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B-mode and Doppler ultrasonographic examination assists male small ruminant fertilizing ability evaluation

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ABSTRACT: Systematic evaluation of male reproductive capacity ensures fertility, enhancing the economics of small ruminant farms. The assessment of semen quality using contemporary and objective laboratory techniques contributes to the evaluation of sperm fertilizing ability, while in combination with the clinical examination, supports the prognosis of ram and buck fertility. B-mode and Doppler ultrasonographic examination of male reproductive system can be applied in addition to clinical examination, since various non clinically identified pathological conditions adversely affect fertility. Over the past two decades, numerous researchers indicated a variable but notable correlation between seminal quality and B-mode and Doppler ultrasonographic indices in small ruminants. The present review aims to provide updated knowledge on the prospect of applying B-mode and Doppler ultrasonographic examination to support ram and buck fertilizing ability evaluation.

Keyword: buck; ram; semen quality; testicular echotexture; testicular blood flow

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THE SIGNIFICANCE OF RAM/ BUCK REPRODUCTIVE CAPACITY EVALUATION

Small ruminant fertility is exceptionally important, as it contributes to farm sustainability. Systematic evaluation of male reproductive capacity ensures fertility, supporting the productivity and the financial status of a farm. The selection of the appropriate rams/bucks is based on their genetic value, as well as on the results of the clinical and laboratory evaluation.

Impact of semen quality assessment on ram/ buck fertility evaluation

The estimation of semen quality via contemporary and objective laboratory techniques contributes to the assessment of sperm fertilizing ability (Rodríguez-Martínez et al., 2003), while in combination with the clinical examination, an accurate prognosis of male fertility can be achieved (Ganter et al., 2008, Farquharson 2009).

Semen volume, concentration, viability, motility, and morphology are the most substantial and routine characteristics, that contribute to male fertilizing ability assessment. The last decades, except for the basic semen traits, there have been developed more advanced techniques for semen evaluation, such as sperm DNA fragmentation, sperm membrane biochemical functionality by hypo-osmotic swelling test (HOST), sperm mitochondrial functionality, sperm oxidative stress (e.g., reactive oxygen species-ROS), sperm proteins identification as markers of apoptosis (e.g., caspases 3 and 7), capacitation status of spermatozoa, etc.

Despite the fact that the basic semen quality characteristics assessment provides useful information, male fertility cannot be guaranteed, since fertilization is a complex, multifactorial process that is confirmed by pregnancy rate and birth.

It has been reported that 20% of rams can be characterized subfertile, due to low or diverse semen quality (Campbell et al., 2003). Normally, semen traits can be affected by various factors, such as breed, age, season (Ntemka et al., 2019), frequency and semen collection method (Jiménez-Rabadán et al., 2012, Palacin-Martinez et al., 2022), libido, and nutrition (Fernandez et al., 2004). Besides, semen quality can be affected by congenital or acquired pathological cases of genital system (Van Camp 1997, Karaca et al., 1999, Ahmad et al., 2000).

The majority of pathological conditions that adversely affect ram/buck fertility can be identified during clinical examination (Boundy, 1992, Bruere et al., 1993). The application of imaging methods, such as the ultrasonographic examination of male reproductive system can be applied in addition to clinical examination.

B-mode ultrasonographic examination, a useful tool for the assessment of ram/buck reproductive system

Ultrasonographic examination is a particularly useful method for assessing male reproductive system. Both B-mode and Doppler ultrasonographic techniques are non-invasive, accurate, rapid, and painless.

Testicular biometry (length, width and thickness), testicular and epididymal function, microstructure, and chemical composition of the parenchyma can be revealed via B-mode. Moreover, ambiguous clinical findings can be interpreted and early lesions unidentified through clinical examination can be detected (Ahmad and Noakes, 1995). Additionally, ultrasonography can be applied in small ruminants for auxiliary diagnostic purposes, such as testicular fine needle aspiration cytology (Vencato et al., 2017), which is considered as a determinant technique for the diagnosis of azoospermia and severe oligozoospermia in human medicine.

In the past decades, B-mode ultrasonography was providing information on qualitative characteristics of testis and epididymis (Gouletsou, 2017). In recent years, there has been growing interest in quantitative computer-assisted image analysis of ultrasonograms. In the case of black and white images, there are 256 tons of the gray scale, ranging from completely black (0) to completely white (255). Human eye can distinguish only 18 to 20 tons. Hence, ultrasonograms interpretation could not be objective (Pierson and Adams, 1995).

The analysis of two-dimensional black and white digital images is based on texture analysis, since texture is related to image brightness levels and to the way that they are allocated in space. Moreover, except for brightness, each image provides information related to uniformity, density, roughness, linearity, frequency, phase, directionality, randomness, detail, smoothness, etc., defining the term of image texture (Materka and Strzelecki, 1998).

