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Ultrasonographic Imaging in Small Ruminant Reproduction

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Contents

Ultrasonography is a simple, reliable and non-invasive imaging technique without secondary effects. Application of ultrasonography in veterinary practice, and specifically in small ruminants, has developed to become the most efficient diagnostic tool for managing reproduction. The objectives of current revision are to offer an overview, after a short reminder of equipment and techniques for imaging genitalia in small ruminants, of the uses and utility of ultrasonography for the evaluation of physiological and pathological conditions in males and females and for the application of assisted reproductive technologies.

Introduction

First use of ultrasonography in small ruminants was described in 1983, for pregnancy diagnosis both in sheep and goats, by Tainturier et al. (1983a,b). Ultrasonographic evaluation of the male genital system in small ruminants was first reported by Buckrell (1988); however, unlike in the bovine, application of ultrasounds in small ruminants was associated with the female and not extended to the male. Currently, around 25 years later, ultrasonography is no longer an elite technique for few selected breeders but a widely recognized and used key-tool in reproductive management and research.

Ultrasonography is a simple, reliable, non-invasive imaging technique without side effects. Thus, applications of ultrasonography in veterinary practice has developed, from a limited use for pregnancy diagnosis and detection of some pathological conditions like hydrometra or metritis, to the most efficient diagnostic tool for checking the reproductive health of both males and females and for planning and performing the reproductive management of the flock. Moreover, in the last years, the use and the yields of assisted reproduction techniques have found a substantial benefit in the implementation of high-resolution transrectal ultrasonographic techniques. First, in research, for the study and determination of the limiting factors and for the adjustment of the protocols in artificial insemination and embryo production and transfer. Second, in practice, for the election and management of both donors and recipients. An additional advantage is that in all the aspects related to management and improvement of reproduction, ultrasonography permits a close relationship between research and practice, as findings in research can be directly translated to the field. On the other hand, the most restrictive factors of ultrasonography are that its efficiency is always dependent on the expertise of the operator, and that the learning-time is slow. Alleviation of these issues, being an image-based technique, can be only obtained by training, helped by reading of specific guides, like the very recent one by Viñoles et al. (2009).

Obviously, the use of ultrasonography in small ruminant reproduction has generated a lot of information by now. The objective of this review is not a detailed analysis of such previous literature, but to offer an overview of the use and usefulness of ultrasonography in the assessment of reproduction in small ruminants. Thus, we first provide a brief overview of equipment and techniques for imaging the genitalia of small ruminants, followed by an overview of the utility of ultrasonography for the evaluation of physiological and pathological conditions in males and females and in the application of assisted reproductive technologies (ART).

Equipment for Imaging the Reproductive Tract in Small Ruminants

The first election to be done is the type of ultrasonographic equipment; which is crucial, both from the technical and the economical point-of-view. Main questions are the expected use of the instrument (clinical practice or research) and the need for mobility (non-portable, portable or ultraportable equipment). Non-portable equipment have a top-quality image and are very useful in research centres and veterinary hospitals for detailed examinations using different probes (transducers), frequencies and software; however, it is not possible to transport them and they are very expensive. Portable instruments are easy to carry, weighing from 6 to 20 kg, and may be powered either by batteries or main electric line; hence, they emerge as an economically advantageous option for ambulatory work. Most of the portable equipment in the market offer a high quality image and different probes and software, so they can also be used in research and hospitals with adequate results. Ultraportable instruments are the choice for ambulatory activity since they are extremely light (<3 kg), are powered by batteries and have a good quality of image; on the other hand, the selection of probes is limited and the software is minimal.

The second choice is the probe. Ultrasonographic probes may be classified by mode of emission-reception, arrangement of the piezoelectric crystals and frequency. The first probes functioned in pulsatile mode (alternating emission of ultrasound waves and reception of echoes by the same crystal); current probes use continuous mode (some crystals are emitting at the same time that others are receiving), so the quality of the image is enhanced. Probes may be classified according to the arrangement of the crystals as linear array, sector-array or convex array probes. Sector-array transducers are currently being replaced by convex phase-array transducers. Linear transducers are rectangular and crystals are arranged in a line (up to 500 elements spaced over 75–120 mm), whilst convex probes are shaped in a curve. Linear probes are cheaper and the created image is easier to visualize; convex transducers allow the scanning of a larger area with a smaller array, so they require less surface contact. The frequencies used for diagnostic imaging can be in the range of 1–18 MHz, but the usual is 3.5–12 MHz. Higher frequencies have a correspondingly smaller wavelength, so the quality of the image is enhanced; however, the attenuation of the wave is higher, so the tissue-penetration is diminished. In reproductive ultrasonography of small ruminants, probes of 3.5–5 MHz are commonly used for transabdominal imaging and probes of 7.5–10 MHz are normally used for transcutaneous and transrectal scanning.

Irrespective of which scanning technique is applied, the result is shown on the screen of the console as an image in grey-scale, varying from white to black. The different echotextures of the evaluated tissues have specific denominations depending on tissue characteristics (echogenicity). Thus, tissues or substances such as urine, follicular fluid or other fluids that permit sound waves to penetrate them do not produce echoes. These tissues or substances are called anechoic or anechogenic and their images appear black on the screen. At the opposite end of the spectrum, tissues such as bone or the uterine cervix intensely reflect ultrasound waves, thereby producing an intense echo; they are called hyperechoic or hyperechogenic and their images appear white on the screen. Tissues, such as ovary or uterine stroma, that reflect intermediate proportions of ultrasound waves are called echoic or echogenic and their images appear in different shades of grey on the screen (Pierson et al. 1988).

Ultrasonographic Imaging in the Reproductive Management of Ewes and Does

Main objectives of ultrasonographic imaging in the female of small ruminants, like in other species, are the visualization of ovaries and uterine horns in animals that have not been bred to determine whether or not there are pathological conditions of the tract, whether or not animals are cyclic and, if they are, at what stage of the cycle they are. In animals that have been bred, the main objective is to determine the pregnancy status (including the evaluation of viability, number, age and sex of conceptuses). The technique of scanning depends on the objective aimed.

Techniques for imaging the reproductive tract

The objective of the ultrasonographic evaluation will determine the scanning technique to be applied (transrectal or transabdominal). Transabdominal ultrasonography is easier to perform but a detailed study of the genital system requires the use of high-resolution probes used transrectaly. Therefore, the transrectal technique is the method of choice for ultrasound examination of non-pregnant and early-pregnant females. Transabdominal ultrasonography can be used for diagnosing pregnancy from Day 25 to 30 of pregnancy onwards, and its use is obligatory from Day 60 as 7.5 MHz transrectal probes do not have enough penetration to permit seeing the entire uterus.

Transrectal observations may be conducted with the female standing or restrained in dorsal recumbency either manually or in a cradle; a very practical alternative is scanning the animals in the milking parlour. Faeces can be removed from the rectum for improving the image and a hydrosoluble gel needs to be introduced into the rectum for avoiding damage of the mucosa and improving the transmission of the sound waves. Thereafter, the probe is placed into the rectum, orientating the transducer perpendicularly to the ventral abdominal wall. The urinary bladder is located first; it is easily visualized as an anechogenic structure (Fig. 1). The body of the uterus will be located dorsal to the bladder, and each uterine horn will be found on its respective side. The position of the uterus varies according to the amount of fluid in the bladder, size of the uterus and the age and number of parities of the female. When the bladder is surpassed, the probe has to be rotated laterally clockwise and counter-clockwise to observe the uterine horns entirely and both ovaries. The usual images of the uterine horns are circular and longitudinal shapes, caused by the different cross-sections generated at scanning.

