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# Impact of increasing twining rate in Barki ewes on milk yield, milk composition and lambs performance up to weaning

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**ABSTRACT**

The objectives of this research were to study the maternal response including productive performance, biochemical parameters and milk yield and composition as affected by type of birth. Fifty Barki ewes aged 2.5- 3.5 years and averaged (48.10±0.72 kg) live body weight were used according to fetus type. The estrous cycle of all ewes was synchronized by intravaginal progestagen impregnated sponge and super-ovulated doses of PMSG were used. Singleton and twin pregnancies were established by ultrasound scanning at 55 days, giving two experimental groups: group 1 ewes carrying singleton fetuses (36 ewes); group 2 ewes carrying twin fetuses (14 ewes). Ewes were weighed monthly during pregnancy and weighed again at pre-partum and at post-partum to calculate conceptus weight. Maternal blood samples were taken at monthly intervals from day of mating until the first month post-partum. Blood biochemical (total protein, albumin, glucose, total lipids, ALT, AST, urea and creatinine) and hormonal (progesterone, T3 and T4) pattern were determined. Milk yield was recorded at bi-weekly intervals starting from the second week of lambing till weaning age. Chemical composition of milk in terms of fat, protein, lactose, total solids and solids not fat was determined using milko scan. Results of the present study demonstrated that birth weight was ($P<0.05$) lower in twin bearing ewes as compared with singleton bearing ones. However, weaning weight was comparable. Twin bearing ewes had insignificant higher values of P4 and milk yield. Twin bearing ewes had comparable levels of total protein, albumin, total lipids, glucose, T3, T4, ALT, AST, urea and creatinine with singleton bearing ewes. In conclusion, Barki ewes were able to cope with increasing twining rate without any significant differences observed in weaning weight, blood biochemical parameters and milk yield..

## INTRODUCTION

In Egypt, desert constitutes the majority (95%) of its total area. Barki sheep, with population of 470,000 heads (11% of the total Egyptian sheep population), considered the main breed dominates under the harsh desert conditions and food shortage prevailed in the north western coast of Egypt (El-Wakil *et al.*, 2008). Purebred Barki is the breed of choice for Bedouins in the desert and mainly kept for meat production (Abdel-Moneim, 2009 and Hashem *et al.*, 2015). Thus, lamb crop is an important economic trait which could be maximized by applying the accelerating-lamb production system, three successive lambings in two years.

Total kilograms of lamb weaned each year from a flock of sheep is the best single measure of the meat productive ability of that flock, since it combines both ewe and ram fertility, as well as mortality and growth rate of lambs into one index (Younis *et al.*, 1990). Moreover, Barki sheep has a lower mortality rate than another local breed (El-Tarabany, 2012). Another scenario for accelerating lamb crop production is increasing the twining rate by using the hormonal protocol for synchronization and superovulation (Abdel-Khalek *et al.*, 2014).



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The interest in synchronization of estrus and superovulation in domestic animals has increased as one of the major steps towards the enhancement of reproductive and productive performance (Ezzo and EL-Tohamy, 1992, Akos *et al.*, 2006 and Abdel-Khalek *et al.*, 2014).

Pregnancy and lactation develop metabolic stresses that perform changes dependent upon the reproductive status of the animals (Ceylan *et al.*, 2009). Although twins have lower birth weights than singletons, they may not experience the increased disease risk in adulthood reportedly associated with low birth weight. Thus, the physiology of twin pregnancy is quite different from that of singleton pregnancy and is probably determined by a combination of factors operating in both early and late gestation. Furthermore, the maternal metabolic and endocrine environment, fetal and placental growth are all different in twin pregnancies from those in singleton pregnancies, making it essential that twins and singletons are addressed separately in any study of fetal physiology or its postnatal consequences (Rumball *et al.*, 2008).

Those negative phenomena are more pronounced in ewes nursing more than one lamb, which is due to the stimulating effect of lambs on milk production. Lactating ewes are usually group-fed, regardless of the number of suckling lambs, and the amount of feed they receive is dependent on their average body weight and fecundity (Sato *et al.*, 2005). Such a solution, although simple, may lead to nutrient deficiencies in the mothers of twins. This suggests that lactation may have a different influence on the body condition of ewes suckling singles or twins, reflected in the chemical composition of milk (Sato *et al.*, 2005).

This problem, addressed by few authors to date, is of particular importance in animals live under harsh desert condition like Barki sheep known for low twining rate. So, the present study was carried out to evaluate the ability extent of Barki ewes in the production and care of the newborn twin lambs reaching the weaning age safely. Therefore, the physiological and productive performance of ewes and their lambs are intensively evaluated and discussed.

## MATERIALS AND METHODS

### *Experimental region*

The present study was carried out during the period from June 2015 to March, 2016 at the Animal Production Unit, Sustainable Development Center for Matrouh Resources, Matrouh Governorate, which belongs to the Desert Research Center (DRC), Ministry of Agriculture and Land Reclamation. The station is located at 240 Km West of Alexandria and 222 Km Egyptian western boarders.

