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Use of a sanitary sheath at artificial insemination by nonprofessional technicians does not markedly improve pregnancy rates to artificial insemination in pasture-based dairy cows

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ABSTRACT

Plastic sanitary sheaths over artificial insemination (AI) guns have been used at the time of AI to improve hygiene at AI and fertility in cattle, but fertility responses have been variable in studies when AI was performed by professional inseminators. The aims of this study were to investigate whether the use of a sanitary sheath at the time of AI carried out by nonprofessional (do-it-yourself, or DIY) inseminators improves pregnancy rates to AI in pasture-based dairy cows and whether effects of sheaths are greater in cows with contaminated vulvas and in cows at increased risk of extended calving to conception intervals. Lactating dairy cows located in 10 pasture-based herds in a subtropical region of northern Australia were inseminated by herd-based DIY inseminators and assigned to be inseminated with (n = 3,655) or without (n = 3,969) a sanitary sheath, with potential effects assessed using multivariable logistic regression. Overall, use of a sheath at the time of AI did not significantly affect pregnancy rates to AI (36.3% for those inseminated without a sheath vs. 36.8% for those inseminated with a sheath; odds ratio: 1.02; 95% confidence interval: 0.92–1.11). Effects of using a sheath on pregnancy rates to AI varied by herd, with lower pregnancy rates with the use of sheaths in 1 herd and some evidence of increases in 3 herds. Unexpectedly, there was evidence that the effect of sheath on pregnancy rates was less positive (or more negative) when the vulva was classified as dirty before any cleaning of the vulva before insemination compared with when the vulva was classified as clean (interaction odds ratio: 0.75; 95% CI: 0.56–1.00). Interactions between sheath and other explanatory variables that could affect fertility were not significant; thus, there was no compelling evidence that the effect of using a sheath was modified by any of these variables. We conclude that the use of sheaths during AI of pasture-based dairy cows by DIY inseminators does not, on average, markedly improve pregnancy rates to AI. However, responses may vary between herds, and the response to sheaths may be inferior (i.e., less positive or more negative) when a cow's vulva is contaminated with feces or discharge at the time of AI compared with when the vulva is clean.
Key words: artificial insemination, fertility, sheath, dairy cow

INTRODUCTION

Considerable variation in pregnancy rates to AI have been recorded between inseminators (Buckley et al., 2003a; Morton, 2004) and can contribute to suboptimal reproductive and economic performance within dairy herds. Professional inseminators also achieved higher pregnancy rates compared with so-called do-it-yourself (**DIY**) inseminators in one substantial Australian study (Morton, 2004). Do-it-yourself inseminators are those who conduct inseminations only within herds that they work in and typically undertake fewer inseminations per year compared with professional inseminators. Differences in pregnancy rates between inseminators and between professional and DIY inseminators may be due in part to suboptimal AI practices. In a survey of Australian dairy herds, 36% of DIY inseminators performed fewer than 100 inseminations per year (Hope et al., 2013). Commonly, DIY inseminators do not undergo refresher training. Half of all DIY inseminators in the Australian survey had not received any AI training for at least 12 yr, and 29% of inseminators did not take adequate precautions to ensure adequate hygiene at the time of AI (Hope et al., 2013). In an Irish study, only 32% of the DIY operators had attended refresher courses since their initial training, whereas professional inseminators attended refresher courses annually and

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received extra training if reduced performance was noted (Buckley et al., 2003a). These results indicate that lack of experience and training and poor hygiene at the time of AI for some DIY inseminators could result in suboptimal AI practices and compromised pregnancy rates.

In cattle, bacterial contamination of semen and the vagina during the process of AI can reduce pregnancy rates to AI (Morrell, 2006). Inadequate attention to hygiene could also facilitate colonization of the reproductive tract with pathogens (Givens and Marley, 2008), detrimentally affecting fertility (Dubuc et al., 2010), ovarian function, and oocyte viability in high-producing dairy cows (Sheldon et al., 2014). These risks to fertility reinforce the need to maintain hygiene at the time of AI in cows. Fitting sanitary sheaths over AI catheters during AI has been shown to reduce the risk of uterine contamination of bacteria at the time of AI (Bas et al., 2011), but it has had variable effects on pregnancy rates to AI. Use of sheaths at the time of AI has failed to significantly improve nonreturn rates in dairy cattle (King et al., 1984; Richards et al., 1984) and pregnancy rates to AI in beef cattle (Kasimanickam, 2016) when experienced insemination technicians were used, whereas Bas et al. (2011) observed increased pregnancy rates to AI for second or greater services postpartum. Use of sanitary sheaths at the time of AI by DIY inseminators within pasture-based dairy cows has not previously been investigated. It is possible that use of sheaths by DIY inseminators improves pregnancy rates to AI because these inseminators are likely to have had less experience in inseminating cows and less training than professional inseminators.