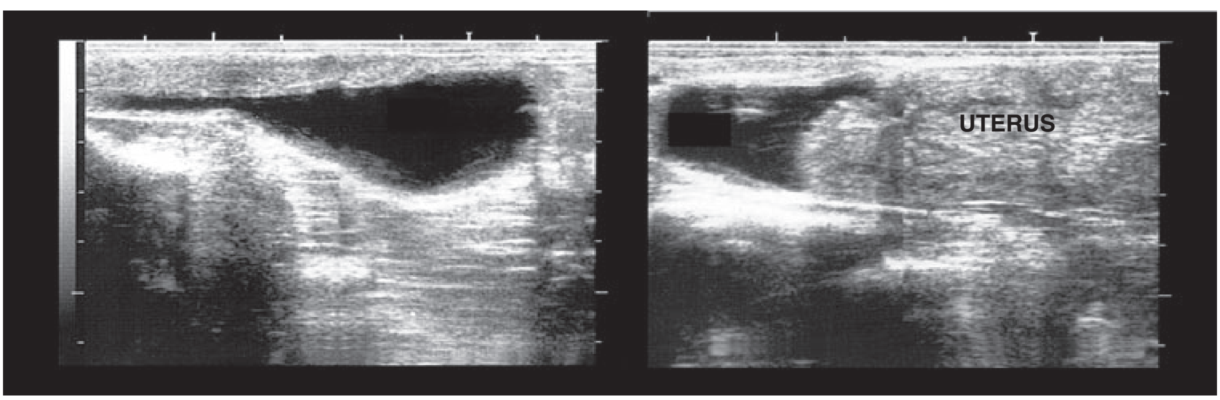

Fig. 1. Ultrasonographic images of the urinary bladder (left image) and of the uterus cranially to the bladder (right image)

Although transabdominal ultrasonography is usually performed with the animal in a standing position, it can also be performed in a sitting position. The transducer should be placed in the right inguinal region of the female because the rumen displaces the pregnant uterus to the right. To eliminate the presence of air and promote contact between the transducer and skin, the wool or hair of the inguinal area may be clipped in some breeds and the probe may be abundantly covered with contact gel.

Evaluation of physiological conditions in non-pregnant females

During transrectal ultrasonography, the first structures to be evaluated are the body of the uterus and the uterine horns. The non-gravid, fully involuted, uterus is a muscular structure and generates an echogenic image which echogenicity depends on the uterine tone and luminal contents. Therefore, echogenicity differs during the luteal and follicular stages of the oestrous cycle.

The ovaries appear elliptic, with a hyperechoic outline, of 10×15 mm in diameter depending on the reproductive stage. Identification of ovarian structures depends on the expertise and experience of technicians. The difference in accuracy between operators may vary up to a 20%, especially for corpora lutea detection (Dickie et al. 1999; Simoes et al. 2005).

In anoestrous females, the ovary is small and contains follicles between one and five millimetres (Fig. 2). Owing to the fluid in the antrum, follicles are identified as black structures with a smooth spherical outline. The diameter of a follicle is measured by freezing the image and placing the electronic callipers of the ultrasound machine in the borderline between the follicular wall and the ovarian stroma. In cycling females, preovulatory follicles may reach 6–7 mm in sheep (Bartlewsky et al. 1999) and 8–9 mm in goats (Cueto et al. 2006; Vázquez et al. 2010). The accuracy of detecting follicles has been measured in sheep; being always higher than 90% for follicles larger than 4 mm, but varying between authors (93.2%, Schrick et al. 1993; 93; % for follicles with 4–5 mm in size and 100% for follicles larger than 6 mm, Gonzalez-Bulnes et al. 1994; 90–95%, Viñoles et al. 2004). Errors in counting large follicles are mainly caused by underestimation but, surprisingly, errors in counting smaller follicles are caused by overestimation. Viñoles et al. (2004) hypothesized that possible causes of overestimation may be more related to errors in the post-mortem examination to validate the ultrasonographic findings. First, the scale interval of the calliper used to measure the follicles during post-mortem examination may have increased the inaccuracy of measuring small follicles and, second, small follicles identified ultrasonographically may have collapsed because of changes in the elasticity of ovarian tissue after death, rendering them invisible during post-mortem examination.

Immediately after ovulation, corpora haemorrhagica are anechoic as they are mainly composed of blood that filled the antrum; later, as luteinization process advances, corpora lutea appear as grey structures. The presence of a central cavity showing an irregular shape surrounded by a hypoechogenic pattern is observed in approximately one-third of the animals (Gonzalez-Bulnes et al. 2000). Presence of cavities is more abundant during the early luteal phase (80% of the animals in goats treated with progestagen and gonadotrophins; Gonzalez-Bulnes et al. 1999), as cavity tends to disappear later. The cavity is usually hypoechoic, but occasionally exhibits a slightly increased and diffuse pattern or the presence of echogenic lines; the former are the images of accumulations of haemolysed blood, whereas the latter are fibrin-like strands (Pierson and Ginther 1987). It is, thus, necessary to distinguish between cavitated corpora lutea and luteinized follicles and luteinized cysts; evaluation of the ratio between cavity diameter and total luteal-tissue diameter is useful, as differed between the two (0.3 vs 0.6, Gonzalez-Bulnes et al. 1999).

Observation of corpora lutea is useful for determining ovulation rate, with a mean of 80–90% of accuracy through the oestrous cycle (Gonzalez-Bulnes et al. 1994; Viñoles et al. 2004; Simoes et al. 2005). Errors in detection are caused by underestimation, by difficulties in discerning the limits between adjacent corpora lutea at the beginning and end of the luteal phase and by presence of central cavities at early luteal phase. In fact, efficiency of detection without prior scanning is at its

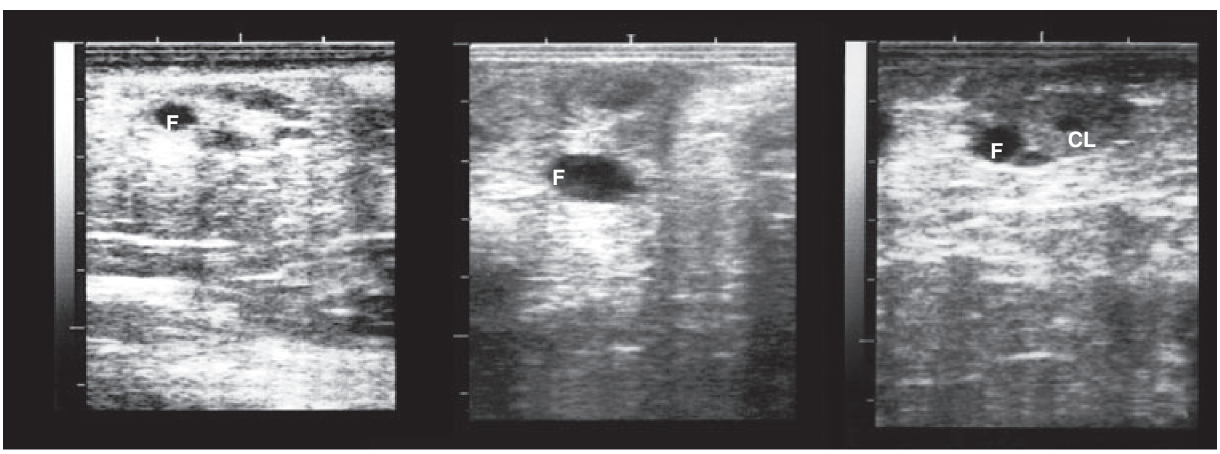

Fig. 2. Ultrasonographic images of sheep ovaries showing small follicles during anoestrous (F; left picture) and preovulatory follicles (F; middle image) and corpora lutea (CL; right image) in cycling females

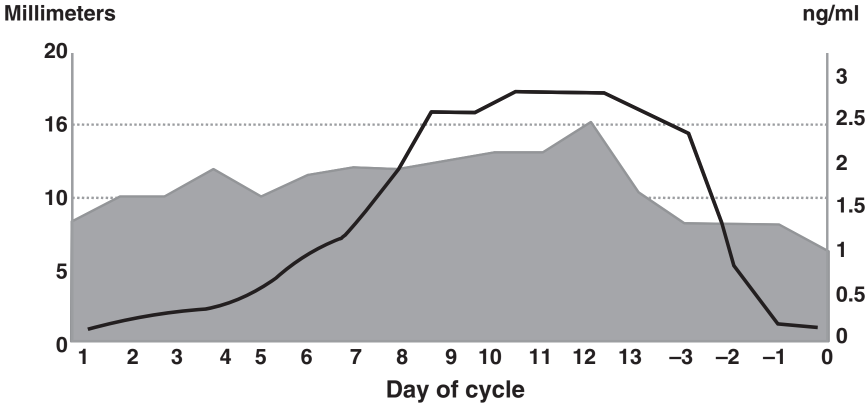

Fig. 3. Mean cross-section of corpus luteum area in mm^2 (shadowed area) and mean plasma progesterone concentration in ng/ml (continuous line) on each day of oestrous cycle of monovular sheep (adapted from Gonzalez-Bulnes et al. 2000)

lowest on the day after ovulation (50%), increases to 62% at Day 4 and to 100% from Day 5 (Gonzalez-Bulnes et al. 1994, 1999). Thus, the data of choice for measuring ovulation rate is around Day 10 of the cycle.

