Comparison of diagnostic methods for uterine health in dairy cattle on different days postpartum

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Abstract

Background Uterine health status is a key factor for dairy management.

Methods The uteri of 28 Holstein cows were assessed weekly by three different methods: ultrasonography (UT), Metricheck device (MT) and cytobrush (CYT), during two weeks to seven weeks postpartum.

Results The percentage of cows with an MT-positive, UT-positive and CYT-positive result decreased dramatically at three weeks, four weeks and five weeks postpartum, respectively. The agreement of the results was poor or weak within six weeks postpartum (κ=0.09–0.35), and only at seven weeks moderate or good agreement was achieved (κ=0.60–0.70). When comparing the results obtained by UT and MT, 30 per cent or more of the total cows had conflicting results from postpartum three weeks to six weeks. The uterine health of cows was re-evaluated by the combined usage of UT and MT referring to CYT. The highest sensitivity was achieved when positive individuals were identified as either MT-positive or UT-positive, whereas the highest specificity occurred when results were positive by both MT- and UT- methods.

Conclusion The accuracy of early postpartum uterine health status by three methods depended heavily on the time postpartum. The combined use of UT and MT is a practical candidate for cow-side monitoring method of uterine health.

Introduction

Reproductive performance is a key factor for the profitability of the dairy industry.[1 2] For improving the fertility of a dairy herd, postpartum genital organs need to be restored completely within the voluntary waiting period. After calving, the uterus contracts immediately and rapidly, the entire genital tract returns to the normal size, and uterine involution continues morphologically until five weeks postpartum.[3] Bacterial contamination is almost sufficiently eliminated by six weeks to seven weeks postpartum,[4] and the uterine endometrium is fully regenerated within eight weeks.[3]

Although almost all dairy cows usually acquire a functionally normal uterus within approximately eight weeks postpartum, 20 per cent to 40 per cent or more of cows may develop one or more uterine diseases during the early lactation period, resulting in economic loss because of decreased milk production, impaired fertility, premature culling and death.[5]

To distinguish abnormal uterine status from normal status, several monitoring methods have been developed and applied in the field conditions. In addition to conventional rectal palpation of the uterus, ultrasonography (UT) has been applied for monitoring the genital status as a non-laboratory method, which can directly confirm the presence or absence of echogenic fluid in the uterus. It has been shown that the intrauterine echogenic fluid might be associated with bacterial growth and delayed clearance of the inflammatory response substances[6] and could be a promising indicator for subsequent reproductive performance.[7–9]

To detect vaginal discharge, a Metricheck device (MT) has been developed as a practical tool, which is as an alternative to traditional vaginoscopy.[10] Vaginal discharge is the most evident indicator to determine

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the presence or absence of the genital inflammatory response for veterinary practitioners. The influence of abnormal vaginal discharge, such as that containing pus, on subsequent fertility has been clearly revealed.[11–13] The device is easy to insert and to clean between cows and is as efficient as vaginoscopy for detecting vaginal materials.[14]

Recently, the cytobrush (CYT) technique has been recommended as a more accurate method to evaluate uterine endometrial health status in cattle. By observing cells on the endometrial surface, this method enables to assess the severity of endometrial inflammation by determining the percentage of polymorphonuclear cells (PMN%).[7 15] However, CYT is a laboratory method and requires more skilful sampling of veterinarians. Referring to CYT, the practical availability of two non-laboratory methods, UT and MT, has been investigated.[7 10 13 16–18] In these studies, only a pair of methods have been compared after parturition, but no comparison among the three methods has been conducted. Moreover, those studies estimated the availability from only one or two examinations conducted with a wide range of sampling timing such as four weeks to six weeks or three weeks to seven weeks postpartum.

The first objective of this study was to describe and compare weekly postpartum uterine status by monitoring three different methods: UT, MT and CYT, during two weeks to seven weeks postpartum. The second objective was to evaluate the practical potential of combined usage of two non-laboratory methods: UT and MT.

Materials and methods

Cows and herd management

Data presented in this study were collected from 28 (7 primiparous and 21 multiparous) lactating Holstein cows. The cows calved between January 2016 and December 2017 at the Livestock Research Institute of the Aomori Prefectural Industrial Technology Research Centre (Noheji, Aomori, Japan). Cows were housed in a free-stall barn after parturition, fed a diet formulated according to Japanese feeding standards (Agriculture, Forestry and Fisheries Research Council Secretariat, 2006), and milked twice a day (at 08:30 and 17:00). The rolling herd average of milk yield was 10,320 kg per cow.

