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IMPACT OF RUMEN PROTECTED L-ARGININE AND EQUINE CHORIONIC GONADOTROPIN ON THE OVARIAN ACTIVITY, FERTILITY RATE, LAMB'S BIRTH WEIGHT AND BLOOD BIOCHEMICAL PARAMETERS OF SYNCHRONIZED EWES IN UPPER EGYPT

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SUMMARY

The current study aimed to investigate rumen-protected L-arginine (ARG) and equine Chorionic Gonadotropin (eCG) treatments effect on ovarian activity, fertility parameters, lamb birth weight, and certain blood parameters in synchronized ewes under Upper Egypt condition. Thirty six native adult and clinically healthy ewes were divided into three equal groups (12 ewes/each), G1: (CG) control group, G2: (eCG), the animals received a vaginal sponge impregnated with 40 mg medroxy progesterone acetate (MAP) for 14 days and injected with 400 IUeCGat the day of sponges withdrawal, and G3: (ARG) ewes were treated with vaginal sponges for 14 days during the same time animals were treated orally with 20 mg/kg body rumen-protected L-Arginine. An ultrasonic device was used to monitor ovarian activity and serum samples were taken 24^{hrs}, 48^{hrs}, and 72^{hrs} after therapy ended, as well as once a month for the first three months of pregnancy to determine glucose, urea, AST and ALT levels. Natural mating was used with fertile rams when estrus behavior was detected. The results demonstrated significant ($P<0.05$) changes in the diameter of large-follicles on the right and left ovaries after 24^{hrs} of treatment among the treated and control groups. After 72^{hrs} of treatments, the ARG group had a considerably higher ($P<0.05$) diameter of small and large follicles on the right ovary than the other groups .the diameter of large follicles and CL in the ARG group on the left ovary were significantly larger ($P<0.05$) than that of the other animals. However, comparing the treatment and control groups, there were significant differences ($P<0.05$) in estrus response, conception, lambing and fecundity rate. After the 7^{th} and 15^{th} days of lambing the ARG group had heavier ($P<0.01$) lambs than that of control group. The concentrations of the serum glucose, urea, ALT and AST were higher in the treated groups than that in the control groups. In conclusion, after progestagine synchronization therapy with eCG or rumen protected L-arginine improved reproductive indices in ewes and lamb birth weight under Upper Egypt conditions.

Keywords: L-Arginine, eCG, ovarian activity, conception rate, lambing rate

INTRODUCTION

According to the Ministry of Agriculture and Land Reclamation (2015), Egypt has about 5463169 sheep, which are mostly utilized for lamb and mutton production. In Egypt, efforts have been made to improve the efficiency of native sheep breeds form eat and milk production, skin, and organic fertilizer by increasing reproductive rates (Galal *et al.,* 2005). However, nutritional and/or hormonal therapies can increase the efficiency of most sheep production systems (Kridli*et al.,* 2003). Because ARG is a precursor to nitric oxide (NO), it has the potential to influence ovarian function (Lassala *et al.,* 2011). Also, NO has been found in the follicular fluid of several animal species. The presence of an intra-ovarian NO-generating system (for example, resident ovarian macrophages) supports the presence of an intra-ovarian NO-generating system, emphasizing its crucial role in modulating follicular growth (Basini and Grasselli, 2015).

The administration of eCG at the end of the progestagensprotocols increases follicular growth (Cline *et al.,* 2001). The follicle on the other side accumulates many layers of granulosa cells as it matures, allowing it to make estradiol-17 (Oktem *et al.,* 2008) and the indications of estrus developed sooner and became more apparent and prolonged (Ustuner *et al.,* 2007). Ovarian ultrasonography becomes a useful technique in detecting the advancement of antral follicular dynamics, ovulation, and corpus luteum (CL) production (Duggavathi *et al.,* 2003).

MATERIALS AND METHODS

Animals and Managements:

The current study was carried out at the experimental farm, Faculty of Agriculture, Al-Azhar University, Assuit, Egypt. The ewes were housed in open barns with sheds during the experimental periods. The animals were clinically healthy, free from reproductive disorders and fed thefarm ration(14 % protein)., water, and a mineral supplement were available *ad libitum* A total of 36 ewes were divided into three groups, each with 12

ewes; group 1: control group, group 2: (eCG) animals were given vaginal sponges impregnated with 40 mg.medroxyprogesterone-acetate (MAP, Pfizer produced, NV/SA, Puurs, Belgium) for 14 days and injected with 400IUeCG, I/M, equine chorionic gonadotropin (freeze-dried serum gonadotrophin 500IU, gonaser, Hipra, Girona) at the time of sponge removal, and group 3:(ARG): animals were given 40mg MAP for 14 days, during this time; the animals were given 25gm./ewes/day/oral, rumen-protected l-Arginine (l-arginine pure, $C_6H_{14}N_4O_2$, Edappally, India, imported by El-goumhouria Co, Cairo, Egypt).

