Koshevoy, V., Naumenko, S., Skliarov, P., Fedorenko, S., & Kostyshyn, L. (2021). Male infertility: Pathogenetic significance of oxidative stress and antioxidant defence (review). *Scientific Horizons, 24*(6), 107-116.

Koshevoy, V., Naumenko, S., Skliarov, P., Syniahovska, K., Vikulina, G., Klochkov, V., & Yefimova, S. (2022). Effect of gadolinium orthovanadate nanoparticles on male rabbits' reproductive performance under oxidative stress. *World's Veterinary Journal, 12*(3), 296-303.

Koshevoy, V.I., Naumenko, S.V., Zhukova, I.O., & Orobchenko, O.L. (2024). Prospects for the use of resveratrol – a polyphenol phytoantioxidant in veterinary reproduction (review). *Veterinary biotechnology, 44*, 50-58.

Liang, Y., Xu, M.L., Gao, X., Wang, Y., Zhang, L.N., Li, Y.C., & Guo, Q. (2023). Resveratrol improves ovarian state by inhibiting apoptosis of granulosa cells. *Gynecological endocrinology, 39*(1), article number 2181652.

Markowska, A., Antoszczak, M., Markowska, J., & Huczyński, A. (2023). The Role of Selected Dietary Factors in the Development and Course of Endometriosis. *Nutrients, 15*(12), article number 2773.

Mendes, T.B., Simas, J.N., Fischer, L.W., Paccola, C.C., de Oliva, S.U., Vendramini, V., & Miraglia, S.M. (2022). Resveratrol benefits on sperm DNA, chromatin structure and reproductive outcomes of varicocelized rats. *Andrologia, 54*(6), article number e14417.

Nishigaki, A., Tsubokura, H., Tsuzuki-Nakao, T., & Okada, H. (2021). Hypoxia: Role of SIRT1 and the protective effect of resveratrol in ovarian function. *Reproductive medicine and biology, 21*(1), article number e12428.

Novakovic, R., Rajkovic, J., Gostimirovic, M., Gojkovic-Bukarica, L., & Radunovic, N. (2022). Resveratrol and Reproductive Health. *Life (Basel, Switzerland), 12*(2), article number 294.

Ochiai, A., & Kuroda, K. (2019). Preconception resveratrol intake against infertility: Friend or foe? *Reproductive medicine and biology, 19*(2), 107-113.

Pyo, I.S., Yun, S., Yoon, Y.E., Choi, J.W., & Lee, S.J. (2020). Mechanisms of Aging and the Preventive Effects of Resveratrol on Age-Related Diseases. *Molecules (Basel, Switzerland), 25*(20), article number 4649.

Shetty, R., Joshi, P. D., Mahendran, K., Jayadev, C., & Das, D. (2023). Resveratrol for dry eye disease – Hope or Hype?. *Indian journal of ophthalmology, 71*(4), 1270-1275.

Vašková, J., Klepcová, Z., Špaková, I., Urdzík, P., Štofilová, J., Bertková, I., Kľoc, M., & Rabajdová, M. (2023). The Importance of Natural Antioxidants in Female Reproduction. *Antioxidants (Basel, Switzerland), 12*(4), article number 907.

Wang, Y., Zhang, M., Chen, Z.J., & Du, Y. (2018). Resveratrol promotes the embryonic development of vitrified mouse oocytes after in vitro fertilization. In vitro cellular & developmental biology. *Animal, 54*(6), 430-438.

ON-FARM DETECTION OF LOW-FERTILITY RAMS: IMPLEMENTATION OF TESTICULAR ULTRASOUND/ECOTEXTURE, SPERMIOGRAM AND BACTERIOLOGY IN SEMEN

Pérez-Marín C.C.[1], PhD (Veterinary Sciences), Professor
Arrebola F.A.[2], Senior Researcher
[1]*University of Córdoba, Spain*
[2]*Instituto de Formación Agraria y Pesquera de Andalucía, Cordoba, Spain*

Introduction. Small ruminants are an important sector in Spain, being Asia the main productive continent jointly with Africa. These animals have seasonal reproduction, and it is absolutely important the reproductive successful in order to reach optimal production (milk, meat, etc). In relation to the male's ability for reproduction, it should be checked, but it is not always carried out on farm. Different

tools are available for the breeding soundness evaluation in rams, as semen assessment, ultrasonography, palpation, among others. The selection of optimal sires will improve the productive efficiency in the farms.