Observation of corpora lutea is also useful for estimating their activity. Both the ultrasonographic appearance and size vary from ovulation to luteolysis; the size being correlated with day of cycle and plasma progesterone levels (Fig. 3; Gonzalez-Bulnes et al. 2000).

The appearance of the uterus, and mainly of the uterine lumen, is also affected by stage of oestrous cycle (Fig. 4; Gonzalez-Bulnes et al. 1996). As a general rule, during the luteal phase, the limits of the different sections of the uterine horns are well defined and easy to differentiate whilst the uterine lumen contains very little fluid. Conversely, during the follicular phase, the uterus becomes swollen and oedematous because of the effect of the oestradiol on the endometrium. Accordingly, the ultrasonographic appearance is poorly defined and predominantly hypoechogenic.

Evaluation of physiological conditions in pregnant females

Buckrell et al. (1986) implemented transrectal ultrasonography for pregnancy diagnosis; thereafter, Schrick and Inskeep (1993) adapted high-frequency probes These techniques and equipment allow determination of pregnant status by visualization of embryo vesicles as fluid-filled dilatations in the uterine lumen from Day 12 of gestation. Visualization of the conceptus (concept that includes the embryo or the foetus as well as the extraembryonic membranes) may be performed from Day 16 in goats and from Day 19 in sheep (Santiago-Moreno et al. 1995a,b; Gonzalez-Bulnes et al. 1998; Fig. 5). The accuracy at these early stages is, however, low. First, because of false negative diagnoses when the trophoblastic vesicle or the embryo are not detected; second, because of false positive diagnoses caused by intrauterine accumulation of fluids because of causes other than pregnancy (Buckrell et al. 1986) or by confusion of embryo vesicles with intestinal loops, blood vessels or pathological conditions. Moreover, the incidence of embryo losses in early

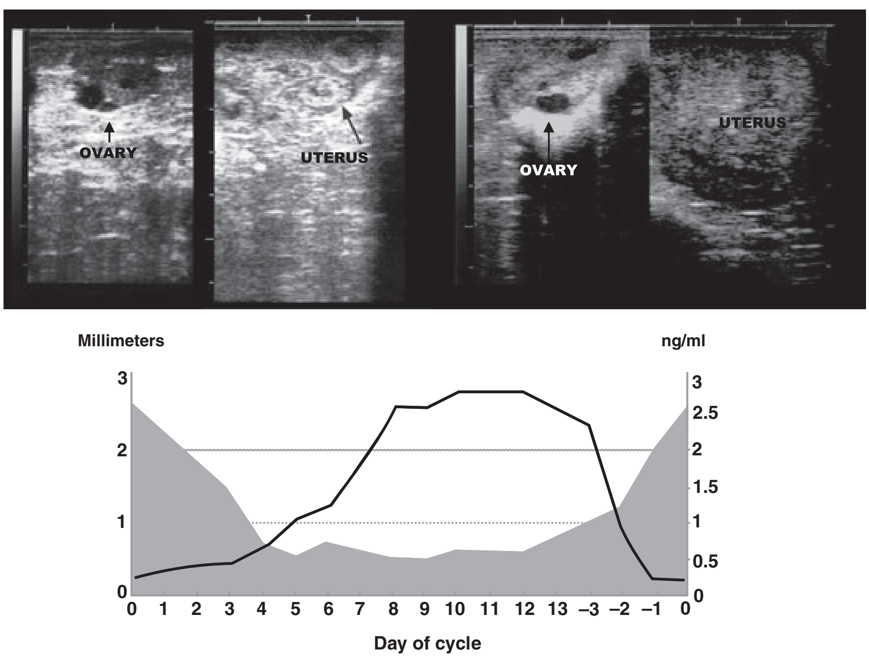

Fig. 4. Ultrasonographic images of the ovary and uterus during the luteal (left image) and the follicular phase (right image) of the oestrous cycle. The lower picture describe changes in mean cross-sectional of uterine lumen in mm^2 (shadowed area) and mean plasma progesterone concentration in ng/ml (continuous line) during the oestrous cycle of sheep

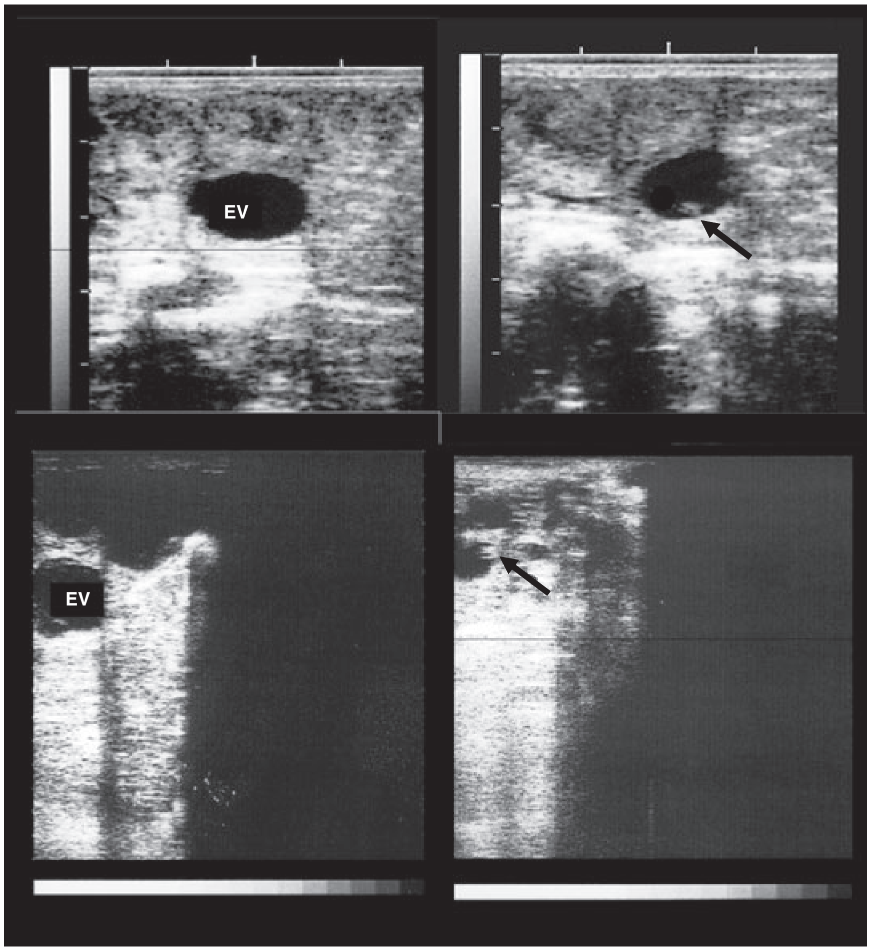

Fig. 5. Pregnancy diagnosis by transrectal (upper images) and transabdominal ultrasonography (lower images), based on visualization either of the embryonic vesicle (EV in left images) or of the conceptuses (arrows in right images)

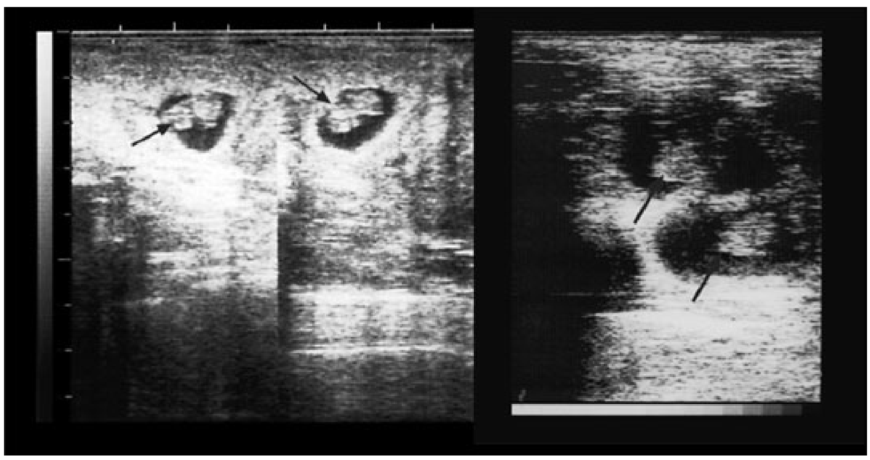

Fig. 6. Determination of twins by transrectal (left image) and transabdominal ultrasonography (right image)

pregnancies is high, increasing the number of false positive diagnoses. Thus, the pregnancy diagnosis should be carried out from Day 24 (Martinez et al. 1998); in practice, it is better to wait for Days 32–34, when efficiency reaches 85–100%, depending on operator experience.

