



## Ultrasonographic detection of fetal sexing in nanny goat

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### Abstract

We aimed to evaluate the accuracy of early fetal sexing by sonography in nanny goats during three periods of gestation and their correlation with the number of fetuses. A total of 50 pregnant nanny goats were included in this study. The B mode of real-time scanner with 3.5-5 MHz transabdominal transducer was used to identify the fetal external genital organs and to evaluate the relative placement of genital tubercle (G.T.) within three different periods were the first period at 45-55 days of pregnancy, the second period at 55-56 days of pregnancy, and the third period at 65-75 days of pregnancy. The result showed that, in the signal fetus cases, the accuracy of fetal sexing was more accurate in the second period of pregnancy (62.5%) than that recorded in the first (56.5%) and third (40%) periods of pregnancy. The results in twine fetus cases showed that the accuracy of fetal sexing was more accurate in the first period of pregnancy (34.7%) than that recorded in the second (27%) and third (20%) periods of pregnancy. The results also showed that, in triple fetus cases, the accuracy of fetal sexing was more accurate in the 1st period of pregnancy (26%) than that recorded in the second (15.4%) and third (6.7%) periods of pregnancy. The results of the fetal sex determination showed that a total of 15 male fetuses were accurately detected, with three fetuses wrongly detected. Similarly, a total of 11 female fetuses were accurately diagnosed, and four fetuses were incorrectly detected. The sensitivity of fetal sex determination was 78.94%, and the gender detection accuracy, sensitivity, specificity, positive predictive value, and negative predictive value were 78.78, 78.57, 83.33, and 73.33%, respectively. We conclude that the transabdominal transducer at B mode used in the second period (55-56 days) of pregnancy was highly accurate for early fetal sex determination. Also, it is the best way to determine the number of fetuses in small ruminants.

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### Introduction

Ultrasonography is a straightforward, dependable, non-invasive imaging method that has no negative effects; it was first used in 1983 to diagnose pregnancy in ovine species (1). As a result, ultrasonography's uses in veterinary medicine have grown from its restricted use for diagnosing pregnancy and identifying certain pathological conditions like metritis or hydrometra to its current status as the most effective diagnostic tool for assessing both male and female reproductive health as well as for organizing and carrying out the flock's reproductive management (2,3). The transrectal or

transabdominal scanning technique will be chosen based on the goal of the ultrasonographic evaluation (4,5). Although transabdominal ultrasonography is simpler, high-resolution transrectal probes are necessary to thoroughly examine the genital system (6). Early pregnancy detection is crucial for animal production systems as it reduces costs associated with feeding non-pregnant females, improves reproductive management, and aids selection (7). From days 25 to 30, transrectal probes can be used to diagnose pregnancy, and its use is required starting on day 60 since transabdominal ultrasonography operating at 7.5 MHz is not able to see the entire uterus (8). Early fetal sex determination is crucial for

the animal breeding sector. Numerous techniques exist for sexing embryos and fetuses, producing highly reliable results at an embryo's early developmental stage (9,10). Diagnosing with either single fetuses or even multiple fetuses enables effective ratio management, particularly in the last period of the pregnancy, leading to improved newborn weight and survival in addition to gaining weight (10). The fetal sex is determined depending upon the examination of the genital tubercle, which is clearly detectable on ultrasonography due to the hyperechoic properties of the genital tubercle, which is clearly evident during ultrasonography (11). The fetuses of small ruminants have one disadvantage: they are more difficult to manipulate during ultrasonography than fetuses of horses and cattle, leading to reduced accuracy of the diagnosis (12,13).

We aimed to investigate the accuracy of early fetal sexing by sonography in nanny goats during the first period (45-55) days, the second period (55-65) days, and the third period (65-75) days of gestation and their correlation with the number of fetuses.

## **Materials and methods**

### **Ethical approve**

All protocols agreed with regulations and orders by SECAE guidelines numbered U.M.VET.2024.069, dated 18/3/2024.

### **Animals**

Fifty goats were used with an average age of 2-4 years and a body weight of 45-55 kg. They were housed on a farm in the Makmour area. They were fed hay and green fodder and had free access to drinking water and mineral supplements. The estrus was monitored carefully, and the goats naturally mated twice on the first and second days of estrus. We considered the first day of estrus designed to be day zero of gestation.

