RD-01

Effect of treatment of phantom cows with a progesterone-based synchrony programme

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Objectives: To determine the effect of a progesterone-based synchrony programme on the daily hazard of conception and the probability of being pregnant at the end of the seasonal mating period in cows not observed in oestrus within 35–49 days of insemination and that were diagnosed non-pregnant (phantom cows) on seasonally-calving New Zealand dairy farms. Secondary aims were to determine the phantom cow prevalence and estimate the proportion of phantom cows with a functional corpus luteum (CL) at enrolment.

Material & Methods: Phantom cows from 14 New Zealand commercial dairy farms were enrolled in a randomised controlled trial. Cows that were artificially inseminated ≤14 days after mating start date and were not subsequently detected in oestrus were presented for pregnancy diagnosis approximately 49 days after mating start date. Non-pregnant cows were diagnosed as phantom cows and randomly allocated to treatment and control groups. A milk sample was collected for progesterone assay to determine the presence of a functional CL. Treatment consisted of an injection of buserelin and insertion of an intravaginal device containing progesterone on day 0, injections of dinoprost and equine chorionic gonadotrophin and removal of the intravaginal device on day 7, injection of buserelin on day 9, and fixed time artificial insemination on day 10. Treatment group cows were then mixed with bulls for the remainder of the seasonal mating period. Cows allocated to the control group were mated naturally by bulls. Statistical models were constructed to determine the effect of treatment on the daily hazard of conception and the probability of being pregnant at the end of the seasonal mating period.

Results: A total of 378/4,214 (9.0%) cows presented for pregnancy diagnosis were diagnosed as phantom cows. A functional CL was diagnosed in 257/362 (71.0%) phantom cows. Median predicted enrolment to conception intervals were 33 (95% CI= 30-45) and 30 (95% CI= 28-33) days respectively for cows in the control and treatment groups respectively. The odds of being pregnant at the end of mating were 1.70 (95% CI= 1.34-2.17) times greater for treated phantom cows than untreated phantom cows. Estimated marginal mean proportion pregnant at mating end date were 59.5 (95% CI= 47.9-70.1) % and 71.5 (95% CI= 62.6-79.0) % for control and treatment group cows respectively.

Conclusion: Treatment with a progesterone-based synchrony programme significantly increased the probability of phantom cows being pregnant at the end of the seasonal mating period.

Keywords: Phantom cow, dairy cattle, reproduction.

RD-02

Evaluating the cost-effectiveness of diagnosing and treating phantom cows in seasonal-calving dairy herds

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Objectives: The objective of this study was to explore the cost-effectiveness of using a progesterone-based synchrony program to manage phantom cows on seasonal-calving dairy farms. Phantom cows were defined as cows that had been artificially inseminated ≤14 days after mating start date (MSD), were not subsequently detected in estrus, and were diagnosed non-pregnant at a pregnancy diagnosis conducted approximately 49 days after MSD.

Material & Methods: Decision-tree analysis was applied to data from a previous randomized controlled trial in which phantom cows (n=378) from spring-calving dairy farms were randomly allocated to an untreated control group or were immediately treated with a 10-day progesterone-based synchrony program with fixed-time artificial insemination. The net economic return of treating all cows presented by the farmer for pregnancy diagnosis that were diagnosed non-pregnant was compared to no intervention. The net return was therefore calculated per cow present at MSD because the decision trees followed all cows present at MSD through to mating end date to account for farmers inadvertently presenting ineligible cows for pregnancy diagnosis and possible treatment. Probabilities, costs and benefits of reproductive outcomes were based on published data and expert opinion. The effects of key parameters on the economic return were tested by sensitivity analysis.

Results: Phantom cow intervention delivered a net return of NZ $4.51 per cow present at MSD. The sensitivity of pregnancy diagnosis, proportion of ineligible cows presented by the farmer for pregnancy diagnosis and the prevalence of phantom cows were highly influential on the net economic return from phantom cow intervention.

Conclusion: These findings suggest that treatment of phantom cows in seasonal-calving dairy farms using a progesterone-based synchrony program is economically viable based on the current model assumptions. Accurate cow selection and pregnancy diagnosis are essential to success. Veterinarians can improve the net economic return by selecting farms likely to have a higher prevalence of phantom cows based on the presence of observable risk factors.

Keywords: Phantom cows, Reproduction, Estrus Synchronization, Economics, Decision Tree Analysis.

Embryo production in dairy cattle after superovulation with gonadotrophin preparation- effect of variability in FSH/LH ratio-

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Introduction and objectives: Ovarian superstimulation of dairy heifers and cows is a practical tool to increase offspring from females of high genetic merit. The success of superovulation and embryo recovery in cattle depends on numerous factors but one of the most significant limiting factor has been the high between-individual variability, in the ovarian response to gonadotropin stimulation. (Mikkola & Taponen, 2017).

Currently the gonadotrophins preparations approved for veterinary use derive from porcine and ovine pituitary tissue. One of the disadvantage resulting from the nature of the origin of the products is inconsistency among batches. This variation in bioactivity affects embryo yield (Kanitz et al. 2002).

The more important question is the possible biological effect of the so called FSH/LH ratio. Variability in FSH:LH ratios among different batches of one product of commercially available gonadotrophins is a very important challange that deserves to be considered. Because of this, the objective of this study was to compare the outcome of embryo recovery after superovulation with purified porcine pituitary extracts with different FSH:LH ratios, using a standard protocol of eight injections in a decreasing dose in cows and heifers.

Materials and methods: Data of 465 superovulations and subsequent embryo collections from Spanish dairy farms were analyzed. Donor animals were cows (n= 222) (47.74%), and heifers (n= 243) (52.26%) of Holstein breed.

Superovulation was induced by eight intramuscular injections of gonadotrophin preparation, at 12-hour intervals over 4 days. Declining doses of 650 to 1000 IU in total was administered for cows, whereas heifers received 500 to 750 IU. Embryos were collected 7 days after inseminations by transcervical uterine flushing according to the IETS guidelines.

Donors were randomly divided into three groups (I, II and III), according to treatment they had received because of different FSH:LH ratio in follicle-stimulating hormone preparation:

Group I (T1): (n= 362) Donors stimulated with a batch in which FSH:LH ratio was ≥ 1

Group II (T2): (n= 56) Donors stimulated with a batch in which FSH:LH ratio was <1 to ≥ 0.9

Group III (T3): (n= 47) Donors stimulated with a batch in which FSH:LH ratio is < 0.9

Statistical analyses: With the aim of studying factors that influence on number total recovered embryos variation; number viable embryos and number of quality 1 embryos variation, statistical analyses were carried out using lineal models (GLM procedure in SAS version 9.1.3, SAS Institute Inc., Cary, NC). The variance factors analyzed were: type of donor (heifer or cow) and treatment (T1, T2, T3).

Results: The average values for the analyzed variables were: number of total embryos recovered (10.28±0.32), number of viable embryos (5.66±0.23) and number of quality 1 embryos (2.83±0.12).

The donor type factor (heifer of cow), was not significant for the three variables analyzed, however the treatment effect was significant for the number of viable embryos (p <0.05) and for the number of quality 1 embryos.

There was no effect (P=0.08) of different FSH:LH ratios of gonadotrophins on mean of **total embryos recovered** between Groups I, II, III (10.53 ± 0.37 vs. 9.73 ± 0.94 vs. 9.25 ± 1.03 respectively).

There was a main effect of different FSH:LH ratios used for superovulation in present trial (P = 0.0004), on **viable embryos** obtained between Group I (FSH:LH ratio ≥1), with highest value (6.06 ± 0.26) and group III (FSH:LH ratio < 0.90) with lowest (3.26 ± 0.73). The mean showed of Group II (FSH:LH ratio: <1 to ≥0.90) (5.07±0.67) tended (P=0.07), respect to Group III value.

There was also a significant effect of the treatment on the number of **quality 1 embryos:** donors of Group III had lesser number (1.81 ± 0.38) than Group II (2.68 ± 0.35) (P=0.09) and Group I (2.98 ± 0.13) (P=0.0043).

Conclusions: The number of viable and quality 1 embryos recovered resulted in significantly increased in donors stimulated with a batch of gonadotrophin preparation in which FSH:LH ratio was ≥ 1 respect to those obtained from females superovulated with a batch which ratio of FSH:LH was < 0.9.

These results suggested that relative high LH contamination in gonadotrophin preparations impairs the ovarian response to superstimulation, resulting in decreased yield of viable and quality 1 embryos.

Keywords: FSH/LH ratio, SOV, embryo, cattle.

