Determination of Fetal Gender by Ultrasound in Ewes and its Correlation with Maternal Serum Levels of Calcium, Phosphorus and Iron

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Abstract: The purpose of this study was to evaluate the accuracy of ultrasonography for determination of the fetal gender and also to study the effect of fetal gender on the maternal serum levels of calcium, phosphorus and iron. Twenty four cyclic ewes were used in the present study. Estrus was detected two times daily at the morning and evening and the ewes detected in estrus were mated with fertile rams 3 times 12 hours interval. Blood samples were collected from each ewe on the day of mating and every 15 days until lambing or 150 days after mating for determination of calcium, phosphorus and iron. For detection of fetal gender, ultrasonographic examinations were performed transrectally or transabdominally using a real-time B-mode scanner equipped with a multiple-frequency (5, 7.5 or 10 MHz) transducer. Ultrasonographic examinations confirmed pregnancy of all ewes at day 30 post-mating and then all ewes examined two times weekly until taking decision of fetal gender type. The results showed that ultrasonography is a reliable method for pregnancy diagnosis in ewes with accuracy 100% at day 30 post-mating. Fetal gender identification was possible at day 50.95 post-mating with accuracy 92.3% for males and 87.5% for females. There were no significant effects of fetal gender on blood (calcium, phosphorus and iron) levels. On the other hand, there was a significant ($P \leq 0.01$) drop in calcium level from mid- to late gestation, and a significant elevation ($P \leq 0.05$) in iron level during the last month of gestation.In conclusion, trans-rectal or trans-abdominal B-mode ultrasonography was efficient in pregnancy diagnosis and determination of fetal gender in ewes. There was no obvious effect of fetal gender on the maternal serum levels of calcium, phosphorus and iron.

Keywords: Ultrasound, fetal gender, ewes, calcium, phosphorus, iron

INTRODUCTION

Accurate diagnosis of pregnancy in addition to determination of fetal gender is essential for the maintenance of high levels of reproductive efficiency (Goel and Agrawal, 1992). A reliable technique for pregnancy diagnosis would allow early re-breeding of barren ewes before the end of the breeding season. Ultrasonography (Haibel, 1990) and progesterone assay (Engeland *et al.*, 1997) are the most useful methods utilized for pregnancy diagnosis. Ultrasonography is quicker and more accurate than other methods and so, real-time B-mode ultrasonography has been used more frequently for pregnancy diagnosis in small ruminants. Ultrasonic imaging of the heartbeat is regularly used to detect the embryo and to evaluate embryo viability (Ishwar, 1995). Heartbeats were first detected between days 19 and 29 in pregnant ewes (Schrick and Inskeep, 1993 and El-Gayar *et al.*, 2006) and goats (Martinez *et al.*, 1998).

Fetal sex can be determined by ultrasonography between days 55 and 75 of pregnancy in horses (Merkt *et al.*, 1999) and from day 50 onward in cattle (Curran *et al.*, 1989 and Stroud, 1996). Barros and Visintin (2001) emphasized that in cattle fetal sex can be accurately established around day 60 of pregnancy. In sheep, Coubrough and Castell (1998) first reported 89% accuracy for early fetal sex determination between days 60 and 69.

In small ruminants, fetal sexing has been used in basic research after artificial insemination with sexed semen (Johnson, 2000 and Garner, 2001) and after sexed embryo transfer (Gutierrez -Adan *et al.*, 1997) or by *in vitro* produced embryos by the intracytoplasmatic sperm injection technique (ICSI). Application of fetal sexing in goats is more likely suited for dairy herds because it would allow better planning of acquirement and commercialization of animals (Chalhoub and Ribeiro Filho, 2002), allowing the marketing of male and female fetuses while still in utero. This primary application would allow a greater concentration of females in milk herds and males in meat herds.

Mineral analysis of body tissues and fluids is often a useful parameter for evaluating mineral status to diagnose some pathological conditions. Pregnancy especially with more than one fetus and lactation are considered as physiological stresses on the mother animal. Therefore, determination of the maternal levels of calcium, phosphorus and iron is important to provide the pregnant mother needs during pregnancy.