### *Experimental animals, feeding and management*

Fifty, non-pregnant and non-lactating, Barki ewes averaged 48.10±0.72 kg Live Body Weight (LBW) and averaged 2.5 – 3.5 years old were used in this study. Ewes were clinically examined for any reproductive disorders as well as general health status. Vaccination against the major prevailing epidemic diseases, internal and external parasites were controlled in proper time good way.

Experimental ewes were weighed before starting the experiment, kept under an intensive production system and housed in semi-open yards throughout the experimental period. Lambs were left all day time with their dams for suckling to the weaning age at three months. All groups were daily fed on a concentrate feed mixture (0.75 kg) and berseem hay (0.5 kg) per head during experimental period to cover their nutrient requirements during different physiological status according to Kearl (1982). Lambs were daily fed only on their dams' milk from birth to weaning age at 3 months of age. The daily ration was offered in a certain mode of feeding starting with concentrate mixture at (8:30 h) followed by chopped rice straw at (12:00 h) and continuing to the next morning feeding. Fresh water was available to sheep during all day.

### *Experimental design*

A fortnight before mating with rams, the estrous cycle of all ewes was synchronized and super-ovulated doses of PMSG were used as follows. Animals were treated using an intravaginal progestagen impregnated sponge (20 mg cronolone, Chronogest® CR, product of Intervet International B.V. and manufactured in the European Union (EU) was inserted. Sponges remained in situ for 12 days and were removed in the $12^{th}$ day. On the ninth day, all ewes received an intramuscular injection of prostaglandin F2α (1 ml Synchromate each 1 ml solution containing 0.250 mg Cloprostenol, Bremer Pharma GMBH, Germany). While on the $10^{th}$ day, all ewes received an intramuscular injection of 750 IU PMSG (Laboratorios Hipra, S.A. Avda. Ia Selva, 135, 17170 Amer (Girona) Spain). PMSG injections were used in gradual decreasing doses for three days that each ewe received 275 IU at the $10^{th}$ day, the second dose (250 IU) was injected at the $11^{th}$ day, while the last dose (225 IU) was injected at the $12^{th}$ day at the time of sponges' removal. Meanwhile, on the $14^{th}$ day, all ewes received an intramuscular injection of 500 IU/ewe of hCG hormone (Epifasi lyophilized ampoule contains 5000 I.U. of Human Chorionic Gonadotrophin (hCG) and 10 mg of lactose, manufactured by Egyptian Int. Pharmaceutical Industries Co. (EIPICO), Egypt). During the estrus synchronization period, all synchronized animals were subjected to a twice daily check (morning and evening) to ensure that sponges remained in their position during the treatment period. Nine fertile Barki rams were introduced to the ewes for estrus detection and mating; started 24 hours after removing the sponges and left to one week.

Singleton and twin pregnancies were established by ultrasound scanning at 55 days, giving two experimental groups: group (1) ewes carrying singleton fetuses (36 ewes); group (2) ewes carrying twin fetuses (14 ewes). Ewes were weighed monthly during pregnancy, at pre-partum and at post-partum to calculate conceptus weight.

***Milk yield and compositions***

Milk yield (MY) was recorded at bi-weekly intervals starting from the second week of lambing till weaning age (3 months) using lamb-suckling technique (Mousa and Shetawi, 1994) plus hand milking. Lambs were separated from their mothers at 8.00 p.m. on the day before measuring milk production. In the following day, lambs were weighed at 8.00 a.m., and left to suckle their dams till satisfaction, then reweighed and kept away from their mothers. While, the residual milk in the udder of each ewe was hand milked and weighed. Milk samples (50 ml) were taken biweekly from individual ewes within the respective groups during the 12-week lactation period. Samples were stored in plastic bags and kept under -20 °C for further analyses. Milk yield was determined to represent early (up to the $4^{th}$ week), mid (up to the $8^{th}$ week) and late lactation periods (up to the $12^{th}$ week). Chemical composition of milk in terms of fat, protein, lactose, total solids and solids not fat was determined using milk scan (MilkoScan, Bentley, Belguim).

***Metabolites and hormonal assays***

Maternal blood samples were taken by jugular puncture into EDTA (ethylenediamine tetra-acetic acid) containing tubes. Blood samples were collected monthly from day of mating until the first month post-partum. Blood plasma was pipetted into Eppendorf tubes and then stored at -20 ºC until further analyses.

Progesterone ($P_4$), triiodothyronine ($T_3$) and thyroxine ($T_4$) hormones were quantified by ELISA method using BIOS kit provided by Chemux Bioscience Corporation, 385 Oyster Point Blvd Suite 5-6., South San Francisco, CA 94080, USA. The standard curve ranged between 0-50 ng/ml for $P_4$, 0-10 ng/ml for $T_3$ and 0-30 µg/dl for $T_4$. The corresponding values for sensitivity of the curve were 0.2 ng/ml, 0.25 ng/ml and 0.5 µg/dl, respectively.

