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# Performance and Puberty of Ram Lambs Produced From Ewes Treated with Arginine

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## ABSTRACT

Aim of this study was to investigate performance and puberty of ram lambs produced from pregnant ewes treated with different levels of arginine. This study was carried out at Sakha Animal Production Research Station, belonging to the Animal Production Research Institute in a cooperation with Animal Production Department, Faculty of Agriculture, Mansoura University. During November 2016, total of 45 Ossimi ewes (September mating season), 3-4 years of age and 39.59±0.15 kg live body weight (LBW ) were divided into three groups (15 head/each). Ewes in the 1st group (G1) served as a control, ewes in the 2nd (G2) and 3rd (G3) groups were weekly treated with oral dose of 20 and 30 mg arginine (AR), respectively, for the duration of the treatment from the second month of pregnancy up to lambing. After lambing, total of 8 ram lambs produced from each group were taken and allotted in three groups similar to treatment of their dams. LBW of ram lambs was recorded at birth and monthly up to 12 month of age. In blood plasma of ram lambs, concentration of IgG and IgM at 2 days and 1, 3 and 4 wk of age, while concentration of total proteins**,** albumin, creatinine and glucose, and activity of aspartate (AST) and alanine (ALT) aminotransferase at 2, 4, 6 and 8 month of age were determined. Age and plasma testosterone concentration (PTC) of each ram lambs were determined at three pubertal stages, while semen of 1st ejaculates (at puberty) was evaluated. Results showed that ram lamb LBW was higher (P<0.05) only in G3 than G1 at birth and at 5 to 12 month of age. Final ram lamb LBW at 12 month of age was higher by 10.2 and 15.9% in G2 and G3 than in G1, respectively. Plasma concentration of Immunoglobulin G (IgG) and Immunoglobulin M (IgM) after 2 days, 1, 3, and 4 wk of lambing was highest (P<0.05) in G3, followed by G2, while, G1 showed the lowest values. Plasma concentration of total proteins at 4 month of age and of albumin at 4 and 6 month of age increased (P<0.05), while glucose decreased (P<0.05) at 2 and 8 month of age in G2 and G3 as compared to those of G1. Creatinine increased (P<0.05) only in G3 as compared to G2 and G1 at 6 and 8 month of age, while activity of AST and ALT was not affected at all ages. At puberty, age of ram lambs was earlier (P<0.05) by 51.4 and 33.0 days and PTC was higher (P<0.05) by 22.5 and 18.8% in G2 and G3 than in G1. Ram lambs in G3 showed the best (P<0.05) semen characteristics of the 1st ejaculation (volume, sperm motility and live sperm output / ejaculate), followed by G2. PTC was higher (P<0.05) in G2 and G3 than in G1 at 6 and 8 month of age, showing significantly (P<0.05) an opposite trend at 7 month of age, while insignificantly different at 5, 9 and 10 month of age. In conclusion, ram lambs of ewes treated with weekly oral dose of L-arginine-HCL (30 mg / ewe) from the second month of pregnancy up to lambing achieved early age and higher weight at puberty.

**Keywords**: Ram lambs, arginine, puberty, semen, testosterone.

## INTRODUCTION

Quality of nutrition is one of the important reasons that have a direct impact on the productivity of farm animals. In this way, there are some nutrients playing an important role in regulating the growth and immunity (Al-Dabbas *et al.,* 2008). Supplementation with amino acids (e.g. glutamine, arginine, and N-acetyl-cysteine) improves oxidative defense (Wu *et al.*, 2004) and immune function (Wu and Meininger, 2002) in animals. L-arginine (L-AR) is a nutritionally essential amino acid for several reproductive processes including spermatogenesis in males, and embryonic survival, fetal development and maintenance of vascular tone and hemodynamic (Morris, 2007; Rhoads *et al.*, 2008 and Yao *et al.*, 2008).

Supplementation of L-AR as dietary or intravenous administration has vital role in improvement of reproductive, cardiovascular, pulmonary, kidney, gastrointestinal and liver functions, and immune-response (Wu *et al.*, 2009). L-AR was found to enhance implantation and development of embryos in sheep (Lassala *et al.*, 2011). It has promoting role on physiological activity in the animal body and on the secretion of growth hormone and prolactin and insulin (Li *et al.*, 2007). It serves as a precursor to nitric oxide (NO) and polyamines (Kwon, 2003). The nitric oxide (NO) produced from L-AR plays an important role in vasodilatation by preventing the adhesion of blood cells and platelets along the endothelial cell layer of blood vessels and inhibiting vascular smooth muscle cell proliferation (Ignarro and Napoli, 2004). So, NO increases blood flow to most organs including those in the reproductive system which increased the transport of metabolites and hormones to organs (Vonnahme *et al.,* 2005). Also, NO has the potential to affect ovarian function (Wu *et al.*, 2013).

