

CONTENTS

| | Page |
|--|-------------|
| INTRODUCTION | 1 |
| REVIEW OF LITERATURE | 3 |
| 1. Arginine biochemistry | 3 |
| 2. Physiological functions of arginine and its metabolites | 3 |
| 3. Effect of arginine supplementation on treated animals | 5 |
| 3.1. Arginine concentration | 5 |
| 3.2. Live body weight | 6 |
| 3.3. Placenta efficiency during pregnancy | 7 |
| 3.4. Blood flow | 8 |
| 3.5. Amino acids profile in blood | 9 |
| 3.6. Reproductive performance | 10 |
| 3.7. Progesterone (P ₄) concentration | 12 |
| 3.8. Blood parameters | 13 |
| 4. Effect of arginine supplementation on offspring performance | 14 |
| 4.1. Live body weight at birth | 14 |
| 4.2. Sex-specific and twinning effect on lamb birth weight | 15 |
| 4.3. Embryonic losses and mortality rate | 16 |
| 4.4. Blood proteins profile | 17 |
| MATERIALS AND METHODS | 19 |
| 1. Animals | 19 |
| 2. Feeding system | 19 |
| 3. Treatment of ewes | 20 |
| 4. Experimental procedures | 20 |
| 4.1. Placental weight and live body weight of ewes and their lambs | 20 |
| 4.2. Milking and milk samples of ewes | 20 |
| 4.3. Physiological response parameters | 21 |
| 4.4. Blood sampling | 21 |
| 5. Analytical procedures | 21 |
| 5.1. Amino acids content | 21 |
| 5.2. Blood biochemicals and enzyme activity | 21 |
| 5.3. Blood immunoglobulins | 22 |
| 6. Reproductive performance | 22 |
| 6.1. Ewes | 22 |
| 6.2. Ram lambs | 22 |
| 6.2.1. Semen evaluation of the 1st ejaculate | 23 |
| 6.3. Ewe lambs | 23 |
| 7. Statistical analysis | 23 |
| RESULTS AND DISCUSSION | 24 |
| 1. Live body weight of ewes during September and May breeding season | 24 |
| 2. Milk production of ewes mated during September breeding season | 27 |
| 2.1. Milk yield and composition | 27 |
| 2.2. Amino acid profile in ewe milk | 29 |
| 3. Physiological response of ewes during September and May breeding season | 32 |
| 3.1. Blood pressure | 32 |
| 3.2. Pulse rate (min) | 33 |
| 3.3. Respiration rate (min) | 35 |

| | |
|--|----|
| 3.4. Rectal temperature (°C) | 37 |
| 4. Blood parameters of ewes during September and May breeding season | 39 |
| 4.1. Blood biochemicals..... | 39 |
| 4.1.1. Total proteins and albumin concentration | 39 |
| 4.1.2. Glucose Concentration..... | 41 |
| 4.1.3. Creatinine Concentration | 43 |
| 4.2. Activity of aminotransferases (AST and ALT) | 45 |
| 5. Reproductive performance of ewes | 48 |
| 5.1. Mated during September breeding season | 48 |
| 5.1.1. Placental and birth weights | 48 |
| 5.1.2. Lambing rate and litter size | 50 |
| 5.2. Mated during May breeding season..... | 53 |
| 5.2.1. Ovarian activity before and during May breeding season | 53 |
| 5.2.2. Estrous and mating rates | 55 |
| 6. Progesterone profile during September and May seasons | 59 |
| 7. Effect of treatment of ewes with arginine on their ram lambs (September breeding season) | 62 |
| 7.1. Live body weight of ram lambs | 62 |
| 7.2. Blood immunoglobulines..... | 65 |
| 7.3. Blood biochemicals and enzyme activity | 67 |
| 7.4. Enzyme activity | 69 |
| 7.5. Puberty stages | 71 |
| 7.6. Physical semen characteristics of the 1st ejaculation | 72 |
| 7.7. Blood serum testosterone concentration | 75 |
| 8. Effect of treatment of ewes with arginine on their ewe lambs | 77 |
| 8.1. Live body weight and placental weight at birth..... | 77 |
| 8.2. Live body weight at different stages | 78 |
| 8.3. Serum immunoglobulins concentration (IgG and IgM) | 81 |
| 8.4. Blood biochemicals..... | 83 |
| 8.5. Enzyme activity | 85 |
| 8.6. Age of puberty | 87 |
| SUMMARY | 89 |
| CONCLUSION | 96 |
| REFERENCES | 97 |
| ARABIC SUMMARY | - |