### **Ultrasound equipment**

Ultrasonography was done using the B mode (ImaGO veterinary ultrasound scanner) with a 3.5-5 MHZ transabdominal transducer. The ultrasonography was applied in 45, 55, and 65 days of gestation. Every identified fetus had its sex determined in addition to the scan that counted the number of fetuses (14). In the beginning, we identify the fetal orientation accurately by visualization of the fetus's anatomic structure as reference points, such as the head and tail, to precisely determine the position of the genital tubercle (14-16). The identification of the genital tubercle with relation to the umbilical cord and insertion site of the tail, in addition to the examination of the external genitalia anatomical features, led to the classification of a fetus as male when the genital tubercle was found close to the umbilical cord or when features like the penis, foreskin, and

scrotum were seen, on the other hand, the fetus was classified as females when the genital tubercle was found close to the tail or when features like the vulva, clitoris, and teats were seen (17).

Low light levels were maintained during the evaluations, and the ultrasound equipment was placed close to the evaluator's eyes. The animal's abdomen was suspended to increase the fetus's visibility when required. In order to validate the fetal sex diagnosis, births were monitored, and the number and sex of infants were appropriately documented, in addition to an estimation of the number of fetuses (18). Fetal quantification was not taken into consideration when a female fetus was diagnosed with a single gestation, but two subsequent female births were noted. Instead, the diagnosis of gestation and sexing was taken into consideration. The same thing happened when male single gestations were diagnosed (19).

### **Statistical analysis**

The Chi-square test was used to compare the accuracy, sensitivity, and specificity results and predicted positive and negative values performed by SPSS software (IBM SPSS Statistics, Version 29.0.2.0). The P values equal to and less than 0.05 were considered significant.

## **Results**

The result of fetal sex detection in this study showed that single gestation had a greater significant value at fetal gender determination in the second period of gestation than in the first and third periods of gestation (Table 1). Of fifty pregnant goats, there were ten single pregnancies, with three pregnant goats due to wrong diagnostics, but twenty-three pregnant goats had not been estimated in the first pregnancy period. In second period of pregnancy, there were twelve single pregnancies with three pregnant goats due to wrong diagnostics. However, twenty-four pregnant goats have not been estimated in this period. However, in the third period of pregnancy, there were eight single pregnancies with 4 pregnant goats due to wrong diagnostics, but thirty pregnant goats have not been estimated (Figure 1-3).

In twine, gestational age had a higher significant value for fetal sexing in the first period of gestation than obtained in the second and third periods of gestation (Table 2). Five twine pregnancies were recorded from fifty pregnant goats, three of which were due to wrong diagnostics, but twenty-three pregnant goats have not been estimated in the first pregnancy period. In the second period, 55-65 days of pregnancy, there were 5 twine pregnancies, with 2 pregnant goats due to wrong Diagnostics, but 24 pregnant goats were not estimated during this period. In the last period, 65- 75 days of pregnancy, there were 4 twine pregnancies, with 2 pregnant goats due to wrong diagnostics, but 30 pregnant goats were not estimated (Figure 4).

Table 1: Accuracy of gender detection obtained by transabdominal ultrasonography at different periods of gestation in single fetuses of nanny goats

Study criteria	Gestation period (days)		
	1 <sup>st</sup> period 45-55	2 <sup>nd</sup> period 55-65	3 <sup>rd</sup> period 65-75
Single fetuses sexed correctly	10	12	8
Single fetuses sexed incorrectly	3	3	4
Fetuses not estimated	23	24	30
Accuracy	56.5% <sup>b</sup>	62.5% <sup>a</sup>	40% <sup>c</sup>
Total	13/50	15/50	12/50

<sup>a-c</sup> Letters differ in the raw mean and have significant differences (P = 0.001).

Table 2: Accuracy of gender detection obtained by transabdominal ultrasonography at different periods of gestation in twine fetuses of nanny goats

Study criteria	Gestation period (days)		
	1 <sup>st</sup> period 45-55	2 <sup>nd</sup> period 55-65	3 <sup>rd</sup> period 65-75
Twine fetuses sexed correctly	5	5	4
Twine fetuses sexed incorrectly	3	2	2
Fetuses not estimated	23	24	30
Accuracy	34.7% <sup>a</sup>	27% <sup>b</sup>	20% <sup>c</sup>
Total	8/50	7/50	6/50

<sup>a-c</sup> Letters differ in the raw mean and have significant differences (P = 0.001).