Most studies that have examined the effects of using sanitary AI sheaths have assessed pregnancy rates across all study cattle pooled. This may have resulted in beneficial effects in some subgroups of cows going undetected. In one study conducted in dairy cattle, Bas et al. (2011) observed no significant improvement in first-service pregnancy rates to AI in dairy cattle when sheaths were fitted but observed an 11.5% increase in pregnancy rates to AI for second or greater services when a sheath was used at the time of AI. Negative associations between disease and time from calving to conception (Loeffler et al., 1999) and between disease and ovarian function (Opsomer et al., 2000) have been reported in dairy cows, as have weak negative associations between milk yield and reproductive performance (Rearte et al., 2018). Fecal contamination of the vulva may also increase the risk of bacterial contamination of the vagina and ascending infection from the vagina to the uterus, which could decrease pregnancy rates to AI (Dubuc et al., 2010; Galon et al., 2010; Salasel et al., 2010; Gilbert, 2011). It is possible that some subpopulations of cows at risk of poor reproductive performance are more susceptible to the detrimental effects of uterine contamination with bacteria at the time of AI and that cows with vulvas contaminated with feces may be at increased risk of subfertility at the time of AI. Accordingly, responses to fitting a sanitary sheath at the time of AI may vary with cow status for these and other variables. Extent of vulval contamination can also vary with housing design, bedding, and management (Cook, 2002; Lombard et al., 2010) and between seasons (Wagner et al., 2017). As a result, differences in these factors could also affect the risk of conception failure by influencing hygiene at the time of AI and so contribute to variations in responses to the use of sheaths in different studies.

The aims of this study were to investigate whether the use of a sanitary sheath at the time of AI carried out by DIY inseminators improves pregnancy rates to AI in pasture-based dairy cows and whether effects of sheaths are greater in cows with contaminated vulvas and in cows at increased risk of extended calving to conception intervals. We hypothesized that pregnancy rates increase with the use of a sheath at the time of AI of pasture-fed dairy cows by DIY inseminators and that sheaths have larger beneficial effects on pregnancy rates in cows with contaminated vulvas and in cows at increased risk of extended calving to conception intervals.

MATERIALS AND METHODS

Location of Experimental Herds and Insemination Practices

Predominantly Holstein-Friesian dairy herds (n = 10) from the Atherton Tableland region of northern Queensland were selected for this study. Herds were selected from clients of Tableland Veterinary Service who participated in a herd health program, used AI, and performed more than 250 inseminations per year and were willing to participate and record data required for the study. Cows grazed pastures that consisted predominantly of tropical (*Setaria sphacelata*) and temperate (*Lolium multiflorum*) pastures and were fed grain-based supplements at milking and, in some herds, preserved forages when pastures were limiting. Herds were visited at least monthly for veterinary monitoring of reproductive performance, maintained accurate records of calvings and reproductive and disease events, and entered data into commercial herd recording software (DairyWin, Epicenter, Massey University, NZ). Cows within herds were bred to AI according to the routine breeding practices for the herd. Voluntary waiting periods varied from 42 to 52 d (Table 1).

Within study herds, cows calved and were inseminated throughout the year with the exception of herds 2 and 9, which did not breed cows during some summers (January and February) to help avoid negative aspects of environmental heat load on fertility. Within herds, cows were subjected to AI following detection of estrus by herd workers or at a predetermined time following the application of treatments that synchronized estrous cycles. Ethics approval for procedures conducted in this study was provided by the James Cook University Experimentation Ethics Review Committee (approval no. A2242).

Sample Size Calculation

It was estimated that the number of AI needed to detect a 3% increase in pregnancy rate to AI (35% vs. 38%) with 2-sided α = 0.05, equal-sized groups, and statistical power of 0.80 was 4,042 cows in each treatment group (WINPEPI version 11.65; Abramson, 2011). This calculation disregarded clustering of AI within cows within herds.

Allocation of Treatment

Cows were selected for AI by herd staff according to the routine practices for the herd. Artificial inseminations were allocated to treatment groups based on cow identification number, with odd-numbered cows assigned to the no sanitary sheath (N) group and even-numbered cows assigned to the sanitary sheath (S) group.

AI Training and Process

Before commencement of the study, procedures for undertaking inseminations were discussed with each technician using a DIY inseminator checklist (Dairy Australia, 2013) as a training tool to help ensure that procedures associated with AI were standardized. Inseminators were also instructed to classify the vulva before any cleaning of the vulva before insemination as either dirty (D), where there was visual evidence of fecal contamination of the vulva that was likely to contaminate an AI gun, or clean (C), where visually there was little evidence of fecal contamination of the vulva and the risk of contamination of the AI gun with fecal material was low. On completion of an AI, the gun (for no sanitary sheath AI) or sheath was graded as either clean or dirty (no or distinct appearance of contamination with fecal or inflammatory material after AI, respectively). To facilitate the ease of recording, dirty sheaths or guns were classed as bad (B), and clean sheaths or guns were classed as good (G). Thus, each AI was classified as having 1 of 8 possible 3-digit codes: NCG, NCB, NDG, NDB, SCG, SCB, SDG, or SDB. Prior to the study, each of the participating DIY inseminators were trained in how to classify the vulva

Table 1. Descriptors of 10 herds used to assess effects of sheaths at AI on pregnancy rates in dairy cows and pregnancy rates to AI with no sheath or with a sheath at the time of AI

