# Application of Transvaginal Ultrasonography for Estimation of the Fetal Number in Bulgarian White Milk Goats

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## ABSTRACT

The present study aimed to evaluate the possibilities for fetal number estimation in Bulgarian White milk goats by transvaginal ultrasonography. The experiment was carried out with 64 animals submitted to estrus synchronization by intravaginal sponges for 12 days and PMSG injection at the time of sponge removal. All goats were twice mated by a fertile buck 12 hours apart after heat detection by a teaser animal. Serial transvaginal ultrasound examinations with endocavity probe with a frequency of 7.5 MHz were performed at days 25, 30, 35, 40, and 45 after the last mating (Day 0). Ultrasonographic criteria for pregnancy were visualization of enlarged uterine lumen and embryo. The results were compared with the parturition data. The accuracy (Ac), sensitivity (Se), specificity (Sp), positive (PPV), and negative predictive value (NPV) of the method were calculated and processed by computer statistical software. The influence of the factor time of examination on the parameters of the method was determined by correlation analysis, and the comparison of the values of the parameters during the individual periods was performed by a non-parametric statistical method for comparison of proportions. From all pregnant goats (92.2%), 59.4% were diagnosed with singletons and 32.8% with twins. On Day 25 the values of Ac, Se, Sp, PPV, and NPV were 67.2%, 82%, 14.3%, 77.3%, and 18.2%, respectively. On Day 35, Ac (87.5%), Se (96.3%), Sp (40.0%), PPV (89.6%) and NPV (66.7%) differed statistically ($P<0.05$) with values for the same findings on Day 25. Similar differences were recorded for day 40 and 45, with the highest significance ($P<0.001$) between days 25 and 45. In conclusion, transvaginal ultrasonography can be recommended for estimation of the foetal number in goats. The most suitable period for correct diagnosis is day 45 after mating or artificial insemination.

## INTRODUCTION

B-mode ultrasonography allows monitoring and control of the reproductive status in small ruminants (Scott, 2012). The introduction of this technology is crucial for modern science, and the fact that ultrasound diagnostics is a routine and modern component in the reproductive management of animals makes the method an integral part of up-to-date reproductive technologies (King, 2006; Medan and Abd El-Aty, 2010).

In human medicine, the transvaginal ultrasonography is widespread for early pregnancy determination and allows visualization of the uterine contents during the earliest gestational period (Goldstein and Timor-Tritsch, 2007). Transvaginal ultrasound is used in obstetrics and gynecology for examination of ovarian and uterine structures, identification of various markers of the fetal development such as gestational sac, yolk sac, fetal heart rate, crown-rump length, and fetal morphology in normal and pathological pregnancies (Itskovitz et al., 1990; Clement et al., 2003; Kaur and Kaur, 2011). The main advantages of the method are the elimination of the requirement for a full bladder, use in overweight patients, bypassing obstacles such as bones, gas-filled intestines, and extensive adhesions of tissues in the pelvic area. The disadvantages are mainly related to limitations in the manipulation of the probe and difficulties in the initial orientation due to the unorthodox position and angle of the transducer (Kaur and Kaur, 2011).

In animal reproduction, travaginal ultrasonography has been used in assisted reproductive technologies for observation of ovarian structures after superovulation and oocyte production after follicular aspiration (Pieterse et al., 1990; Rajamahendran et al., 1994; Velazquez et al., 2014). The method for obtaining bovine oocytes by follicular aspiration using transvaginal ultrasonography and a 5.0 MHz sector probe was first described by Pieterse et al. (1988). It has been found that the follicles of superovulated cows are easier to grasp and aspirate, while the puncture of small follicles is often difficult and increases the risk of injury to the cow's rectum or even the operator's hand. (Pieterse et al., 1988; Pieterse et al., 1991; Rajamahendran et al., 1994).

