

COMPARATIVE BETWEEN DIFFERENT TECHNIQUES FOR PREGNANCY DIAGNOSIS IN SHEEP

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ABSTRACT

The aim of this study was to compare the accuracy of five different methods to diagnose pregnancy in sheep. A total of 466 of crossbred (Finnish x Rahmani) ewes were mated naturally in September (n=382) and May (n=84) mating season. Serum P₄ concentrations at day 18 after mating were determined by RIA test. The ewes which showed blood P₄ >1 ng were considered pregnant. Based on lambing data, the accuracy for diagnosing pregnant (positive) and non-pregnant ewes (negative) were 96.59 and 100% at day 18, respectively. By day 30 after breeding, an accuracy of 80 and 94.2% has been obtained for pregnant ewes using non-return to estrus as pregnancy diagnostic method at May and September mating season, respectively. Pregnancy diagnosis is possible using ultrasonic-technique (A-scan) 42-85 day post breeding with an accuracy of 92.6 and 69% for pregnant and nonpregnant ewes, respectively. Abdominal palpation is effective during late pregnancy provides accuracy of 84.8 and 57.8% after 60-95 days of gestation in pregnant and nonpregnant ewes, respectively. The present results indicated that ewes pregnant in triple had significant increase in live body weight followed by ewes pregnant in twins and singleton- carrying ewes, with percentage of change in live body weight 6.87-10.29 and 11.52-16.51 after 60 and 90 days post breeding, respectively. It could be concluded that ovine pregnancy can be reliably with high accuracy and safety under field condition by using non-return to estrus, ultrasonic technique (A-scan), abdominal palpation and increase in body weight. The advantage of these methods over the P₄ test using RIA method is overcoming lab expenses.

INTRODUCTION

Reliable techniques for early detection of pregnancy in sheep and goats aid in culling or rebreeding of barren ewes and does, and provides a valuable tool for controlled breeding programs. Inability to detect early pregnancy can result in economic losses in milk and lamb production due to long kidding and lambing intervals (Ishwar, 1995). Moreover, special attention and supplementary feed can then be given to the pregnant animals (Padilla-Rivas *et al.*, 2005).

Many methods have been described to diagnose pregnancy in sheep and goats, however, most of them are not adaptable to field condition and are expensive. Therefore, it the objective of the present work to study the accuracy of some practical and economic techniques for pregnancy diagnoses in herds of ewes under field condition.

MATERIALS AND METHODS

This study was carried out at Sakha Experimental Farm, belonging to the Animal Production Research Institute, Ministry of Agriculture, located in the Northern part of Delta (latitude 31°N and longitude 31°E).

Experimental Design:

The aim of this work was to compare the accuracy of five different methods for pregnancy diagnosis.

A total of 466 mature Finn. crossbred ewes were available for this study, 382 of them mated in September and 84 ewes mated in May mating season. The ewes were examined for pregnancy at different stages of gestation period using five different methods as following:

1. Non-return to estrus:

Detection of heat was done using two teaser rams, twice a day (at 8 a.m. and 4 p.m.) for 30 days post mating in ewes mated in September (n=348) and May (n=84) mating seasons. Ewes showing estrous were recorded and considered as failed to conceive.

2. Progesterone (P₄):

Blood samples (about 5 ml) were taken from the jugular vein at day 18 after mating. Samples were collected (n=94) in vacotainer tubes to determine serum progesterone level. After 30 minutes samples were centrifuged for 15 minutes at 2500 rpm for serum separation. Serum samples are stored at -20°C until the assay was carried out at the RIA Laboratory of the Sheep and Goats Research Division, APRI.

Progesterone assay was carried out using (COAT-A-COUNT) kits. The coat-A-count progesterone procedure is a solid phase radioimmunoassay, produced by coat-A-count Co., USA. The standard curve ranged between 0 and 40 ng/ml. The sensitivity of the procedure was as little as 0.02 ng/ml.

Ewes with progesterone levels of 1 ng/ml or higher at day 18 after mating were recorded as being pregnant.

3. Ultrasound:

Ultrasonic technique (A-scan) was used for pregnancy diagnosis in 382 ewes by uterine detection during 42-85 days after mating. HK-pregnancy detector for sows and ewes, (Rheinechnid, West-Germany) was used. Detected animals were not less than 30 days pregnant. Ewes were deprived of food and water for 12-16 hours prior to diagnosis. The transducer was placed on the lower right flank in front of the udder and directed toward the uterus of the standing ewes. Wool of this area was clipped to facilitate optimal contact. A commercial ultrasonic gel or vegetable oil was applied to the transducer to eliminate air spaces between the skin and transducer head. When continuous sound occurred for 1-2 second, the ewe was defined as pregnant (positive diagnosis), but split sound mean that ewe was not pregnant (negative diagnosis).

