

—Technical Note—

Use of Contrast Sonography to Test for Tubal Patency in Dairy Cattle

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Abstract. Hysterosalpingo-contrast sonography is an accurate first-line method used to test for tubal patency in human gynecology. Tubal pathology occurs in dairy cattle and is a reason for infertility, but easy and accurate methods to test for tubal patency are not available in the living cow. In this study it was thus investigated if contrast sonography (CS) using Echovist[®]-200 as the echo-contrast medium is a feasible procedure to test for tubal patency in dairy cattle. In eight repeat breeder Holstein Frisian cows, all of them being in behavioral estrus, Echovist[®]-200 was given into the uterus and its exit into the abdominal cavity then imaged by transrectal conventional B-mode ultrasound, and used as indicator for tubal patency. Animals were slaughtered one day later and the genital tracts subjected to gross morphology and histology in order to confirm the results of CS. In two cows, CS was prematurely terminated after examination of one oviduct because of rectal bleeding, while CS completely failed in another cow because of poor image quality. In five cows, both oviducts could be examined by CS, respectively. A total of five oviducts were found patent by CS and confirmed by post mortem examination. Two out of five oviducts diagnosed as occluded by CS were morphologically intact and thus misdiagnosed. Of the three non-patent oviducts, two were occluded because of a hydrosalpingis, respectively, while the third was inflamed. In conclusion, CS has been shown a feasible procedure to test for tubal patency in dairy cattle. Further studies with more animals are however recommended to warrant this result.

Key words: Contrast sonography, Repeat breeder cow, Tubal patency

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Between 5 and 25 % of dairy cows are culled for repeat breeding each year [1–3]. Repeat breeder cows are usually referred as to infertile animals that are bred several times but do not conceive and return to estrus in a regular interval, in the absence of signs that can be associated with not conceiving [4]. Repeat breeding is a multifactorial problem involving a multitude of extrinsic as well as intrinsic factors including subtle genital disorders [5–7]. Tubal pathology has been associated with infertility in dairy cattle [8–10], but its contribution to the repeat breeder syndrome has yet not been determined. This is due, at least in part, to the difficulty in the assessment of the oviducts in the living cow. Rectal palpation, laparotomy and laparoscopy have been advocated as viable methods for this purpose [11], however these are either inappropriate to test for tubal patency and/or unreliable, laborious and expensive. In contrast, tubal patency in cattle can be tested using the red dye phenolsulfonphthalein (PSP). When it is given into the uterus as a solution, it flows into the abdominal cavity in case of tubal patency, where it is resorbed and then excreted in urine [11, 12]. Using the PSP test false positive results are however occurring [11], and testing of individual oviducts is impossible. In woman, where tubal dysfunction or obstruction accounts for approximately 35% of all the causes of infertility [13], hysterosalpingo-contrast sonography is a widely used first-line screening method to test for tubal patency [14–16]. An echo-contrast medium is instilled into the uterus through a balloon catheter and is imaged transvaginally as it exits

the patent tube. This method is considered reliable, is well-tolerated and can be performed separately to test individual oviducts. In veterinary medicine, contrast sonography is still in its infancy, and has yet not been used to test for tubal patency in either farm or companion animal species. This pilot study was conducted to investigate if contrast sonography is a feasible procedure to test for tubal patency in dairy cattle on the example of the repeat breeder Holstein Frisian cow.

Materials and Methods

Animals and synchronization of estrus

A total of 8 repeat breeder Holstein Frisian cows (parity 1 and 2) of good general health and body condition (BCS 3.0–3.5) from one farm were used. Repeat breeders were defined as animals having at least one calving record, were mated or inseminated ≥ 3 times before culling (range: 4–9), returned to estrus in regular intervals (days 17–25 after last service [17]), in the absence of clinical signs indicative for infertility [5]. The animals were treated with 500 μg of the prostaglandin $F_{2\alpha}$ analogue cloprostenol (PGF Veyx[®]; Veyx Pharma, Schwarzenborn, Germany) given intramuscularly twice in an interval of 11 days in order to synchronize estrus.