LIST OF TABLES

| | Page |
|--|-------------|
| Table (1): Average live body weight of ewes in experimental groups at different periods of the experimental period. | 25 |
| Table (2): Effect of treatment on average daily milk yield (g) and milk composition (%) of ewes during the suckling period | 28 |
| Table (3): Amino acid (mg%) in milk of ewes at 2 weeks after lambing..... | 30 |
| Table (4): Average of upper and lower blood pressure of ewes in the experimental groups at different stages | 32 |
| Table (5): Average of pulse rate/min of ewes in the experimental groups at different stages before and 2 h post-treatment..... | 34 |
| Table (6): Average of respiration rate (min) of ewes in the experimental groups at different stages before and 2 h post-treatment..... | 36 |
| Table (7): Average of rectal temperature (°C) of ewes in the experimental groups at different stages | 38 |
| Table (8). Total proteins and albumin concentration in blood serum of ewes in the experimental groups during different stages..... | 40 |
| Table (9). Glucose concentration in blood serum of ewes in the experimental groups during different stages..... | 42 |
| Table (10): Creatinine concentration in blood serum of ewes in the experimental groups during different stages..... | 44 |
| Table (11): Activity of AST and ALT (U/l) in blood serum of ewes in the experimental groups during different stages..... | 46 |
| Table (12): Average weight of placenta and lambs in experimental groups at birth..... | 47 |
| Table (13): Reproductive traits of ewes in different experimental groups during September breeding season..... | 51 |
| Table (14): Ovarian structure of right and left ovaries of ewes (n=5) before and during May breeding season | 54 |
| Table (15): Reproductive traits of ewes in different experimental groups during May breeding season | 56 |
| Table (16): Progesterone concentration (ng/ml) in blood serum of ewes during different stages of the experimental period..... | 60 |
| Table (17): Effect of treatment of ewes with arginine on live body weight of their ram lambs from birth to 12 months of age..... | 63 |
| Table (18): Effect of treatment of ewes with arginine on IgG and IgM concentration in blood serum of their ram lambs at different ages | 66 |
| Table (19): Effect of treatment of ewes with arginine on some biochemicals in blood serum of their ram lambs at different ages (month) | 68 |
| Table (20): Effect of treatment of ewes with arginine on activity of AST and ALT in blood serum of their ram lambs at different ages (month) | 70 |
| Table (21): Effect of treatment of ewes with arginine on age and serum testosterone concentration of their ram lambs at different pubertal stages..... | 72 |

| | Page |
|--|-------------|
| Table (22): Effect of arginine treatment on semen characteristics of ram lambs in the experimental groups..... | 73 |
| Table (23): Effect of treatment of ewes with arginine on placental and birth body weight (kg) of their ewe lambs | 77 |
| Table (24): Average monthly body weight of ewe lambs affected by treatment of their dams with arginine | 79 |
| Table (25): Effect of arginine treatment of ewes on IgG and IgM concentration in blood serum of their ewe lambs at different ages (week)..... | 82 |
| Table (26): Effect of treatment of ewes with arginine on some biochemicals in blood serum of their ewe lambs at different ages (month) | 84 |
| Table (27): Effect of treatment of ewes with arginine on activity of AST and ALT in blood serum of their ewe lambs at different ages (month) | 86 |
| Table (28): Effect of treatment of ewes with arginine on age and progesterone level at puberty of their ewe lambs | 88 |

SUMMARY

This study was carried out at Sakha Animal Production Research Station, belonging to the Animal Production Research Institute (APRI) in cooperation with Physiology and Biotechnology Lab., Animal Production Department, Faculty of Agriculture, Mansoura University, during the period from November 2016 to January, 2018 to investigate the effect of treatment of ewes with different levels of L-arginine (20 or 30 mg/weekly/ewe) during pregnancy on productive and reproductive performance of ewes during September 2016 up to May 2017 breeding seasons. Also, performance and puberty of their ram and ewe lambs were studied.