Quantification of reproductive hormone use in Dutch dairy herds: "From no treatment to excessive use"

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Objectives: Globally, reproductive hormones are used in the dairy industry to increase reproductive efficiency and, hence, to increase the longevity of dairy cows. The number of treatments seems to vary significantly between herds, but exact numbers on reproductive hormone use are lacking for most countries. Variation between countries appears to be highly dependent on a farm's management system, including the type of calving pattern used. In the Netherlands, the

calving pattern is year-round and, consequently, hormonal treatments tend to be prescribed at the cow level based on diagnostics rather than by using timed artificial insemination protocols at herd level.

Consumers' growing interest in food safety and cases of pharmaceutical product misuse have resulted in scepticism among the public regarding the use of antimicrobials and hormones in modern farming, although direct negative effects of reproductive hormones are not described. Transparency in the process of milk production, including insight into key performance indicators and medicinal treatments, is a strong and effective tool in influencing social opinions and acceptance of modern dairy farming.

The aim of this study was to estimate reproductive hormone use on year-round calving dairy farms under Dutch circumstances and to obtain insight into a number of herd-level factors that are associated with this reproductive hormone use.

Materials and methods: The use of three commonly used reproductive hormones, namely prostaglandins, GnRH and progesterone, and associated herd-level factors were studied on 760 Dutch dairy farms selected from 5 veterinary clinics. From 2017 to 2019, data on the registered sales of reproductive hormones were collected and converted into the number of reproductive hormone doses based on the prescribed amount of product per treatment on the package leaflet. The total number of doses was expressed as the annual number of reproductive hormone doses per 100 adult dairy cows. Additional herd-level information, including milking system and participation in veterinary herd health management (VHHM) programs, was available for 2019. Due to the excess of zeros in the dataset (i.e., a substantial number of nonusers), zero-inflated negative binomial models were used to associate herd-level factors with the use of reproductive hormones.

Results: In the entire study period from 2017 to 2019, 5.8% of the dairy farms did not use any reproductive hormones, varying between 0.0 and 10.3% per veterinary clinic. This proportion of nonusers was around 13.5% on an annual basis. Prostaglandins were the most frequently used reproductive hormone in Dutch dairy cows (62.9%), followed by GnRH (33.1%) and progesterone (4.0%). In 2019, the total number of reproductive hormone doses per 100 adult dairy cows ranged from 0 to 248. The mean number of doses per 100 adult dairy cows was 40.6, and its median was 32.8. Similar observations were made regarding the distribution of reproductive hormone use in 2017 and 2018. Furthermore, farms participating in a VHHM program used more reproductive hormones than farms that did not participate in such a VHHM program and these farms were also a minority in the nonuser group. Technologies, such as pedometers and automatic milking systems, also had an effect on reproductive hormone use. The presence of pedometers or activity monitors on farms was more common in the group of farmers with reproductive hormone use. Farms with an automatic milking system used more reproductive hormones than farms equipped with a conventional milking system, while organic herds used less reproduction hormones. Lastly, hormone use was more common in larger herds compared to smaller herds.

Conclusions: The veterinary clinics and farms varied regarding their use of reproductive hormones, indicating room for optimization regarding the use of hormones on a number of farms. Farm characteristics were associated with greater use of reproductive hormones. This study is a first step to achieve transparency in the Dutch dairy industry by providing an objective overview of reproductive hormone use on dairy farms and its association with a number of herd-level factors. Further research will be needed to estimate the effect of reproductive hormone interventions on cow level and, hence, the impact on reproductive performance of the herd.

Keywords: Hormone treatment, reproduction, dairy.

RD-05

Progesterone based heifer re-synchrony in an extensive pasture grazing system: A case study

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Objective: The objective of this study was to determine if a synchrony/re-synchrony programme would prove effective in achieving a pregnancy rate of >85% in 3 cycles.

New Zealand dairy heifers are typically raised in extensive pasture based grazing systems. These systems often preclude artificial insemination (AI) from being used throughout the mating period due to the inability to carry out extended periods of daily heat detection. However, with the eradication of Mycoplasma bovis currently underway in New Zealand, some farmers are reluctant to use bulls of often unknown provenance for their heifers.

Materials and Methods: 140 nulliparous approximately 15-month old dairy heifers from a single farm were used for the study. All heifers received gonadotrophin-releasing hormone (GnRH) intramuscularly (I/M) on Day -9, a progesterone (P4)-releasing intravaginal device [1.38g CIDR®] from Days -9 to -2, prostaglandin F2α (PGF) I/M on Day -2 and a second dose of GnRH I/M as well as fixed time artificial insemination (FTAI) on Day 0 (Co-Synch). On Day 14, all heifers were re-synchronized with insertion of a new progesterone device, which was removed at Day 21. From Day 22-26 heifers were mated to detected heat. On Day 35, all heifers were pregnancy tested using rectal ultrasonography, and divided into three groups. All heifers diagnosed as pregnant were removed from the programme (Group 1). All heifers not diagnosed pregnant, but which had been mated at Days 22-25 (Group 2: possibly pregnant) were treated with a new progesterone device from Day 35 to Day 42. These devices were removed at day 42, and heifers were mated to detected heat for Days 43-46. Heifers which were not visibly pregnant at Day 35 and which had also not been mated on day 22-26 (Group 3) received another Co-Synch programme followed by FTAI on day 44. The final pregnancy test was carried out on Day 74.

Results: 123/135 (91%) of heifers which completed the trial conceived over the 46-day mating period. The submission rate (heifers mated/heifers not pregnant and available for mating) was >90% in both re-synchrony cycles. The conception

rate (heifers pregnant/heifers mated) in cycle 1 (Day 0) was 64%, in cycle 2 (Days 22-26) was 52% and in cycle 3 (Days 42-46) was 67%.

Conclusions: The heifer re-synchrony programme used was successful in achieving a >85% pregnancy rate in three cycles of mating in an extensive pasture based grazing system.

This study was approved by the Kaiawhina Animal Ethics Committee, Palmerston North, New Zealand. CIDR is a registered trade mark of InterAg. ACVM No. A4559

Keywords: Dairy cattle, synchrony, re-synchrony, heifer, progesterone device.

RD-06

Association between infectious diseases with late embryonic losses in supplemented grazing dairy cow

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Objectives: To evaluate the association between Bovine Viral Diarrhea (BVD) and Infectious Bovine Rhinotracheitis (IBR) with the odds for late embryonic losses (LEL) in supplemented grazing dairy cows in Argentina.

Materials and Methods: A prospective cohort study was carried out in a commercial dairy herd, located in Carlos Casares, Buenos Aires Province, Argentina (35°37′ S, 61°22′ W) to assess the associations between seroprevalence and seroconversion to BVD and IBR with the odds for LEL. Cows having embryo with no heartbeat, or with detached membranes or floating structures including embryo remnants detected by ultrasonography (US) at 28-42 days post-AI were defined as LEL, whereas cows diagnosed with positive pregnancy by US 28-42 d post-AI were considered as non-LEL. A total of 92 cows were selected for the study (46 LEL and 46 Non-LEL). All the cows were bled twice 28 days apart (0 and 28 d). The day 0 corresponded to the day when every cow with LEL or non-LEL was diagnosed. Serological titers were determined by Virus Neutralization Test (titers were from 1:2 up to 1:2048, and from 1:2 up to 1:512 for BVDV and IBR, respectively). Seroconversion was considered when titers at d 28 were 3X higher than at d 0. The conceptus from LEL cows was sampled (day 0) with an insemination pistol attached to a 10 mL syringe, stored in a vial with RNAlater, and transported to the laboratory. Subsequently, the aspirated conceptuses from LEL cows that seroconverted were processed for virus identification by PCR. Logistic regression models were used to assess the association between serological titers for BVD and IBR (as continuous predictors) with the odds for LEL, and the association between seroconversion to BVD and IBR (yes vs. no) with the odds for LEL. Logistic models were run with Proc Glimmix of SAS with binomial distribution and logit link function.

Results: At d 0, the seroprevalences for BVD and IBR were 100% (46/46) for LEL and non-LEL cows. At d 28, the seroprevalence for BVDV was also 100% (46/46) for LEL and non-LEL cows, whereas the seroprevalence for IBR was 93.4% (43/46) and 82.6% (38/46) for LEL and non-LEL cows, respectively. Seroconversion to BVD was 20.0% (9/46) and 6.6% (3/46) for LEL and non-LEL cows, whereas seroconversion to IBR was 4.4% (2/46) and 2.2% (1/46) for LEL and non-LEL cows. The odds for LEL was associated with BVD at d 28 (*P* = 0.03) given that the risk increased 3.44 times per 1 SD of increment in titer over the mean. The odds for LEL was also associated with seroconversion to BVD (*P* = 0.09) given that the risk was 3.27 times higher in cows that seroconverted than in herd-mates that did not. Conversely, neither IBR at d 28 nor seroconversion to IBR were associated with the odds for LEL. Finally, the BVD virus was identified in all the conceptuses from LEL cows that seroconverted (9/46).

