SCIENTIFIC CULTURE

TRANSRECTAL ULTRASONOGRAPHY FOR GESTATIONAL DIAGNOSIS IN SHEEP

Rene Antonio Hinojosa Benavides[1]*, Adelfa Yzarra Aguilar[2], Edwin Rojas Felipe[3], Brian Jaime Ordoñez Mulato[4], Karla Inés Zúñiga Chambilla[5], Rufino Paucar Chanca[6], Juan Pablo Castro Illesca[7]

[1]Universidad Nacional Autónoma de Huanta, Ayacucho, Perú.
ORCID iD: 0000-0002-0452-3162, Email: rhinojosa@unah.edu.pe
[2]Universidad Nacional Autónoma de Huanta, Ayacucho, Perú.
ORCID iD: 0000-0002-6442-6065, Email: ayzarra@unah.edu.pe
[3]Universidad Nacional de Huancavelica, Huancavelica, Perú.
ORCID iD: 0000-0003-2284-7934, Email: edwin.rojas@unh.edu.pe
[4]Universidad Nacional de Huancavelica, Huancavelica, Perú.
ORCID iD: 0000-0002-4272-124X, Email: brianhvca@gmail.com
[5]Universidad para el Desarrollo Andino, Huancavelica, Perú.
ORCID iD: 0000-0002-8741-7380, Email: kzuniga@udea.edu.pe
[6]Universidad Nacional de Huancavelica, Huancavelica, Perú.
ORCID iD: 0000-0001-6820-6185, Email: rufino.paucar@unh.edu.pe
[7]Universidad Nacional de Huancavelica, Huancavelica, Perú.
ORCID iD: 0000-0001-8221-1124, Email: juan.castro@unh.edu.pe

Corresponding Author: Rene Antonio Hinojosa Benavides
(rhinojosa@unah.edu.pe)

ABSTRACT

In the diagnosis of pregnancy in sheep, ultrasound has been gaining more and more ground among sheep farmers, due to its safety and practicality in quantifying the number of foetuses and assessing gestational age in order to improve the reproductive management of the flock. The objective is to determine the efficacy of transrectal ultrasonography in the accuracy of gestational diagnosis of sheep from Pampalca, Huanta, Ayacucho. A total of 60 Creole ewes were used, whose heat was synchronized with sponges impregnated with medroxyprogesterone acetate, removed after 12 days and 100 IU of eCG (Novormon) applied. On the same day, two males were incorporated into the group with marker devices, which made it possible to know exactly the day on which mating took place (day 0). The evolution of the ultrasound images was monitored from day 15 to day 44 of gestation, on an interdaily basis, using a portable veterinary multifrequency ultrasound machine ESAOTE PIE MEDICAL and a 6.5 MHz multifrequency transducer transrecally, with the help of an ultrasound conducting gel. This gestational diagnosis used transrectal ultrasound, identifying the first and last day of visualization of eleven gestation characteristics between the 15th and 44th day of gestation. The earliest and latest identification of signs of gestation occurred between days 15 and 19 (16.42 ± 1.01) for intrauterine fluid, 16 and 22 (18.68 ± 1.77) for the embryonic vesicle, 18 and 26 (21.65 ± 1.92) for embryos, 24 and 32 (27.15 ± 2.11) for the amniotic membrane, 20 and 29 (24.10 ± 2.25) for placentomas, 24 and 29 (26.22 ± 0.92) for heartbeat, 32

and 39 (35.28 ± 1.56) for the uterine horn, 30 and 37 (33.73 ± 1.55) for head and trunk differentiation, 34 and 39 (36.43 ± 1.18) for limb outbreak, 30 and 38 (35.38 ± 2.28) for fetal movement and between days 39 and 44 (41.27 ± 1.02) for the eyeball; concluding that the first signs of pregnancy can be identified from day 15 of gestation; however, it is prudent to diagnose pregnancy after day 24, when it is possible to visualize the heartbeat.

