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Association Between Lambing Performance and Reproductive Hormones in Mongolian and Poll Dorset Ewes

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Abstract: Association between lambing performance (single lamb and twins) and reproductive hormones has been explored. Twenty four ewes divided into 4 groups of each 6 individuals were sampled according to the number of lambs in two breeds (Mongolian ewes and Poll Dorset ewes) blood samples from each individual ewe were collected at the three periods: estrus (day 0) estrus (day 11) and post-partum anoestrus. All the seven hormones concentration of the Poll Dorset ewes was higher than that of the Mongolian ewes in the three periods. And the hormones secretion shown different patterns in two breeds. Five hormones concentrations of ewes with twins were higher than that of ewes with single lamb, the reverse situation found in MT and T concentration even a few of them were statistically significant ($p<0.05$) at the three periods. Finally, the highly positive and negative significant correlations between hormones were detected, this relationship also shown the differences in two breeds.

Kew words: Mongolian ewes, lambing performance, reproductive hormones, correlation, breeds

INTRODUCTION

Sheep is one of the major livestock breeding in the world. Sheep industry is also the important industry and the economic source of the farmers and herdsmen. China is the world's biggest sheep country not only on the number of sheep breeds but also the sheep varieties. Sheep breeding performance is one of the most important economic characters. Among which lambing performance is the main factor affecting the production efficiency. Ewes lambing performance exists three ways: a birth of a lamb (single tire ewes lambing trait, ewe life every tire production one lamb), a births with double lambs (lambing trait of double ewe lambs also known as twins or double lamb traits) more than double lambs (lambing trait of ewe lamb, ewes had once in a lifetime in a births three or more than three sheep lambs said multiples ewes) (Piper *et al*., 1985). In China, there exists big difference in lambing performance among those different sheep breeds. For example, Small Tail Han sheep and Hu sheep produce three or more than three sheep lambs in a birth others are mainly single fetal sheep breeds (Mongolian sheep), among which double lambs rate is below 5%. What's more, the latter breeds are well adapted to the local environment. Taking all factors into consideration, either little efficiency in single lamb or poor condition in more than double lambs. With the intensification of sheep production systems, the twinning trait is regarded as the ideal lambing performance favoring by sheep industry (Zhang *et al*., 2012).

Several factors determine and influence the lambing performance in sheep. Nutrition and genetics play an important role in follicular development through a variety of endocrine, neural, metabolic and genetic mechanisms (Mandiki *et al*., 1990; Mitchell *et al*., 2003; Davis, 2005; Mateescu *et al*., 2009; Abecia *et al*., 2012; Sejian *et al*., 2012; Long *et al*., 2013). Reproductive hormones play important roles in the estrous cycle while the estrous cycle of the ewe is essentially analogous to the ovarian cycles of other spontaneously ovulating species (Nett, 1987). Considerable attention has been given to reproductive hormonal patterns that occur throughout the estrous cycle (Jackson and Davis, 1979; Casao *et al*., 2010). In Romney ewes, some experience twin ovulations show higher blood concentrations of FSH than others have one during the luteal phase for a 24 h interval within the 48 h before the onset of luteolysis (McNatty *et al*., 1984). Evidence in support of this notion is provided in several studies which show that the reproductive hormones levels with the ovulation. However, little information is available on the relationship between lambing performance and reproductive hormones levels in

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many other breeds. Currently, it is still unclear how well the reproductive hormones levels work in predicting lambing performance although, this may be evaluated by empirically. The aims of this study were to explore the relationship between lambing performance and reproductive hormones levels. The difference in different breeds was also tested. And the correlations effects of the reproductive hormones were also assessed.

MATERIALS AND METHODS

Animals and treatments: Healthy multiparous (2-3 years) Mongolian ewes and Poll Dorset ewes were collected from the Mongolian sheep Stock Seed Farm, Gansu Province, China. The average body weight was 35-40 kg. All animals were handled in accordance with the Guidelines for Care and Use of Experimental Animals (Gansu Agricultural University). The ewes were grouped in four groups accord to the lamb numbers (one and double) of the last produce. Group 1: 6 Mongolian ewes with one lamb in last produce; Group 2: 6 Mongolian ewes with double lambs in last produce; Group 3: 6 Poll Dorset ewes with one lamb in last produce; Group 4: 6 Poll Dorset ewes with double lambs in last produce. The development of a technique for monitoring hormones secretion in the hypophyseal portal blood of unanesthetized sheep has produced considerable information on the pattern of endogenous hormones secretion during the ovine estrous cycle. Therefore, researchers have chosen to describe secretion of these hormones in tandem, rather than sequentially. All 24 ewes entered a second natural estrus, healthy animals were selected at estrus (day 0) estrus (day 11) and post-partum anoestrus for sampling.

