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Fetal sexing in small ruminants through visualization of the genital tubercle

Sexagem fetal em pequenos ruminantes por meio da visibilização do tubérculo genital

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SUMMARY

The objective of this study was to verify the accuracy of sexing and fetal number estimation in small ruminants by ultrasonographic examination. Fifty fetuses from 36 sheep and 23 fetuses from 11 goats were evaluated. In the case of sheep, twenty-four, ten, and two pregnancy were single, double, and triple, respectively. Regarding the goats, three, five, two, and one simple pregnancy were single, double, triple, and quadruple, respectively. The evaluations were performed on days 55 and 65 of gestation, by means of transrectal ultrasonographic examination, using a dual-frequency linear transducer, (6.0 and 8.0 MHz). Fetal sex was diagnosed by the location of the genital tubercle or visualization of external genitalia. The accuracy of fetal sexing was evaluated using the Chi-square test (X^2) considering a 5% significance level. The gestational period interfered in the accuracy of fetal sexing in both species ($P < 0.05$). Obtained results showed 30% and 82.61% accuracy on day 55 of gestation, and 90% and 95.83% on day 65 for goats and sheep, respectively. The fetal sex and the type of pregnancy did not interfere in the sexing accuracy in both species during the evaluated periods ($P > 0.05$). Thus, B-mode ultrasonography is an efficient method to perform an early diagnosis of fetal sex and to determine the number of fetuses in the studied small ruminants on day 65 of gestation. In addition, the accuracy of fetal sexing is not influenced by the type of pregnancy or fetal sex.

Keywords: B-mode ultrasonography, goat, sheep, fetal quantification, pregnancy

RESUMO

O objetivo com este estudo foi verificar a acurácia da sexagem e da estimativa do número fetal em pequenos ruminantes por meio de exame ultrassonográfico. Foram avaliados 50 fetos de 36 ovelhas e 23 fetos de 11 cabras. No caso das ovelhas, 24, 10 e duas gestações foram simples, duplas e triplas, respectivamente. Enquanto nas cabras, três, cinco, duas e uma gestação foram simples, duplas, triplas e quádrupla, respectivamente. As avaliações foram realizadas aos 55 e 65 dias de gestação, por meio de exame ultrassonográfico transretal, utilizando-se um transdutor linear de dupla frequência (6,0 e 8,0 MHz). O sexo fetal foi diagnosticado pela localização do tubérculo genital ou visibilização da genitália externa. A acurácia da sexagem fetal foi avaliada por meio do teste Qui-quadrado (X^2) considerando 5% de significância. O período gestacional interferiu ($P<0,05$) na acurácia da sexagem fetal. Obteve-se 30% e 82,61% de acurácia aos 55 dias de gestação, e 90% e 95,83% aos 65 dias, para cabras e ovelhas, respectivamente. O sexo fetal e o tipo de gestação não interferiram ($P>0,05$) na acurácia da sexagem em ambas as espécies nos períodos avaliados. Desta forma, a ultrassonografia em modo-B é um método eficiente para realização de diagnóstico precoce do sexo fetal e para determinação do número de fetos nos pequenos ruminantes no 65º dia da gestação, além disso, a acurácia da sexagem fetal não é influenciada pelo tipo de gestação, nem pelo sexo fetal.

Palavras-chave: ultrassonografia modo B, cabra, ovelha, quantificação fetal, gestação

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INTRODUCTION

The early diagnosis of pregnancy has great economic and practical importance within animal production systems because, in addition to minimizing the additional costs associated with feed supplementation to non-pregnant females, it facilitates reproductive management and contributes to the selection process, since unproductive females are identified (PAULA et al., 2003). According to Santos et al. (2007b), the diagnosis of single and multiple gestations allows performing adequate nutritional management, especially during the final third of gestation time, guaranteeing better birth weight, weight gain, and survival of the newborn.