Experimental design

All cows were examined once a week for six weeks by one veterinarian. First, vaginal discharge was collected by MT, followed by UT and CYT at two weeks (mean±SD: 14.1±0.8 days in milk), three weeks (21.0±0.9), four weeks (28.0±0.9), five weeks (35.1±1.0), six weeks (42.2±0.9) and seven weeks (49.2±0.9) after normal calving.

Vaginal discharge collection by the MT

Vaginal discharge was collected using MT (Simcro Tech, Hamilton, New Zealand) as previously described,[10] with some modifications. The material within the concave surface of the device and/or adherent to the convex surface was assessed for categories on a 0–5 scale (0=no discharge, 1=clear mucus, 2=flecks of purulent material within otherwise clear mucus, 3=mucopurulent but less than 50 per cent purulent material, 4=mucopurulent with more than 50 per cent purulent material and 5=mucopurulent with more than 50 per cent purulent material and with an odour). The cows with the categories at least 2 were determined as MT-positive, and the others as MT-negative.[10] The devices were rinsed in an antiseptic solution between cows.

UT examination

Intrauterine fluid was monitored using a real-time linear array ultrasound scanner equipped with a 7.5 MHz rectal probe (HS-1600; Honda Electronics, Tokyo, Japan). Scanning was performed carefully and slowly along the dorsal/lateral surface of the uterus, and the equipment was supplied with image freeze for observation of intrauterine fluid at the base of each horn (approximately 5 cm anterior to the uterine body). The intrauterine fluid was categorised on a 0–2 scale (0=anechogenic, 1=echogenic compact contents, 2=echogenic fluctuant contents).[9] The cows evaluated with the categories at laest 1 by UT examination were determined as UT-positive, and the others as UT-negative.[9]

Endometrial cytology by the CYT

Endometrial samples for cytological examination were collected using a CYT (Fujihira Industry, Tokyo, Japan). Endometrial cytology was conducted as previously described[7] with some modifications. The instrument was placed in a sanitary plastic sleeve (IMV-Technologies, L'Aigle, France), and was sterilised by ethylene oxide gas before use. The covered instrument was passed through the vagina to the external cervical os, the sanitary sleeve punctured, and the instrument advanced through the cervix into the body of the uterus where the stainless-steel tube was retracted to expose the brush. Endometrial cytology samples were collected by rotating the brush while in contact with the uterine wall. The brush was retracted into the stainless-steel tube before removal from the uterus. Cytology slides were prepared by rolling the brush on a clean glass microscope slide for each sample.

Slides were stained with Diff-Quick stain (SYSMEX Corp, Hyogo, Japan), and air dried. Three hundred cells were counted under a microscope (magnification ×400) to determine the percentage of polymorphonuclear leucocytes (PMN%). The threshold value used for the proportion of PMN% indicating the presence of endometrial inflammation for each week was determined by referring to previous studies (two weeks and three weeks ≥18 per cent, four weeks ≥8 per cent,

five weeks to seven weeks ≥6 per cent).[7 8 16 19] The cows that exceeded the threshold of PMN% were determined as CYT-positive, and the others as CYT-negative.

Statistical analysis

All statistical analyses were performed using the JMP statistical software (JMP Statistics and Graphics guide, V.13.0; SAS Institute, Cary, North Carolina, USA).

A weekly descriptive analysis for the indications monitored by three methods was performed. The distribution of PMN% was illustrated as a box plot diagram. Data for the PMN% for each week were not normally distributed. Therefore, the data were transformed to ranks and multiple comparisons of the mean values for each week were performed using the Kruskal-Wallis test, and the differences between weeks were analysed by the Steel-Dwass test. By using the decisions obtained by three different methods, the level of agreement between the tests was examined using the κ statistic.[20] For κ value agreement, less than 0.00, between 0.01 and 0.20, between 0.21 and 0.40, between 0.41 and 0.60, between 0.61 and 0.80 and greater than 0.81 was interpreted as poor, very weak, weak, moderate, good, and excellent agreement, respectively.[20]

Using CYT as a reference, sensitivity and specificity were determined for the single usage of UT and MT. For the combined usage, both the UT and MT results had to be positive for the final decision to be positive or either the UT or MT result had to be positive for the final decision to be positive. Sensitivity and specificity of non-laboratory methods (UT, MT and combinations) were defined as follows; the sensitivity is the proportion of positive cows determined also positive by CYT, and the specificity is the proportion of negative cows determined also negative by CYT.