1. INTRODUCTION

Sheep farming in the Peruvian highlands has intensified in recent years with the introduction of genetically superior animals, and genetic improvement is possible if the environment is improved to sustain it, becoming sustainable as long as the current technical support from research centers continues (Mirkena et al., 2023). This new orientation has changed the production model from being only a subsistence activity to becoming a technological activity dependent on the adoption of biotechniques that guarantee the genetic improvement of herds and allow obtaining a return on the capital invested, certainly the continuous development and application of technology for genetic improvement is a key element for the advancement of sheep production. In every agricultural production unit, the livestock process is developed in two processes: it begins with the work carried out on the farms, carrying out the production of livestock to obtain live cattle; continuing with a second process, which is the Industrial (Vergara et al., 2023; Thorne et al., 2021).

Pregnancy begins with the fertilization phase, which is the fusion of the male and female gametes, until the development of the membranes giving rise to the formation of the egg, which in turn undergoes a cell division, giving rise to the embryo (embryonic phase), between days 11 and 34 of gestation there is a rapid growth and differentiation of the main tissues, organs and systems, establishing the external characteristics of the body; then continuing the fetal phase that lasts from day 35 of gestation until birth, in an average of 150 days, and is characterized by the growth and development of the fetus (Parraguez et al., 2022; Usuda et al., 2017; Sen et al., 2016). Gestational diagnosis by transrectal ultrasonography is a timely and efficient method for sheep production, since it allows adding value to the animal trade by defining the type of gestation, makes it possible to rationalize management actions, facilitates the implementation of correct feeding of pregnant wombs, as well as involves changes in management. but the benefits that can be achieved, in terms of productive and economic results, are sufficiently interesting for the producer, to assume the cost of incorporating this technology, being able to carry out multiple evaluations throughout the gestational period (Sandoval et al., 2022; Valasi et al., 2017; Crilly et al., 2017).

Additional information on gestational age and the number of fetuses allows for better nutritional management of the herd in the peripartum period in order to prevent metabolic diseases such as toxemia

of pregnancy (Roberts, 2022), being a non-invasive and highly accurate method in pregnancy diagnosis, alternatively offering the diagnosis of uterine anomalies; even when the date of mating is known, gestational ultrasound monitoring allows characterizing embryonic and fetal development, in addition to the counting and sexing of fetuses, but this ultrasound diagnosis of gestation is useful if performed early (Jones et al., 2016), due to advantages such as monitoring cyclicity in follicles, corpus luteum, uterine involution, as various events can affect the normal development of the fetus and the viability of the offspring (Del Águila et al., 2021; Khedri et al., 2018).

The main ultrasound images that characterize a pregnancy are the presence of intrauterine fluid, visualization of the bladder embryonic vesicle, detection of the embryo, visualization of the heartbeat, identification of the amniotic membrane, visualization of placentomas, differentiation of the head and trunk, identification of the germinal bud of the limbs, embryonic/fetal movement, delimitation of fetal movement, delimitation of the umbilical cord and visualization of the eyeball (Barbagiani et al., 2017); however, the strongest indicator of pregnancy is the visualization of the fetus with a heartbeat, there are also other indicators of pregnancy such as the development of placentomas and uterine fluid, the latter containing a large amount of nutrients, along with regulatory proteins, lipids and RNA remnants (Zhang et al., 2017; Hong et al., 2016); likewise, the fetal number can also be determined from the first half of gestation, which allows predicting the size of the litter (Jones & Reed, 2017); the quality of the image depends on factors such as the presence of body fat or gas accumulations, the skill of the operator and the size of the window available to examine an organ, for example the heart (Meinecke, 2017).