Comparative studies performed by Altun and Gürbulak (2011) show that the transvaginal ultrasound method cannot be recommended for pregnancy diagnosis in dairy cows until the fifth week of pregnancy. However, the authors report that this technique can be an accurate, rapid, and an alternative method of transrectal ultrasonography and rectal palpation if the study is performed during 40-55th day of pregnancy. The pregnancy diagnosis by transvaginal ultrasound approach is insufficiently studied in animals. The investigations in goats indicate that this method is more hygienic and safer than transrectal ultrasonography. In addition, the presence of faeces and the gentler rectum in goats are potential disadvantages of transrectal ultrasound, and the lack of a starvation diet before the examination may reduce the sensitivity of the method (Aria et al., 2004; Koker et al., 2012; Philip et al., 2017).

Despite routine use in humans, data for application of transvaginal ultrasonography in animals, especially in small ruminants, are scantly (Aria et al., 2004; Moraes et al., 2007; Koker et al., 2012; Philip et al., 2017).

The aim of the present study was to determine the possibilities for estimation of the foetal number in Bulgarian White milk goats by transvaginal ultrasonography.

## MATERIALS AND METHODS

### Experimental Animals

In accordance with Ordinance № 20 of 1 November 2012 of the Ministry of Agriculture, Food, and Forestry, for the minimum requirements for protection and welfare of experimental animals and the requirements for sites for use, breeding and/or delivery, the experiment was approved by the Animal Ethics Committee to the Faculty of Veterinary Medicine, Trakia University - Stara Zagora. In this regard, we performed our

investigation with a minimum number of experimental animals, although one of the great advantages of ultrasonography compared to other imaging diagnostic methods is the lack of invasiveness and ionizing radiation.

The experiment was carried out with 64 Bulgarian White milk goats, aged 2-6 years, weighing 41-52 kg The goats were raised on a private farm in the Stara Zagora region. The food ration included meadow hay, alfalfa hay and concentrated feed in the form of granules consisting of corn, wheat, barley, sunflower meal, vitamins and minerals, and water ad libitum. All goats were subjected to antiparasitic and immunoprophylactic measures before their inclusion in the experiments.

**Clinical Examination**

The clinical examination of the animals included control of the health status – measurement of rectal temperature, heart rate, respiratory rate, rumen movements, and condition of the visible mucous membranes. Animals with deviation in general health status were excluded from the experiment.

**Estrus Synchronization and Insemination**

Estrus synchronization was performed with intravaginal sponges containing 30 mg flurogeston acetate (Syncro-part® 30 mg, Ceva Sante Animale, France). The external genitalia of the goats were cleaned with a dry paper towel. The sponges were inserted into the bottom of the vagina using a precleaned, disinfected and lubricated vaginal applicator. They remained into the vagina of the goats for 12 days, and during this time spontaneous shedding was monitored. On the 12th day, they were removed by monitoring for the presence of non-specific secretions leaking from the external genitalia. Immediately after removal of the sponges, each animal was treated intramuscularly with 500 IU serum gonadotropin (Syncropart® PMSG, Seva Sante Animale, France). A sexually active buck teaser was used to detect goats in oestrus. Specific behavioral responses such as increased motor activity, vulvar hyperaemia, characteristic tail movement, increased vocalization, and manifestation of a standing estrus were recorded. All animals were tested twice daily (morning and evening) using the buck teaser. The goats were mated by fertile buck twice – after determination of standing estrus and twelve hours later. The date of the last mating was accepted as Day 0 of pregnancy.

Transvaginal Ultrasonography and Criterions for Pregnancy

Ultrasound examinations were performed through ultrasound machine SonoScape S2 Vet (SonoScape, China), with multifrequency (4.0-8.0 MHz) endocavity transducer. The technique involved preplacing the probe in a latex condom filled with ultrasound gel (Eco-Ultra gel, Milano, Italy). After careful insertion into the vagina of the animal, the transducer reached to the external os of the cervix. Then the ultrasound rays were directed to different parts of the uterus. The bladder was considered the main reference point in the ultrasound examination (anechogenic structure with an oval shape) then the area was scanned laterally and cranioventrally from it (Fig. 1). Ultrasonographic criterions for early pregnancy diagnosis were visualization of the enlarged uterine lumen, presence of anechogenic amniotic fluid and embryo/embryos with visible cardiac activity. All goats were examined on the 25th, 30th, 35th, 40th, and 45th day after the last mating (Day 0).