In females during gestation period, pregnant animals need more nutrients for growth and development of fetuses. In sheep, AR supplementation during late pregnancy increased lamb birth weight (de Boo *et al.* 2005 and Zeitoun *et al.*, 2016), survival rate (Lassala *et al.*, 2011 and Zeitoun *et al.*, 2016), improved maternal health (Zeitoun *et al.*, 2016). Similarly, AR supplementation increased the number and litter weight of live-born piglets by 22 and 24%, respectively (Mateo *et al.* 2007).

The available information about the effect of AR on puberty or reproductive performance of males is rare. The objective of the current study was to study performance and puberty of ram lambs produced from pregnant ewes treated with different levels of arginine.

## MATERIALS AND METHODS

This study was carried out at Sakha Animal Production Research Station, belonging to the Animal Production Research Institute (APRI) in a cooperation with Animal Production Department, Faculty of Agriculture, Mansoura University, during the period from November 2016 to January, 2018.

**Ewes and treatment:**

During November 2016, total of 45 Ossimi ewes (September season), having 3-4 years of age and averaged 39.59±0.15 kg live body weight (LBW) were divided into three groups (15 head/each) according to their age and live

body weight were used in this study. Ewes in the 1st group (G1) served as a control group without treatment. However, ewes in the 2nd (G2) and 3rd (G3) groups were weekly treated with oral dose of 20 and 30 mg arginine, respectively. Ewes were kept under similar management conditions and housed in collective pens. Water and mineral salt were permanently available.

L-arginine-HCl (L-AR-HCl) was purchased from Nutrients Scientific (Diamond Bar, CA, USA) in crystalline powder with 99.6% purity. Capsules (ARGIPOWER™ 1500 MEGA Caps® MEGA) containing 1500 mg / capsule of highest-quality pharmaceutical L-AR-HCL. Content of each capsule was dissolved in 750 ml distilled water (2 mg/ml) and stirred, then each ewe in G2 and G3 was weekly treated with oral dose of 10 and 15 ml containing 20 and 30 mg L-AR-HCL / ewe, respectively according to Luther *et al.* (2008) and Saevre *et al.* (2011). Each of AR-treated animals was given an oral dose of the designed formula for the duration of the treatment from second month of pregnancy up to lambing.

**Experimental lambs groups:**

After lambing, total of 8 ram lambs produced from each control or treated ewes group were taken and allotted in three groups similar to treatment of their dams. Lambs of each group were weighed at birth and monthly during the experimental period from birth up to 12 month of age.

**Reproductive performance of lambs:**

Ram lambs in each group were subjected to observation to detect changes in sexual behavior, once at 10 day-interval during the period from 5 month of age till the onset of puberty (first successful ejaculate with motile sperm). To ensure the availability of at least two ewes in estrus at each time of libido test. Ewes were subjected to estrus synchronization by intramuscular injection of 25 mg progesterone (Lutone, Misr Co., for Pharm Ind. SAA, Cairo) for five successive days followed by a signal injection of 5 mg estradiol benzonate (Follone, Misr Co, for Pharm Ind. SAA, Cairo) 24 hours after the last progesterone injection. Treated ewes were subjected to estrus detection 24-48 hours after last hormonal injection using intact ram. Treatment for estrus synchronization was planned at a time suitable for the time of libido test. Age, body weight and plasma testosterone concentration of each ram lamb were determined at 1st mounting, mounting with erection (1st penile protrusion) and puberty.

**Semen evaluation:**

After the occurrence of puberty (ejaculation), semen was collected by means of an artificial vagina biweekly until 12 months of age. Before ejaculate, ram lambs were sexually stimulated by allowing two false mounts followed by 5 minutes restrain. In the 1st ejaculate, semen volume and initial gross motility of sperm cells (without extension) was estimated on a percentage score basis as described by Melrose and Loing (1970). Also, percentages of live, dead and abnormal spermatozoa as well as sperm cell concentration (Neubauer Haemocytometer) were determined in semen. Sperm output per ejaculate was calculated by multiplying sperm cell concentration by ejaculate volume.

**Blood sampling of ewes and their lambs:**

Concentration of immunoglobulines (IgG and IgM) was determined in blood plasma of ram lambs at 2 days and 1, 3 and 4 wk of age by ELISA (enzyme-linked immune sorbent assay) technique of the Immuno Tech-Beckman Coulter Company according to Mancini *et al.* (1965). At 2, 4, 6 and 8 month of age, blood samples were taken from ram lambs of each group for determination of total proteins (Henry, 1964), albumin (Hill and Wells, 1983) and creatinine (King, 1951) concentrations. Glucose concentration was estimated according to the colorimetric method of Sacks (2008). Also, activity of aspartate (AST) and alanine (ALT) aminotransferase were determined spectrophotometrically in blood plasma, on a Helios gamma UV visible spectrophotometer, Thermo spectronic UK. Blood plasma testosterone was quantified by the use of a commercial ELISA kit (HUMAN, Germany) according to Joyce *et al.* (1977). Intra-assay coefficient of variation was 6.3%.

