

Changes in some serum hormonal profiles during pregnancy in single- and twin foetus-bearing Akkaraman sheep

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Summary

This study was conducted to determine the changes in the blood serum concentrations of T_3 , T_4 , TSH, E_2 and P_4 hormones at days 60, 100 and 150 of gestation in single- and twin foetus-bearing Akkaraman sheep. Serum P_4 and E_2 concentrations (on day 150) significantly increased ($P < 0.001$); meanwhile there were significant decreases ($P < 0.05$) in T_4 and insignificant decreases in T_3 ($P > 0.05$) while there were no significant changes in TSH during the sheep's pregnancy. Results of the tests of the twin pregnancy revealed a significant increase ($P < 0.001$) in P_4 and E_2 while there was a significant decrease ($P < 0.05$) in T_3 and T_4 when compared to the single bearing sheep. It may be concluded that the number of foetuses significantly influences the hormone profile during pregnancy. Predicting single or twin pregnancies in sheep is possible when basing on P_4 values and taking into account the time of mating.

Keywords: oestradiol, progesterone, thyroid hormones, sheep, pregnancy

Endocrine functions frequently are overlooked when examining pregnant state of animals. The several hormones have an important role in embryogenesis and foetal development during pregnancy. Determination of plasma progesterone gives important clues about the reproductive activities of animals (14). Pregnancy-dependent variations are observed for triiodothyronine (T_3), thyroxin (T_4), thyroid stimulating hormone (TSH), estradiol-17 β (E_2) and progesterone (P_4) hormones (1, 4, 11, 12, 20). There are numerous reports in several animals, such as the goat (17, 35), the sheep (26) and the horse (25) concerning foetal number on the concentrations of hormones. However, there is a clear need to understand the effect of twinning on the differences in some serum hormone profiles at various stages of gestation more completely in the sheep.

Therefore, this study was conducted to determine the changes in the blood serum concentrations of T_3 , T_4 , TSH, E_2 and progesterone hormones at days 60, 100 and 150 of gestation in single- and twin foetus-bearing Akkaraman sheep.

Material and methods

Animals. In the present study, a total number of 30 apparently healthy pregnant Akkaraman sheep (15 single pregnancies, 15 twin pregnancies), aged 3-5 years and

weighing 40-50 kg were used. The experiment was conducted between October and March. Between 5 and 10 days after oestrus, each ewe was treated with two intramuscular injections of PGF2- α (5 mg), 4 hours apart, to synchronize oestrus. Ewes were observed for oestrus for every 12 hours, and two different fertile rams mated those exhibiting oestrus. Both groups of animals were fed a constant diet composed of mineral and vitamin premixes, wheat straw and a concentrate feed consisted of 14% crude protein, 15% fibres and 3% fat to secure intake of nutrients required for maintenance in accordance with NRC (27). About 0.5 kg/animal of concentrate was distributed twice a day in addition to 2 kg wheat straw. Water was available *ad libitum* in the shed. On day 30, pregnancy and number of fetus were determined on a per sheep basis by transabdominal ultrasonography using a B mode ultrasound (Scanner 200 Vet) console fitted with a five MHz transducer and the sheep were assigned to two groups by single or twin pregnancy (15).

Serum and assay procedures. The blood samples were collected in vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium) from the jugular vein on days 60, 100 and 150 of pregnancy for determination of serum hormonal concentrations. Blood samples were allowed to clot; the serum was removed, and then stored in single test tubes at -30°C until the assayed. The samples were analysed for triiodothyronine (T_3) and thyroxin (T_4), thyroid

stimulating hormone (TSH), estradiol-17 β and progesterone concentrations.

Serum samples were thawed at room temperature, and T₃, T₄ and TSH concentrations were determined using commercially available Chemiluminescent Enzyme Immunoassay DPC kits (Diagnostic Products Corporation). Concentration of serum estradiol-17 β in duplicate was measured by radioimmunoassay (Diagnostic Products) (22). Serum was assayed for progesterone by radioimmunoassay as described in detail by Yildiz (40).