In small ruminants, ultrasound is a precise, fast, and non-invasive method routinely used to diagnose gestation (DOIZÉ et al., 1997, KARADAEV et al., 2016). In addition to an early diagnosis of gestation, fetal sexing through ultrasound analysis could add commercial value to pregnant animals.

Fetal sexing through ultrasound examination is based on the location of the genital tubercle (COUBROUGH & CASTELL, 1998) and/or the differentiation of the external genitalia (CURRAN et al., 1989). According to these authors, since the genital tubercle is a hyperechoic anatomical structure, it is easily visible on ultrasonography. However, Santos et al. (2007a) point out an important obstacle affecting the accuracy of the diagnosis due to the inability to manipulate the uterus of small ruminants during the examination, especially compared with the accuracy of the method in equine and bovine species.

Taking all this into account, and considering the lack of research on the diagnosis of fetal sex in goats and sheep under field conditions, it becomes important to improve our knowledge in order to contribute to livestock activity, as well as to academic research. The objective of the present study was to verify the accuracy of sexing and fetal number estimation in small ruminants by means of ultrasonographic examination.

MATERIAL AND METHODS

The study was carried out at the Experimental Farm of the Center for Agrarian, Environmental and Biological Sciences of the Federal University of Recôncavo da Bahia (UFRB), in the city of Cruz das Almas-BA, located at 12º 40' 12" S latitude and 39º 06' 07" W longitude and at the Experimental Farms of the Federal University of Bahia (UFBA), in the cities of Entre Rios and São Gonçalo dos Campos — BA, located at 11º 56' 31" S and 38º 51' 04" W, and 12º 26' 00" S and 38º 58' 00" W, respectively. The procedures adopted were approved by the Committee on Ethics in the Use of Animals (CEUA) of UFRB (nº 23007.025914 / 2016-94).

Eleven pregnant goats of the Anglo-Nubian breed were evaluated, with a total of 23 fetuses, corresponding to three single pregnancies, five double pregnancies, two triple pregnancies, and one quadruple pregnancy. Females were between 2 and 5 years old.

Moreover, a total of 36 pregnant Santa Inês sheep were used, with a total of 50 fetuses, corresponding to twenty-four single pregnancies, ten double pregnancies, and two triple pregnancies. Females were between 2 and 7 years old.

Females were randomly divided into three experimental groups (G) according to the number of fetuses. G1 was composed of females with single gestation (goats n = 3 and sheep n = 24), G2 — double gestation (goats n = 5 and

sheep n = 10) and G3 — triple gestation (goats n = 2 and sheep n = 2).

The ultrasound examination was performed twice in each female, on days 55 and 65 of gestation, simulating a field examination routine. An AlokaTM (model SSD-Prossound 2), Mode B, equipped with a transrectal linear transducer (6.0 and 8.0 MHz) adapted to a rigid support of polyvinyl chloride (PVC) was used to facilitate manipulation in the rectum of the animal, as directed by Oliveira et al. (2005). In order to introduce the transducer into the rectum, feces were digitally removed when necessary. During the procedure, a transmission gel was used to increase the contact between the transducer and the rectal mucosa, while also acting as a lubricant. The gestational age was calculated considering the day of the coverage as the zero day of pregnancy.

The effects of gestational age and pregnancy type on fetal sexing and quantification were evaluated for both species.

After insertion of the transducer, the observation of the fetus, as well as its heart beats, were used to confirm gestation and fetal viability. Once gestation was confirmed, a careful scan was conducted in order to evaluate the presence of other fetuses and, consequently, to determine and quantify the number of them.

Together with the scan performed to determine the number of fetuses, the sexing of each identified fetus was also performed. During the examination, in order to determine with precision the position of the genital tubercle (GT), care was taken to identify the correct orientation of the fetus, either through the complete observation of the fetus or through the visualization of anatomical structures that served as a reference point, such as: head, heart, stomach, and tail. The determination of sex was based on the identification and location of the GT in relation to the place of insertion of the umbilical cord or the tail, as well as the observation of the structures of the external genitalia. Thus, fetuses were identified as males when the GT was positioned near the umbilical cord or structures such as penis, foreskin, and scrotum were observed; and as females when the GT was observed near the tail or structures as vulva, clitoris, and teats were identified.