The sheep industry is mainly limited to poor small and marginal farmers who do not have cost-effective technological aids to diagnose pregnancy early in the field (Yadav et al., 2019). Sheep producers in Huanta, Ayacucho, lack technological advances that help them make efficient decisions for selection and reproduction, counting and sexing of fetuses, in such a way that the late diagnosis of pregnancy does not allow them to feed the mother adequately during most of the pregnancy, nor does it allow them to calculate the dates of delivery. making it difficult to monitor females susceptible to pregnancy toxemia, during its last stage, without being able to monitor the abnormal growth of the fetus in a non-invasive way; therefore, there is inadequate gestational

management that has a negative impact on the health and development of animals (Dwyer et al., 2015); For this reason, the present study was carried out with the aim of determining the first precise signs of pregnancy in sheep, by means of transrectal ultrasound, to encourage the use of ultrasound by producers, doctors and researchers, in conditions of Huanta, Ayacucho, Peru.

2. MATERIALS AND METHODS

60 sheep of the Creole breed between two and six years old belonging to the community of Pampalca, located in the district of Uchuraccay, province of Huanta, department of Ayacucho, Peru, - 12.82413634972328, -74.1123177023261, of latitude and longitude, respectively, were used. The community adopted a semi-intensive system and the sheep were subjected to controlled natural mating. When they were in the pastures they had access to native pastures, as well as water and mineral salt *ad libitum*. In the pen, they were offered alfalfa (*Medicago sativa*) and barley hay (*Hordeum vulgare*); animal health assistance was also provided, applying to each sheep an injectable solution with vitamin A, D and E, at a rate of 1 ml with a concentration of 500 000, 75 000 and 500 IU, respectively. Real-time ultrasounds were performed from July to December 2022, between the 15th and 25th days of gestation, every 24 hours, between the 26th and 35th days, every 48 hours, and every 72 hours between days 36 and 44, to visualize the following characteristics: intrauterine fluid, embryonic vesicle, embryo, amniotic membrane, placentomas, heartbeat, uterine horn, differentiation of head and trunk, budding of limbs, fetal movement and eyeball.

Ultrasound observations were made with an ESAOTE PIE MEDICAL portable veterinary multifrequency ultrasound machine, equipped with a 6.5 mhz multifrequency transducer via transrectal (Fig. 2), with the ultrasound conductive gel attachment to facilitate the introduction of the transducer and avoid air interference between the rectum and the probe. The images were saved with a digital camera. The RStudio software was used to systematize the mean, standard deviation and coefficient of variation of the data of the first visualizations of each characteristic evaluated; as well as the box and whiskers diagram (*boxplot*) was also used, which was constructed from five values: the minimum value, the first quartile, the median, the third quartile and the maximum value, in order to compare the proximity of other values of the other pregnancy characteristics. Minimal mild outliers and maximum mild outliers were searched, using the formulas $Q1-1.5*IQR$ and $Q3+1.5*IQR$, respectively.

3. RESULTS AND DISCUSSION

Table 1 shows the average number of days of first viewing of the evaluated pregnancy characteristics and their standard deviation, as well as the first and last days of viewing with its corresponding coefficient of variation. Figure 1 shows the boxplot comparison in R on the ultrasound visualization of pregnancy characteristics in sheep from Pampalca.

Table 1: Moment of the first ultrasound visualization of the characteristics of gestation in 60 sheep of the Creole breed, in Pampalca, Huanta, Ayacucho.

Pregnancy characteristics	Day of First Viewing ☐ ± s	Amplitude of variation (days)	CV (%)
Intrauterine fluid	16.42 ± 1.01	15-19	6,0
Embryonic vesicle	18,68 ± 1,77	16-22	9,0
Embryo	21,65 ± 1,92	18-26	9,0
Amniotic membrane	27,15 ± 2,11	24-32	8,0
Placentomas	24,10 ± 2,25	20-29	9,0
Heartbeat	26,22 ± 0,92	24-29	4,0
Uterine horn	35,28 ± 1,56	32-39	4,0
Head and trunk differentiation	33,73 ± 1,55	30-37	5,0
Limb Outbreak	36,43 ± 1,18	34-39	3,0
Fetal movement	35,38 ± 2,28	30-38	6,0
Eyeball	41.27 ± 1.02	39-44	2,0