**Statistical analysis:**

Data obtained from this study were statistically analyzed using a software package (SAS, 2004) by one way ANOVA using GLM procedures. Duncan's Multiple Range Test were set at $P<0.05$ to determine the significant difference according to Duncan (1955). The percentage values were subjected to arcsine transformation before performing the analysis of variances. Means were presented after being recalculated from the transformed value to percentages. Data were expressed as mean ± standard error.

## RESULTS AND DISCUSSION

**Live body weight of ram lambs:**

Live body weight (LBW) of ram lambs significantly ($P<0.05$) increased in G3 as compared to G1 at birth and at 5 to 12 month of age, but LBW of G2 did not different from that in G1 or G3. However at 1 to 4 month of age, the differences in LBW of lambs among the experimental groups were not significant. It is of interest to note that the effect of AR is dose-dependent, being more pronounced with AR at 20 than 30 mg/ewe. Generally final LBW of ram lambs in G2 and G3 was higher by about 10.2 and 15.9% than that of the control group (G1), respectively (Table 1).

**Table 1. Effect of arginine treatment of ewes on live body weight (kg) of their ram lams from birth to 12 months of age.**

| Live body weight | G1 | G2 | G3 |
|---|---|---|---|
| At birth | 2.99±0.27$^{b}$ | 3.56±0.12$^{ab}$ | 4.02±0.22$^{a}$ |
| 1 month | 4.06±0.63 | 5.54±0.54 | 5.42±0.59 |
| 2 month | 8.94±0.43$^{b}$ | 7.32±0.94 | 9.38±0.52 |
| 3 month | 12.80±0.38 | 13.5±1.03 | 14.40±0.54 |
| 4 month | 14.20±0.51 | 15.20±0.98 | 17.15±0.50 |
| 5 month | 17.00±0.66$^{b}$ | 17.70±1.38$^{b}$ | 19.80±0.59$^{a}$ |
| 6 month | 18.50±1.03$^{b}$ | 20.70±1.40$^{ab}$ | 22.30±0.77$^{a}$ |
| 7 month | 22.30±1.09$^{b}$ | 24.20±1.53$^{ab}$ | 27.10±0.91$^{a}$ |
| 8 month | 24.40±1.04$^{b}$ | 27.60±2.05$^{ab}$ | 30.10±0.84$^{a}$ |
| 9 month | 26.10±0.94$^{b}$ | 30.20±1.83$^{ab}$ | 34.90±1.49$^{a}$ |
| 10 month | 28.80±1.21$^{b}$ | 33.50±1.83$^{ab}$ | 36.00±1.43$^{a}$ |
| 11 month | 30.30±1.13$^{b}$ | 35.40±1.64$^{ab}$ | 38.60±1.43$^{a}$ |
| 12 month | 33.20±1.12$^{b}$ | 36.80±1.57$^{ab}$ | 40.80±1.32$^{a}$ |

**a, and b: Means denoted within the same row with different superscripts are significantly different at $P<0.05$.**

In accordance with the present results, Zeitoun *et al.* (2016) found that lamb birth weight of Najdi pregnant ewes treated with L-AR (75 mg/kg/head/day) at first 56 d of pregnancy increased as compared to those treated with high L-AR level (150 mg/kg/head/day). Treatment of ewes with AR from day 100-121 of pregnancy increased birth weights of lambs born from mothers carrying multiple fetuses by about 23% (Lassala *et al.* 2011). In pig, dietary supplementation (0.83 % AR) between 90 and 114 days of pregnancy increased average LBW of live-born piglets at birth by 16% (Wu *et al.,* 2012).

Uterine capacity is a major factor limiting fetal survival and growth in sheep as demonstrated by the inverse relationship between fetal number and birth weight (Gootwine *et al.*, 2007). Also, placental size, function and utero-placental transfer of materials between dam and fetus were the major factors to affect growth and development of the fetus (Gude *et al.,* 2004 and Wu *et al.,* 2006, 2010). A crucial factor for survival, growth, and development of fetus is a functional placenta for transporting nutrients, respiratory gases, and metabolism products between the maternal and fetal circulations (Wang *et al.* 2012). AR is a nutritionally essential amino acid for the fetus and is a precursor for the synthesis of nitric oxide and polyamines, which are essential for placental growth and function, and growth of new vessels from the existing vasculature and, therefore, for increasing uterine and placental–fetal blood flow (Wu *et al.*, 2004 and 2009; Lassala *et al.*, 2010 and 2011 and McCoard *et al.*, 2013). Polyamines play important roles in placental health and development by regulating angiogenesis or new blood vessels formation (Kwon, 2003). These physiological processes are critical for growth and development of fetuses (Wu *et al.*, 2004 and 2008). In addition, AR treatment during pregnancy could result in changes in hormonal secretions, which may affect fetal and maternal metabolism (Chew *et al.,* 1984 and Kensinger *et al.,* 1986).

