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Neonatal lamb behaviour and thermoregulation with special reference to thyroid hormones and phosphorous element: Effect of birth weight and litter size

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New-born lambs have limited energy reserves and need a rapid access to colostrum to maintain homeothermy and survive. The object of this study was to investigate the importance of neonatal lamb behaviour in the maintenance of its body temperature and survival. The time taken to stand and suck after birth and rectal temperatures were determined in 72 crossbred lambs (progeny of crossing 1/2 Rhamani and 1/2 Finnish). In addition, blood samples were collected at 1, 24 and 72 h after birth. The obtained sera were assayed for thyroid hormones (T_3 and T_4), known to be involved in heat production and phosphorous element (ph) which is essential for energy metabolism. The obtained results revealed that, low birth weight and triplet lambs were behaviourally less active at birth and had less T_3, T_4, Ph and body temperatures values over the first 3 days of life than lambs of heavy or medium birth weight and single or twin lambs. Thus, light weight and triplet lambs were highly susceptible to hypothermia and were exposed to high rate of mortality (18.75 and 16.66%) during the neonatal period than other lambs (3.84, 10.00, 4.55, and 9.38 %, respectively). Therefore, the present study concludes that neonatal lamb behaviour, specifically success in standing and obtaining colostrum are extremely important for thermoregulation and survival of the neonate.

The world wide rate of lamb mortality is typically around 15% (Purser and Young, 1983; Wassmuth *et al.,* 2001; Darwish, 2004). This rate of mortality is of concern both from an economic and welfare perspective **(**Christley *et al.,* 2003). Most lamb deaths result from a failure in bonding between the ewe and lamb (Dwyer and Lawrence, 1998; Dwyer, 2003), thus research in ewe and lamb behaviour could help to improve lamb survival.

The majority of lamb deaths occur within the first three days after birth (Rowland *et al.,* 1992; Nowak *et al.,* 2000; Darwish, 2004). Traditionally, lamb mortality has been considered to be due to failures in ewe maternal care (Gubernick, 1981). However, the role of neonate behaviour in ensuring survival becomes increasingly important and may be at least as important as that of the mother (Nowak and Lindsay, 1990; Dwyer and Lawrence, 1999; Morris *et al.,* 2000). Lamb birth weight and type of birth significantly influenced the pre- weaning growth rate and mortality of the lamb (Tuah and Baah, 1985; Bekele *et al.,* 1992).

Low birth weight lambs from higher litter sizes were at greater risk for most causes of death than heavier lambs from smaller litters (Hinch *et al.,* 1986).

The ability to survive is crucially dependent on the response of the lamb to the climatic environment into which it is born (Alexander *et al.,* 1980; Slee, 1981; Slee and Springbett, 1986; Symonds *et al.,* 1989; Mellor and Stafford, 2004). Lambs are born, often into cold or wet conditions, with low fat cover and have a high surface area to body weight ratio, which exacerbates heat loss (Eales and Small, 1995; Stephenson *et al.,* 2001). Therefore, the newborn lamb must produce as much heat as it loses to maintain its body temperature (Mellor and Stafford, 2004). This could be supplied by oxidation of fat from brown adipose tissue (BAT) by a process under the control of triiodothyronine (T_3) which produced from thyroxine (T_4) in BAT by the enzyme 5-monodeiodinase (Dauncey, 1990; Brent, 1994) and also by showing the appropriate neonatal behaviour to obtain colostrum which is extremely important (particularly important for lambs born into cold, wet areas where its body reserves will be rapidly used up; Nowak and Poindron, 2006). In addition to its immunoglobulin, colostrum provides the lamb with fuel to maintain body temperature (Hall *et al.,* 1992; Al-Jassim *et al.,* 1999 Charismiadou *et al.,* 2000).

Developing young organisms have high requirements for phosphorus for the mineralization of the skeleton and maintenance of metabolism and growth (Scott *et al.,* 1994). Animals that are able to stand up and suck rapidly within a short time after birth, implies a well-developed bone mineralization and adequate energy metabolism for mobility and thermogensis (Scott *et al.,* 1997; Huber *et al.,* 2002). Thus, it seems that rapid access to the udder and early suckling are intimately associated with the ability to thermoregulate. Therefore, the current study aimed to investigate the role of neonatal lamb behaviour in its thermoregulation and survival.