Intrauterine fluid

It was visualized from days 15, 16, 17, 18 and 19 of gestation, in 15, 50, 15, 19 and 1% of the ewes examined, respectively; although there was one sheep that was detected on day 19, constituting an atypical data (*outlier*); similar to that indicated by Kusama et al. (2018) who indicate that intrauterine fluid can be diagnosed by transrectal ultrasound from day 17 of gestation. Its presence is usually the first sign of pregnancy (Bragança et al., 2016); however, it does not always occur in the form of vesicles, and can be confused with estrogenic phase fluid (Lone et al., 2016).

Ultrasonographically, intrauterine fluid is anechoic (Rajashri et al., 2017). Fetal urine production plays a key role in amniotic fluid volume after 24 weeks of gestation (Azarkish et al. (2022), as the latter

tends to increase as fetal urine increases (Barbagiani et al., 2017; Soni et al., 2016), so the bladder is often confused with the fluid-containing uterus, especially when it is filled with urine, although in the present study there was no possibility of this confusion, so the bladder served as an important reference point to observe and determine the parameters studied here. It is possible that the difference between the quality of the equipment and the access route helps to facilitate the differentiation between structures with similar characteristics, a fact that, in some situations, leads authors to diverge on the same characteristic.

Embryonic vesicle

It was visualized from days 16, 17, 18, 19, 20, 21 and 22 of gestation, in 12, 13, 27, 20, 10, 8 and 10% of the ewes examined, respectively; similar to what was reported by (Paige et al., 2017) who visualized the embryonic vesicle with embryo inside it on day 17 of gestation in sheep of the Merino breed.

Embryo

The initial visualization of the embryo was already possible from days 18, 19, 20, 21, 22, 23, 24, 25 and 26 of gestation, in 7, 8, 12, 8, 44, 8, 5, 3, 5% of the ewes examined, respectively; in agreement with Paige et al. (2017) who visualized the embryo on day 19 of gestation in sheep of the Merino breed, and with Sandoval et al. (2022) who express that the observation of the embryo, which by the way is an echogenic structure, is possible around 20 days of gestation; these results are similar to other studies in which the visualization of the embryo was reported only on day 21 of gestation in sheep (Brzozowska et al., 2022).

Amniotic membrane

The detection of the amniotic membrane was possible from days 24, 25, 26, 27, 28, 29, 30, 31 and 32 of gestation, in 10, 20, 12, 12, 19, 13, 10, 3 and 1% of the sheep examined, respectively, although there was one sheep that was detected on day 32, constituting an atypical data (*outlier*); coinciding with Sadi (2022) who indicates the visualization of the amniotic membrane from the day 25 of gestation, becoming clearer on day 39; in contrast to what was reported by (Paige et al., 2017) who visualized the amniotic membrane on day 29 of gestation in sheep of the Merino breed. Considering the data from the literature consulted, it is possible to comment that in some animals it was possible to identify the amniotic membrane early, possibly due to the quality of the equipment, since the dual-frequency transducer offers greater options for obtaining images.

Placentomas

The earliest identification of placentomas was possible from days 20, 21, 22, 23, 24, 25, 26, 27, 28 and 29 of gestation, in 7, 13, 7, 8, 5, 42, 7, 5, 5, 1% of the ewes examined, respectively, (Fig. 3) although there was one sheep that was detected on day 29, constituting an atypical data (*outlier*); it should be noted that the data obtained here are very early compared to other authors, since according to Sadi (2022), the earliest detection of placentoma by transrectal ultrasound can be visualized as a circular structure oriented towards the fetus on day 34 of gestation in pregnant sheep of the Kurdish breed; these results are corroborated by Sandoval et al. (2022) who assert that the onset of the formation of placentomas can be detected after 30 days of gestation in the sheep.