Bypass L-arginine pellets:

L-Arginine was changed in the lab to prevent ruminal degradation; this alteration was made up of two layers (Julio *et al.*, 2015). L-Arginine and barium sulphate made up the first layer (El-Goumhouria Co, Cairo, Egypt) L-Arginine, a nonfunctional polymer, was added to the cellulose acetate phthalate in a 1: 1 ratio and thoroughly mixed before adding the barium sulphate to the preceding mixture. The use of barium sulphate served to give the pellets solidity, which prevented them from being destroyed by rumen microorganism. The exterior layer was made of – Eudragit® S100, a functional polymer (imported by Memphis pharm and chemical Co, Cairo, Egypt), will be made from Ether solution, which has the ability to dissolve at pH levels above 7.2, allowing for the release of L-Arginine in the intestineaccording to themethod of Moore and Stein (1954) and Maurer*et al*. (2015). To make the billet, the L-Arginine and polymer were mixed in a 4:1 ratio. Moore and Stein (1954) approach was used to determine the amount of L-Arginine in the pellets.

Ovarian Ultrasonography Examination in ewes:

Blood sampling:

Blood samples were collected by venipuncture from the jugular vein into collection non-heparinized tubes and centrifuged at 4000 r.p.m for 15 minutes, and then serum was harvested and stored at -20C° till assay. The blood samples were collected during 24, 48 and 72 hours after intra vaginal sponge's removal and during 2nd, 3rd and 4st month's pregnancy. Plasma glucose and urea were determined according to Caraway and Watts (1987) using assay kits supplied by Diamond Chemical Company, Germany. Plasma AST and ALT were determined according to Young (1990) using assay kits supplied by Spectrum Chemical Company, Egypt.

Statistical Analysis:

The SPSS computer programs (2006), technique of analysis, were used to perform statistical analysis of the data gathered in the study (Snedecor and Cochran, 1982). Duncan's multiple range tests were used to look for significant differences between sub-class means (Duncan 1955). The ANOVA test was used to determine the differences between the groups. The chi-squared test was used to compare the groups' estrus, conception, lambing, fecundity rates, and estrus symptoms.

Using a real-time, B-mode diagnostic scanner with a trans-rectal 5/7.5 MHz linear array transducer, ewes ovarian structures were monitored ultra-sonographically (Hitachi, EUB-405B, Japan). From the 15th to the 17th day of the estrous cycle, ultrasound tests were done once a day. Images were frozen on the ultrasound scanner's monitor, and the diameters of these structures were measured at their maximum with the ultrasound device's integrated caliper. All follicles with a diameter of ≤ 2 mm and CL were measured and mapped individually for ewes. When a tracked large, developing antral follicle was no longer visible, it was considered ovulation (Ginther*et al.*, 1997). The following ovarian characteristics were measured and compared among groups: Ovulation rates after the vaginal sponge's removal; Number and diameter of small follicle (diameter = 2-3mm) and large follicle (diameter ≤3) of the ovulatory follicles; Number and diameter of the CL; and Cross section in uterus.

Measures of fertility:

Fertility was measured according to the following formula (Zeleke *et al*., 2005):

Estrus response in ewe lambs (percentage of animals comes into heat after each treatment.

$$- \quad \text{Conception} \quad \text{rate} = \frac{ewes\ \ conseved}{ewes\ \ inse\min ated} x100$$

$$\text{- Lambing} \quad \text{rate} = \frac{\text{lambs}\ \ born}{ewes\ \ inse\min ated} x100$$

$$\text{- Fecundity} \quad \text{rate} = \frac{number\ \ of\ \ lambs\ \ born}{number\ \ of\ \ pregnant\ \ ewes} x100$$

RESULTS AND DISCUSSION

Ultrasonic appearance of the ovaries and uterus:

There were no statistical differences in the number of follicular categories on the right ovary atthe end of the first day of therapy. The diameter of the largefollicles (mm) were larger (P<0.05) in the eCG and ARGgroups than in the control one. There were no statistical differences in the number of follicular groups on the left ovary, while there were significant differences in the diameter of small follicles (P<0.01) and large follicles (P<0.05) in the eCG and ARG groups, as showed in table (1) and figure (1).