Blood biochemical parameters including total proteins (TP), albumin (Alb), total lipids (TL) and glucose (Glu) concentrations were determined by commercial kits supplied by Biodiagnostic Company for Laboratory Services, Egypt. Blood plasma aspartate (AST) and alanine aminotransferases (ALT) activities as indicators for liver functions, as much as, blood urea nitrogen (BUN) and creatinine (CR) concentrations as indicators for kidney functions were determined using commercial kits supplied by Biostc Company for Laboratory Services, Egypt. All determinations were executed according to the standard methods as outlined by the respective manufacturers.

***Statistical analysis:***

Data were analyzed using General Linear Model procedure (SAS, 2004). Differences among means were tested according to Duncan’s Multiple Range Test (Duncan, 1955).

## RESULTS AND DISCUSSION

***Productive efficiency***

Measuring and recording the body weight (BW) in each stage of the productive cycle of sheep is very important to evaluate the body condition and corporal reserves in preparation to high demand nutrient stages like final gestation and early lactation. The obtained resulted demonstrated that ewes procreated twins had a comparable weight with those lambed one, since there were no significant difference between the two groups in body weight from mating until post-partum (Table 1).

Although, body weights of the twin-bearing ewes were higher than those in the singleton-bearing ewes, but these differences found to be not significant. On the same trend Gardner (2007) reported that litter size does not have a significant effect on body weight of the studied sheep. On the other hand, the conceptus weight of ewes lambed twins was heavier ($P<0.05$) than that of one lamb group by about 42.6% (Table 1).

**Table 1. (LSM±SE) of the body weight (kg) of Barki ewes as affected by birth type during pregnancy period**

| Month of pregnancy | Singleton-bearing ewes | Twin-bearing ewes | Overall mean |
|---|---|---|---|
| At mating | 48.58±1.05 | 49.46±1.68 | 48.83±0.88 |
| 1st month | 50.38±1.00 | 50.81±1.61 | 50.49±0.84 |
| 2nd month | 52.17±0.98 | 52.86±1.58 | 52.36±0.83 |
| 3rd month | 53.67±0.99 | 54.51±1.58 | 53.91±0.83 |
| 4th month | 55.07±1.04 | 56.48±1.65 | 55.47±0.88 |
| 5th month (pre-partum) | 58.55±0.92 | 61.73±1.46 | 59.46±0.80 |
| Post-partum | 51.19±0.89 | 51.23±1.41 | 51.20±0.74 |
| **Conceptus weight** | **7.36±0.28[B]** | **10.50±0.55[A]** | **8.26±0.33** |

[A-B] Least square means with different superscripts differ significantly ($P<0.05$).

This significant difference between the two experimental groups might be explained since the total fetal weight/ewe, weight of fetal membranes/ewe, and total number of placentomes/ewe increased with the increase in fetal number. Although, individual fetal weight, the number of placentomes/fetus and the weight of placentomes/fetus were greater for single pregnancy than for multiple pregnancies (Grazul-Bilska *et al.*, 2006 and Gardner, 2007).

Regarding the lambs weight from birth to weaning, the present findings declared that average birth weight of the progeny was 3.87±0.11 kg ranged from 3.11 kg in twin lambs to 4.47 kg in single lambs (Table 2). On average, at birth, singles were about 1.36 kg heavier ($P<0.05$) than twins. This result is in harmony with that found by Mellado *et al.* (2011) since carrying multiple fetuses lead to a decline in kid birth weight in the present study. This is explained by the fact that uterine space has a finite capacity to gestate offspring; consequently, as the litter size increases, individual birth weights decline, because the mother does not have the physiological capacity to adequately supply fetuses with metabolic substrates. Moreover, decline in litter birth weight with increasing litter size in sheep, has been reported by many authors (Freetly and Leymaster 2004; Gootwine 2005 and Gardner *et al.* 2007). In sheep, the acute reduction in birth weight as the litter size increases apparently is due to the failure of the uterus–placenta complex to support full growth of multiple fetuses (Gardner *et al.* 2004 and MacLaughlin *et al.* 2005). In fact, the single greatest effect on birth weight of lambs is litter size (Gardner *et al.* 2007). A negative correlation exists between the number of young in a new-born litter of polytocous mammals and the birth weight of the offspring (Freetly and Leymaster 2004 and Mellado *et al.*, 2011),

**Table 2. (LSM±SE) of body weight (kg) of Barki lambs as affected by birth type during post-partum periods**

| Days of age | Singleton-bearing ewes | Twin-bearing ewes | Overall mean |
|---|---|---|---|
| 0 (Birth weight) | 4.47±0.08[a] | 3.11±0.12[b] | 3.87±0.11 |
| 15 day | 7.04±0.17[a] | 5.85±0.17[b] | 6.52±0.14 |
| 30 day | 9.75±0.22[a] | 7.96±0.23[b] | 8.97±0.19 |
| 45 day | 12.89±0.32[a] | 10.34±0.28[b] | 11.77±0.27 |
| 60 day | 14.87±0.29[a] | 12.59±0.39[b] | 13.87±0.28 |
| 75 day | 16.78±0.35[a] | 15.11±0.43[b] | 16.05±0.29 |
| 90 (weaning weight) | 18.58±0.42[a] | 17.42±0.49[a] | 18.07±0.32 |
| **Average daily gain** | **156.94±4.56[a]** | **159.1±5.71[a]** | **157.9±3.56** |

[a-b] Least square means within each row, with different superscripts differ significantly ($P<0.05$).