Total of 45 Ossimi ewes (September breeding season 2016), 3-4 years of age and 39.59 kg LBW were divided into three groups. Ewes in the 1st group (G1) served as a control without treatment. 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 5 week of pregnancy up to next May breeding season (2017).

After lambing, total of 10 ram lambs and 10 ewe lambs produced from each ewes group were taken and allotted in three groups similar to treatment of their dams. LBW of ewes, ram lambs and ewe lambs was recorded at birth and monthly up to 12 month of age. In blood serum of ram and ewe 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 AST and ALT was determined in blood serum of ewes at early, mid- and lat pregnancy, at lambing, before and after suckling, and during start, mid- and end of May breeding season, as well as at 2, 4, 6 and 8 month of age in blood serum of ram or ewe lambs.

Age and serum testosterone concentration of each ram lamb as well P₄ concentration in ewe lambs were determined at puberty. Also, semen of 1st ejaculates (at puberty) of ram lambs was evaluated.

The obtained results could be summarized as the following:

1. Live body weight of ewes mated during September breeding season:

Effect of AR on LBW of ewes was not significant up to the 1st half of pregnancy period. During the 2nd half of pregnancy period (13-14 week), LBW of ewes was higher ($P<0.05$) in G2 and G3 than G1.

Effect of AR on LBW of ewes at lambing and up to three weeks post-partum, was not significant, but LBW of ewes increased ($P<0.05$) in G2 and G3 as compared to G1 at 5, 7 and 9 weeks of suckling period, at weaning and during next May breeding season.

2. Milk yield and composition of ewes mated during September breeding season:

Average daily milk yield (ADMY) was higher ($P<0.05$) in G3 (790 g) than in G1 (615 g), while ADMY of G2 (728 g) did not differ significantly in G1 and G2. ADMY increased by 15.5 and 22.2% in G2 and G3 as compared to G1, respectively.

Milk contents, including fat, lactose, solid not-fat (SNF) and total solids (TS) were the highest ($P<0.05$) in G3, followed by G2, but the differences between G2 and G3 was not significant. Protein content was higher ($P<0.05$) in G2 than in G1 and G3.

Treatment with AR in G3 showed marked increase in all essential and most non-essential amino acids, while milk of G2 showed slight increase in most amino acids in milk.

Arginine acid showed moderate values in amino acid profile, being the highest in G3, moderate in G2 and the lowest in G1. Generally total non-essential amino acids were higher than total essential amino acids.

3. Physiological response of ewes during September and May breeding season:

The present results revealed insignificant effect of AR treatment on upper and lower blood pressure, respiration rate and rectal temperature of ewes in different

experimental groups at early and late pregnancy period, lambing, early and late suckling period, pre-breeding May season (April), and early and late May breeding season.

The differences in pulse rate of ewes among the experimental groups were insignificant at early and late pregnancy period, late suckling period, pre-breeding May season (April), and early and late May breeding season. However, pulse rate was lower ($P<0.05$) lower in G2 and G3 at lambing, and only in G3 at early suckling.

4. Blood parameters of ewes during September and May breeding season:

AR treatment in G2 and G3 decreased ($P<0.05$) total proteins concentration as compared to G1 at mating season (September and end of May) and at early pregnancy of September season. AR treatment in G2 decreased ($P<0.05$) total proteins concentration as compared to G1 before suckling. AR treatment in G3 increased ($P<0.05$) of total proteins as compared to G1 during mid-pregnancy period. The differences in albumin concentration were not significant.

Glucose concentration in blood serum only at early and mid-pregnancy was higher ($P<0.05$) in G2 and G3 than in G1. The differences in serum glucose concentration at mating and lambing, during suckling period and May breeding season, were not significant.

Serum creatinine concentration was higher ($P<0.05$) in G2 and G3 than in G1 before suckling, and in G3 than in G2 at the end of May breeding season. Effect of AR on creatinine concentration at mating, pregnancy, lambing, after suckling and during early and mid-May breeding season was not significant.