Materials and methods

Animals. This study was carried out in Sakha Animal Production Research Station, Kafr El-Sheikh Governorate, Egypt. Data were collected from 72 crossbred lambs (progeny of crossing 1/2 Rhamani and 1/2 Finnish) born to 44 crossbred multiparous ewes, in parity 4. Ewes were pregnant by natural insemination and had an average body weight of 47.43 kg prior to pregnancy and 51.71 kg at parturition with 5.57 years an average age. Ewes were given access to green fodder (Trifolium Alexandrium) and fresh drinking water *ad-libitum.* Concentrates (cotton seed cake, Soya bean cake, corn, limestone and mineral mixture) containing 16.58 % crude protein, 12.68 % crude fiber and 73.38% TDN were provided during the last 6 weeks of pregnancy at levels appropriate for body weight and stage of pregnancy. In week 17^{th} of pregnancy, ewes were vaccinated with a clostridial vaccine (covexin, 2ml, s/c, Schering-plough co.).

At approximately 2 weeks prior to the expecting lambing time, ewes were transported into large straw-bedded pens (7x10 m), in groups of approximately 10-12 ewes / pen for lambing. Ewes were habituated to the presence of observer in the central walk ways between pens before parturition and as lambing approaches, the pregnant ewes were kept under 24-h surveillance for the exact time of lambing. Ewes need assistance at delivery were ignored from the current study. Ewes were remained in the lambing pens for 3 days after birth. Afterwards, they were moved outside to the pasture and lambs were clearly identified by ear tag.

Data recording. Data of the present study were collected from lambs born in the period between 2.00 to 6.00 a. m. during the winter season of lambing (February-March, 2007) with an average ambient temperature of 9.4 C^o (range = 7 to 12 C^o). These data including:

Behavior. Neonatal lamb behaviour was recorded for the first 2 h after birth using a video camera. The definitions of behaviours recorded were given in table 1 according to Dwyer (2003).

Table (1): Definitions of the lamb behaviours recoded.

Behaviour element	Definition
Stands	Lamb supports itself on all four feets for at least 5 seconds.
Successful suck	Lamb holds teat in its mouth and appears to be sucking with appropriate mouth and head movements, may be tail-wagging, remains in this position for at least 5 seconds.

Birth weight. Lambs were weighed by digital weighing machine at 2 h of birth. Lamb birth weights were divided into 3 categories based on the number of standard deviations (SD) above and below the mean birth weight (Dwyer and Morgan, 2006).

Heavy lambs.Weighing more than 1 SD above the group mean (including lambs weighing more than 3.25 kg).

Medium lambs. Weighing 1 SD above and below the group mean (including lambs weighing in between 2.62 - 3.25 kg).

Light lambs. Weighing more than 1 SD below the group mean (including lambs weighing less than 2.62 kg).

Neonatal lamb mortality: Lamb mortality was recorded during the neonatal period of first week of lamb's life.

Blood assay.

Sampling. One hour after birth, the lamb was caught and a 2-ml blood sample was taken by jugular venipuncture into non heparinized vacutainer. Rectal temperature was also recorded at this time. To minimize the disruption to ewe and lamb bonding, all samples were collected within the home pen of the animal, which allowed the ewe to continue to interact with the lamb during sampling, and the sample was collected within 2 minutes of entering the pen. Blood samples were then centrifuged (3000 g / 20 minutes). The obtained sera were separated and stored frozen at–20°C until assayed for T_3, T_4 and Ph. The blood sampling and temperature recording procedures were repeated at 24 and 72 h after birth (Cabello and Levieux, 1981 and Schermer *et al.,* 1996).