Echotexture indices are mathematically equated with statistical quantities that can be statistics related to the gradient of image brightness, first-order, second-order or higher-order gray-level statistics. Gradient of image brightness is calculated by comparing the brightness of each pixel with the adjacent pixels (Nailon, 2010). Parameters of this category are mean value of gradient (mean of different brightness values of adjacent pixels) and variance of gradient (variance of different brightness values of adjacent pixels). First-order gray-level statistics refer to the overall echogenicity of an anatomical region. They are defined based on the brightness values of the pixels, regardless of their location in space (Nailon, 2010). Mean numerical pixel values (NPVs) is an echotexture index commonly measured in ultrasonograms, which refers to the different tones of gray scale, whereas pixel heterogeneity represents the standard deviation of mean numerical pixel values. Second-order gray-level statistics are more complex parameters. The parameters of this category take into account both the position of the pixel and its relation in space with the adjacent pixels (Nailon, 2010). Contrast (brightness variance between adjacent pixels) and correlation (brightness relation between adjacent pixels) are second-order values. Higher-order gray-level statistics can be extracted through route length to reveal the spatial organization of the image texture. Route lengths indicate the directionality and roughness of the image texture (Nailon, 2010). Gray value distribution is a characteristic higher-order parameter. In fact, ultrasonograms can be analyzed by several software, consequently the echotextural indices are various.

Small ruminant testicular and epididymal echogenicity can be affected by various factors, such as puberty (Andrade et al., 2014), age (Chandolia et al., 1997), season (Ntemka et al., 2021), and pathological conditions (Gouletsou, 2017).

Doppler ultrasonographic examination, a useful tool for the assessment of ram/buck reproductive system

Doppler ultrasonography evaluates the vascular changes in testicular parenchyma and provides information on vascular architecture, direction and speed of blood flow, resistance etc. Blood perfusion must be maintained constant and normal since it ensures the supply of nutrients to the testicular parenchyma. Moreover, the study of blood flow in testicular artery via the Doppler technique, aiming to identify pathological conditions, such as focal or diffuse hyperemia, has already been subject of research (Gouletsou, 2017, Cook and Dewbury, 2000).

Blood flow recorded by Doppler technique can be assessed qualitatively and quantitatively. In qualitative assessment of Doppler curves, waves are estimated descriptively. In particular, parameters that are used are presence or absence of diastolic blood flow and the direction of flow.

In quantitative assessment, Blood flow volume (BFV) is determined per unit of time, at a specific point in the vessel, based on the formula BFV=V x 60 x A, where: BFV=blood flow volume (ml/min), V=mean velocity of blood flow (cm/sec) and A=vessel diameter (cm) (Fendel and Gans, 1993). Regarding vessels examination, the measurement of vessel resistance aims at calculating the observed vasodilation during the development of an occlusive condition (reduction of resistance) and the detection of a random hemodynamic obstruction (maximization of resistance). The assessment of vascular resistance is based on indices that express the continuous (dilation) and alternating (contraction) blood flow (Figure 1).

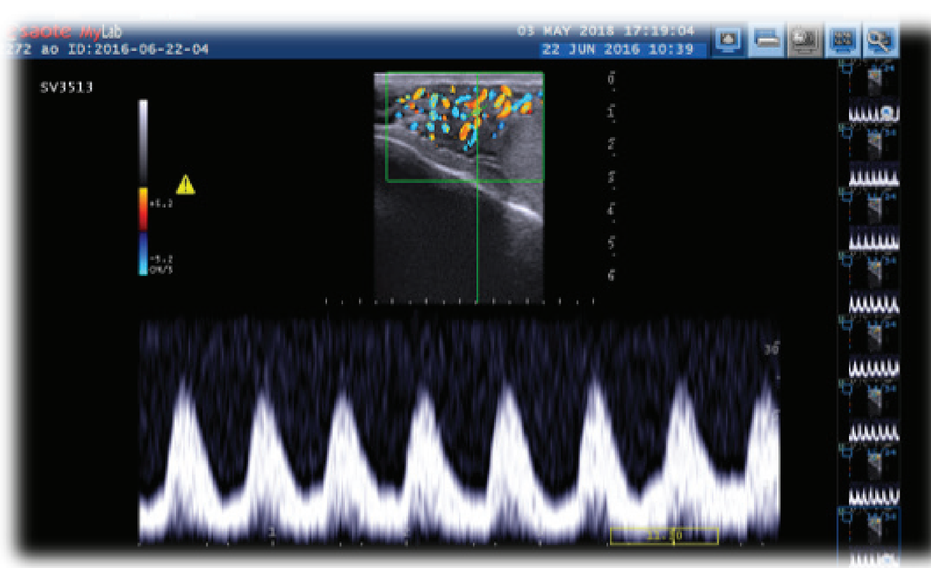

Figure 1. Pulsed wave doppler ultrasonographic examination of testicular artery (Ntemka, 2020).