Conclusion: We concluded that seroconversion to BVD is associated with increased risk for LEL in grazing dairy cows. In addition, the BVD virus can be detected in conceptuses from cows diagnosed with LEL that seroconverted to BVD 28 d later.

Keywords: Bovine Viral Diarrhea, Infectious Bovine Rhinotracheitis, Late embryonic loss, Dairy cows.

RD-07

Risk factors for anovulation and its association with reproductive performance in a herd of grazing dairy cows

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Objective: The objectives of this study were to assess the risk factors for anovulation and its association with reproductive performance in a herd of grazing dairy cows.

Material and Methods: A retrospective cohort study, including a total of 15,846 records of cows calving from January 1st, 2010 to December 31st, 2016, from a dairy farm in Argentina (~2800 milking cows) was run. Included records were those having an episode of anovulation diagnosed by ultrasonography at 50-64 day postpartum (dpp, n=3,975) and those not having an episode of anovulation that were considered as cyclic (n=11,871). The odds for anovulation at 50-64 dpp was tested with a Logistic Regression Model that included the fixed effects of calving season (spring [SP], summer [SU], autumn [AU], winter [WI]), parity (PAR 1, 2, 3+), and disease status during the first 50-64 dpp (healthy [HEALTHY, no health events], reproductive [REPRO, retention of fetal membranes, puerperal metritis, clinical endometritis, and pyometra],

non-reproductive [NOREPRO, clinical mastitis, clinical lameness], both [BOTH]). Additionally, the association between anovulation (yes vs. no) with reproductive performance was tested with a Kaplan-Mayer survival analysis through calving to conception interval (DOPEN).

Results: The occurrence of anovulation at 50-64 dpp was 25.1%. The risk for anovulation (odds ratio [OR], 95 percent confidence interval [95%CI]) was higher in cows that calved in autumn (1.388 [1.235-1.558]), winter (1.717 [1.525-1.929]) and spring (2.339 [2.080-2.631])than in cows that calved in summer (P<0.001). The risk was lower in cows of parity 2 (0.845 [0.765-0.935]) and 3+ (0.988 [0.898-1.087])than in those of parity 1 (P <0.001); and lastly, the risk for anovulation tended to increase with REP events (1.088 [0.977-1.211]), and increased with NREP events (1.609 [1.454-1.780]) and with both events (1.715 [1.501-1.960]; P <0.001). Regarding reproductive performance, anovulatory cows had a more DOPEN than cyclic cows (164.56±2.32 vs. 123.20±1.11; P<0.001). The REPRO events (126.08±1.84), NOREPRO events (144.23±1.84), and BOTH events (149.51±3.03) had more DOPEN than HEALTHY cows (123.20±1.11, P<0.001).

Conclusions: In conclusion, the risk for anovulation is affected by season, given that it is higher in cows calved in autumn, winter, and spring than in those calved in summer; and by parity, due to the risk is higher in primiparous cows multiparous ones. In addition, anovulatory cows have a longer calving to conception interval than cyclic herd mates.

Keywords: Anovulation, risk factors, reproductive efficiency, days open, grazing dairy cows.

Relationship between reproductive performance and Q-fever in dairy herds in Brittany (France)

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Objectives: *Coxiella burnetii* is a small intracellular bacterium responsible for Q Fever in animals and humans. In cattle, Q fever is a major cause of abortion: 2nd cause according to the French observatory of the causes of abortion in ruminants (OSCAR). But this disease is also responsible for low fertility.

GDS Bretagne is a farmer association helping farmers to manage sanitary issues, including abortions and fertility disorders. Evolution is an Artificial Insemination Company operating in the same area. These two companies together with Ceva Santé Animale, carried out a study to compare reproduction performances in dairy herds depending on the reason for abortion.

Material and methods: 831 herds with abortions were included between November 2018 and May 2020. Causes of abortions were determined using a combination of PCR on vaginal discharge and serology on blood of animals from the same cohort. In the case of two or more aborted animals had

positive PCR results for *Coxiella burnetii*, Q fever was considered as a highly probable cause of abortion. If only one PCR result was positive, the blood serology of other animals was taken into account. If at least 50% of serology tests were positive, Q fever was considered the cause of abortion with medium probability.

An annual summary of reproductive performances was made for each herd and crossed with the reason for abortion. Fertility of cows and heifers, stillbirth, culling rate, calving interval, age at first calving were studied.

ANOVA was applied to explain reproductive criteria.

Results: Finally, data were available for 642 herds.

12 % of the abortion events were due to Q fever with medium or high probability.

In herds where *Coxiella burnetii* was detected as a highly probable cause of abortion, the fertility rate at first insemination was 6.99 points lower than the average for herds in the same area and during the same period (40.16% and 47.16% respectively, p=0.023). If herds with a medium probability of Q fever abortion are also included, the fertility rate remains lower than that of herds without Q fever abortion (41.42% and 47.16% respectively, p=0.013). In herds with low probability of Q fever, the first insemination was almost twice as likely to be successful as in herds with a medium or high probability of Q fever (OR=1.92 CI95% [1.07 - 3.45]).

The study also highlighted that the calving interval was extended by 4.7 days in Q fever infected herds (420 d vs 415 d).

No difference was highlighted for the other criteria (fertility of heifers, culling rate, stillbirth, and age at first calving).

From an economic point of view, the cost simulator developed by the Vets school of Nantes (Bilan de santé du troupeau laitier - bioepar.org) showed that this drop in fertility rate was 23 euros per cow per year, excluding the cost of abortions.

Conclusion: Dairy herds affected by Q fever abortions have lower cow fertility than others. We showed that the disease has a global impact on reproduction and not only on abortions. This finding is in accordance with some data already published on the involvement of *Coxiella burnetii* in fertility, metritis/endometritis and retained placenta.

It appears that a control of the disease including both biosecurity measures and vaccination could be of interest in such herds to improve fertility.

We focused herds with abortions due to Q fever. This study could be complemented by another one which would consist in the detection of *Coxiella burnetii* in herds with degraded fertility, even in the absence of abortions.

Keywords: Q fever, fertility, cattle, reproductive performance.

RD-09

Evaluation of the impact of multifactorial parameters to improve activity-based heat detection systems

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Objectives: Heat detection devices have traditionally been using changes in the activity pattern of female cows. This is either done via recording of simple parameters such as the number of steps, detection of specific movements (mounting behavior), or by detailed analysis of intensity and direction of the animal's movements. Inclusion of other parameters could lead to an improvement in heat detection algorithm accuracy. The restless behavior of cows in estrus is associated with a decline in their rumination time. This was demonstrated after the introduction of rumination monitoring collars (Bar and Solomon, 2010) and confirmed in later studies (Reith and Hoy, 2012). Inclusion of the normal estrous cycle pattern and duration of behavioral heat should further increase the accuracy of heat detection and monitoring. In order to increase heat detection accuracy, Heat Index algorithm, based on the above mentioned parameters, was developed for an automated monitoring system. The objective of this study was to compare the accuracy of estrus detection in dairy cows using Heat Index versus activity data only algorithm.

Materials & Methods: A total of 15,000 activity increase alerts with a very low threshold (about 25% activity index increase) were collected from about 3,000 cows in 7 commercial farms, with all cows on farm wearing monitoring collars (Allflex® Livestock Intelligence™) throughout the lactation.

A novel notation method was developed defining a "gold standard" to evaluate heat detection efficiency under field conditions. All heat alerts resulting in inseminations leading to a confirmed pregnancy were classified as True Positives. All episodes of increased activity recorded in pregnant cows were classified as True Negatives. It is important to note that the system user does not receive the false positive alerts because the system does not present pregnant cows in the "Cows ready for AI" report. From these two populations, a Receiver Operating Characteristic (ROC) curves were drawn for an algorithm based on activity change alone, or Heat Index, combining several parameters.

Results: The analysis of True Positives (TP) against False Positives (FP) revealed a significant added benefit of using the Heat Index algorithm compared to the one based on the activity increase only. At the relevant interval (95-98% sensitivity), the use of Heat Index algorithm led to a reduction of false heats by 28% and 36%, respectively. At 95% specificity, activity only algorithm discarded 51% (1839/3617) FP heats while Heat index algorithm discarded 79% (2857/3617) TP heats. At 98% specificity, activity only algorithm discarded 26% (940/3617) FP heats while Heat index algorithm discarded 62% (2242/3617) TP heats; Heat Index ROC curves also demonstrated an improvement of 1% in sensitivity of heat detection, at the default detection level threshold.

