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Reproduction in Domestic Animals

Influence of Practitioner Expertise During Early Pregnancy Diagnosis on Pregnancy Loss Rate: a Controlled, Blinded Trial

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1 ***Influence of Practitioner Expertise During Early***
2 ***Pregnancy Diagnosis on Pregnancy Loss Rate: a***
3 ***Controlled, Blinded Trial***

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15 **Running head: PD-US practitioner experience and pregnancy loss**

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SUMMARY

A controlled field trial was conducted to assess the potential influence of practitioner inexperience during early pregnancy diagnosis with ultrasound (PD-US) on the risk of pregnancy loss. A veterinarian with more than 10 years' experience in PD-US (Vet-A) and a veterinarian with fewer than 12 months' experience at the start of the study (Vet-B) visited the same dairy farm once a week for 33 and 26 weeks, respectively. The two veterinarians did not interact with each other at any time during the study, nor did they know that their data would later be used in this study. Using the same farm scanner, they performed PD-US at 28-34 d after breeding, together diagnosing 915 pregnancies. All cows were re-checked at 49-56 d after artificial insemination, and cows no longer pregnant were recorded as having suffered pregnancy loss. Although Vet-A and Vet-B diagnosed a similar proportion of pregnancies (58.44±16% vs 56.96±18%, $P > 0.05$), the rate of pregnancy loss was significantly higher among cows diagnosed by Vet-B (10.41±11.2% vs 4.87±9, $P = 0.029$). In addition, among cows diagnosed by Vet-B, the rate of pregnancy loss was significantly higher among cows diagnosed while he had fewer than 12 months' PD-US experience (11.17±12.14%) than among cows that he diagnosed later (7.14±11.01%, $P = 0.038$); in fact, this latter loss rate was comparable to that among cows diagnosed by Vet-A during the same period (3.51±9.83%, $P = 0.620$). These results suggest that inexperience with PD-US during the late embryonic period can increase risk of early pregnancy loss, supporting the need for proper training.

Key words: ultrasound, reproductive management, bovine reproduction

48 INTRODUCTION

49 For technical and economic reasons, bovine practitioners perform early pregnancy
50 diagnosis (PD) during the late embryonic period (Romano et al. 2007), frequently using
51 transrectal amniotic sac visualization with ultrasound (US) (Gandy et al. 2001).
52 Typically, the PD-US technique is conducted 28-35 days after artificial insemination,
53 and pregnancies are confirmed 4-6 weeks later (Fricke et al., 2016).

54 PD-US provides immediate results, is less invasive than transrectal palpation
55 (Vaillancourt et al. 1979), is accurate when performed by trained veterinarians, and can
56 be used to age fetuses and detect twin pregnancies (Romano et al. 2016). While it is
57 assumed not to harm the conceptus (Kahn 1992, Ball and Logue 1994, Miller 2008),
58 several researchers have suggested that proper training in PD-US is important for
59 ensuring the safety as well as accuracy of the technique (Romano et al. 2016, Fricke et
60 al. 2016). Nevertheless, little is known about how the expertise of the person
61 performing PD-US affects risk of pregnancy loss.

62
63 Therefore, we performed a controlled, blinded field trial to assess the effect of
64 practitioners' PD-US experience on the rate of pregnancy loss.

66 MATERIAL AND METHODS

67 The study was conducted on a commercial dairy farm in eastern Spain with 470 cows in
68 lactation with the following characteristics: mean age at first calving, 23.9 mo; mean
69 number of lactations, 2.3; mean milk yield per cow per year, 10,580 L. On this farm
70 during the study period, estrus was synchronized after a waiting period of 70 days after
71 parturition using the G6G/ovsynch protocol for first artificial insemination (Bello et al.
72 2006). This protocol involves presynchronization with PGF, followed 2 d later with