Doppler indices that are most commonly measured are Resistance index (RI) and Pulsatility index (PI) (Gosling and King, 1975). The advantage of their use is the fact that the size of the vessel is not required for their calculation. RI is expressed by the formula RI=(PSV-EDV) / PSV, where PSV=Peak systolic velocity and EDV=End-diastolic velocity. On the other hand, PI is expressed by the formula PI=(PSV-EDV)/ TAVM, where PSV=Peak systolic velocity, EDV=End-diastolic velocity and TAVM=-Time averaged mean velocity during one cardiac cycle. PI and RI values increase when vascular resistance increases, consequently blood flow within the vessels decreases (Figure 2).

In addition, Stuart et al. (1980) described the simpler index S/D ratio=Mean systolic velocity/Mean diastolic velocity.

Small ruminants' testicular blood flow can be affected by several factors, such as puberty (Camela et al., 2019), age, season (Ntemka et al., 2021), administration of hormones (Shahat et al., 2022) and different pathological conditions (Gouletsou, 2017).

According to Herwig et al. (2004), sperm fertilizing ability of mammals is related to testicular blood flow, while spermatogenesis is affected by disorders of testicular parenchyma and by blood flow disorders in testicular artery. Hence, numerous researchers studied the possible correlation between small ruminant semen quality traits and B-mode as well as Doppler ultrasonographic parameters. This review will present the interesting results of previous studies on this specific topic, indicating the different results and the factors that could affect the surveys' outcome. Thus, the present review aims to record the application perspectives of B-mode and Doppler ultrasonographic examination in ram and buck fertilizing ability evaluation.

CORRELATION BETWEEN RAM/ BUCK SEMEN PARAMETERS AND B-MODE INDICES

Several studies demonstrated considerable results concerning the correlation between small ruminants' semen characteristics and echotexture indices. Interesting enough was the research of Ahmadi et al. (2012) who studied the correlation between ram semen parameters and B-mode indices of testicular and epididymal parenchyma. Although the experiment was conducted separately during breeding and non-breeding seasons, results were recorded independently of the season effect. On the day of ultrasonographic examination, morphologically normal spermatozoa were negatively correlated with epididymal NPVs ($r=-0.46$, $p<0.05$) and epididymal pixel heterogeneity ($r=-0.46$, $p<0.05$). However, epididymal pixel heterogeneity was positively correlated with sperm tail abnormalities ($r=0.43$, $p<0.05$). On the other hand, sixty days after ultrasonographic examination, a correlation was recorded as positive between testes pixel heterogeneity, and head and tail sperm abnormalities ($r=0.79$ and $r=0.72$,

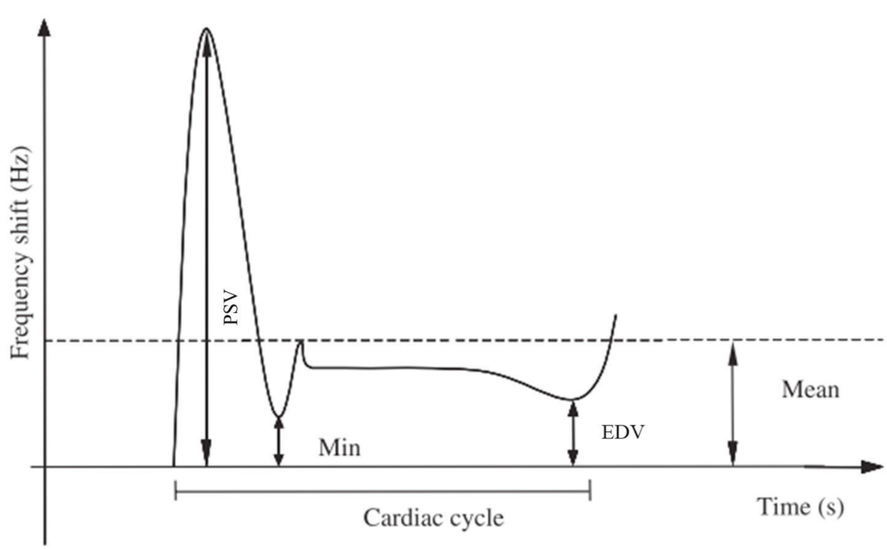

Figure 2. Schematic representation of a Doppler wave. PSV: maximum (peak) systolic velocity, Min: minimum diastolic velocity, EDV: end-diastolic velocity and Mean: mean frequency shift during one cardiac cycle. (Maulik, 2005).

