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SENSITIVITY AND SPECIFICITY OF REAL-TIME ULTRASONOGRAPHY FOR PREGNANCY DIAGNOSIS AND LITTER SIZE DETERMINATION IN SAANEN GOATS (CAPRA HIRCUS)

(with 4tabls & 5Figs)

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حساسية ونوعية الموجات الصوتية الحية لتشخيص الحمل وتحديد حجم المواليد فى أغنام السعانين

بواسطة

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الملخص العربى

الهدف من إجراء الدراسة الحالية هو وصف حساسية ونوعية الموجات فوق الصوتية الحية لفحص الحمل وتحديد عدد المواليد فى أغنام السعانين. إستخدمت لهذه الدراسة ٢١ بكر و٣٩ متعددة الولادات من أغنام السعانين . تمت مراقبة الحيال بحرص وذلك بإدخال ذكر حيوان كاشف مرتين فى اليوم. الانثى التى تظهر أعراض الحيال سرعان مايتم تلقيحها بإستخدام منى طازج تم تجميعه من ذكر مثبت الخصوبة، وبالتالى يعتبر يوم إدخال الذكر هو اليوم صفر فى الحمل.تم إستخدام جهاز موجات صوتية حية من نوع (بايميدكال ،إيسوت، هولندا) مزودج بتردد (٣-٣,٥) ميجاهيرتز مجس محدب و(٥ -٧,٥) ميجاهيرتز مجس محدب صغير لتحديد الحمل وكان الحيوان مكبوح جماحه جيدا فى وضع الرقاد الظهرى فى طاولة خاصة. من ٦٠ أنثى، ٤٤ تم تشخيصهم كحامل (حساسية ١٠٠%) و١٦ تم تشخيصهم غير حامل (نوعية ١٠٠%) وذلك بنسبة دقة ١٠٠%. فيما يخص حجم المواليد فان الدقة الكلية لكل التخمينات وجدت ٩٢,٥%. الحساسية والنوعية لتحديد عدد الأجنة المفردة كانت ١٠٠% لكليهما. نسبة الحساسية، النوعية، القيمة التخمينية الموجبة والقيمة التخمينية السالبة لتحديد عدد الأجنة التوائم كانت ١٠٠، ٨٦,٣، ٨٥,٧، و ١٠٠% على الترتيب. الحساسية،

النوعية، القيمة التخمينية الموجبة والقيمة التخمينية السالبة لتحديد عدد الأجنة الثلاثية كانـــت ٢٥، ١٠٠، ١٠٠ و ٩٢,٣ % على الترتيب. من اربع إناث تحمل أجنة ثلاثية واحدة فقط تم تشخيصها صحيحا على انها تحمل أجنة ثلاثية بينما الثلاث الأخريات تم تشخيصها على انها تحمل توائم. فى الختام وجد ان الموجات فوق الصوتية الحية فعالة،يعتمد عليها ولا تـــستهلك وقت فى تشخيص الحمل وتحديد عدد الأجنة المفردة والتوائم بينما فشلت الطريقة فى تحديـــد عدد الأجنة الثلاثية بكفاءة.

SUMMARY

The aim of the present study was to report the sensitivity and specificity of real-time ultrasonography for pregnancy diagnosis and litter size determination in Saanen goats. Null (n=21) and multiparous (n=39) Saanen goats were involved in the present study. Estrus was carefully observed by introducing a teaser buck twice a day. A doe exhibiting estrus signs was immediately inseminated artificially using fresh semen collected from a proven fertile buck; thus the day of introducing the buck for detection of heat was considered as day 0 of gestation. Real-time ultrasound machine (Pie medical, Easote, Holland) equipped with dual frequency (3.5-5) MHz curvilinear probe and (5-7.5) MHz micro convex probe was used, while the animal was well restrained on a dorsal recumbancy on special table. Out of 60 does, 44 (Multiparous=31, Nillparous=13) were diagnosed as pregnant (Sensitivity =100%) and 16 as non-pregnant (Specificity =100%) with accuracy reaching 100%. Concerning litter size the overall accuracy for all predictions was found to be 92.5%. The sensitivity (Se) and specificity (Sp) for determining single fetuses was 100% for both. The Se, Sp, positive predictive value (PPV) and negative predictive value (NPV) for determining twin fetuses were 100, 86.3, 85.7 and 100 % respectively. The Se, Sp, PPV and NPV for determining triplet fetuses were 25, 100, 100 and 92.3% respectively. Out of four does bearing triplet fetuses only one doe was correctly diagnosed as bearing triplets and the other three ones were diagnosed as bearing twins. In conclusion real-time ultrasound was found to be efficient, reliable and non-time consuming tool in diagnosing pregnancy and determining single and twin fetuses in Saanen goats, however the method failed to determine triplets effectively.