Table 1. Effect of eCG and ARG on ovarian activity (mm), at 24hrs of the treatments in ewes using Ultrasonography

Treatment*	Right Ovary				Left Ovary				CL	Uterus
	NSF	DSF	NLF	DLF	NSF	DSF	NLF	DLF		
G1	3.00	0.22	1.16	0.31^{b}	2.33	0.20^{b}	1.00	0.28^{b}	0.81	1.85
G2	2.83	0.26	1.16	0.37^{a}	2.16	0.33^{a}	1.50	0.30^{b}	0.84	2.01
G3	2.88	0.27	1.50	0.45^{a}	2.66	0.37^{a}	1.33	0.38^{a}	0.87	1.78
SEM	0.02	0.01	0.01	0.02	0.02	0.03	0.21	0.02	0.02	0.09
Sig	NS	NS	NS	0.05	NS	0.01	NS	0.05	NS	NS

a, b Means with different superscripts in the same column are significantly different (P<0.05).
NSF= Number small Follicle, DSF= Diameter small follicle, NLF= Number large Follicle, DLF= Diameter large follicle, CL=Corpus luteum.
*G1: (CG) control group, G2: (eCG), 40 mg MAP and injected with 400 IU eCG, and G3: (ARG) ewes 40 mg MAP and 20 mg/kg body rumen-protected L-Arginine orally.

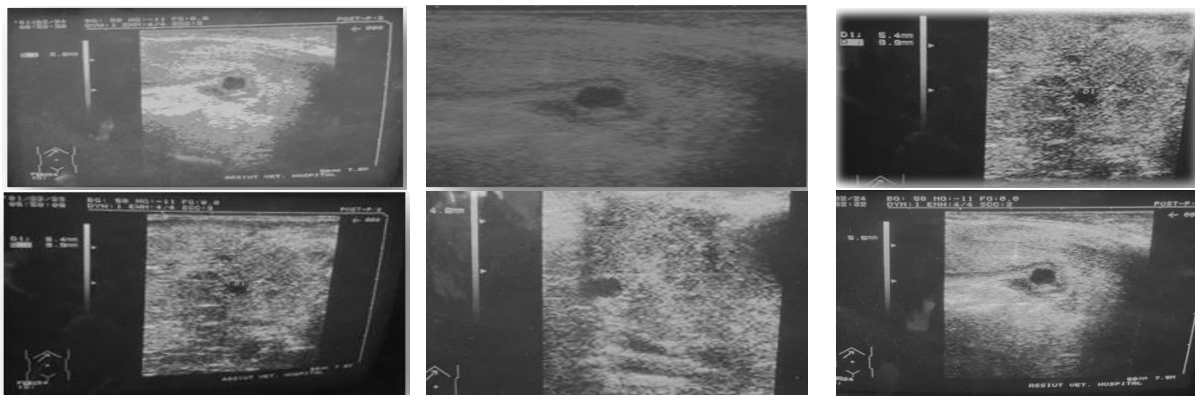

Fig. 1.The follicular diameters in ovarian ewes by ultrasonography.

The current findings are consistent with the previous findings of Duggavathi *et al*. (2003) who found that the follicular diameter increased to approximately 1 to 2 mm before ovulation and that follicles emerge every 3–5 days from a constant pool of 2 to 3 mm in mature ewes. Furthermore, Tassell and Kennedy (1980) observed that ewe's ovarian follicles grow in size in response to an exogenous gonadotropin stimulus. Nutritional direct activities at the ovarian level could be another route for the immediate nutritional effect on follicle formation. According to previous study by Ying *et al.* (2013) indicated that sheep ovarian follicles development was altered by high- and low-intake during the luteal phase for six days. This was accompanied by changes in the intra-follicular microenvironment as well as changes in the reproductive hormone, urea, and fat concentrations in the blood. In the same line, follicularatresia can be prevented by giving antral follicles enough GNRH exposure especially when gonadotropins (eCG) injected after the progestagens treatment cause stimulates follicular development and improve the ovulation rate in small ruminants (Cline *et al*., 2001).

The ARG group had a significantly (P<0.01) larger diameter of the small and large follicle on the right and left ovaries at the second day of treatment than the other groups (Table 2 and Figure 1). Contreras-Solis *et al.* (2008) discovered a follicular diameter of 3.90 mm, which was consistent with this data. While this finding was higher than those of Tarek and Ashmawy, (2012) who reported that when synchronized Rahmani ewes by GnRH, the follicular diameter was 2.33mm before ovulation.