$p<0.05$, respectively), but as negative between pixel heterogeneity, and individual motility and sperm normal morphology (r=-0.76 and r=-0.73, $p<0.05$, respectively).

The day of ultrasonographic examination had an effect on the results of this study. Correlation coefficient values were lower when semen quality assessment was applied in the day of ultrasonographic examination, compared to 60 days after it. This difference could be attributed to the fact that semen collection is a procedure accompanied by peripheral vasoconstriction, increased metabolic activity and elevated body temperature.

Moreover, in the day of B-mode performance, the correlation was recorded between semen and epididymal B-mode parameters. In contrast, 60 days after B-mode performance, the correlation was noticed between semen and testicular B-mode parameters. Actually, spermatogenesis is a complex process that lasts 47 days and is constantly evolving, consequently sperm maturation in epididymis is indispensable to ensure semen quality (Fournier-Delpech and Thibault, 1991, König and Liebich, 2004).

Ntemka et al. (2018) investigated the correlation between adult and old ram semen characteristics and echotexture parameters of testicular parenchyma in breeding and non-breeding seasons, providing different results from Ahmadi et al. (2012). Ultrasonographic examination was performed separately for each testis, because of statistically significant differences, while the number of the studied B-mode ultrasonographic parameters was notably higher. Semen was collected via artificial vagina, a stressless and painless method, compared to electroejaculation that Ahmadi et al. (2012) performed. Moreover, Ntemka et al. (2018), used CASA (computer-assisted sperm analyzer) for sperm motility and kinetics evaluation, which is a more objective method, whereas DNA fragmentation and HOST assays enhance semen quality assessment. Concerning the results of Ntemka et al. (2018) study, sperm DNA fragmentation was negatively correlated with right testis Gray Value Distribution (GVD), Run Length Distribution (RunLD), and Long Run Emphasis (LRunEm) of old rams, in breeding period (r=-0.9, $p<0.05$). Sperm chromatin integrity has been closely linked to reproductive indices, providing useful information that cannot be available by conventional laboratory tests. Chromatin integrity ensures embryonic development, reducing return to estrus rates (Gillan et al., 2005, Waterhouse et al., 2006). In addition, it has significant predictive value for fertility, especially when it is studied in combination with sperm morphological abnormalities (Tsakmakidis et al., 2010). Among the total results of the study, Ntemka et al. (2018) also noticed that in non-breeding season, left testis LRunEm of adult rams was negatively correlated with sperm tail and total abnormalities (r=-0.8 and r=-0.7, $p<0.05$, respectively). The above-mentioned semen variables were positively correlated to old rams' left testis Contrast (Con) during breeding period (r=0.5, $p<0.05$).

Montes-Garrido et al. (2022) investigated the semen collection frequency effect on the correlation between several ram semen parameters and testicular echotextural characteristics. These researchers also used objective and contemporary methods in semen quality evaluation process. In the period of standard semen collection frequency, sperm rapid progressive motility, BCF (beat cross frequency) and VSL (straight line velocity) were positively correlated with tubular area (r=0.504, r=0.521, r=0.432, $p<0.05$, respectively). Moreover, tubular diameter was positively correlated with progressive and rapid progressive motility, BCF and VSL (r=0.407, r=0.460, r=0.504, r=0.569, $p<0.05$, respectively). Different enough were the results of intense semen collection frequency, since tubular density was positively correlated with caspases 3 and 7 activity (r=0.476, $p<0.05$), but negatively with rapid progressive motility, VSL, ROS production and viability (r=-0.417, r=-0.425, r=-0.532, r=-0.504, $p<0.05$, respectively). Caspases 3 and 7 have been characterized as protein markers of sperm apoptosis, while ROS production is an indicator of sperm oxidative stress. It is well-known that intense ejaculation has a negative effect on semen quality, mainly semen volume, concentration, and motility, fact that is in line with Montes-Garrido et al. (2022) findings.

