; Coleson *et al*., 2015; Afri-Bouzebda *et al*., 2015; Fernandez *et al*., 2018) and improve the reproductive performance (Moeini *et al*., 2013; Kaya *et al*., 2013; Mirzaei *et al*., 2014)

The objective of the present study was to compare the effectiveness of hormonal treatment (GnRH and HCG) on plasma proteins and urea concentrations in Cyprus does during the non-breeding season.

Materials and Methods

Animals

The study was conducted in Ruminant Research in the Department of Agricultural Research Station / Ministry of Agriculture, Abu Ghraib /Baghdad (latitude 33 20º N). Thirty three mature Cyprus does, averages 45 kg and aging 2-5 years old were used in this trial. Goats were kept indoors at night, and allowed field grazing pasture near the station on day. Indoors, the goats were fed hay and concentrated diet .All does have free access to water and trace mineral salt blocks.

Experimental design

During the non-breeding season in 22 June does were divided randomly into three groups (11 does per group). The first group (47.6 ± 1.24 kg) control (G1) 0.9% NaCl intramuscular injections are divided into two doses (2 ml / animal / dose). Second group 44.7 ± 2.49 kg (G2) GnRH intramuscular injection (Receptal, Intervet International BV, Boxmeer, Holland) 20 µg/ animal and divided two doses.

Third group 43.04 ± 3.00 kg (G3) HCG intramuscular injection (Chorulon, Intervet International BV, Boxmeer, Holland) 250 IU/ animal, divided two doses. All goats received all the treatments and carried out two doses at 2-h intervals.

Six fertile bucks introduced to the does of all groups, two bucks for each group for estrus detection and mating for two consecutive estrus cycles; started 24 hours after treatments and left to ten days. Estrus checked continuously by observation.

Blood sampling and assay:

Blood samples collected 24 hrs (pretreatment), 60 min after the second dose injected and on day when does showed estrus. The blood samples collected from the jugular vein using vacutainer tubes, container inhibitor coagulant (EDTA). The blood sample immediately (5ml) collected via vacutainer tubes, and plasma was harvested following centrifugation of the samples (3000 RPM for 20 minutes) and stored under -20°C until assay.

The plasma biochemical measured were total protein (mg/dl) by biuret, albumin (mg/dl) by method BCG, urea (mg/dl) by Berthelot enzymatic method. Globulin (mg/dl) was calculated from the values obtained for total protein and albumin concentrations and then, A/G ratio was estimated by dividing albumin on globulin.

Statistical analyses

Statistical analysis was performed with the SPSS Statistics 24.0 (2016). Statistical significance was declared at P<0.05.

Results and Discussion

A new method for the manipulation of reproduction by using hormonal treatments carried out two doses at 2-h intervals in Cyprus does during the non-breeding season for investigation value changed for proteins and urea concentrations during post-treatments (GnRH and HCG) and led to induce estrus in Cyprus does.

In the present study, there was significant difference (P< 0.05) in plasma total protein in G2 and G3 groups compared with G1 group (Table 2). On the other hands, no significant difference in plasma total protein during estrus in all groups (Table 3).

Analysis of plasma protein in blood, which giving indicator as changes of hormonal treatments due to proteins has many functions including transport, metabolic control, contraction, and catalysis of chemical transformations (Devlin, 1997). Therefore, hormonal treatments might elevate total protein concentration during post-treatment. The previous studies that applied GnRH and HCG with various protocols to induce estrus and ovulation in goats during the non-breeding season as a result improve the reproductive performance (Medan *et al*., 2002; Rodríguez-Martínez *et al*., 2018). Therefore, goats responded following hormonal treatments to occur estrus within some hours by endocrine events to release estrogen. Singh and Dutt, (1974); Ishwar and Pandey, (1994) provides evidence that the positive relation between increased total protein and increased levels of estrogen. On the other hand, Wani *et al*., 2018 reported that total protein decreased in ewes at induced estrus when using progesterone vaginal sponge. Imasuen and Otoikhian, (2011) pointed out that administered modroxyl-progestrone acetate in West African Dwarf does might a delayed effect led to decrease total protein during post-treatment.

Albumin of the same total protein pattern during post-treatment (P< 0.05) in G2 and G3 groups compared with G1 group. However, plasma albumin concentration decreased significant (P<0.05) in G2 group than G1 and G3 groups. Albumin, the major serum protein (Baker, 1998) and it binds steroid hormones (Hammond, 2016) to regulate of their contact to receptors (Baker, 1998). The previous studies showed that no significant effect was observed after treatments on albumin in goats (Imasuen and Otoikhian, 2011) ewes (Hassanein *et al*., 1999; Juma, 2010; Wani *et al*., 2018). On the other hands, Singh and Dutt, (1974) reported that increased albumin during estrus than di-estrus due to the effect of elevated levels of estrogen. Moreover, increased total protein and albumin concentrations during post-treatment in G2 and G3 groups due to increased demand for amino acids and protein for steroidogenesis (Stocco *et al*., 2017). On the other hands, decreased albumin concentration in G2 group during estrus suggested that may be delay effect to require time for steroidogenesis.

The plasma globulin concentration during pre-treatment was found to be 4.34±0.09, 4.31±0.27 and 4.30±0.11 g/dl in G1, G2 and G3groups respectively. The plasma globulin was significant (P<0.05) in G1 group as compared G2 and G3groups (Table 1). However, there was similar observed in G2 and G3groups with G1group. Regarding the effect of treatments data observed that, the plasma globulin was significant (P<0.05) in G2 and G3 groups as compared G1. However, there was similar observed G1 group with G2 and G3 groups (Table 2). The results in table 3 regarding occur estrus indicate that plasma globulin concentration was significant (P<0.05) in G2 and G3 groups compared with G1.

