

Controlling the success of superovulatory treatments in ewes: Comparing laparoscopy to transrectal and transvaginal ultrasonography

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RESEARCH ARTICLE



ABSTRACT

In small ruminants, assessing the success of superovulatory treatments can be challenging, as there is considerable variability between individual animals. The current „gold standard” for examination of the superovulated ovaries is laparoscopy. B-mode ultrasonography with a transrectal or transvaginal transducer can also be used to locate the ovarian structures.

In this study, 23 Merino ewes were used to test two types of ultrasonographic examination techniques against laparoscopy.

The ewes were treated with long-term (12 days) progesterone treatment followed by a single injection of eCG (1000 IU) at removal of the progesterone sponge. The animals were examined two days after sponge removal and 7 days after the first examination.

The chance of localizing the ovaries was significantly higher with the transrectal method than with the vaginal ultrasonography ($P > 0.05$). In cases when the ovary was successfully visualized, there was a significant difference between the number of ovarian structures (antral follicles and corpora lutea) detected by transrectal ultrasonography and laparoscopy ($P < 0.01$). There was no significant difference between intravaginal examination and laparoscopy in this regard.

The current results are promising in the usefulness of vaginal ultrasonography as an alternative to the more commonly used transrectal technique.

However, more research is needed to evaluate if either the transrectal or the vaginal ultrasound examination can be completely reliable in assessing the results of a superovulatory treatment.

KEYWORDS

laparoscopy, superovulation, antral follicle, ultrasound

INTRODUCTION

In sheep, individual reactions to superovulatory treatments vary significantly (Pinto et al., 2018). Some examples of factors influencing the number of ovulated follicles are breed, age, reproductive state, time of year (in-season or out of season) and hormonal treatments (Torrès et al., 1987; Gonzalez-Bulnes et al., 2004). Minimizing the influence of these factors is an important part of multiple ovulation and embryo transfer programs; however, in small ruminants, the ovarian response remains highly variable even when using the most precise techniques possible. Another course of action is determining the success of the treatments before the embryo collection. Assessing the antral follicle count (AFC) and the number of corpora lutea (CL) is a useful procedure to evaluate the success of the treatment (Bartlewski et al., 2016).

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Contrary to cattle, B-mode ultrasound (US) is not routinely used to examine the ovaries of ewes. Currently, the „gold standard” for assessing the number of ovarian structures after superovulatory treatments in sheep is laparoscopy (Oliveira et al., 2018). However, laparoscopy is not available for all practitioners, and it also requires anaesthesia, which may affect the reproductive performance of ewes and takes considerable time to perform (Bartlewski et al., 2016). Animal welfare questions could also be considered when executing laparoscopy.

B-mode ultrasound can be used to examine the reproductive tract of ewes, but the accuracy of this technique is influenced by multiple factors, such as the type of transducer used (linear, sector, convex), the individual anatomy of the animal and the experience of the practitioner (Viñoles-Gil et al., 2009). In sheep, 3.5–5 MHz transducers are used for transabdominal scanning, while 5–10 MHz transducers are more appropriate for examining smaller structures transrectally, vaginally or transcutaneously (Meinecke-Tillmann, 2017).

Veterinary practitioners dealing with cattle likely have access to a 3.5–8.0 MHz linear transducer (Crilly et al., 2017). Transrectal ultrasonography with 7.5 MHz linear transducers has been used for multiple purposes in examining the reproductive tract of ewes, such as early pregnancy diagnosis (Scott, 2012), monitoring the pattern of follicular waves (Bartlewski et al., 1999; Seekallu et al., 2010) and detecting ovarian structures (Dickie et al., 1999; Barrett et al., 2002, 2004). This can be done in dorsal recumbency or in standing position (Viñoles et al., 2004). Visualisation of the ovaries might be challenging, as the size of a normal ovary in ewes is approximately 10 × 15 mm (Gonzalez-Bulnes et al., 2010).

Vaginal ultrasound is a less commonly used, alternative approach for the examination of the reproductive tract (Meinecke-Tillmann and Meinecke, 2007). It can be carried out with a convex transducer that is of appropriate size (Viñoles-Gil et al., 2009).

