



## Research Article

# Litter Number Modulates Some Behavioral and Physiological Aspects in Egyptian Native Breed Ewes (*Ovis aries*)

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## Abstract

**Background and Objectives:** Lactation is considered as one of the most stressful conditions along the entire life of female mammals. From the welfare point of view, nursing several lambs represents emerging health challenge both on the levels of dam and offspring. Therefore, this study was designed to explore the effects of litter number on Egyptian native breed sheep welfare by evaluating some physiological and behavioral parameters in ewes from 0D-30D after giving birth. **Materials and Methods:** Twelve multiparous ewes were identified into 2 groups based on their litter number as follow: Single Lamb Ewe (SLE) group include 6 ewes, each one had birth for one lamb and Twin Lamb Ewe (TLE) group include 6 ewes, 5 ewes each one of them had birth for two lambs and one ewe had birth for 3 lambs). **Results:** TLE group ewes had significantly higher total movement and maternal rejection behavioral activities and significantly lower resting and total ingestive behavioral activities than SLE group ewes. In addition, results showed that TLE group at days 0 and 30 postpartum had significantly higher levels of cortisol and prolactin (PRL) and lower calcium level than SLE group. At day 0, the plasma progesterone (P4) and glucose levels were significantly higher in TLE group than that of SLE group. **Conclusion:** Increased litter number may affect negatively on the behavioral and physiological conditions of ewes as it put them under stress.

**Key words:** Sheep, behavior, litter number, physiology, maternal activities, lactation stress, cortisol

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Lactation is considered as the most energy consuming phase along the entire life of female mammals<sup>1</sup>. The modulation in the hormonal secretory patterns in suckling ewes may be a central dominator in determining the rate of mobilization of energetic resource and milk yield due to differences in demand for milk based on differences in litter sizes<sup>2</sup>. The negative energy balance in the term of changes in blood biomarkers is more obvious in ewes nursing more than one lamb and this is may be due to corresponding increase in the milk production<sup>3,4</sup>. Therefore, investigation of changes in the metabolic profiles of lactating ewes in relation to the number of suckling lambs is of outmost significance in directing the attention towards specifying ration formulation according to the dam requirements.

From the welfare point of view, nursing several lambs represents emerging health challenge both on the levels of dam and offspring. Ewes experience higher degree of stress and individually, lambs receive less maternal care and this issue becomes more obvious when triplets and quadruplets are involved<sup>5</sup>.

Ewes rearing twine lambs had higher milk yield, fat mobilization rate and non-esterified fatty acids, 3-hydroxybutyrate, growth hormone and cortisol concentrations and lower insulin concentration than those rearing one lamb without obvious differences in prolactin concentration<sup>2,6</sup>. The changes in the parameters of acid-base balance and liver and kidney functions indicated that nursing twine causes greater challenge to ewes than nursing single lamb<sup>3</sup>. The increase in litter size from two to ten pups in rats was associated with increase in maternal food consumption, prolactin concentration and lipogenic enzymes activity in mammary glands and decrease in insulin concentration<sup>7</sup>. Glucose concentration decreased and progesterone and 3-hydroxybutyrate concentrations increased in crossbred ewes during 24 h post-parturient period with increasing litter size<sup>8</sup>. Although the previous data showed the modulating effects of litter size on maternal lactogenesis and stress indicators, they are quiet insufficient and completely ignore the maternal behavioural and physiological changes in the native Egyptian breeds.

A wide variety of factors influences the expression pattern of maternal care including the sex and age of offspring, genotype and parity of mother, the interval between first and second birth and whether the litters are natural un-manipulated or artificially altered<sup>9-12</sup>. Thus, the aim of this study was to highlight the differences in behavioural and physiological responses of ewes nursing single lamb versus

those nursing multiple ones at day 0 and 30 postpartum. We hypothesized increased stress indicators and hormones involved in milk yield together with depletion of calcium reserve in ewes possess large litter size.