4. Abdominal palpation and ballotement:

Abdominal palpation and ballotement was used for pregnancy diagnosis in 360 ewes by balloting at the base of the udder (Sane *et al.*, 1982). Ballotement was done by keeping the ewes in standing position and lifting the abdomen repeatedly in front of the udder. If the ewe is pregnant

the fetus is felt to drop on to the palpating hand (Arther *et al.*, 1982). Ewes were deprived of food and water for at least 12 hours prior to examination.

5. Increase in body weight (kg):

A total of 348 ewes were weighted at mating and thereafter monthly intervals till 90 days post mating to determine body weight changes.

6. Statistical analysis:

Chi square (χ^2) of independence was used to compare the accuracy of different methods of pregnancy diagnosis recorded as percentages. The obtained data of body weight was statistically analyzed using general linear models procedure adapted by SPSS (1997) for user's guide.

RESULTS AND DISCUSSION

1. Estrous return:

Number of ewes did not return or returned to estrus within 30 days post breeding at May and September mating seasons for this study are presented in Table 1.

Table (1): Accuracy of non-returning to estrus (within 30 days post-breeding) as a pregnancy diagnostic method.

Mating season	Total number	No. of ewes		Accuracy %
		Non-returned to estrus	Lambded	
		Ve+	Ve+	
May	84	80	64	80.0 ^b
September	348	309	291	94.2 ^a

a and b: the different superscripts within column are significant at ($P < 0.05$)

Results of this study showed (Table 1) that percentage of ewes mated at season of May and lambded represented 80.0% of those non returned to estrous and were considered to be pregnant. The corresponding value for those mated at September season was 94.2% with a significant ($P < 0.05$) superiority of 14.2%. Such superiority might be due to seasonal effect on ovarian and estrous activity. Results showed that 68.42% of the ewes mated at the September failed to be pregnant compared with a percent of 25% (4/16) in May season.

Percentage of non return to estrous (NR%) within 23-25 days has been reported by several authors (Machado and Simplicio, 1990 ; Schakell *et al.*, 1990 ; Cappai *et al.*, 1997 and El-Maghraby, 2003) to express conception rate.

El-Maghraby (2003) reported that the overall accuracy of non-return to estrus (within 23 days post breeding) as pregnancy diagnostic method in ewes was 96.55%.

Khalifa (1993) considered ewes as pregnant when they did not exhibited signs of estrus during the next two estrous cycles post insemination

Percentage of ewes lambled, on this basis were 75.7% of those considered as conceived.

2. Serum progesterone (P₄) level:

Serum progesterone determination was carried out on bred ewes on day 18 following mating to test the accuracy of the technique to detect ewes in early pregnancy. A progesterone level of 1 ng/ml or higher on day 18 post mating was considered as a sign of pregnancy. Number of ewes showed positive or negative progesterone test compared with those lambled or not are presented in Table 2.

Table (2): Serum progesterone (P₄) level on day 18 as early pregnancy diagnostic method.

No. of tested ewes	P ₄ level (ng/ml)		No. of ewes lambled	Accuracy %
	N	mean±SE		
Pregnant (+)	88	3.78±0.49	85	96.59
Non-Pregnant (-)	6	0.31±0.07	-	100

Eighty five ewes out of ninety four used in this test, proved to be pregnant and lambled. When the 94 ewes were tested for pregnancy using serum progesterone (P₄) 88 ewes were predicted to be pregnant on day 18 post mating. Accuracy of positive response to the test was 96.59% on day 18 post mating. All ewes diagnosed as non pregnant on the tested day, actually failed to conceive. Results showed that accurate positive response to pregnancy test could be obtained early (day 18 post breeding).

The level of P₄ for pregnancy diagnosis (>1 ng/ml) had been considered by Mukasa-Mugerwa *et al.* (1990) to be an indication of anoestrous or early luteal phase of estrous cycle. It was also considered by Halbert *et al.* (1990a) as the minimum level, on day 18 following mating indicating pregnancy. However, Robertson and Sarda (1971); Thimonier *et al.* (1977) and Langford *et al.* (1983) reported that ewes could be considered as pregnant if they had plasma P₄ concentration more than 0.5 ng/ml on day 18. Longford *et al.* (1983) obtained 72% accuracy of positive pregnancy diagnosis

However, El-Magharby (2003) obtained an accuracy of positive pregnancy diagnosis on base of 1 ng/ml to be 96% on that base Aly Karen *et al.* (2003) obtained an accuracy of positive and negative pregnancy diagnosis 95.4 and 100%, respectively, which are similar to our results .