The aim of this study was to compare the two types of ultrasonography (transrectal and vaginal) to laparoscopy in their reliability at detecting ovarian structures.

MATERIALS AND METHODS

Animals, hormonal treatment, experimental design

Twenty-three clinically healthy Hungarian Merino ewes from different genetic backgrounds were used in the

experiment. The animals were 2–6 years old, and all have lambed previously at least once. Their body condition score ranged from 2.5 to 4 on a scale of 1–5. Their nutrition was based on grazing natural grass, a supplement of meadow hay and grain (barley, 300–350 g*day⁻¹) was offered during the breeding season and from lambing to weaning. The experiment was carried out in the spring, during April. The ewes last lambed in the previous fall, during September and their lambs were weaned at the end of November. All animals belonged to the Teaching Farm of University of Veterinary Medicine, Budapest.

A hormonal protocol was implemented, which consisted of 12 days of progesterone treatment (Chronogest CR 20 mg sponge, MSD Animal Health, US). The Chronogest sponge was inserted at Day 0, and removed at Day 12. On Day 12, a single injection of 1000 IU of eCG (Folligon, MSD Animal Health, US). On Days 14 and 21, ultrasonography and laparoscopic examinations were carried out to record the number of ovarian structures (antral follicles and corpora lutea). The timeline of the experimental procedures is shown in Fig. 1.

The results of the examinations were compared in assessing the success of the superovulatory treatment. The treatment was considered successful in case of >3 antral follicle/corpus luteum on the ovaries (Bari et al., 2001).

Ultrasound examination

The procedure was carried out in standing position, with manual restraint. The type of ultrasound used was Logiq V2 (GE Healthcare, US). Linear rectal transducer (5–7.5 MHz) was used for transrectal ultrasonography, with manual control. Once the ovary was located, it was scanned twice, with two examiners recording the number of structures. Transvaginal examination was performed with a microconvex endovaginal transducer (7.5–10 MHz). The transducer was moved in a dorsoventral motion to scan the pelvic cavity and the caudal part of the abdominal cavity. 184 individual examinations were performed: every animal was examined on Days 14 and 21, rectally and vaginally, the structures were recorded on both ovaries. The number of ovarian structures (antral follicles on Day 14 and corpora lutea on Day 21) were recorded immediately as assessed by the examiner and video recordings were also taken for later evaluation if needed. Follicles ≥2 mm were categorized as antral follicles.

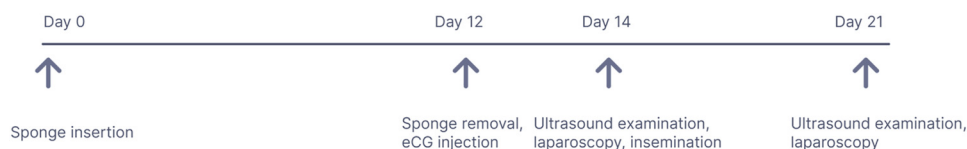


Fig. 1. Timeline of the experiment: At Day 0, an FGA-sponge was inserted. At Day 12, the sponge was removed, and 1000 IU eCG (Folligon, Intervet) was injected. At Day 14 (theoretical day for artificial insemination) ultrasound examinations were carried out, which were repeated at Day 21 (theoretical day for embryo recovery). Twenty-three Merino ewes were examined on two occasions. A linear rectal transducer (5–7.5 MHz) was used for transrectal ultrasonography. Transvaginal examination was performed with a microconvex endovaginal transducer (7.5–10 MHz). Laparoscopy was performed under general anaesthesia

Laparoscopic examination

Feed was withheld from the animals 24 h prior to the examinations. The laparoscopy was carried out immediately after the ultrasound examination. The animals were put under general anaesthesia via TIVA (total intravenous anaesthesia) for the process. 0.1 mg*kg⁻¹ BW xylazine (Nerfasin 20 mg*mL⁻¹ injection, Produlab Pharma B.V., Netherlands) was given intravenously (i.v.) as premedication, and general anaesthesia was induced with 2.2 mg*kg⁻¹ BW i.v. zolazepam-tiletamin combination (Zoletil 100 inj. A.U.V., Virbac, France). The abdominal wall was isolated, surgically prepared and 2 trocars were inserted into the abdominal cavity. The equipment used was a 5-mm rigid laparoscope (Olympus Corporation, Japan) with the corresponding trocars. The trocars were placed cranially to the position of udder, approximately 5–10 cm (depending on the individual anatomy of the animal) laterally to the linea alba on each side. The ovaries were located and the number of structures were recorded. If fixation was needed for counting the number of structures on the ovary, a pair of atraumatic grasper forceps were placed at the tip of the uterine horn. After the removal of the trocars, the skin incision was sutured with horizontal mattress sutures. 92 individual examinations were performed (right and left ovary, two occasions).