Herd no.	Herd size[1]	Peak daily production[2]		VWP[3] (d)	No. of AI		Days from calving to service[4]
		Milk volume (L)	Milk solids[5] (kg)		No sheath	Sheath	
1	458	31.7 (7.5)	2.1 (0.5)	49	234	232	97.5 (57–191)
2	918	24.3 (5.6)	1.7 (0.4)	46	1,569	1,568	97.0 (58–169)
3	200	28.4 (5.2)	1.9 (0.4)	50	405	306	104.0 (54–208)
4	253	—[6]	—[6]	51	41	32	91.0 (62–184)
5	309	26.5 (5.8)	1.7 (0.4)	47	341	287	93.0 (54–193)
6	262	25.4 (5.8)	1.6 (0.4)	45	286	254	119.0 (62–239)
7	187	—[6]	—[6]	52	78	71	102.0 (61–214)
8	316	28.5 (6.2)	1.9 (0.4)	43	614	596	91.0 (51–190)[7]
9	153	29.7 (5.4)	2.1 (0.4)	50	358	281	115.0 (58–319)[7]
10	226	23.3 (5.6)[8]	1.6 (0.4)[8]	42	43	28	97.0 (56–194)
Pooled					3,969	3,655	

[1]Average number of calvings per 12 mo, averaged over period from Apr. 1, 2014, to Aug. 31, 2017.

[2]Mean (SD in parentheses) of peak test-day production values for test days recorded in the first 120 d of lactation for all lactations in herd that commenced from Apr. 1, 2014, to Aug. 31, 2017.

[3]Voluntary waiting period. Estimated as the third percentile of all calving to service intervals (as validated by J. M. Morton, unpublished data) for all lactations in the herd that commenced from Apr. 1, 2014, to Aug. 31, 2017.

[4]Median (10th to 90th percentile in parentheses). Calving to service intervals for AI used in statistical analyses.

[5]Fat plus protein yield.

[6]No test data were available for cows calved from Apr. 1, 2014, to Aug. 31, 2017.

[7]Excluding 1 AI where the recorded calving to service interval was 1 d.

[8]Excluding 1 test day record where the recorded milk volume and milk solids were 101.3 L and 8.9 kg, respectively.

before AI and AI guns after AI. Herd managers were requested to record all services (AI and bull services) per standard procedures within herds, date of AI, sire of semen used, AI technician, and 3-digit codes.

The insemination procedure involved loading a thawed straw of frozen semen into an AI gun, fitting a standard plastic sheath securely over the AI gun, and then, where required, inserting the AI gun into a sanitary sheath (sanitary sheaths, 533 mm; IMV Technologies, L'Aigle, France). Artificial insemination was performed using the standard rectovaginal technique (Ax et al., 2000). The vulva was first wiped with a paper towel to remove any visible fecal material, the lips of the vulva were spread, and the AI gun was inserted into the vagina to the level of the external cervical os. Once the AI gun engaged the external cervical os, the sanitary sheath, when used, was manually retracted about 3 cm and the AI gun was directed cranially through the end of the sheath, through the cervix, and into the body of the uterus, where the semen was deposited.

Pregnancy Diagnosis and Assigning of Conception Dates

Inseminations were classified as resulting in pregnancy if the animal's first conception date for the lactation was the same as the AI date. Conception dates for each lactation were defined based on results of either transrectal ultrasound examination (Ibex Pro, Ibex, E.I. Medical Imaging, Loveland, CO) or manual pregnancy diagnoses by veterinarians, with most cows examined 35 to 63 d after AI. If animals had 1 or more services recorded from 14 d before to 10 d after the estimated conception date, the most recent of those services was chosen as the conception date.

Other Data Collection and Data Management

Dates of birth, calving dates, disease events, test-day data, AI data, and details of pregnancy diagnoses were extracted from the herd recording software package and imported and manipulated in Microsoft Excel (Microsoft Corp., Redmond, WA) and Stata (version 15; StataCorp, College Station, TX). Each cow was classified as having had 1 or more clinical diseases in the first 30 d of lactation if the calving was an abortion or premature calf or if the cow was recorded as having dystocia, milk fever, retained placenta, or vaginal discharge in the first 30 d of lactation.

Milk production was assessed for each lactation as the cow's highest daily milk volume (L) and milk solids (kg; i.e., fat plus protein) yield of recorded test-day results in the first 120 d of lactation. Herds 3, 4, 7, and 10 were excluded from statistical analyses of the influence of milk production on the effect of sheaths on cow pregnancy rate because there were no or few inseminations in cows in some milk production categories in these herds.

Total numbers of inseminations performed by each AI technician in their study herd in the 12 mo preceding the herd's study commencement date were calculated. Each included AI was classified as either performed within the period when the AI technician had performed fewer than approximately 50 inseminations with a sheath (including inseminations that were ineligible or lost to follow-up) or performed after that period (i.e., after the day of the technician's 50th insemination with a sheath).

Eligibility of Inseminations for Analyses

Study inseminations were selected from those with N or S (signifying no sheath and sheath, respectively) as the first character in the AI's 3-digit code. Inseminations were ineligible if they were performed in nulliparous heifers, if they were after the cow's first conception date for the lactation, or if any intervals from the recorded conception date for the lactation to the date of pregnancy diagnoses were less than 29 d. Inseminations were lost to follow-up (i.e., the success of the AI could not be assessed) if there were no pregnancy tests (positive or negative) between 29 and 281 d after AI in the same lactation.