On the other hand, the body weight of the single lambs continued to exceed significantly the body weight of their counterparts of twin lambs from birth to 75 days of age by 43.73, 20.34, 22.49, 24.66, 18.11 and 11.05% at birth, 15, 30, 45, 60 and 75 day of age, respectively (Table 2). These results could be explained as the singleton lambs received more amount of milk than twin lambs even though the higher milk production observed in twin bearing ewes. Lower BW is associated with reduced postnatal growth rate (Greenwood and Bell, 2003). Rumball *et al.* (2008) reported that twins develop in a different environment to singletons from conception at least until weaning. Throughout gestation twin fetuses compete for maternal nutrients, have smaller placentae than singletons, and restricted physical space. After delivery twins compete for milk supply and parental attention. Coop *et al.* (1972) reported that in addition to restricting lamb growth, failure to produce sufficient high-quality milk, in extreme cases, can cause lamb mortality, with twin-born lambs being at most risk. After the 1st month, lambs increasingly consume solid foods (Lane *et al.*, 2000), but milk continues to be important and can determine as much as 70% of their growth up to 12 weeks of age (Doney *et al.*, 1984), although postweaning compensatory growth can overcome some set-backs in twin-reared progeny (Thompson *et al.*, 2011). However, the absence of any significant differences between the experimental lambs at weaning (Table 2) might refer to the ability of Barki ewe to rear their progeny.

***Progesterone concentration***

The obtained findings revealed that twin bearing ewes had higher progesterone levels all the way through the pregnancy and post-partum stages. However, there weren't any significant differences between the two experimental groups (Table 3). The corpus luteum is the only source of progesterone for the maintenance of pregnancy in goats. The presence of more placental tissue due to twinning was related to more placental lactogen secretion to stimulate mammary gland growth (Anderson *et al.*, 1981). An increase number of corpora lutea may contribute more estrogen and progesterone secretion, which leads to stimulation of mammary gland development in goats or sheep bearing twin fetuses. The increased plasma progesterone during pregnancy is due to increase litter size in sheep (Butler *et al.*, 1981).

**Table 3. (LSM±SE) of blood progesterone concentration (ng/ml) in Barki ewes as affected by birth type**

| Month of pregnancy | Singleton-bearing ewes | Twin- bearing ewes | Overall mean |
|---|---|---|---|
| 1st month | 11.43±0.86 | 11.49±0.44 | 11.46±0.46$^{c}$ |
| 2nd month | 13.03±0.95 | 14.42±1.40 | 13.73±0.83$^{b}$ |
| 3rd month | 14.03±0.67 | 15.28±0.68 | 14.65±0.49$^{ab}$ |
| 4th month | 16.03±0.67 | 16.49±0.62 | 16.26±0.44$^{a}$ |
| 5th month | 15.19±0.66 | 16.33±1.09 | 15.76±0.72$^{ab}$ |
| Post-partum | 2.90±1.01 | 3.50±0.98 | 3.20±0.67$^{d}$ |
| **Overall mean** | **11.90±0.84$^{B}$** | **12.92±0.91$^{A}$** | |

$^{a-d}$ least square means within each column, with different superscripts differ significantly (P<0.05).
$^{A-B}$ least square means within each row, with different superscripts differ significantly (P<0.05).

***Milk yield and composition***

The obtained results of milk yield revealed that twin bearing ewes had higher milk yield than that of singleton bearing ones throughout the lactation period (early, mid and late stages) with insignificant differences (Table 4). In similar, ewes rearing twins produced more milk than those suckling singles and the differences were significant (Abd Allah *et al.*, 2013). Reiad *et al.* (2010) found that the total milk yield increased with high twinning rates. Moreover, Sobiech *et al.* (2008) found that ewes suckling twins were characterized by a higher milk yield and higher (by 22.09%) milk production during 70-d lactation than ewes nursing singles.