At the same time, the optimal period for determining single and multiple pregnancies by transrectal ultrasounds starts around Day 27–30 of gestation (Fig. 6), as the effectiveness for identifying single embryos reaches 100%, accuracy is 85% for twins and 70% for triplets (Santiago-Moreno et al. 1995a). Determination of number of conceptuses by transrectal ultrasonography should be performed prior to Days 50–55; later, the large size of the conceptuses impedes a correct visualization of the entire uterus.

In the field, transabdominal ultrasonography is preferred because of the combination of speed, accuracy for evaluation of pregnancy and number of conceptuses and easier management of animals (Levy et al. 1990),. Using this technique, gestation can be diagnosed from 24 to 25 days of pregnancy by visualization of embryo vesicle (Mialot et al. 1991a; Picazo et al. 1991). Embryos may be observed from Day 28 (Picazo et al. 1991); however, it is recommended to delay the examination until Day 35. In practice, pregnancy diagnosis is recommended to be performed after days 40–55 as efficiency in counting the number of conceptuses in multiple pregnancies reaches 100% (Dawson et al. 1994).

We recommend, whichever the technique used, always to check viability of embryos at the same time that pregnancy diagnoses and number of conceptuses are evaluated. Mistakes caused by embryo losses can be limited if the presence of heart beat is checked.

Assessment of embryo/foetal development is also a key tool in reproductive management. When the exact date of mating is unknown, monitoring conceptus development allows estimation of the gestational age and planning of parturition, colostrum feeding and early postnatal care (Martin et al. 2004). However, when the date of mating is known, it could be useful to assess the normality or abnormalities in foetal growth. Estimation of the gestational age is more accurate during early pregnancy as the values obtained in later gestation are more representative of the individual characteristics of the foetus, and mainly determined by genetic (Stephenson and Lambourne 1960) or nutritional factors (Richardson 1976).

The first measurements to be recorded (Gonzalez-Bulnes et al. 1998) are the internal transverse diameter of the embryonic vesicle (from Day 12 of pregnancy), the crown-rump length and the trunk diameter of the embryo (from Days 19 and 23 of gestation, respectively).

At foetal stage (from Days 32 to 35 of pregnancy), occipito-nasal length, biparietal diameter, as well as the diameter of the trunk at the level of the last rib and the stomach can be determined (Haibel and Perkins 1989; Kelly and Newnham 1989; Sergeev et al. 1990; Picazo et al. 1991; Gonzalez-Bulnes et al. 1998). All these measurements have a high correlation with foetal age, but the head can be measured over a long period, from 36 to 38 days to almost delivery, when combining transrectal and transabdominal scanning. The skull usually remains in a good position for observation; occipito-snout length and biparietal diameter are easy to measure as it is easy to place the callipers on the hiperechogenic limits of the bones bordering the soft tissues (Fig. 7).

Other measurements – such as the width of the vertebrae and ribs, the longitudinal diameters of the stomach, kidney and urinary bladder, and the length of the femur – have lower correlations with age.

Finally, ultrasonography is increasingly used for sexing the foetuses, by identifying the genital tubercle. Foetuses are identified as males when the genital tubercle is found next to the umbilical cord and female when the genital tubercle is found next to the tail. Studies performed by transabdominal ultrasonography on ewes between 60 and 69 days of pregnancy show that accuracy for detecting males is 100%, but diminish to 76% for females (Coubrough and Castell 1998). Studies by transrectal ultrasonography have reported an earlier mean time of sexing, and earlier for sheep than for goats (41 vs 47 days), with no difference between sexes (Azevedo et al. 2009). Accuracy of sexing decreases as the number of foetuses increase, from 100% for singletons to 92.8% for twins and 62.5% for triplets (Santos et al. 2007).

Determination of pathological conditions in females

Ultrasonography is especially useful for the diagnoses of pathological conditions in either pregnant or non-pregnant females, with that may compromise fertility and reproductive health. In animals that failed to conceive, the main causes of infertility are follicular and luteal cysts, at ovarian level, and hydrometra and metritis, at uterine level.

Evidences and description of ovarian cysts in small ruminants, in contrast to the cow, are scarce. Possible causes may be related to differences in reproductive management of the flock and the limited duration and lower incidence of ovarian cysts in small ruminants

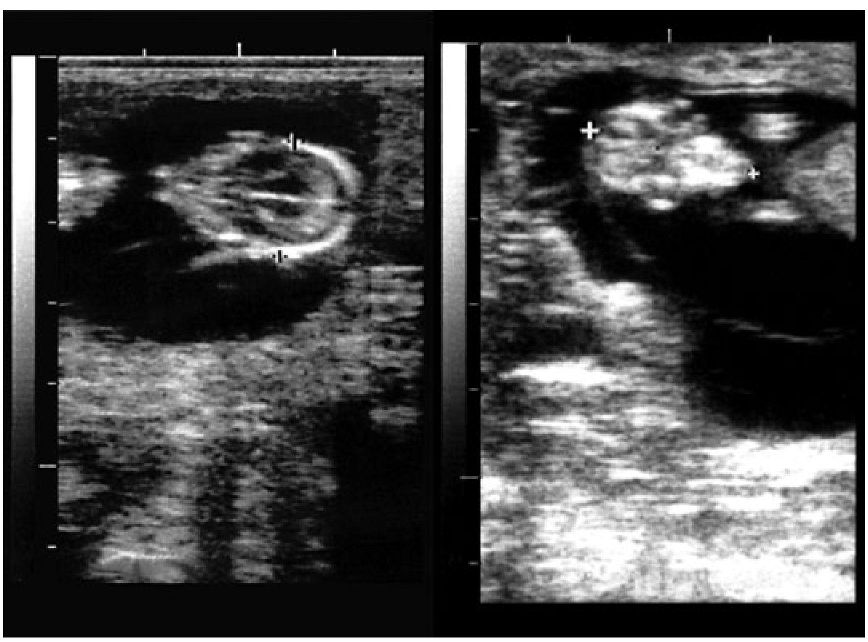

Fig. 7. Measurement of biparietal diameter (left image) and occipito-nasal length (right image)

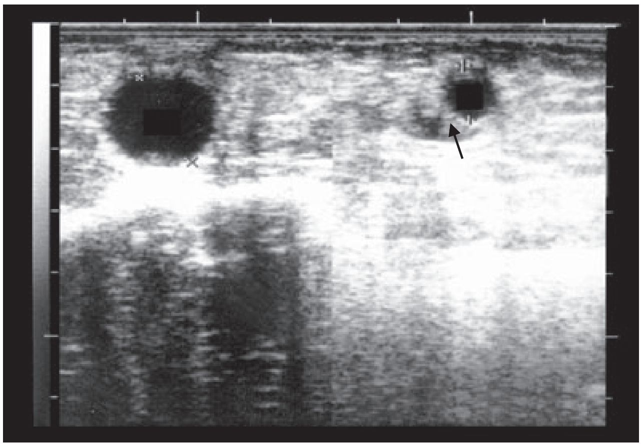

Fig. 8. Ultrasonographic image of follicular cysts (left image) and luteal cysts (right image) with luteinization (arrow)

(5–8%). Ultrasonographically, follicular cysts appear the same as follicles but with a diameter of 10 mm, although they may become as large as 30 mm, and with little or no visible luteinization (Fig. 8); luteal cysts are fluid-filled ovarian structures with a diameter of 10 mm or greater and with a thick luteal wall which may be up to 3 mm wide, indicating luteinization. Follicular cysts may be differentiated from paraovarian cysts; the later are anechoic spherical structures lying close to but outside of the ovary and has no negative effects on fertility.