## MATERIALS AND METHODS

**Sheep, housing and management:** This research was conducted from December, 2018 to April, 2019 at private farm in Elwan Village, Assiut, Egypt. The farm contains small sheep flock (20 female and one male). All the study procedures including sheep were approved by the institutional use and care committee, faculty of veterinary medicine, Assiut University, Egypt. The study involved randomly selected (12) multiparous Egyptian native breed (*Ovis aries*) (Ossimi) sheep of the same body scores (4). Twelve multiparous ewes were identified into 2 groups based on their litter number as fellow (Single Lamb Ewe (SLE) group include 6 ewes, each one had birth for one lamb (n = 6) and Twin Lamb Ewe (TLE) group include 6 ewes, 5 ewes each one them had birth for two lambs and one ewe had birth for 3 lambs (n = 6)). All mothers were not separated from the flock along the study, to avoid the effect of isolation on ewe involved in the study. Animals were provided with wheat straw, some green fodders, corn, water and mineral blocks *ad libitum*. The owner of the farm informed us when a sheep give birth in the first 3 days of each month to include it in the experiment and start the data collection from her. To avoid the effect of environment on the animal, we tried to use a fixed number of ewes/group for each month (Table 1). All ewes nursed only their own litter. Ewes were housed in (4 × 10 m) pens (night housing), that is connected with (8 × 10 m) yard where ewes were exposed to normal daylight, fresh air was provided with food and water.

### Welfare measurements

**Behavioral observations:** Definitions of ewe behaviors 'ethogram' recorded during the study represented in Table 2. Observation period extend from the day following lambing day (0D) and ends at the 30D. Two behavioral observation

Table 1: Number of ewes included for the experiment/month

Groups		
SLE	TLE	Month
<b>Ewe number</b>		
1	1	December
2	2	January
2	2	February
1	1	March

Table 2: Detailed ethogram for the behavioral activities recorded in the study

Behaviour	Description	Units
Total movement	Include walking (Ewe walk more than 2 steps), running (run from one place to another) and standing (Ewe stand immobile without doing any other activities)	Percentage
Resting (lying down)	Ewe sit or sleep, eyes closed or opened, do not do any other activities	Percentage
Feeding	Ewe feed plants or concentrates or any feed staff from ground or feeding trough	F h <sup>-1</sup>
Drinking	Ewe drink water from water trough	F h <sup>-1</sup>
Rumination	Ewe ruminate (regurgitation of a food bolus, followed by chewing, swallowing and regurgitation)	F h <sup>-1</sup>
Total ingestive	Sum of feeding, drinking and rumination	F h <sup>-1</sup>
Maternal acceptance	Ewe allows lamb sucking, usually accompanied with willingness	F h <sup>-1</sup>
Maternal rejection	Ewe rejects lamb sucking, usually accompanied with aggression	F h <sup>-1</sup>

techniques were used according to the type of behaviors: (1) instantaneous scan sampling by direct observation, for posture behaviors (%), 2 h daily/3days/week, (2) continuous sampling by direct observation, for ingestive and maternal acceptance (frequency/hour) activities of each ewe was done for 1 h daily/3 days/week. For identification and differentiation of the experimental ewe from the other sheep, colored livestock spray was applied on the head, neck, back and tail; where each ewe and its lamb had the same color. Behavioral observation carried out from 10:00-14:00 h. The observer was sitting (laying down extending his whole body) on the roof of the shedding room, to avoid its effect on the sheep and allow him to see the yard from a top view, giving him the chance to see all animals and their activities in the yard.

**Physiological analysis:** Blood samples were taken twice, one at the first day of the experiment which is first day after parturition (0D) and the second at 30 day of experiment (30D). Twenty milliliter of blood were collected from jugular vein in tubes containing heparin at 10-11 am. For plasma collection, blood samples kept on ice and refrigerated for 30 min, then centrifuged for 12 min at 3000 rpm at 4°C. Plasma samples were stored at -80°C until analyses.

Cortisol level was determined as described previously by Watts and Tindall<sup>13</sup> using cortisol ELISA kit (Diagnostics Biochem Canada Inc., Canada) Cat. No. CAN-C-270. Glucose and calcium (Ca) levels were estimated by commercially available reagent kits (Egyptian Company for Biotechnology, Cairo, Egypt) according to the manufacturer's instructions. Prolactin (PRL) was estimated following Thorner *et al.*<sup>14</sup> by prolactin ELISA kit (PishtazTeb Diagnostics, Germany) Cat. No. MA-PRO-48-02. Progesterone (P4) was analyzed according to Shepard and Senturia<sup>15</sup> as described in the progesterone ELISA kit manufacturer's instructions (ICN pharmaceuticals, Costa Mesa, California, USA) Cat. No. 07B70102.