Heartbeat

Heartbeats were visualized from days 24, 25, 26, 27, 28, 29 of gestation, in 3, 5, 74, 7, 8 and 3 % of the ewes examined, respectively; agreeing with Sandoval et al. (2022) who state that, from 25 days of gestation, it is possible to see the heart beating, an unequivocal sign of vitality and viability; these results are similar to those reported by Quintela et al. (1999) who consider the heartbeat as the first certain characteristic of pregnancy, which is observable from day 23; although contrary to what was reported by Paige et al. (2017) who visualized the heartbeat on day 19 of gestation in sheep of the Merino breed.

Uterine horn

Dilation of the uterine horns began to be seen from days 32, 33, 34, 35, 36, 37, 38 and 39 of gestation, in 3, 9, 10, 50, 6, 10, 9 and 3% of the ewes examined, respectively; however, it is necessary to take into consideration Quintela et al. (1999), who mention that this characteristic of pregnancy is not necessarily adequate as an indicator of the existence of pregnancy, since it can also be due to alterations that imply a concentration of fluid inside.

Head and Trunk Differentiation

The differentiation of the embryo into head and trunk was possible from days 30, 31, 32, 33, 34, 35, 36, and 37 of gestation, in 3, 7, 10, 12, 47, 8, 10, and 3% of the sheep examined, respectively, in contrast to what was observed by Sadi (2022) who claims to have visualized the fetus in the uterine lumen with an easily identifiable head. ears and trunk adequate on examination on day 42 of gestation.

Limb Outbreak

With respect to the day of identification of the

forelimbs and hindlimbs, in this study the appearance of the limb outbreak was detected from days 34, 35, 36, 37, 38 and 39 of gestation, in 3, 7, 63, 7, 10 and 10% of the ewes examined, respectively; in contrast to Sadi (2022) who mentions the first visualization of folded forelimbs on day 42 of gestation.
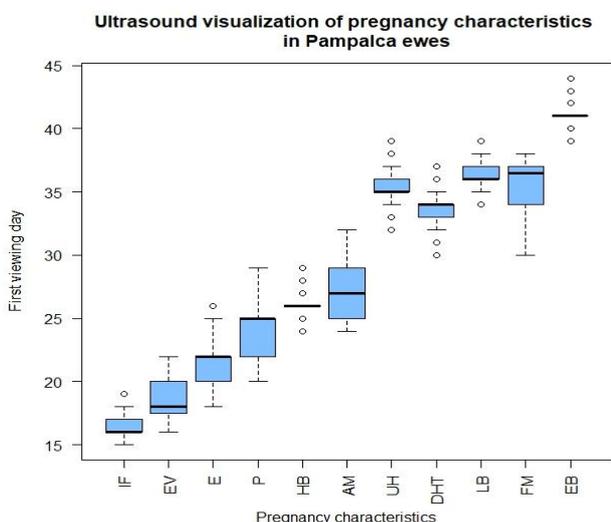
Fetal Movement

In this study, it was possible to verify embryonic movement from days 30, 31, 32, 33, 34, 35, 36, 37 and 38 of gestation, in 5, 5, 5, 10, 8, 12, 43 and 7% of the ewes examined, respectively, in accordance with Roberts (2022), who states that this visualization is possible before 40 days of gestation in the ewe. since the fetus enlarges to the point of exceeding the limits

of the ultrasound screen, a fact that reinforces the opinion of Sandoval et al. (2022) who indicate that, in advanced pregnancies greater than 100 days, it is possible that, on transrectal ultrasound, the fetus is not seen, because it is outside the range of the ultrasound emitted by the transducer, or, because it is covered by the placentomas.

Eyeball

The eyeball began to be seen from days 39, 40, 41, 42, 43 and 44 of gestation, in 3, 5, 7, 7 and 7% of the ewes examined, respectively; The results of the present study coincide with Barreto et al. (2008) who state that the first visualization of the eyeball occurs from day 39 of gestation in 13.3% of the animals and day 43 in 8.3 of the sheep of the Santa Inés breed.