3. Ultrasonic diagnosis:

A total of 382 ewes were tested ultrasonically for pregnancy 42-85 days post mating. Ewes were classified into 2 categories according to their pregnancy stage at time of testing. Response of ewes to ultrasonic pregnancy test are presented in Table 3. Results showed that accuracy of ultrasonic diagnosis increases as stage of pregnancy advances.

Table (3): Accuracy of ultrasonic techniques as "pregnancy diagnosis for ewes

Days post mating	No. of ewes		Accuracy %
	ultrasonically examined	lambled	
Pregnant ewes:			
42-60 days	120	102	85.0 ^b
61-85 days	220	213	96.8 ^a
overall	340	315	92.6
Non pregnant ewes:			
42-60 days	28	10	64.3 ^b
61-85 days	14	3	78.6 ^a
overall	42	13	69.0

a and b: the different superscripts within column are significant at ($P < 0.05$) for pregnant and non-pregnant ewes.

Accuracy of positive diagnosis was 85% during the stage from 42-60 days of gestation, increased to 96.8% during the period 61-85 days post mating. For ewes diagnosed as non-pregnant, accuracy of the test increased also as pregnancy advanced being 64.3 and 78.6% during the same two stages, respectively.

Lindahl (1969) reported that in ewes the earliest time at which pregnancy could be detected in ewes by using A-scan is between 40 and 50 days after mating.

Dinc *et al.* (1989) found that percentage of true positive pregnancies detected were 97.7% at 38-81 days post mating which is higher than the present results (92.6%).

Early work with externally applied A-Scan indicated that an accuracy of at least 95% is possible between 60 and 80 days of gestation in ewes (Haible, 1990) which is almost similar to the present result (96.8%) during the same period for pregnancy and 78.6% for non pregnancy.

El-Maghraby (2003) recorded accuracies of positive diagnosis to be 96.3 and 100% in ewes at 38-60 and 61-99 days of pregnancy, respectively. However, in non pregnant ewes it was 25 and 50% during the same stages.

An extended urinary bladder, hydrometer or pyometra may give false positive. False negatives may occur in early gestation or in late gestation because of decrease in the ratio of uterine fluid to fetal (Ishwar, 1995).

4. Abdominal palpation and ballotement:

A total of 360 ewes were examined by using abdominal palpation and ballotement for pregnancy after 60-95 days of mating. Ewes were classified into two categories according to their pregnancy stage at time of testing. The accuracy of this method as a pregnancy test are presented in Table (4).

Results showed that accuracy of abdominal palpation diagnosis increases as stage of pregnancy advances.

Accuracy of positive diagnosis was 77.8% during the stage from 60-75 days of gestation, increased to 90% during the period 76-95 days after mating. For ewes diagnosed as non-pregnant, the accuracy of the test was 53.6 and 64.7% during the two stages, respectively.

Table (4). Accuracy of abdominal palpation as a pregnancy diagnostic method

Days post mating	No. of ewes		Accuracy %
	Examined	lambled	
Pregnant ewes:			
60-75 days	135	105	77.8 ^b
76-95 days	180	162	90.0 ^a
overall	315	267	84.8
Non pregnant ewes:			
60-75 days	28	13	53.6 ^b
76-95 days	17	6	64.7 ^a
overall	45	19	57.8

a and b: the different superscripts within column are significant at ($P < 0.05$) for pregnant and non-pregnant ewes.

Pregnancy can be diagnosed to an acceptable accuracy after 80 days of gestation by the abdominal palpation method. Pratt and Hopkins (1975) recorded accuracy of 80 to 90% in ewes at 90-130 days of pregnancy. However, the present work indicated that reasonable accuracy (77.8 and 90%) in predicting pregnancy in ewes could be obtained at earlier stages (60-75 and 76-95 days post breeding), respectively by using abdominal palpation method. The accuracy of abdominal palpation method was recorded by Goel and Agrawal (1990) to be 70%.

Ewes and does in the late stages of pregnancy can be examined by these technique. These become easier and more reliable as pregnancy advances. It is easier in thin ewes and does than in fat animals (Ishwar, 1995)

5. Increase in body weight (kg):

There are lack in information regarding live body weight changes as a pregnancy diagnosis in sheep.

Table (5) indicated the percentage of change in the live body weight of ewes according to pregnancy status (days post mating) and type of pregnancy (single, twins and triple). It was 2.53, 6.87, 8.38 and 10.29% for non pregnant ewes, pregnant in single, twins and triple after 60 days post breeding, while it was 4.23, 11.52, 11.70 and 16.51% after 90 days post breeding, respectively.

Table (5): Body weight changes of ewes as a pregnancy diagnostic method.