Data analysis. Data was presented as mean \pm standard error of the mean (s.e.m.). At the each time intervals, data were analysed by One-way analysis of variance (ANOVA) (32). T-test was used to evaluate the relationships of serum some biochemical levels to single and twin pregnancy in observed periods (32).

Results and discussion

The mean levels with standard error of the mean of concentrations of serum T₃, T₄, TSH, estradiol-17 β and progesterone of single and twin pregnant sheep and interactions between evaluated parameters are given in tab 1.

Serum progesterone and estradiol-17 β levels were found to be significantly higher ($P < 0.001$) for the evaluated periods of pregnancy in twin pregnant sheep comparison with single ones. Serum progesterone concentrations in single and twin pregnant sheep showed a significant increase ($P < 0.001$) among all the periods of pregnancy. For the concentrations of estradiol-17 β in single and twin pregnant sheep there were not significant increases between days 60 and 100, but highly significant increases ($P < 0.001$) of estradiol-17 β concentrations were observed on day 150. At all the periods of pregnancy, T₃ and T₄ values were significantly lower in twin pregnancies when compared with single pregnancies ($P < 0.05$). There were no significant changes in TSH values of animals bearing single or twin foetuses at all periods. In the sheep bearing single and twin foetuses, no significant decrease occurred in the level of serum T₃ from day 60 to day 150. In sheep bearing single- and twin foetuses, no significant decrease was found in the level of serum T₄ between day 60 and day 100, whereas a significant decrease ($P < 0.05$) was observed on day 150. In both groups, the concentrations of TSH did not change significantly for the evaluated periods.

The changes in the blood serum concentrations of hormones concerning the number of foetuses and the stage of pregnancy in domestic animals are important to understand, because strategic manipulation of those hormonal changes may offer insights into therapeutic strategies to improve conceptus survival and reproductive health in domestic animals.

Tab. 1. Blood serum concentrations of some hormones on days 60, 100 and 150 of in single ($n = 15$) and twin pregnant sheep ($n = 15$) ($\bar{x} \pm$ SEM)

Hormones	Animals	Pregnancy (day)			F-test
		60	100	150	
P ₄ (ng/ml)	Single	1.38 \pm 0.04 ^a	1.72 \pm 0.06 ^b	3.12 \pm 0.08 ^c	$P < 0.001$
	Twin	2.11 \pm 0.08 ^a	3.31 \pm 0.18 ^b	4.98 \pm 0.10 ^c	$P < 0.001$
	t-test	$P < 0.001$	$P < 0.001$	$P < 0.001$	
E ₂ (pg/L)	Single	146.33 \pm 8.78 ^a	192.67 \pm 11.85 ^a	656.67 \pm 13.68 ^b	$P < 0.001$
	Twin	287.36 \pm 8.86 ^a	313.33 \pm 17.77 ^a	958.67 \pm 29.42 ^b	$P < 0.001$
	t-test	$P < 0.001$	$P < 0.001$	$P < 0.001$	
T ₃ (ng/dl)	Single	155.40 \pm 9.83	144.78 \pm 9.77	130.54 \pm 8.96	$P > 0.05$
	Twin	123.20 \pm 7.75	110.11 \pm 8.02	103.00 \pm 7.36	$P > 0.05$
	t-test	$P < 0.05$	$P < 0.05$	$P < 0.05$	
T ₄ (μ g/dl)	Single	8.26 \pm 0.18 ^a	7.99 \pm 0.17 ^a	7.50 \pm 0.16 ^b	$P < 0.05$
	Twin	7.39 \pm 0.27 ^a	7.03 \pm 0.30 ^a	6.62 \pm 0.29 ^b	$P < 0.05$
	t-test	$P > 0.05$	$P > 0.05$	$P > 0.05$	
TSH (μ IU/ml)	Single	0.02 \pm 0.003	0.03 \pm 0.004	0.03 \pm 0.007	$P > 0.05$
	Twin	0.02 \pm 0.001	0.04 \pm 0.002	0.03 \pm 0.004	$P > 0.05$
	t-test	$P > 0.05$	$P > 0.05$	$P > 0.05$	