At uterine level, hydrometra is possibly the most common uterine pathology affecting fertility. Hydrometra is characterized by the accumulation of non-septic fluids in the uterine lumen concurrently with a persistent corpus luteum (Pieterse and Taverne 1986). Hydrometra is also called pseudopregnancy because the abdomen distendes and resembles advanced pregnancy. Fluid accumulation in uterus is responsible for the abdominal enlargement and large volumes up to 7 l may accumulate (Mialot et al. 1991b) It is regarded as an important cause of anoestrous (Taverne et al. 1988), being more common in goats than in sheep. The estimated incidence varies between 3% and 25% in goats (Mialot et al. 1991b; Hesselink 1993), but it is lower in sheep (3%, Bretzlaff 1993). Hydrometra occurs more frequently in high-producing animals and the incidence increases with age.

Ultrasonographic findings are related to volumes of fluid accumulated in the uterus; thus, the image corresponds with anechogenic areas, with different degrees of extension depending on the amount of fluids. The anechoic areas may traversed by hyperechogenic lines representing the thinly stretched uterine walls (Fig. 9). Sometimes, it is possible to observe hyperechogenic dots moving in the uterine fluid ('snow-storm'); these dots originate from desquamated endometrial cells. One or more corpora lutea may be identified in cases with hydrometra. Hydrometra should be differentiated from pregnancy, in the former there will neither be an embryo or a foetus nor placentomes.

The ultrasonographic image of the uterine content in a metritis, which allows a differential diagnosis, may be classified as anechoic, hypo-normoechoic and hyperechoic. Initially, metritis is characterized by a little anechoic uterine content and an increase in the thickness of the uterine wall (Fig. 10); with endometritis, the myometrium is hypoechogenic because of presence of oedema. The presence of an abundant quantity of anechoic fluid with areas of diffuse hyperechogenicity suggests pyometra. Observation of an ultrasonographic image characterized by a hypoechogenic-normoechoic uterine content, more or less homogeneous, is the most frequent ('dirty uterus'); this process is usually consequence of embryo mortality or placental retention. Finally, metritis with hyperechoic uterine content correspond to chronic processes caused either by evolution of a pyometra or by foetal death and mummification. In the latter, it is possible to observe the foetal bones as very hyperechogenic structures inside the hyperechogenic content of the uterus. In foetal maceration, it is possible to observe decreases of the echogenicity in areas of the foetal body.

Fig. 9. Ultrasonographic image of the anechoic content of the uterus in a case of hydrometra; the hyperechogenic dots inside the uterine fluid ('snow-storm') represent endometrial desquamation

Ultrasonographic Imaging in the Reproductive Management of Rams and Bucks

Main objective of ultrasonographic imaging in the male of small ruminants, like in other species, is the evaluation of external (mainly testis and epididymedes) and internal genitalia (accessory glands) for determining presence or absence of pathological conditions that may compromise the fertility of the animal. In practice, ultrasonography complements clinical work ultrasound and it is used to confirm the diagnosis, as clinical examination of male animals always precedes ultrasonography and remains as the primary avenue of evaluation. The technique of ultrasonographic scanning, again, depends on the objective aimed.

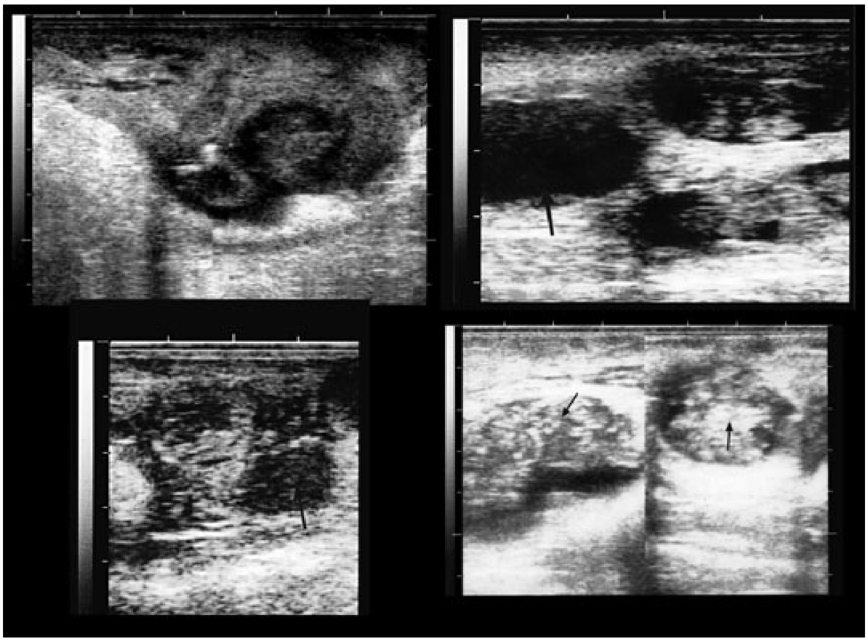

Fig. 10. Ultrasonographic image of early metritis with little anechoic contentin the lumen and an increase in thickness of the uterine wall (upper left image); diffuse hyperechogenic areas indicative of pyometra (upper right image); hypoechogenic normoechoic, quite homogeneous, uterine content is the most commonly resulting from embryo mortality or placental retention (lower left image); metritis with hyperechoic content corresponding to a chronic process either caused by a progression to pyometra or by foetal death and mummification (lower right image)

Techniques for imaging the reproductive tract

Imaging of both external and internal genitalia in rams and bucks should be easier to perform with the male sedated depending on his temperament. Thereafter, scanning may be performed in standing position or after restraining in dorsal recumbency on a metallic cradle or on the floor.

External genitalia are examined by transcutaneous, trans-scrotal, ultrasonography. For trans-scrotal imaging, the transducer may be firmly pressed against the skin over the area to be explored. Previously, to eliminate the presence of air and to promote the contact between the transducer and the skin, the area of the scrotum to be studied may be clipped in certain breeds –

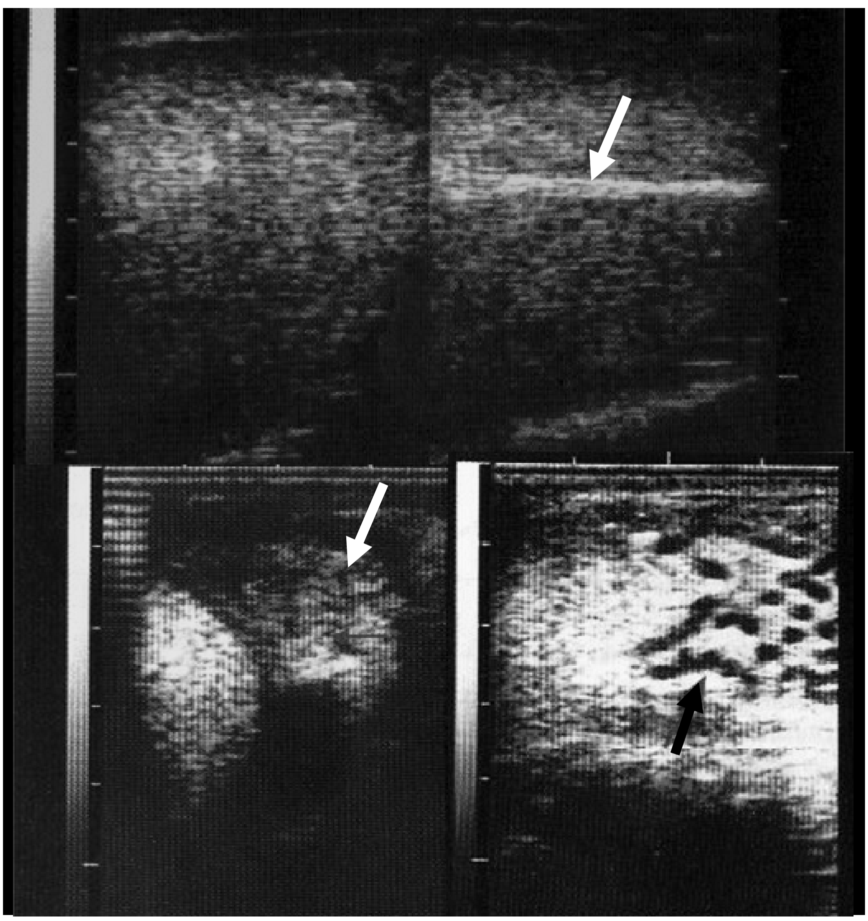

Fig. 11. Ultrasonographic images of longitudinal view of a testis (upper left image) longitudinal view of the mediastinum (upper right image), head of the epididymus (lower left image) and plexus pampiniformis (lower right image)