Testing for tubal patency

Animals were tested for tubal patency 2 days after the second cloprostenol injection when they were all in behavioral estrus. This stage of estrous cycle was chosen for the reason of standardization and to allow for ease with manipulations. Prior to testing, animals received an epidural anesthesia using 4 ml of 2% procaine (Proca-

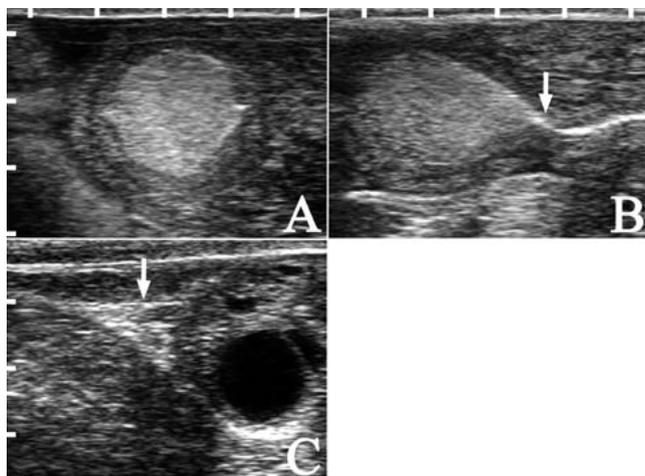


Fig. 1. Images are generated with conventional B-mode ultrasound using a linear probe, a frequency of 7.5 MHz and Echovist®-200 as the echo-contrast medium. (A) Cross-section of a uterine horn immediately cranial the tip of the balloon catheter and filled with echo-contrast medium. (B) Diagonally imaged slightly distended uterus at the horn tip with echo-contrast medium. The echo-contrast medium passes through the utero-tubal junction (arrow) into the oviduct. Note the hyperechoic line of echo-contrast medium within the oviductal lumen. The oviduct itself is not discernible. (C) Picture taken immediately after the echo-contrast medium has entered the tubal infundibulum but before being dispersed into the abdominal cavity. Note the cloud of echo-contrast medium with funnel-like appearance (arrow) adjacent to the ovary which is right to the cloud and has a few but only one big follicle. Scale bars on the top and left margin in cm.

sel®; Selectavet, Weyarn-Holzolling, Germany). A 18FR, 650 mm long balloon catheter used for embryo flushing in cattle (Minitüb Abfüll- und Labortechnik, Tiefenbach, Germany) was gently inserted into one uterine horn, the balloon placed approximately 5 cm cranial the uterine bifurcation, filled with air and checked for tight fitting. The flow of the echo-contrast medium was monitored by conventional B-mode ultrasound. For scanning, a HS 1201 ultrasound unit (Honda Electronics, Tokyo, Japan) and a dual frequency linear probe Model HLS-275VWF (5.0 MHz/ 7.5 MHz/ 50 mm; Honda Electronics) was used. The probe was inserted rectally to allow for visualization of the flow of the echo-contrast medium. As echo-contrast medium, Echovist®-200 (Bayer Schering AG, Berlin, Germany) was used. Echovist®-200 is designated for use in human gynecology and licensed in several European and Arabian countries as well as in South Africa and Argentina, but not in Japan and the United States. The active substance in Echovist®-200 is D-galactose, that comes as a granulate (3 g/ bottle) and has to be dissolved in 13.5 ml of a dissolving solution to be ready for use. In this experiment, the 3 g granulate/ bottle were also dissolved using the 13.5 ml dissolving solution but then, however, further extended with sterile NaCl to get a larger volume of finally 30 ml. This solution was then vigorously shook and the entire 30 ml immediately but slowly injected into the uterine horn via the balloon catheter to test for patency of the unilateral oviduct. Prior to injection, the ultrasound probe was placed above the tip of the balloon catheter in order to monitor first appearance of the echo-contrast medium

within the uterine horn, and was then moved forward toward the uterine horn tip and the oviduct as the echo-contrast medium flowed forward. Tubal patency was diagnosed if the echo-contrast medium that initially could be monitored in the uterus and subsequently appeared in the oviduct disappeared from the genital tract and dispersed into the abdominal cavity (Fig. 1A–C). If echo-contrast medium was retained and dispersion not detected, tubal occlusion was assumed. After tubal patency was tested on one oviduct, the balloon was emptied and the catheter relocated into the other uterine horn to test the contralateral oviduct for patency following the same procedure as described above. Retrograde reflux of echo-contrast medium was not detected.