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3 73 GnRH (100 µg) and then 6 d later with the first GnRH injection of Ovsynch, after which
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5 74 the Ovsynch program is completed. The entire protocol from presynchronization to
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7 75 timed artificial insemination takes 18 d. A pedometer system was used to detect estrus
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9 76 in subsequent inseminations. The farm was visited weekly by two practitioners, who
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11 77 performed routine reproductive explorations. PD-US was performed at 28-34 days after
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13 78 artificial insemination using an Ibex Pro portable ultrasound machine equipped with a
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15 79 7.5-MHz linear transducer. Pregnancy was diagnosed based on the presence or absence
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17 80 of fetal heartbeat (Kastelic and Ginther 1989) and confirmed at days 49-56 after
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19 81 artificial insemination.
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25 83 All activities within the study were routine farm practises, with no ethical approval
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27 84 required following the European Union Directive 2010/63/UE.
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32 86 Between March 2014 and December 2015, a controlled, blinded field trial was
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34 87 conducted. During this period, the farm was visited once weekly by Veterinarian A
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36 88 (Vet-A), who had more than 10 years' PD-US experience, and by Veterinarian B (Vet-
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38 89 B), who had fewer than 12 months' PD-US experience at the start of the study. Vet-B
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40 90 had obtained his veterinary medicine degree 18 months before participation in this
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42 91 study, and during that time, he had been working in the field. Vet-B received training
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44 92 from Vet-A in reproductive check-ups and pregnancy diagnosis over a 1-month period,
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46 93 when they performed routine reproduction check-ups together at commercial farms.
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48 94 Vet-B performed all explorations and diagnoses on his own under the supervision of
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50 95 Vet-A. After 1 month, Vet-A considered that Vet-B was capable of performing PD-US
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52 96 as well as other reproductive health procedures entirely independently.
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3 98 PD-US was performed as described (Baxter and Ward, 1997; Romano et al., 2006). In
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5 99 brief, the practitioner removed the feces from the rectum, introduced the lubricated
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7 100 transducer and positioned it dorsal to the genital tract, then advanced it cranially. The
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9 101 uterus was not manipulated unless absolutely necessary. The ovaries were scanned only
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11 102 if problems arose when imaging the conceptus. A positive diagnosis of pregnancy was
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13 103 made if the allantochorion and embryo in the uterine lumen were visualized and a
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15 104 heartbeat was detected. The reproductive tract was not examined further once the
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17 105 embryo was found, in order to minimize risk of additional trauma to the uterus and
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19 106 conceptus.
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23 107 The two veterinarians did not interact with each other, nor did they know that their data
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25 108 would later be used. After the conclusion of the study period, the veterinarians
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27 109 consented to have their data analyzed and published.
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29
30 110 Vet-A visited the farm 33 times and Vet-B 26 times, diagnosing a total of 915 early
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32 111 pregnancies at similar gestational ages (Vet-A, 31.76 ± 2.09 d; Vet-B, 31.59 ± 1.89 d) on
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34 112 cows with similar lactations (Vet-A, 2.33 ± 1.24 lactations/cow; Vet-B, 2.11 ± 0.95
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36 113 lactations/cow). All pregnant cows were re-evaluated at 49-56 d after artificial
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38 114 insemination; those no longer pregnant were considered to have suffered early
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40 115 pregnancy loss. Neither veterinarian had access to pregnancy loss data from the other
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42 116 colleague during the study.
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47 118 By the end of May 2015, Vet-B had acquired 12 months' PD-US experience, so we
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49 119 conducted an additional analysis to compare pregnancy loss rates of Vet-A and Vet-B
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51 120 before and after this point. Before this point, Vet-A visited the farm 26 times and
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53 121 diagnosed 425 pregnancies, while Vet-B visited the farm 19 times and diagnosed 296
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3 122 pregnancies. After this point, Vet-A visited the farm 7 times and diagnosed 102
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5 123 pregnancies, while Vet-B visited the farm 7 times and diagnosed 92 pregnancies.
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11 125 **Statistics**

12 126 SPSS® 22.0 (IBM, New York, NY) was used. Since data showed a skewed distribution
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14 127 based on the Kolmogorov-Smirnov test, differences between practitioners were assessed
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16 128 for significance using non-parametric Mann-Whitney analyses. Threshold of
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18 129 significance was defined as $P < 0.05$.
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22 131 **RESULTS**