Effect of AR on AST and ALT was not significant at most stages studied. AR treatment decreased ($P<0.05$) activity of AST and ALT at start and mid of May breeding season.

5. Reproductive performance of ewes mated during September breeding season:

Placental and birth weight of lambs was higher ($P<0.05$) in G2 and G3 than in G1, being higher ($P<0.05$) in G3 than in G2. Similar trend was recorded for placental weight based on sex of lambs either male or female lambs, but placental weight of males were higher than that of females in all experimental groups.

Lambing rate was lower ($P<0.05$) in G2 than in G1 (80 vs. 100%), but did not differ in G3 (93.3%) from that in G1 or G2. Litter size was higher ($P<0.05$) in G2 (1.25/ewe) and lower in G3 (1.0/ewe), but both did not differ from G1 (1.13/ewe).

Ewes in G2 showed the highest ($P<0.05$) twinning births and the lowest single births as compared to G1, while those in G3 showed ($P<0.05$) an opposite trend as compared to G1. Ewes in G2 and G3 produced higher ($P<0.05$) females and the lowest males than in G1, while the sex ratio showed an opposite trend in G2 and G3. Viability rate at weaning was the highest in G3 (100%), followed by G2 (93.3%), being significantly ($P<0.05$) lower than in G1 (76.5%).

6. Reproductive performance of ewes mated during May breeding season:

Before May breeding season, the ovarian activity of ewes was higher in G2 and G3 than in G1. The ovarian activity was higher on the left than on the right ovaries in all groups, being the highest in G3, in term of the highest CL number on both left and right ovaries.

During May breeding season, number of follicles (0.2-0.4 cm) was greater in G1 than in G2 and G3 (10 vs. 8 and 7), number of antral follicles (>0.4 cm) was lower in G3 than in G1 and G2 (6 vs. 8 for each), but diameter of antral follicles was lower in G2 and G3 than in G1 (0.57 and 0.55 vs. 0.61 cm). Number and diameter of CL was higher in G2 and G3 than in G1 (5 and 7 vs. 4 for CL number and 0.35 and 0.36 vs. 0.24 cm), respectively, on both right and left ovaries.

Estrus rate was 30% in G2 and G3 showed estrous activity during 1-15 May or 16-30 May versus 0% in G1. Up to the interval (1-15 June), other 30% of ewes in G2 plus 40% of ewes in G3 showed estrus versus the 1st 20% of ewes in G1. At 16-30 June, 70 and 90% of total ewes in G2 and G3 showed estrus versus 40% of total ewes in G1. Estrus/mating rate at the end of June month was the highest ($P<0.05$) in G3 (90%), moderate in G2 (70%) and the lowest in G1 (40%).

Ewes in G3 showed the highest ($P<0.05$) lambing rate, litter size and twinning rate with ideal sex ratio (50:50%), and the highest viability rate of lambs at weaning.

7. Progesterone profile during September and May seasons:

During September breeding season, P_4 concentration was higher ($P<0.05$) in G2 than in G1, but did not differ in G3 from that in G1 or G2. Concentration of P_4 was higher ($P<0.05$) in G3 (=1.0 ng/ml) than in G1 and G2 (0.068 and 0.79 ng/ml, respectively) one month post-lambing.

During May breeding season, the differences in P_4 concentration among the experimental groups were not significant on days 7 or 30 post-mating, although there was a tendency of increasing P_4 concentration in G2 and G3 than in G1, being almost more than 1 ng/ml in all groups.

8. Effect of treatment of ewes with arginine on their ram lambs (September breeding season):

Ram lambs were heavier ($P<0.05$) in G3 than in G1 at birth and at 5 to 12 mo of age, but did not differ in G2 from that in G1 or G3. At 1 to 4 mo of age, the differences in LBW of lambs were not significant. Final LBW of lambs was higher in G2 and G3 by 10.2 and 15.9% than in G1, respectively.

Ram lambs in G3 showed the highest ($P<0.05$) immune response (IgG and IgM) at all studied ages (after 2 day, 1, 3, and 4 wk of lambing), followed by those of G2, while, G1 showed the lowest response at all ages.