The present study aims to **s**tudy the percentage of subfertile rams in the analyzed population based on semen quality, to identify testicular ultrasound alterations, and to determine the main bacteria associated with decreased fertility in farms.

Material and methods. This study involved a total of 542 rams from 27 sheep farms in the Southern Spain (Cordoba, Spain), reared under Mediterranean environmental conditions. The animals were managed in semi-extensive or semi-intensive systems, basing their feeding on pastures or concentrates, respectively. A breeding soundness evaluation of rams was conducted at the beginning of the breeding season. For this purpose, testes were checked by palpation and ultrasonography, semen was collected and assessed, and microbiological tests were carried out to identify pathogens in subfertile rams.

Sperm collection and assessment. Rams were sedated using xylazine (0.2 mg/kg, Rompun 2%, Bayer Hispania S.L., Barcelona, Spain). The measurement of the scrotal circumference (SC) was performed. For semen collection, an electric pulse electroejaculator (Electro Jack 6, IDEAL Instruments, USA) was used. After the semen collection, the seminal quality parameters (color, volume, sperm concentration, and masal motility) were recorded. The criteria for classifying stallions as subfertile were established based on testicular abnormalities and semen quality. In this study, a ram was considered suspicious for subfertility if it met any of the following criteria: testicular lesions (abscesses, granulomas, nodules, etc.), transparent-colored semen, semen concentration <1300 $x10^6$ ml, and mass motility (MM) <1.5. Semen was diluted in TRIS-based extender and the total motility (TM) and progressive motility (PM) of each sample were evaluated using a phase-contrast microscope (Nikon Eclipse E400) with the Integrated Semen Analysis System computer system (ISAS).

Ultrasonography and echotexture of testis. For testicular ultrasonographic evaluation, a scan equipped with 7-mHz curved linear probe (Easi-Scan Go Curve, BCF, Ireland) was used. Different cross-sections were evaluated in the transverse (proximal, middle and distal) and longitudinal planes. The ultrasound images were analyzed to determine their echotexture value. Echotexture is defined as the appearance of the gray-scale framework obtained from a tissue. Specifically in the testicle, it refers to the appearance of the parenchyma and the development of the seminiferous tubules that compose it. The images were processed using a computer software (Image J 1.5, USA). The color of the pixels corresponded to intensity values that range from 0 (anecogenic) to 255 (hyperechoic) (Adriana et al., 2012).

Blood sampling. A blood sample were obtained from the jugular vein from those rams considered as low-fertility suspicious were taken to determine the presence of any pathogen that could be associated with the decrease in fertility. Vacuum tubes (BD Vacutainer® tubes) for whole blood and plasma were used.

Bacteriological analysis. Samples obtained from testicular lesions and from semen were analyzed, an aliquots was stored at -80ºC. The samples were cultured on TSA agar (Oxoid) along with a streak of *Staphylococcus aureus* (source of factor V) and incubated at 37ºC in an atmosphere enriched with 15% CO_2 for a period of 24-72 hours. The representative colonies were subcultured following the same procedure until pure cultures were obtained for identification. The pure isolates were classified using rapid biochemical techniques such as catalase, oxidase, latex particle agglutination test for the confirmation of capsular groups, or the CAMP (Christie-Atkins-Munch-Peterson) test against *Staphylococcus aureus*.

The samples were also cultured in a specific medium for the isolation of *mycoplasmas* (Mycoplasma agar base enriched with *Mycoplasma* Supplement-G, Oxoid) and incubated at 37ºC in a modified atmosphere with 5% CO_2 for 7 days. The semen samples were analyzed for the detection of *Ureaplasma* sp. using qPCR (EXOone Ureaplasma urealyticum). The serological analysis was performed using commercial ELISA kits (INgezmin *Brucella ovis*, Eurofins Technologies Ingenasa)

on centrifuged serum samples for the detection of specific antibodies against B. ovis. The semen samples from seropositive animals were evaluated using qPCR (EXOone *Brucella ovis*).