Explanation: a, b, c – means with different superscript letters differ significantly in horizontal direction

The results of our study reveal that the number of foetuses and the different stages of pregnancy can be effective on the changes of some hormone concentrations during gestation. This study has shown that both the different periods of pregnancy and number of fetus in sheep affect maternal progesterone and oestradiol-17 β concentrations. As reported by most investigators (3, 10, 19, 28, 38), in the present study, plasma progesterone level increased during pregnancy and reached its highest level on day 150. Regardless of whether sheep were bearing single or twin foetuses, progesterone concentration in serum increased steadily from 60 to 100 to 150 days in gestation. Progesterone concentration rapidly elevated in the stages of pregnancy, and was significantly greater in twin- than single-bearing ewes. The number of fetus had a significant effect on progesterone concentration during the different stage of pregnancy. Serum progesterone concentration has been demonstrated to be significantly higher in ewes carrying two and three foetuses than those carrying one fetus (6). It has been showed that average maternal serum progesterone concentrations during the last two months of the gestation period for twins was almost twice as much as found for single-bearing does (23). Lower progesterone concentration was shown in sheep carrying a single fetus compared to those carrying twins by many authors (2, 33). Similarly, a research showed that plasma progesterone concentrations in sheep giving birth to only one lamb were lower than those with two or three lambs (12). The distinctly higher progesterone concentrations noted in sheep carrying two foetuses than those carrying one

fetus in the present study are in agreement with the above researchers. Concerning the estimation of the foetal number, a quantitative assay of plasma progesterone was demonstrated to could be used for predicting the number of foetuses carried by the ewes (9). It has been suggested that the number of foetuses can be estimated with 88% accuracy in ewe lambs and with 74% accuracy in mature ewes on Days 100 ± 9 after breeding (31). It was reported that there was a positive relationship between the number of foetuses and the mean plasma progesterone concentrations ($P < 0.001$) after the second half of pregnancy (14). Similar results were observed in other study (26) and all these theses are also in good agreement with the data reported here. However, these conclusions have not shared by other workers (7, 30).

In this study, in both groups, there was no significant difference in the level of oestradiol on Days 60 and 100 of gestation. Oestradiol was considerably greater on day 150 than those found on days 60 and 100. In general, oestradiol concentrations increased with the advance of pregnancy approaching parturition date (29, 37). It has been observed that the release of estradiol- 17β was greatest on the day of parturition in ewes (21). Dwyer et al. (8) reported that plasma oestradiol concentration did not differ from nonpregnant ewes until mid-gestation (week 10), thereafter, that oestradiol increased rapidly to term, being 15-fold higher than nonpregnant ewes at week 20. It has been demonstrated that oestradiol concentrations in peripheral plasma remained at a steady level for the majority of pregnancy (12). Challis (5) explained that unconjugated estrogens remained at low values for the majority of pregnancy in the sheep. Tsang (36) reported that the level of estrone sulphate increased steadily till 2 days before parturition when an upsurge was seen. Our results are in agreement with the above findings.

The level of serum oestradiol was significantly higher in ewes carrying twin than those carrying single foetus at the same period of gestation, which agrees with observations of values in serum for the sheep (13) and in serum for does (23). Manalu et al. (22) determined that oestradiol concentrations could be used as a parameter to predict whether the sheep are carrying single or twin foetuses. However, our results showed that there were differences in mean levels of oestradiol in sheep carrying single and twin foetuses, but the variation was too great for use as a predictive test. A similar finding observed by Worsfold et al. (39) who demonstrated that the determination of the hormone concentrations in ovine blood might not be reliable for prediction of foetal numbers due to the high variation between individuals.