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

Results

Description of the population

All cows had a normal delivery with no clinical complications and a total of 3 (10.7 per cent) of the 28 cows delivered twins. Data from 1, 2 and 5 cows were eliminated from the 28 cows at four weeks, six weeks and seven weeks postpartum, respectively, because of missing values for the uterine examination.

A weekly descriptive analysis for the indications monitored by the three methods

Metricheck device

Table 1 shows the percentage of the cows with MT categories on a 0–5 scale from two weeks to seven weeks postpartum. At two weeks postpartum, more than 90 per cent of cows were assessed with a category at least 2, whereas there was only one cow with a category less than 2. From postpartum two weeks to three weeks, the frequency of the cows with a category of 0 or 1 increased rapidly and those with a category of 4 or 5 decreased. Thus, at three weeks postpartum, the percentage of MT-positive cows declined dramatically by 50.0 points (from 96.4 per cent to 46.4 per cent). There was no significant change in the distribution of the categories from three weeks to six weeks postpartum. Then, the frequency of cows with categories of 2 and 3 decreased by 22.1 points, and that of the cows with categories of 0 and 1 increased by 21.6 points at seven weeks postpartum.

Table 1 Percentage (number) of the cows with Metricheck device (MT) categories on a 0–5 scale from two weeks to seven weeks postpartum

		% (n) of MT-category*						
		0	1	2	3	4	5	Total No.
Week	2	3.6 (1)	0.0 (0)	25.0 (7)	25.0 (7)	32.1 (9)	14.3 (4)	28
	3	14.3 (4)	39.3 (11)	28.6 (8)	17.9 (5)	0.0 (0)	0.0 (0)	28
	4	18.5 (5)	44.4 (12)	14.8 (4)	18.5 (5)	3.7 (1)	0.0 (0)	27
	5	10.7 (3)	60.7 (17)	17.9 (5)	10.7 (3)	0.0 (0)	0.0 (0)	28
	6	15.4 (4)	50.0 (13)	19.2 (5)	11.5 (3)	3.8 (1)	0.0 (0)	26
	7	34.8 (8)	52.2 (12)	4.3 (1)	4.3 (1)	0.0 (0)	4.3 (1)	23

* 0=no discharge, 1=clear mucus, 2=flecks of purulent material within otherwise clear mucus, 3=mucopurulent but less than 50% purulent material, 4=mucopurulent with more than 50% purulent material, and 5=mucopurulent with more than 50% purulent material and with an odour.

Ultrasonography

The distribution of the number of UT categories on a 0–2 scale from two weeks to seven weeks postpartum is shown in table 2. At two weeks postpartum, only 7.2 per cent of the cows had a category of 0 or 1, and most of the cows were assessed as category of 2. From two weeks to three weeks postpartum, the number of cows with a score of 0 was constant, although the distribution of cows with a category of 1 increased and those with a category of 2 decreased. At four weeks postpartum, the frequency of cows with a category of 0 exceeded 50.0 per cent, resulting in the percentage of UT-positive cows rapidly reducing by 41.2 points (from 89.3 per cent to 48.1 per cent). Then, the number of cows having a category of 1 or 2 gradually decreased.

Cytobrush

Figure 1 shows the PMN% evaluated by CYT from two weeks to seven weeks postpartum as a box plot diagram. The mean PMN% decreased continuously, and the values at two weeks and three weeks postpartum were significantly higher compared with those at five weeks to seven weeks postpartum. At five weeks postpartum, the percentage of CYT-positive cows decreased rapidly by 31.0 points (from 66.7 per cent to 35.7 per cent). As summarised in figure 2, the percentage of cows with an MT-positive, UT-positive and CYT-positive result decreased dramatically at three weeks, four weeks and five weeks postpartum, respectively.