Statistical analysis. The statistical program IBM SPSS Statisticals V. 25 was used (Chicago, IL, EEUU). A descriptive analysis was carried out regarding the percentage of fertile rams based on seminal quality values. Testicular injuries detected by ultrasonography were described. In order to determine if testicular echotexture varied between fertile and subfertiler rams, an analysis of variance (ANOVA) was conducted. The correlation between scrotal circumference measured with a tape measure (orchidometer) and testicular perimeter by ultrasound was analyzed. A graphical representation was made using a scatter plot, and then a bivariate correlation analysis was conducted, calculating the Pearson statistic. Finally, the coefficient of determination was determined to understand how the testicular perimeter influences the measurement of scrotal circumference. To compare the percentages related to seminal quality and ultrasound anomalies and reproductive performance, Fisher's test or, as appropriate, the Chi-squared test was used. The differences were considered significant when $p<0.05$. The results are expressed as mean ± standard deviation.

A descriptive analysis of the data was conducted for the type of isolated pathogens. The data were subjected to the Kolmogorov-Smirnov test to determine their normality. None of the studied variables exhibited a normal distribution, so non-parametric tests were used to analyze the data. The Mann-Whitney test was used for comparison of means, considering that significant differences existed when $p <0.05$. The data are expressed as mean ± standard deviation.

Results and discussion. Of the 542 rams examined, 465 (85.79%) were considered suitable and 77 (14.21%) were considered as subfertile, according to the established criteria. Among the total number of rams suspected of subfertility, 22.08% showed testicular lesions (epididymitis, nodules, orchitis, abscesses, hydrocele, hypoplasia, microlithiasis, cysts), 25.97% exhibited transparent-colored semen, 35.06% had a concentration < 1300 million sperm/ml, and 71.43% had an MM < 1.5%. Three age groups were established (under 1.5 years, from 1.5 to 3.0 years, and over 3 years). Testicular size showed significant differences associated with age, so in younger animals, the average perimeter was significantly lower (30.6 ± 2.9 cm; mean ± SD) than in the other groups (35.1 ± 3.5 and 35.6 ± 3.0 cm, respectively). It is deduced that the greatest growth occurs when the animals are between 18-30 months old. As in cattle (Adriana et al., 2012), it was observed that the phase of greatest growth of the testicular perimeter was from 15 to 30 months.

In order to evaluate whether testicular size was associated with the loss of semen quality, the young and adult groups were compared. No significant differences were observed, suggesting that testicular size could not be used as a predictor for rams with poor semen quality. The longitudinal and transverse echotexture of each testicle was analyzed, and a comparison was made between rams showing good or low semen quality. To avoid the effect of age, the study was conducted separately in both age groups. Significant differences were observed in most of the measures analyzed. The average values of rams classified as subfertile were below 80 pixels, while in ram with optimal semen this measure was above 90 pixels. These results demonstrate that the determination of testicular echotexture can be a good indicator of reproductive performance, in terms of seminal quality, due to the development of the seminiferous tubules and spermatogenesis in healthy parenchyma (Chandolia et al. 1997). The correlation between changes in the cellularity of the seminiferous tubules and sperm production has also been studied by Omer et al. (2013), and they even confirmed that it can provide information about future seminal quality. On the contrary, in bulls, Tomlinson et al. (2017) did not find a significant relationship between pixel intensity and semen quality, although according to them, ultrasound remains a useful tool for the detection of macroscopic pathologies. When the parenchyma presents heterogeneous pixels pattern with hyperechoic foci, it is related to degeneration and the presence of granulomas, according to Karaka et al. (1999). It is described that infection by *Pasteurella multocida* presents microscopic microcalcifications in the testis (García-Pastor et al., 2009), pattern that has been identify in this study. Therefore, ultrasonography and echotexture could help to the identification of certain disease in sheep farming.