**Calculation of the Parameters Characterizing the Ultrasound Method**

The possibilities of transvaginal ultrasonography for estimation of the foetal number were based on the calculation of the parameters characterizing the method. After comparing the diagnoses with the results obtained at parturition, the parameters accuracy (Ac), sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were calculated by the method described by Martin et al. (1987).

**Statistical Analisys**

The obtained data were processed by a computer statistical program StatSoft (Statistica

***Table 1.*** *Parameters of the transvaginal ultrasonography for estimation of the foetal number, depending on the gestational age in goats (n=64)*

| Parameter | Day of pregnancy | | | | |
|---|---|---|---|---|---|
| | 25th | 30th | 35th | 40th | 45th |
| **a** | 41 | 46 | 52 | 56 | 58 |
| **b** | 12 | 9 | 6 | 2 | - |
| **c** | 2 | 3 | 4 | 5 | 5 |
| **d** | 9 | 6 | 2 | 1 | 1 |
| **Ac%** | 67.2 | 76.6 | 87.5* | 95.3*** | 98.4*** |
| **Se%** | 82.0 | 88.5 | 96.3* | 98.2** | 98.3** |
| **Sp%** | 14.3 | 25.0 | 40.0** | 71.4*** | 100.0*** |
| **PPD%** | 77.3 | 83.6 | 89.6* | 96.5** | 100.0*** |
| **NPD%** | 18.2 | 33.3* | 66.7*** | 83.3*** | 83.3*** |

*a: correct positive diagnosis, b: incorrect positive diagnosis, c: correct negative diagnosis, d: incorrect negative diagnosis, Ac: accuracy, Se: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value.*
** Statistically significant difference compared to 25th day of pregnancy at P<0.05*
*** Statistically significant difference compared to 25th day of pregnancy P<0.01*
**** Statistically significant difference compared to 25th day of pregnancy P<0.001*

7, Microsoft Corp. 1984-2000 Inc.). The influence of the factor time of examination on the parameters of the method was determined by correlation analysis, and the comparison of the values of the parameters during the individual periods was performed by a non-parametric statistical method for comparison of proportions. The differences were considered statistically significant at P<0.05. The ultrasound images were processed using a computer program Microsoft Power Point 2016 (Microsoft Corp.).

## RESULTS

From all goats with a proven pregnancy (92.2%), 59.4% had a singleton and 32.8% had a twin pregnancy (Fig. 2).

In cases of singleton pregnancy, an enlarged uterine lumen, an anechoic amniotic fluid and a hyperechoic embryo with visible cardiac activity were visualized (Fig. 3).

Transvaginal ultrasonography in goats with twin pregnancies was characterized by clear visualization of two embryos (Fig. 4), with the presence of cardiac activity, and to exclude the diagnostic error, it was used B-B mode of the ultrasound device.

The correlation analysis showed that the factor time of examination has significant influence on the values of the recorded parameters. With advance of the gestational age of the fetus, the values of all parameters were increased significantly, as the correlation coefficient R varied from 0.95 to 0.98, with statistical significance from P<0.021 to P <0.001.

On the 25th day of pregnancy, the values of the parameters Ac, Se, Sp, PPV and NPV were 67.2%, 82%, 14.3%, 77.3%, and 18.2%, respectively.

On the 35th day of pregnancy, Ac (87.5%), Se (96.3%), Sp (40.0%), PPV (89.6%), and NPV (66.7%) differed significantly (P<0.05), compared to the values for the relevant parameters established on the 25th day of pregnancy. Similar statistical dependences were reported on the 40th and 45th pregnancy day, with the highest significance of differences (P<0.001) recorded between 25th and 45th day of pregnancy (Table 1).