Statistical Analyses

Statistical analyses were performed using logistic regression models fitted using Stata. Effects of sheath on pregnancy (cow did or did not become pregnant to that AI) were first assessed with herd also fitted as a fixed effect using Stata's logistic command. The interaction between sheath and herd was then fitted. For each model, the significance of all interaction terms jointly was assessed using likelihood ratio test *P*-values. As there was strong evidence of interaction between sheath and herd (large and relatively precise interaction terms; low *P*-values for interaction terms), we retained these interaction terms in models when assessing interactions between sheath and each of vulval status pre-AI, post-AI classification, cow parity, clinical disease in the first 30 d of lactation, calving to service interval, service number within lactation, peak daily milk volume, or peak daily milk solids. The terms for interactions between sheath and these variables provided estimates of the change in effect of sheath on odds of conception at various values of the variable relative to the effect of sheath at the reference level of the variable. For these analyses, 9 variables were either categorical (vulval

status pre-AI, post-AI classification, clinical disease in the first 30 d of lactation, whether the AI was within the first 50 sheath inseminations by that technician, cow parity, service number, and total number of inseminations performed by the AI technician before study commencement date, with categories further collapsed for the latter 3 variables) or continuous variables that we categorized (peak daily milk volume and peak daily milk solids). For calving to service interval, linear and quadratic terms were both fitted, along with terms for interactions between each of these and sheath. The total number of inseminations performed by the AI technician before the study commencement date and whether the AI was within the first 50 sheath inseminations by the technician were used to assess effects of AI technician experience on the effect of sheaths. The results indicated that, in herd 8, conception rates were lower for AI where sheaths were used; this was further explored by assessing interaction between sheath and AI technician within this herd. The same approach was used as described above but without herd fitted.

For all of these analyses, clustering of AI within herd was accounted for, but the actual hierarchical clustering (AI within lactation, lactation within cow, and cow within herd) was more complex. To explore effects of disregarding this complexity, a 3-level, multilevel model was also fitted using Stata's melogit command, with AI nested within cow and cow nested within herd, with sheath fitted simultaneously as a fixed effect and with a random slope at herd level. An unstructured variance–covariance structure was used to allow for any correlation between the random intercept for herd and the random slope. We also attempted to fit a 4-level, multilevel model with AI nested within lactation, lactation nested within cow, and cow nested within herd, but this model did not converge after 100 iterations.

RESULTS

Herd Demographics

Descriptors of the study herds in relation to herd size, mean peak daily milk yield, number of study inseminations, and calving to service intervals are presented in Table 1.

Numbers of Study Inseminations and Adherence to Allocation of Treatment

Study inseminations were performed from April 7, 2014, to August 31, 2017. In total, 13,564 inseminations were performed in cows in the 10 study herds within the study period. Of these, 8,597 inseminations were selected by the farmer for the study (63.4%; range between herds: 27.0–93.3%), with 8,124 being eligible study inseminations. Of those, 500 (6.2%; range between herds: 2.2–15.4%) were lost to follow-up as there were no pregnancy tests (positive or negative) between 29 and 281 d later in the same lactation. The distribution of the remaining 7,624 inseminations by herd is shown in Table 1. These 7,624 inseminations were performed in 3,924 lactations (mean of 1.94 inseminations per lactation; range: 1–16) in 3,062 cows (mean of 1.28 lactations per cow; range: 1–3), so the average number of inseminations per cow was 2.49 (range: 1–16).

Of the 7,624 inseminations, virtually all had the correct treatment recorded. The incorrect treatment (i.e., inseminations with a sheath in odd-numbered cows or inseminations with no sheath in even-numbered cows) was recorded as having been used for 280 inseminations (3.7%; range between herds: 0.0–11.0%). Of these 7,624 inseminations, 3,655 were performed with sheaths, with the inseminator identified for 3,253. These 3,253 AI were performed by 26 inseminators with a median of 36 AI per inseminator (range: 1–578).

Baseline Values for Treatment Effects

Baseline values by treatment (sheath or no sheath) are shown in Table 2. All variables were similar for AI with no sheaths and AI with sheaths except post-AI classifications, where a greater percentage of AI with sheaths were classified as bad.

Pregnancy Rates to AI

Numbers of inseminations and pregnancy rates to AI by treatment (no sheath or sheath) are shown by herd in Figure 1. The odds ratio for the effect of sheath at AI in all herds pooled was 1.02 (95% CI: 0.92–1.11; $P = 0.754$). If the true effect is 1.02, the increase in conception rates to AI associated with use of sheaths from 36.3% (the crude conception rate in the no-sheath group) would be 0.5 percentage points, to 36.8%. If the true effect is 0.92, the decrease in conception rates would be 1.9 percentage points, to 34.4%. If the true effect is 1.11, the increase in conception rates would be 2.4 percentage points, to 38.7%. The effect of sheath varied by herd (sheath × herd interaction, $P < 0.001$), with lower conception rates for sheaths in 1 herd (herd 8; Figure 1) and some evidence of increases in 3 herds (herds 1, 4, and 5; Figure 1). To assess for any bias in overall results due to farmers selecting only some AI in cows for the study from all AI performed on the day or allocating incorrect treatment, we also assessed effects of sheaths using only inseminations on days where all AI in cows were enrolled in the study and the correct treatment was allocated (n = 6,372 AI). Results were

generally similar to when all 7,624 AI were used. The odds ratio for the effect of sheath at the time of AI in all herds pooled was 1.01 (95% CI: 0.91–1.12; P = 0.844), and similarly, an interaction between sheath and herd was detected ($P < 0.001$). The estimated effects of sheath by herd were also similar (within 11%) except for herd 4, where the odds ratio estimate for the effect of sheath declined from 2.43 to 1.72 (a 32% reduction). The similarity of these results to those when all 7,624 AI were used indicates that there was no important bias in overall results due to farmers selecting only some AI in cows for the study from all AI performed on the day or allocating incorrect treatments.