Fig. 1

IF: Intrauterine fluid
EV: Embryonic vesicle
E: Embryo
P: Placentomas
HB: Heart beat
AM: Amniotic membrane
UH: Uterine horn
DHT: Differentiation of head and trunk
LB: Limb budding
FM: Fetal movement
EB: Eye ball

Fig. 2. Use of ESAOTE PIE MEDICAL portable veterinary multi-frequency ultrasound system, equipped with a 6.5 mhz multi-frequency transducer.
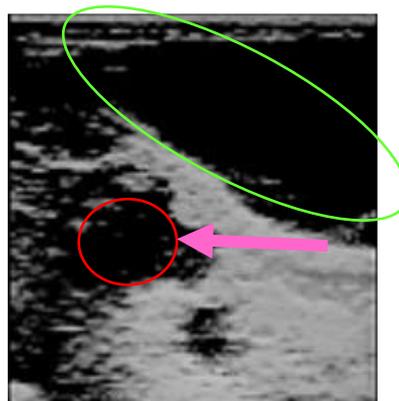

Fig. 3. On day 25 of pregnancy, placentomas were identified in 42% of the ewes

4. CONCLUSIONS

Transrectal ultrasonography improves the accuracy of gestational diagnosis of sheep, optimizing the reproductive efficiency of sheep production; whereas, the early onset of gestation characteristics may be due to dietary factors, transducer frequency and access pathway, ease of operation, and operator practice.

Certainly, the quality of the equipment and the review route are variables that can contribute to the diversity of results among authors; however, it is also necessary to take into account the breed and size of the animals that can determine a differentiated embryonic-fetal development.

The precocity of transrectal ultrasonography makes it possible to diagnose pregnancy reliably from day 26.22 ± 0.92 when the heartbeat is visualized, with greater accuracy in 74% of the sheep examined

The results obtained allow us to conclude that in the Creole sheep of Pampalca it is possible to identify the first signs of gestation from the 15th day of gestation; however, it is recommended to issue a diagnosis only from day 24 given the possibility of visualizing both the embryo and the heartbeat.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Azarkish, F., Janghorban, R., Bozorgzadeh, S., Arzani, A., Balouchi, R. y Didehvar, M. (2022). The effect of maternal intravenous hydration on amniotic fluid index in oligohydramnios. *BMC Res Notes 15*, 95. https://doi.org/10.1186/s13104-022-05985-6

Barbagianni, M., Ioannidi, K., Vasileiou, N., Mavrogianni, V., Orfanou, D., Fthenakis, G. y Valasi, I. (2017). Ultrasonographic examination of pregnant ewes: From early diagnosis of pregnancy to early prediction of dystocia. Small Ruminant Research, 152, 41-55. https://doi.org/10.1016/j.smallrumres.2016.12.008

Bragança, G., Balaro, M., Fonseca, J., Pinto, P., Rosa, R., Ribeiro, L., Almeida, M., Souza, J., Garcia, A., & Brandao, F. (2016). Doppler ultrasound in the diagnosis of early pregnancy in sheep. *Anim Reprod, 13*(3), 587. https://n9.cl/oalcn

Brzozowska, A., Stankiewicz, T., Błaszczyk, B., Chundekkad, P., Udała, J. y Wojtasiak, N. (2022). Ultrasound parameters of early pregnancy and Doppler indices of blood vessels in the placenta and umbilical cord throughout the pregnancy period in sheep. *BMC Vet Res 18*, 326. https://doi.org/10.1186/s12917-022-03424-z

Crilly, J., Politis, A. y Hamer, K. (2017). Use of ultrasonographic examination in sheep veterinary practice. Small Ruminant Research, 152, 166-173. https://doi.org/10.1016/j.smallrumres.2016.12.021