Analytical procedures. Concentration of T_3 and T_4 hormones was determined using a solid phase competitive chemiluminescence immuno-assay system (Elecsys 2010, Roche, Diagnostic, Mannheim). Concentrations were determined using kits, controls, mono-clonal mouse antibodies and reagent supplied by Roche, Diagnostic, 2005 .The intra – and inter assay coefficients of variation (C.V. %) were 3.6 and 5.4% for T_3 and 4.7 and 6.9 % for T_4.`The minimum detectable levels of the assay were 0.195 ng /ml and 0.42 µg /dl for T_3 and T_4 respectively. Phosphorus concentration was measured by a colorimetric test using commercial kits (Spin react, S.A., Spain), according to the manufactures instructions .The sample absorbance was measured in spectrophotometer (Spectonic, USA) at wave length of 710 nm. The results were expressed in mg /dl. All samples were assayed in triplicate. The intra- and inter assay C.V. % were 2.3 and 1.2 respectively. Detection limit ranged from 0.008 -15 mg /dl .The sensitivity was 1 mg /dl.

Statistical analysis. The least square analysis procedure (SAS, 1996) was used to test the effect of litter size (single, twin and triplet) and birth weight (heavy, medium and light) on neonatal lamb behaviour, T_3, T_4 and phosphorous. The difference in temperature was studied by using Duncan Multiple Range test while the relationship between neonatal lamb behaviour and T_3, T_4, Ph and body temperature was investigated by regression analysis.

Results and Discussion

Neonatal lamb behavioural progress.

Litter size effect. In the current study, the behavioural data demonstrated that, triplet lambs were clearly compromised in comparison with single or twin lambs (Table 2), and this was most evident in the more co-ordinated behaviours of standing (15.21±0.48, 12.67±0.82 and 11.63±0.70 min, respectively, $P<0.01$), and sucking (37.40±1.55, 23.59±3.34 and 21.94±1.19 min, respectively, $P<0.001$), independent of the effects of litter size on lamb birth weight or birth difficulty. Moreover, twin and triplet lambs were observed to get less colostrum from the ewes (13.31±1.53 and 8.58±1.28 min / 2 h, respectively, $P<0.05$) than single lambs (15.54±1.61 min), due to competition and limited supply, as single lamb had higher oppournity for more successive overall sucking attempts than the individual twin or triplet lamb (O'Connor and Lawrence, 1992; Dwyer, 2003).

To suck successfully, the new born lamb must be able to stand and move to the udder (Cloete *et al.,* 1993; Nowak and Poindron, 2006). Triplet lambs have subjected to placental insufficiency (Darwish *et al.,* 2007) and foetal hypoxia before birth, which will affect the maturation of neural development, resulting in brain function deficits (Mallard *et al.,* 1998; Rees *et al.,* 1998). These factors may contribute to the greater behavioural retardation of these lambs than can be accounted for by their low birth weight alone (Kaulfuss *et al.,* 2000).

Birth weight effect. The behaviour of the neonatal lambs recorded in this study (Table 3) will support previous results in a different population of lambs (Darwish, 2004). Lambs with higher birth weight had higher vigour and suckling drive than smaller ones. An increase in lamb birth weight was associated with an increase in speed of standing (16.15±0.26, 12.93±0.37 and 10.02±0.84 min, respectively, $P<0.001$) and sucking (40.81±0.28, 25.10±1.85 and 19.72±0.85 min, respectively, $P<0.001$). Consequently, total time spent sucking / 2 h could also increased (7.43±0.61, 12.65±1.12 and 17.15±2.31 min / 2 h, respectively, $p<0.05$), as has been shown before (Rathray, 1987; Kuchel and Lindsay, 1999).This suggests that there was a delay in behavioural and physical development associated with low birth weight (Mourad *et al.,* 2001).

Thermoregulation of neonatal lamb.

Litter size effect. In the present study, in lambs which survived, serum concentrations of T_3, T_4 and Ph were reduced with increasing litter size (Table 4). Triplet lambs had less concentration in comparison with single or twin lambs. Moreover, these lambs had lower temperatures (hypothermia) over the first 3 days of life, which was in addition to any influence of their lower birth weight (Barlow *et al.,* 1987; Mellor and Stafford, 2004). Phosphorus is known to be essential for growth and energy metabolism (Huber *et al.,* 2002).To meet the higher Phosphorus requirements of developing lamb, there must be adequate phosphate absorption or reabsorption (Schroder and Breves, 1996).The higher concentration of Phosphorus recorded in single and twin lambs compared with triplets may be attributed to higher incidence of absorption of Phosphorus from colostrum intake in these lambs after birth.