Separately for each of 10 variables—vulval status pre-AI, post-AI classification, cow parity, clinical disease in the first 30 d of lactation, service number, calving to service interval, peak daily milk volume, peak daily milk solids, AI technician experience (total number of AI performed by the AI technician in the 12 mo before study commencement date in their herd), and whether the AI was within the first 50 sheath AI by the technician—the variable was fitted along with the interaction between sheath and that variable. The interaction between sheath and herd was not accounted for by any of these 10 potential explanatory variables and the interaction between sheath and that variable. After separately adjusting for each of these variables along with the interaction between sheath and that variable, the sheath × herd interaction term odds ratios changed by 29% or less for vulval status pre-AI and by 9% or less for the remaining 9 variables, and P-values for the interaction between sheath and herd remained low (<0.001 to 0.002).

Results were broadly similar after accounting for clustering of both AI within cow and cow within herd. The odds ratio for the effect of sheath at the time of AI at zero intercept random effect was 1.07 (95% CI: 0.88–1.29; P = 0.498), and there was evidence that the effect of sheath varied by herd (random slope variance estimate on logit scale: 0.05; 95% CI: 0.01–0.019).

The reduced pregnancy rate to AI where sheaths were used in herd 8 was further assessed by AI technician. Of the 1,210 inseminations used in analyses, 1,205 were performed by 3 technicians. The magnitudes of the reductions in pregnancy rate were similar for each of these 3 technicians. Pregnancy rates for inseminations where sheaths were not used and were used, respectively, were as follows: technician 1, 34.5% (78/226) and 24.1% (52/216); technician 2, 36.3% (66/182) and 26.1% (41/157); technician 3, 39.6% (80/202) and 28.4% (63/222). There was also no evidence that odds of pregnancy differed markedly by technician. The P-value for interaction between sheath and technician was 0.994, and the interaction odds ratio point estimates for the added effects of sheath within each of technicians 2 and 3 (over the effect of sheath within technician 1) were very close to 1 (1.03 and 1.00).

Pregnancy rates by treatment (sheath or no sheath) are shown by vulval status pre-AI and post-AI classification in Table 3. For vulval status pre-AI, unexpectedly, there was evidence that the effect of sheath on pregnancy rate is less positive (or more negative) when the vulva was classified as dirty compared with when the vulva was classified as clean. The interaction odds ratio estimate for the term for interaction between sheath and vulval status where "clean" was the refer-

Table 2. Baseline values by treatment (no sheath or sheath) for AI in dairy cows used to assess the effects of sheaths at AI on pregnancy rates to AI in dairy cows

Variable	No sheath	Sheath
No. of study inseminations[1]	3,969	3,655
Vulval status pre-AI: dirty[2]	24.4 (938/3,845)	24.3 (878/3,615)
Post-AI classification: bad[2]	5.5 (199/3,638)	7.2 (242/3,367)
Cow parity[3]	2 (1–5; 3,512)	2 (1–5; 3,225)
Clinical disease in first 30 d of lactation[2]	15.6 (619/3,961)	15.5 (565/3,648)
Service number within lactation[2]		
1	51.6 (2,047/3,969)	51.2 (1,872/3,655)
2	26.5 (1,050/3,969)	25.6 (935/3,655)
≥3	22.0 (872/3,969)	23.2 (848/3,655)
Calving to service interval[3] (d)	97.5 (57–188; 3,968)	100.0 (57–192; 3,654)
Peak daily milk volume[4] (L)	27.7 (6.5; 3,453)	27.6 (6.4; 3,233)
Peak daily milk solids[4,5] (kg)	1.87 (0.45; 3,453)	1.86 (0.44; 3,233)

[1]Denominators are less than these where data are not available.

[2]Percent (no.).

[3]Median (10th to 90th percentile; number of AI where data available).

[4]Mean (SD; number of AI where data available).

[5]Fat plus protein yield.