**Statistical analysis:** The data were expressed as mean ± standard error of the mean (SEM) and tested for normality using Shapiro Wilk test and for variances homogeneity to be sure that the data are normally distributed and variances would be homogenous. Independent samples T test was used for exploring the statistical differences between groups at the same time point. Differences were considered significant at  $p < 0.05$ . The statistical package SPSS for Windows version 16 (SPSS, Richmond, VA, USA) was used for these calculations.

## RESULTS

Figure 1a-c represents the effect of litter number on the postural, ingestive and maternal activities of ewe. Results clarified that TLE group had significantly ( $p < 0.05$ ) higher total movement activities and lower resting activities in compare to SLE group (Fig. 1a). Feeding, drinking and rumination were not significantly different between the groups. While, in sum the total ingestive activities were in TLE group significantly lower in compare to SLE group (Fig. 1b). TLE group had significantly lower ( $p < 0.05$ ) maternal acceptance and higher rejection activities in compare to SLE group (Fig. 1c).

Figure 2a-e represents the effect of litter number on the physiological parameters, TLE group at days 0 and 30 postpartum had significantly higher levels of cortisol ( $p < 0.01$ ) than SLE group (Fig. 2a). While, the plasma glucose level at day 0 was significantly ( $p < 0.05$ ) higher in TLE group than SLE group and the reverse outcome pattern was observed at day 30 postpartum (Fig. 2b). TLE group at days 0 and 30 postpartum had significantly higher levels of PRL ( $p < 0.05$ ) than SLE group (Fig. 2c). At day 0, the plasma P4 level was significantly ( $p < 0.01$ ) higher in TLE group than that of SLE group (Fig. 2d). In comparison with TLE group, the SLE group were characterized by a significant hypercalcemia at days 0 and 30 postpartum ( $p < 0.01$  and  $p < 0.05$ , respectively) (Fig. 2e).

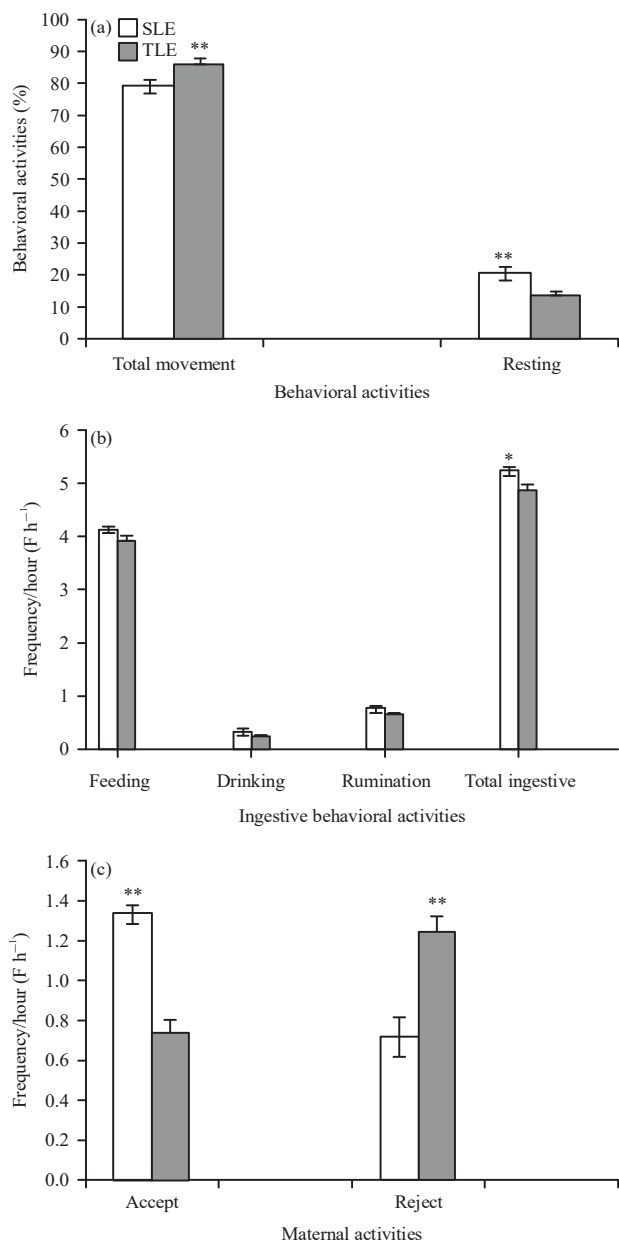


Fig. 1(a-c): Effect of litter size on (a) Ewe posture behaviors, (b) Ingestive behaviors and (c) Maternal behaviors. Plotted values are Mean  $\pm$  SEM, treatments are significantly different at \* $p < 0.05$ , \*\* $p < 0.01$