Conclusions: The Heat Index algorithm (Allflex® Livestock Intelligence™) shows a better performance in heat detection in dairy cows compared to algorithms based on activity patterns only, with significantly superior specificity while preserving, and even slightly improving the detection sensitivity.

This brings significant benefits to the dairy farmers using monitoring system with heat detection ability. The use of Heat Index algorithm decreases the risk that animals that are not in heat are selected for insemination, which reduces wastage of semen and unnecessary expenses on service. Although more limited, improved sensitivity of heat detection with Heat Index algorithm means fewer cows in heat and eligible for insemination are missed. Consequently, improved heat detection accuracy would contribute to improved farm reproduction performance, reduction of days open and intervals between calvings.

Keywords: Heat detection, monitoring, heat index, estrus.

RD-10

Pastoral dairy bull procurements and management effects on fertility

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Objective: Dairy herd bulls are utilised on most farms as a backup to AI in the southern Australian dairy system. The current recommendations for their management include the purchasing and movement of bulls onto farm 2-3 months prior to their use. This recommendation is from the Bull Power study completed in Queensland & New South Wales, Australia which assessed beef bull reproductive performance following transportation to new properties and changes in management conditions.

We aimed to assess dairy bulls' reproductive performance; both prior to sale and following movement onto their destination property under new management conditions. Links between stress associated with transportation & management changes, semen motility, morphology and scrotal circumference will be investigated.

Methods: Holstein-Friesian & Jersey bulls (n=34) were examined using a veterinary bull breeding soundness evaluation (VBBSE) on their farms. Bulls were then transported to new properties and a VBBSE was repeated at 20 days, 50-70 days and 120-150 days following arrival. At each VBBSE semen samples were collected via electroejaculation and submitted for morphological examination by a registered morphology laboratory.

Management practices on the property of origin and new property were noted for each of the bulls. Recorded management practices included mixing with existing bull teams, workload, and nutrition.

Results: Preliminary results show that following movement onto a new property and changes to management (BBSE 20d) sperm morphology & scrotal circumference are temporarily negatively affected, these deficits are resolved in the follow-

ing BBSEs (50-70d, 120-150d). Some correlation between distance travelled and the effect on semen morphology and scrotal circumference is evident in preliminary assessment of the data set.

Conclusion: The current recommendations for dairy herd bulls throughout Australia are based on data obtained from a study of beef bulls completed in 2005. This work aims to give appropriate guidelines for minimum timing of procurement prior to use as well as an indication of the likely stress associated with mixing of bull teams.

Keywords: Bull, fertility, scrotal circumference, semen morphology, dairy herd bull.

The effect of integrin binding domain peptide and C-terminal fragment of osteopontin on restoring the endometrial EGF abnormality in repeat breeder dairy cows

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Objectives: In fertile cows, epidermal growth factor (EGF) concentrations in the endometrium show two peaks on days 2-4 and 13-14 of the estrous cycle. The absence of EGF peaks in the endometrium has been linked related to reduced fertility and around 70% of repeat breeder (RB) dairy cows lost endometrial EGF peaks. Intravaginal infusion of seminal plasma osteopontin (OPN) restored the endometrial EGF profile and fertility in RB cows. However, the mechanism by which OPN exerts this effect is unknown. OPN can be cleaved by thrombin, dividing into N-terminal and C-terminal fragments. The N-terminal fragment has integrin binding domain (RGD sequence and SVAYGLK sequence adjacent to RGD). While the C-terminal fragment interacts with CD44. The objective of this study was to evaluate whether the integrin binding domain peptide and the C-terminal fragment of OPN restore the endometrial EGF abnormality in RB cows.

Material & Methods: 271 RB dairy cows were used in this study. They were examined for endometrial EGF concentrations on day 3 (day 0 = estrus) and cows showing low concentration (< 4.70 ng/g tissue weight) were used. Then, all cows were synchronized for estrus. Each cow was infused with either 10 ml phosphate-buffered saline (PBS) alone (n = 85) or one of the following samples prepared in 10 ml PBS into the vagina within 4 to 12 hours after detection of estrus; recombinant full length OPN (rOPN, 32 nmol, n = 121), recombinant C-terminal fragment (C-rOPN S162-N278, 46 or 53 nmol, n=25) and synthetic peptide (peptide G151-K161, 32 nmol or 320 nmol, n = 20). Then, endometrial EGF concentrations on day 3 of the estrous cycle were examined for the

second times.

Results: The normalization rates of the endometrium EGF profile (≥ 4.70 ng/g tissue weight) were higher in the cows infused rOPN (59.5%, n = 72) and 320 nmol peptide G151-K161 (55.0% n = 11) than PBS (27.1%, n = 23) (P<0.05). The normalization rates were similar between rOPN and 320 nmol peptide G151-K161. While the normalization rates were similar in the cows infused C-rOPN S162-N278 (12.0%, n = 3) and 32 nmol peptide G151-K161 (30.0%, n = 6) to PBS. Endometrial EGF concentrations after treatment were higher in the cows infused with rOPN [5.91 (3.15-7.05), median (interquartile range)] than PBS [2.71 (1.91-5.34)] (P<0.05), but were similar in the cows infused with C-rOPN S162-N278 [1.91 (1.71-2.62)], 32 nmol [3.15 (1.81-5.20)] and 320 nmol [5.30 (1.94-6.35)] peptide G151-K161 to PBS. In the cows infused with peptide G151-K161, endometrial EGF concentrations after treatment tended to be higher in the cows infused 320 nmol peptide than 32 nmol peptide (P<0.1).

Conclusion: Integrin binding domain peptide of OPN has function to restore the endometrial EGF abnormality in RB dairy cows. However, the peptide needed a larger amount in mole than rOPN to restore the endometrial EGF profile. Cells having integrin might have a role to normalize the endometrial EGF profile by OPN. On the other hands, the C-terminal OPN fragment may have less or no function to normalize the endometrial EGF profile in RB dairy cows. This may indicate that CD44 might not be a main targeting receptor for normalization of the endometrial EGF profile by OPN.

Keywords: Osteopontin, Epidermal growth factor (EGF), Endometrial biopsy, Repeat breeder dairy cows.

Reproductive Prolapse in Cows in Western Thailand: Grades, Severity and Treatment

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Objectives: This study determined grades, severity and treatment of reproductive prolapse in cows in western Thailand.

Materials and methods: This study investigated the reproductive prolapse cases submitted to Kasetsart University Veterinary Teaching Hospital Kamphaengsaen, from January 2016 to November 2019. The data were collected from out-patient department records and a questionnaire and analyzed by STATA.

Results: There were 90 cases submitted during the study period, 72 cases (80%) were beef and 18 cases (20%) were dairy cattle. Forty-nine cows (54%) were raised in the free stall system and 32 cows (36%) were raised in the grazing system. Only 11 of affected cows (13%) were heifers. One-fourth of the reproductive prolapse cases found before calving occurred during the last trimester of pregnancy.

Grade of prolapse was divided in 5 grades. There were 20

vaginal prolapse (grade 1 and 2) cases (22.22%), 52 cervical prolapse (grade 3) cases (57.78%) and 18 uterine prolapse (grade 4 and 5) cases (20%). A few factors were associated with the severity of reproductive prolapse. More severe prolapse cases were found in beef cattle less than in dairy cattle (P < 0.001). Most of the severe cases were found after calving (P < 0.001). All uterine prolapse (grade 4 and 5) cases were found after calving and almost all of these cases (17/18) occurred within the first day after calving.

The treatment protocols were cleaning the reproductive tract to decrease risk of infection, performing epidural anesthesia, replacing the reproductive organs and suturing the vulva lips with Bühner technique, applying tincture-iodine at the suture site and injecting an anti-inflammatory drug. Calcium solution was administered before replacement in cows suspected hypocalcemia. Every case was rechecked 1-7 days after treatment depended on severity of the reproductive prolapse, and there was no recurrent prolapse.

Conclusions: The reproductive prolapse cases submitted in this study were beef cattle more than dairy cattle. However, most reproductive prolapse cases in dairy cattle were uterine prolapse which is related to post-partum hypocalcemia.

Keywords: Reproductive prolapse, cows, grade of prolapse.

RD-14

Comparison of three different reproductive management strategies for lactating dairy cows emphasizing detection of oestrus or synchronization of ovulation and Timed Artificial Insemination (TAI)

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Objectives: The aim of this study was to compare three reproductive management strategies for first insemination of lactating dairy cows: Ovsynch; PRID-synch, both using oestrus detection and subsequent timed artificial insemination (TAI); and Double-Ovsynch at TAI.