Blood collection and ELISA: Before sampling, to keep ewes in same estrous phases, blood samples from each individual ewe were collected aseptically via jugular venipuncture into 5 mL Vacutainer K3 Ethylene Diamine Tetraacetic Acid (EDTA) tubes of the day at 9 a.m. All the hormones (Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), Prolactin (PRL), Testosterone (T), Estradiol (E_2), Progesterone (PROG), Melatonin (MT)) were tested in 3 days after collection. Standards or samples were tested using the LH, FSH, PRL, T, PROG, E_2 and MT ELISA kit (Beijing Gershong Biotechnology Ltd., China, Catalogue No. QS49012, QS48268, QS49721, QS49357, QS48982, QS458242 and QS48694, respectively) according to the manufacturer protocol and the OD of plates was read at 450 nm using a microtiter plate reader.

Statistical analysis: All data were analyzed by one-way ANOVA of Least Significant Difference (LSD) Post Hoc test. The correlations between the data from real-time PCR and ELISA were analyzed by two-tailed Pearson's correlation. Statistical analyses were performed using the SPSS 19.0 Statistical Software Package. Values of $p<0.05$ were considered to be statistically significant.

RESULTS

Hormone differences between sheep breeds: The seven hormones average concentration analyzed by one-way ANOVA, the results showed that there were significant differences in mean hormones secretion between Mongolian ewes and Poll Dorset ewes in the three periods (Table 1). Mean±SEM hormone concentrations during post-partum anoestrus, 1st and 11th days after oestrus shown in Table 1. All the seven hormones concentration of the Poll Dorset ewes was higher than that of the Mongolian ewes in the three periods. However, there is no difference ($p>0.05$) in some hormones (Table 1).

The hormones dynamics summary statistics shown in Fig. 1. The hormone PRL is high in post-partum anoestrus, decreases to a nadir late in the estrous period of the 1st day and then increases progressively throughout the estrous phase. The hormone E_2 follows

Table 1: Mean±SEM hormone concentrations during the three periods

Periods	PRL	E_2	T	MT	FSH	PROG	LH
G1							
N	2.90±0.23^{f}	37.97±2.52^{de}	112.29±8.350^{de}	8.86±0.65^{c}	2.90±0.23^{h}	12.03±1.01^{i}	3.45±0.28^{g}
0 day	2.50±0.13f	28.82±1.49^{g}	155.57±4.440^{ab}	11.01±0.36^{b}	4.07±0.18^{g}	15.26±0.82^{hi}	8.64±0.20^{d}
11 days	6.07±0.30^{cd}	43.95±1.45^{cd}	111.43±5.980^{de}	6.40±0.37^{de}	4.52±0.07^{fg}	38.82±0.84^{c}	10.19±0.62^{c}
G2							
N	3.99±0.15^{e}	40.89±1.86^{de}	131.95±8.670^{cd}	10.47±0.42^{b}	3.99±0.15^{g}	15.14±0.95^{hi}	4.53±0.20^{f}
0 day	4.02±0.24^{e}	30.81±2.02^{fg}	165.00±8.950^{a}	8.74±0.88^{c}	4.86±0.16^{ef}	20.86±0.76^{fg}	9.79±0.27^{c}
11 days	6.70±0.10^{bc}	40.97±1.66^{de}	73.98±3.820^{g}	5.11±0.24^{ef}	5.76±0.12^{cd}	43.24±0.73^{b}	11.53±0.20^{b}
G3							
N	5.72±0.14^{d}	43.46±2.09^{cd}	145.72±6.180^{abc}	16.24±0.65^{a}	5.72±0.14^{cd}	18.68±0.88^{gh}	5.44±0.23^{f}
0 day	4.12±0.24^{e}	35.20±1.66^{ef}	136.06±9.070^{bc}	7.09±0.27^{d}	5.26±0.26^{de}	27.52±1.32^{e}	9.94±0.31^{c}
11 days	6.99±0.54^{b}	55.15±4.14^{a}	82.16±12.18^{fg}	4.20±0.05^{fg}	6.06±0.25^{bc}	40.76±2.42^{bc}	12.44±0.61^{ab}
G4							
N	6.80±0.18^{b}	47.25±2.18^{bc}	158.11±8.490^{ab}	17.65±0.38^{a}	6.80±0.18^{a}	23.62±0.72^{f}	6.74±0.20^{e}
0 day	4.35±0.21^{e}	39.81±1.18^{de}	97.52±3.800^{ef}	6.55±0.50^{de}	5.78±0.27^{cd}	32.94±2.46^{d}	10.50±0.22^{c}
11 days	7.90±0.24^{a}	52.73±1.26^{ab}	65.89±6.410^{g}	3.32±0.35^{g}	6.58±0.33^{ab}	52.72±1.25^{a}	13.14±0.28^{a}