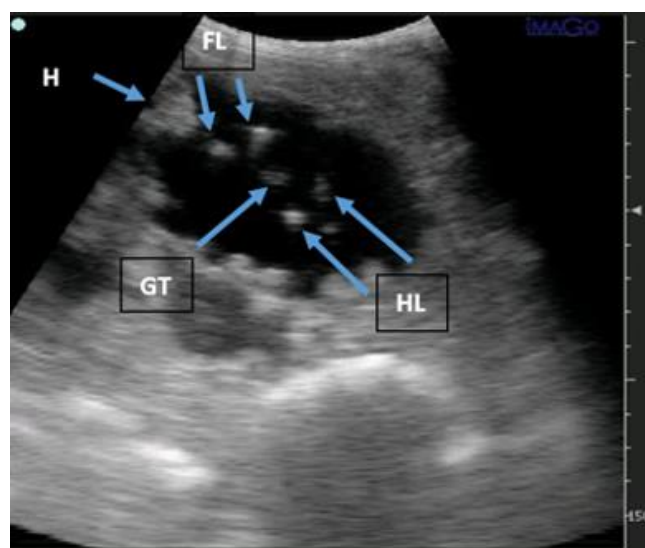


Figure 1: A single male fetus showing forelimb (F.L.), Hind Limbe (H.L.), genital tubercle (G.T.), Head (H), and fetuses. 3.5 MHz is used in ultrasonography.

The accuracy of gender detection in gestation in triple fetal results showed a higher significant value in the first period of gestation than that obtained during the second and third period of gestation (Table 3). There were two triple pregnancies from fifty pregnant goats, with four pregnant goats due to wrong diagnostics, but twenty-three pregnant goats were not estimated in the first period of pregnancy. In the second period of pregnancy, there were two triple

pregnancies with two pregnant goats due to wrong diagnostics. However, twenty-four pregnant goats have not been estimated in this period. However, in the third period of pregnancy, there was one triple pregnancy with one pregnant goat due to wrong diagnostics, but thirty pregnant goats were not estimated (Figure 5).

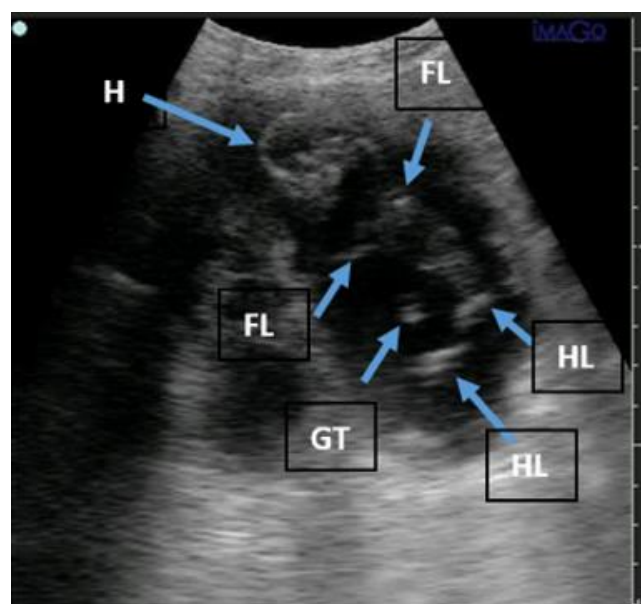


Figure 2: A single male fetus showing forelimb (F.L.), Hind Limbe (H.L.), genital tubercle (G.T.), Head (H), and plain view fetuses. 3.5 MHz is used in ultrasonography.

Table 3: Accuracy of gender detection obtained by transabdominal ultrasonography at different periods of gestation in triple fetuses of nanny goats

Study criteria	Gestation period (days)		
	1 <sup>st</sup> period 45-55	2 <sup>nd</sup> period 55-65	3 <sup>rd</sup> period 65-75
Triple fetuses sexed correctly	2	2	1
Triple fetuses sexed incorrectly	4	2	1
Fetuses not estimated	23	24	30
Accuracy	26% <sup>a</sup>	15.4% <sup>b</sup>	6.7% <sup>b</sup>
Total	6/50	4/50	2/50

<sup>a-c</sup> Letters differ in the raw mean and have significant differences (P = 0.001).