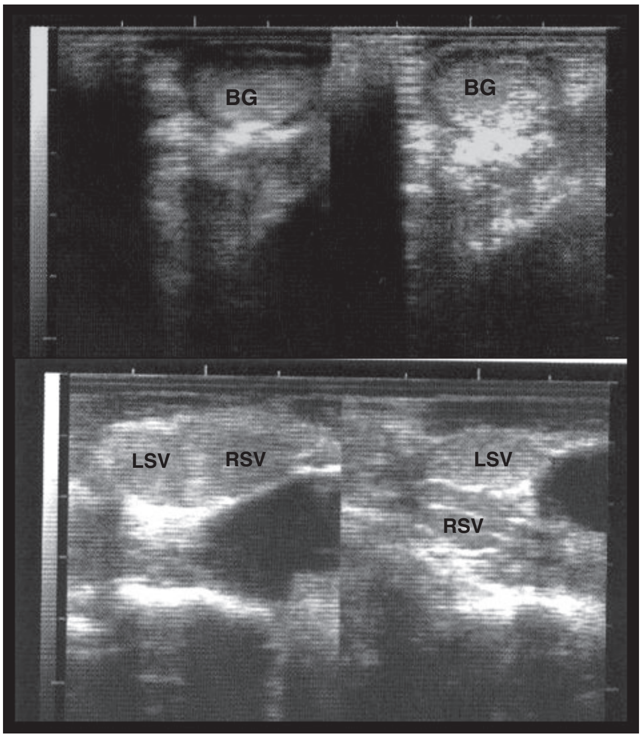

Fig. 12. Ultrasonographic images of the bulbourethral (BG in upper pictures), and transversal (lower left image) and longitudinal shapes (lower right image) of left and right vesicular glands (LVG and RVG, respectively)

taking care not to cause injury to the scrotum – before gently applying a hydrosoluble contact gel. Thereafter, sagittal, transverse and oblique images can be obtained through the scrotum, allowing the visualization of the testis, mediastinum of the testis, rete testis, epididymis and ductus deferens.

The internal genitalia, the accessory glands, are examined by transrectal ultrasonography. In a similar way to female's examination, thereafter, the probe is placed into the rectum, orientating the transducer in such a way that the ultrasound beam is directed perpendicular to the ventral abdominal wall. The first structures to be located are the urinary bladder and the urethra; thereafter, the probe may be slightly rotated laterally to observe internal glands. The bulbourethral glands are paired and located dorsal and lateral to the pelvic urethra, at the level of the ischiatic arch. The prostate only has the disseminate component and is found diffusely spreading along the wall of the pelvic urethra. The paired vesicular glands are visualized deeper, near the bladder neck, dorso-lateral to the bladder.

Evaluation of physiological and pathological conditions in males

The ultrasonographic evaluation of testis is easy to perform. First planes correspond to skin and the two tunica testis (albuginea and vaginalis); the three structures are visualized like echoic lines surrounding the testis, with anechoic spaces between them. The ultrasonographic image of the normal parenchyma of the testis is homogeneous (Fig. 11), with hypoechoic and normoechoic mixed patterns; the hypoechoic pattern corresponds to the seminiferous tubules, whilst the normoechoic or slightly hyperechoic pattern corresponds to the interstitial connective tissue. In the middle of the testis, the mediastinum is visualized as a hyperechoic structure.

The epididymis is visualized laterally to the testis. The head of the epididymis is a well-defined structure with a pattern isoechoic or slightly more echogenic than the testis, but more coarse-grained; conversely, the body and tail are difficult to identify because of its smaller size. Close to the head of the epididymis, the rete testis may be visualized as a formation composed of anechoic tubules and hyperechoic interstitial tissue. The ductus deferens is continuous to the tail of the epididymis and may be visualized like an anechoic tubular structure with hyperechoic walls.

Ultrasound can be used for identifying undescended testis, testicular torsion, inguinal hernia and other pathological conditions of the scrotum or its contents, with or without presence of mass, pain, swelling or trauma to the scrotal area. The possible findings are calcifications (scrotal and albuginea calcifications, epididymal calcifications, testicular tumours and chronic inflammatory reactions with calcification of testis), cystic appearance (intratesticular varicocoeles, hydrocele, epididymis cysts, albuginea cysts, haematoma, abscesses), solid nodular testicular lesions (infarcts, neoplasms) and diffuse lesions (orchitis, hyperplasia). However, the most common findings are acute and chronic orchitis and epididymitis. In acute orchitis, the peripheral extent of the testis cannot be discerned because of a general lack of echogenicity, the testis has a diffuse hypoechoic pattern, because of oedema and fluid accumulation in the tissue; in orchitis of infectious origin, it is habitual to find anechoic focus. In chronic orchitis, there is a generalized hyperechoic pattern as the inflammation of the testis is usually accompanied by fibrosis; when the evolution of the disease is testicular degeneration and multifocal calcification, the image is filled of hyperechoic spots. Epididymitis is usually linked to bacterial infection (mainly *Brucella ovis*). Ultrasonographic image coincides with inflammation of tissues; acute epididymitis, as a rule, are hypoechoic; chronic epididymitis are hyperechoic.

The ultrasonographic evaluation of the accessory glands in small ruminants is mainly focused in bulbourethral and vesicular glands because the prostate only has a disseminate component. The bulbourethral glands have ovoid shape and a slightly hyperechoic pattern, whilst vesicular glands have an isoechoic pattern with hyperechoic limits (Fig. 12).

The most common findings in the accessory glands of small ruminants are acute and chronic inflammations, usually associated to orchitis and epididymitis. The most common pattern of inflammations in both bulbourethral and vesicular glands is mixed and heterogeneous, with hyperechogenic and anechogenic foci indicating abscesses.

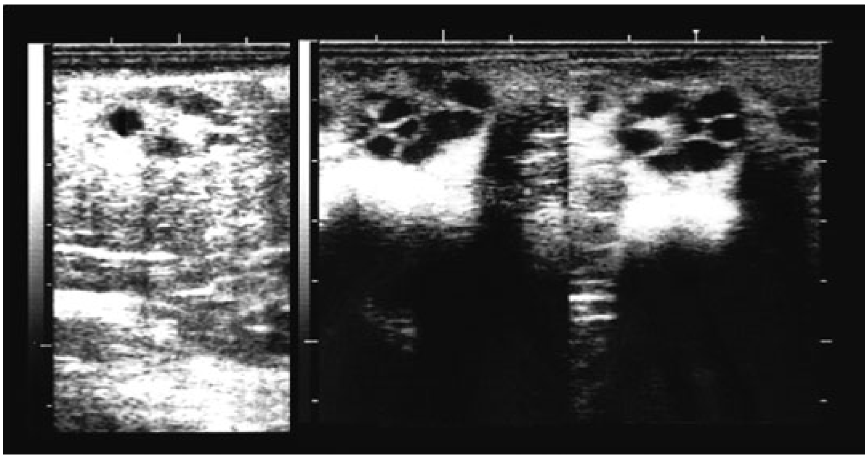

Fig. 13. Ultrasonographic image of the follicular population at 0 (left image) and 60 h (middle and right images) of a superovulatory gonadotrophin treatment

Application of Ultrasonography in ART in Small Ruminants

The ART mainly used by small ruminants by sheep and goat producers are oestrus synchronization and fixed-time artificial insemination; in addition, international commerce of embryos and genetic improvement are also based in superovulation and embryo production.