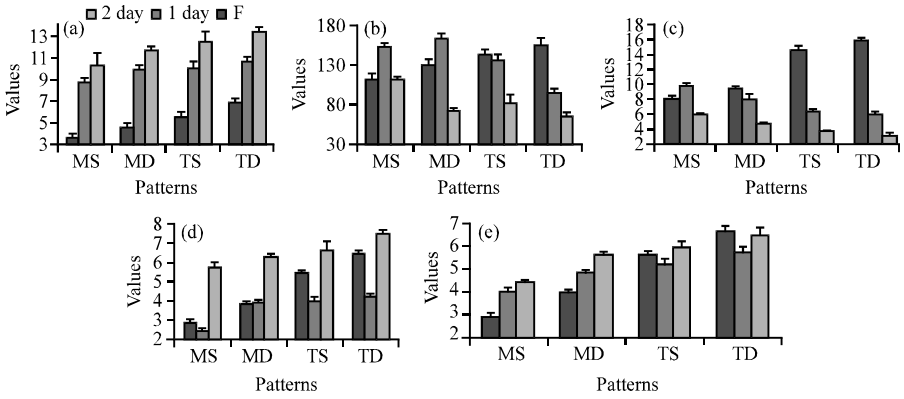

Fig. 1: Change patterns of LH, T, MT, PRL and FSH secretion in sheep blood; a) LH; b) T; c) MT; d) PRL; e) FSH

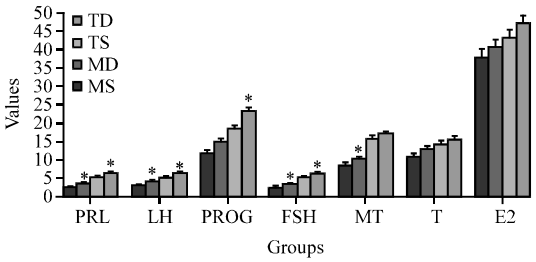

Fig. 2: The hormones relationships between lamb performances of two breeds at post-partum anoestrus

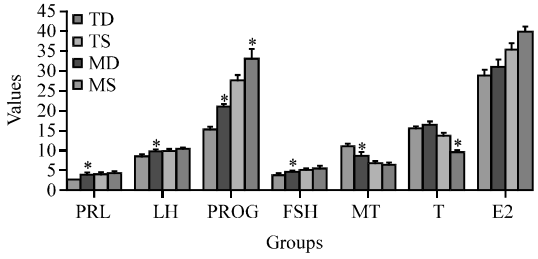

Fig. 3: The hormones relationships between lamb performances of two breeds at the estrous period of the 1st day

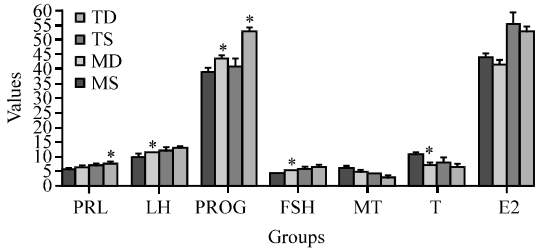

Fig. 4: The hormones relationships between lamb performances of two breeds at the estrous period of the 11th day

the same pattern of the PRL (Fig. 2). While the T secretion shown the different pattern: it is low in post-partum anoestrus, increases to a peak in the the estrous period of the 1st day and then decreases progressively throughout the estrous phase. The pattern of PROG, LH secretion increases progressively throughout the follicular phase both keep low level during the post-partum period. While the MT secretion decreases progressively throughout the follicular phase, it keeps high level during the post-partum period although discordance was found in single lamb of Mongolian ewes. Unexpectedly, the FSH secretion shown different patterns in two breeds. In Mongolian ewes, it follows the pattern of the PROG and LH pattern but T pattern in Poll Dorset ewes (Fig. 1).