Table 4: Results of gender detection by transabdominal ultrasonography in nanny goats

Diagnosis	No. of goats	Transabdominal ultrasonography
Males	18	15a/3b
Females	15	11c/4d
Accuracy $(a+c)/(a+b+c+d)*100$		78.78 %
Sensitivity $a/(a+d)*100$		78.94%
Specificity $c/(c+b)*100$		78.57%
Positive predictive value $a/(a+b)*100$		83.33%
Negative predictive value $c/(c+d)*100$		73.33%

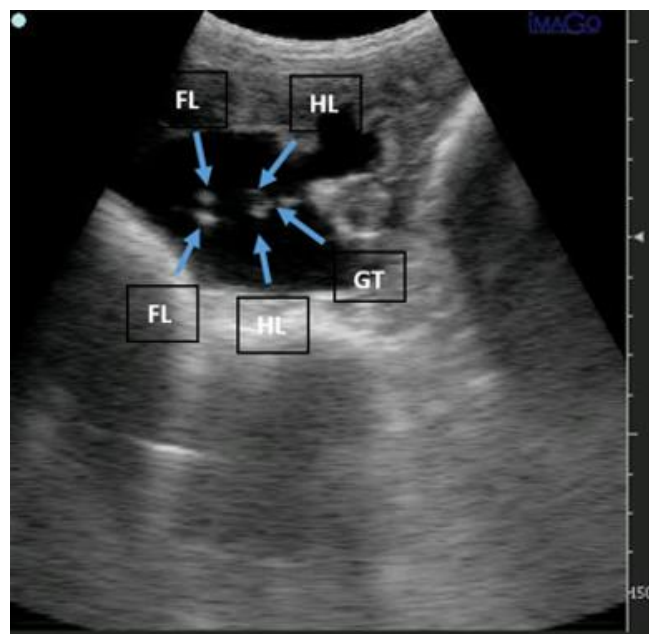


Figure 3: Images of a single female fetus showing forelimb (F.L.), Hind Limbe (L), and genital tubercle (G.T.). 3.5 MHz is used in ultrasonography.

The results of the gender detection analysis showed that fifteen male fetuses were accurately detected, and only three were wrongly diagnosed; similarly, eleven female fetuses were correctly diagnosed, and only four were incorrectly diagnosed. The gender detection accuracy, specificity, positive predictive value, and negative predictive value for

the species were 78.78, 78.57, 83.33, and 73.33%, respectively, with a sensitivity of approximately 78.94%. Table 4 displays the number of goats falsely identified as males, true positives diagnosed as females, true negatives diagnosed as females, and false negatives diagnosed as females.

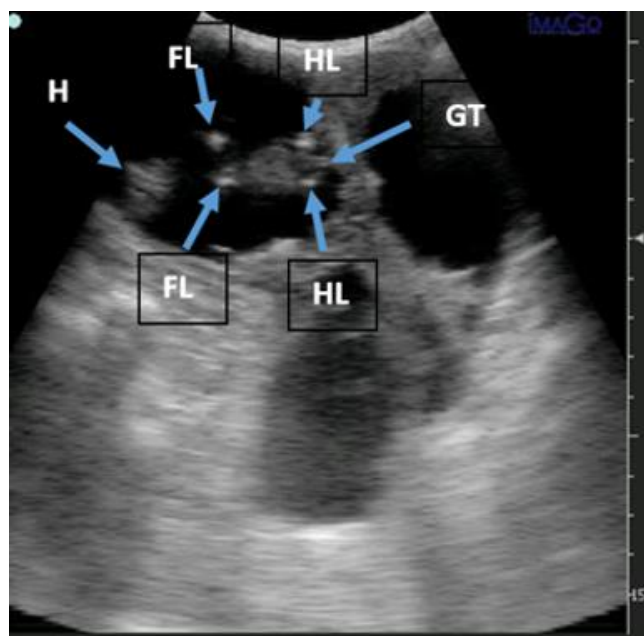


Figure 4: A twine female fetus showing forelimb (F.L.), Hind Limbe (H.L.), genital tubercle (G.T.), Head (H), 3.5 MHz used in ultrasonography.