Measuring serum levels of calcium, phosphorus and iron in pregnant ewes carrying males or females is important to study the relationship between their levels and the type of gender and if we can use them as indicators of the type of the gender.

The objectives of the present study were to estimate the accuracy of real-time B-mode ultrasonography in determining fetal gender in ewes, and to study the effect of fetal gender on the maternal levels of calcium, phosphorus and iron.

MATERIALS AND METHODS

This study was carried out on 24 ewes aged 2-5 years and weighed 35-45 Kg. The ewes were housed and managed at the experimental farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. The diets contained concentrated mixed rations and rice straw. The concentrated mixed rations were formulated and offered to meet the essential nutrient requirements

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of sheep according to NRC (1985). Rice straw was offered to the animals *ad libitum* and fresh water was available all times.

Estrus was detected two times daily and ewes in estrus were mated three times 12 hours interval. Blood samples were collected on the day of mating and every 15 days until lambing or 150 days post insemination for determination of calcium, phosphorus and iron in the maternal blood.

Ultrasonographic examinations were performed either transrectally or transabdominally. Ewes were examined in a standing position and the transducer was lubricated with carboxymethylcellulose gel (Perfect Ultrasound Gel). In transrectal examination, the transducer was fastened to a rod (30 cm length and 20 mm in diameter) by adhesive tape to manipulate it externally into the rectum. Transabdominal examination was done according to Haibel (1990).

Ultrasonography was performed two times weekly using a real-time B-mode scanner equipped with a 5, 7.5 or 10 MHz transducer starting from day 30 post-mating for determination of fetal gender. Confirmation of fetal gender was assured at lambing. The fetal heart and chest were initially located, as they are easy structures to identify. Fetal head and neck indicated cranial orientation. Gender determination is made by scanning the caudal fetal abdomen, hindquarters, and buttocks to identify primary sex organs. Once the fetal hindquarters are located and fetal orientation is determined, a frontal plane of scanning is obtained. By moving in a ventral direction, the scrotum is visualized between the two hind limbs.

Blood samples were collected from ewes' jugular veins into Eppendorf tubes every 15 days. Blood samples were left for 45-60 minutes until coagulation and then centrifuged at 3000 g for 20 minutes to obtain serum. Serum samples were stored deep frozen at minus 20 °C until subjected to mineral analysis (calcium, phosphorus and iron) using specific kits produced by ELITech Company (SEPPIM S.A.S. – Zone Industrielle – 61500 SEES France) according to the procedure outlined by the manufacture.

Statistical analysis

Statistical analysis was computed using the General Linear Model (GLM) procedure of Statistical Analysis Software (SAS Institute Inc., 1998), and the significant differences between means were detected according to Duncan's multiple range test (Duncan, 1955).

The accuracy of determination of fetal gender was estimated by dividing the number determined by ultrasonography divided by the total number determined male or female after birth.

RESULTS

All ewes were confirmed pregnant at day 30 post-mating with accuracy 100 % as compared at lambing. The embryo appeared as an area of high echogenicity in the fetal fluids (non-echogenic) and surrounded with the fetal membranes as shown in Fig. 1.

Fetal gender results

The accuracy of determination of fetal gender by real-time B-mode ultrasonography is shown in TABLE (1). The fetal gender detected by ultrasonography was compared with the actual lambs born. Fetal gender determined in the present study in sheep was done by ultrasonic location of the fetal scrotum as shown in Fig. 2. Scanning plane is very important and the frontal plane is the best. Sagittal plane (Fig. 3) is not good for locating fetal scrotum.

As shown in Table (1) out of the 24 lambs examined by ultrasound, 92.3 % of males and 87.5 % of females lambs were diagnosed correct. On the other hand, 3 cases of the 24 cases could not be diagnosed. The gender of the fetus was decided around day 50.95 ± 1.5 post-mating.