The study of Carvajal-Serna et al. (2022) demonstrated quite diverse results, as semen traits evaluation was performed the day of B-mode testicular ultrasonographic examination, thirty and sixty days after it. The day of testes ultrasonographic examination ROS level was positively correlated with number of white pixels (Ec2) but negatively with lumen area (r=0.201, r=-0.238, $p<0.05$). Besides, tubular density was positively correlated with progressive motile spermatozoa and normal morphology (r=0.371, r=0,233, $p<0.05$, respectively), but negatively with semen concentration, ROS level, proximal and distal cytoplasmic droplet (r=-0.218, r=-0.225, r=-0.338, r=-0.263, p<0.05, respectively). Also, a correlation was observed between testicular echotexture and semen quality assessment performed thirty days later. Among all, the authors reported that tubular density was positively correlated with total and progressive motility (r=0.234, r=0.295, p<0.05, respectively), but negatively with viability, DNA fragmentation, proximal and distal cytoplasmic droplet (r=-0.226, r=-0.216, r=-0.291, r=-0.312, p<0.05, respectively). Lumen area was positively correlated with motility, but negatively with distal cytoplasmic droplet (r=0.229, r=-0.207, p<0.05, respectively). Limited were the results sixty days after testes ultrasonographic examination. The duration of spermatogenesis could play a key role in this fact. Echotexture has been positively correlated with seminiferous tubules diameter (Giffin et al., 2014). Besides, testis tissue consists of 70-80% seminiferous tubules and certainly testicular volume is associated with spermatogenesis.

There are four studies that demonstrated no significant correlation between semen characteristics and parenchymal echotexture of the examined organs. Camela et al. (2017) studied the correlation between ram semen traits and B-mode indices of accessory sex glands parenchyma, whereas Urt et al. (2018), Camela et al. (2019) and Yotov et al., (2020) applied the ultrasonographic examination in testicular parenchyma. Urt et al., (2018), measured pixels intensity, while the other researchers measured NPVs and pixel heterogeneity of the respective organ parenchyma. Rams in these surveys were divided into two age groups and were younger, either before or after puberty, compared to the above-mentioned surveys. Testicular ultrasonographic images of pre-pubertal males appear low echogenic, while as males move to the stage of puberty, testes acquire normal echogenicity (Chandolia et al., 1997, Gouletsou et al., 2003, Ülker et al., 2005, Andrade et al., 2014, Ribeiro et al., 2017). Therefore, testicular echogenicity appears to be affected by changes in parenchymal microstructure, which are directly related to the total number of undifferentiated spermatogonia, as well as the percentage of seminiferous tubules containing spermatogonia (Giffin et al., 2014). Additionally, it has been indicated that the percentage of live sperm is increasing by the increase of age, while optimal semen motility is observed at the age of 3 years old compared to younger rams (Hassan et al., 2009, Chella et al., 2017).

CORRELATION BETWEEN RAM/BUCK SEMEN PARAMETERS AND DOPPLER INDICES

Different studies on the correlation between small ruminants' semen traits and Doppler parameters revealed various results. El-Kon et al. (2004) investigated the correlation between young and old bucks' semen traits and blood flow inside testicular artery. Semen volume was positively correlated with RI (r=0.709, p<0.05) and S/D (r=0.597, p<0.05). The examined blood flow parameters (PI, RI, S/D) were positively correlated with sperm concentration (r=0.382, r=0.368, r=0.347, p<0.05, respectively), but negatively correlated with morphologically abnormal spermatozoa (r=-0.254, r=-0.289, r=-0.262, p<0.05, respectively). A positive correlation was recorded between motility, PI, RI and S/D (r=0.287, r=0.233, r=0.287, p<0.05, respectively). Viability was the only semen parameter not correlated with any blood flow index. Although viability is an indirect indicator for evaluating the integrity of the cytoplasmic membrane of spermatozoa, it is an important trait of semen quality that affects fertility, because it ensures the propulsive movement and functionality of spermatozoa (Holt et al., 1997, Pintado et al., 2000). Besides, viability is positively correlated with progressive motility (Moghaddam et al., 2012). Consequently, absence of correlation between viability and hemodynamic parameters was not expected.

Batissaco et al. (2013) assessed the vascular perfusion in testicular artery and in testicular parenchyma separately for each testis. A positive correlation of total sperm abnormalities with PI and RI of left testicular artery (r=0.3 and r=0.31, p<0.05, respectively) and with parenchymal vascular perfusion score of right and left testes, was observed (r=0.29 and r=0.32, p<0.05, respectively). Estimating the extent of perfusion as indicated by the number of colored spots or areas on the tissue image can be done by scoring (e.g., 0-4 for none to maximal). This Doppler technique allows the evaluation of an entire structure or part of a structure in real time, while the area is being scanned systematically. However, the big disadvantage of parenchymal vascular perfusion estimation is that the scoring system is subjective and depends on the sonographer.