The prevalence of bacteriospermia in subfertile rams was 80%. The main microorganisms isolated were *Corynebacterium sp.*, *Enterobacteriaceae sp*., *Mycoplasma sp*., *Staphylococcus sp*.,

Streptococcus sp., *Ureaplasma sp*., *Actinobacillus seminis*, *Brucella ovis*, *Histophilus somni* and *Trueperella pyogenes*. In 68% of subfertile rams were isolated one or more types of pathogens; in 26%, there was no significant growth; and in 6% of them, there was microbial contamination, and no pathogens could be isolated. All cultures resulted negative for the isolation *Mycoplasma sp*. Finally, a qPCR was performed on semen samples to determine *Ureaplasma diversum*, detecting a 46.8% of positive cultures. When comparing the scrotal circumference in rams showing with or without bacteria in semen, it was observed a significantly lower value in the rams with positive bacterial culture (30.07 ± 4.27 cm vs 32.77 ± 3.75 cm; $p<0.013$).

The most prevalent bacteria in the study were *Ureaplasma diversum,* followed by *Brucella ovis*, *Trueperella pyogenes*, *Corynebacterium sp.* and *Streptococcus pluranimalium*. In reference to the type of samples, *Actinobacillus seminis* and *Histophilus somni* were more commonly isolated in semen, while *Corynebacterium pseudotuberculosis* and *Trueperella pyogenes* were isolated in abscess and nodule samples. 71% of the rams were negative for *Brucella ovis* serology.

Conclusions. It is confirmed the high incidence of subfertility in rams from the studied farms, and the semen assessment is an essential, quick, and simple method to detect reproductive failures in rams. Ultrasonography is a useful tool for detecting injuries/pathologies at testicular or epididymal level, with high diagnostic ability than palpation/observation. The most frequent pathologies observed by ultrasonography were microlithiasis, testicular hypoplasia, abscesses, epididymitis, orchitis, cysts and hydrocele. Results support that testicular ecotexture can be a good indicator of reproductive performance, although there is no practice equipment to carry out this measure. Few information exists about the pathogens involved in subfertile rams in our area. These data give us new information, showing a high incidence of *Brucella ovis*, *Trueperella pyogenes* and *Corynebacterium sp*.

References

Adriana Santana DC., Jair P.O., F.M, Luiza, DL, Luiz Alberto, H. Marc, y C.J Liliana (2012). Aspectos biométricos y ultrasonográficos del desarrollo testicular en bovinos de la raza guzerat (Bos taurus indicus). Rev. Cienc. Anim 5; 51-62.

Chandolia R.K., P.M. Bartlewski; B.C. Omeke. A.P. Beard, N.C. Rawlings, R.A. Pierson. (1997) Ultrasonography of the developing reproductive tract in ram lambs: effects of a GnRH agonist. Theriogenology 48: 99-117.

García-Pastor, L.M. (2006). Alteraciones testiculares en moruecos: Estudio clínico, serológico, microbiológico y anatomopatológico. [Tesis doctoral, Universidad de Zaragoza].

Karaca F., M., Kaya A., Ataman M.B., Tekeli T. (1999) Spermatic granuloma in the ram: diagnosis by ultrasonography and semen characteristic. Veterinary Radiology & Ultrasound 40(4); 402-6.

Omer R., Giffin J., Hahnel A., Bartlewski P., (2012) Relationships of ultrasonographic and magnetic resonance image attributes to the histomorphology of ram testes. Reproductive Biology, 12, 355-361.

Tomlinson M., Jennings A, Macrae A., Truyers I., (2017) The value of trans-scrotal ultrasonography at bull breeding soundness evaluation (BBSE): The relationship between testicular parenchymal pixel intensity and semen quality. Theriogenology 89, 169–177.

THE DEPENDENCE OF QUALITY INDICATORS OF COWS' MILK ON THE AGE OF THEIR CALVING

Yemets Z.V.[1], Cand. Sci. (Agric.), Ass. Professor
Honcharova I.I.[2], Cand. Sci. (Agric.), Ass. Professor
[1]*Odesa State Agrarian University, Odesa, Ukraine*
[2]*State Biotechnological University, Kharkiv, Ukraine*

Introduction. Competition in agribusiness and the instability of cattle keeping conditions, milk prices, and the factor of prolonging the productive use of dairy cattle are becoming more and more