Effect of fetal gender on calcium, phosphorus and iron levels

Serum calcium, phosphorus and iron levels during pregnancy in ewes carrying either male or female feti are shown in figures 4, 5 and 6 and Table (2). As shown in Table (2), the overall mean serum calcium concentrations in ewes carrying either male or female feti are not significantly different. On the other hand, there is a significant drop in the maternal calcium level from mid- to late gestation. In addition, serum calcium level was significantly higher ($P < 0.0001$) in ewes carrying males than those carrying females at days 15 and 30 of gestation. However, serum calcium level was significantly lower in ewes carrying males at day 45 and 60 of gestation (Fig. 4).

Regarding serum phosphorus concentrations, the results do not show any significant changes between ewes carrying males or females (Table 2). From mid- to late gestation, there is a non significant decrease in phosphorus level in the maternal blood as shown in Fig. 5.

The serum iron level in ewes showed a significant decrease around day 90 of gestation. In the last month of gestation, iron levels significantly increased in ewes carrying either males or females (Fig. 6). However, serum iron level was significantly lower ($P < 0.03$) at day 105 of gestation in ewes carrying males.

Table (1): Accuracy of determination of fetal gender in ewes by real-time B-mode ultrasonography.

	Male	Female	No decision
Number determined by ultrasound	13	8	3
Number confirmed correct at lambing	12	7	
Percentage correct	92.3 %	87.5 %	

Table (2): Mean (± SEM) serum concentrations of calcium, phosphorus and iron in ewes carrying males or females during pregnancy.

Parameter	Ewes carrying males	Ewes carrying females
Calcium (mmol/L)	3.45 ± 0.52	3.39 ± 0.47
Phosphorus (mmol/L)	2.58 ± 0.09	2.59 ± 0.10
Iron (µmol/L)	28.02 ± 2.60	27.93 ± 1.82

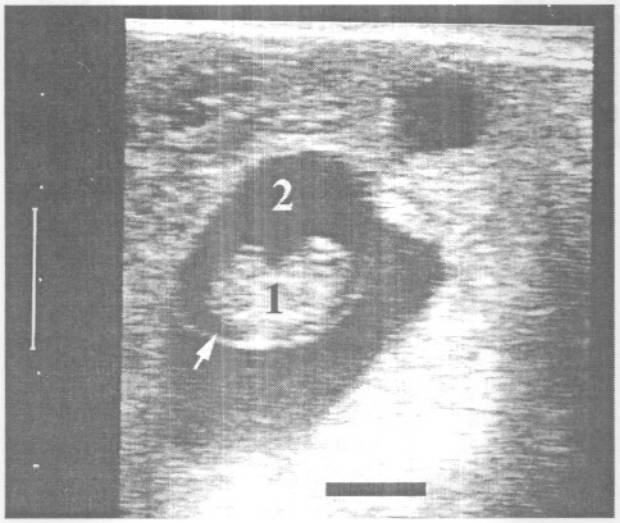

Fig. (1): Ultrasound images of an embryo on day 30 of gestation obtained using a 7.5 MHz transrectal transducer. The embryo (1) is observed as an area of high echogenic density in the fetal fluids (non-echogenic area; 2) and surrounded by fetal membranes (arrow). Scale bar represents 10 mm.

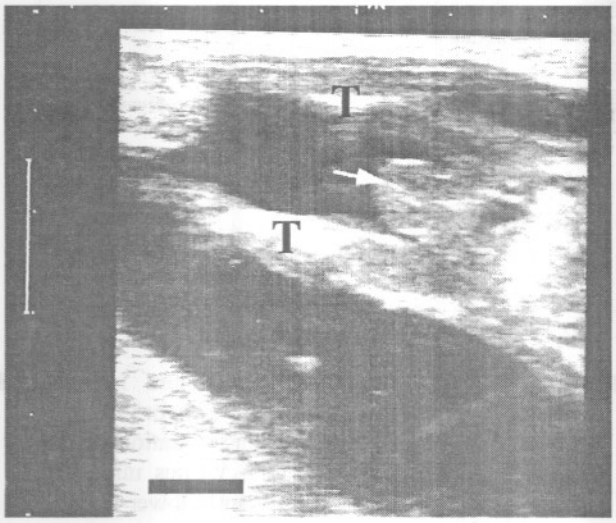

Fig. (2): Ultrasound image (frontal scan) showing a male scrotum on day 60 of gestation. The scrotum (arrow) can be clearly seen between the fetal thighs (T). Scale bar represents 10 mm.