The first use of ultrasonography in ART was the assessment of fertility by means of pregnancy detection after treatments. Thereafter, the implementation of transrectal ultrasonography for screening of follicular dynamics (Schrick et al. 1993) represented a key step in the determination of the ovarian response to administration of hormones used in ART and the study of the factors modifying such response (revised by Gonzalez-Bulnes et al. 2004a).

Protocols for inducing and synchronizing oestrus and ovulation are commonly applied world-wide, both in sheep and goats, either based in male-effect or in pharmacological treatment using prostaglandins or progestagen-based protocols (Kusina et al. 2000); these practices may be combined with artificial insemination. In the different activities developed by different researchers, ultrasonography was primarily used for assessment of fertility throughout detection of pregnancies and, thereafter, it was apply for the evaluation of follicular characteristics, determining the efficiency of the treatment and(or) for the assessment of the onset of ovulation which defines the time of artificial insemination (Cardwell et al. 1998; Ungerfeld et al. 1999; Evans et al. 2001; Viñoles et al. 2001; Rubianes and Menchaca 2003; Gonzalez-Bulnes et al. 2004b, 2005, 2006; Ungerfeld et al. 2005; Kaulfuss et al. 2006; De Santiago-Miramontes et al. 2008; Fernandez-Moro et al. 2008). In practice, ultrasonography is a useful tool for clinical evaluation of selected males and for diagnosis of pregnancies and possible pathological conditions in the females.

However, the most significant contribution of ultrasonography to ART has been related to the field of superovulation, embryo production and transfer in both sheep and goats. The screening of follicular status at start the superovulatory gonadotrophin treatments, in terms of presence of large dominant follicles and size-distribution of the remaining follicles, and the changes during treatment (Fig. 13) has allowed researchers to identify the important role of ovarian factors on superovulatory and embryo protocols (Rubianes et al. 1997; Gonzalez-Bulnes et al. 2004a; Veiga-Lopez et al. 2005, 2008; Ammoun et al. 2006; Mossa et al. 2007) providing key information for the design and implementation of protocols enhancing embryo yields. In practice, ultrasonography is a useful tool for the clinical evaluation of both donor and recipient females prior to treatments, for the assessment of follicular status at the onset and during the execution of superovulatory programmes in donor females, and for the assessment of the quality of corpora lutea at embryo transfer and, later, pregnancy, in recipient females.

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

All the authors contributed equally to the review.

References

Ammoun I, Encinas T, Veiga-Lopez A, Ros JM, Contreras I, Gonzalez-Añover P, Cocero MJ, McNeilly AS, Gonzalez-Bulnes A, 2006: Effects of breed on kinetics of ovine FSH and ovarian response in superovulated sheep. Theriogenology **66,** 896–905.

Azevedo EM, dos Santos MH, Filho CR, Freitas Neto LM, Bezerra FQ, Neves JP, Lima PF, Oliveira MA, 2009: Migration time of the genital tubercle in caprine and ovine fetuses: comparison between breeds, sexes and species. Acta Vet Hung **57,** 147–154.

Bartlewsky PM, Beard AP, Cook SJ, Chandolia RK, Nonaramooz A, Rawlings NC, 1999: Ovarian antral follicular dynamics and their relationship with endocrine variables throughout the oestrous cycle in breeds of sheep differing in prolificacy. J Reprod Fertil **115,** 111–124.

Bretzlaff KN, 1993: Development of hydrometra in a ewe flock after ultrasonography for determination of pregnancy. J Am Vet Med Assoc **203,** 122–125.
Buckrell BC, 1988: Applications of ultrasonography in reproduction in sheep and goats. Theriogenology **29,** 71–84.
Buckrell BC, Bonnett BN, Johnson WH, 1986: The use of real-time ultrasound rectally for early pregnancy diagnosis in sheep. Theriogenology **25,** 665–673.
Cardwell BE, Fitch GQ, Geisert RD, 1998: Ultrasonic evaluation for the time of ovulation in ewes treated with norgestomet and norgestomet followed by pregnant mare's serum gonadotropin. J Anim Sci **76,** 2235–2238.
Coubrough CA, Castell MC, 1998: Fetal sex determination by ultrasonically locating the genital tubercule in ewes. Theriogenology **50,** 263–267.
Cueto M, Gibbons A, Alberio R, Taddeo H, Gonzalez-Bulnes A, 2006: Timing of emergence of ovulatory follicles in polyovulatory goats. Anim Reprod Sci **91,** 275–284.
Dawson LJ, Sahlu T, Hart SP, Detweiler G, Gipson TA, Teh TH, Henry GA, Bahr RJ, 1994: Determination of fetal numbers in Alpine does by real-time ultrasonography. Small Rumin Res **14,** 225–231.
De Santiago-Miramontes MA, Rivas-Muñoz R, Muñoz-Gutiérrez M, Malpaux B, Scaramuzzi RJ, Delgadillo JA, 2008: The ovulation rate in anoestrous female goats managed under grazing conditions and exposed to the male effect is increased by nutritional supplementation. Anim Reprod Sci **105,** 409–416.
Dickie AM, Paterson C, Anderson JLM, Boyd JS, 1999: Determination of corpora lutea numbers in Booroola-Texel ewes using transrectal ultrasound. Theriogenology **51,** 1209–1224.
Evans AC, Flynn JD, Quinn KM, Daffy P, Quinn P, Madgwick S, Crosby TF, Bolan MP, Beard AP, 2001: Ovulation of aged follicles does not affect embryo quality or fertility after a 14-day progestagen estrus synchronization protocol in ewes. Theriogenology **56,** 923–936.
Fernandez-Moro D, Veiga-Lopez A, Ariznavarreta C, Tresguerres JA, Encinas T, Gonzalez-Bulnes A, 2008: Preovulatory follicle development in goats following oestrous synchronization with progestagens or prostaglandins. Reprod Domest Anim **43,** 9–14.
Gonzalez-Bulnes A, Santiago-Moreno J, García-López M, Gomez-Brunet A, López-Sebastián A, 1994: Observación del ovario en la oveja y eficacia en la detección de folículos y cuerpos lúteos mediante ecografía transrectal. Invest Agr **10,** 319–329.
Gonzalez-Bulnes A, Santiago-Moreno J, Gómez-Brunet A, García-López M, López-Sebastián A, 1996: Determinación ecográfica de la morfología uterina durante el ciclo sexual de la oveja y relación con los niveles de progesterona plasmática. Invest Agr **11,** 243–252.
Gonzalez-Bulnes A, Santiago-Moreno J, Lopez-Sebastian A, 1998: Estimation of fetal development in Manchega dairy ewes by transrectal ultrasonographic measurements. Small Rumin Res **27,** 243–250.
Gonzalez-Bulnes A, Osoro K, López-Sebastián A, 1999: Ultrasonographic assessment of the ovarian response in eCG-treated goats. Small Rumin Res **34,** 65–69.
Gonzalez-Bulnes A, Moreno JS, Brunet AG, Lopez-Sebastian A, 2000: Relationship between ultrasonographic assessment of the corpus luteum and plasma progesterone concentration during the oestrous cycle in monovular ewes. Reprod Domest Anim **35,** 65–68.
Gonzalez-Bulnes A, Baird DT, Campbell BK, Cocero MJ, García-García RM, Inskeep EK, López-Sebastián A, McNeilly AS, Santiago-Moreno J, Souza CJH, Veiga-López A, 2004a: Multiple factors affecting the efficiency of MOET in sheep and goats. Reprod Fertil Dev **16,** 421–435.
Gonzalez-Bulnes A, Díaz-Delfa C, Urrutia B, Carrizosa JA, López-Sebastián A, 2004b: Ultrasonographic screening of ovulatory process in goats. Small Rumin Res **52,** 165–168.
Gonzalez-Bulnes A, Veiga-Lopez A, Garcia P, Garcia-Garcia RM, Ariznavarreta C, Sanchez MA, Tresguerres JA, Cocero MJ, Flores JM, 2005: Effects of progestagens and prostaglandin analogues on ovarian function and embryo viability in sheep. Theriogenology **63,** 2523–2534.
Gonzalez-Bulnes A, Carrizosa JA, Urrutia B, Lopez-Sebastian A, 2006: Oestrous behaviour and development of preovulatory follicles in goats induced to ovulate using the male effect with and without progesterone priming. Reprod Fertil Dev **18,** 745–750.
Haibel GK, Perkins NR, 1989: Real-time ultrasonic biparietal diameter of second trimester Suffolk and Finn fetuses and prediction of gestational age. Theriogenology **32,** 863–869.
Hesselink JW, 1993: Incidence of hydrometra in dairy goats. Vet Rec **132,** 110–112.
Kaulfuss KH, Giucci E, Süss R, Wójtowski J, 2006: An ultrasonographic method to study reproductive seasonality in ewes isolated from rams. Reprod Domest Anim **41,** 416–422.
Kelly RW, Newnham JP, 1989: Estimation of gestational age in Merino ewes by ultrasound measurement of fetal head size. Aust J Agric Res **40,** 1293–1299.
Kusina NT, Tarwirei F, Hamudikuwanda H, Agumba G, Mukwena J, 2000: A comparison of the effects of progesterone sponges and ear implants, PGF2alpha, and their combination on efficacy of estrus synchronization and fertility of Mashona goat does. Theriogenology **53,** 1567–1580.
Levy I, Emery P, Mialot JP, 1990: Echographie et gestion des tropeaux ovins. Rec Mèd Vet **166,** 751–764.
Martin GB, Milton JT, Davidson RH, Banchero Hunzicker GE, Lindsay DR, Blache D, 2004: Natural methods for increasing reproductive efficiency in small ruminants. Anim Reprod Sci **82,** 231–245.
Martinez MF, Bosch P, Bosch RA, 1998: Determination of early pregnancy and embryonic growth in goats by transrectal ultrasound scanning. Theriogenology **49,** 1555–1565.
Mialot JP, Levy I, Emery P, 1991a: Echographie et gestión des troupeaux caprins. Rec Med Vet **167,** 399–406.
Mialot JP, Saboreau L, Gueraud JM, Prendere E, Parizot D, Pirot G, Duquesnel R, Petat M, Chemineau P, 1991b: La pseudogestation chez la chévre. Rec Med Vèt **167,** 383–390.
Mossa F, Duffy P, Naitana S, Lonergan P, Evans AC, 2007: Association between numbers of ovarian follicles in the first follicle wave and superovulatory response in ewes. Anim Reprod Sci **100,** 391–396.
Picazo RA, Barragan ML, Valenciano M, Lopez Sebastian A, 1991: Evolución de la imagen ecográfica durante la gestación de la oveja. Med Vet **8,** 300–315.
Pierson RA, Ginther OJ, 1987: Ultrasonographic appearance of the bovine uterus during the estrous cycle. J Am Vet Med Assoc **190,** 995–1001.
Pierson RA, Kastelic JP, Ginther OJ, 1988: Basic principles and techniques for transrectal ultrasonography in cattle and horses. Theriogenology **29,** 3–19.
Pieterse MC, Taverne MAM, 1986: Hydrometra in goats: diagnosis with real-time ultrasound and treatment with prostaglandins or oxytocin. Theriogenology **26,** 813–821.
Richardson C, 1976: Morphological parameters of intrauterine growth retardation in the newborn lamb. Vet Rec **101,** 151–152.
Rubianes E, Menchaca A, 2003: The pattern and manipulation of ovarian follicular growth in goats. Anim Reprod Sci **78,** 271–287.