The difference in follicular diameters between our results andthe results obtained by Tarek and Ashmawy, (2012) may be attributed to variances in ewe breed. The enormous antral and pre-ovulatory follicles, which are heavily vascularized, make up the third stage of folliculogenesis, however, large ovarian follicles are also capable of having an effect on residual follicles due to their ability to achieve ovulation size (Gonzalez-Bulnes *et al.,* 2004). Furthermore, arginine therapy increases ovarian blood flow, which increases ovarian diameter follicle size (Saevre *et al.,* 2011). On the other side, the application ofeCG has been demonstrated to improve the daily follicular growth rate, the diameter of the dominant follicle, and ovulation rates in cows (SáFilho *et al.,* 2005).

The ARG group showed a significant difference (P<0.01) in diameter of the small and large follicles on the right ovary at 72^{hrs} of therapy compared to the other groups (Table 3 and Figure 1). On the left ovary, the diameter of large follicles and CL in the ARG group were considerably (P<0.05) larger than in other treatments. These findings are consistent with those of Contreras-Solis *et al.* (2008) who discovered that the follicular diameters at the end of waves 1 and 2 were 5.0±0.2 and 3.9±0.1 mm, respectively, and the follicular diameter at wave 3was 5.7±0.2mm.

Table 2. Effect of eCG and ARG on ovarian activity (mm), at 48[hrs] of the treatment in ewes using Ultrasonography

Treatment*	Right Ovary				Left Ovary				CL	Uterus
	NSF	DSF	NLF	DLF	NSF	DSF	NLF	DLF		
G1	2.00	0.21[b]	1.17	0.38[b]	2.17	0.23[b]	1.17	0.39[b]	0.66	1.71[b]
G2	3.17	0.28[a]	1.33	0.43[b]	2.50	0.27[a]	1.17	0.49[a]	0.70	2.36[a]
G3	3.00	0.31[a]	1.50	0.55[a]	3.17	0.32[a]	1.33	0.53[a]	0.81	2.20[a]
SEM	0.37	0.01	0.33	0.03	0.03	0.01	0.17	0.03	0.05	0.01
Sig	NS	0.01	NS	0.05	NS	0.01	NS	0.05	NS	0.01

a, b Means with different superscripts in the same column are significantly different (P<0.05).
NSF= Number small Follicle, DSF= Diameter small follicle, NLF= Number large Follicle, DLF= Diameter large follicle, CL=Corpus luteum.
*G1: (CG) control group, G2: (eCG), 40 mg MAP and injected with 400 IUeCG, and G3: (ARG) ewes 40 mg MAP and 20 mg/kg body rumen-protected L-Arginine orally.

Table 3. Effect of eCG and ARG on ovarian activity (mm), at 72[hrs] of the treatment in ewes using Ultrasonography

Treatment*	Right Ovary				Lift Ovary				CL	Uterus
	NSF	DSF	NLF	DLF	NSF	DSF	NLF	DLF		
G1	2.17	0.21[b]	1.33[b]	0.41[b]	2.33	0.23	1.17	0.43[b]	0.65[b]	1.77[b]
G2	2.67	0.28[a]	1.33[b]	0.48[ab]	2.33	0.27	1.17	0.48[ab]	0.69[b]	1.99[ab]
G3	2.67	0.29[a]	2.33[a]	0.51[a]	2.83	0.29	1.50	0.53[a]	1.06[a]	2.33[a]
SEM	0.12	0.02	0.017	0.04	0.21	0.02	0.17	0.02	0.013	0.14
Sig	NS	0.01	0.05	0.05	NS	NS	NS	0.05	0.05	0.05

a, b Means with different superscripts in the same column are significantly different (P<0.05).
NSF= Number small Follicle, DSF= Diameter small follicle, NLF= Number large Follicle, DLF= Diameter large follicle, CL=Corpus luteum.
*G1: (CG) control group, G2: (eCG), 40 mg MAP and injected with 400 IUeCG, and G3: (ARG) ewes 40 mg MAP and 20 mg/kg body rumen-protected L-Arginine orally.

Furthermore, arginine administration improved ovarian blood flow, which resulted in an increase in the number and diameter of follicles in the left and right ovaries compared to the control group, where increased blood flow velocity is also linked to the terminal development of ovulatory follicles. The formation of several ovulatory-sized follicles in combination with a large increase in ovarian blood flow was a good predictor of super-ovulatory responses in mares (Witt *et al.,* 2012). However, in order to maintain pregnancy, the CL must be maintained and continue to produce high levels of progesterone (Weems *et al.,* 2007). Unfortunately, because a technical issue with an ultrasonic instrument, the blood supply of the uterus and ovaries was not carried out in this study.