Materials and methods: A total of 1681 Holstein cows from two commercial Scottish dairy farms were randomised by parity to three different reproductive strategies for first AI. For the first two protocols, cows were eligible to be inseminated by detected oestrus between 50 and 70 days in milk (DIM) and were subsequently enrolled in either one of the following two protocols:

1. Ovsynch (n=541), GnRH (G1) at 70±3 DIM, PGF2α (PG1) 7 d later, PGF2α (PG2) 24h later and GnRH (G2) 32h later, followed by FTAI 16 h after;
2. PRID-synch (n=562), GnRH (G1) + PRID at 70±3 DIM, PGF2α (PG1) 7 d later, PGF2α (PG2) + PRID removal 24h later and GnRH (G2) 32h later, followed by FTAI 16 h after.

For the third protocol, Double-Ovsynch (n=578), 53±3 days after parturition, cows received an injection of GnRH, PGF2α 7 d later and GnRH 3 d after, as presynchronization. Then at 70±3DIM, the breeding Ovsynch including an injection of GnRH (G1), PGF2α (PG1) 7 d later, PGF2α (PG2) 24h later and GnRH (G2) 32h later, followed by FTAI 16 hours later.

Ovarian monitoring was performed by transrectal ultrasonography at 43±3 and 50±3 DIM in all animals, and 70±3 (G1) and 77±3 (PG1) DIM only for the cows that were synchronized. Pregnancy was diagnosed by transrectal ultrasonography between 28 and 35 days after AI.

Results: Twenty-eight-day pregnancy rates for first AI was similar among treatment groups: Ovsynch (44%; 162/365), PRID-synch (41%; 158/383) and Double-Ovsynch (45%; 160/353). Cows with a corpus luteum (CL) at 77±3 DIM (PGF1) were 3.23 times more likely to be pregnant regardless of the treatment group (p<0.001). Primiparous cows were more likely to be pregnant at 28 days than multiparous cows in all treatment groups (Ovsynch 59% vs 35%; PRID-synch 47% vs 38%; and Double-Ovsynch 55% vs 39%) (p<0.001).

Conclusions: There was no significant difference in pregnancy rate at 28 days among treatment groups, which is consistent with previous comparable studies (Fricke et al., 2014, Robichaud et al., 2016). This may have been due to cows in the first two protocols being mated to observed oestrus on commercial farms where heat detection efficiency and P/AI on standing oestrus were good (57% and 46% respectively); which may have over-inflated the 28 days pregnancy rate in these two groups; however, these results are reflective of the expected performance of the protocols in a commercial setting. Primiparous cows submitted to Double-Ovsynch did not have higher pregnancy rate compared with the other two strategies in contrast to other published reports (Souza et al., 2008; Borchardt et al., 2016).

Keywords: Dairy, reproduction, oestrus, synchronization, TAI.

RD-15

Effect of puerperal metritis occurrence on reproductive efficiency and milk production in grazing dairy cow

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Objective: The objective of this study was to estimate the effect of puerperal metritis (PM) occurrence on reproductive efficiency and milk production in a dairy farm in Argentina (~2800 milking cows).

Material and Methods: An observational retrospective study, including a total of 16856 records of cows calving from January 1st, 2010 to December 31st, 2016, was used. At parturition, calving assistance was recorded (ACAL; normal, slight or severe assistance, c-section, abortion), and retention of fetal membranes (RFM) was recorded. All cows were exam-

ined for the diagnosis of PM (0-3) during the first two weeks after calving. Only cows diagnosed with PM3 (enlarged uterus and a fetid watery red-brown uterine discharge and signs of systemic illness [decreased milk yield, dullness, or other signs of toxemia]) were treated (TRT) with ceftiofur (6.6 mg/kg BW, SC; Excede, Zoetis Argentina). Cows were rechecked a week later, and cows with PM0 were diagnosed as clinically cured (CCURE), and cows with PM1-2 were diagnosed as partially cured (PCURE). Only cows with PM3 were treated again with the same protocol until clinically cured or culled from the herd. After three weeks, post-partum cows were examined for diagnosis of clinical endometritis (CE, 0-3). After having a 50 days voluntary waiting period (VWP), cows were AI at detected heat. Reproductive efficiency was measured by percent of IA cows by 80 (AI80) days postpartum (dpp), percent of pregnant cows by 100 (PRE100) and percent of open cows by 200 (OPE200) dpp, percent of pregnancy losses to first pregnant AI (LOSS1P), and interval from calving to first IA (C1AI) and to conception (ICC). Also, milk production (kg) at 150 (MP150) and 305 (MP305) days of lactation were measured.

Results: During the first week of calving, the occurrence of PM0-PM3 was 47.72%, 14.04%, 22.99%, and 15.25% (8043, 2366, 3874, and 2571/16854, respectively). First lactation cows had higher occurrence of PM3 compared to 2+ lactation cows (22.96% [1510/6578] vs 10.32% [1061/10276]; P<0.01), and cows calving during the spring had higher occurrence of PM3 compared to cows calving the rest of the year (17.45% [634/3634] vs 11.94% [1937/16218]; P<0.01). Of a total 2571 cases of PM3 diagnosed, 2439 had registered TRT information and were used for further analysis. The CCURE rate 24.89% (607/2439) and the PCURE rate was 53.59% (1307/2439). About 18.49% (451/2439) of the cases did not respond to TRT and remained PM3; and 3.03% (74/2439) of the cases were TRT but were not rechecked by the veterinarian. Furthermore, 39.66% of PM3 persisted as CE after 21 dpp. The interval calving to PM3 TRT was 8.81±0.77 d. Retrospectively, cows with PM3 had more ACAL and RFM compared to cows without PM0 (52.98% [1362/2571] vs 72.63% [5842/8043], P<0.01; 14.16% [364/2571] vs 2.00% [161/8043], P<0.01). Also, more cows with PM3 failed to persist in the herd for a subsequent lactation compared to PM0 cows (27.15% [698/2571] vs 26.00% [2091/8043], P<0.01). Cows with PM3 had a lower IA80 and PRE100, and higher OPE200 compared to PM0 cows (58.37 [1465/2510] vs 64.55 [5036/7802], 30.89 [767/2483] vs 37.59 [2897/7706], 30.74% [710/2310] vs 25.64 [1843/7188]; P<0.01, respectively). Furthermore, cows with PM3 had 4% more pregnancy losses compared to PM0 cows (30.12% [635/2108] vs 25.97% [1742/6709], P<0.01). Pregnancy rate to first IA (IC1AI) and day open (ICC) were 3 and 10 days longer for PM3 compared to PM0 cows (76.43±0.41 [n=2260] vs 73.67±0.22 [n=7108]; 134.42±1.43 [n=1982] vs 124.34±0.77 [n=6379]; P<0.01, respectively). Cows with PM3 produced 460 kg and 675 kg less of milk compared to PM0 cows (5055.91±11.82 [n=6961] vs 4595.62±19.55 [n=2335]; 9016.34±37.35 [n=1750] vs 9691.85±22.58 [n=4834]; P<0.01, respectively).

Conclusions: Cows with PM3 have more pregnancy losses, more days open, and produced less milk at 150 and 305 days of lactation compared to PM0 cows. The clinical cure rate and partial cure rate for PM3 cows were low, and many PM3 cows developed CE after 21 dpp.

RD-16

Purulent vaginal discharge score and its association with subsequent reproductive performance in seasonal-calving, pasture-based dairy cows

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Objectives: Uterine infection diminishes reproductive performance in cattle. The use of Metricheck® to assess purulent vaginal discharge (PVD) may help to identify uterine pathological condition. The objective of this study was to evaluate PVD as a predictor of failure of reproductive performance in pasture based dairy cows to simplify diagnostic tools for uterine abnormalities and to reduce costs. Secondary objective was to determine the agreement between Metricheck® with ultrasound examination of the uterus as a tool to identify post-partum uterine abnormalities in dairy cattle. Materials&Methods: First and second lactation dairy cows (n=2,600) located in the province of Munster in Ireland were enrolled in the study. Postpartum examinations were performed at week 3 (wk3,14 to 27 days in milk) and week 7 (wk7, 42 to 55 days in milk) after calving. Purulent vaginal discharge was established as follows: PV1= clear mucus; PV2= flecks of pus within clear mucus; PV3= 50% purulent material. Ultrasound uterine score examination (8.5 MHz transrectal transducer Ibex Pro, Ibex®, Colorado, USA) was assessed as follows: G1: spoke wheel-shaped lumen; G2: small volume (>2 mm, ≤5 mm) of fluid of mixed echogenicity; G3: Moderate volume (>5 mm, ≤10 mm) of fluid of mixed echogenicity; G4: Large volume (>10 mm) of mixed echogenicity fluid. During the breeding season all animals were served after estrous detection. A post-breeding visit was carried out once between 34-50 days after farm mating end date to determine pregnancy status and foetal age. The reproductive parameters included in the study were: Submission rate (SR21), pregnant to first service (P/AI1), pregnant within 21, 42 and 84 days of onset of breeding (P21, P42, P84, respectively) and the Mating Start day Conception Interval (MSD-CI). Fisher's Exact Test was used and was supplemented by multiple logistic regression using the LOGISTIC procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC) to calculate odds ratios and predicted probabilities. The concordance between PVS and USE was assessed using Cohen's Kappa Coefficient with the FREQ procedure of SAS. Results: At wk 3, 18.7% (486/2600), 30.4% (790/2600), 26.9% (699/2600) and 24.0% (625/2600) of animals were diagnosed as having PV1, PV2, PV3 and PV4; and 2.2% (59/2600), 25.1% (653/2600), 60.5% (1574/2600) and 12.0% (314/2600) of animals were diagnosed as having G1, G2, G3 and G4 ultrasound uterine score, respectively. At wk7, 40.4% (1051/2600),