Serum values of T_3 and T_4 decreased during pregnancy in both groups. In addition, the decline of T_4 level became significant ($P < 0.05$) on day 150. T_3 and T_4 productions appear to be related to the number of fetuses. Serum T_3 and T_4 concentrations in twin-

-bearing sheep were lower ($P < 0.05$) than those in single bearing sheep. The results are in agreement with those Khan and Ludri (16), who reported that the levels of triiodothyronine (T_3) were significantly higher ($P < 0.01$) during all the periods of sampling in single- than in twin-bearing goats and that plasma T_4 was significantly lower ($P < 0.01$) in twin- than in single-bearing goats. Similar results were found for does by Manalu et al. (24). Moreover, a significant decrease of hormone concentrations, greater for T_4 than T_3 , was observed at the end of gestation as was found in other article (1). In contrast, Studzinski et al. (34) reported that the highest levels of blood plasma T_3 and T_4 were observed in the two last months of gestation in cows. We think that this difference may be due to species.

Kumar et al. (20) reported that mean TSH levels were seen to rise progressively through the three trimesters of pregnancy in women. However, Khandakar et al. (18) found that the serum TSH level of pregnant mother was within normal limit and was similar to that of non-pregnant. In current study, it was observed that the serum TSH level of single pregnant sheep was closely similar to that of twin pregnant sheep showing no significant difference during the whole period examined. There were no significant effects of the numbers of foetus on TSH concentration at the different stages of pregnancy. The mean TSH concentrations in twin pregnancy did not differ from those in singleton pregnancy in each period.

It can be concluded that the number of fetuses significantly influences the hormone profile during pregnancy. It was shown that there were significant variations for serum T_4 , E_2 and P_4 concentrations in the single- or twin bearing sheep during the period of pregnancy. Based on values of the P_4 taking into account the time of mating in relation to the normal breeding season, prediction of single or twin pregnancies in sheep is possible. Dating the age of the fetus in sheep may be possible as well. This information could be used in formulating appropriate feeding strategies for pregnant sheep to meet the nutrient requirements of the mother and the fast-growing foetuses during late pregnancy.

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TING L. J., LEE M. S., HUANG T. S., HUANG C. C., KUO S. T., LEE F., JONG M. H., SHIAU J. R., Y LIN S.: Identyfikacja wirusa choroby niebieskiego języka u kóz na Tajwanie. (Identification of blue-tongue virus in goats in Taiwan). Vet. Res. 156, 52, 2005 (2)

W maju 2003 r. na wyspie Kinmen zbadano testem ELISA w kierunku choroby niebieskiego języka (BT) stada kóz. W jednym stadzie reagowało pozytywnie 33, w drugim 83, a w trzecim i czwartym 100% badanych zwierząt. Do izolacji wirusa użyto surowic wszystkich 22 kóz reagujących dodatnio. Wyizolowany wirus, określony jako BTV/KM, serotyp 2BTV, wywierał działanie cytopatogenne w hodowli komórek nerki płodu chomika (BHK-21). Znajdował się on w tej samej grupie z serotypem 2 BTV wyizolowanym na Korsyce, USA i Chinach. Wykazywał on 95% identyfikacji nukleotydowej ze szczepem wyizolowanym w Chinach (V440).

G.

MAZURKIEVICH A., VASYLYK N., AVRAMENKO T., VELICHKO S., TARELLO W., VARODI E.: Dojrzałe nicienie *Dirofilaria repens* u kota w Kijowie, Ukraina. (Adult *Dirofilaria repens* nematodes in a cat from Kiev, Ukraine). Vet. Rec. 155, 638-639, 2004 (20)

Dirofilaria (Nochtiella) jest zoonocycznym nicieniem pasożytującym u psów i kotów przenoszonym przez komary. Na Ukrainie opisano 57 przypadków zarażenia człowieka przez *Dirofilaria repens*. U kota w wieku 3 lat z Kijowa operacyjnie usunięto dwa pasożyty płci męskiej z części wewnętrznej moszny, jednego z powrózka nasiennego. Rozmiary pasożytów wynosiły: 54,5 mm × 350 μm, 60,0 mm × 400 μm i 167 mm × 490 μm. Na Ukrainie koty mogą być rezerwuarem *Dirofilaria immitis*. Dotychczas stwierdzono obecność tego pasożyta u kotów w Sri Lance i w Afryce Południowej. Tam też występuje u ludzi podskórna dirofilarioza.

G.