The uterine diameter after 48[hrs] of the end of treatment was significantly (P<0.01) larger in eCG and ARG groups than that in control animals (2.36±0.13 and 2.20±0.14 Vs. 1.71±0.13, respectively). The uterine diameter has significant (P>0.05) differences between ARG and other groups (2.33±0.17 vs. 1.99±0.19 and 1.77±0.07, respectively), after 72[hrs] at the end of treatments. Arginine (ARG) supplementation reported to promote a beneficial uterine environment for the maintenance of pregnancy in sheep (Wu *et al.,* 2013); to our knowledge, there are no available studies about the effect of short term treatment of arginine on uterus of the non-pregnant ewes.

Fertility parameters:

The onset of estrus was not documented for the control group because they did not receive treatment. While the arginine group had an earlier beginning of estrus than the eCG group (36.83±1.97[hrs]Vs 40.70±1.87[hrs], respectively). Similarly, the onset of estrus observed after 39.5[hrs] of treatment, in synchronized sheep with vaginal sponges impregnated with MAP followed by eCG injection (Zeleken*et al.,* 2005). Short-term supplementation, such as rumen protected L-arginine, has been developed to improve the reproductive performance of small ruminants (Jena*et al*., 2020). There is some evidence that a short period of elevated metabolic function can stimulate follicular growth and increase the ovulation rate in sheep (Zabuli*et al.,* 2010). Also, eCG stimulates follicular growth in small ruminants at the end of the progestagens treatment (Cline *et al.,* 2001),further than that, the hypothesis was that the assayable amount of estrogens is the resultant of the contribution of each estrogenically active follicle; thus, the larger the number of follicles developed in response, the higher the estradiol concentration (Irene Valasi *et al.,* 2007).

In the present study (Table 4), the estrus length of the treated groups (ARG and eCG) was significantly (P<0.01) longer than that of the control group (36.66±1.84 and 41.00±2.64 vs. 29.33±0.79[hrs], respectively). In sheep synchronized with MAP plus eCG, Santos *et al.* (2011), found that estrus response,

pregnancy rates and fecundity rate were 88 %, 79 % and 120 %, respectively. The injection with eCG at the end of treatment stimulates follicular growth and induces more exact synchronization of estrus with tighter synchrony of ovulation, as well as increases in ovulation and fertility rates for anestrous and cycling small ruminants (Cline *et al.*, 2001).

In comparison to the control group, the ARG and eCG groups exhibited significantly increased estrus responses, conception rates, lambing rates, and fecundity rates (P<0.05). The Nitric oxide system's favorable effect on improving the fertility rate, embryo implantation, survival and growth, and pregnancy maintenance in sheep could be linked to arginine treatment (Wu *et al*., 2013).

Table 4. Effect of eCG and ARGon onset of estrus, estrus duration and fertility parameters in ewes

Treatment*	Onset of estrus (hours)	Estrus duration (hours)	Estrus response (%)	Conception rate (%)	Lambing rate (%)	Fecundity rate (%)
G1	-	29.33±0.79^{b}	50%	41.7%	41.7%	100%
G2	40.70±1.87	41.00±2.64^{a}	91.7%	91.7%	91.7%	109.09%
G3	36.83±1.97	36.66±1.84^{a}	100%	91.7%	91.7%	136.36%
Sig	NS	0.01	0.01	0.05	0.05	0.05

a, b Means with different superscripts in the same column are significantly different (P<0.05).
NSF= Number small Follicle, DSF= Diameter small follicle, NLF= Number large Follicle, DLF= Diameter large follicle, CL=Corpus luteum.
*G1: (CG) control group, G2: (eCG), 40 mg MAP and injected with 400 IUeCG, and G3: (ARG) ewes 40 mg MAP and 20 mg/kg body rumen-protected L-Arginine orally.