Key words: Sensitivity, Specificity, Pregnancy diagnosis, Ultrasound, Goats

INTRODUCTION

Early and accurate diagnosis of pregnancy in livestock is useful to make decisions for food allotment, and allows separating the flock into pregnant and non-pregnant females to permits scheduling breading technology (Wani *et al*., 1998, Yotov 2005, Anwar *et al*., 2008). In addition to that accurate information on the stage of pregnancy would be useful to dry off lactating females and to monitor females near term (Karen *et al*., 2001). Until recently there was no reliable way of pregnancy detection in goats (Padilla and Holtz, 2000). For the past few years two dimensional diagnostic ultrasonic methods have become available to veterinary medicine (Taverne, 1984). Since its introduction it has been used in large scale to monitor the reproductive status of sheep and goats (Azevedo *et al*., 2007). It has been used successfully to diagnose pregnancy in domestic and non-domestic animals as well as, appears feasible for diagnosing pregnancy where immediate determinations are required (Kemble canon *et al*., 1997). Prediction of the number of fetuses is of considerable value in reproductive management, it could provide earlier treatment for prevention of lambing difficulties (Fukui *et al*., 1986, Martinez *et al*., 1998).

Although there are many reports on transcutaenous and transrectal ultrasonography in sheep, there is a paucity of information on the suitability of this technique in goats (Padilla-Rivas *et al*., 2005). In Sudan there are only few reports concerning uses of ultrasound techniques for pregnancy diagnosis and determination of fetal numbers in Saanen (Abdelghafar *et al*., 2007b) and Damascus goats (Abdelghafar *et al*., 2009), prediction of the gestational age in Saanen goats (Abdelghafar *et al*., 2007a). The objective of the present study was to report the sensitivity and specificity of real-time ultrasonography for pregnancy diagnosis and litter size determination in Saanen goats.

MATERIALS AND METHODS

All procedures were performed with the approval of the General Directorate of Animal wealth, Ministry of Agriculture, Animal wealth and Irrigation, Khartoum State, Sudan. Null (n=21) and multiparous (n=39) Saanen goats (total= 60) were used in the present study. Their ages were between 1-5 years and weighing between 23-60 kgs. They were kept and managed under closed system at Khartoum Livestock Genetics Improvement Center, Ministry of Agriculture, Animal wealth and Irrigation, Khartoum State. They were fed Alfa Alfa hay *ad libitum*

as roughages and a mixture of (Sesame cake, Groundnut cake, Sorghum and Wheat brand) as a concentrate ration in the amount of 1/2 kg per day per doe. The animals had free access to water and minerals blocks supplement. Estrus was carefully observed by introducing a teaser buck twice a day. A doe exhibiting estrus signs was immediately inseminated artificially using fresh semen collected from a proven fertile buck. Thus the day of introducing the buck was designated as day 0 of gestation.

Ultrasound scanning:

Animals were kept off food for 12 hours prior to scanning. The ventral abdomen was clipped and shaved carefully using manual clippers. Animals were turned on their backs (dorsal docubitus) and well restrained on a special table designed for this purpose as recommended previously by Abdelghafar (2006).

Sufficient amount of ultrasonic gel was applied to the ventral abdomen prior to scanning. Real time ultrasound machine (Pie-medical Easote, Holland) equipped with dual frequency (5-7.5) micro convex probe and (3.5-5 MHz) curvilinear probe was used. Only a single ultrasound scanning was performed on each doe simulating field examination studies. Images were stored in a memory card attached to the scanner and later were printed in thermal paper, Sony corporation type 1 (Normal), UPP-110S, 1-7-1, Konan, Minato-KU, Tokyo, Japan) using video graphic printer UP-895EC (Sony- Japan). Sagittal, parasagittal, cross and cross oblique sections were taken to ascertain accurate diagnosis.

Statistical analysis

The experiment was designed in a complete randomized design; the chi square test for independency was used to evaluate the statistical association between the recorded prenatal observations of fetal number (P value 0.000) using real-time ultrasonography and the actual numbers at delivery using SPSS.

Statistical analysis was carried out by evaluating the accuracy of the real-time ultrasonography, Sensitivity (Se), specificity (Sp), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) (Table 1). Sensitivity of a diagnostic method is defined as the proportion of true positives that are detected by the method and specificity is defined as the proportion of true negatives that are detected by the method (Thrusfield, 1995). The positive predictive value or precision rate is defined as a proportion of positive test results that are correctly diagnosed and negative predictive value is a proportion of negative test results that are correctly diagnosed.