Furthermore, Dos Santos Ribeiro et al. (2020) and Abdelkhalek et al. (2022) also applied parenchymal vascular perfusion score of testes. Specifically, they investigated the correlation between buck semen parameters and blood flow indices of testicular artery and testicular blood perfusion. Dos Santos Ribeiro et al. (2020) did not observe any correlation between the studied parameters. On the contrary, Abdelkhalek et al. (2022) demonstrated that only sperm individual motility was negatively correlated with PI (r=-0.877, p<0.05) and RI (r=-0.544, p<0.05). Actually, Abdelkhalek et al., (2022) objectively evaluated sperm motility by CASA, while Dos Santos Ribeiro et al. (2020) used a conventional light microscope.

In the study of Ntemka et al. (2018), results were recorded taking into consideration the effect of specific factors. In detail, all semen and blood flow parameters were assessed in adult and old ram groups, and in breeding and non-breeding seasons. Moreover, ultrasonographic examination was performed separately for each testis. Left testis PI of adult rams was negatively correlated with semen concentration, sperm total and tail abnormalities, in breeding season (r=-0.5, p<0.05). Also in breeding season, sperm total and tail abnormalities were negatively correlated with right testis RI (r=-0.5, p<0.05) and left testis RI (r=-0.4 and r=-0.5, p<0.05, respectively) of adult rams. In this study, correlation findings refer only to breeding and not to non-breeding season. Seasonal variations in testicular perfusion may be associated with seasonal variation of testosterone concentration, which has been positively correlated with both the diameter of the testicular artery and the blood flow (Pozor, 2007). Additionally, the increased blood flow could be attributed to the increase in testicular volume observed in breeding period (Sarlós et al., 2013).

Camela et al. (2017) reported a positive correlation between semen volume and PSV and EDV of internal iliac artery (r=0.79 and r=0.67, p<0.05, respectively). Moreover, Camela et al., (2019) also indicated that semen volume is correlated with blood flow, but negatively (r=-0.57, p<0.05). This specific difference is attributed to the fact that the blood flow index correlated to semen volume was PI and the examined vessel was testicular artery. A negative correlation between semen characteristics and testicular artery PI (r=-0.89 and r=-0.759, p<0.05, respectively) and RI (r=-0.896 and r=-0.752, p<0.05, respectively) was also indicated by Hedia et al. (2019). These researchers performed their experiments in older rams, compared to the previously mentioned studies. In other species, EDV index was reported as decreased, whereas RI was increased in older males (Pozor and McDonnell, 2004). In general terms, it is supposed that testicular volume is decreased in very old males since degenerative changes of the seminiferous tubules can be observed. However, according to Turner et al. (2007) these processes may not be reflected in the ultrasonographic evaluation of testes.

Montes-Garrido et al. (2022) conducted a correlation study under standard and intensive semen collection frequency, having numerous results. Among them, rapid progressive motility, BCF and VSL were negatively correlated with TABF (total artery blood flow) (r=-0.568, r=-0.544, r=-0.578, p<0.05, respectively) in the period of standard semen collection frequency. On the other hand, in the period of intense semen collection frequency, caspases' 3 and 7 activity was positively correlated with PI and RI (r=0.648 and r=0.615, p<0.05, respectively). ROS production and BCF were negatively correlated with PI (r=-0.684 and r=-0.503, p<0.05, respectively) and RI (r=-0.661 and r=-0.48, p<0.05, respectively). During the intensive semen collection frequency, spermatozoa remain for a short period in epididymis, so they are not exposed to damage due to oxidative, osmotic, and thermal stress, or due to the depletion of adenosine triphosphate (ATP) reserves (Austin, 1985, Bedford and Hoskins, 1990, Brooks, 1990, Aitken and Baker, 2004). Hence, the correlation between ROS production and testicular blood flow was negative in this research.

Finally, Carvajal-Serna et al. (2022) investigated the correlation between Doppler indices of testicular artery and ram semen traits on the day of ultrasonographic examination, thirty and sixty days after it. The day of testes ultrasonographic examination, semen concentration and ROS level were negatively correlated with PI (r=-0.31 and r=-0.304, p<0.05, respectively) and RI (r=-0.303 and r=-0.317, p<0.05, respectively). Although thirty days after testes ultrasonographic examination only RI was negatively correlated just with ROS level (r=-0.206, p<0.05), sixty days after Doppler no correlation was observed between semen and blood flow parameters. At that time, stress of semen collection is not present. This process increases blood flow resistance in testicular artery, negatively affecting fertility, due to hypoxia and the production of reactive oxygen species (Balci et al., 2008). On the other hand, reduced blood flow leads to oxygen deficiency, accumulation of toxic metabolites in testes, and disruption of the heat exchange mechanism between the vessels and the testis, negatively affecting Leydig cell's function and spermatogenesis (Velickovic and Stefanovic, 2014).