**Table 4. (LSM±SE) of milk yield and composition in Barki ewes as affected by birth type**

| Items | Singleton-bearing ewes | Twin- bearing ewes | Overall mean |
|---|---|---|---|
| **Milk yield (ml/day)** | | | |
| Early | 586.3±19.8 | 680.8±23.3 | 618.8±16.3$^{B}$ |
| Mid | 635.6±25.9 | 773.3±32.4 | 683.1±21.9$^{A}$ |
| Late | 367.1±14.3 | 429.7±20.9 | 388.7±12.3$^{C}$ |
| **Overall mean** | **529.7±16.1$^{a}$** | **627.9±23.9$^{a}$** | |
| **Colostrum composition (%)** | | | |
| Fat | 10.46±0.65$^{a}$ | 9.91±0.68$^{a}$ | 10.26±0.48 |
| Protein | 12.13±0.56$^{b}$ | 13.88±0.34$^{a}$ | 12.75±0.41 |
| Lactose | 2.79±0.28$^{a}$ | 2.64±0.16$^{a}$ | 2.74±0.19 |
| Total solids (TS) | 28.39±0.78$^{a}$ | 27.89±0.80$^{a}$ | 28.21±0.57 |
| Solids not fat (SNF) | 16.78±0.44$^{a}$ | 17.88±0.23$^{a}$ | 17.18±0.31 |
| **Milk composition (%)** | | | |
| Fat | 4.22±0.17$^{a}$ | 4.02±0.19$^{a}$ | |
| Protein | 4.74±0.08$^{b}$ | 5.12±0.14$^{a}$ | |
| Lactose | 5.57±0.09$^{b}$ | 5.81±0.16$^{a}$ | |
| Total solids (TS) | 15.10±0.16$^{b}$ | 16.02±0.32$^{a}$ | |
| Solids not fat (SNF) | 11.07±0.16$^{b}$ | 11.65±0.27$^{a}$ | |

$^{a-b}$ Least square means within each row, with different superscripts differ significantly (P<0.05).
$^{A-C}$ Least square means within each column, with different superscripts differ significantly (P<0.05).

The difference between the two groups was more pronounced in the second half of lactation. This corresponds to the opinion of Snowder and Glimp (1991) who claim that milk production in the mothers of twins is stimulated to a higher degree in later phases of lactation. These results may be owing to that increase of litter size and subsequently that number of corpora lutea increasing the secretion of progesterone in blood during pregnancy. The increase fetal number would increase hormonal stimulation for growth and development of mammary gland for preparation of more milk synthesis for the newborn (Anderson *et al.*, 1981). Manalu *et al.* (1996) reported almost twice higher maternal serum progesterone concentration during the last two months of gestation period for twins compared to single bearing goats. From another point of view, the increasing milk yield may be due to the ability of twin lambs to empty the udder of their dams completely especially of the early lactation period. More frequent suckling's were observed by twins compared to single lambs.

Regarding the milk composition, the twin bearing ewes had lower fat percentage than singleton bearing ewes. However; they had higher protein, lactose, total solids (TS) and solids not fat (SNF) than singleton bearing ewes during all stages of lactation (Fig. 1 and Table 4).

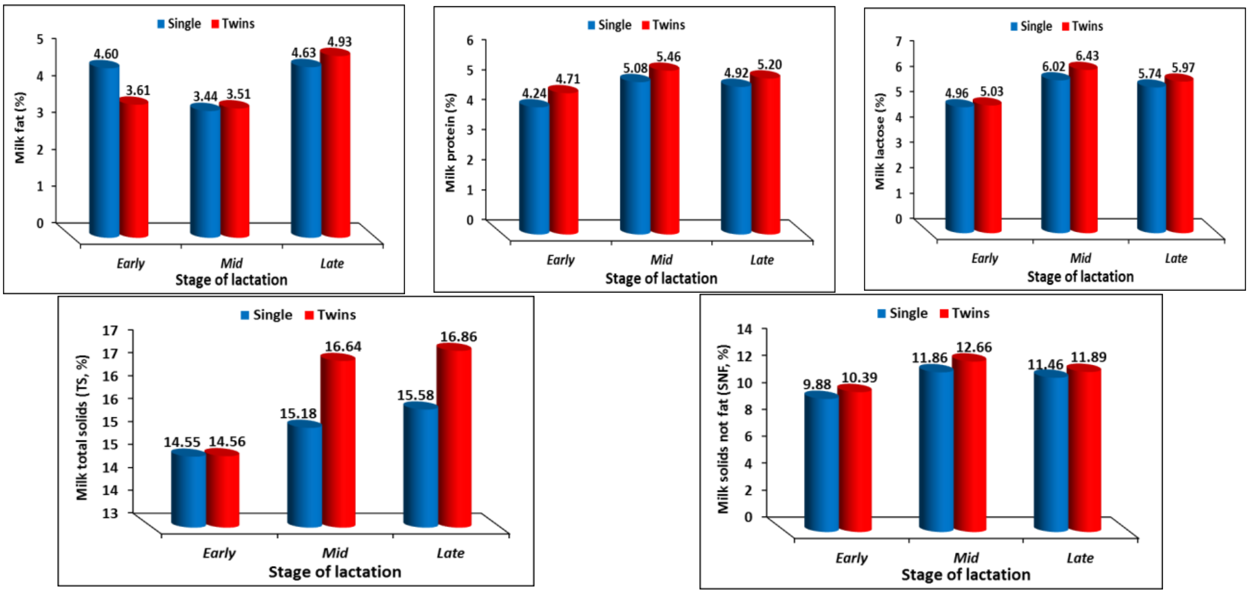


**Figure 1.** Milk composition of Barki ewes as affected by the type of birth (single or twins) during different lactation stages.