Collection of reproductive tissues and morphological examination

The morphological examination was done in order to confirm the results of contrast sonography. One day after testing for tubal patency the cows were slaughtered, the genital tracts collected, placed on ice, transported to the laboratory within 1 hr and immediately processed. Initial examination included assessment of the oviducts for gross appearance and of the ovaries to confirm that all animals were equally in the immediate post-ovulatory phase. All patent and occluded oviducts that appeared grossly normal were subjected to histology. A 1 cm long piece of the tubal segments ampulla and isthmus, respectively, were fixed in 4% formaldehyde, dehydrated and then embedded in paraffin before being cut into sections (3–5 μ m) and stained with haematoxylin-eosin. Histological slides were assessed considering the stage of estrous cycle with particular consideration given to inflammatory cells within the tubal epithelium [18–20]. The oviducts were classified as either “normal” or as “inflamed” in case inflammation was observed in one or both oviductal segments. A normal morphological appearance of the oviduct was used for confirmation of tubal patency, while gross abnormalities or inflammation was used for occlusions.

Results

Results are documented in Table 1. Contrast sonography of both oviducts could be performed in five cows. In two cows, only one oviduct could be tested, respectively. This was because the cows showed rectal bleeding after testing of the first oviduct, and all manipulations were then immediately terminated. In another cow, results of contrast sonography were inconclusive for both oviducts because of poor image quality that did not allow for unambiguous monitoring of the flow of the echo-contrast medium.

Genital tracts of the five cows with complete testing records were collected and subsequently examined morphologically. Accordingly, in cows #82991 and #01332 both oviducts were patent and confirmed by morphological examination. Cow #83037 had bilaterally occluded oviducts, that were however morphologically intact. Cows #93919 and #81966 had a unilateral hydrosalpingis, respectively (Fig. 2A and B), which were both found occluded when examined by contrast sonography. While the contralateral oviduct of cow #81966 was patent and morphologically intact, it was occluded and inflamed in cow #93919.

Table 1. Results of testing of repeat breeder Holstein Frisian cows (n=8) for tubal patency using contrast sonography, and of post mortem examination of the oviducts

Cow ID	Contrast sonography		Gross morphology		Histology	
	Left OD	Right OD	Left OD	Right OD	Left OD	Right OD
83037	occluded	occluded	normal	normal	normal	normal
82991	patent	patent	normal	normal	normal	normal
01332	patent	patent	normal	normal	normal	normal
93919	occluded	occluded	HS	normal	n.a.	inflamed
81966	patent	occluded	normal	HS	normal	n.a.
82467	patent	not tested ^a	n.a.	n.a.	n.a.	n.a.
82940	patent	not tested ^a	n.a.	n.a.	n.a.	n.a.
93665	inconclusive ^b	inconclusive ^b	n.a.	n.a.	n.a.	n.a.

n.a.: not applicable. OD: Oviduct. HS: Hydrosalpingis. ^a Due to premature termination of manipulations because of rectal bleeding.

^b Both uterine horns were catheterised and the echo-contrast medium properly installed, but its flow could not be monitored unambiguously because of poor image quality.

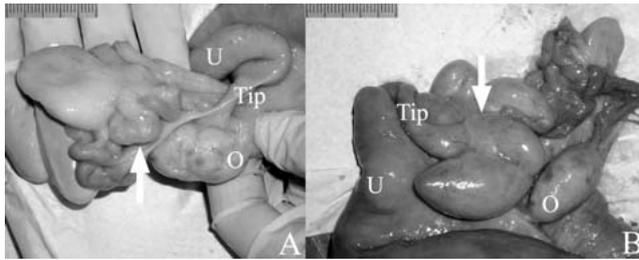


Fig. 2. Hydrosalpingis (arrows) in (A) cow #93919 and (B) cow #81966. O: Ovary. U: Uterine horn. Tip: Tip of the uterine horn. Scale bar on the top in mm.