Rubianes E, Ungerfeld R, Viñoles C, Rivero A, Adams GP, 1997: Ovarian response to gonadotropin treatment initiated relative to wave emergence in ultrasonographically monitored ewes. Theriogenology **47,** 1479–1488.

Santiago-Moreno J, Gonzalez-Bulnes A, García-López M, López-Sebastián A, 1995a: Diagnóstico precoz de gestación y determinación del número de embriones mediante ecografía transrectal en la cabra. ITEA **91A,** 37–43.

Santiago-Moreno J, Gonzalez-Bulnes A, García-López M, López-Sebastián A, 1995b: Valoración de estadíos precoces de gestación en oveja y cabra mediante ecografía transrectal. Invest Agr **10,** 53–61.

Santos M, Rabelo HB, Guido MC, Torreao SI, Lopes Junior NC, Freitas ES, 2007: Determination of the genital tubercule migration period in Morada Nova sheep foetuses by ultrasonography. Reprod Domest Anim **42,** 214–217.

Schrick FN, Inskeep EK, 1993: Determination of early pregnancy in ewes utilizing transrectal ultrasonography. Theriogenology **40,** 295–306.

Schrick FN, Surface RA, Pritchard JY, Dailey RA, Townsend EC, Inskeep EK., 1993: Ovarian structures during the estrous cycle and early pregnancy in ewes. Biol Reprod **49,** 1133–1140.

Sergeev L, Kleemann DO, Walker SK, Smith DH, Grosser TI, Mann T, Seamark RF, 1990: Real-time ultrasound imaging for prediction ovine fetal age. Theriogenology **34,** 539–601.

Simoes J, Potes J, Azevedo J, Almeida JC, Fontes P, Baril G, Mascarenhas R, 2005: Morphometry of ovarian structures by transrectal ultrasonography in Serrana goats. Anim Reprod Sci **85,** 263–273.

Stephenson SK, Lambourne LJ, 1960: Prenatal growth in RomneyxSouthdown cross and Australian Merino sheep. Aust J Agric Res **2,** 1044–1063.

Tainturier D, Lijour L, Chaari M, Sardjana KW, Denis B, 1983a: Diagnostic de la gestation chez la brebis par échotomographie. Rec Méd Vét **134,** 523–526.

Tainturier D, Lijour L, Chaari M, Sardjana KW, Lenet JL, 1983b: Diagnostic de la gestation chez la chèvre par échotomographie. Rec Méd Vét **134,** 597–599.

Taverne MA, Lavoir MC, Bevers MM, Pieterse MC, Dieleman SJ, 1988: Peripheral plasma prolactin and progesterone levels in pseudopregnant goats during bromocryptine treatment. Theriogenology **30,** 777–783.

Ungerfeld R, Pinczak A, Forsberg M, Rubianes E, 1999: Response of Corriedale ewes to the ''ram effect'' after priming with medroxyprogesterone, fluorogestone, or progesterone in the non-breeding season. Acta Vet Scand **40,** 299–305.

Ungerfeld R, Carbajal B, Rubianes E, Forsberg M, 2005: Endocrine and ovarian changes in response to the ram effect in medroxyprogesterone acetate-primed Corriedale ewes during the breeding and nonbreeding season. Acta Vet Scand **46,** 33–44.

Vázquez MI, Blanch MS, Alanis GA, Chaves MA, Gonzalez-Bulnes A, 2010: Effects of treatment with a prostaglandin analogue on developmental dynamics and functionality of induced corpora lutea in goats. Anim Reprod Sci, **118,** 42–47.

Veiga-Lopez A, Gonzalez-Bulnes A, Garcia-Garcia RM, Dominguez V, Cocero MJ, 2005: The effects of previous ovarian status on ovulation rate and early embryo development in response to superovulatory FSH treatments in sheep. Theriogenology **63,** 1973–1983.

Veiga-Lopez A, Dominguez V, Souza CJ, Garcia-Garcia RM, Ariznavarreta C, Tresguerres JA, McNeilly AS, Gonzalez-Bulnes A, 2008: Features of follicle-stimulating hormone-stimulated follicles in a sheep model: keys to elucidate embryo failure in assisted reproductive technique cycles. Fertil Steril **89,** 1328–1337.

Viñoles C, Forsberg M, Banchero G, Rubianes E, 2001: Effect of long-term and short-term progestagen treatment on follicular development and pregnancy rate in cyclic ewes. Theriogenology **55,** 993–1004.

Viñoles C, Meikle A, Forsberg M, 2004: Accuracy of evaluation of ovarian structures by transrectal ultrasonography. Anim Reprod Sci, **80,** 69–79.

Viñoles C, Gonzalez-Bulnes A, Martin GB, Sales F, Sale S, 2009: Sheep and goats. In: DesCôteaux L, Colloton J, Gnemmi G (eds), Practical Atlas of Ruminant and Camelid Reproductive Ultrasonography. Wiley-Blackwell, Iowa (United States), pp. 181–210.

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