During the evaluations, the environment was maintained in low light and the ultrasound device was near and at eye level of the evaluator. When necessary, the abdomen of the animal was suspended to improve the visibility of the fetus.

To confirm the diagnosis of fetal sex, as well as to estimate the number of fetuses, deliveries were followed up and the number and sex of newborns were duly recorded.

When a single gestation of a female fetus was diagnosed, but a subsequent double birth of females was recorded, the diagnosis of gestation and sexing was considered, but fetal quantification was not considered. The same occurred with diagnoses of single gestations with male fetuses, and double births of males.

When a single gestation of a female fetus (or a male fetus) was diagnosed, but with a later double birth of a male and a female, only the diagnosis of gestation was considered correct.

In the case of unquantified fetuses, that is, those whose births were not expected, as well as in cases in which the fetus was quantified, but sexing was not possible, it the recorded data were not considered in the sexing accuracy analysis.

The accuracy of the diagnosis of fetal sexing was evaluated using the Chi-square test (X^2). Normality was checked with the Shapiro-Wilk test. A 5% significance level was considered in all tests.

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RESULTS AND DISCUSSION

Gestational age had a significant influence (P <0.05) on fetal sexing with the diagnosis on day 65 of gestation having greater efficiency than that obtained on day 55 (Table 1).

The results obtained on day 55 of gestation may have been due to wrong diagnostics, possibly because the GT migration occurred later in two male fetuses, which were initially diagnosed as females. As mentioned by Santos et al. (2006c), the GT of some fetuses can complete the migration after day 55 of gestation and, according to the study carried by Aguiar Filho et al. (2010) with caprines, male fetuses complete the GT migration later than female fetuses, possibly due to the greater distance to be traversed by the GT in males.

Table 1. Effect of gestation period on the accuracy of fetal sexing in caprines

Gestation period	Fetuses sexed correctly	Fetuses sexed incorrectly	Fetuses not estimated	Number/total (Accuracy)
Day 55	11	6	3	11/17 (64.71%) [b]
Day 65	18	0	2	18/18 (100%) [a]

[a, b] Values followed by different letters in the same column presented significant difference (P = 0.006), by chi-square test (X^2), considering 5% of significance.

In addition, four female fetuses were wrongly diagnosed as males possibly due to structures of the same echogenicity that had been confused with the GT, such as the tip of the folded hind limbs or even the umbilical cord wrapped in the abdomen. Wrong diagnosis may commonly occur when diagnoses are made earlier.

Although the sexing of goat fetuses can be conducted before day 55 of gestation, Aguiar Filho et al. (2010) recommended performing it only after the structures of the external genitalia are visible in order to achieve greater accuracy in the identification of sex. According to observations made by Santos et al. (2006a), the identification of the sex was easier in gestations in which the GT had already differentiated in external genital structures due to the greater number of components to be observed, especially in males.

Although the GT migration in goats occurred from day 40 to 45 of gestation, Santos et al. (2007d) and Amer (2010), respectively, recommended sexing between days 55 and 70 of gestation, thus avoiding errors in diagnosis resulting from specific variations.

Therefore, it is believed that in the present study the GT differentiation in the external genitalia favored the fetal sexing on day 65 of gestation, providing better accuracy, with 100% of the fetuses correctly sexed. These results were superior to those of Santos et al. (2006c), who obtained a total accuracy of 87.2% in a single evaluation between days 45 and 60 of gestation, and Amer (2010), who obtained 82% accuracy for gestational ages between days 61 and 70.

There was no significant difference (P> 0.05) in the accuracy of fetal sexing in function of the types of gestation (single, double, and triple) for both gestational periods (Table 2), corroborating the results of Santos et al. (2008) in single and double gestations.