**Immuno response:**

***Blood immunoglobulines of ram lambs:***

Ram lambs in G3 showed significantly (P<0.05) the highest immune response, in terms of the highest plasma concentration of immunoglobulines (IgG and IgM) at all studied ages (after 2 day, 1, 3, and 4 wk of lambing), followed by those of G2, while, control ram lambs (G1) showed significantly (P<0.05) the lowest IgG and IgM concentrations at all ages. It is of interest to note that IgG and IgM concentrations in all groups showed the maximum levels on day 2 post-lambing, and then markedly decreased by advancing lamb age, particularly after one week of age (Table 2).

**Table 2. Effect of arginine treatment of ewes on IgG and IgM concentration in blood plasma of their ram lambs at different ages (week).**

| Age | G1 (control) | G2 | G3 |
|---|---|---|---|
| **Concentration of IgG (mg/dl) in blood plasma:** | | | |
| 0 wk | 52.16±1.19$^{b}$ | 52.57±0.87$^{b}$ | 57.77±0.77$^{a}$ |
| 1 wk | 42.64±1.34$^{c}$ | 48.90±1.87$^{b}$ | 57.36±0.95$^{a}$ |
| 3 wk | 28.79±1.27$^{b}$ | 31.74±0.87$^{ab}$ | 33.77±1.38$^{a}$ |
| 4 wk | 23.78±0.97$^{b}$ | 28.81±1.22$^{a}$ | 29.28±1.13$^{a}$ |
| **Concentration of IgM (mg/dl) in blood plasma:** | | | |
| 0 wk | 1.83±0.16$^{b}$ | 2.51±0.23$^{a}$ | 2.60±0.08$^{a}$ |
| 1 wk | 1.16±0.03$^{b}$ | 1.62±0.02$^{b}$ | 2.68±0.17$^{a}$ |
| 3 wk | 1.13±0.05$^{b}$ | 1.54±0.03$^{a}$ | 1.61±0.13$^{a}$ |
| 4 wk | 1.04±0.06$^{b}$ | 1.61±0.03$^{a}$ | 1.60±0.09$^{a}$ |

**a, b and c: Means denoted within the same row with different superscripts are significantly different at P<0.05.**
**0 wk: 2 days after lambing.**

According to Barta (1993), concentration of IgG in blood serum at birth steeply increases in lambs after colostrum intake until the second day to the maximum value, and then it decreases until 16 days of age. This decrease continues until the minimum value at 60 days of age. In the subsequent period until 180 days of age, a recurrent increase occurred, but at value does not reach the level of adult individuals. The syndesmochorial placenta of ruminants does not allow the transfer of high-molecular-weight proteins. Ruminants suffer from a gammaglobulinaemia immediately after birth before the first intake of colostrum (Aldridge *et al.*, 1992). This is in accordance with the present results during 2 weeks post-lambing. On the other hand, Maden *et al.* (2003) observed that IgG concentration in the blood serum dropped on day 1 post partum as compared to at parturition, then increased again on day 15 post partum.

**Blood parameters:**

**Blood biochemicals and enzyme activity:**

Ram lambs of treatment ewe groups (G2 and G3) significantly (P<0.05) increased only plasma concentration of total proteins at 4 month of age and of albumin at 4 and 6 month of age, while significantly (P<0.05) decreased plasma glucose concentration at 2 and 8 month of age as compared to those of G1. Only ram lambs of G3 significantly (P<0.05) increased plasma creatinine concentration at 6 and 8 month of age. However, treatment had insignificant effect on plasma enzyme activity (AST and ALT) at all ages studied (Table 3).

Concentrations of blood total proteins and globulin can be used as an indicator for monitoring passive transfer and estimating the amount of circulating IgG in newborns. In addition, total proteins concentration in blood and colostrum are important for lamb growth (O'Brien and Sherman, 1993). Increasing albumin level, which represents about 35 and 50% of total proteins (Kaneko, 1997), and subsequently increasing total proteins level in ram lambs of treatment groups (G2 and G3), significantly at 4 and 6 month of age and insignificantly at other ages, indicated high liver function and good healthy status of these lambs. These results could be in consistent with the 1$^{st}$ intake of colostrum immediately after lambing during

the first hours of life of lambs (Piccione *et al.*, 2009). This finding indicated the tendency of high concentration of total proteins and albumin in blood plasma of lambs of treatment groups (G2 and G3) as a result of increasing plasma immunoglobulin levels (Baranowski *et al.*, 2000). Piccione *et al.* (2012) observed that the total blood proteins levels were significantly affected by the physiological period and increased during Lactation. This may reflect the maternal needs of proteins for milk production and providing immunoglobulin (Bell *et al.,* 2000; Mohri *et al.,* 2007).