In consistence, Fuerst-Waltl *et al.* (2005) found that yield and protein percentage increased with higher number of lambs whereas fat percentage slightly decreased. Ewes born as singles had significantly higher protein percentages in higher lactations and were in tendency superior to ewes born as multiples for other production traits. Niżnikowski *et al.* (1994) noted only a higher lactose concentration in the milk of the mothers of twins. However, Snowder and Glimp (1991) and Sormunen-Cristian *et al.* (1997) demonstrated that the milk of ewes nursing twins contained more fat. On the other hand, lower lactose content in the milk of ewes suckling twin lambs was reported by Snowder and Glimp (1991).

***Maternal blood metabolites***

***Total proteins***

The results of total protein and albumin demonstrated that singleton bearing ewes exceeded insignificantly the total proteins levels of their counterparts of twin bearing ones throughout all the experimental stages (Table 5) which might be owing to the higher need protein to cope with the growth of twin than singleton lambs. Previous studies (Firat and O¨ zpinar, 2002 and Balikci *et al.*, 2007) reported twin bearing ewes had lower plasma total protein levels than single-bearing ewes.

On the other hand, the TPs concentrations decreased significantly in advance with gestation and postpartum periods. This decrease in TPs in late-pregnancy could be explained where the fetus synthesizes all its proteins from the amino acids derived from the mother, and fetus growth increases exponentially reaching a maximum level, especially in muscles, during late pregnancy (Jainudee and Hafez, 1994 and Safsaf *et al.*, 2012). Moreover, it could be attributed to the rapid extraction of immunoglobulin from plasma during the last few months of pregnancy, when colostrum formed in the mammary gland, as well as the increase needs to proteins for the fetus development (Castillo *et al.*, 1999, Kaneko, 1997, Antunovic *et al.*, 2004 and Kaneko *et al.*, 2008). The lower levels of serum total protein at postpartum/suckling may be attributed to the passive transfer of maternal protein via colostrum from dams to offspring (Antunovic *et al.*, 2004 and Teleb *et al.*, 2009).

**Table 5. (LSM±SE) of blood biochemical parameters in Barki ewes as affected by birth type during early, mid, late and post-partum periods**

| Items | Singleton-bearing ewes | Twin- bearing ewes | Overall means |
|---|---|---|---|
| **Total protein (g/dl)** | | | |
| Early | 7.01±0.31 | 6.64±0.14 | 6.83±0.17[a] |
| Mid | 6.89±0.29 | 6.43±0.15 | 6.66±0.17[a] |
| Late | 6.73±0.19 | 6.31±0.16 | 6.52±0.13[ab] |
| Post-partum | 6.37±0.07 | 5.78±0.07 | 6.08±0.11[b] |
| **Overall mean** | **6.80±0.13[A]** | **6.36±0.08[B]** | |
| **Albumin (g/dl)** | | | |
| Early | 4.36±0.32 | 3.96±0.18 | 4.16±0.18[a] |
| Mid | 3.98±0.25 | 3.75±0.08 | 3.86±0.13[ab] |
| Late | 3.95±0.12 | 3.50±0.24 | 3.72±0.14[ab] |
| Post-partum | 3.78±0.15 | 3.18±0.21 | 3.48±0.16[b] |
| **Overall mean** | **4.05±0.12[A]** | **3.66±0.10[B]** | |
| **Glucose (mg/dl)** | | | |
| Early | 80.67±3.89 | 78.66±4.11 | 79.67±2.76[a] |
| Mid | 82.92±2.39 | 73.46±3.15 | 78.19±2.21[a] |
| Late | 78.16±3.44 | 69.99±5.56 | 74.07±3.32[ab] |
| Post-partum | 71.93±3.49 | 63.55±5.71 | 67.74±3.45[b] |
| **Overall mean** | **79.35±1.75[A]** | **72.54±2.37[B]** | |
| **Total lipids (mg/dl)** | | | |
| Early | 259.5±13.6 | 301.3±13.5 | 280.4±10.5[b] |
| Mid | 286.46±8.88 | 279.9±13.7 | 283.2±7.9[b] |
| Late | 278.4±18.9 | 310.3±14.3 | 294.3±12.1[b] |
| Post-partum | 322.6±13.1 | 365.7±9.6 | 344.2±10.5[a] |
| **Overall mean** | **281.6±7.8[B]** | **306.9±8.1[A]** | |

[a-b] least square means within each column, with different superscripts differ significantly (P<0.05).
[A-B] least square means within each row, with different superscripts differ significantly (P<0.05).

***Glucose***

Concerning the effect of litter size on the concentrations of glucose, the present results revealed that twin bearing ewes had insignificant lower glucose levels as compared with singleton bearing ewes (Table 5). Lower plasma glucose levels in twin-bearing sheep compared with single-bearing sheep in late pregnancy may be ascribed to the larger metabolic demand in the twin-bearing ewes (Firat and O¨ zpinar, 2002 and Balikci *et al.*, 2005).