The obtained results in this study for day 65 of gestation (Table 2), corroborate

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those of Santos et al. (2007a), who monitored daily goat fetuses from day 40 to 60 of gestation, obtaining 100% accuracy in the diagnosis of fetal sex, independent of gestation being single, double or triple. However, they are superior to the findings of Santos et al. (2006c), who, performing a single examination in Boer goat fetuses between days 45 and 60 of gestation, obtained a precision of 94.0%, 80.8%, and 100% for single, double, and triple gestations, respectively.

Table 2. Effect of gestation type on the accuracy of fetal sexing in goats on day 55 and 65 of gestation

Gestation type	Day 55		Day 65	
	Gestations/Fetuses sexed	Correct sexing	Gestations/Fetuses sexed	Correct sexing
Single	4/4	3/4 (75%)	3/3	3/3 (100%)
Double	5/10	5/10 (50%)	5/10	10/10 (100%)
Triple	1/3	2/ 3 (66.67%)	1/3	3/3 (100%)
Total	10/17	7/17	9/16	16/16

For diagnosis at day 55 of gestation, P = 0.055; for diagnosis at day 65 of gestation, P = 0.72 (Chi-square test (X^2), considering 5% of significance).

The results were also superior to those reported by Oliveira et al. (2005), who obtained 85.7% and 80.0% for single and double gestations, respectively, in American Alpine goats with gestational ages between 45 and 70 days in a single evaluation; and 100%, 72.7%, and 66.7% accuracy in single, double, and triple gestations, respectively, evaluating Saanen fetuses between 47 and 77 days of gestation in a single examination.

In relation to the effect of fetal sex on the accuracy of sexing, no significant difference (P> 0.05) was observed in any of the gestational periods evaluated (Table 3).

Table 3. Effect of sex on the accuracy of fetal sexing in goats on day 55 and 65 of gestation

Sex	Diagnosis at day 55		Diagnosis at day 65	
	Diagnosed/born	Accuracy	Diagnosed/born	Accuracy
Male	8/7	87.50%	8/8	100%
Female	9/11	81.82%	10/10	100%
Total	17/18	-	18/18	-

For diagnosis at day 55 of gestation, P = 0.54; for diagnosis at day 65 of gestation, P = 1.00 (Chi-square test (X^2), considering 5% of significance).

Means of 84.66% and 100% of accuracy were obtained on day 55 and day 65 of gestation, respectively, corroborating the results reported by Santos et al. (2008) when performing fetal sexing in single gestations.

Two goats with double gestations had their fetuses diagnosed as females on day 55 of gestation; however, on day

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65, the fetuses were diagnosed as a couple and later confirmed at birth. It is assumed that, as reported by Santos et al. (2006c), the GT migration occurred later after day 55.

There was no significant difference (P> 0.05) in the accuracy of fetal quantification among single, double, and triple gestation of goats during the evaluated periods (Table 4).

Of the 10 evaluated females, eight (80%) had their fetuses correctly quantified on day 55 of gestation, while on day 65, nine (90%) gestations were correctly quantified, thus showings higher efficacy in fetal quantification on day 65 (Table 4).

Table 4. Effect of gestation type on the accuracy of fetal quantification in goats on the days 55 and 65 of gestation

Gestation type	Diagnosis at day 55		Diagnosis at day 65	
	Diagnosed/real	Accuracy	Diagnosed/real	Accuracy
Single	4/3	75%	3/3	100%
Double	5/4	80%	6/5	83.33%
Triple	1/1	100%	1/1	100%
Total	10/8	-	10/9	-

For diagnosis at day 55 of gestation, P = 0.87; for diagnosis at day 65 of gestation, P = 0.67 (Chi-square test (X^2), considering 5% of significance).

In one of the 10 goats, a single gestation was initially diagnosed; however, on day 65 of gestation, another fetus was recorded and, a double gestation was confirmed later at birth. A goat with quadruple gestation was mistakenly diagnosed as a double gestation during the two evaluated periods. The inaccuracy was verified only at delivery, which commonly occurs in multiple gestation, as noted by Dias et al. (2009) who also reported errors in fetal quantification, especially in the case of multiple gestation.