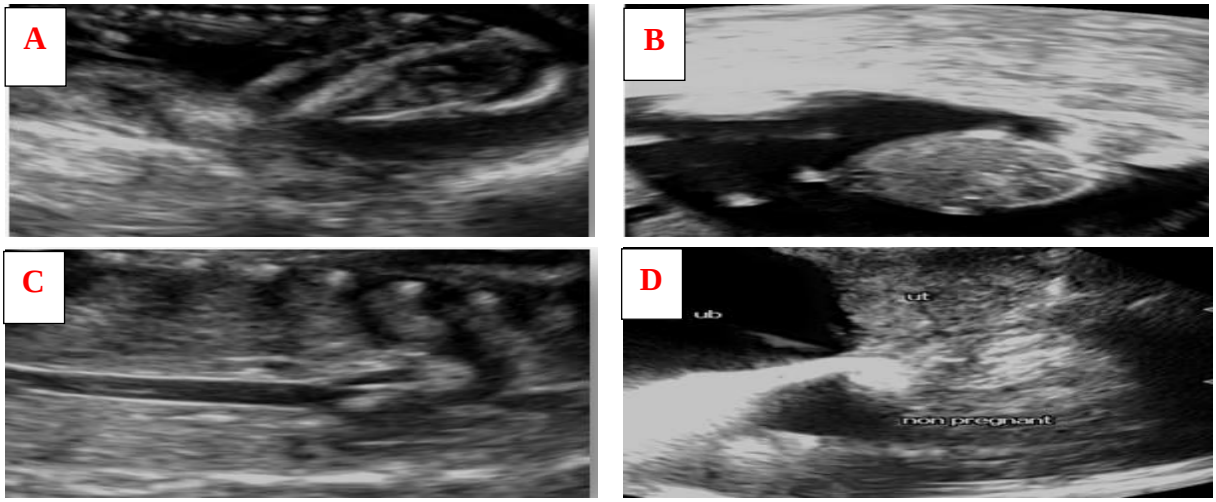

Fig. 2. Sonograms showing pregnant uterus, A: clear an echogenic fetal fluid with fetal parts clear (fetal head); B: the fetus appeared from dorsal view and the cephalic diameter is clear; C: the fetus chest is clear with the vertebral column and shadows of the ribs, D: non-pregnant uterus cross section without any contents.

Lambs birth weight and its weight after 7, and 15 days of birth:

There were significant (P<0.05) changes in birth weight between the ARG and the control group (3.15± 0.13 vs. 2.67±0.10, respectively). However, there were extremely significant (P<0.01) differences in lamb body weight at the 7th and 15th days of birth between the treated groups (ARG and eCG) and the control one (4.85±0.13 and 5.11±0.19 Vs. 4.16±0.06), and (8.75±0.17 and 8.14±0.28 Vs. 7.23±0.21), respectively (Table 5).

Table 5. Effect of eCG and ARG on birth weight, after 7th, and 15th days of birth (Kg.) in the ewes

Treatment*	**birth Weight**	**After 7 days**	**After 15 days**
Control	2.67±0.10^{b}	4.16±0.06^{b}	7.23±0.21^{b}
eCG	2.93±0.08^{ab}	5.11±0.19^{a}	8.14±0.28^{a}
ARG	3.15±0.13^{a}	4.85±0.13^{a}	8.75±0.17^{a}
Sig	0.05	0.01	0.01

a, b Means with different superscripts in the same column are significantly different (P<0.05).
NSF= Number small Follicle, DSF= Diameter small follicle, NLF= Number large Follicle, DLF= Diameter large follicle, CL=Corpus luteum.
*G1: (CG) control group, G2: (eCG), 40 mg MAP and injected with 400 IUeCG, and G3: (ARG) ewes 40 mg MAP and 20 mg/kg body rumen-protected L-Arginine orally.

The ARG treatment group had a higher birth weight than the control group, and the lambs' body weight improved after 7 and 15 days. Enhance fetal protein accretion, thus boosting lamb birth weight (de Boo *et al.,* 2005), and enhance the proportion of lambs born alive by acting through the nitric oxide system (Lassala *et al.*, 2011). Also, Ali *et al.* (2015) found that the lamb's weight was 3.29±0.15 when synchronized ewes with progestagen plus eCG. However, this result was lower than that recorded by Khan.(2009) who found that the birth weight was 4.01±0.21 and 4.13±0.5Kg in synchronized ewes.

Glucose, urea, AST and ALT concentrations:

There were significant (P<0.01)differences in the plasma glucose and urea concentrations between treated groups (ARG and eCG) and the control after 48^{hrs} and 72^{hrs} the end of treatment, plasma urea concentration was also slightly low during the three months of pregnancy (Table 6). There were significant (P<0.01) differences in the plasma urea concentrations among eCG, ARG and control groups. However, there were significant (P<0.01) differences in the plasma glucose concentration among eCG, ARG and control groups during the second and third months of pregnancy. It is possible, that plasma glucagon responses to arginine may be representative of pancreatic alpha cell responses to amino acids in general (Davies-Morel and Beck. 2003). Moreover, higher glucose concentrations during estrus in ewe have resulted in an increased rate of lipolysis and reduced glucose utilization.