In sheep, entry rate of glucose was reported to positively correlate with energy supply level (Landau, 1994). At late pregnancy, Landau (1994) found positive correlation between lamb birth weight and glucose entry rate of their mothers. Also, availability of colostrum to new-born lambs was affected by glucose entry rate (Barry and Manley, 1985). These finding may reflect the highest LBW of lambs in treatment groups as compared to control group.

Creatinine level is considered as an indicator of glomerular filtration rat in the kidney. The present creatinine levels of lambs in all groups are within the normal range (Keenan and Allardyce, 1986), indicating normal kidney function of ram lambs in treatment and control groups. It is of interest to note that to note that increasing creatinine level in lambs of G3 was attributed to higher protein utilization of lambs in this group, reflecting the highest live body weight from 5 up to 12 month of age (Table 1).

Activity AST has some relations with liver and heart functions in cows (Kaneko *et al.,* 2008), and it is one of the common indicators of liver failure (Sattler and Fürll, 2004). However, ALT activity is important for protein catabolism by catalyzing alanine and alpha ketoglutarate and transfer them into generates pyruvate and glutamate (Ray *et al.,* 2008). The obtained insignificant differences among the experimental groups at all ages studied in plasma aminotransferases activities, being within the normal ranges, may reflect intact structure of body animal cells and normal liver function (Milinković-Tur *et al.,* 2005).

**Table 3. Effect of arginine treatment on blood plasma biochemicals and enzyme activity in blood serum of ram lambs at different ages (wk).**

| Item | G1 (control) | G2 | G3 |
|---|---|---|---|
| Total proteins (g/dl): | | | |
| 2 mo of age | 6.99±0.71 | 7.68±1.21 | 7.74±1.22 |
| 4 mo of age | 6.50±0.47$^{b}$ | 7.14±0.14$^{a}$ | 7.20±0.25$^{a}$ |
| 6 mo of age | 6.20±0.41 | 6.64±0.61 | 7.15±0.42 |
| 8 mo of age | 5.93±1.57$^{b}$ | 6.68±0.31$^{ab}$ | 6.65±0.57$^{a}$ |
| Albumin (g/dl): | | | |
| 2 mo of age | 3.60±0.42$^{b}$ | 4.22±0.10$^{a}$ | 4.41±0.36$^{a}$ |
| 4 mo of age | 3.10±0.47$^{b}$ | 4.16±0.26$^{a}$ | 4.22±0.08$^{a}$ |
| 6 mo of age | 3.02±0.51$^{b}$ | 4.07±0.57$^{a}$ | 3.92±0.50$^{a}$ |
| 8 mo of age | 3.92±0.44 | 3.83±0.73 | 3.80±0.42 |
| Glucose (mg/dl): | | | |
| 2 mo of age | 105.33±5.23$^{a}$ | 86.88±5.76$^{b}$ | 85.88±4.38$^{b}$ |
| 4 mo of age | 94.44±0.29$^{a}$ | 85.77±10.03$^{b}$ | 84.11±5.42$^{b}$ |
| 6 mo of age | 92.77±3.89$^{c}$ | 99.33±11.75$^{b}$ | 103.8±5.89$^{a}$ |
| 8 mo of age | 127.7±4.19$^{a}$ | 111.3±3.89$^{b}$ | 110.8±2.74$^{b}$ |
| Creatinine (mg/dl): | | | |
| 2 mo of age | 0.47±0.04 | 0.35±0.15 | 0.56±0.04 |
| 4 mo of age | 0.91±0.20 | 0.99±0.10 | 0.99±0.09 |
| 6 mo of age | 0.94±0.17$^{b}$ | 0.82±0.00$^{b}$ | 1.21±0.24$^{a}$ |
| 8 mo of age | 0.84±0.05$^{b}$ | 0.94±0.09$^{ab}$ | 1.10±0.08$^{a}$ |
| AST (U/l): | | | |
| 2 mo of age | 70.86±5.12$^{a}$ | 62.06±5.22$^{b}$ | 58.00±0.74$^{b}$ |
| 4 mo of age | 59.56±1.26$^{c}$ | 72.50±2.49$^{a}$ | 68.30±2.53$^{b}$ |
| 6 mo of age | 66.83±3.40$^{a}$ | 57.40±3.78$^{b}$ | 60.83±2.53$^{b}$ |
| 8 mo of age | 70.10±3.14$^{b}$ | 64.80±3.90$^{c}$ | 84.10±3.63$^{a}$ |
| ALT (U/l): | | | |
| 2 mo of age | 17.50±0.50$^{ab}$ | 18.50±2.38$^{a}$ | 15.00±1.50$^{b}$ |
| 4 mo of age | 19.50±3.96 | 17.00±3.27 | 16.50±5.26 |
| 6 mo of age | 17.00±1.32 | 17.00±3.50 | 16.00±4.27 |
| 8 mo of age | 25.93±2.71 | 26.00±2.00 | 23.00±0.50 |

**a, b and c: Means denoted within the same row with different superscripts are significantly different at P<0.05.**

**Puberty stages:**

Reproductive performance of ram lambs in the experimental groups, in terms of age and plasma testosterone concentration was studied and compared at three main pubertal stages (Table 4). At the 1$^{st}$ stage (1$^{st}$ mounting), age of ram lambs was significantly (P<0.05) the earliest in G2 and the latest in G3, but did not differ in G3 from that in G1 and G3, but the differences in plasma testosterone concentration were not significant among the experimental groups.