There was a significant difference (P <0.05) in the accuracy of fetal sexing in relation to the gestational age. Higher accuracy was obtained when the technique was performed on day 65 of gestation (Table 5). This result was similar to the one observed for goats and, as mentioned by Aguiar Filho et al. (2010) and Santos et al. (2006a), can be attributed to better visualization of the anatomical structures that relate to fetal sex on day 65.

Table 5. Effect of the gestation period on the accuracy of fetal sexing in sheep

Gestation period	Fetuses sexed correctly	Fetuses sexed incorrectly	Fetuses not estimated	Number/total (Accuracy)
Day 55	38	8	1	38/46 (82.61%) b
Day 65	46	2	1	46/48 (95.83%) a

[a, b] Values followed by different letters in the same column presented significant difference (P = 0.029), by chi-square test (X^2), considering 5% of significance.

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Of the 47 fetuses examined on day 55 of gestation, it was not possible to diagnose sex in 2.12% of them, whereas of the 49 fetuses examined on day 65 of gestation, 2.04% of the fetuses did not have their sex diagnosed. The results of the present study are superior to those obtained by Coubrough & Castell (1998), who diagnosed sex in 93% of the examined animals and failed to determine fetal sex in 7% of the animals when testing between days 60 and 69 of gestation.

Regarding the influence of fetal sex on the accuracy of fetal sexing, no significant difference (P> 0.05) was observed in any of the evaluated gestational periods. This result reinforces the observations made by Santos et al. (2006a). However, sexing of female fetuses was more efficient in both evaluated periods (Table 6). This is contrary to the results obtained by Coubrough & Castell (1998) and Dias et al. (2009), who reported higher efficiency in the diagnosis of male fetuses. Dias et al. (2009) attributed the difference to the difficulty associated with the visualization of the GT when it was located near the tail, possibly due to poor fetal position and thus not allowing good images of the pelvic region. This problem was also observed in the present study.

Table 6. Effect of fetal sex on the accuracy of fetal sexing in sheep

Sex	Diagnosis at day 55		Diagnosis at day 65	
	Diagnosed/born	Accuracy	Diagnosed/born	Accuracy
Male	17/20	85%	18/20	85.71%
Female	29/28	96.55%	30/28	93.33%
Total	46/48	-	48/48	-

For diagnosis at day 55 of gestation, P = 0.62; for diagnosis at day 65 of gestation, P = 0.44 (Chi-square test (X^2), considering 5% of significance).

In both studied periods there was no significant difference (P> 0.05) over the accuracy of fetal sexing in relation to the type of gestation (Table 7). The findings corroborate those of Santos et al. (2006c), who did not find differences in the precision of the determination of fetal sex between single and multiple gestations.

Table 7. Effect of gestation type on the accuracy of fetal sexing in sheep on days 55 and 65 of gestation

Gestation type	Diagnosis at day 55		Diagnosis at day 65	
	Gestations / Fetuses sexed	Correct sexing	Gestations / Fetuses sexed	Correct sexing
Single	27/27	23/27 (85.18%)	25/25	24/25 (96%)
Double	7/14	8/14 (57.14%)	9/18	16/18 (88.89%)
Triple	2/5	5/6 (83.33%)	2/5	5/6 (83.33%)
Total	36/46	36/47	36/48	45/49

For diagnosis at day 55 of gestation, P = 0.23; for diagnosis at day 65 of gestation, P = 0.24 (Chi-square test (X^2), considering 5% of significance).

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The results obtained on day 65 of gestation (Table 7) resemble those of Santos et al. (2006b) who obtained an accuracy of 100% and 85.7% in single and double gestations, respectively, in a study performed to identify fetal sex in Santa Inês sheep with gestations of 55 to 75 days. Moreover, Santos et al. (2007c) obtained an accuracy of 100% and 83.3% in single and double gestation, respectively, in serial tests from day 30 to 60 of gestation in Santa Inês sheep. A comparison of the results of the present study and those of Santos et al. (2007c), indicates that repeated exams in a short period do not increase the accuracy of fetal sexing, as previously proposed.