Del'Aguila, P., Cirino, F., Correia, V., Rodrigues, A., Ramirez, R., Padilha, L., Amoroso, L., Russiano, W., Rossi, M. (2021). B-mode ultrasound and ecobiometric parameters to assess embryonic and fetal development and estimate gestational age in goats. Theriogenology, 175, 123-133. https://doi.org/10.1016/j.theriogenology.2021.09.002

Dwyer, C., Conington, J., Corbiere, F., Holm, I., Muri, K., Nowak, R., Rooke, J., Vipond, J. y Gautier, J. (2015). Invited review: Improving neonatal survival in small ruminants: science into practice. *Animal, 10*(3),

449–459. https://doi.org/10.1017/S1751731115001974

Hong, P., Simpson, R., Salamonsen, L., Greening, D. (2016). Extracellular Vesicles in the Intrauterine Environment: Challenges and Potential Functions. *Biology of Reproduction, 95*(5), 1-12, https://doi.org/10.1095/biolreprod.116.143503

Jones, A. y Reed, S. (2017). Benefits of ultrasound scanning during gestation in the small ruminant. *Small Ruminant Research, 149*, 163-171. https://doi.org/10.1016/j.smallrumres.2017.02.008

Jones, A., Gately, R., McFadden, K., Steven A., Govoni, K. y Reed, S. (2016). Transabdominal ultrasound for detection of pregnancy, fetal and placental landmarks, and fetal age before Day 45 of gestation in the sheep. Theriogenology, 85(5), 939-945. https://doi.org/10.1016/j.theriogenology.2015.11.002

Khedri, A., Khodja, S., Semai, A. and Mimoune, N. (2018). Peculiarities of the diagnosis of pregnancy in sheep species [Tesis de veterinario, École Nationale Supérieure Vétérinaire, Argelia]. http://archive.ensv.dz:8080/jspui/handle/123456789/1790

Kusama, K., Nakamura, K., Bai, R., Nagaoka, K., Sakurai, T., Imakawa, K. (2018). Intrauterine exosomes are required for bovine conceptus implantation. *Biochemical and Biophysical Research Communications, 495*(1), 1370-1375. https://doi.org/10.1016/j.bbrc.2017.11.176

Lone, S., Gupta, S., Kumar, N., Prakash, K., Ganaie, B., Rather, H. y Kumar, S. (2016). Recent technologies for pregnancy diagnosis in sheep and goat: an overview. *International Journal of Environmental Science and Technology, 3,* 1208-1216. https://n9.cl/slu15

Meinecke, S. (2017). Basics of ultrasonographic examination in sheep. *Small Ruminant Res.152*, 10-21. http://dx.doi.org/10.1016/j.smallrumres.2016.12.023.

Mirkena, T., Solkner, J., Haile, A., Rischkowski, B. y Okyo A. (2023). Implementation of Community-based Sheep Breeding Programs in Different Production Systems in Ethiopia. *Revista de Agricultura, Alimentación y Recursos Naturales*, 1 (1), 71–79. https://doi.org/10.20372/afnr.v1i1.72

Paige, J., Ryan, G., Hall, E., De Graaf, S. y Hermes, R. (2017). Using transrectal ultrasound to examine the effect of exogenous progesterone on early embryonic loss in sheep. *PLoS ONE 12*(8): e0183659. https://doi.org/10.1371/journal.pone.0183659

Parraguez, V.H.; Sales, F.; Peralta, O.; De los Reyes, M.; Gonzalez-Bulnes, A. Oxidative Stress and Fetal Growth Restriction Set Up Earlier in Undernourished Sheep Twin Pregnancies: Prevention with Antioxidant and Nutritional Supplementation. *Antioxidants, 11*, 1287. https://doi.org/10.3390/antiox11071287