## DISCUSSION

In addition to the early pregnancy diagnosis, estimation of the foetal number is an important part of the reproductive management of

goats. The grouping of flocks, depending on the number of foetuses allows proper nutrition and adequate parturition management, which will optimize feed costs and improve the management of the reproductive process (Dawson et al., 1999; Erdogan, 2012).

B-mode real-time ultrasonography has a clear advantage in identification the foetal number compared to other available methods of examination (Karen et al., 2014; Medan and Abd El-Aty, 2010). It is the most commonly used method because it provides the ability to simultaneously determine the number, vitality and gestational age of the foetuses (Karadaev et al., 2018; Karadaev et al., 2019).

Our results showed that on day 25 of pregnancy, the sensitivity has the highest values (82%), compared to all parameters characterizing transvaginal ultrasonography for estimation the foetal number in goats. According to different studies, the number of embryos/foetuses can be determined up to day 100 of pregnancy, but the period with the highest recorded accuracy is between gestational days 40 and 70, and during this time is possible visualization of the entire uterine contents (Medan and Abd El-Aty, 2010; Vinoles-Gil et al., 2010).

After the 70th day of pregnancy, sometimes the fetuses are located at a greater depth than the penetrating capacity of the probe, which makes difficult the determination of their number, especially in multiple pregnancy (Dawson, 2002). To minimize positive and negative false diagnoses, some authors recommend animals be subjected to a preliminary diet and the abdominal wall to be raised during the examination (Vinoles-Gil et al., 2010; Karen et al., 2014).

On the 35th day of pregnancy, all parameters had significantly higher values compared to the first examination. This trend was even clearer on the 40th and 45th gestational days. Suguna et al. (2008) reported that a differentiation between singleton and twin pregnancies could be made at the earliest on the 35th day of gestation with transrectal ultrasonographic approach, and on the 42nd day with transabdominal approach. The authors accepted that the most appropriate period for distinguishing singleton from twin pregnancies in goats is between 5-7 gestational week. The results of Dawson et al. (1994) also showed that the prediction of foetal numbers is more accurate in the 7th week of pregnancy than in the 5th week.

In contrast, Padilla-Rivas et al. (2005) distinguished singleton and twin pregnancies between the 28th and 40th pregnancy days, using transrectal approach and a probe with frequency 7.5 MHz. Other authors determining the number of embryos/foetuses in Saanen goats by transvaginal approach and a probe with a frequency of 5.0-7.5 MHz reported 17% accuracy on the 3rd week and 60% on the 8th week of pregnancy. It was indicatior that transvaginal approach is not the most appropriate for determining multiple pregnancy in goats (Koker et al., 2012).

Our data showed that through transvaginal ultrasound approach, it is possible to detect singleton and twin pregnancies most accurately on the 40th and 45th day of pregnancy. Many researchers stated that the type of pregnancy (single or multiple) has significant effect on the accuracy of the method and it is difficult to distinguish between twins, triplets, or quadruplets at each stage of pregnancy, both in goats and sheep (Padilla-Rivas et al. 2005; Abdelgafar et al., 2007; Goel et al., 2009).

Increased motor activity of the embryo makes it difficult to differentiate twin and multiple pregnancies, especially until the 40th day of pregnancy. Counting twice the same embryo is also an error, which in order to avoid it is necessary to scan in B/B mode of the ultrasound device, allowing differentiation of different foetuses (Yotov, 2011; Yotov, 2020), as the operator should slowly scan the examination area from left to right at the same level until the uterine section is located. The scan direction must then be changed so that it is perpendicular to the previous one and covers the entire scanned area sequentially (Vinoles-Gil et al., 2010). In this regard, in our opinion, the use of a

transvaginal endocavity probe allows simultaneous scanning of both uterine horns in goats, which allows a more accurate estimation of the foetal number in these animals.