Foetal adipose tissue development is known to be very sensitive to foetal nutrition in late gestation (Shrestha and Heaney, 1990; Budge *et al.,* 2003). Triplet lambs have experienced some degree of under nutrition in utero (Kaulfuss *et al.,* 2000). Thus, in triplet, foetal adipose tissue development would impaired associated with low T_3, T_4 and less ability to produce heat by non sheivering thermogensis after birth and were thus very susceptible to hypothermia (Cabello and Levieux, 1981 ; Wrutniak *et al.,* 1990; Budge *et al.,* 2004). In addition, they were slower to stand and suck after birth. For that, they were less able to make use of behavioural strategies to maintain their body temperature (Nowak and Poindron, 2006), due to less colostrum intake recorded in these lambs that is extremely important for energy production supplied by oxidation of its fat content by thyroid hormones (Dauncey, 1990; Brent, 1994).

Birth weight effect. This study has shown that, low birth weight lambs had less T_3, T_4, Ph and also were at greater risk of hypothermia over the first 3 days of life than heavier lambs (Table, 5) and this was markedly observed after birth and at 24 h of life, although, there was also still a tendency for birth weight to influence these measures at 72 h after birth. The greater concentration of serum T_3, T_4 and Ph recorded in heavy lambs, suggests that both their thyroid and brown adipose tissue (BAT) were more active than light lambs and thus cold thermogensis was more efficient in heavy lambs (Symonds *et al.,* 1989; Stephenson *et al.,* 2001

The lesser ability of low birth weight lambs to maintain body temperature can be at least partly attributed to their small body size and therefore greater relative surface area for heat loss and reduced body reserves of lipid and glycogen but mainly attributed to poor thermogenic capacity due to delay in access to the udder and suckling to obtain colostrum that provides the lamb with fuel to maintain body temperature (Rathray *et al.,* 1987 ; Gama *et al.,* 1991; Budge *et al.,* 2000; Mourad *et al.,* 2001). This finding was underlined by low concentrations of thyroid hormones (T_3 and T_4) and Phosphorus element recoded in these lambs which known to be essential for energy metabolism (Huber *et al.,* 2002).

The relationship between neonatal lamb behaviour and thermoregulation. In the present study, neonatal lamb behaviours of standing and sucking were negatively correlated with measures of thermoregulation (T_3, T_4, Ph and body temperature) while the time spent sucking during the first 2 h following birth was positively correlated with these measures (Table, 6), demonstrating that behavioural progress and body temperature appear to be linked. Lambs with a low T_3, T_4, Ph and rectal temperatures were more likely to fail to reach the udder after birth than those with normal temperatures (Slee and Springbett, 1986). Thus, behavioural mechanisms may operate to prevent falls in temperature. Standing rapidly after birth helps to reduce convective heat loss of the wet lamb to the ground and sucking bouts or feeding raise body temperature (Bird *et al.,* 2001).Thus, the rapid behavioural progress of single and twin lambs compared with triplets and of the heavier lambs compared with low birth weight lambs seen in the current study will have contributed directly to their greater temperatures (Rathray *et al.,* 1987; Hancock *et al.,* 1996; Mellor and Stafford, 2004).

Neonatal lamb behaviour and mortality. Lamb deaths are known to be highest in the first few days of life, and may be related to behavioural deficiencies in neonate lambs (Nowak *et al.,* 2000) as is also shown in the present study (Table, 7), suggesting events occurring at this time are of particular importance in lamb survival (Dwyer, 2003).

The single greatest contributor to lamb mortality is lamb birth weight (Lindsay *et al.,* 1990; Fogarty *et al.,* 2000). Increasing birth weight was associated with decrease in lamb mortality (18.75, 10.00 and 3.84 %, respectively, $P<0.001$). Other contributor to lamb mortality is litter size, which can not be totally explained by birth weight (Knight *et al.,* 1988). Litter size had a major effect on mortality rate, as single and twin lambs were much less likely to die (4.55 and 9.38%) than triplets (16.66%, $P<0.01$). Therefore, low birth-weight and born in multiple litters were two significant risk factors for lamb deaths (Christley *et al.,* 2003). In addition, the effects of placental insufficiency (Kaulfuss *et al.,* 2000; Mellor and Stafford, 2004; Darwish *et al.,* 2007) and insufficient plasma levels of thyroid hormones and phosphorus element with hypothermia demonstrated in these lambs due to slower neonatal behavioural progress could explain their higher rate of deaths (Cabello and

Table (2): Effect of litter size on neonatal lamb behavioural progress.