Hormones differences in single and double lambs: The hormones relationships between lamb performances of two breeds are summarized in Fig. 2-4. There were significant differences ($p<0.05$) in PRL, LH, FSH concentrations in both breeds and MT concentrations in Mongolian ewes and PROG in Poll Dorset ewes during post-partum anoestrus (Fig. 2). While in the estrous period of the 1st day, there were significant differences ($p<0.05$) in PROG concentrations in both breeds, PRL, LH, FSH, MT concentrations in Mongolian ewes, T concentrations in Poll Dorset ewes. Interestingly, the MT and T concentrations of ewes with single lamb ewes were higher than that of ewes with twins (Fig. 3). In the estrous period of the 11th day, only PROG concentrations was significant differences ($p<0.05$) in both breeds. There were significant differences ($p<0.05$) in LH, FSH and T concentrations in Mongolian ewes, PRL concentrations in Poll Dorset ewes, there were no differences in other hormones' (Fig. 4).

Correlation of hormones levels among three stages of two breeds: The relationships between hormones are summarized in Table 2 and 3. The results showed a highly

Table 2: Correlation analysis of hormones levels among three stages in Mongolian ewes

Levels	LH	PROG	FSH	MT	T	E2
PRL	0.993**	0.900**	1.000**	0.838**	0.479	0.272
(N/1/11 days)	0.808**	0.808**	0.564	-0.625*	0.279	0.433
	0.717	0.354	0.741	-0.151	-0.388	-0.251
LH		0.906**	0.993**	0.822*	0.463	0.265
		0.810**	0.337	-0.674*	0.630*	0.366
		0.494	0.664	-0.383	-0.667	-0.676
PROG			0.900**	0.942**	0.452	0.246
			0.609	-0.774*	0.479	0.369
			0.834*	-0.912**	-0.848*	-0.350
FSH				0.838**	0.479	0.272
				-0.564	0.318	0.207
				-0.694	-0.813*	-0.397
MT					0.289	0.063
					-0.555	-0.135
					0.835*	0.300
T						0.948**
						0.414
						0.739

Table 3: Correlation analysis of hormones levels among three stages in Poll Dorset ewes

Levels	LH	PROG	FSH	MT	T	E2
PRL	0.933**	0.916**	1.0000**	0.698	0.695	0.706
(N/1/11 days)	0.580	0.169	0.7840*	0.032	-0.299	0.362
	0.399	0.879*	-0.0080	-0.313	-0.346	-0.392
LH		0.962**	0.0933**	0.868**	0.737*	0.717*
		-0.103	0.6860	0.276	-0.405	0.617
		0.533	0.5450	-0.514	-0.055	-0.457
PROG			0.0916	0.893**	0.791*	0.801*
			0.4650**	-0.875**	-0.453	0.344
			0.3890	-0.561	-0.551	-0.042
FSH				0.698	0.695	0.706
				-0.281	-0.301	0.611
				-0.913*	-0.565	-0.589
MT					0.837**	0.823*
					0.383	-0.352
					-0.637	0.349
T						0.979**
						-0.731*
						0.149

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

positive and negative significant correlation between hormones. In Mongolian ewes, the significant positive correlation ($p<0.01$) were found between LH and PRL, PROG and PRL, LH, FSH and PRL, LH, PROG, MT and PRL, PROG, FSH, E_2 and T. A significant negative ($p<0.01$) correlation was found between MT and PROG in post-partum anoestrus. And the negative correlation ($p<0.05$) were also found between MT and PRL, LH, PROG in the estrous period of the 1st day. Few correlations were found in the estrous period of the 11th day (Table 2). And there was no association ($p>0.05$) between other hormones (Table 2).