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25 132 Based on subsequent pregnancy diagnosis at the gold standard time point of 49-56 days,
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27 133 Vet-A diagnosed pregnancy with a sensitivity of 100%, specificity of 93.59%, positive
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29 134 predictive value of 95.13% and negative predictive value of 100%. The corresponding
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31 135 diagnosis parameters of Vet-B were 100, 87.89, 89.59 and 100%. The conception rate at
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33 136 first diagnosis by both veterinarians was 57.8% (386/915), and it was similar for Vet-A
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35 137 (58.44±16%) and Vet-B (56.96±18%, $P > 0.05$; Figure 1).
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41 139 The early pregnancy loss rate during the study period was 7.18% (38/529), and this rate
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43 140 was significantly higher among cows diagnosed by Vet-B (10.4±11.2%) than among
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45 141 cows diagnosed by Vet-A (4.87±9%, $P = 0.029$; Figure 1).
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50 143 Next, we compared the pregnancy loss rates between Vet-A and Vet-B for diagnoses
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52 144 made before or after June 2015, when Vet-B had accumulated 12 months' PD-US
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54 145 experience. Before this point, the pregnancy loss rate was significantly greater for Vet-B
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56 146 (11.17±12.14%) than for Vet-A (5.18±8.95%, $P = .039$), similar to the results obtained
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3 147 over the entire study period. After this point, the pregnancy loss rate was similar
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5 148 between Vet-B (7.14±11.01%) and Vet-A (3.51±9.83%, $P=0.620$; Figure 1). This
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7 149 difference before or after June 2015 was not associated with differences in conception
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9 150 rates, which were similar between Vet-B and Vet-A before June 2015 (60.86±16.9 vs
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11 151 57.43±15.2%, $P=0.620$) and after June 2015 (47.38±19.9 vs 51.86±17.9%, $P=0.669$).
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16 153 **DISCUSSION**

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20 155 This study provides some of the first evidence from a controlled field trial that the
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22 156 expertise of the PD-US practitioner can significantly affect the risk of early pregnancy
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24 157 loss in cows. These preliminary data suggest that at least 12 months' PD-US experience
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26 158 are needed to minimize risk of pregnancy loss. It is likely that proper PD-US training
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28 159 can reduce this "learning curve" and therefore should become routinely available.
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34 161 The present study highlights the importance of experience for PD-US safety, which may
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36 162 have been masked in previous comparisons of early pregnancy diagnosis techniques.
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38 163 For example, one study found a similar mean pregnancy loss rate of 5.3% between PD-
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40 164 US and other techniques (Baxter and Ward, 1997), and another study found a mean
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42 165 pregnancy loss rate of 5.4% between palpation and non-palpation techniques
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44 166 (Alexander et al., 1994), but the practitioners in both studies were "highly experienced"
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46 167 in their respective techniques. This literature suggests that several techniques for early
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48 168 pregnancy diagnosis can give similar results, but as the present study implies, this may
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50 169 be true only when the practitioners are highly experienced.
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3 171 Although the results of the current study are based on only two veterinarians working
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5 172 on a single farm, the observed difference in pregnancy loss is likely to reflect the
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7 173 difference in experience and not other confounding variables. The two veterinarians
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9 174 worked with the same herd for longer than 20 months using the same US machine. In
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11 175 addition, they diagnosed pregnancies at a similar rate throughout the study period as
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13 176 well as during the period when Vet-B had fewer than 12 months' PD-US experience
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15 177 These results suggest that less PD-US experience is required to diagnose pregnancy
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17 178 accurately than to diagnose it safely.
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23 180 The correlation between PD-US inexperience and higher risk of early pregnancy loss
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25 181 may reflect rough handling that damages the conceptus, perhaps through altered
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27 182 vascularization of placenta and other systems (Johnson 1986, Maiorka et al. 2015), or
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29 183 through mechanical trauma to the heart (Abbitt et al. 1978, Franco et al. 1987), which is
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31 184 located near the conceptus surface at days 28-35 of development (Pierson and Ginther
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33 185 1984). Whether such mechanical trauma was a major factor in the present study is
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35 186 unclear, since the cows were young and the veterinarians did not need to retract the
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37 187 uterus into the pelvis for scanning. This should reduce the difficulty and riskiness of the
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39 188 PD-US procedure, suggesting that other experience-related factors may have
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41 189 contributed to our results.
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47 191 The total pregnancy loss on our farm during the study period was 7.2%, which is
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49 192 slightly lower than the 11.9% reported in an extensive study involving more than 20,000
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51 193 pregnancies (Wiltbank et al. 2016). Our lower loss rate may reflect how we diagnosed
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53 194 pregnancy: in another study reporting a similar loss rate (Fricke et al. 2016), pregnancy
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3 195 was diagnosed based on fetal heartbeat as in the present work. Our lower loss rate may
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5 196 also reflect other farm factors (Wiltbank et al. 2016).

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9 198 This small field trial provides clear evidence that PD-US inexperience can increase risk
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11 199 of early pregnancy loss. Practitioners should receive adequate training before
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13 200 implementing this technique on farms in order to shorten the “learning curve”.

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18 202 **ACKNOWLEDGMENTS**

19
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22 204 Department of the UCM, Madrid, Spain) for statistical analyses, and to the farm staff
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24 205 and veterinarians for their work.

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29 207 **CONFLICT OF INTEREST**

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31 208 None of the authors have any conflict of interest to declare.