Even though there was no significant difference between the sexing of fetuses from single, double, and triple gestation, important difficulties occurred in the sex diagnosis of double and triple gestations. These examinations required more meticulous evaluations since multiple gestations increase the chances of incorrectly determining the sex of a fetus mainly due to the proximity and overlap of other fetuses, thus reinforcing the considerations made by Santos et al. (2005) and Santos et al. (2006b).

The influence of the type of gestation was not significant (P> 0.05) in the accuracy of the fetal quantification for the evaluated periods (Table 8). One sheep was diagnosed with a single gestation in both evaluated periods, but at birth it was found that it was a double gestation. The error in fetal quantification may be attributed to the fact that the two fetuses were females.

Table 8. Effect of gestation type on fetal quantification in sheep on the days 55 and 65 of gestation

Gestation type	Diagnosis at day 55		Diagnosis at day 65	
	Diagnosed/real	Accuracy	Diagnosed/real	Accuracy
Single	27/24	88.89%	25/24	96%
Double	7/10	70%	9/10	90%
Triple	2/2	100%	2/2	100%
Total	36/36	-	36/36	-

For diagnosis at day 55 of gestation, P = 0.35; for diagnosis at day 65 of gestation, P = 0.82 (Chi-square test (X^2), considering 5% of significance).

Of the 50 fetuses born, three were not diagnosed for fetal quantification on day 55 of gestation; however, on day 65, only one fetus was not diagnosed, these fetuses were from double gestations mistakenly diagnosed as single gestations. In this study, in accordance with the findings by Dias et al. (2009), the accuracy of fetal quantification in single gestations was higher than in double gestations (Table 8).

Depending on the period and type of gestation, it is not always possible to quantify or determine the sex of all fetuses in small ruminants. This observation corroborates the points raised by Santos et al. (2007b). According to Dias et al. (2009), the female's tolerance to the examination is a factor that can lead to errors in the quantification and determination of fetal sex. In the present study, during the evaluations, it was observed that some animals were less tolerant to the test, being restless and making the diagnosis more difficult.

The GT in goat and ovine fetuses was seen as a hyperechogenic anatomical structure, usually bilobular in appearance, corroborating the findings of Azevedo et al. (2009a). However, these results contradict the observations of Azevedo et al. (2009b) in both species and Coubrough & Castell (1998) in sheep, who reported the GT in both sexes only as an echogenic point.

During the examinations, the inadequate position of the fetuses made it difficult to visualize the anatomical structures responsible for the diagnosis of sex, limiting the ultrasound examinations, especially in multiple pregnancies. Under these circumstances the pregnant female's abdomen was manually raised in an attempt to improve the ultrasound image. Santos et al. (2008) also reported this problem.

This study demonstrated that in single gestations of small ruminants, the accuracy of the diagnosis of fetal sex by means of transrectal ultrasound examination is around 100% in the evaluated periods (day 55 and day 65 of gestation). These observations are in accordance with the results of Santos et al. (2006b) and Santos et al. (2007b) who obtained 100% accuracy for single gestations during a single examination between days 55 and 75, and days 55 and 60 of gestation, respectively.

The identification and location of the GT by means of ultrasonographic examination allowed an early diagnosis of fetal sex. The same results were previously obtained by authors such as Coubrough & Castell (1998), Santos et al. (2006b), and Azevedo et al. (2009a).

In this study, corroborating the considerations of Freitas Neto et al. (2010), it was observed that the practical experience and ability of the operator, as well as the quality of the ultrasound device, influence the efficiency of the fetal sexing technique.

The results demonstrated that, in small ruminants, B-mode ultrasonography is an efficient method for the early diagnosis of fetal sex and the determination of the number of fetuses when performed on day 65 of gestation. The accuracy of fetal sexing in goats and sheep is not influenced by the type of gestation, and neither by the fetal sex.

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