At the 2$^{nd}$ stage (1$^{st}$ mounting with erection), age was significantly (P<0.05) earlier and plasma testosterone concentration was higher in G2 and G3 than in G1, being the earliest and highest in ram lambs in G2 (Table 4).

The same trend was observed at the 3$^{rd}$ stage (1$^{st}$ ejaculation, puberty), whereas age of ram lambs was

significantly (P<0.05) earlier by 51.4 and 33.0 days and plasma testosterone concentration was significantly (P<0.05) higher by 22.5 and 18.8% in G2 and G3 than in G1 (control). It is worthy noting that testosterone level showed marked development in all groups by advancing pubertal stage, particularly from the 2nd stage to 3rd stage (puberty, Table 4).

**Table 4. Effect of arginine treatment of ewes on age and plasma testosterone concentration of their ram lambs at different pubertal stages.**

| Pubertal stage | Item | G1 (control) | G2 | G3 |
|---|---|---|---|---|
| | Age (day) | 172.4±2.23[a] | 150.3±1.13[b] | 162.1±1.06[ab] |
| 1st mounting | LBW (kg) | 18.43 | 17.70 | 19.80 |
| | Testosterone (ng/ml) | 1.15±0.06 | 1.13±0.03 | 1.03±0.02 |
| 1st mounting with erection | Age (day) | 224.8±1.98[a] | 182.3±0.98[b] | 191.4±1.22[b] |
| | LBW (kg) | 23.50 | 20.60 | 22.40 |
| | Testosterone (ng/ml) | 1.53±0.01[b] | 2.15±0.03[a] | 2.21±0.04[a] |
| 1st ejaculation (puberty) | Age (day) | 274.8±1.15[a] | 223.4±1.03[b] | 241.8±1.11[b] |
| | LBW (kg) | 26.10 | 24.80 | 29.89 |
| | Testosterone (ng/ml) | 2.71±0.02[b] | 3.32±0.04[a] | 3.22±0.01[a] |

**a and b: Means denoted within the same row with different superscripts are significantly different at P<0.05.**

In agreement with the obtained results, feed ration supplemented with 0.2 gm protected AR / kg body weight to pre-pubertal Hebsi male goat kids resulted in earlier age at puberty than in controls, probably through the indirect effect of AR on testosterone secretion and consequently spermatogenesis (Basiouni, 2010).

**Physical semen characteristics of the 1st ejaculation:**

Ram lambs in G3 showed significantly (P<0.05) the best semen characteristics of the 1st ejaculation, in terms of the highest ejaculate volume, sperm motility percentage and live sperm output per ejaculate. However, ram lambs in G2 showed significantly (P<0.05) higher ejaculate volume only than in G1, while live sperm output/ejaculate did not differ from that in G3 and G1. On the other hand, the differences in percentage of live and abnormal sperm cells, and sperm cell concentration were not significant among the experimental groups (Table 5).

It is expected that these starting values will increase and reach normally by the advance of age and consequently the complete development of the sex organs. It is believed that any comparison at such early stage might be meaningless due to the quick change in the values of the characters and the variability of body weight between tested groups at puberty. However, the value of first ejaculate in this study was used as a sign to indicate that animals reached puberty.

**Table 5. Effect of arginine treatment on semen characteristics of ram lambs in the experimental groups.**

| Pubertal stage | G1 (control) | G2 | G3 |
|---|---|---|---|
| Ejaculate volume (ml) | 0.21±0.01[b] | 0.32±0.01[a] | 0.36±0.02[a] |
| Sperm motility (%) | 45.5±2.15[b] | 45.4±1.98[b] | 50.6±1.66[a] |
| Live sperm (%) | 41.9±1.89 | 41.8±1.46 | 44.6±1.23 |
| Abnormal sperm (%) | 18.1±1.78 | 16.7±1.13 | 17.3±1.02 |
| Sperm concentration ($x10^9$/ml) | 1.01±0.06 | 1.15±0.04 | 1.07±0.04 |
| Live normal sperm output ($x10^9$/ejec.) | 0.404±0.03[b] | 0.689±0.06[ab] | 0.869±0.04[a] |

**a, and b: Means denoted within the same row with different superscripts are significantly different at P<0.05.**

The results obtained concerning the semen quality at first ejaculate (puberty) are in agreement with the results of El-Shamaa (2002), who found that semen quality at puberty were low and reach normality by the advance of age and consequently the complete development of the sex organs. In this respect, several authors reached to marked improvement in ejaculate volume with age progress (El-Saidy *et al.*, 2004).