In Poll Dorset ewes, more significant positive correlation ($p<0.01$) were found between hormones in post-partum anoestrus. Both a significant negative ($p<0.01$) and a negative ($p<0.05$) correlation were found between MT and PROG, E_2 and T in the estrous period of the 1st day. A positive correlation ($p<0.05$) was also found between FSH and PRL in the estrous period of the 1st day. Again, only a positive correlation ($p<0.05$) was also found between FSH and PROG, a negative positive correlation ($p<0.05$) was also found between FSH and MT in the estrous period of the 11th day, there was no association ($p>0.05$) between other hormones (Table 3).

DISCUSSION

Lambing performance of ewes is the most important economic trait and is regarded as a critic factor affecting the productivity in sheep industry in which the twinning trait is regarded as the ideal lambing performance favoring by sheep industry. Reproductive hormonal secretion highly, influenced the cyclic ovarian activity in ewes. In this study, all the seven hormones concentration of the Poll Dorset ewes were higher than that of the Mongolian ewes in the three periods, even some of them were statistically significant ($p<0.05$). There are significant differences in reproductive hormone concentrations among sheep breeds. Even there are some differences in cycle lengths among breeds and with age (Hafez, 1952). Still there are also the difference hormones concentration dynamics in breeds although these differences are relatively small (1 day). An increase of ~8-25% in plasma FSH concentrations for 24 h within the 48 h before the onset of luteolysis is known to be sufficient to increase the ovulation rate of Romney ewes from 1-2 (McNatty *et al*., 1984). In addition, the breeds characteristics are also important factors in determining the hormones levels in plasm. The results obtained here provided the basic data for the two breeds.

The results also showed that there were significantly difference between the plasma hormone concentrations in single embryo and double embryo. These results show that there were significant differences in the mean plasma concentrations of PRL, LH, FSH in both breeds, MT in Mongolian ewes and PROG in Poll Dorset ewes during post-partum anoestrus. While in the estrous period of the 1st day, PROG concentrations in both breeds, PRL, LH, FSH, MT concentrations in Mongolian ewes, T concentrations in Poll Dorset ewes were statistically significant. The significant differences were also detected in PROG concentrations of the two breeds and LH, FSH and T concentrations of Mongolian ewes and PRL concentrations of Poll Dorset ewes during the estrous period of the 11th day. In Romney ewes, some experience twin ovulations show higher blood concentrations of FSH than others have one during the luteal phase for a 24 h interval within the 48 h before the onset of luteolysis (McNatty *et al*., 1984). The FSH concentrations pattern is

consistent with the earlier studies of Falchi *et al*. (2012). As the difference between the breeds may be partly contributed to the long breeding history and its breeding practice. Dorset ewes originated in a herd at North Carolina State University, Raleigh, North Carolina and a registry of the polled Dorset was established in 1956 while Mongolian ewes is a native local sheep, shorter breeding history and simple and scare breeding practice in Mongolian ewes.

The results showed a highly positive and negative significant correlation between hormones. The relationships among the hormones also show the differences in two breeds. In sheep, a short-day seasonal breeder, the annual patterns of gonadotropin and Prolactin (PRL) secretion are inversely correlated with highest concentrations of PRL detected during the long days of Summer, coinciding with the nonbreeding season (Jackson and Davis, 1979). Tonic LH secretion occurs in a pulsatile pattern at a low level throughout the cycle and is important for ovarian steroidogenesis; the LH surge occurs around estrus and induces ovulation and formation of the corpus luteum. Prenatal Testosterone (T) excess compromises the Estradiol (E_2) positive feedback (Salloum *et al*., 2012). The dramatic fall in circulating oestrogen concentrations at the time of parturition leads to an immediate post-partum increase in FSH secretion which initiates a wave of ovarian follicular growth (Mandiki *et al*., 1990). As the more complicated relationships in those reproductive hormones further, studies needed to clarify those relationships.

CONCLUSION

The seven hormones concentration of the Poll Dorset ewes were higher than that of the Mongolian ewes in the three periods this study provide evidence that significantly difference between the plasma hormone concentrations in single and double lambs. The highly positive and negative significant correlation between hormones and the differences in two breeds were found. Those findings provided the important theoretical and practical basis for improving fine ewes twinning traits.

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