Table 2 Percentage (number) of the cows with ultrasonographic (UT) categories on a 0–2 scale from two weeks to seven weeks postpartum

		% (n) of UT- category *			
		0	1	2	Total No.
Week	2	3.6 (1)	3.6 (1)	92.9 (26)	28
	3	10.7 (3)	46.4 (13)	42.9 (12)	28
	4	51.8 (14)	29.6 (8)	18.5 (5)	27
	5	67.9 (19)	21.4 (6)	10.7 (3)	28
	6	73.0 (19)	26.9 (7)	0.0 (0)	26
	7	78.2 (18)	13.0 (3)	8.7 (2)	23

*0=anechogenic, 1=echogenic compact contents, 2=echogenic fluctuant contents.

Agreement between the UT, MT and CYT results

The agreement of each pair of tests was poor or weak, excluding seven weeks postpartum (table 3), at which the κ values indicated moderate or good agreement. The disagreement between the two non-laboratory methods is summarised in figure 3. From postpartum three weeks to six weeks, results from 30 per cent or more of the total cows disagreed. The cows with the UT-positive and MT-negative results accounted for 43.0 per cent of the total cows at three weeks postpartum, while the cows with the UT-negative and MT-positive accounted for 23.0 per cent of the total at postpartum six weeks.

Sensitivity and specificity for UT, MT and combinations

Using CYT as a reference technique, the sensitivities and specificities for single and combined usages of UT and MT from two weeks to seven weeks postpartum are shown in table 4. The highest sensitivity was achieved when positive individuals were identified as either MT-positive or UT-positive, whereas the highest specificity occurred when results were positive by both MT and UT methods.

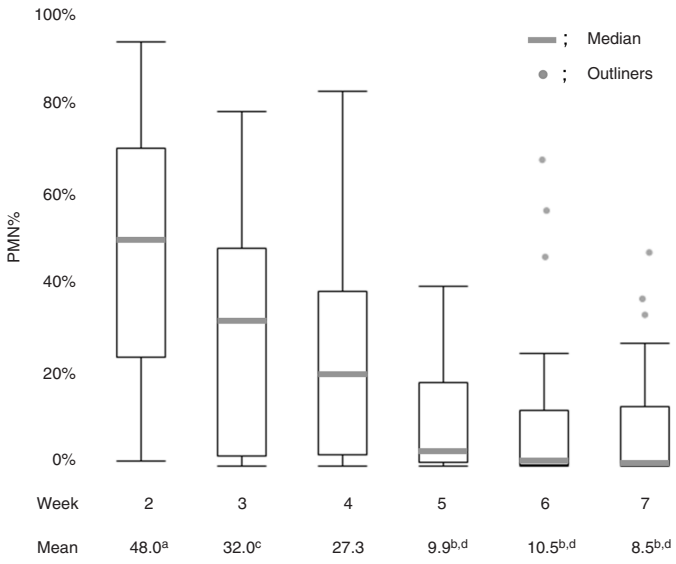

Figure 1 Box plot diagram showing the median, minimum, maximum and interquartile range values for the weekly percentage of polymorphonuclear leucocytes (PMN%) evaluated by the cytobrush technique from two weeks to seven weeks postpartum.[a, b]; values with different letters are significantly different (P<0.01).[c, d]; values with different letters are significantly different (P<0.05).

