ACCURACY OF EARLY PREGNANCY DIAGNOSIS AND DETECTION OF TWINS USING REAL-TIME ULTRASONOGRAPHY COMPARED TO PROGESTERONE CONCENTRATION IN BARKI GOATS

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SUMMARY

Pregnancy diagnosis was carried out in 53 Barki goats after 45, 55, and 75 days of the start of the mating season (September) using a real-time ultrasound. The calculated corresponding gestational ages were 20, 35, and 50 d for the scanning on days 45, 55, 75 of mating season, respectively. Transrectal ultrasound (TRU) data were compared to serum progesterone concentrations in pregnant and non-pregnant does. Pregnancy rate was differed insignificantly among the tested days ranging from 86 to 96%. Detection of twin pregnancy increased (P<0.01) from 9.9% at day 45 after the onset of mating season to 45.3% in days 55 and 75. Pregnant does carrying ≤19 days-old embryos either correctly or incorrectly diagnosed by TRU had high serum progesterone concentrations. Progesterone concentrations were almost similar in open does and those pregnant in singles or twins (10.9, 12.2, and 12.9 ng/ml, respectively). Progesterone concentrations tended to increase with the advance of pregnancy (10.4, 11.7, 15.0 ng/ml in days 45, 55, and 75, respectively) however insignificant. The present study revealed that early diagnosis of pregnancy and determination of number of fetuses based on serum progesterone concentrations proved to be inconclusive. Pregnancy diagnosis using ultrasound technique is recommended for higher accuracy after day 20 onwards after mating. Accurate detection of single or multiple fetuses in goat does should be carried out starting from day 30 after mating.

keywords: Goats, ultrasound, progesterone, pregnancy, twins

INTRODUCTION

Early identification of pregnant does either by serum progesterone or by ultrasound scanning of uterine contents enables herdsmen to cull non-pregnant does and give more managerial attention to pregnant ones (Padilla-Rivas *et al.*, 2005). Determination of serum progesterone (P4) concentration at 21-22 day post-breeding has been widely used to detect pregnancy in goats (Corteel *et al.*, 1982). Low progesterone concentrations (<1.5 ng/ml) has a 100% accuracy in detecting non-pregnant does (Boscos *et al.*, 2003). However, the accuracy of identifying pregnant does was reported to be 82.8%. High (1.5-2.5 ng/ml) P4 concentrations on day 22

after breeding in non-pregnant does may be due to the extension of CL lifespan for physiological disorders resulting in 34.4% of false diagnoses (Thibier *et al.*, 1982; and Williams, 1986).

The use of ultrasound to detect pregnancy in goats is limited to dairy goats (Padilla-Rivas *et al.*, 2005) and exclusively to transabdominal type of scanning (Buckrell, 1988; Lavoir and Taverne, 1989; Haibel, 1990; Russel, 1990; Hesselink and Taverne, 1994; and Kaulfuss *et al.*, 1996). Few studies have been conducted on the use of transrectal ultrasound in pregnancy diagnosis in meat goats (Martinez *et al.*, 1998; Padilla-Rivas *et al.*, 2005).

The purpose of the present work was to compare the accuracy of transrectal ultrasound technique and serum progesterone assay in early diagnosis of pregnancy and identification of twins in Barki goat does.

MATERIALS AND METHODS

This study was carried out at Bourg El-Arab Research Station for Animal Production, Ministry of Agriculture and the Department of Animal Production, Faculty of Agriculture, Alexandria University. The Research Station is located at 50 km west of Alexandria (31° 15′ N and 30° 10′ E).

Animals and management

A total of 53 Barki goat does were used during the breeding season, autumn (mean temperature was 28°C). The animals were kept in open barns and had free access to feed and water. Mating groups (n = 5) consisted of 11 does with a fertile buck. Bucks were rotated among mating groups every other week throughout the mating season. Fertile bucks were introduced to the does throughout September then pulled-out. Pregnancy diagnosis by ultrasonography was executed three times at 15, 25, 45 days after removing the bucks. Pregnant does were gathered in separate groups and open does were isolated to be re-bred in the following mating season. Dates of fertile mating were calculated retrospectively by subtracting 148 days from the kidding dates and gestational ages were then calculated at every scanning.