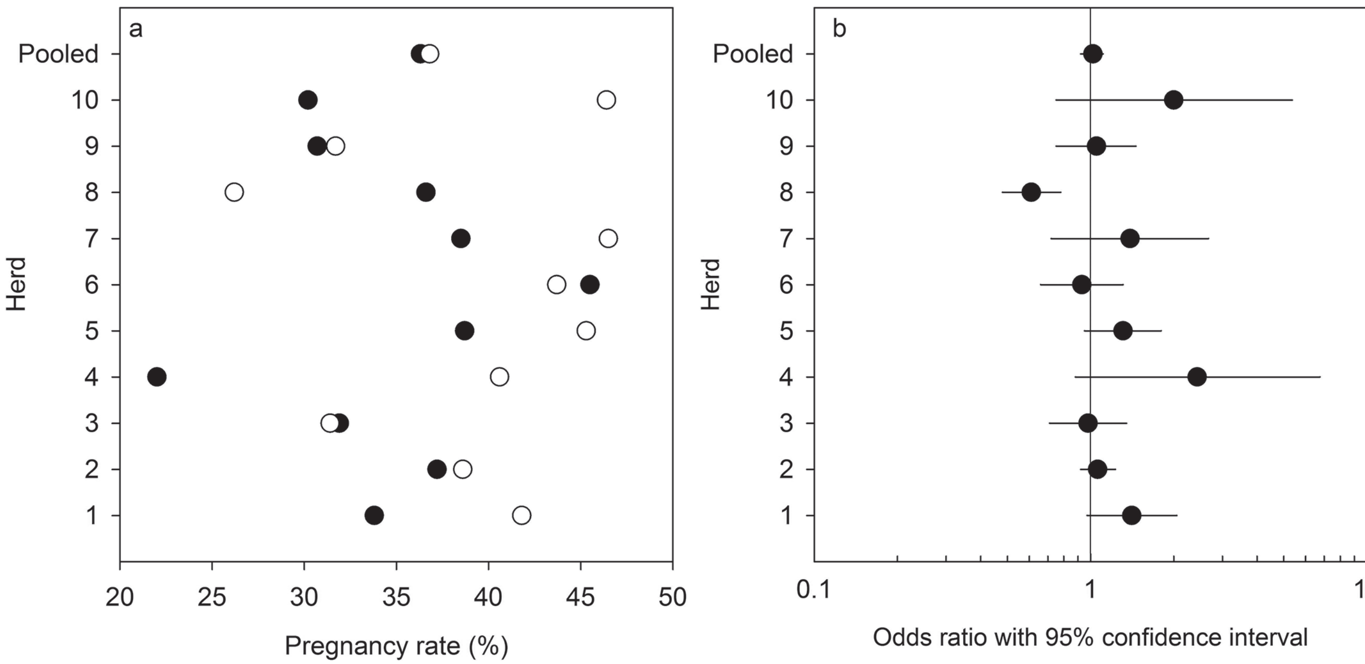

Figure 1. (a) Pregnancy rates when no sheath (●) or a sheath (○) was used at the time of AI and (b) adjusted odds ratios for effects of sheath (sheath relative to no sheath; ●) and 95% confidence intervals (horizontal lines) within herds (1 = estimate is no effect). Where the 95% confidence interval crosses 1 (vertical line), the pregnancy rates do not differ significantly at the 0.05 level between treatments within the herd. Where the confidence interval does not cross 1, the pregnancy rates differ significantly at the 0.05 level between treatments within the herd.

ence category was 0.75 and significantly less than 1.00 ($P = 0.049$).

For post-AI classification (Table 3), cow parity, clinical disease in the first 30 d of lactation, service number, and peak daily milk production P-values for interaction odds ratios were high ($P \geq 0.172$), thus providing no evidence that the effect of sheaths varied between categories of these variables. For calving to service interval, the P-value for joint interaction terms (sheath by linear and quadratic term for calving to service interval) was 0.067, providing some evidence that the effect of sheath on conception rate differed by calving to service interval. However, the odds ratios for the effect of sheath at the 10th and 90th percentiles of calving to service interval (57 and 190 d) were only slightly higher than at the median calving to service interval of 99 d; interaction odds ratios at these intervals were 1.052 and 1.039. The P-value for interaction between

Table 3. Pregnancy rates for AI in cows where no sheath or a sheath were used by vulval status pre-AI and by post-AI classification

	No sheath		Sheath				
Item	No. of AI	% resulting in pregnancy	No. of AI	% resulting in pregnancy	Interaction odds ratio[1]	95% CI	P-value
Vulval status pre-AI							
Clean	2,907	36.5	2,737	37.8			
Dirty	938	35.4	878	33.1	0.75	0.56–1.00	0.049
Not recorded	124	37.9	40	47.5			
Pooled	3,969	36.3	3,655	36.8			
Post-AI classification							
Good	3,638	36.3	3,367	37.3			
Bad	199	36.7	242	28.5	0.74	0.49–1.14	0.172
Not recorded	132	36.4	46	43.5			
Pooled	3,969	36.3	3,655	36.8			

[1]Odds ratios for terms for interaction between sheath and each of vulval status pre-AI and post-AI classification in their effects on pregnancy rate. For example, for vulval status pre-AI, the interaction odds ratio of 0.75 estimates the ratio of the odds ratio for the effect of sheath when the vulva is dirty relative to the odds ratio for the effect of sheath when the vulva is clean. Thus, this result provides evidence that the effect of sheath on pregnancy rate is less positive (or more negative) when the vulva is dirty compared with when the vulva is clean. Wald P-values are shown; likelihood ratio test P-values for interaction terms were the same as these.

sheath and total number of inseminations performed by the AI technician before study commencement date was 0.490 and for interaction between sheath and AI within the first 50 sheath AI by technician was 0.412, thus providing no evidence that the effect of sheaths varied between categories of these variables.

DISCUSSION

The results of this study, involving 7,624 inseminations performed by DIY inseminators, demonstrate that there was no large advantage, on average, in using a sheath at the time of AI in pasture-based dairy cattle or in cows with contaminated vulvas at the time of AI. As a result, we found no compelling reason to recommend the routine use of sheaths at the time of AI in pasture-based dairy cows that are inseminated by nonprofessional technicians.

The results of this study support the results of studies conducted in dairy (King et al., 1984; Richards et al., 1984) and beef (Kasimanickam, 2016) cattle in which the use of a sanitary sheath at the time of AI did not significantly affect nonreturn and pregnancy rates to AI. We estimate that a 2 to 3% or more increase in pregnancy rates would justify routine use of sheaths, and the upper limit of the 95% confidence interval for the estimated effect of sheaths was 1.11, which equated to an approximately 2.4% increase. Assuming that there was no prior information about the effect of sheaths in cows such as those in the current study, the 95% confidence interval can be interpreted as if it were a Bayesian 95% credible interval, and the true effect would be unlikely to be near the upper limit of the 95% confidence interval. Thus, we conclude that it is unlikely that sheaths increase pregnancy rates by enough to justify their routine use across all herds.