Table 1: Evaluation of the accuracy of ultrasound compared to delivery

		Delivery (Gold standard)		
		positive	***negative***	
ultrasound outcome	***positive***	**(TP)**	**(FP)**	**→ TP+FP**
	Negative	**(FN)**	**(TN)**	**→ FN+TN**
		↓ TP+FN	**↓ FP+TN**	

TP = True positive, TN = True negative, FP = False positive, FN = False negative

$$Sensitivit \quad y = \frac{TP}{TP + FN} \qquad Specificit \quad y = \frac{TN}{TN + FP}$$

$$PPV = \frac{TP}{TP + FP} \qquad NPV = \frac{TN}{TN + FN}$$

RESULTS

Animal was said to be pregnant when fluid-filled gestational sac (Fig. 1) and/ or fetus (Fig. 2) were recognized. In the present study out of 60 does, 44 (Multiparous=31, Nillparous=13) were diagnosed as pregnant (Sensitivity=100%) and 14 as non-pregnant (Specificity=100%); with 100% accuracy (The gold standard test was delivery).

Regarding litter size determination only 40 does were subjected to litter size determination. The overall accuracy of ultrasound for all predictions was found to be 92.5%. Out of 40 does; only three animals were incorrectly diagnosed. The sensitivity and specificity in determining single fetuses was found to be 100% for both. Out of 40 does, 18 does were diagnosed as bearing single fetus with 100 % accuracy (Table 2). The sensitivity, specificity, PPV and NPV in determining twins (Fig.3 a, b) were 100, 86.3, 85.7 and 100% respectively. Out of 40 does, 18 were correctly diagnosed as bearing twins with 100% accuracy (Table 3). The sensitivity, specificity, PPV and NPV in determining triplet fetuses (Fig. 4) were 25, 100, 100 and 92% respectively. Out of 4 does bearing triplet fetuses; only 0ne doe was correctly diagnosed as bearing triplets and the other three ones were incorrectly diagnosed as bearing twins (Table 4). All fetuses were alive

due to their heart beats and movements. The mean gestational length in this study was found to be 146.03 ± 12 days.

Table 2: Sensitivity and Specificity of determining single fetuses

		Delivery (Gold standard)		
		One fetus	***Different than one fetus***	
ultrasound outcome	***One fetuses***	**(TP) ١٨**	**0 (FP)**	**→ 18**
	Different than one fetus	**(FN) 0**	**22 (TN)**	**→ 22**
		↓ 18	**↓ 22**	**40**

Sensitivity = 100%
Specificity =100%

Table 3: Sensitivity, Specificity, PPV and NPV of determining twins

		Delivery (Gold standard)		
		Two fetuses	***Different than two fetuses***	
Ultrasound outcome	***Two fetuses***	**(TP) 18**	**3 (FP)**	**→ 21**
	Different than two fetuses	**(FN) 0**	**19 (TN)**	**→ 19**
		↓ 18	**↓ 22**	**40**

Sensitivity (Se) =100 %
Specificity (Sp) =86.3 %
PPV = 85.7 %
NPV = 100 %

Table 4 :Sensitivity, Specificity, PPV and NPV of determining triplet fetuses

		Delivery (Gold standard)		
		Three fetuses	***Different than three fetuses***	
ultrasound outcome	***Three fetuses***	**(TP) 1**	**0 (FP)**	**→ 1**
	Different than three fetuses	**(FN) 3**	**36 (TN)**	**→ 39**
		↓ 4	**↓ 36**	**40**

Sensitivity (Se) =25 %
Specificity (Sp) = 100%
PPV = 100%
NPV= 92.3%

DISCUSSION

In the present study the accuracy of ultrasound for pregnancy diagnosis was found to be 100 % , this is in accordance with our previous studies in Saanen goats (Abdelghafar *et al*., 2007b) and Damascus goats (Abdelghafar *et al*., 2009); and with Dias *et al*., (2009) and Medan *et al*., (2004) who reported 100 % accuracy in ewes and Shipa goats respectively. Gearhart *et al*. (1988) and White *et al*. (1984) found similar results.