Ultrasonographic examination

Ultrasonographic examination of does was done via transrectal route in the standing position using a Concept MLV ultrasound device (Dynamic Imaging, Scotland UK) equipped with a linear 5.0 and 7.5 MHz probe (dual frequency probe) for early diagnosis of pregnancy and detection of number of fetuses. The system was connected to portable recording machine to record scanning sessions. The transducer was fitted to a plastic rod as an adaptor to enable manipulation of the probe after being inserted in the rectum (Garcia *et al.*, 1993). The rectum was evoked from feces if necessary and the lubricated probe was gently inserted into the rectum approximately 10cm deep until the anechoic content of the bladder was visible on the screen. The probe was rotated clockwise 90° and anti-clockwise 180° to scan the entire reproductive tract. Detection of the anechoic uterine fluids, embryo proper, or fetus was considered a positive sign of pregnancy (Fig. 1). Ventral abdominal lifting was done while conducting the scanning by the operator or by an assistant if necessary. No feed withholding was done to avoid further strain on the does.
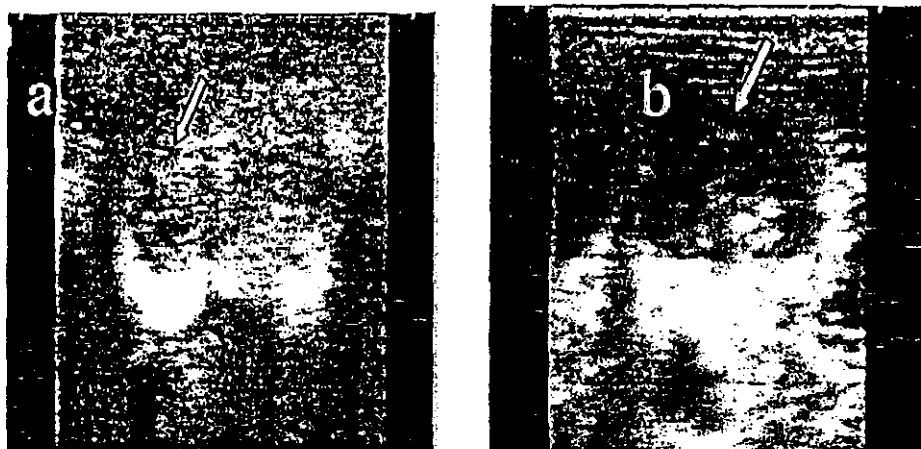
Figure 1. Ultrasound images of uterine contents of goat does showing (a), non-pregnant uterus (arrow) and (b), early pregnancy with a fluid-filled uterus, embryo proper and amnion sac (arrow).

Blood samples were collected by jugular venipuncture every time animals were handled for ultrasonography. Serum was harvested and stored at -20°C until assayed. Serum progesterone (P4) concentrations were determined by enzyme immunoassay ELISA using commercial kits (Calbiotech, Inc., Spring Valley, CA). The range of the standard used was 0.0 - 40.0 ng/ml. The inter- and intra-run precision had coefficients of variation of 2.9 and 4.8%, respectively.

Statistical analysis

Ultrasound data including pregnancy and twinning rates were analyzed using the procedure GLM (SAS, 1996). Percentages of pregnant and open does that were either correctly or incorrectly diagnosed by TRU were determined using the frequency procedure. Progesterone concentrations in open does, and in does with single and twin-pregnancy were compared using "*t*" test.

RESULTS AND DISCUSSION

Data in Table 1 indicate that the accuracy of pregnancy diagnosis using transrectal ultrasonography increased with pregnancy development however, no significant differences were observed among the given scanning days 45, 55, and 75. Furthermore, transrectal ultrasound diagnosis of pregnancies in the current study was done with satisfaction within 1 min in pregnant does in the standing position with older pregnancies being done in less time and effort. Others (Padilla-Rivas *et al.*, 2005) reported that the time required for the transrectal and transabdominal measurements in goats was, on average, 2.5 and 1.5 min, respectively.

In the current study, reliable detection of pregnancy via the transrectal ultrasound was made possible as early as 20 d after fertile mating in Barki goats which agrees with the findings of Buckrell *et al.* (1986); Garcia *et al.* (1993); Schrick and Inskeep (1993). It has also been reported that the embryo could be observed frequently but

not consistently on day 24 post-breeding in does (Bretzlaff *et al.*, 1993). The accuracy of using ultrasound scanning in twins identification was higher (p<0.01) on days 55 and 75 compared to day 45 after onset of mating season.

Table 1. Pregnancy and twining rates (%) and gestational age (day) in Barki goat does detected by transrectal ultrasound scanning of uterine contents after the onset of mating season (M±SE).

Item	Days after onset of mating season		
	45	55	75
Gestational age (days)	20±1.07[c]	35±1.07[b]	50±1.07[a]
Pregnancy rate (%)	86±0.04	90.2±0.04	96±0.04
Twining rate (%)	9.9±0.06[b]	45.3±0.06[a]	45.3±0.06[a]

Values with different superscripts within rows differ (P<0.05).