Quintela, L., C. Díaz, Peña, A., Becerra, J. y Herradón, P. (1999). Early pregnancy diagnostic by transrectal ultrasonography in the ewe. *Arch. Zootec. 48* (181), 13-20. https://dialnet.unirioja.es/descarga/articulo/4123.pdf

Rajashri, M., Prasad, K., Surabhi, K. y Reddy, M. (2017). Hydrometra in a deccani ewe after oestrus synchronization and artificial insemination. *Haryana Veterinarian, 56*(1), 113-114. https://www.luvas.edu.in/haryana-veterinarian/download/harvet2017/32.pdf

Roberts, J. (2022). Pregnancy diagnosis in small ruminants. *AABP PROCEEDINGS, 55* (2), 156-158. https://doi.org/10.21423/aabppro20228636

Sadi, F. (2022). Diagnosis of Pregnancy and Embryonic Development by 2D Ultrasound in Kurdish Sheep. Journal of Alternative Veterinary Medicine. Spring, 5(12). 687-694. https://joavm.kazerun.iau.ir/article-1-89-en.pdf

Sandoval, C., Sales, F., & Parraguez, V. (2022). Diagnosis of Pregnancy by Ultrasonography in Sheep. Institute of Agricultural Research. INIA-Kampenaike. Punta Arenas, Magallanes. Chile. https://n9.cl/285fmo

Sen, U., Sirin, E., Ensoy, U., Aksoy, Y., Ulutas, Z. y Kuran, M. (2016). The effect of maternal nutrition level during mid-gestation on postnatal muscle fibre composition and meat quality in lambs. *Animal Production Science*, 56, 834-843. https://doi.org/10.1071/AN14663

Soni A, et al. Role of l-arginine in oligohydramnios. *J Obstet Gynecol, 66*(1):279–83

Soto, A., Gómez, M., & Sota, R. (2022). Ultrasonographic inspection of the reproductive system of female sheep and goats. Libros de Cátedra.

Thorne, J., Murdoch, B., Freking, B., Redden, R., Murphy, T., Taylor, J. y Blackburn, H. (2021). Evolution of the sheep industry and genetic research in the United States: opportunities for convergence in the twenty-first century. Animal Genetics, 52(4), 395-408.

Usuda, H., Watanabe, Sh, Miura, Y., Saito, M., Musk, G., Rittenschober, J., Ikeda, H., Sato, Sh., Hanita, T., Matsuda, T., Jobe, A., Newnham, J., Stock, S. y Kemp, M. (2017). Successful maintenance of key physiological parameters in preterm lambs treated with ex vivo uterine environment therapy for a

period of 1 week. American Journal of Obstetrics and Gynecology, 217(4), 457.e1-457.e13. https://doi.org/10.1016/j.ajog.2017.05.046

Valasi, I., Barbagianni, M., Loannidi, K., Vasileiou, N., Fthenakis, G. y Pourlis, A. (2017). Developmental anatomy of sheep embryos, as assessed by means of ultrasonographic evaluation. Small Ruminant Research, 152, 56-73. https://doi.org/10.1016/j.smallrumres.2016.12.016

Vergara, A., Gómez, R., Hinojosa, R., Quispe, U., Machaca, J., Llacchua, F., Huamancaja, R. (2023). Livestock Production Chains: Influence On The Social Dimension Of Sustainable Agricultural Development Huando, Huancavelica. Journal of Namibian Studies, 33 S1, 2156-2169. https://doi.org/10.59670/jns.v33i.4227

Yadav, V., Kumar, R., Bisla, A., Saini, G., Dutt, R. Y Ranga, L. (2019). *International Journal of Livestock Research, 9*(10), 170-178. https://doi.org/10.5455/ijlr.20190821041723

Zhang, Y., Wang, Q., Wang, H., & Duan, E. (2017). Uterine fluid in pregnancy: a biological and clinical outlook. *Trends in molecular medicine, 23*(7), 604-614. https://doi.org/10.1016/j.molmed.2017.05.002