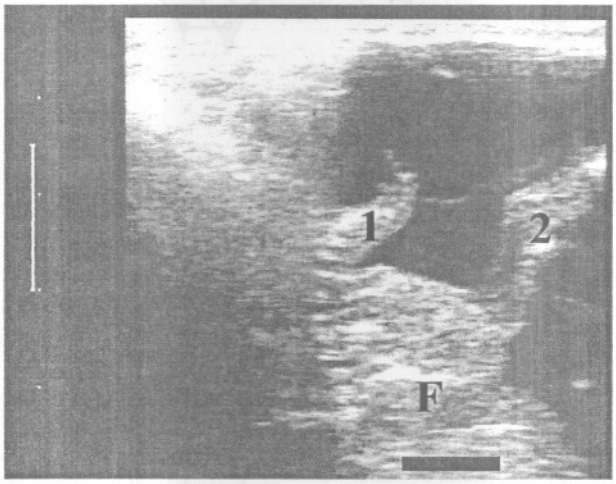

Fig. (3): Ultrasound image (sagittal scan) of a fetus (F) on day 60 of gestation showing the tail (1) and hind limbs (2). Note that this position is not good for examination of fetal gender through seeing the scrotum. Scale bar represents 10 mm.

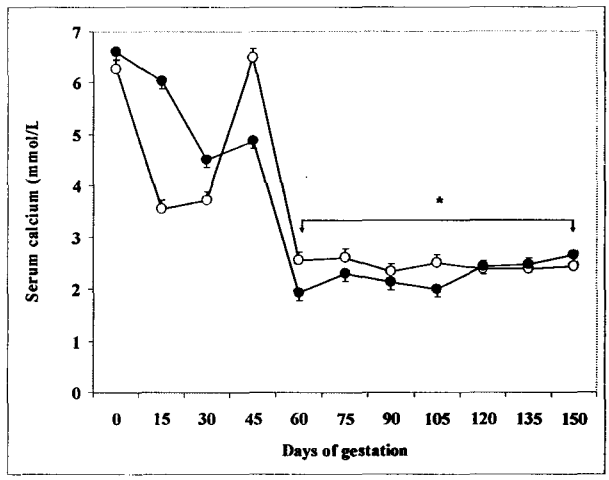

Fig. (4): Serum concentration of calcium in ewes carrying males (—●—; n=12) or carrying females (—○—; n=12) during gestation period. Values are means ± SEM. Values under the line (*$P < 0.01$) are significantly lower than the other data.

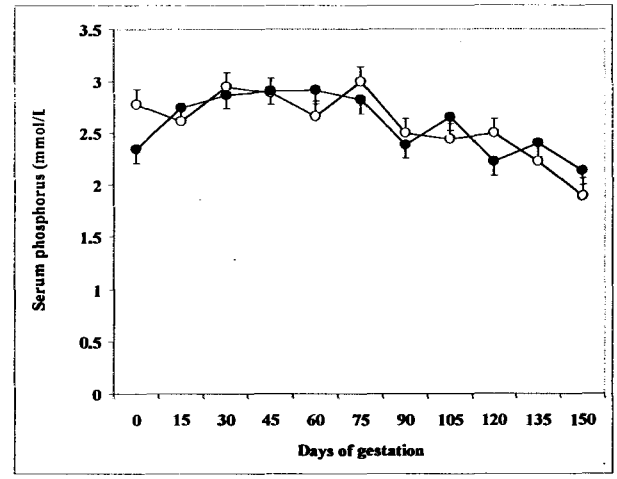

Fig. (5): Serum concentration of phosphorus in ewes carrying males (—●—; n=12) or carrying females (—○—; n=12) during gestation period. Values are means ± SEM.