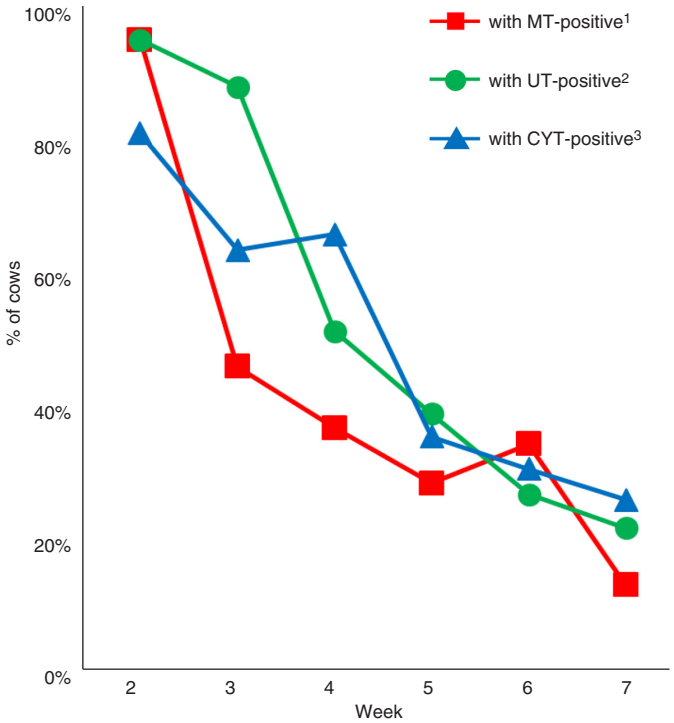

Figure 2 Percentage of cows MT-positive[1], UT-positive[2] and CYT-positive[3] results from two weeks to seven weeks postpartum.[1] MT-positive; the cow with Metricheck category at least 2 was defined as positive.[2] UT-positive; the cow with ultrasonography category at least 1 was defined as positive.[3] CYT-positive; the threshold values at weeks 2 and 3, 4, 5 to 7 were ≥18 %, ≥8 %, ≥6 %, respectively, and the cows that exceeded the threshold for PMN% were defined as CYT-positive. CYT, cytobrush; MT, Metricheck device; UT, ultrasonography.

Discussion

This is the first study to compare the three different methods: UT, MT and CYT, for monitoring uterine health and to describe the details of weekly indications during the early postpartum period. It is generally considered that almost all methods for evaluating

Table 3 κ value agreement of the tests from two weeks to seven weeks postpartum

Week	Combination of methods*	κ† (95% CI‡)	P value
2	MT * UT	−0.04 (-0.09 to 0.01)	0.84
	MT * CYT	−0.06 (-0.17 to 0.04)	0.63
	CYT * UT	−0.06 (-0.17 to 0.04)	0.63
3	MT * UT	0.19 (-0.01 to 0.39)	0.09
	MT * CYT	0.09 (-0.25 to 0.43)	0.61
	CYT * UT	−0.01 (-0.29 to 0.26)	0.92
4	MT * UT	0.33 (-0.02 to 0.65)	0.08
	MT * CYT	0.18 (-0.12 to 0.48)	0.26
	CYT * UT	0.34 (0.01 to 0.67)	0.06
5	MT * UT	0.24 (-0.13 to 0.62)	0.20
	MT * CYT	0.35 (-0.01 to 0.71)	0.06
	CYT * UT	0.28 (-0.08 to 0.65)	0.13
6	MT * UT	0.10 (-0.28 to 0.49)	0.59
	MT * CYT	0.21 (-0.17 to 0.60)	0.27
	CYT * UT	0.34 (-0.05 to 0.74)	0.08
7	MT * UT	0.70 (0.32 to 1.00)	<0.01
	MT * CYT	0.60 (0.20 to 0.99)	<0.01
	CYT * UT	0.64 (0.27 to 1.00)	<0.01

*MT, Metricheck device; UT, ultrasonography; CYT, cytobrush.
†Kappa value (κ) agreement was interpreted as: less than 0.00, between 0.01 to 0.20, between 0.21 to 0.40, between 0.41 to 0.60, between 0.61 to 0.80, greater than 0.81 was poor, very weak, weak, moderate, good and excellent agreement, respectively.
‡95% CI; 95 per cent confidence interval.

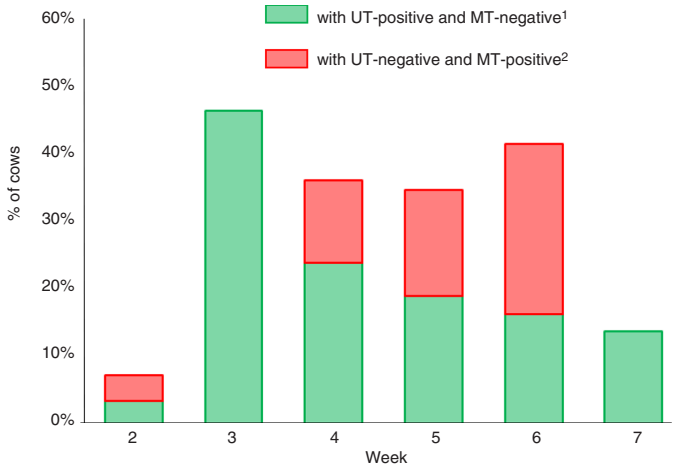

Figure 3 Percentage of the cows with the disagreement results between the two non-laboratory methods from two weeks to seven weeks postpartum. [1]with UT-positive and MT-negative; the cow with ultrasonography category at least 1 and with Metricheck category less than 2. [2]with UT-negative and MT-positive; the cow with ultrasonography category less than 1 and with Metricheck category at least 2.