Variable / Litter size	N	Time to first stand (min)	Time to first suck (min)	Time spent sucking / 2 h (min)
Single	22	11.63±0.70[B]	21.94±1.19[B]	15.54±1.61[a]
Twin	32	12.67±0.82[B]	23.59±3.34[B]	13.31±1.53[ab]
Triplet	18	15.21±0.48[A]	37.40±1.55[A]	8.58±1.28[b]
S.O.V.			**Mean square**	
Treatment		30.36**	438.435***	71.20*

Capital letters means within the same column carry different letters are significantly different at $P<0.01$ or $P<0.001$.
Small letters means within the same column carry different letters are significantly different at $P<0.05$.
* $P<0.05$ ** $P<0.01$ *** $P<0.001$ S.O.V. Source of variance

Table (3): Effect of lamb birth weight on neonatal behavioural progress.

Variable / Birth weight	N	Time to first stand (min)	Time to first suck (min)	Time spent sucking / 2 h (min)
Heavy	26	10.02±0.84C	19.72±0.85B	17.15±2.31a
Medium	30	12.93±0.37B	25.10±1.85B	12.64±1.12b
Light	16	16.15±0.26A	40.81±0.28A	7.43±0.61c
S.O.V.			**Mean square**	
Treatment		56.45***	452.2***	95.38*

Capital letters means within the same column carry different letters are significantly different at P<0.001.
Small letters means within the same column carry different letters are significantly different at P<0.05.
* P<0.05 *** P<0.001 S.O.V. Source of variance

Table (4): Effect of litter size on lamb thermoregulation measures.

Variable / Lamb age		T_4 (µg /dl)	T_3 (ng /ml)	Phosphorous (mg /dl)	Temperature (ºC)
At 1h	**Single**	9.55±0.67A	3.77±0.12A	7.83±0.09A	39.55±0.23a
	Twin	8.86±0.18A	3.65±0.07A	7.67±0.19A	39.45±0.08a
	Triplet	6.80±0.39B	3.1±0.11B	6.67±0.13B	39.06±0.07b
		P<0.001	P<0.01	P<0.01	P<0.05
At 24h	**Single**	10.62±0.70A	4.52±0.25a	9.67±0.92a	39.41±0.15a
	Twin	9.55±0.43A	4.19±0.26a	8.91±0.39a	39.32±0.15a
	Triplet	6.42±0.15B	3.54±0.23b	7.85±0.53b	38.30±0.30b
		P<0.001	P<0.03	P<0.05	P<0.03
At 72h	**Single**	10.88±0.47A	4.92±0.26a	9.49±0.94a	39.46±0.13a
	Twin	10.03±0.37A	4.77±0.28ab	8.75±0.44ab	39.40±0.06a
	Triplet	7.41±0.20B	4.34±0.06b	7.68±0.57b	38.93±0.03b
		P<0.001	P<0.05	P<0.05	P<0.06

Capital letters means within the same column carry different letters are significantly different at P<0.01 or P<0.001.
Small letters means within the same column carry different letters are significantly different at P<0.05.

Table (5): Effect of lamb birth weight on its thermoregulation measures.

Variable / Lamb age		T_4 (µg /dl)	T_3 (ng /ml)	Phosphorous (mg /dl)	Temperature (ºC)
At 1h	Heavy	9.60±0.43A	3.80±0.11A	7.94±0.13a	39.7±0.15a
	Medium	8.39±0.34A	3.55±0.08A	7.77±0.13a	39.52±0.11a
	Light	6.76±0.51B	3.10±0.15B	6.71±0.24b	39.11±0.21b
		P<0.001	P<0.001	P<0.02	P<0.04
At 24h	Heavy	11.03±0.33A	4.89±0.11A	10.1±0.50A	39.62±0.08a
	Medium	7.58±0.62B	3.97±0.04B	8.45±0.23B	39.22±0.21ab
	Light	7.53±0.59B	3.36±0.21C	7.67±0.24B	38.88±0.23b
		P<0.001	P<0.01	P<0.01	P<0.03
At 72h	Heavy	11.12±0.16A	5.38±0.12a	10.03±0.48a	39.55±0.11a
	Medium	8.52±0.66B	4.41±0.05b	8.81±0.21b	39.35±0.10ab
	Light	8.28±0.44B	4.26±0.05b	7.90±0.24b	39.23±0.08b
		P<0.01	P<0.02	P<0.05	P<0.05