Concerning litter size determination, the overall accuracy of ultrasound for all predictions was found to be 92.5%. The results of the present study demonstrated clearly that real-time ultrasonography could accurately detect single (Sensitivity =100%) and twin fetuses (Sensitivity=100%); however the method failed to detect triplet fetuses in 75% of the cases (sensitivity =25%). Out of four does bearing triplets only one was correctly diagnosed as bearing triplets and the other three ones were incorrectly diagnosed as bearing twins; this could be due to the stage of pregnancy as the correctly diagnosed doe was scanned at 47 days post insemination while the other three does were scanned 90 days post insemination; at this time only two fetuses could be depicted in the

screen. This is in accordance with Abdelghafar *et al*. (2007b) who diagnosed triplet fetuses in Saanen and Damascus goats (Abdelghafar *et al*. 2009) at about 8 wks of gestation and with Dawson *et al*. (1994) who reported 100% accuracy in determining triplets at 7wks of gestation in alpine does using transabdominal approach. Haibel *et al*. (1989) reported that accuracy in differentiating singleton from twin pregnancy is higher than differentiating twins from triplets and depends on the stage of pregnancy, equipment and operator experiences. In the present study although the operator is so experienced but the low sensitivity of ultrasonography in detecting triplets (25%) was due to the stage of pregnancy; this claims were supported by many authors. Karen *et al*. (2001) reported that the accuracy of ultrasound in detecting ewes carrying two fetuses or more was disappointed; while Padilla-Rivas, *et al* (2005) reported that in Boer goats it is some times difficult to identify more than two kids. Goel and Agrawal (1992) reported that it is difficult to differentiate twins from triplets or quadruplets at any stage of pregnancy.

Good preparation of the animal (fasting, clipping and shaving of the skin), putting the animal on a dorsal recumbancy and using the transabdominal approach participated clearly in obtaining high accuracies in the present study.

In conclusion, ultrasound was found to be of high accuracy, reproducible, non- invasive and potential tool for pregnancy diagnosis and litter size determination in Saanen goats. Scanning the does on a dorsal recumbancy on special table using transabdominal approach is highly recommended.

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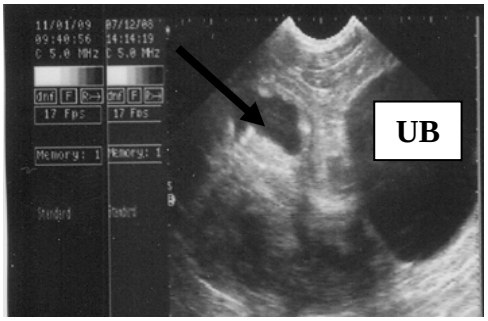

Fig. 1. Gestational sac (Arrow) at day 28 of gestation UB =Urinary bladder

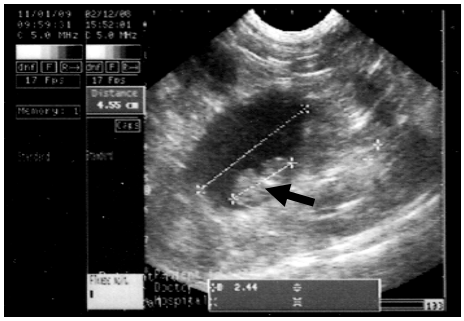

Fig.2. Gestational sac measuring 4.55 cm with fetus Measuring 2.44 cm (Arrow) at day 42 of gestation

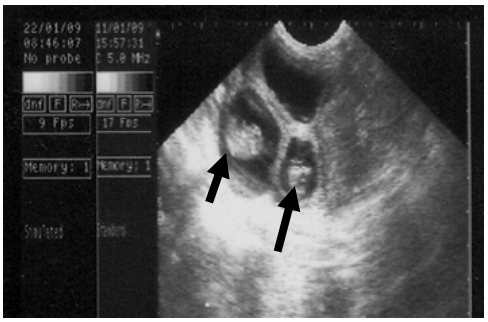

Fig. 3a. Twins at day 35 of gestation (Arrows)

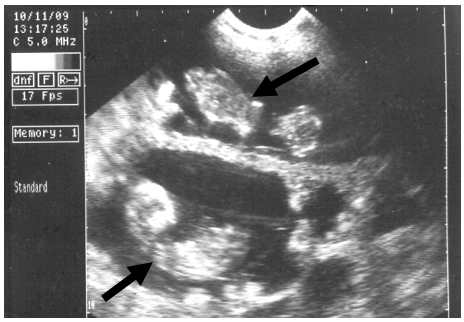

Fig. 3b. Twins at 54 days of pregnancy (Arrows)

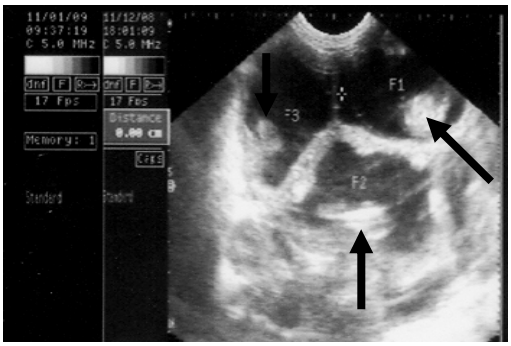

Fig. 4. Triplet fetuses at day 47 of gestation (Arrows)