SPECIFIC FEATURES, FACTORS, AND DIFFERENCES AMONG STUDIES ABOUT RAM/BUCK SEMEN TRAITS AND B-MODE/ DOPPLER ULTRASONOGRAPHIC CORRELATION

International bibliography includes several studies about the correlation between small ruminants' semen characteristics and ultrasonographic parameters. The majority of them refer to ram, while a few researchers studied buck (El-Kon et al., 2004, Dos Santos Ribeiro et al., 2020, Abdelkhalek et al., 2022).

Most of the published studies provide positive or negative r values between 0.4 and 0.6. In many studies, no statistically significant correlation was recorded, whereas several researchers indicated positive or negative r values below 0.4. Only in a very few studies the correlation between a small number of parameters was above 0.8 (Ntemka et al., 2018, Hedia et al., 2019, Abdelkhalek et al., 2022).

This notable diversity of results could be attributed to different factors. Experimental design is a general factor that could affect the survey's outcome. In addition, ram/buck nutrition is an important parameter that could be related to the diversity of research results. Numerous studies have proven that male nutrition affects testicular volume, serum hormones concentration (e.g., testosterone), seminal plasma hormones concentration (e.g., insulin-like growth factor), fresh semen quality characteristics, semen freezing ability, etc. (Fernandez et al., 2004, Selvaraju et al., 2012, Esmaeili et al., 2014).

Male breed could also play a role on research dissimilar results. Native breeds and purebred males were involved in most of the studies. Ahmadi et al. (2012) and Dos Santos Ribiero et al. (2020) were an exception including crossbreeds. The specific characteristics of each breed and the genetic improvement that has been succeeded could certainly diversify the surveys.

Geographic coordinates and climatic conditions of the experimental location should be also considered because these features affect the gene expression of each breed's characteristics. In addition, seasonality has already been demonstrated as a great effective factor on semen traits (D'Alessandro and Martemucci, 2003, Chella et al., 2017), B-mode and Doppler ultrasonographic parameters (Hedia et al., 2019, Ntemka et al., 2021).

Moreover, the examined tissue or vessel during ultrasonographic examination could contribute to differentiated results. Almost all researchers investigated the echotextural parameters on testicular parenchyma, except for Ahmadi et al. (2012) and Camela et al. (2017), who performed the B-mode ultrasonographic examination on both testis and epididymis or on accessory sex glands, respectively. Concerning Doppler technique, it was mainly applied on testicular artery, and in some studies on testicular blood perfusion. However, Camela et al. (2017) performed this examination on internal iliac artery.

Furthermore, the approach of separate testes' evaluation can affect the survey process. B-mode indices were usually presented as an average of left and right testes measurements, apart from Ntemka et al. (2018), who recorded the indices separately for each testis, due to statistically significant differences between them. Regarding the Doppler indices, Batissaco et al. (2013) also evaluated the parameters separately for each testis. Statistically significant differences between the testes can be related to testicular asymmetry. Within the scrotum, the one testis may be located in an inferior point, compared to the other one. This fact may be attributed to the different anatomy of the vascular system. This size and position difference between testes has also been indicated in stallion (Davies Morel, 2008). Moreover, contralateral diseases of testes, such as varicocele, inflammation, abscess, can negatively affect seminal traits (Basioura et al., 2022).

The manner of semen collection also varies among the surveys. Even though artificial vagina was mainly used, electroejaculation was observed in many studies (El-Kon et al., 2004, Ahmadi et al., 2012, Batissaco et al., 2013, Dos Santos Ribiero et al., 2020, Yotov et al., 2020, Abdelkhalek et al., 2022). Electroejaculation is a stressful process which definitely affects semen quality characteristics and cryopreservation resistance (Jiménez-Rabadán et al., 2012). Besides, it has been indicated that semen collection method has also an impact on testicular blood flow (Rodriguez et al., 2023).

Concerning semen assessment assays, the majority of researchers evaluated just a few conventional semen traits. Only a small number of surveys (Carvajal-Serna et al., 2022, Montes-Garrido et al., 2022, Ntemka et al., 2018) included further parameters, which were objectively assessed. It is well known that the objectiveness and accuracy of the results become higher when the number of examined traits is greater (Amman and Hammerstedt, 1993).

Some further key factors are the ultrasonographic device, the type and frequency of probe and the settings on the device. All investigators used a linear probe, except for Batissaco et al. (2013) who used a convex one. The linear transducer presents a rectangular view field. The advantage of this probe is the possibility of depicting large structures in one view. On the other hand, the convex transducer presents a pie-shaped view field, imaging a narrowest section of a structure (Ginther and Utt, 2004). Frequency of transducers was variable enough, too. In addition, settings of the ultrasound machine for both B-mode (focus, gain, brightness, contrast etc.) and Doppler examination (gate, main gain, angle between the Doppler beam and the long axis of testicular artery, filter, velocity range etc.) differ among the studies or are not even mentioned. Gain is one of the most important settings, as it can reduce the noise, which is often high-frequency signals. Also, artifacts such as aliasing can be avoided with the application of the appropriate settings (e.g., by increasing the velocity scale).