Several factors can influence fertility in cows (Buckley et al., 2003a,b; Chebel et al., 2004; Morton, 2004) and could modify effects of sheaths. We therefore included in various logistic regression models interaction terms between sheath and each of herd and 10 other factors that could have potentially interacted with the use of sanitary sheaths in their effect on pregnancy rates to AI. Significant interactions with treatment (use of a sanitary sheath or not) within these multivariable models were detected only with herd and whether the vulvas of cows were classified as dirty or clean pre-AI. Our results provide evidence that the effect of sheath on pregnancy rate is less positive (or more negative) when the vulva is dirty compared with when the vulva is clean and indicate that the use of an AI sheath may be contraindicated when the vulva is dirty.

In cattle, vulval contamination may increase the risk of bacterial contamination of the vagina and ascending uterine infection, which could in turn decrease pregnancy rates to AI (Dubuc et al., 2010; Galon et al., 2010; Salasel et al., 2010; Gilbert, 2011). It is plausible that any increase in bacterial contamination of the vulva could increase the risk of conception failure. Risk and extent of vulval contamination with feces can be increased by low fecal consistency, cranial sloping of the vulva, anatomical defects such as perineal lacerations, and an incompetent vulval seal (Hillman and Gilbert, 2008). Vulval conformation in mares has been reported to affect pregnancy and live foal rates (Hemberg et al., 2005). We hypothesized that using a sheath at the time of AI increases pregnancy rates based on the assumption that sheaths result in improved hygiene. Therefore, our result that the effect of the use of a sheath on pregnancy rate was less positive (or more negative) when the vulva was classified as dirty compared with when the vulva was classified as clean was unexpected. This result may have been because when the vulva is contaminated with fecal material or there is evidence of discharge, the use of a sanitary sheath may inadvertently increase bacterial contamination of the cranial vagina and thereby decrease the probability of pregnancy. Bas et al. (2011) demonstrated that the use of sanitary sheaths at the time of AI reduced bacterial contamination of the tips of AI catheters after AI, indicating that the use of a sanitary sheath improves hygiene at the time of AI. In our study, perhaps some inseminators paid reduced attention to hygiene at the time of AI when sheaths were used, instead relying on the sheath to maintain hygiene. It is also possible that when a vulva is dirty before AI, use of a sanitary sheath inadvertently results in the transport of more bacteria into the vagina than when an AI gun is not covered with a sheath. Although the causative factor for a reduction in pregnancy rates to AI when sanitary sheaths were used in cows with dirty vulvas cannot be determined, we conclude that there is no advantage to using a sheath when a cow's vulva is dirty at the time of AI, and, in fact, the probability of pregnancy may be reduced if a sheath is used in those circumstances.

The site of retraction of a contaminated sheath at the level of the cranial vagina or after engaging the external cervical os could potentially affect the number of bacteria deposited into the uterus at the time of AI and influence pregnancy rates to AI. We are, however, not aware of studies that have examined the site of retraction on uterine contamination or pregnancy rates. Some differences exist between studies on the site of sheath retraction, with Bas et al. (2011) and Kasimanickam (2016) describing retracting the sheath in the cranial portion of the vagina, adjacent to the os, whereas King et al. (1984) retracted the sheath 2 to 3 cm before the external cervical os and Richards

et al. (1984) retracted the sheath when the opening of the cervix was reached, which was similar to our study. The type of sheath also varied between studies, with more rigid polyvinyl chloride sheaths being used in some studies (King et al., 1984; Bas et al., 2011; Kasimanickam, 2016); both polyvinyl chloride and thinner and more flexible sheaths were used in other studies (Richards et al., 1984), and a thin, flexible sheath was used in this study. Although some potential exists for differences in the site of sheath retraction or type of sheath to influence fertility, it would seem that if such an effect existed it is likely to be small. When taken together, despite minor differences in location of where sheaths were retracted or use of different sheaths, most studies reported no significant effects of using a sheath on pregnancy rates to AI. For 9 other factors that could influence pregnancy rates to AI, we found no evidence that they modify responses to sheaths. We hypothesized that factors that decrease the risk of pregnancy at AI may interact with the use of sanitary sheaths to confer a fertility advantage in cows that are at increased risk of being subfertile at AI. It could be that any potential disadvantages associated with factors such as clinical disease in the first 30 d after calving were overcome by the time cows were presented for AI in this study. It is also possible that effects of sheaths on pregnancy rates are not influenced by some of these 9 factors. However, we cannot rule out such interactions because small to modest interactions could be economically important, and our study had low statistical power for detecting weak interactions.