39.6% (1030/2600), 16.6% (434/2600) and 3.2% (85/2600) of animals were diagnosed as having PV1, PV2, PV3 and PV4; and 27.3% (669/2445), 48.3% (1182/2445), 23.3% (572/2445) and 0.8% (22/2445) of cows were diagnosed by ultrasonography as having G1, G2, G3 and G4, respectively. There was no association between PVD at wk3 (P=0.54) and wk7 (P=0.84) and SR21. Conversely, animals classified as having a PV4 at wk 3 and wk7 had reduced P/AI1 (both *P*<0.05; - 8 and -11 percentage points) compared with animals classified as having PV1. On the same way, cows classified as having PV3 and PV4 were less likely to achieve P21(P=0.01; -9.2 and -10.6 percentage points), P42 (*P*=0.008; 4.8 and 7.9 percentage points) P84 (*P*=0.003; only PV4, -5.6 percentage points) and the mean MSD-CI was 4.19±0.24 and 5.03±0.19 d longer (*P*<0.0001) compared with animals diagnosed as having PV1, respectively. Similarly, reproductive performance and MSD-CI were associated with PVS at wk7. Animals classified as having PV4 had less likelihood of P42 (*P*=0.005; -15.35, -12.25 and -14.11 percentage points) and P84 (*P*=0.01; -8.61, -6.67 and -7.11 percentage points) compared with animals having PV1, PV2 and PV3, respectively. MSD-CI was longer (*P*=0.04) in animals classified as having G4 uterine score compared with cows classified as having PV1, PV2 and PV3 (7.4 ± 0.2, 6.1 ± 6.18 and 6.4 ± 0.15 d, respectively).

The Kappa coefficients at week 3 and week 7 postpartum were 0.17 and 0.13, respectively, indicating slight to fair agreement between PVD and ultrasound uterine score examination as diagnostic techniques for uterine abnormalities.

Conclusion: The observations arising from this study provide evidence that PVD was strongly associated at both wk3 and wk7 postpartum in seasonal-calving, pasture-based lactating dairy cows. Purulent vaginal score evaluation at wk3 and wk7 post-partum identified an unfavorable association between PV3 and PV4 and reproductive performance. On average, the PVD scores were lower than ultrasound examination scores in the early postpartum period.

Keywords: Uterine-infection, dairy-cattle, fertility, ultrasonography.

RD-17

Effects of thermal stress on pregnancy characteristics in dairy cows

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Objectives: Due to global warming, the adverse effects of heat stress (HS) on dairy cattle welfare and production are gradually extending in northern latitudes. The combination of high ambient temperatures and relative humidity (THI) in dairy cows decreases appetite, milk yield, estrous expression, estrus length and pregnancy rates of inseminated animals. The extensive use of cooling systems for the abatement of the negative effects of HS, proved to be successful in preserving milk production, but their impact on fertility records are rather negligible. This is related to the high sensitivity of the maturing oocyte and the early embryo to the elevated temperatures as well as to the modifications that increased temperatures bring about at follicular, oviductal and uterine environment.

The trophoblastic cells of the bovine placenta express a series of glycoproteins named Pregnancy Associated Glycoproteins (PAGs) whose concentration is steadily increased with pregnancy progression, culminating at calving. PAG concentration of inseminated cattle is used as a biochemical marker of pregnancy and a reliable indicator for the wellbeing and functionality of the placenta. Progesterone (P4) is of paramount importance for pregnancy establishment and preservation, however, the limits between optimal and suboptimal concentrations is difficult to be accurately defined, as P4 concentrations are related to various environmental and metabolic factors.

Here, we focused to estimate the association between circulating concentrations of PAG, P4 and the early embryo mortality rates during summer and winter.

Materials and Methods: Two trials were conducted in two Holstein dairy farms of central Greece. Both farms are equipped with effective cooling systems comprising electronic assessment of THI and automatic activation of fans and sprinklers. In addition, according to thermal load all lactating cows had intensive cooling (soaking–drying) two to four times daily.

The purpose of the first trial was the quantification of the early embryonic mortality rates under HS or thermoneutral conditions. In 2018, a total of 279 cows were used (144 during the winter, group W, and 135 during the summer, group S). On days 24-25 post AI, all cows were diagnosed as pregnant by transrectal ultrasonography; on days 34-36 they were re-examined, and a blood sample was withdrawn for PAG determination.

The second trial lasted from 2014 to 2019 and aimed at PAG levels evaluation in 6109 blood samples submitted for routine pregnancy diagnosis. All samples were collected 29 to 36 days after breeding and assayed by a commercial ELISA kit (DG29, Conception Animal, Canada). According to THI, the samples were allotted into group S THI≥76 n=2388, or into group W THI≤68, n=3721. A sample was characterized as positive, ambiguous, or negative (PAG ≥1000pg/ml, 500 to 999pg/ml or <500, respectively).

Progesterone concentration was also determined in 50 pregnant animals from each group in serum samples collected on days 31 to 33 post AI by a commercial ELISA kit (DRG, Germany).

Results: In trial 1, the pregnancy maintenance rate differed significantly (p=0.03) among groups (136/144 - 94.4% and 81.5% - 110/135, for groups W and S, respectively). According to the PAG concentrations all animals were characterized as pregnant.

In trial 2, PAG levels on days 33 to 36 after breading were significantly higher (p=0.043) in group S (2546.8±63.1 ng/ml) compared to those of group W (2385.51±42.4 ng/ml).

Progesterone was lower (p<0.05) in group S (6.76±0.96ng/ml) compare to W (8.66±1.27).

The proportions of ambiguous pregnancies were lower

(p<0.001) in group S (7.88%) compared to W (11.10%).

Conclusions: From the data presented here we infer that during summer months the embryos that can survive HS develop a well-functioning placenta. This is in concert with our in vitro studies where we provided evidence that blastocysts produced from oocytes exposed to HS have high expression of genes related to placentation. On the other hand, the combined results of embryo mortality and progesterone might be indicative that during the winter, slow developing or weak embryos have more chances to survive.

Keywords: Heat stress, progesterone, PAGs, early embryonic death, dairy cattle.

RD-18

The effect of *coxiella burnetii* inactived vaccine on reproductive parameters and milk yield in Holstein cows

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Objectives: The aim of this study was to determine the effect of *Coxiella burnetii* inactivated vaccine on reproductive parameters and milk yield in Holstein cows.

Material & Methods: A total of 575 pregnant cows (165-170 days of pregnancy) were examined serologically and distributed to the research groups. While the cows were serologically positive formed the Positive Control (n=174, PosC) group, the cows that were serologically negative for *Coxiella brunetii* were randomly assigned into two groups: The cows were vaccinated formed the Negative Vaccine (n=175, NegV) and were not vaccinated served as Negative Control (n=226, NegC) The cows in the NegV were vaccinated 3 weeks apart with *Coxiella burnetii* inactivated vaccine (4 ml, s.c. Coxevac®, Ceva, Istanbul, Turkey) and serological examinations were repeated 3-4 weeks later to determine the efficacy of the vaccine. During the study, pregnancy, birth, postpartum, and lactation processes of the cows were monitored. At the end of voluntary waiting period, the cows were inseminated with modified G6G protocol for the first service and the cows whose unable to get pregnant were received resynchronisation protocol. Pregnancy examinations performed by ultrasonography.