## CONCLUSION

The analysis of the obtained data indicate that transvaginal ultrasonography can be used successfully for estimation of the foetal number in goats. The most appropriate period for an accurate diagnosis is the 45th day after mating or artificial insemination.

### Conflict of Interest Statement

The authors have no conflict of interest to declare.


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## REFERENCES


1. Altun, Ö., Gürbulak, K., 2011. A comparison of diagnosis of early pregnancy in dairy cows via transrectal and transvaginal ultrasound scanning. *Erciyes Üniv. Vet. Fak. Derg*. 8 (1), 17–21.
2. Aria, G., Shau, Z., Botta, R, Giuliotti, L., Rota, A., 2004. Transvaginal echographic approach to early pregnancy diagnosis in small ruminants. *Ann. Fac. Med. Vet. Pisa* 57, 99–105.
3. Clement, S., Candy, B., Heath, V., To, M., Nicolaides, K.H., 2003. Transvaginal ultrasound in pregnancy: its acceptability to women and maternal psychological morbidity. *Ultrasound Obstet. Gynecol.* 22 (5), 508–514. https://doi.org/10.1002/uog.893
4. Dawson, L., 1999. Pregnancy diagnosis in goats. In Proceeding 14th Ann. Goat fielo Day, Langston University, Langston, OK, 97–103.
5. Dawson, L.G., Sahlu, T., Hart, S.P., Detweiler, G., Gipson, T.A., Teh, T.H., Henry, G.A., Bahr, R.J., 1994. Determination of fetal numbers in Alpine does by real-time ultrasonography. *Small Rumin. Res*. 14 (3), 225–231. https://doi.org/10.1016/0921-4488(94)90045-0
6. Erdogan, G., 2012. Ultrasonic assessment during pregnancy in goats – a review. *Reprod. Domest. Anim.* 47, 157–163. https://doi.org/10.1111/j.1439-0531.2011.01873.x
7. Goel, A.K., Kharche, S.D., Jindal, S.K., 2009. Determination of early pregnancy and embryonic development by transrectal ultrasonography in goats. *Indian J. Anim. Sci*. 79, 476–478.
8. Goldstein, S.R., Timor-Tritsch, E.T., 2007. Early pregnancy. In: Timor-Tritsch, E.T., Goldstein, S.R. (Eds.), *Ultrasound in Gynecology. Churchill Livingstone* Elsevier, Philadelphia, pp. 139–150.
9. Itskovitz, J., Boldes, R., Levron, J., Thaler, I., 1990. Transvaginal ultrasonography in the diagnosis and treatment of infertility. *J. Clin. Ultrasound* 18 (4), 248–256. https://doi.org/10.1002/jcu.1870180407
10. Karadaev, M., Fasulkov, I., Yotov, S., Atanasova, S., Vasilev, N., 2018. Determination of the gestational age through ultrasound measurements of some uterine and foetal parameters in Bulgarian local goats. *Reprod. Domest. Anim.* 53, 1456–1465. https://doi.org/10.1111/rda.13305
11. Karadaev, M., Fasulkov, I., Vasileva, R., Vasilev, N., 2019. Use of hormonal and ultrasonographic examinations to determine the fetal number in Bulgarian local goats. *Mac. Vet. Rev.* 42 (1), 35–42. https://doi.org/10.2478/macvetrev-2018-0029
12. Karen, A., Samir, H., Ashmawy, T., El-Sayed, M., 2014. Accuracy of B-mode ultrasonography for diagnosing pregnancy and determination of fetal numbers in different breeds of goats. *Anim. Reprod. Sci.* 147, 25–31. https://doi.org/10.1016/j.anireprosci.2014.03.014
13. Kaur, A., Kaur, A., 2011. Transvaginal ultrasonography in first trimester of pregnancy and its comparison with transabdominal ultrasonography. *J. Pharm. Bioall. Sci.* 3 (3), 329–338. https://doi.org/10.4103/0975-7406.84432
14. Koker, A., Ince, D., Sezik, M., 2012. The accuracy of transvaginal ultrasonography for early pregnancy diagnosis in Saanen goats: A pilot study. *Small Rumin. Res.* 105, 277–281. https://doi.org/10.1016/j.smallrumres.2012.02.013
15. Martin, S.W., Meek, A.H., Willeberg, P., 1987. Veterinary Epidemiology, Principles and Methods. Iowa State University Press, Ames, IA, pp. 63–71.
16. Medan, M.S., Abd El-Aty, A.M., 2010. Advances in ultrasonography and its application in domestic ruminants and other farm animals reproduction. *J. Adv. Res.* 1, 123–128. https://doi.org/10.1016/j.jare.2010.03.003
17. Padilla-Rivas, G.R., Sohnrey, B., Holtz, W., 2005. Early pregnancy detection by real-time ultrasonography in Boer goats. *Small Rumin. Res.* 58, 87–92. https://doi.org/10.1016/j.smallrumres.2004.09.004
18. Philip, L.M., Abhilash, R.S., Bastin, P.F., 2017. Accuracy of transvaginal ultrasonography for early pregnancy diagnosis in Attappady Black goats. *Malays. J. Vet. Res*. 8 (2), 35–41.
19. Pieterse, M.C., Kappen, K.A., Kruip, T.A., Taverne, M.A., 1988. Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries. *Theriogenology* 30 (4), 751–762. https://doi.org/10.1016/0093-691X(88)90310-X
20. Pieterse, M.C., Szenci, O., Willemse, A.H., Bajcsy, C.S., Dieleman, S.J., Taverne, M.A., 1990. Early pregnancy diagnosis in cattle by means of linear array real time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. *Theriogenology* 33, 697–707. https://doi.org/10.1016/0093-691X(90)90547-7
21. Pieterse, M.C., Vos, P.L., Kruip, T.A., Wurth, Y.A.,