## DISCUSSION

Current experiment reported that twinning significantly declines the resting and total ingestive behavioral activities of ewes. As well as induce marked increase in standing and total movement which may be attributed to ewes with twins spend more time in nursing and hindering sucking<sup>16</sup>. The present

study shows that twinning is positively related to high rate of maternal rejection. These results harmonized with Teke and Akdag<sup>17</sup>, Hafez and Hafez<sup>18</sup> and Hafez, E. and B. Hafez *et al.*<sup>19</sup>. This may be attributed to ewes with twins receive more sucking attempts than ewes with singles, so udder refusal behavior rate increases with higher sucking attempts. At days 0 and 30 postpartum, the cortisol level in multiple neonate-nursing ewes was higher than single neonate-nursing one in correspondence with the findings of Rhind *et al.*<sup>2</sup> and Bass<sup>6</sup>. This outcome response indicated that exposure of ewe to vigorous stressful condition such as excessive contact between dam and neonates, struggling between the litter and extra stimulation of udder resulted in a profound stimulation of hypothalamic-pituitary-adrenal (HPA) axis. According to our findings, high cortisol level in these ewes might a response to hypocalcemia which also was found in this study as cortisol helps in recovery of Ca homeostasis by increasing<sup>20</sup> synthesis of 1, 25-dihydroxy vitamin D<sub>3</sub>. Cytokines and lipopolysaccharide binding proteins are strong inducers that signal HPA axis to release cortisol during dehydration, feed restriction and chronic restraint stresses<sup>21-23</sup> which were observed in the ewes nursing multiple lambs during examination of ingestive and posture behaviour in the current study. In contrast, a previous investigation found that twinning had no effect on cortisol level and its elevation at the first post-parturient 15 days may be attributed to lambing process; suggesting that cortisol level could not be used as a reliable indicator for assessing stress caused by the twinning in lactating ewes<sup>5</sup>. These conflict data may be related to differences in the time of sampling or number of lambs, single versus twine in the previous study and single versus multiple (2-4 lambs) in our study.

Hyperglycemia in ewes nursing multiple lambs versus those nursing singlet at the day 0 is parallel to the decrease in insulin levels in twin-rearing ewes in the studies of Rhind *et al.*<sup>2</sup> and Bass<sup>6</sup>, but is contradictory to Quesnel *et al.*<sup>24</sup> who demonstrated absence of changes in glucose profile in pigs nursing different litter size. The hyperglycemia in the current study may be secondary to stress-associated cortisol hypersecretion. Cortisol is responsible for hyperglycemia by inhibition of insulin secretion and glucose uptake in peripheral tissues, activation of key enzymes involved in hepatic gluconeogenesis and increase of the glucogenic precursors as amino acids through muscle proteolysis<sup>25-27</sup>. High levels of PRL in multiple-nursing ewes according to our finding could be also implicated in shifting glucostatic homeostasis towards the hyperglycemic side by increasing hepatic insulin resistance<sup>28</sup>.