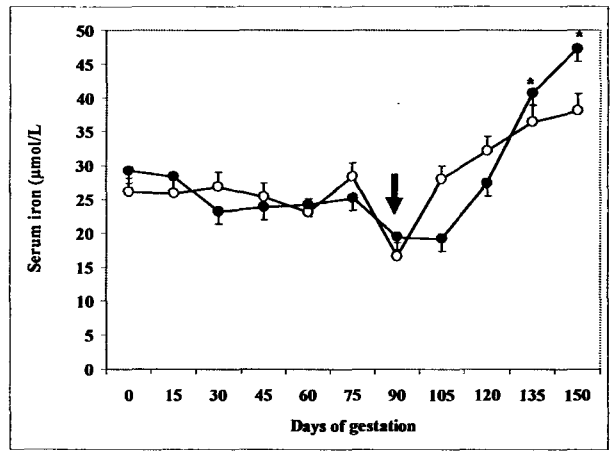

Fig. (6): Serum concentration of iron in ewes carrying males (—●—; n=12) or carrying females (—○—; n=12) during gestation period. Values are means ± SEM. The value at 12 weeks of gestation (↓*$P < 0.05$) is significantly lower than the other values while there is a significant increase (**$P < 0.01$) during the last month of gestation.

DISCUSSION

Accurate pregnancy diagnosis in profitable commercial farms is important for effective herd management and re-breeding of ewes before the end of the breeding season. Also, determination of fetal gender would allow producers the opportunity for fulfillment of market needs of specific demand. Although determination of progesterone on days 20 to 22 after mating in ewes is accurate for detection of pregnancy, it is inaccurate for detecting pregnant ones when the corpus luteum lifespan is extended for reasons other than pregnancy (Bretzlaff, 1981). Ultrasonography is an important tool for pregnancy diagnosis in ewes. In the present study, we found that the technique is easily applicable with accuracy of 100% at day 30 post mating.

Studies on ewes have reported the detection of an embryo heartbeat from 18 (Schrick and Inskeep, 1993) to 30 days of gestation (Buckrell *et al.*, 1986 and El-Gayar, *et al.*, 2006). The ability to determine fetal gender with real-time ultrasonography is a clear advantage. The results showed that fetal gender in ewes could be determined around days 50.95±1.5 post mating and these results are in agreement with that recently reported study by Santos *et al.* (2007*a*, *b*) who reported that fetal sexing can be done from the 50th day onwards in sheep fetuses and from 55 days onward in Saanen goats. In buffaloes, fetal sex determination results also revealed that the best time frame window for fetal

sexing was between the 10th and 18th week of gestations, with an overall accuracy of 97.1% (Ali and Fahmy, 2008). Also, in equine, fetal gender can be easily determined by ultrasound between days 120 and 210 (Bucca, 2005).

One of the early reports of visualization of male and female characteristics of bovine fetuses by real-time ultrasound was done by Muller and Wittkowski (1986). Eighty two cows were studied between 57 and 120 day gestation using both 3.0 MHz and 5.0 MHz transducers. The scrotal swelling in male and mammary glands in female fetuses were the references for sex determination with an accuracy of 94% between days 70 and 120. Kahn (1990) reported that accurate sex determination can be performed after day 60 and that the gender of a male fetus was less difficult to determine than that of a female, based on the presence or absence of a scrotum. Curran and Ginther (1991) reported up to 100% accuracy in sex determination by ultrasonography between days 50 and 100. However, considerable experience was found to be essential for the accurate determination of sex. The average time required to determine fetal sex varied from 2-15 min per cow (Curran and Ginther, 1991; Wideman *et al.,* 1989).