Capital letters means within the same column carry different letters are significantly different at *P*<0.01 or *P*<0.001.
Small letters means within the same column carry different letters are significantly different at *P*<0.0

Table (6): The relationship between neonatal lamb behaviour and thermoregulation.

	Time to first stand	Time to first suck	Time spent sucking / 2 h
T_4	-0.578**	-0.65***	0.556**
	P<0.01	*P*<0.001	*P*<0.01
T_3	-0.43*	-0.52**	0.588**
	P<0.05	*P*<0.01	*P*<0.01
Phosphorus	0.468*	-0.66***	0.498*
	P<0.03	*P*<0.001	*P*<0.03
Temperature	-0.409*	-0.597**	0.47*
	P<0.05	*P*<0.01	*P*<0.03

* *P*<0.05 ** *P*<0.01 *** *P*<0.001

Table (7): Neonatal lamb behaviour and mortality over the first week of life.

Treatment	Litter size			Birth weight		
Variable	Single	Twin	Triplet	Heavy	Medium	Light
Mortality %	4.55	9.38	16.66	3.84	10.00	18.75
Chi-square		10.15**			14.75***	

** P<0.01 *** P<0.001

Levieux, 1981; Barlow *et al.,* 1987; Symonds *et al.,* 1989; Hancock *et al.,* 1996; Schermer *et al.,* 1996; Budge *et al.,* 2000).

In conclusion, this study has demonstrated that lambs that are behaviouraly less active at birth are also less able to maintain their body temperatures after birth. Although part of their lower body temperature might be attributable to behavioural influences on thermoregulation, the data also suggests that physiological and biochemical differences exist between these animals. These differences may be related to different degree of maturity at birth.

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علاقة سلوك الحملان عقب الولادة بثبات درجة حرارتها ومعدل و فياتها و بيان تأثرها بنوع الولادة ووزن الحملان عند الولادة.

يعتبر سلوك الحملان بعد الولادة أحد أهم العوامل التي تؤثر على ارتباطها بنعاجــها ووصولها إلى الضرع للحصول على السرسوب الغنى بمكوناتة ومن ثم يؤدى ذلك الى ثبات درجة حرارة هذة الحملان وتقليل معدل وفياتها٠ ولهذا أجريت هذة الدراسة لبيان أهمية سلوك الحملان وما يصاحبه من بعض التغيرات الفسيولوجيه والكيميائيه ومدى تاثره بعدد ووزن الحملان عند الولادة وقد أسفرت النتائج عن الآتي :-

١- تأثر سلوك الحملان عقب الولادة بنوع الولادة ووزن ولادة الحملان حيث كانت الحملان الناتجة من الولادة الثلاثية و الحملان ذات الوزن المنخفض أقل نشاطاً عن بــاقي الحملان واستغرقت وقت أطول في الوقوف والرضاعة مما أدى إلى تأخر وقلة رضاعتها ٠

٢- أدى تأخر وقلة الرضاعة في الحملان الناتجة من الولادة الثلاثية والحملان ذات الوزن المنخفض إلى انخفاض مستوى هرمونات الغدة الدرقية وعنصر الفوسفور المرتبطين بإنتاج الطاقة مما سبب إنخفاضاً في درجة حرارتها على مدى الثلاث أيام الأولى من الولادة وكان لذلك أثرة في ارتفاع نسبة وفيات هذة الحملان (١٦,٦٦ و ١٨,٧٥%) خـــلال الأسبوع الأول من الولادة عن باقي الحملان سواء الفردي (٤,٥٥%) أو الـــــتوأم (٩,٣٨%) وأيضا عن الحملان ذات الوزن المرتفع (٣,٨٤%) أو متوسطة الوزن (١٠%) ٠