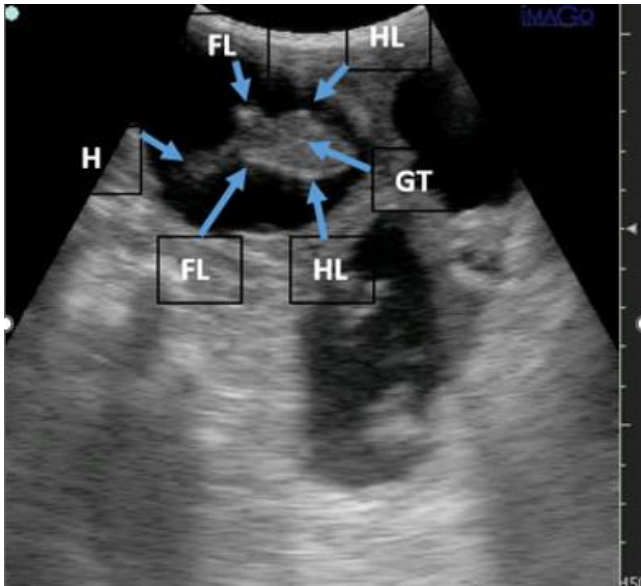


Figure 5: A triple male fetus showing Forelimb (F.L.), Hind Limb (H.L.), genital tubercle (G.T.), Head (H), 3.5 MHz used in ultrasonography.

## Discussion

The study's gender detection results revealed that the diagnosis was more significantly influenced by the single gestational age at fetal gender. The accuracy of fetal gender detection was 56.5% in the first gestation period, 40% in the third period, and 62% in the second period. The results in the first gestation period of gestation might have been the result of incorrect diagnosis, perhaps due to the G.T. migrating later in male fetuses (20), perhaps because the male G.T. has a longer distance to travel (21).

In addition, incorrect diagnoses of female fetuses as males could have originated from structures with similar echogenicity that were confused for the G.T., such as the tip of the folded back limbs or even the umbilical cord wrapped within the belly. As (20) pointed out, certain fetuses' G.T. can finish the migration after day 55 of gestation. Better visibility of the anatomical components related to fetal sex on day 65 is likely the reason for this similar outcome to the one noted in goats (21,22). With 62.5% of the fetuses correctly sexed, these results were inferior. It is, therefore, thought that in the current study, the G.T. distinction in the external genitalia favoured fetal sexing on day 65 of gestation, providing superior accuracy. Between days 45 and 60 of gestation, those (20) who achieved a total accuracy of 87.2% in a single evaluation might have done so because male fetuses finish the G.T. migration later than female fetuses.

In the current study, twin pregnancies had a higher significance for fetal sex determination in the first period of gestation, which was 37% of gestation, than in the third

period of gestation, which was 20%, or in the second period of gestation, which was 27% days of gestation. On (23), the outcomes were different. The cause is because Ultrasound scanning is restricted by the fetus's improper posture, which prevents the imaging of anatomic structures used for identifying sex, especially during numerous pregnancies, and an additional mistake in two males and female determines occurred. Conclusion: While transabdominal ultrasonography using the sector transducer was used in this study, it was not as effective as transrectal ultrasonography using the linear transducer for sexing goat fetuses between days 40 and 60 of pregnancy in both single and multiple gestations (24).

In the first period of gestation, 26%, significantly greater than in the second period of gestation, 15.4%, and 65-72 was 6.7% of gestation, were found to have a greater accuracy of gender prediction in triple pregnancies. These outcomes supported the findings that the presence of three fetuses during this phase is evident before the fetuses develop and become larger, which explains why there are noticeable distinctions between this period and the others due to the difficulty and misunderstanding that arises during the examination due to the growing cotyledons and larger fetuses (25-27). In addition to employing the sector transducer for transabdominal ultrasonography in this investigation, the findings of this study were inferior to those of (28). In line with (29), the longitudinal plane provided the greatest images. It made it simple to determine the gender when identifying the G.T., particularly in the early stages of pregnancy with triple fetal development. These examinations required more thorough evaluations even though there was no significant difference in the sexing of fetuses. In days of gestation, 55-65 was 15.4%, and 65-72 days was 6.7%. This is because multiple gestations increase the likelihood of incorrectly determining a fetus's sex, primarily due to the proximity and overlap of other fetuses (30,31). The time of G.T. migration, which usually occurs between days 45 and 50 of pregnancy, as well as the accuracy, sensitivity, specificity, and positive and negative predictive values of transrectal ultrasonography-all of which were obtained in this study at 78.78, 78.94, 78.57, 83.33, and 73.33%, respectively-all have a significant impact on the accuracy of fetal sexing in small ruminants. For (31), it was comparable in some ways (32).