Table 6. Plasma Glucose, urea, AST and ALT concentrations (ng/ml) within days after the end of treatments and first three months of pregnancy in ewes

Treatment*		Days after the end of treatment			3months of pregnant		
		After 24^{hrs}	After 48^{hrs}	After 72^{hrs}	2^{nd}month	3^{rd}month	4^{st}month
Glucose	Control	74.65±4.71^{b}	63.33±4.59^{b}	78.50±3.05^{b}	47.50±8.47^{b}	28.46±0.32^{b}	39.92±7.83
	eCG	94.18±3.18^{a}	92.98±2.94^{a}	82.46±5.18^{b}	79.39±6.17^{a}	31.70±0.60^{b}	44.91±3.85
	Arg	85.63±3.54^{ab}	88.34±3.45^{a}	100.28±1.84^{a}	53.20±1.39^{b}	63.90±3.01^{a}	35.34±3.41
	Sig	0.05	0.01	0.01	0.01	0.01	NS
Urea	Control	36.22±2.08	29.21±1.93^{b}	33.27±0.49^{b}	21.68±0.57^{c}	27.24±1.40^{b}	26.21±1.56^{b}
	eCG	38.28±0.57	41.16±3.33^{a}	33.66±1.48^{b}	29.01±1.07^{b}	32.31±1.45^{a}	31.46±0.45^{a}
	Arg	44.22±5.34	44.73±1.67^{a}	56.48±2.13^{a}	35.54±1.71^{a}	33.26±1.35^{a}	28.52±1.43^{ab}
	Sig	NS	0.01	0.01	0.01	0.05	0.05
AST	Control	22.20±1.99	22.80±1.20^{b}	23.40±1.81^{b}	25.00±2.90^{b}	27.20±2.06^{b}	27.20±1.46^{b}
	eCG	24.80±0.37	29.40±1.91^{a}	33.40±2.87^{a}	30.80±3.35^{ab}	39.60±1.81^{a}	34.80±1.66^{a}
	Arg	21.60±1.47	25.80±1.66^{ab}	33.60±3.31^{a}	35.60±1.97^{a}	38.60±3.09^{a}	37.20±2.40^{a}
	Sig	NS	0.05	0.05	0.01	0.01	0.01
ALT	Control	47.20±1.02^{b}	47.20±1.02^{ab}	47.20±1.02	40.40±1.28^{b}	39.20±0.37^{b}	38.60±0.51^{b}
	eCG	43.60±1.03^{b}	44.25±0.73^{b}	47.00±1.18	48.20±1.35^{a}	46.25±0.85^{a}	40.60±0.81^{a}
	Arg	54.80±2.57^{a}	49.40±1.83^{a}	44.60±1.60	41.40±1.20^{b}	46.25±0.85^{a}	38.80±0.37^{ab}
	Sig	0.01	0.05	NS	0.05	0.05	0.05

a, b Means with different superscripts in the same column are significantly different (P<0.05).

NSF= Number small Follicle, DSF= Diameter small follicle, NLF= Number large Follicle, DLF= Diameter large follicle, CL=Corpus luteum.

*G1: (CG) control group, G2: (eCG), 40 mg MAP and injected with 400 IUeCG, and G3: (ARG) ewes 40 mg MAP and 20 mg/kg body rumen-protected L-Arginine orally.

Most obvious was the fact that arginine-stimulated glucagon secretion according to Jerry *et al*. (1976). However, Mateo *et al.* (2007) reported that ARG supplementation might reduce whole-body amino acid degradation, thus, increased plasma urea production as well as the endogenous synthesis of glutamine from branched-chain amino acids and ammonia. The obtained results in this study agreed with that reported before, where there was the significant difference in urea concentration (35.3±1.15ng/ml and 23.9±2.00ng/ml) between control and arginine groups, respectively (Hazim *et al*., 2012). In addition, plasma glucose is the major metabolite used by the sheep fetus and the energy requirements of the ewe increase during late pregnancy due to the rapid growth of the fetus (Firat and Ozpinar, 2002).