Globulins are a group of proteins made in liver and it can be used to diagnose liver function and disorders of the immune system. In our results increased globulin significantly in G2 and G3 groups during post-treatment and estrus than G1 group. Other studies mentioned that decreased globulin with using various protocols (Hassanein *et al*., 1999; Yaqub *et al*., 2011; Wani *et al*., 2018). However, administered modroxyl –progestrone acetate not impact alters globulin concentration (Imasuen and Otoikhian, 2011). On the other hands, variations in globulin concentration may be due in subtropical regions to breed variation, management practices, sex, over hydration and low fodder protein quality (Ahmad *et al*., 2014). Therefore, Rufai *et al.* (2013) reported that globulin in follicular liquid can be fundamental for ensuring the follicle from outside conditions. To prove these results, plasma globulin concentration in Cyprus does during pre-treatment was significant (P<0.05) in G1 group as compared G2 and G3groups suggested there were variation in globulin concentration that may be agreed with found (Ahmad *et al*., 2014).

In the present study, data in table 2 and 3 cleared that, there were significant differences (P<0.05) in plasma urea concentration in G2 and G3 groups compared to G1 group during post-treatment and estrus showed. Researchers studied different physiologic statuses gives better pictures about urea concentration. Imasuen and Otoikhian, (2011) reported that increment or diminish in the urea during treatment and post-treatment due to any endogenous toxic substance could develop by reducing the use of proteins. However, Yaqub *et*

al. (2011) reported that increased urea during early estrus indicated that efficient digestion of dietary protein compared to luteal phase of the estrus. On contrary, Sitaresmi *et al*. (2017) found that increased urea in the luteal phase due to reduce energy diets as result of decreased protein synthesis microbes. Though, increased urea associated increased rate of protein catabolism (Naqvi *et al*., 2013). On the other hands, Sitaresmi *et al*. (2019) reported that no contrast in urea between short estrus cycles does and normal estrus cycle does. Moreover, urea concentration increased that may lead to effect on fertility in cows (Pathan *et al*., 2011; Rasolomboahanginjatovo *et al*. 2014). However, the repeat breeding buffaloes that decreased urea then pregnant and/or regularly cycling as result decreased protein level (Sabasthin *et al*., 2012). On contrast, (Barson *et al*., 2019) reported that increased concentration of urea in repeat breeding cows then in normally cyclic cows lead to fertility disorder.

Conclusion

It can be concluded that the hormonal treatment (GnRH and HCG) had pronounced effect on induction of estrus as revealed by changing in plasma proteins and urea concentrations in Cyprus does during the non-breeding season.

Table 1 : Plasma proteins and urea concentrations during pre-treatment of three groups (Mean ±SE).

Parameters	Treatments		
	Control G1	GnRH G2	HCG G3
Total protein (g/dl)	7.15±0.12a	7.14±0.21a	7.12±0.12a
Albumin (g/dl)	2.81 ±0.02a	2.82±0.09a	2.81± 0.03a
Globulin (g/dl)	4.34±0.09a	4.31±0.27ab	4.30±0.11ab
A/G (g/dl)	0.64 ±0.01a	0.66±0.05a	0.64±0.02a
Urea (mg/dl)	29.95±0.04a	30.08±0.00a	29.94±0.07a

Means within the row with different letters are significantly different ($p < 0.05$).
G1= 2ml (0.9% NaCl) (control), G2= (20 µg/ animal GnRH) and G3= (250 IU HCG / animal)

Table 2 : Plasma proteins and urea concentrations during post-treatment in three groups (Mean ±SE).

Parameters	Treatments		
	Control G1	GnRH G2	HCG G3
Total protein (g/dl)	7.00±0.04b	8.05±0.30a	8.01±0.09a
Albumin (g/dl)	2.76±0.07b	3.26± 0.26a	3.20±0.03a
Globulin (g/dl)	4.23± 0.06ab	4.79±0.51a	4.80±0.12a
A/G (g/dl)	0.65±0.02a	0.72±0.14a	0.66±0.02a
Urea (mg/dl)	29.95±0.03b	32.62±0.14a	32.62±0.12a

Means within the row with different letters are significantly different ($p < 0.05$).
G1= 2ml (0.9% NaCl) (control), G2= (20 µg/ animal GnRH) and G3= (250 IU HCG / animal)

Table 3 : Plasma proteins and urea concentrations during estrus in three groups (Mean ±SE).

Parameters	Treatments		
	Control G1	GnRH G2	HCG G3
Total protein (g/dl)	7.05±0.04a	7.12±0.01a	7.14±0.02a
Albumin (g/dl)	3.00±0.04ab	2.78±0.06b	2.99±0.04ab
Globulin (g/dl)	4.04±0.03b	4.33±0.05a	4.14±0.04a
A/G (g/dl)	0.74±0.01a	0.64±0.02a	0.71±0.01a
Urea (mg/dl)	29.18±0.04b	29.57±0.09a	29.55±0.07a

Means within the row with different letters are significantly different ($p < 0.05$).
G1= 2ml (0.9% NaCl) (control), G2= (20 µg/ animal GnRH) and G3= (250 IU HCG / animal)

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