Results: The abortion rate was 2.7% (6/226) in NegC, 1.1% (2/175) in NegV, 0.6% (1/174) in PosC group. Due to the different reasons 21 cows were culled thus 554 cows were monitored for postpartum period; The incidence of stillbirth was 1.9% (4/210) in NegC and 3.5% (6/170) in PosC groups, no

stillbirth cases were observed in the NegV (0/169) group. Fetal membrane retention was 14.5% (31/214), 10.0% (17/170) and 9.4% (16/170) in NegC, NegV and PosC groups, respectively. The incidence of metritis was 8.4% (18/214) in NegC, 10.6% (18/170) in NegV and 7.1% (12/170) in PosC groups. The total of 477 cows were able to be evaluated for reproductive parameters since there were cows (n=98) that culled for different reasons. First service conception rate was 29.8% (54/181) in NegC, 30.1% (43/143) in NegV, 34.4% 52/151 in PosC groups. Number of inseminations per pregnancy were 1.56 (128/82) in NegC, 1.39 (96/69) in NegV and 1.44 (89/88) in PosC groups. Embryonic loss rates were determined as 6.0% (5/83) in NegC, 9.2% (7/76) in NegV and 11.1% (11/99) in PosC groups. The rate of repeat breeder cows was determined as 4.9% (9/181) in NegC, 2.8% in NegV (4/144) and 4.6% (7/152) in PosC groups. The data belongs to the cows were completed for the first 100 days; In the NegC (n=184), NegV (n=147) and PosC (n=154), the total milk yield in the first 100 days was recorded as 4739.65 ± 46.56 kg, 4772.81 ± 55.27 and 4752.82 ± 47.92 kg; mean peak yield was recorded as 56.21 ± 0.50 kg/day, 56.39 ± 0.53, and 55.91 ± 0.56 kg/day. While the mean peak day was 64.03 ± 2.13 DIM in NegV, it was 67.07 ± 2.43 DIM in PosC, which was higher (P<0.05) than in Neg C (61.28 ± 1.74 DIM) groups.

Conclusion: In conclusion, it was found that the cows have no *Coxiella burnetii* antibody at the beginning of the study (NegC) had numerically higher abortion and retention of fetal membrane rates compare to other cows in NegV and PosC groups which included the cows whether had natural or acquired immunity. Stillbirth and embryonic loss rates were numerically higher in PosC group compare to the other groups. Also it was found that the milk peak day of the cows in PosC group was seen later than of the cows in other groups.

Keywords: Cow, Coxiella brunetii, inactivated vaccine, reproductive parameters, milk yield.

RD-19

HH1 double-carrier embryos develop normally to elongated conceptuses

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Objectives: Intensive genotyping of Holstein population has identified deleterious haplotypes impacting fertility. These haplotypes are never found in homozygosity indicating that double-carrier (i.e., homozygous) embryos die before birth. However, the developmental stage when embryonic or fetal loss occurs remains unknown. Timing of developmental arrest is relevant for reproductive management, as embryonic losses occurring before maternal recognition of pregnancy lead to luteolysis and recovery of estrus cycle, whereas later losses ex-

ert a much greater impact on conception intervals. The objective of this study was to determine if homozygous embryos for Holstein Haplotype 1 (HH1) are able to develop to elongated conceptuses, which trigger maternal recognition of pregnancy.

Material & Methods: *In vivo* produced embryos were recovered at Days 9 (D9, expanded blastocysts), 11 (D11, ovoid conceptuses) or 14 (D14, elongated conceptuses) post-fertilization from superovulated HH1-carrier cows (2 cows/stage) inseminated with semen from a HH1-carrier bull. Embryos were fixed in 4 % paraformaldehyde for 10 min and kept at 4 °C until analysis. D9 embryos were subjected to immunostaining with anti-CDX2 to determine trophectoderm (CDX2+) and inner cell mass (CDX2-) cell number. Total and epiblast cell numbers were determined on D11 conceptuses by immunostaining for SOX2. Conceptus and embryonic disc length were measured on D11 and D14 conceptuses. Finally, Sanger sequencing was performed to determine the genotype of each embryo: non-carrier (NC), carrier (C) or double-carrier (DC).

Results: Day 9 embryos showed Mendelian distribution of alleles (5:8:4 for NC:C:DC). At that stage, genotype did not determine blastocyst cell counts (TE: 98±7 vs. 106±4 vs. 105±5; ICM: 20±1 vs. 21±1 vs. 22±2; for NC, C and SC, respectively, ANOVA p>0.05). Day 11 conceptuses also showed Mendelian distribution of alleles (4:12:5 for NC:C:DC). Conceptus or embryonic disc size was also similar across genotypes (conceptus length 535±84 vs. 546±89 vs. 446±68 µm, disc length 106±13 vs. 107±12 vs. 105±7 µm, for NC, C and DC, respectively, ANOVA p>0.05) and no differences were noted on total or SOX2+ (epiblast) cells (total cells 1262±209 vs. 1291±352 vs. 951±282; SOX2+ cells 50±4 vs. 54±10 vs. 50±7; for WT, Hz and KO, respectively, ANOVA p>0.05). Finally, Mendelian distribution was also unaltered by D14 (2:3:3 for NC:C:DC) indicating that DC embryos are able to develop to elongated conceptuses. A significant cow effect was noted on conceptus and embryonic disc length at D14, but genotype did not influence any of these parameters (conceptus length 7.9±6.1 vs. 5.6±5 vs. 5.7±2.7 cm; disc length 0.52±0.11 vs. 0.44±0.16 vs. 0.57±0.13 cm, for NC, C and DC, respectively, two-way ANOVA p<0.05).

Conclusion: HH1 double-carrier embryos develop normally to elongated conceptuses, suggesting that the developmental arrest induced by the causative mutation occur after maternal recognition of pregnancy, leading to a significant increase in days open.

Keywords: Embryo; haplotype; fertility; elongation; HH1.

Effect of clinical and subclinical mastitis treatment with meloxicam on clinical cure and fertility of dairy cows in Ecuador

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Objective: To study the effect of clinical and subclinical mastitis treatment with meloxicam on clinical cure rate and fertility of dairy cows in Ecuador.

Material and Methods: Holstein cows from two dairy herds from Pichincha, Ecuador, were used in a study that began in March 2020 and ended in October 2021. Cows with 35-150 days in milk (DIM) had a California Mastitis Test (CMT) done every two weeks and cows with a CMT score 2 (CMT2), score 3 (CMT3), or with clinical mastitis (CM) were enrolled. Cows with odd ear tags were assigned to the control group (CON, n=204), and cows with even tag numbers were assigned to the treatment group (TRT, n=189). Cows with CMT2 in the TRT group were administered meloxicam (0.5 mg/kg BW, Metacam® Boehringer Ingelheim, Ecuador), whereas cows in the CON group remained untreated. Cows with CMT3 and CM in the TRT group were administered an intramammary antibiotic treatment (tetracycline 200mg, neomycin 250 mg, bacitracin 2000 UI, prednisolone 10 mg, Mastijet Fort®, MSD Salud Animal, Mexico) and meloxicam (0.5 mg/kg BW); whereas cows in the CON group were administered the intramammary antibiotic but not the meloxicam treatment. After treatment, cows had a CMT done every two weeks to determine the clinical cure rate (complete, CMT0; partial, CMT reduced; worst, CMT increased or maintained). After a voluntary waiting period of 55 days (d), cows were synchronized with cloprostenol (Ciclase DL®, Zoetis, Ecuador) and AI at detected heat. Cows not detected in heat by 80 DIM were synchronized and timed AI. In addition, for a cow to enroll in the study, the CMT2-3 and CM event had to take place in a window of time of 30 d before 45 d post-AI. Transrectal ultrasonography was used to diagnose pregnancy status at 30±3d post-AI, and those cows diagnosed pregnant were reconfirmed pregnant at 60±3d post-AI. The clinical cure rate (CCR) to CMT2, CMT3+CM at 30 d post-treatment were examined. Pregnancy rate at 30d (PREGR), pregnancy losses at 60 d (PLOSS), calving to conception interval-days open (CCI), number of services per conception (SPC), and culling rate (CULLR) were also analyzed with logistic regression (PROC GLIMMIX, SAS 9.04).

Results: The CCR for CMT3+CM in the TRT group was higher compared to the CON group (72.5% [58/60] vs. 57.3% [43/75], P=0.036), but was similar between CMT2 TRT and CON groups (62.5% [152/243], P=0.73). The PREGR and PLOSS were similar in TRT and CON groups for CMT3+CM (41.9%, P=0.44; 36.76%, P=0.53), and CMT2 (47.22%, P=0.56; 32.35%, P=0.35). The CCI for CMT3+CM in the TRT group was shorter than the CON group (100.66±11.08 vs. 133.23±11.79 d, P=0.04). Also, the SPC for CMT3+CM in the TRT group was lower than the CON group (1.69±0.32 vs. 2.62±0.34, P=0.05). Conversely, the CCI and the SPC were similar between CMT2 TRT and CON groups (118.50±10.95 d, P=0.46; 2.19±0.24, P=0.21). The CULLR was reduced in CMT2 TRT cows compared to CON cows (5.50% [6/109] vs. 15.5% [20/129], P=0.01), but was similar in CMT3+CM TRT and CON cows (14.19% [22/155], P=0.52).