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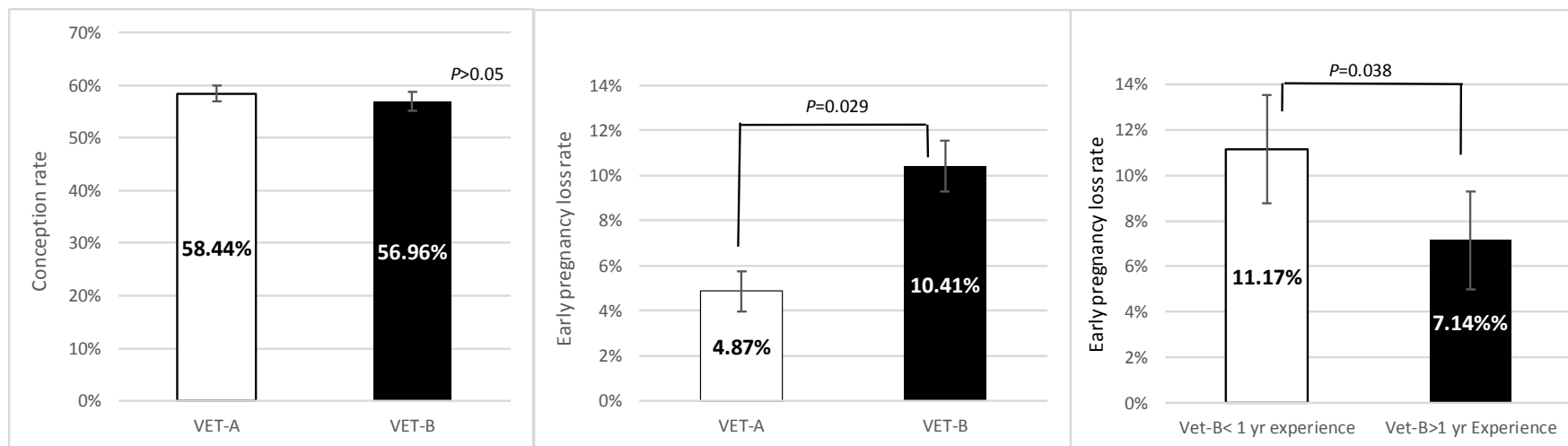


Figure 1. Comparison of the early loss of pregnancies diagnosed with ultrasound by a more experienced veterinarian (Vet-A) or less experienced veterinarian (Vet-B) in the same herd. **(Left)** Conception rate after early pregnancy diagnosis at 28-34 d after artificial insemination. **(Middle)** Rate of early pregnancy loss based on re-evaluation of pregnant cows at 49-56 d after artificial insemination. **(Right)** Rate of early pregnancy loss in cows diagnosed as pregnant by Vet-B before or after he had accumulated 12 months' experience with the ultrasound diagnosis procedure.



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Editor-in-Chief, *Reproduction in Domestic Animals*

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Title: Influence of practitioner expertise during early pregnancy diagnosis on pregnancy loss rate: a controlled, blinded trial

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Dear Prof. Dr. Rodriguez-Martinez,

Please find enclosed the revised version of the manuscript "Influence of practitioner expertise during early pregnancy diagnosis on pregnancy loss rate: a controlled, blinded trial", which we have modified according to the last reviewer's comments.

We thank you and the reviewer for help improving the manuscript. Below we provide detailed responses to the reviewer comments, and we highlight in yellow the corresponding changes in the text.

We hope this revised version can be considered suitable for publication.

RESPONSES TO REVIEWER COMMENTS

It had better mention the accuracy of pregnancy diagnosis for each vet in terms of sensitivity, specificity and predictive values based on the 2nd pregnancy diagnosis at Days 49 to 56 (Gold standard in the study). The authors have already used the gold standard for calculating the rate of embryonic mortalities. Also, it is more accurate to mention the accuracy of each vet for pregnancy diagnosis than to mention the pregnancy rate.

We apologize for having earlier misunderstood the reviewer's comments. This information has now been added (lines 131-134).

1
2
3 *Specific comments*

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5 *Discussion*

6
7 *Page 8 line line 175 -180: the explanation of higher pregnancy loss made by Vet B are*
8 *logic, because the vet B had known the proper methodology of scanning and the the young*
9 *age and small parity of cows used in the study. 1.e. he did not have to retract the uterus int*
10 *the pelvis for scanning of the uterus.*

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12 We are confused about what the reviewer would like us to add or modify in the
13 manuscript. We have added relevant information to the text (lines 184-188).
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