Nevertheless, the findings of studies (33-35) were superior to those of this one, and the explanation for this was that the two earlier investigations used the linear transducer for transrectal ultrasonography and selected days 40 of pregnancy (36). The conceptus's age and size could be the cause of this discrepancy. The placenta, various fetal annexes, and the fetus itself may be mistakenly understood as hyperechoic organs connected to sexual differentiation due to the fetus's increased growth in the final two months of pregnancy, which decreases the amount of space in the uterine environment.



## Conclusions

We conclude that transabdominal transducer B mode ultrasound, when used in the second period (55-56 days) of pregnancy, was highly accurate and effective for early identification of fetal sex and the number of fetuses in small ruminant species.

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## Conflict of interest

None.

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## الكشف بالموجات فوق الصوتية عن جنس الجنين في الماعز

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فرع الجراحة وعلم تناسل الحيوان، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

### الخلاصة

هدفنا إلى تقييم دقة تحديد جنس الجنين المبكر باستخدام الموجات فوق الصوتية في إنث الماعز خلال ثلاث فترات من الحمل وارتباطها بعدد الأجنة. وقد تم تضمين ما مجموعه ٥٠ من الماعز الحامل في هذه الدراسة. تم استخدام الوضع بينا للماسح الضوئي في الوقت الحقيقي مع محول عبر البطن ٣،٥-٥ ميجا هرتز لتحديد الأعضاء التناسلية الخارجية للجنين وتقييم الوضع النسبي للدرنة التناسلية، ضمن ثلاث فترات مختلفة كانت الفترة الأولى عند ٤٥-٥٥ يوما من الحمل، والفترة الثانية عند ٥٥-٥٦ يوما من الحمل، والفترة الثالثة عند ٦٥-٧٥ يوما من الحمل. أظهرت النتيجة أنه في حالات الجنين الإشارة، كانت دقة تحديد جنس الجنين أكثر دقة في الفترة الثانية من الحمل (٦٢,٥٪) من تلك المسجلة في الفترتين الأولى (٥٦,٥٪) والثالثة (٤٠٪) من الحمل. بينما أظهرت النتائج في حالات الأجنة الخيطية أن دقة تحديد جنس الجنين كانت أكثر دقة في الفترة الأولى من الحمل (٣٤,٧٪) من تلك المسجلة في الفترتين الثانية (٢٧٪) والثالثة (٢٠٪) من الحمل. وأظهرت النتائج أيضا أنه في حالات الأجنة الثلاثية، كانت دقة تحديد جنس الجنين أكثر دقة في الفترة الأولى من الحمل (٢٦٪) من تلك المسجلة في الفترتين الثانية (١٥,٤٪) والثالثة (٦,٧٪) من الحمل. أظهرت نتائج تحديد جنس الجنين أنه تم اكتشاف إجمالي ١٥ جنينا ذكرا بدقة مع اكتشاف ثلاثة أجنة بشكل خاطئ. وبالمثل، تم تشخيص إجمالي ١١ جنينا أنثى بدقة مع اكتشاف أربعة أجنة بشكل غير صحيح. كانت حساسية تحديد جنس الجنين ٧٨,٩٤٪، وكانت دقة تحديد الجنس والحساسية والنوعية والقيمة التنبؤية الإيجابية والقيمة التنبؤية السلبية ٧٨,٧٨ و ٧٨,٥٧ و ٨٣,٣٣ و ٧٣,٣٣٪ على التوالي. نستنتج أن جهاز الاستشعار عبر البطن في الوضع بينا المستخدم في الفترة الثانية (٥٥-٥٦ يوما) من الحمل كان دقيقا للغاية لتحديد جنس الجنين في وقت مبكر، كما أنه الأفضل في تحديد عدد الأجنة في المجترات الصغيرة.