uterine health are poor alternatives for each other. Previous studies comparing results obtained by MT and CYT indicated that the agreement of the tests was poor or fair (κ=0.30 during four weeks to six weeks,[17] κ=0.14 at five weeks[16]), and similarly low κ values were also obtained when comparing the results using UT and CYT (κ=0.33 during three weeks to seven weeks,[18] κ=0.11–0.25 during four weeks to six weeks[8]). In this study, the agreement of each pair of methods was also poor or weak (κ=0.09–0.35) within six weeks postpartum, and at seven weeks moderate or good agreement was achieved (κ=0.60–0.70).

Table 4 Sensitivity and specificity for single and combined usages of ultrasonography (UT) and Metricheck device (MT) from two weeks to seven weeks postpartum

Week	Single or combined techniques*	Sensitivity %	95% confidence interval	Specificity %	95% confidence interval
2	MT	95.7	0.78 to 0.99	0.0	0.00 to 0.00
	UT	95.7	0.78 to 0.99	0.0	0.00 to 0.00
	Both MT and UT	91.3	0.72 to 0.98	0.0	0.00 to 0.00
	Either MT or UT	100.0	1.00 to 1.00	0.0	0.00 to 0.00
3	MT	50.0	0.26 to 0.73	60.0	0.26 to 0.87
	UT	88.9	0.65 to 0.98	10.0	0.00 to 0.44
	Both MT and UT	50.0	0.26 to 0.73	60.0	0.26 to 0.87
	Either MT or UT	88.9	0.65 to 0.98	10.0	0.00 to 0.44
4	MT	44.4	0.21 to 0.69	77.8	0.20 to 0.97
	UT	61.1	0.35 to 0.88	77.8	0.20 to 0.97
	Both MT and UT	33.3	0.13 to 0.59	88.9	0.51 to 0.99
	Either MT or UT	72.2	0.46 to 0.90	66.7	0.29 to 0.92
5	MT	50.0	0.18 to 0.81	83.3	0.58 to 0.97
	UT	50.0	0.18 to 0.81	77.8	0.52 to 0.93
	Both MT and UT	30.0	0.01 to 0.65	94.4	0.72 to 0.99
	Either MT or UT	70.0	0.36 to 0.93	66.7	0.44 to 0.88
6	MT	50.0	0.15 to 0.84	72.2	0.46 to 0.90
	UT	50.0	0.15 to 0.84	83.3	0.58 to 0.96
	Both MT and UT	37.5	0.01 to 0.75	100.0	1.00 to 1.00
	Either MT or UT	62.5	0.24 to 0.91	55.6	0.30 to 0.78
7	MT	50.0	0.11 to 0.88	100.0	1.00 to 1.00
	UT	66.7	0.22 to 0.95	94.1	0.71 to 0.99
	Both MT and UT	50.0	0.11 to 0.88	100.0	1.00 to 1.00
	Either MT or UT	66.7	0.22 to 0.95	94.1	0.71 to 0.99

*MT, Metricheck device; UT, ultrasonography; Both MT and UT, both methods being positive as final positive; Either MT or UT, either of the methods being positive as final positive.

To improve the low level of agreement, one previous study proposed to optimise more clearly the timing of examination determining uterine status relative to calving.[21] The weekly uterine health examinations in the present study demonstrated that it is difficult to standardise the examination timing because each indication obtained by the three different methods frequently conflicted within six weeks postpartum.

Results between three weeks to six weeks postpartum in 30 per cent or more cows disagreed when comparing UT and MT methods. This means that intrauterine echogenic fluid can be detected in the absence of vaginal discharge containing pus, in contrast, vaginal discharge containing pus can be detected in the absence of intrauterine echogenic fluid. In the authors' opinion these complicated results should confuse veterinarians trying to evaluate uterine health status in the field condition.

Most of the previous studies on reliability of UT or MT to estimate uterine status have focused on single usage.[8 16–18] The present study indicates that the combined usage of UT and MT could provide mostly equivalent results as CYT; the highest sensitivity was achieved when positive individuals were identified as either MT-positive or UT-positive, whereas the highest specificity occurred when results were positive by both MT and UT methods. Thus, the former application is recommended when practitioners hope to decrease false-negative results, and the latter is recommended when practitioners hope to decrease false-positive results. Usefulness of this combined methods should be verified in future studies with large number of animals at different times postpartum.