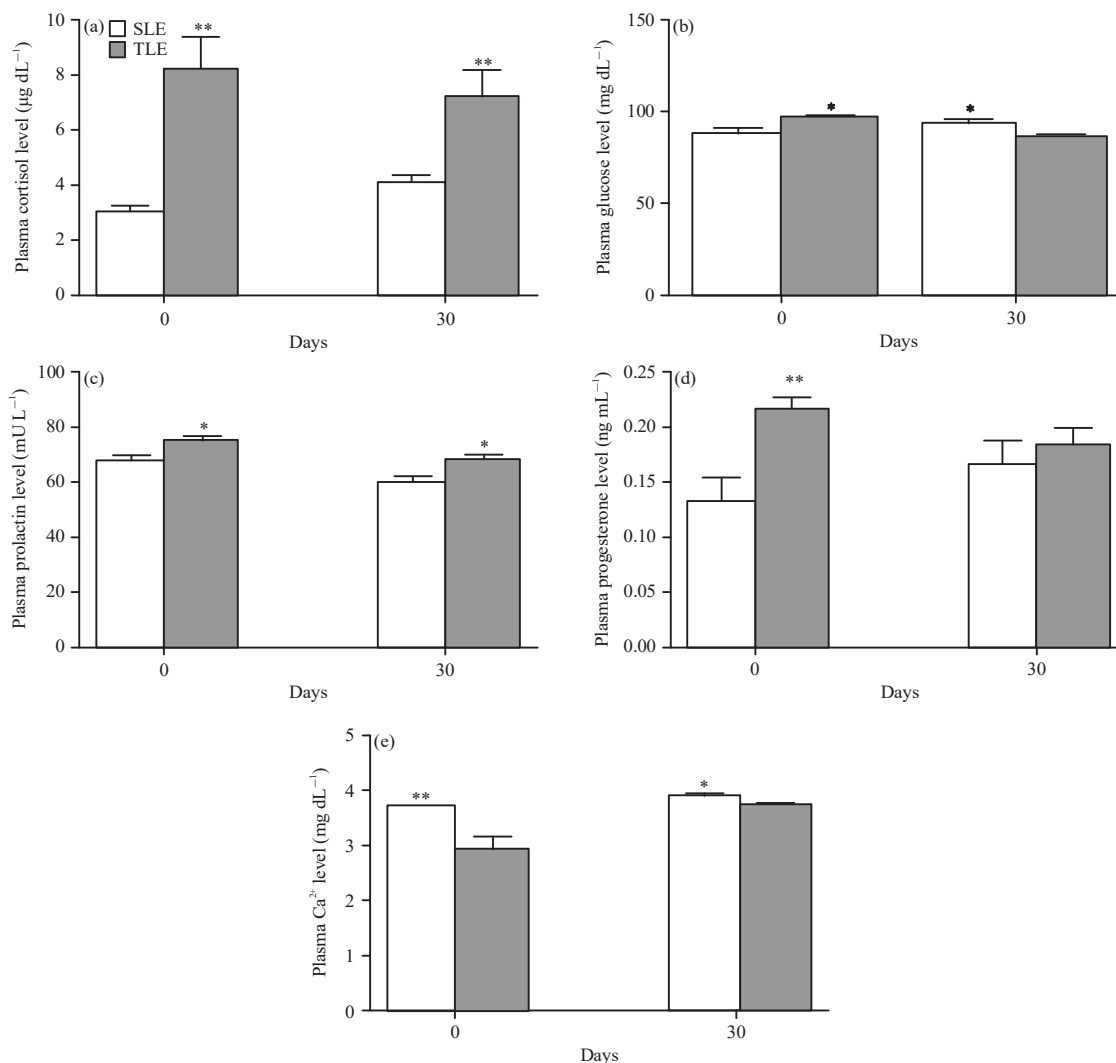


Fig. 2(a-e): Effect of litter size on (a) Plasma cortisol, (b) Glucose, (c) Prolactin, (d) Progesterone and (e) Calcium level in sheep ewes

Plotted values are square Mean ± SEM, treatments are significantly different at \*p < 0.05, \*\*p < 0.01

However, other investigators suggested the anti-diabetogenic nature of PRL through enhancing insulin synthesis, potentiating insulin secretory capacity, protecting pancreatic islets from beta cells inhibitory effectors and accelerating the rate of glucose metabolic utilization<sup>29,30</sup>. According to our findings, a significant elevation of P4 in the blood of multiple lambs-nursing ewes at day 0 could be one of the underlying hyperglycemic factors by impairing insulin sensitivity and suppressing hepatic glucose production, muscle and adipocyte glucose uptake and pancreatic insulin secretion<sup>31-33</sup>. In the contrary, ewes nursing multiple lambs at the day 30 suffered from hypoglycemia. The hypoglycemic effect of prolonged stress as a result of nursing more than one lamb for

one month may surmount the well-known diabetogenic action of the above-mentioned counter-regulatory hormones. This gives insight into exhaustion of energy substrates, mainly glucose, reflecting exposure of these dams to great challenge. Actually, higher demand of milk production in twin-lamb ewes increases the metabolic channeling of glucose towards intense milk yield which may lead to hypoglycemia and increased susceptibility to negative energy balance<sup>4,34</sup>. The observed hypocalcemia in ewes nursing multiple lambs along the whole period of the current study may be involved in decreasing the endogenous production of glucose by enhancing the rate of glucose turnover and suppressing insulin secretion<sup>35-37</sup>. The switching