Despite the observed reduction in ejaculate volume, Rege *et al.* (2000) reported a gradual improvement in ejaculate volume of ram lambs by advancing age. The earlier ejaculation with the highest ejaculate volume of ram lambs in treatment groups (G2 and G3) may indicate the beneficial effects of ewe treatment with AR on growth performance and consequently on the accessory sex glands to produce large volume of the seminal plasma and/or attributed to the significantly (P<0.05) increase in testosterone level in blood plasma of ram lambs. The accessory glands are functionally controlled by testosterone (Martin *et al.*, 1994).

The observed improvement in sperm motility of ram lambs at puberty in G3 as compared to that in G2 or G1 was reported by Hassanpour *et al.* (2007), found that the effects of L-AR on sperm motility are dose-dependent. Some authors recorded a correlation between AR deficiency and decrease in sperm motility (Jungling and Bunge, 1976; Polakoski *et al.*, 1976). L-AR plays a key role in modulating the host's defense and cellular immunity. L-AR administration resulted in an improvement in sperm motility of oligospermic and asthenospermic patients without any side effects (Aydin *et al.*; 1995). Also, AR plays a vital role in the maintenance of sperm motility and sperm metabolism within the reproductive tract or throughout storage under *in vitro* conditions (Mann and Lutwak-Mann, 1981). Moreover, AR stimulates *in vitro* sperm motility in different species (Patel *et al.*, 1998; Srivastava *et al.*, 2006). Generally, the reported increase in sperm motility percentage at mature than at puberty age was attributed to contentious development in testicular and epididymal tissues (Perez-Claring *et al.,* 1998).

L-AR plays a key role in modulating the host's defense and cellular immunity. In this line, Aydin *et al.* (1995) observed that sperm count increased in oligospermic and asthenospermic patients treated with L-AR. Generally, AR takes part in spermatogenesis and

considered as a basic component of the nucleoprotein of sperm cells in several animal species (Adnan, 1970). Also, AR prevents bi-layer phospholipids membrane peroxidation under various peroxidation situations through production of NO to protect integrity of structure and function of sperm cells (Srivastava *et al.*, 2006).

**Blood plasma testosterone concentration (ng/ml):**

Results illustrated in figure (1) revealed that testosterone concentration of ram lambs was significantly (P<0.05) higher in G2 and G3 than in G1 at 6 and 8 month of age, showing significantly (P<0.05) an opposite trend at 7 month of age, while insignificantly different at 5, 9 and 10 month of age. These trends cleared testosterone spikes prior to puberty during the 6th month in treatment groups and during the 7th month in control one, being earlier in treatments than in control group.

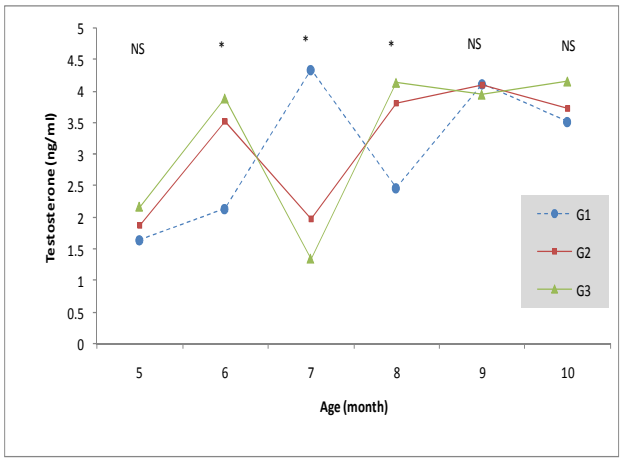


**Fig. 1. Change in plasma testosterone concentration (ng/ml) of ram lambs in different experimental groups at pre- and post-pubertal ages.**

In accordance with the present results, Basiouni (2010) found that testosterone concentration significantly increased in male goat kids fed ration containing 0.2 gm protected AR / kg body weight/day as they approach the age of puberty compared to control (3.15 vs. 1.10 ng/ml). Puberty is associated with a marked increase in testosterone concentration (Chakraborty *et al.*, 1989), which affect the development of the male accessory sex glands, sexual behavior stimulation and spermatogenesis (Hafez and Hafez, 2000). It is well known that testosterone is under the indirect regulation of the anterior pituitary gland LH secretion (Bearden and Fuquay, 1992) and AR treatment in pre-pubertal female sheep, goats and hens was found to increase the rate of LH secretion (Basiouni, 2009). Finally, AR was found to stimulate the release of GH hormone and insulin-like growth factor (Davenport *et al.*, 1995), GnRH and LH (Basiouni, 2009).