On the other side, the levels of glucose decreased (P<0.05) in both experimental groups throughout the gestation and post-partum periods (Table 5). These results are consistent with the findings of Brito *et al.* (2006) and Antunovic *et al.* (2011) who found a decrease in glucose levels at late gestation and lactation periods. This decrease in blood glucose concentrations in lactating ewes have to be considered as a result of constant energy loss with the milk synthesis (Antunovic *et al.*, 2011); and low glucose levels in high pregnancy are associated with fetus development and mobilization of maternal glucose to fetal blood circulation (Jacob and Vadodaria, 2001).

Decreased blood glucose concentrations in lactating ewes have to be considered as a result of constant energy loss with the milk. Specifically, these changes suggest that the combination of increased utilization of glucose for milk lactose synthesis and the low intake of nutrients during investigation was insufficient to maintain blood glucose homeostasis (Pambu—Gollah *et al.*, 2000).

***Total lipids***

Plasma total lipids (TL) showed significant (P<0.05) increases throughout the gestation and post-partum periods (Table 5). This increase could be attributed to the higher levels of free fatty acids (FFA) in pregnant ewes, caused by increased cortisol level as a result to stress induced by pregnancy (Fleming, 1997). Additionally, increased sensitivity of ewes to epinephrine hormone leads to an increase in serum FFA concentrations in late-pregnancy (Revell *et al.*, 2000). Schlumbohm *et al.* (1997) demonstrated that the elevated level of TL in late gestation could be due to the reduced insulin-mediated inhibition of lipolysis observed in late pregnancy. Lipogenesis stimulated by insulin cause the increased values of TL observed in ewes during early lactation.

On the other hand, twin bearing ewes had insignificant higher total lipids levels than those of singleton bearing ewes along with gestation and post-partum stages (Table 5). In similar, El-Tarabany (2012) reported that cholesterol and triglyceride concentrations decreased significantly in ewes conceived single fetus than in ewes conceived twins fetuses.

***Thyroid hormones***

Thyroid hormones play a relatively important role in pregnancy and lactation. They are involved in the metabolic response via maintaining the homeostasis of energy and protein metabolism, thermoregulation, growth and productivity parameters (Huszenicza *et al.*, 2002). Monitoring the concentration of blood parameters as well as thyroid hormones in sheep gives a clear picture of their nutritional and health status before the changes are visible on the animal (Antunovic *et al.*, 2009).

The results revealed that thyroid hormones decreased with advancement of gestation (Table 6). Khaled and Illek (2012) reported that serum $T_3$ and $T_4$ in Barki ewes were significantly declined in the last month of pregnancy and postpartum. They suggested that the decrease in the thyroid hormones around parturition is due to alterations in cardiac output and increase of blood volume, as reported by Illek *et al.* (1998) and Dalvi *et al.* (1995). Colodel *et al.* (2010) suggested that the lower concentrations of $T_3$ and $T_4$ observed during gestation in comparing with non- pregnant ewes could be related to the passage of thyroid hormones through the placenta, since the ovine thyroid becomes functional only between the $6^{th}$ and $8^{th}$ weeks of embryonic life.

Singleton bearing ewes had insignificant higher thyroid hormones than those of twin bearing ewes throughout the gestation period (Table 6). In similar, Khan and Ludri (2002) found that the levels of thyroid hormones were significantly higher during all the periods of sampling in single bearing goats compared to the twin ones.

**Table 6. (LSM±SE) of blood thyroid hormones concentrations in Barki ewes as affected by birth type during early, mid, late and post-partum periods**

| Hormones | Singleton-bearing ewes | Twin- bearing ewes | Overall mean |
|---|---|---|---|
| **$T_3$ (ng/ml)** | | | |
| Early | 1.56±0.14 | 1.39±0.06 | $1.47±0.08^{a}$ |
| Mid | 1.34±0.08 | 1.12±0.07 | $1.23±0.06^{b}$ |
| Late | 1.29±0.09 | 1.01±0.05 | $1.15±0.06^{b}$ |
| Post-partum | 1.42±0.07 | 1.18±0.08 | $1.30±0.06^{ab}$ |
| **Overall mean** | **$1.40±0.05^{A}$** | **$1.18±0.04^{B}$** | |
| **$T_4$ (µg/ml)** | | | |
| Early | 10.09±0.76 | 10.08±0.69 | $10.09±0.50^{a}$ |
| Mid | 13.18±0.64 | 9.41±0.53 | $11.29±0.59^{a}$ |
| Late | 10.78±0.63 | 8.86±0.83 | $9.82±0.55^{a}$ |
| Post-partum | 10.78±0.44 | 8.86±0.46 | $9.82±0.44^{a}$ |
| **Overall mean** | **$11.27±0.39^{A}$** | **$9.37±0.35^{B}$** | |

$^{a-b}$ least square means within each column, with different superscripts differ significantly (P<0.05).
$^{A-B}$ least square means within each row, with different superscripts differ significantly (P<0.05).