Ram lambs in G2 and G3 increased ($P<0.05$) only serum total proteins at 4 mo of age and of albumin at 4 and 6 mo of age, while decreased ($P<0.05$) serum glucose at 2 and 8 mo of age as compared to G1. Only ram lambs of G3 increased ($P<0.05$) serum creatinine at 6 and 8 mo of age.

Treatment of ewes with AR had insignificant effect on serum enzyme activity (AST and ALT) at all ages studied.

At the 1st stage (1st mounting), age of ram lambs was the earliest ($P<0.05$) in G2 and the latest in G3, but did not differ in G3 from that in G1 and G3. The differences in serum testosterone concentration were not significant. At the 2nd stage (1st mounting with erection), age was earlier ($P<0.05$) and serum testosterone was higher ($P<0.05$) in G2 and G3 than in G1, being the earliest and highest in G2. At the 3rd stage (1st ejaculation, puberty), age of ram lambs was earlier ($P<0.05$) by 51.4 and 33.0 days and serum testosterone 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, in terms of the highest ejaculate volume, sperm motility percentage and live sperm output per ejaculate. Ram lambs in G2 showed higher ($P<0.05$) ejaculate volume only than in G1, while live sperm output/ejaculate did not differ from that in G3 and G1.

Testosterone concentration was higher ($P<0.05$) in G2 and G3 than in G1 at 6 and 8 mo of age, showing an opposite trend at 7 mo of age, while insignificantly different at 5, 9 and 10 mo of age.

9. Effect of treatment of ewes with arginine on their ewe lambs:

Ewe lambs at lambing were heavier ($P<0.05$) in G2 (0.376 and 3.30 kg) and G3 (0.352 and 3.36 kg) than in G1 (0.285 and 2.89 kg).

Average of monthly live body weight of ewe lambs was higher in treatment groups than in the control one at each of the 1st five months of age, but the differences were significant ($P<0.05$) only at 3 months of age. During the interval from 6 to 8

months of age, body weight of ewe lambs was higher ($P<0.05$) in G3 than in G1, but did not differ in G2 from that in G1 and G3. During the interval from 9 to 12 months of age, ewe lambs was heavier ($P<0.05$) higher in G2 and G3 than in G1, being the highest in G3. Yearling body weight of ewe lambs was significantly higher in G2 and G3 by 14.5 and 18.3% of that of the control group, respectively.

Immune response (IgG) at all studied ages (after 2 day, 1, 3, and 4 wk of lambing) was the highest ($P<0.05$) in G3, moderate in G2, and the lowest in G1. The differences in IgM concentrations at all ages were not significant.

The differences in concentration of total protein and albumin in blood serum of ewe lambs among the experimental groups were not significant at all ages studied. Serum glucose decreased ($P<0.05$) in G2 and G3 as compared to G1 at 2 and 6 mo of age. Serum creatinine decreased ($P<0.05$) in G2 and G3 as compared to G1 at 4, 6 and 8 mo of age.

Activity of AST in G2 at 2 months of age and in G3 at 4 months of age was higher ($P<0.05$) than in G1, while AST activity in both groups at 8 months of age did not differ significantly from that in G1. Activity of ALT in G3 at 4 and 8 months of age and in G2 at 6 months of age increased ($P<0.05$) as compared to G1. The differences in AST activity at 6 months of age and those in ALT activity at 2 months of age were not significant.

Age at puberty was earlier ($P<0.05$) earlier in G3 (219 days) than in G1 (252 days) and G2 (238 days).

CONCLUSION

Based on the foregoing results of ewes during September and May breeding season, treatment of pregnant ewes of September breeding season with arginine at a level of 30 (G3) during pregnancy period and weekly up to the end of May breeding season had beneficial effects on LBW of born lambs, yield, composition and amino acid profile of milk produced, physiological response of ewes during pregnancy and suckling period, blood parameters, liver function of ewes, without pronounced effect on reproductive perform of ewes during September, but ovarian and estrous activities as well as reproductive performance of ewes before and during May breeding season were improved.

In addition, this treatment of ewes with L-AR-HCL 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.

Furthermore, this treatment showed also impact on their ewe lambs, in term of shorter age at puberty with suitable live body weight at 1st mating and lambing.