The results of blood analysis in the present study showed that the serum levels of calcium, phosphorus and iron are within the normal range as compared with previously reported data (Kaneko, 1997). Moreover, there was no clear effect of fetal gender on the maternal serum level of calcium, phosphorus and iron. On the other hand, the results showed a significant decline in calcium level during pregnancy in ewes which agree with the results of Yokus and his co-workers (Yokus *et al.,* 2004). Another study (Yildiz *et al.,* 2005) showed that there was a significant decrease in serum calcium level at day 100 and 150 of pregnancy in sheep and the calcium level is lower in sheep carrying twins compared with those carrying singles. A previous study (Braithwaite, 1983) in sheep showed that if calcium supplementation in is not enough to meet the high demands during pregnancy and lactation, skeletal stores of calcium will be mobilized to support the calcium deficiency. Supplementation of calcium in late pregnancy and lactation is important. It has been suggested that higher concentrations of calcium increase uptake of calcium by passive absorption and counteract the depletion of calcium (Lean *et al.,* 2003). In a previous study in cow, Serum calcium reached its nadir during the calving period or 1 day after parturition (Abu Damir *et al.,* 1994; Chan *et al.,* 2006).

The authors recommended that the nutritional requirements must be supplemented during certain periods especially from mid- to late pregnancy in sheep. Otherwise, it is apparent that this will cause a decline in the total performance of sheep and, consequently, economic lost. The relationships between serum mineral levels of the pregnant ewes and fetal gender need more investigation.

In conclusion, we found that ultrasonography is reliable for pregnancy diagnosis with an accuracy of 100 % at day 30 post mating. Moreover, determination of fetal gender could be determined at day 50.95 ± 1.5 Post mating with an accuracy of 92.3 % for males and 87.5% for females.

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تحديد جنس الجنين بالموجات فوق الصوتية وعلاقته بمستوى الكالسيوم والفسفور والحديد في سيرم النعاج

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** قسم التوليد والتناسل والتلقيح الإصطناعى- كلية الطب البيطري- جامعة قناة السويس- ٤١٥٢٢ الإسماعيلية- مصر

تهدف هذه الدراسة إلى تقييم دقة الموجات فوق الصوتية في تحديد جنس الجنين في النعاج وكذلك تحديد تأثير نوع جنس الجنين على مستويات الكالسيوم والفسفور والحديد في سيرم الأم. تم استخدام ٢٤ نعجة بالغة في هذه الدراسة وتم ملاحظة الشبق مرتين يوميا عند الصباح والمساء. أيضا تم تلقيح النعجة التي تظهر الشبق ثلاث مرات بينهم ١٢ ساعة. تم تجميع عينات دم من كل نعجة يوم التلقيح ثم كل ١٥ يوما حتى الولادة أو ١٥٠ يوما بعد التلقيح لتحديد مستويات الكالسيوم والفسفور والحديد.

لتحديد جنس الجنين تم استخدام الموجات فوق الصوتية عن طريق المستقيم أو جدار البطن باستخدام ماسح (Transducer) متعدد الترددات (٥، ٧,٥، ١٠ ميجاهرتز). تم تأكيد الحمل بالسونار عند اليوم ٣٠ بعد التلقيح وبعد ذلك تم الفحص مرتين أسبوعيا حتى تم تحديد نوع جنس الجنين.

أظهرت النتائج أن الموجات فوق الصوتية طريقة فعالة وجديرة بالثقة لتشخيص الحمل بدقة ١٠٠% عند اليوم ٣٠ بعد التلقيح. أيضا أمكن تحديد نوع جنس الجنين عند اليوم ٥٠,٩٥ بعد التلقيح بدقة وصلت ٩٢,٣% في الذكور ٨٧,٥% في الإناث. أظهرت نتائج تحليلات السيرم أنه لا يوجد تأثير معنوي لنوع جنس الجنين على مستويات الكالسيوم والفسفور والحديد في سيرم الأم. من ناحية أخرى كان هناك انخفاض معنوي ($P\leq0.01$) في مستوى الكالسيوم من منتصف الحمل وحتى نهايته وزيادة معنوية ($P\leq0.05$) في مستوى الحديد في الشهر الأخير من الحمل.

يمكن استنتاج أن استخدام الموجات فوق الصوتية عن طريق المستقيم أو البطن أثبت أنه طريقة جيدة ليس فقط لتشخيص الحمل ولكن لتحديد نوع جنس الجنين في النعاج. وأنه لا يوجد تأثير معنوي لجنس الجنين على مستويات الكالسيوم والفسفور والحديد في سيرم الأم إلا في بعض مراحل الحمل.