***Transaminase enzymes (ALT and AST)***

The current results revealed that activity of aspartate aminotransferase (AST) increased (P<0.05) on advance with gestation stages and post-partum. Whereas, alanine aminotransferase (ALT) level increased insignificantly from early to late pregnancy then declined again during post-partum period (Table 7). Changes in activities of these enzymes may be related to reduced dry matter intake around parturition, may lead to hepatic lipidosis to alter the normal function of the liver (Greenfield *et al.*, 2000). Concerning the effect of litter size on ALT and AST levels, the present findings declared that twin bearing ewes higher levels of ALT and AST than those observed for singleton bearing ewes throughout gestation stages and post- partum period (Table 7). However, the differences between the two experimental groups were not significant. In constancy, El-Tarabany (2012) reported that ALT and AST activities showed significant decrease in ewes conceived single fetus than those obtained in ewes conceived twins fetuses.

**Table 7. (LSM±SE) of transaminases enzymes activities in Barki ewes as affected by birth type during early, mid, late and post-partum periods**

| Enzymes | Singleton-bearing ewes | Twin- bearing ewes | Overall mean |
|---|---|---|---|
| **AST (IU/L)** | | | |
| Early | 57.06=2.33 | 58.00±1.05 | 57.53±1.25[b] |
| Mid | 59.86±2.42 | 60.36±1.21 | 60.11±1.32[ab] |
| Late | 60.01±1.96 | 60.87±2.55 | 60.44±1.57[ab] |
| Post-partum | 64.89±3.26 | 62.02±3.68 | 63.45±2.37[a] |
| **Overall mean** | **59.82±1.22[A]** | **60.07±0.98[A]** | |
| **ALT (IU/L)** | | | |
| Early | 32.78±1.21 | 30.79±1.09 | 31.79±0.82[a] |
| Mid | 33.88±1.47 | 32.13±0.92 | 33.00±0.87[a] |
| Late | 33.26±1.27 | 33.77±2.47 | 33.52±1.35[a] |
| Post-partum | 30.80±1.79 | 35.05±2.76 | 32.93±1.70[a] |
| **Overall mean** | **32.95±0.69[A]** | **32.63±0.89[A]** | |

[a-b] least square means within each column, with different superscripts differ significantly (P<0.05).
[A-B] least square means within each row, with different superscripts differ significantly (P<0.05).

***Blood urea nitrogen and creatinine***

The results of blood urea nitrogen and creatinine demonstrated increases in their concentrations with gestation stages advancement and in post-partum period (Table 8). In consistence, the highest values of blood urea were also observed in the last trimester of pregnancy in sheep (Antunovic *et al.*, 2002). The high requirements for energy by pregnant sheep during their second half of pregnancy might lead to an increase in serum urea levels (Piccione *et al.*, 2009). Another reason for high urea concentration in pregnant ewes could be related to either high protein metabolism during pregnancy or nutritional management (Gurgoze *et al.*, 2009). It could also be a result of catabolizing muscle protein when large amounts of body reserves are mobilized (Antunovic *et al.*, 2011). El-Sherif and Assad (2001) in Barki ewes found that plasma urea level started rising during week 10 of pregnancy and reached a peak around parturition. The same trend was observed by Durak and Altiner (2006) in Chios ewes.

**Table 8. (LSM±SE) of blood urea nitrogen and creatinine concentrations of Barki ewes as affected by birth type during early, mid, late and post-partum periods.**

| Item | Singleton-bearing ewes | Twin- bearing ewes | Overall mean |
|---|---|---|---|
| **Blood urea nitrogen (mg/dl)** | | | |
| Early | 32.89±2.01 | 35.43±1.40 | 34.16±1.23[a] |
| Mid | 32.17±1.43 | 35.63±2.55 | 33.89±1.47[a] |
| Late | 34.73±2.18 | 34.38±2.51 | 34.56±1.62[a] |
| Post-partum | 35.53±2.96 | 34.59±3.51 | 35.06±2.17[a] |
| **Overall mean** | **33.59±1.01[A]** | **35.07±1.15[A]** | |
| **Creatinine (mg/dl)** | | | |
| Early | 1.84±0.34 | 2.82±0.48 | 2.33±0.31[a] |
| Mid | 2.00±0.33 | 2.85±0.22 | 2.42±0.21[a] |
| Late | 2.48±0.38 | 2.57±0.47 | 2.52±0.29[a] |
| Post-partum | 2.29±0.34 | 2.94±0.59 | 2.62±0.34[a] |
| **Overall mean** | **2.13±0.17[B]** | **2.77±0.21[A]** | |

[a-b] least square means within each column, with different superscripts differ significantly (P<0.05).
[A-B] least square means within each row, with different superscripts differ significantly (P<0.05).

On the other hand, twin bearing ewes recorded insignificant higher urea and creatinine levels than those of singleton bearing ones in all experimental stages (Table 8). The statistical analysis showed that there were no significant differences between experimental groups. Similarly, El-Tarabany (2012) reported that creatinine and urea levels showed significant decreased significantly in ewes conceived single fetus than in ewes conceived twins fetuses.

## CONCLUSION

From the abovementioned results, it could be concluded that Barki breed have the ability for improving fertility using hormonal manipulation. Moreover, Barki ewes were able to cope with increasing twining rate without any significant differences observed in weaning weight, blood biochemical parameters and milk yield.

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