Additionally, the general methodology of ultrasonographic examination and images capturing differs among the studies. In most surveys, males were in a standing position, hairs on testes were razored and gel was applied on the transducer and/or on the organ surface, in order to obtain images with optimal resolution. Concerning the methodology of B-mode technique, Dos Santos Ribiero et al. (2020) mentioned that the probe was placed longitudinally, while Carvajal-Serna et al. (2022) and Montes-Garrido et al. (2022) placed it transversely. Ntemka et al. (2018) scanned testicular parenchyma in both longitudinal and transverse sections. Besides, in most studies there are not any detailed descriptions. Therefore, the standardization of B-mode examination and image capturing method is crucial. On the other hand, regarding Doppler methodology, almost all researchers mentioned thoroughly that the probe was placed to locate accurately the testicular artery, in the caudal pole of testis, in the region of pampiniform plexus.

B-mode images were analyzed by different software, thus the measured echotextural parameters vary and the process of image analysis may differ. The most commonly used software is Image ProPlus, which measures mean numerical pixel values and pixel heterogeneity (Ahmadi et al., 2012, Camela et al., 2017, Camela et al., 2019, Yotov et al., 2020). ImageJ software was used only by Urt et al. (2018) measuring the average pixels intensity. Echovet v.2 was used by Ntemka et al. (2018) to measure numerous indices, while Ecotext software was also used by two researchers to estimate several echotexture parameters (Carvajal-Serna et al., 2022, Montes-Garrido et al., 2022). In fact, all software is standardized, even though quite a few methods for image texture analysis have been developed so far. Since image texture analysis can concern texture statistics, fractals features, Fourier power spectrum, texture spectrum etc., considerably different parameters can be measured (Nailon, 2010).

Finally, regarding Doppler ultrasonography method, most researchers performed pulsed wave Doppler, whereas some of them performed the spectral Doppler technique. Among Doppler indices, PI and RI were measured in the majority of the studies. The values of blood flow indices were determined by the software incorporated into the ultrasound machine, while the examination was performed. Besides, just a few surveys subjectively estimated the vascular perfusion of testicular parenchyma and pampiniform plexus.

FUTURE PERSPECTIVES

Nowadays, we are going through the era of artificial intelligence (AI) in every aspect of life. During the past few years, machine learning and AI were focused on enhancing dairy cows' performance, via genetic data and monitoring cows' health status, feed intake and milk yield. Afterwards, cow reproduction was the principal objective, aiming at detecting the estrus, predicting the probability of conception after insemination, predicting the onset of calving, etc. (De Vries et al., 2023). In addition, the use of AI on B-mode ultrasonography has been reported by a survey (Themistokleous et al., 2022), in which it was demonstrated that echotextural images of cow udder can predict the milk yield and production stage of a dairy cow. Regarding male, the interpretation of bioimage data of boar semen morphology using AI, has been studied (Kerns et al., 2022).

Henceforth, the application of AI will be subject of research on male reproduction, contributing to the rapid and accurate diagnosis of infertility or subfertility. Therefore, the wider application of B-mode and Doppler ultrasonographic examination of male genital system, as a part of breeding soundness evaluation, is essential. The methodology of ultrasonographic examination must be standardized, whereas the incorporation of echotexture and blood flow evaluation software into the ultrasound machines would automate the indices measurement and ensure the accuracy and objectiveness of the process. Moreover, a future aim should be the investigation of normal echotexture and blood flow of a high number of rams of different breeds in different geographical and meteorological conditions. Besides, the determination of the abnormal conditions of rams' genital system through ultrasonographic examination and its evaluation after a probable rehabilitation would also be valuable. Consequently, if a database would be created based on genetic data, clinical examination of genital system, laboratory semen analyses and B-mode and Doppler ultrasonographic examination, male fertility would be ensured.

CONCLUSIONS

Male fertility plays a vital role on small ruminants' herd profitability. Semen analysis is the most objective and irreplaceable method for evaluating male fertility. According to numerous studies, semen traits are correlated with B-mode as well as Doppler ultrasonographic indices. Although studies' results vary, since the factors that affect the ram and buck are several, B-mode and Doppler ultrasonographic examination could definitely assist small ruminants' fertilizing ability evaluation. Future research should focus on improving ultrasonographic techniques in order to be widely applied in the prevention and early diagnosis of ram and buck infertility and other pathological conditions.

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