van Beneden, T.H., Willemse, A.H., Taverne, M.A., 1991. Transvaginal ultrasound guided follicular aspiration of bovine oocytes. *Theriogenology* 35 (4), 857–862. https://doi.org/10.1016/0093-691X(91)90426-E

22. Rajamahendran, R., Ambrose, D.J., Burton, B., 1994. Clinical and research applications of real-time ultrasonography in bovine reproduction: A review. *Can. Vet. J.* 35, 563–572.
23. Scott, P.R., 2012. Applications of diagnostic ultrasonography in small ruminant reproductive management. *Anim. Reprod. Sci.* 130, 184–186. https://doi.org/10.1016/j.anireprosci.2012.01.013
24. Suguna, K., Mehrohta, S., Agarwal, S.K., Hoque, M., Shanker, U., Singh, S.K., Varshney, V.P., 2009. Effect of exogenous insulin administration on ovarian function, embryo-fetal development during pregnancy in goats. *Anim. Reprod. Sci.* 111, 202–213. https://doi.org/10.1016/j.anireprosci.2008.03.021
25. Velazquez, M.A., Kues, W.A., Niemann, H., 2014. Biomedical applications of ovarian transvaginal ultrasonography in cattle. *Anim. Biotechnol.* 25 (4), 266–293.
26. Vinoles-Gil, C., Gonzales-Bulnes, A., Martin, G.B., Zlatar, F.S., Sale, S., 2010. Sheep and Goats. In: Des Coteaux, L., Colloton, J., Gnemmi, G. (Eds.), *Ruminant and Camelid Reproductive Ultrasonography*. Willey- Blackwell, Hong Kong, pp. 181–210.
27. Yotov, S., 2011. Application of two-dimensional (B-mode) ultrasonography in sheep reproduction. Kota Publishing, Stara Zagora, pp. 15–37.
28. Yotov, S., 2020. Optimization of the reproduction in dairy goats. Kota publishing, Stara Zagora, pp. 79–89.