In conclusion, the accuracy of early postpartum uterine health status by the three methods depended heavily on the time postpartum. The combined use of UT and MT is a practical candidate for a cow-side monitoring method of uterine health.

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Competing interests None declared.

Ethics approval The Institutional Animal Care and Use Committee of Kitasato University approved the experimental protocol.

Data availability statement Data are available on request.

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References

1 Plaizier JC, King GJ, Dekkers JC, *et al.* Estimation of economic values of indices for reproductive performance in dairy herds using computer simulation. *J Dairy Sci* 1997;80:2775–83.
2 Krpálková L, Cabrera VE, Kvapilík J, *et al.* Associations between age at first calving, rearing average daily weight gain, herd milk yield and dairy herd production, reproduction, and profitability. *J Dairy Sci* 2014;97:6573–82.
3 Sheldon IM. The postpartum uterus. *Vet Clin North Am Food Anim Pract* 2004;20:569–91.10.1016/j.cvfa.2004.06.008
4 Griffin JF, Hartigan PJ, Nunn WR. Non-Specific uterine infection and bovine fertility. II. infection patterns and endometritis before and after service. *Theriogenology* 1974;1:107–14.
5 Sheldon IM, Price SB, Cronin J, *et al.* Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. *Reprod Domest Anim* 2009;44:1–9.

6 Mateus L, da Costa LL, Bernardo F, *et al*. Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. *Reprod Domest Anim* 2002;37:31–5.
7 Kasimanickam R, Duffield TF, Foster RA, *et al*. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology* 2004;62:9–23.
8 Barlund CS, Carruthers TD, Waldner CL, *et al*. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. *Theriogenology* 2008;69:714–23.
9 López-Helguera I, López-Gatius F, Garcia-Ispierto I. The influence of genital tract status in postpartum period on the subsequent reproductive performance in high producing dairy cows. *Theriogenology* 2012;77:1334–42.
10 McDougall S, Macaulay R, Compton C. Association between endometritis diagnosis using a novel intravaginal device and reproductive performance in dairy cattle. *Anim Reprod Sci* 2007;99:9–23.
11 Williams EJ, Fischer DP, Pfeiffer DU, *et al*. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology* 2005;63:102–17.
12 LeBlanc SJ, Duffield TF, Leslie KE, *et al*. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J Dairy Sci* 2002;85:2223–36.
13 McDougall S, Hussein H, Aberdein D, *et al*. Relationships between cytology, bacteriology and vaginal discharge scores and reproductive performance in dairy cattle. *Theriogenology* 2011;76:229–40.
14 Colin P. Postpartum uterine infection. In: Hopper RM, ed. Bovine reproduction. 1st edn. Mississippi: Starkville, 2015: 442–4.
15 Kasimanickam R, Duffield TF, Foster RA, *et al*. A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. *Can Vet J* 2005;46:255–9.
16 Dubuc J, Duffield TF, Leslie KE, *et al*. Definitions and diagnosis of postpartum endometritis in dairy cows. *J Dairy Sci* 2010;93:5225–33.
17 Peter AT, Jarratt GM, Hanlon DW. Accuracy of diagnosis of clinical endometritis with Metricheck™ in postpartum dairy cows. *Clinical Theriogenology* 2011;3:461–5.
18 Meira EBS, Henriques LCS, Sá LRM, *et al*. Comparison of ultrasonography and histopathology for the diagnosis of endometritis in Holstein-Friesian cows. *J Dairy Sci* 2012;95:6969–73.
19 Couto GB, Vaillancourt DH, Lefebvre RC. Comparison of a leukocyte esterase test with endometrial cytology for diagnosis of subclinical endometritis in postpartum dairy cows. *Theriogenology* 2013;79:103–7.
20 Bernard T, Jean-Pierre V, Barbara D, *et al*. Dictionary of veterinary epidemiology. 1st edn. Iowa, Ames, 1999.
21 de Boer MW, LeBlanc SJ, Dubuc J, *et al*. Invited review: systematic review of diagnostic tests for reproductive-tract infection and inflammation in dairy cows. *J Dairy Sci* 2014;97:3983–99.