## CONCLUSION

In conclusion, weekly treatment of ewes with L-arginine-HCL at a level of 30 mg in 15 ml distilled water as an oral dose during the period from second month of pregnancy up to lambing showed impact on their ram lambs regarding incidence of puberty at early age with appropriate live body weight, sexual desire, and semen characteristics, which may be beneficial for raising breeding rams for natural mating and artificial insemination in sheep farms.

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## الاداء والبلوغ الجنسى لذكور الحملان الناتجة من نعاج عوملت بالارجنين

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يهدف هذا البحث الى دراسة الاداء والبلوغ الجنسى لذكور لحملان الناتجة من نعاج عشار تم معاملتها بمستويات مختلفة من الارجنين . اجريت هذه الدراسة بمحطة بحوث الانتاج الحيوانى بسخا التابعة لمعهد بحوث الانتاج الحيوانى بالتعاون مع قسم انتاج الحيوان ، كلية الزراعة ، جامعة المنصورة. اجريت الدراسة على ٤٥ نعجة اوسيمى خلال شهر نوفمبر ٢٠١٦ بعد موسم تلقيح سبتمبر عمرها ٣-٤ سنوات ووزن جسم حى ٣٩.٥٩±٠.١٥ كجم وقسمت الى ثلاث مجموعات (١٥ رأس / مجموعة). المجموعة الاولى بدون معاملة (كنترول) ، وعوملت نعاج المجموعة الثانية والثالثة اسبوعيا بالتجريع بـ ٢٠ أو ٣٠ مجم ارجنين على التوالى فى الفترة من الشهر الثانى من الحمل حتى الولادة. بعد الولادة اخذ ٨ ذكور حملان من كل مجموعة معاملة وقسمت تبعا لنفس معاملة امهاتها . تم تقدير الوزن عند الميلاد وشهريا حتى ١٢ شهر من العمر. تم اخذ بلازما من دم ذكور الحملان لتقدير IgG, IgM بعد يومين من الولادة ، ١ ، ٣ ، ٤ اسابيع من العمر وتم تقدير البروتين الكلى والالبومين والكرياتنين والجلوكوز ونشاط انزيمى AST, ALT عند ٣ ، ٤ ، ٦ ، ٨ شهور من العمر . تم تقدير العمر وتركيز هرمون التستسيرون فى ذكور الحملان عند الثلاث مراحل للبلوغ وعند الحصول على قذفة سائل منوى (البلوغ) . اوضحت النتائج ان وزن الجسم الحى لذكور الحملان كان اعلى ومعنويا فقط بين المجموعة الثالثة عن المجموعة الاولى عند الميلاد وعند ٥ ، ١٢ شهر من العمر . وكان وزن الجسم الحى عند ١٢ شهر اعلى بنسبة ١٠.٢% و١٥.٩% فى المجموعة الثانية والثالثة عن المجموعة الاولى على الترتيب. كان تركيز IgG, IgM بعد يومين من الولادة ، ١ ، ٣ ، ٤ اسبوع من الولادة عاليا معنويا فى المجموعة الثالثة متبوعا بالمجموعة الثانية بينما كانت اقل القيم فى المجموعة الثالثة. زاد تركيز البروتين الكلى فى بلازما الدم عند ٤ شهور ، الالبومين عند ٤ ، ٦ من العمر بينما تناقص تركيز الجلوكوز عند ٢ ، ٨ شهور من العمر فى المجموعتين الثانية والثالثة مقارنة بالمجموعة الاولى. زاد تركيز الكرياتنين فقط فى المجموعة الثالثة مقارنة بالثانية والاولى عند ٦ ، ٨ شهور من العمر بينما لم يختلف تركيز انزيمى ALT, AST عند مراحل العمر المختلفة. تم الوصول الى البلوغ الجنسى لذكور الحملان مبكرا معنويا بـ ٥١.٤ ، ٣٣ يوم وكان تركيز هرمون التستسيرون عاليا معنويا بـ ٢٢.٥% و١٨.٨% فى المجموعة الثانية والثالثة عن المجموعة الاولى. ذكور حملان المجموعة الثالثة اظهرت افضل خصائص للسائل المنوى عند اول قذفة سائل منوى (الحجم ، الحيوية ، الحيوانات المنوية الحية / قذفة) يليها ذكور حملان المجموعة الثانية. تركيز هرمون التستستيرون كان مرتفعا فى المجموعة الثانية والثالثة عن المجموعة الاولى عند ٦ ، ٨ شهور من العمر بينما كان العكس عند عمر ٧ شهور بينما الفروق لم تكن معنوية عند ٥ ، ٩ و١٠ شهور من العمر.

تخلص هذه الدراسة الى ان ذكور الحملان المولودة من نعاج أوسيمي عوملت بالارجنين في الشهر الثاني من الحمل وحتى الولادة بجرعه قدرها (٣٠ مجم/نعجة/اسبوعيا) قد اظهرت بلوغا جنسيا مبكرا ووزن الجسم اعلى عند البلوغ.