Discussion

In human gynecology, hysterosalpingo-contrast sonography is a widely used first-line screening procedure to test for tubal patency [14–16]. An echo-contrast medium is given into the uterus and its flow through the upper genital tract monitored by transvaginal ultrasound. The progressive accumulation of echo-contrast medium in the retrouterine space is considered indicative for tubal patency, and the absence of accumulation after completion of instillation for tubal occlusion [16, 21]. Using a similar approach in this study it could be demonstrated that contrast sonography is a feasible procedure to test for tubal patency in dairy cattle on the example of the repeat breeder Holstein Frisian cow. Echovist[®]-200 was well tolerated by the cows, as no apparent side effects were observed. This is in line with studies conducted in humans that did also not reveal side effects after use of Echovist[®]-200 including on fertility [21, 22]. However, incidents such as rectal bleeding or poor image quality as observed in this study may occur in individual cows and limits the outcome of contrast sonography. In this study approximately 30 min was needed per cow to perform contrast sonography (data not detailed). Narrowing this time together with gathering more routine may help to reduce the occurrence of those incidents.

In humans, hysterosalpingo-contrast sonography has been shown to be very accurate in the diagnosis of patent oviducts.

While a sensitivity of $\geq 95\%$ was achieved with hysterosalpingo-contrast sonography when compared to laparoscopy and dye test as the gold standard, the method more often failed to detect occluded oviducts [16, 21]. A similar observation has been made in this study. While all five oviducts diagnosed as patent by contrast sonography were confirmed as being patent by the results of the post mortem examination, two (of five) diagnoses “occluded” were not correct (both oviducts of cow #83037). The reasons for the misdiagnoses are not easily to be determined, but may include methodical biases related to both the morphological examination and the contrast sonography. For instance it might be that the tubal sides that were collected for histology did not exactly match with the sides of tubal occlusion. Maybe the volume of Echovist[®]-200 that was installed was inappropriate. It could however also be that the Echovist[®]-200 lost its contrast-giving properties before being completely passed through the oviduct, perhaps due to the lower concentration of D-galactose in the solution used in this study (3 g/30 ml) compared to what is recommended by the manufacturer for use in humans (3 g/13.5 ml). These methodological aspects clearly need further consideration.

This study has shown once more that tubal pathology occurs in dairy cattle [8–12]. The fact that two of five repeat breeders had severely damaged tubes suggests that tubal pathology is more often than expected and is likely being a reason for repeat breeding. Repeat breeding is costly for the dairy industry [23], and a procedure that would allow for rapid and accurate recognition of cows having fatally damaged oviducts is thus highly desirable. Contrast sonography using conventional B-mode and Echovist[®]-200 as the echo-contrast medium may have this potential and would then also allow testing for patency of individual oviducts.

Since the number of cows used in this study was low, another investigation involving more animals is necessary to verify accuracy of contrast sonography as performed in this study. However, contrast sonography is currently very costly, which is mostly due to high expenses for Echovist[®]-200. Thus, studies into the use of other echo-contrast media such as saline with or without air [24, 25] as cost-cutting alternatives are also encouraged. Another ultrasound technology that also bases on the use an echo-contrast medium, i.e. contrast harmonic imaging, would provide a much

better spatial and contrast resolution than conventional B-mode ultrasound, and thus certainly may help to increase diagnosis accuracy [14, 26]. However, this technology is even more expensive than the one used in this study and is therefore most likely, at least not currently, applicable to the dairy veterinary practice for reproduction purposes including salpingo-contrast sonography.

In conclusion, this pilot study has demonstrated that contrast sonography using conventional B-mode and Echovist®-200 as the echo-contrast medium is a feasible procedure to test for tubal patency in dairy cattle on the example of the repeat breeder Holstein Frisian cow. Further investigations are however required using larger numbers of animals in order to verify accuracy of this procedure and to optimize it if appropriate.

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