Statistical analysis

Data were analysed using the R3.5.2 statistical software (R Core Team, 2018). Data were checked for normality using the Shapiro-Wilk test.

A generalized linear model was used to determine the effect of type of examination on the number of ovarian structures found. Difference in the successful detection of structures by rectal and transvaginal US was determined by two sample proportion test. A logistic regression was also run to obtain odds ratios for the detection of ovarian structures using type of examination as a factor. The results of the Day 14 and 21 examinations were also analysed separately.

Detection of superovulation (>3 structures) was explored using logistic regression with type of examination as a factor. Difference in the successful detection of superovulation by rectal and transvaginal US was determined by two-sample proportion tests. The results of Day 14 and 21 examinations were also analysed separately.

A probability of $P < 0.05$ was considered statistically significant.

RESULTS

The mean number of recorded ovarian structures was 4.17 (± 2.35) at Day 14, and 2.30 (± 2.01) at Day 21.

Visualisation of the ovaries was possible in 51.63% of the US examinations (43.48% vaginally, 49.78% rectally). The likelihood of visualising the ovaries was significantly higher with the transrectal transducer ($P < 0.05$) if all examinations were considered. There was no significant difference in the number of visualisations between the two types of transducers on Day 14; however, there was a significant difference at Day 21 ($P < 0.01$) (Table 1).

In cases when the ovary was successfully visualized, there was a significant difference between the number of ovarian structures (antral follicles and corpora lutea) detected by transrectal ultrasonography and laparoscopy ($P < 0.01$). There was no significant difference between vaginal examination and laparoscopy.

In assessing the success of the superovulatory treatment (>3 ovarian structures), the vaginal examination did not differ from laparoscopy significantly (OR 1.43); however, there was a significant difference between the transrectal examination and the laparoscopy ($P < 0.01$, OR 3.42).

When the results of the ultrasonography were compared to those of laparoscopy only at Day 14, there was a significant difference between the transrectal technique and the laparoscopy ($P < 0.01$) and the vaginal ultrasound also differed from the laparoscopy ($P = 0.07$). At Day 21, the rectal ultrasonography differed from the laparoscopy ($P = 0.08$), but the vaginal examination did not differ significantly (Table 2).

DISCUSSION

B-mode ultrasonography is a less widely used method for examinations of the reproductive tract in sheep than in cattle (Scott and Sargison, 2010), albeit it is a non-invasive technique which allows the visualisation of ovarian structures (Meinecke-Tillmann and Meinecke, 2007). This could be an important step in simplifying MOET programs, because it is easier to assess the efficiency of the superovulatory treatment this way and it also improves animal welfare (Bartlewski et al., 2016).

Although ultrasonography has been established as a reliable tool for examinations of the reproductive tract in sheep, its accuracy is determined by many factors

Table 1. Percentage of unsuccessful visualisations of the ovaries with the different examination methods in all cases (Sum) on the day of ovulation (Day 14) and on the day of potential embryo recovery (Day 21). Examination of the ovaries was done with laparoscopy, transrectal and transvaginal ultrasound

	Sum	<i>P</i> -value	OR	Day 14	<i>P</i> -value	OR	Day 21	<i>P</i> -value	OR
Laparoscopy	8.70%			8.70%			8.70%		
Rectal US	40.22%	<0.01	0.14	36.96%	<0.01	0.16	43.48%	<0.01	0.12
Vaginal US	56.52%	<0.01	0.07	34.78%	<0.01	0.18	78.26%	<0.01	0.03