Use of a sanitary sheath was associated with an improvement in pregnancy rates to AI for second or greater services postpartum in dairy cows in one study, but no such improvement was evident for first services (Bas et al., 2011). Pregnancy rates in dairy cattle at the first service postpartum can be greater than that at subsequent services (Silva et al., 2009; Inchaisri et al., 2011), and in a study by Bas et al. (2011), a 9% reduction in pregnancy rates occurred between the first and second or greater services where no sheath was used (43.0%, n = 194 vs. 32.3%, n = 315; $P < 0.007$). However, no such reduction was evident in the present study. Thus, perhaps the use of a sanitary sheath in the study by Bas et al. (2011) conferred a fertility advantage in second or greater services because it in some way ameliorated the cause of the reduction in pregnancy rate with service numbers, whereas in the current study, no major decline in fertility with service number was evident, so no fertility advantage could be conferred by using a sheath. It is also possible that the ability of cows to mount an adequate immune response within the uterus declined after the time of first service in the study population used by Bas et al. (2011); this could explain the responses to sheaths only after the first service in that study. Cows in that study were also less variable in breed, were milked thrice daily (rather than twice daily as in the current study), were fed a TMR (rather than being pasture-based and fed a partial mixed ration as in the current study), had greater milk yields, and, on average, at second or greater services had greater calving to service intervals (171–174 d compared with 148 d in the present study). Another difference between the 2 studies was that in the study by Bas et al. (2011), 1 inseminator performed all 996 study inseminations, more than any individual inseminator in the present study. Statistical power in the present study was greater, with almost 7 times as many inseminations performed across multiple herds, providing a more precise estimate of the overall effect of sheaths across the target population represented by our study population (DIY inseminations in pasture-based dairy cows).

We considered that the experience of inseminators before commencing the study and experience with the use of sanitary sheaths during the study could both influence the results associated with sheaths. Experience with AI and use of sheaths have been implicated as potential causes of variation in AI pregnancy rates (Richards et al., 1984). However, in the present study, the number of inseminations by the AI technician in the 12 mo before the commencement of the study did not significantly affect response to the use of sanitary sheaths. Similarly, pregnancy rates with and without a sanitary sheath were not significantly affected when the number of inseminations with a sheath was within the technician's first 50 sheath AI or after that. Thus, we found no compelling evidence that the experience of the DIY inseminator on enrollment alters the effect of sheaths on pregnancy rates and that most inseminators quickly adapted to the use of sanitary sheaths.

The causes of the interaction between sheath and herd remain undetermined. Each inseminator performed AI in only one of the study herds, so the interaction between sheath and herd could have been due, in part or whole, to interaction between sheath and inseminator. However, a major contributor to the interaction between sheath and herd was a large reduction in pregnancy rates in herd 8 with the use of sanitary sheaths (36.6 vs. 26.2%; $P < 0.001$; Figure 1), and we found no direct evidence for interaction between sheath and inseminator in this herd. The magnitude of the decline in pregnancy rates to AI was similar across all 3 inseminators that performed AI in this herd, with each inseminator performing between 339 and 442 inseminations. In all herds before commencing the study, inseminators received training in how to use sheaths in an attempt to minimize any negative effect of introduc-

ing this technique on pregnancy rates to AI. This training, if uniformly effective, would be expected to reduce any interaction between sheath and inseminator. The interaction between sheath and herd was also not accounted for by 10 potential explanatory variables and their interaction with sheath, including vulval status pre-AI. For example, any differences in the prevalence of dirty vulvas in herd 8 would not have caused a reduction in pregnancy rates to AI in herd 8 with the use of sheaths. Individual sheaths were not inspected before distribution to participating herds, so there remains a possibility that sheaths used in this herd were somehow physically defective or were contaminated with an agent or an organism that could detrimentally affect fertility. These seem unlikely. Another possibility could be reduced hygiene when a sanitary sheath was being used in this herd but not in other herds. It is also possible in a large study such as ours that compliance to the protocol varied within and between herds, which may have affected the results, although training was used to try to ensure that treatments were applied as planned. Nevertheless, these results show that responses to sheaths may vary by herd, with decreases in pregnancy rates to AI in some herds and possibly increases in other herds.

Cows could receive more than 1 insemination in the same or different lactations, and as we allocated treatment based on permanent cow identification numbers, within cows all study inseminations had the same treatment. It is highly unlikely that there was any systematic difference in cow attributes between odd- and even-numbered cows, but this design could cause confounding by service number, for example, if pregnancy rates to first AI differed between treatment groups. There was no evidence of such confounding in our study; pregnancy rates did not vary substantially by service number, and the distributions of study inseminations by service number within lactation were virtually identical for AI in the 2 treatment groups (no sheath or sheath; Table 2).

CONCLUSIONS

The results of this study demonstrated that any positive effect of the use of sheaths on pregnancy rates to AI by nonprofessional inseminators in pasture-based dairy cows is, at most, minimal. Effects of sheaths varied between herds, with lower pregnancy rates per AI accompanying the use of sheaths in 1 herd and evidence of increases in 3 herds. Unexpectedly, when the cow's vulva was visibly contaminated with feces or discharge pre-AI, effects of sheaths were inferior compared with when they were used with cows with clean vulvas. No significant interactions were detected between the use of sheaths and the degree of soiling of AI guns after AI, parity, clinical disease in the first 30 d of lactation, service number, calving to service interval, peak daily milk volume, peak daily milk solids, or AI technician experience before the study and during the study. We conclude that use of sheaths during AI in pasture-based dairy cows by DIY inseminators does not, on average, markedly improve pregnancy rates to AI. However, responses may vary between herds and may be inferior when a cow's vulva is contaminated with feces at the time of AI compared with when the vulva is clean. Our results do not support the routine use of sheaths during AI of pasture-based dairy cattle by DIY inseminators.

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