Days post mating	Body weight (kg)							
	Non-pregnant ewes		Pregnant ewes					
			Single		Twins		Triple	
	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE
(0) at mating	57	50.09±1.07 ^a	118	50.24±0.71 ^c	157	51.53±0.53 ^c	16	55.31±1.74 ^b
60 days	57	51.36±1.03 ^a	118	53.69±0.74 ^b	157	55.85±0.56 ^b	16	61.00±1.77 ^a
90 days	57	52.21±1.05 ^a	118	56.03±0.73 ^a	157	58.59±0.55 ^a	16	65.55±1.74 ^a

a,b and c: the different superscripts within column are significant ($P < 0.05$)

These results agree with the findings of Domaniski and Lipecka (1966), who observed that body weight increased by about 13-16% in ewes carrying twins at the fourth month of gestation. Ewes carrying single lambs had body weight gain of 6-12%. The present data in Table (5) indicated that ewes pregnant in triple had significant increase in live body weight followed by ewes pregnant in twins and singleton-carrying ewes and the least was recorded in non pregnant ewes.

It could be concluded that the non-return to estrus, ultrasonic technique (A-scan), abdominal palpation and increase in body weight are a reliable methods for diagnosing pregnancy in sheep with high accuracy, safety, inexpensive and easy to perform in diagnosis pregnancy in sheep. However, pregnancy can be diagnosed as early as day 18 of gestation by using the P4 test in sheep with high accuracy

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مقارنة طرق مختلفة لتشخيص الحمل في الاغنام

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معهد بحوث الانتاج الحيواني

- اجريت هذه الدراسة بمحطة بحوث الانتاج الحيواني بسخا التابعة لمعهد بحوث الانتاج الحيواني ، وزارة الزراعة التي تقع في الجزء الشمالي من التلثا حط عرض ٣١ ، خط طول ٣١ . استخدم في هذه الدراسة عدد ٤٦٦ نعجة خليط (فنلندي × رحمانى) ، منهم ٣٨٢ اجري لهم تلقيح طبيعي في موسم تلقيح سبتمبر ، وعدد ٨٤ نعجة تم تلقيحهم طبيعي في موسم مايو . تم فحص هذه النعاج لتشخيص الحمل بها باستخدام خمسة طرق مختلفة لمقارنة دقة تشخيص الحمل ويمكن تلخيص أهم النتائج المتحصل عليها فيما يلي:
- تم تقدير مستوى هرمون البروجستيرون عند اليوم ١٨ من التلقيح باستخدام طريق RIA واعتبر تركيز البروجستيرون ١ نانوجرام/ملي او اكثر كدليل للحمل ، دقة الاختبار الموجب للحمل كانت ٩٦,٥٩% ، كل النعاج التي تم تشخيصها على انها غير حامل كانت حقيقيا غير مخصبة كانت بنسبة ١٠٠% .
 - المتوسط العام لدقة عدم العودة للنباح كطريقة لتشخيص الحمل كان ٨٠% ، ٩٤,٢% خلال موسمي مايو وسبتمبر على الترتيب.
 - المتوسط العام لدقة تشخيص الحمل باستخدام الموجات فوق الصوتية (A-scan) كان ٩٢,٦% . ٦٩,٠% للنعاج الحامل وغير الحامل على الترتيب خلال الفترة من ٤٢-٨٥ يوم من التلقيح .
 - دقة تشخيص الحمل باستخدام الجس اليدوي للبطن خلال الفترة من ٦٠-٩٥ يوم من التلقيح كان ٨٤,٨% ، ٥٧,٨% للنعاج العشار وغير العشار على الترتيب.
 - كان معدل الزيادة في وزن الجسم -كطريقة لتشخيص الحمل - بالنسبة للنعاج الحامل في فردى ، ثنائى ، وثلاثى ٦,٨٧ ، ٨,٣٨ ، ١٠,٢٩% من الوزن عند التلقيح بعد ٦٠ يوم من التلقيح في حين ان هذه الزيادة كانت ١١,٥٢ ، ١٣,٧٠ ، ١٦,٥١% بعد ٩٠ يوم من التلقيح على الترتيب.
 - من خلال تلك الدراسة يمكن التوصية باستخدام احدى الطرق الاتية عدم العودة للنباح والموجات فوق الصوتية والجس اليدوي للبطن ومعدل الزيادة في وزن الجسم كطرق لتشخيص الحمل في الاغنام بدقة تشخيص عالية مع سهولة اجرائها وقلة التكاليف وكذلك كطريقة آمنة للحيوانات مقارنة بطريقة تقدير البروجستيرون كطريقة مكلفة لتشخيص الحمل وتحتاج الى معامل خاصة.