There were no significant differences in plasma AST concentration (ng/ml) among groups after 24^{hrs} of the end of treatment, while there were significant differences (P<0.05) in the AST concentration (ng/ml) between treated groups (ARG and eCG) and control one at estrus day after the end of treatment. These results are in agreement with Marta *et al.* (2017)who found no significant difference between control and arginine groups in AST concentration (57.4±1.25ng//ml and 53.0±1.19ng/ml). On the other hand, the present results disagree with that recorded by Mohammed *et al.* (2009), where they reported that the plasma AST concentrations were

(18.92±0.05ng/ml and 18.37±0.82ng/ml) in Rahmani and Chios ewes, respectively. However, plasma AST and ALT levels and their ratio are commonly measured clinically as biomarkers for liver health. Plasma AST is a cofactor to transfer the amino group. Kirsch *et al.*(1984) reported that the amino group transfer catalyzed by this enzyme is crucial in both amino acid degradation and biosynthesis. Also, there was a significant ($P<0.01$) difference in the plasma AST concentration between treated groups (ARG and eCG) and control one during the first three months of pregnancy. The ARG group has a significant higher ALT concentration than the other groups, at the days of estrus at 24^{hrs} ($P<0.01$) and 48hrs ($P<0.05$) of the end of treatment, while there are no significant differences among groups at 72^{hrs}at the end of treatments. However, plasma ALT concentration in ARG and eCG groups were higher ($P<0.05$) than the control, during the three months of the pregnant, (Table 6).

CONCLUSION

From this study, it could be concluded that ovarian activity and reproductive performance of ewes can be improved by using equine chorionic gonadotropin (eCG), and rumen-protected L-arginine under Upper Egypt condition.

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تأثير الأرجنين المحمي وهرمون الافراس العشار علي النشاط المبيضي ومعدلات الخصوبة ووزن الحملان وبعض مقاييس الدم البيوكيميائية للأغنام المزامنة في صعيد مصر

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هدفت هذه الدراسة الي تحسين النشاط المبيضي ومعدلات الخصوبة ووزن المواليد باستخدام هرمون الافراس العشار والارجنين المحمي في النعاج المصرية، ولتحقيق هذا الهدف تم استخدام ستة وثلاثون نعجة صحيحة سريرياً تم تقسيمها الي ثلاثة مجاميع متساوية في العدد (١٢ نعجة لكل مجموعة). اعتبرت المجموعة (١) كمجموعة ضابطة والمجموعة (٢) تم زرع الإسفنجات المهبلية المشربة بهرمون البروجستيرون لمدة ١٤ يوم وعند إزالة الإسفنجات تم الحقن ب ٤٠٠ وحدة دولية من هرمون الأفراس العشار. و المجموعة (٣) زرعت الإسفنجات المهبلية المشربة بهرمون البروجستيرون لمدة ١٤ يوم خلال هذه الأيام تم تجريعها بالأرجينين المحمي (٢٠ مللجرام/كجم وزن حي). تم فحص النشاط المبيضي باستخدام جهاز الموجات الصوتية، وجمعت عينات الدم بعد ٢٤، ٤٨، ٧٢ ساعة بعد سحب الاسفنجات المهبلية، ومرة واحدة شهريا خلال اشهر الحمل لقياس مستوي هرمون البروجيستيرون وبعض مقاييس الدم البيوكيميائية (الجلكوز، اليوريا، وانزيمات الكبد).أظهرت النتائج تغيرات معنوية ($P <0.05$) في قطر الحويصلات المبيضية الكبيرة (التي يذيد قطرها عن ٣ ملليمتر) على المبيض الأيمن والأيسر بعد ٢٤ ساعة من العلاج بين المجموعة (٢، ٣) مقانة بمجموعة (١). وبعد ٧٢ ساعة من العلاج ، كان لدى مجموعة ARG قطر أكبر في الحويصلات المبيضية في المبيض الأيمن ($P <0.01$) من المجموعات الأخرى، وكان قطر الحويصلات المبيضية التي يزيد قطرها عن ٣ ملليمتر و CL في مجموعة ARG في المبيض الأيسر أكبر بشكل ملحوظ ($P <0.05$) من الحيوانات التي في المجموعات الأخري. وعند مقارنة مجموعتي العلاج والضابطة كانت هناك فروق ذات دلالة إحصائية ($P <0.05$) في استجابة الشبق ، والخصوبة ، ومعدل الحمل والولادة.علاوة علي ذلك، بعد اليومين السابع والخامس عشر من الحمل ، كان لدى مجموعة ARG زيادة معنوية ($P <0.01$) في وزن الحملان المولودة عن المجموعة الضابطة. وكانت تركيزات الجلوكوز واليوريا وانزيمات الكبد أكبر في المجموعات المعالجة عنها في مجموعات التحكم. في الختام، يمكن تحسين النشاط المبيضي ومعدلات الخصوبة ووزن المواليد باستخدام هرمون الافراس العشار والارجنين المحمي في الاغنام تحت ظروف صعيد مصر.