Table 2. Percentage of successful (>3 ovarian structures) and unsuccessful (≤3 ovarian structures) superovulatory treatments in all cases (Sum) on the day of ovulation (Day 14) and on the day of potential embryo recovery (Day 21). Examination of the ovaries was done with laparoscopy, transrectal and transvaginal ultrasound

	Sum			Day 14			Day 21		
	>3	≤3	P-value	>3	≤3	P-value	>3	≤3	P-value
Laparoscopy	48.81%	51.19%		71.43%	28.57%		26.19%	73.81%	
Rectal US	21.80%	78.20%	<0.01	34.50%	65.50%	<0.01	7.70%	92.30%	0.08
Vaginal US	40.00%	60.00%	0.36	50.00%	50.00%	0.07	10.00%	90.00%	0.30

(Meinecke-Tillmann and Meinecke, 2007). Transrectal ultrasonography is more commonly used for the visualisation of the ovaries which can also be visualized via endovaginal microconvex probe (Viñoles-Gil et al., 2009).

In this study, transrectal and vaginal ultrasound examination was tested against laparoscopy as a technique for assessing ewes' reaction to superovulatory treatments. Detection of the ovaries was low (43.48% vaginally, 49.78% rectally) compared to the results reported in other studies (Dickie et al., 1999; Ślósarz et al., 2003; Pinto et al., 2018). A lot of practitioners advise to carry out the transrectal examination in a dorsal recumbency (Neal Schrick et al., 1993; Kaulfuss et al., 1997; Ślósarz et al., 2003; Lopez-Alonso et al., 2005); however, others suggest that an experienced examiner can locate the reproductive tract with a satisfactory success rate in standing position as well, though the results vary based on the age, parity and body condition of the animal (Tajik et al., 2001; Karen et al., 2004; Viñoles et al., 2004). Whichever position the animal is placed in, minimizing the distance between the probe and the reproductive tract allows using higher frequency ultrasound, which in turn generates a higher resolution image (Szabo and Lewin, 2013; Meinecke-Tillmann, 2017).

Transrectal ultrasonography was more successful in the visualisation of the ovaries, however, in case of proper visualization, vaginal ultrasonography was more reliable in counting the ovarian structures. This phenomenon can be explained by the anatomical differences between animals and the range of the ultrasound probe used.

In ruminants, the size and shape of the ovaries is highly variable depending on the structures present; their anatomical location can also differ between animals (Jackson and Cockcroft, 2007). In case of a transrectal examination, the proximity of the reproductive tract to the rectum makes the ovaries easier to locate, but there are also additional factors (eg. faeces, peristaltic waves, blood vessels in the connective tissue, fluid accumulation in the uterus, distended bladder) that can hinder the evaluation of the image (Dickie et al., 1999; Meinecke-Tillmann and Meinecke, 2007). At the same time, the vaginal probe might not have an appropriate range to visualize the ovaries, especially in a standing position (Dickie et al., 1999). The microconvex probe used in this study operates at 7.5–10 MHz, which only allows 6–8 cm of penetration. However, if the ovary is within this range, there are fewer structures that can interfere with the interpretation of the results.

The experience of the examiner also plays a significant role in the detection of the ovaries and its structures (Ślósarz et al., 2003; Gonzalez-Bulnes et al., 2010). In the current study, re-evaluation of video recordings was needed in some cases to correctly determine the number of ovarian structures, which points to the possibility of intra- and interobserver variability.

In 2 cases, laparoscopic examination wasn't possible due to life threatening complications during anaesthesia imposing immediate termination of the procedure.

In cases where visualisation of the ovary was successful, the vaginal probe had similar results to the laparoscopy in assessing the success of the treatment, while the transrectal ultrasound differed significantly.

The current study alludes to the fact the BCS (Body Condition Score), body size and parity of the examined animal influences the results of ultrasonographic examinations of the reproductive tract.

Many ultrasound machines can operate with multiple probes, which means that there is an option to choose the optimal way for each animal based on their individual anatomy.

However, more research is needed to evaluate if either the transrectal or the vaginal ultrasound examination can be completely reliable in assessing the results of a superovulatory treatment. A more homogenous population of research animals (especially in terms of body size and BCS) could also improve the reliability of ultrasonography. Further studies are needed to find out which breeds and types are good candidates for using ultrasonography instead of laparoscopy.

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