from hyper- to hypoglycemic status at 0 and 30 days, respectively, may be due to positive correlation of milk production with lamb growth<sup>38</sup>, resulting in depletion of the maternal blood glucose along with progressive increases in both the production of milk and growth rate of lambs. In consistent with a previous result in rats<sup>7</sup>, PRL level in ewes nursing multiple lambs at the two time points postpartum was higher than those nursing single one. Due to the direct causative relationship between suckling stimulus and reflex PRL secretion<sup>39</sup>, higher PRL levels in the blood of multiple neonates-nursing ewes was found owing to frequent suckling stimuli which in turn lead to lower secretion of PRL inhibitory factor from the hypothalamus and increase the response of the pituitary to PRL releasing factor<sup>40</sup>. Increased P4 level at day 0 in ewes nursing multiples neonates are in the same line with that observed by Hall and his colleagues<sup>8</sup>. This is may be corresponding to the utilization of adrenal P4 as a valid reflector of acute stress<sup>41</sup>. Suckling induces high magnitude of pre-estrus P4 peak in postpartum beef cows<sup>42</sup>, so it was hypothesized that intensive suckling by multiple lambs may be one of the causative mediators in magnifying the secretory pattern of P4. According to *in vitro* studies, cortisol stimulates P4 secretion from follicular granulosa cells by inducing  $3\beta$ -hydroxysteroid dehydrogenase and suppressing  $20\alpha$ -hydroxysteroid dehydrogenase<sup>43,44</sup>. Taken into consideration that P4 causes sedative, anxiolytic and HPA-axis-suppressing effects<sup>45,46</sup>, it has been suggested that P4 are secreted during stress in order to down-regulate HPA axis activity and anxiety<sup>47,48</sup>. Therefore, the increase in P4 levels in multiple nursing ewes in parallel with the increase in cortisol levels could imply adaptation to suckling stress by trying to restore the normal level of HPA axis activity. The increase in PRL level could trigger P4 secretion in multiple-nursing ewes based on the fact that PRL can directly stimulate ovarian P4 secretion and acts as a luteotropic hormone by maintaining corpus luteum function<sup>49,50</sup>. Ewes nursing more than one lamb were characterized by hypocalcaemia at day 0 and 30 postpartum in the same line with previous finding in Lori-Bakhtiari ewes<sup>35</sup>. Taken into account that milk production is higher in ewe nursing twin than that nursing single<sup>4</sup>, exhaustion of maternal Ca reserve which directed towards milk synthesis can result in hypocalcaemia. The hypocalcaemia may originate also from insufficient calcium dietary requirement to cope with fetal skeletal needs during pregnancy together with impaired osteolysis due to increased estrogen secretion in late gestation<sup>35</sup> and this decline may continue during the early lactation period especially in ewe carrying more than one fetus. Also, it is noteworthy that cortisol impairs Ca

homeostasis by reducing its intestinal absorption and urinary excretion<sup>51</sup>. Thus, it can be concluded that lactating ewes with multiple lambs debilitate high amount of Ca for bone formation and growth of neonates and production of milk during pregnancy and lactation and these requirements lead to hypocalcaemia if no dietary adjustments is taken into consideration.

## CONCLUSION

The study demonstrates that nursing twins act as stressor at early lactation in ewes. The attitudes of stress fade as lactation progress. This was indicated by the decline in both cortisol and glucose level at the end of the study and coincide with the beginning of lamb eating. Furthermore, twinning seems to increase lactogenesis and milk production indicated by superior plasma prolactin and inferior plasma calcium level when compared with corresponding ewes with singles. In addition, twinning seems have effect on both posture and ingestive behaviors. Twinning was positively related with high maternal rejection frequency which seems to be only a reflection to positive correlation between sucking attempts and udder rejection. But it does not indicate the complete rejection of lambs. Also, it may indicate that suckling twins lead to higher rate of udder emptying.

## SIGNIFICANCE STATEMENT

This study reported the possible effects of nursing several lambs on the behavioral and physiological indicators in ewes. This study will help the researcher to uncover the critical area of stress results from such condition that many researchers were not able to explore. Thus, a new managerial or nutritional strategy may be established in future to improve the ewe's health and reduce the negative effect of such stress condition.

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