Averages of calculated gestational ages of embryos (20, 35, and 50 d) varied (P<0.05) at the scanning days 45, 55, and 75, respectively. Gestational ages were variable among animals at every respective scanning date because no estrous synchronization protocol was applied and consequently mating dates varied among individuals. In the current study, twin pregnancies were best detected by transrectal ultrasonography after 35 days of gestation. These findings are in agreements with those reported by Padilla-Rivas *et al.* (2005) who found that the best time to distinguish between single and twin fetuses with a 7.5MHz transrectal probe is between the 28th and the 40th day of pregnancy.

Accuracy of transrectal ultrasound examination to correctly diagnose pregnancy (TRU = pregnant) were 42.86 and 93.0% in pregnant does carrying ≤19 d and ≥20d-old embryos, respectively. However the percentage of incorrectly diagnosed open does (TRU = open; false positive) was 57.14% in pregnant does carrying ≤19 d-old embryos compared to only 7.0% in pregnant does carrying ≥20d-old embryos. Three does were diagnosed open and remained so until the end of mating season.

Progesterone concentrations were similar in all pregnant does regardless of age of embryos (≤ 19 d or ≥ 20 d-old) and diagnosis (correct or incorrect) of their pregnancy by transrectal ultrasound examination (Table 2). Non-pregnant (n=3) does had an average progesterone concentration of (10.9±2.9 ng/ml) that was comparable to pregnant does' progesterone concentrations. Detection of pregnancy using P4 determination was recommended to be used day 3 or 4 of estrous cycle onwards (Gonzalez de Bulnes *et al.*, 2000) meanwhile, progesterone concentration is not accurate in diagnosing pregnant animals as long as a functional CL is present despite their pregnancy status (Thibier *et al.*, 1982 and Williams, 1986).

Indeed, P4 concentrations more than 1.0 ng/ml on day 22 indicate a functional CL which is associated either with pregnancy, irregular length of the estrous cycle, early embryonic death, hydrometra, or luteal cysts (Fleming *et al.*, 1990; Ishwar, 1992). Other investigators (Thibier *et al.*, 1982) reported that progesterone concentration on day 22 after artificial insemination gave only 87.5% specificity estimating pregnancy in goats.

Table 2. Percentage of detected embryos (≤19 and >19 days old) compared to serum progesterone concentrations in Barki goat does after scanning with transrectal ultrasonography (TRU) of the uterine contents

Item	Gestational age (days)			
	≤19		≥20	
TRU	Pregnant	Open	Pregnant	Open
Pregnant goat does (%)	3 (42.86)	4 (57.14)	40 (93.0)	3 (7.0)
Progesterone concentrations (ng/ml±SE)	14.5±3.5	15.3±3.1	15.5±3.1	14.0±3.5

No significant difference in progesterone concentrations was detected between open, single, and twin-bearing does throughout the three scanning dates (Fig. 2). However, despite the number of fetuses carried, progesterone concentrations in pregnant does tended ($p<0.06$) to increase with the advancement of pregnancy in days 45, 55, and 75 after the onset of mating season (Fig. 2). Similar data were reported by Boscos *et al.* (2003) in Swiss goat breeds. The use of progesterone assay to predict the number of embryos carried during early pregnancy seems to be of no satisfactory accuracy (Eastwood *et al.*, 1976; Boscos *et al.*, 2003).
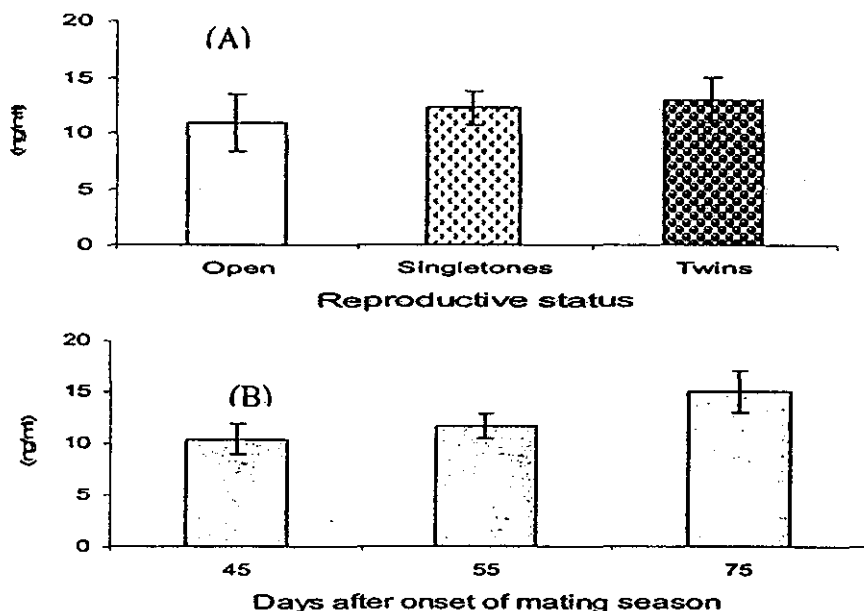

Figure 2. Changes in serum progesterone concentrations in pregnant and non-pregnant Barki goat does (A) and at different intervals after onset of mating season (B)

Data in Table 3 revealed that the accuracy of the TRU to detect pregnant animals was increased with pregnancy advancement, whereas P4 concentrations had accuracy of 100% in all pregnancy stages. However, specificity of TRU to detect non-pregnant

animals was always 100% compared to 33.3% for P4 concentrations in all the scanning dates. Similar results were reported by Corteel *et al.* (1982) who found that progesterone concentrations is very effective in detecting pregnant animals, while had less accuracy in identifying non-pregnant animals.

Table 3. Accuracy of pregnancy diagnosis using TRU compared to P4 based on 50 pregnant and 3 non-pregnant Barki goat does

Days of scanning	Sensitivity (%)		Specificity (%)	
	TRU	P4	TRU	P4
45	86	100	100	33.3
55	90.2	100	100	33.3
75	100	100	100	33.3

Sensitivity: calculated as percentage of pregnant animals correctly diagnosed.
Specificity: calculated as percentage of non-pregnant animals correctly diagnosed.

CONCLUSION

Based on the present results it can be concluded that detection of early pregnancy by determination of progesterone concentrations in goats serum was not consistent because there are many other reasons that causes increased serum P4 concentration. Transrectal ultrasonography (TRU) provides an appropriate alternative.

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دقة التشخيص المبكر للحمل و الكشف عن التوأمية باستخدام الموجات فـوق الــصوتية
مقارنة بتركيز هرمون البروجسترون فى سيرم دم المعز البرقى

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أجرى التشخيص المبكر والكشف عن عدد التوائم فى 53 من اناث الماعز البرقى باستخدام جهاز
الموجات فوق الصوتية (السونار) فى أيام 45 و 55 و 75 بعد بداية موسم التلقيح (سبتمبر). وتــم مقارنــة
نتائج الفحص بالسونار مع نتائج تركيزات هرمون البروجسترون فى سيرم الدم فى المعز الحامـل وغيـر
الحامل ولم توجد اختلافات معنوية فى معدلات الحمل (86 و 90.2 و96%) عند إجراء الفحص فى أيام 45
و 55 و 75 بعد بداية موسم التلقيح على التوالى. لكن كانت هناك اختلافات معنوية فى النسب المئوية للتوائم
عند التشخيص بالسونار (45.3%) فى كل من يومى 55 و 75 مقارنة بـ 9.9% عند الفحص فى يوم 45
بعد بداية موسم التلقيح. وكانت متوسطات أعمار الحمل المحسوبة 20 و35 و 50 يوم فـى أيـام الفحـص
السابق ذكرها. الأناث الحوامل فى أجنة أعمارها أقل من 19 يوم أو أكبر سواء تم تشخيص الحمـل فيهـا
بالسونار بطريقة صحيحة أو خاطئة وجد انها تحتوى على تركيزات مرتفعة من هرمون البروجسترون. لــم
توجد اختلافات معنوية فى تركيزات هرمون البروجسترون بين الماعز غير الحامل و الماعز الحامـل فـى
أجنة مفردة أو توائم (10.9 و 12.2 و 12.9 نانوجرام/مل على التوالى). مع التقدم فى الحمل كانت هنـاك
زيادة تلقائية فى تركيزات هرمون البروجسترون من 10.4 إلى 11.7 إلى 15 نانوجرام/مل فى أيام الفحص
45 و 55 و 75 بعد بداية موسم التلقيح على التوالى. أوضحت النتائج أن تشخيص الحمل مبكراً و تحديـد
عدد الأجنة بالاعتماد على تقدير مستوى هرمون البروجسترون فقط غير دقيق و لا ينصح به. كما توصـى
الدراسة بأن يجرى التشخيص المبكر للحمل فى إناث الماعز البرقى باستخدام تقنية السونار بداية من اليـوم
20 بعد التلقيح المخصب و فى حالة الرغبة فى تحديد عدد الأجنة يتم الفحص بعد اليوم الـ 30 من التلقيـح
المخصب.