Conclusion: Cows with CMT3+CM that received antibiotic treatment plus meloxicam had a higher clinical cure rate than cows that received antibiotic therapy alone. Furthermore,

these cows had fewer days open and lower AI per conception than the untreated cows. In addition, cows with CMT2 had no benefit of meloxicam treatment on clinical cure rate or reproductive performance, but meloxicam treated cows had a lower culling rate during lactation.

Keywords: Clinical mastitis, subclinical mastitis, meloxicam, fertility, clinical cure.

RD-21

Injuries of the bovine birth canal after eutocia

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Objectives: While there is numerous of literature describing the involution of the uterus after unassisted birth, there is little information available about possible alterations of the soft birth canal in cows and heifers. So the aim of this study was to describe potential injuries of the soft birth canal post partum (p.p.).

Material and Methods: For this purpose, 50 Holstein-Friesian cows (25 heifers und 25 cows) were assessed vaginally using a speculum within eight hours after birth and on day 1, 5, 10, 15 and 21 p.p.. Special attention was given to lacerations, hematomas, hyperemia and suffusions in the area of the cervix, vagina, hymenal region, vestibulum vaginae and vulva.

Results: Immediately after birth all animals presented lacerations in the dorsal commissure of the vulva, which had healed off completely until day 15 p.p. in approximately 50% of the heifers, and on day 10 p.p. in approximately 70% of the cows. Additionally, all heifers and 41% of the cows presented with lacerations of the hymenal region, which had healed almost completely by day 15 in 75% and 99%, respectively. Hyperemia and suffusions were present in all heifers in this region. While the hyperemia had disappeared by day 10 p.p, suffusions were still present in approximately 45% of the animals on day 21 p.p..

Conclusions: In summary, there are injuries in the soft birth canal even after unassisted birth and it is possible to describe a unique injury pattern. Particularly heifers have significant injuries in the area between the vulva and the hymenal region; however, these heal entirely within a short period of time.

Keywords: Laceration, hematoma, hymenal region, vulva.

RD-22

Influence of different factors on fertility of dairy cows submitted to fertility protocols for first insemination postpartum and for resynchronization

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Objectives: The use of "fertility protocols" for artificial insemination (AI) at fixed time adding improvements to Ovsynch®, as presynchronization, use of a progesterone device and two doses of prostaglandin F2α (PG), has showed increases of conception rate in comparison to the normal Ovsynch protocol or natural estrus. At commercial farms level, the length of the protocol is something important so it can be long for first insemination postpartum but should be as short as possible in the case of resynchronizing open cows in order to reduce the number of days open.

In this study we tried two protocols for first AI: the G6G protocol, modified to end as Cosynch (with the last GnRH dose at the same time of AI), with 6 handlings, that lasts for 18 days, and a 7 d Cosynch plus progesterone device, with 4 handlings, that lasts for 10 d. For resynchronizing open cows we used a Resynch protocol (modified for administering the last GnRH at the same time of AI) starting with a GnRH 7 d before pregnancy diagnosis, with 4 handlings, that lasts for 3 d from the diagnosis of open cow. We analyzed some factors that could affect these protocols to understand how they can be used to improve reproduction efficiency in comercial dairy farms

Materials and methods: The study was conducted during 2019 in 12 dairy farms located in León, Spain. A total of 994 synchronization protocols were performed and the cows were explored by a veterinarian at the moment of insemination to evaluate the size of the follicle and the signs of estrus at uterine and ovarian level to inseminate only cows with estrus signs.

The cows enrolled for first AI were treated as "Group G6G" (n=421): day 0 PG, day 2 GnRH, day 8 GnRH, day 15 PG, day 16 PG, and day 18 AI plus GnRH; "Group progesterone device" (n= 227): day 0 GnRH + insertion of progesterone device, day 7 PG and progesterone device removal, day 8 PG, day 10 AI plus GnRH. In a subgroup of cows in groups "Progesterone device" and "G6G", blood samples were collected at the beginning of the Cosynch, at the moment of the first prostaglandin of the Cosynch and at the moment of AI. The cows that didn't show return in estrus, were enrolled as "Group Resynch" (n=346) receiving GnRH 7 d before the examination and if diagnosed "open" the day of the pregnancy diagnosis, they were treated: day 0 PG, day 1 PG, day 3 AI plus GnRH.

Data were analyzed with Medicalc® and the different chi-square test were performed for every variable.

Results: The synchronization rate was evaluated as percentage of cows accepted for AI and was high for the three groups: "G6G" 84.03%, "Progesterone device" 88.33% and "Resynch" 94.02% (p=0,057) with progesterone concentration profiles corresponding with that findings: beginning of Cosynch 2,16 ng/ml, first PG 2,90 ng/ml and moment of AI 0,32 ng/ml. The conception rate was high in the three groups: "G6G" 44.89%, "Progesterone device" 40.53% and "Resynch"

49,42%, (p=0,11). The median days to AI were significantly different in inseminations with diagnosis "pregnant" or "open": open 121.47 and pregnant 134.08. (p=0.007). The conception rate of different follicle sizes were 10 to 15: 40.43%, 16 to 20: 40.00% and > 20: 30.77 (p=0.45%). The conception rate of different parities were 1: 49.86%, 2: 44.73%, 3 : 39,13% and cows with 4 or more calvings 49.26%. (p=0.08).

Conclusions: The numerical differences in conception rate between protocols could have been influenced by the days in milk at AI so, taking into account the high synchronization rate and fertility of these fertility protocols, they can be implemented at farm level, the longer ones for first post-partum AI considering their easiness of compliance, and the shorter one for a fast resynchronization of open cows.

The decreasing conception rate by parity have been previously reported, but the high conception rate in cows with 4 or more calvings could be influenced by the retention of the most fertile cows, showing that the retention of some old fertile cows could extend the general longevity of farms.

Keywords: Fertility, protocol, post-partum, resynchronization, insemination.

RD-23

Adiponectin and Chemerin in the endometrium of postpartum dairy cows with cytological endometritis

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Objective: To evaluate the relationship between the gene transcription and expression of Adiponectin (ADIPOQ, ADIPOR1 and ADIPOR2) and Chemerin (RARRES2 and CMKLR1), and the inflammatory status of the uterus of postpartum dairy cows.

Materials and methods: High-yielding postpartum dairy cows (n=36) without puerperal disease were retrospectively allocated to groups: i) Healthy (H; n=6), without cytological endometritis (CE) at 25 and 45 days postpartum (dpp) and pregnant at first AI; ii) CE-H (n=19), with CE at 25 dpp, but that recovered by 45 dpp; iii) CE-CE (n=11), with persistent CE until 45 dpp. Endometrial cytology was assessed from uterine swabs taken with a cytobrush device at 25 and 45 dpp (cut-off values: 18 % and 5 %, respectively). At 45 dpp, a low volume lavage followed by centrifugation, allowed to obtain cellular pellet and supernatant samples of each uterine horn. An endometrial biopsy was also taken from each uterine horn. Gene transcription of *ADIPOQ* and its receptors (*ADIPOR1* and *ADIPOR2*), and of chemerin (*RARRES2*) and its receptor (*CMKLR1*) was analysed in the cellular pellet by quantitative real-time PCR. Protein expression was analysed by immu-nohistochemistry (IHC) in endometrial biopsy samples and protein production (ADIPOQ and RARRES2) by ELISA in the supernatants.

Results: Transcription levels of *ADIPOQ* and *ADIPOR2* were higher (p < 0.001) in CE-CE than in H and CE-H cows, whereas *ADIPOR1* mRNA levels were lower (p < 0.05) in CE-CE cows than in H cows. Transcription levels of *RARRES2* and *CMKLR1* were higher in CE-CE than in H and CE-H cows (p < 0.01). Positive immunostaining for ADIPOQ was observed in the luminal and glandular epithelium, endothelial cells, stroma and inflammatory cells of all cows. However, CE-CE cows exhibited a stronger staining than H and CE-H cows. Staining for ADIPOR1 was only observed in the luminal and glandular epithelium, whereas ADIPOR2 also stained in stroma and inflammatory cells. Staining for RARRES2 and CMKLR1 was observed in all endometrial compartments of all cows. However, RARRES2 staining showed a stronger signal in CE-CE than in H and CE-H cows. Uterine fluid ADIPOQ and RARRES2 concentrations were higher (p < 0.001) in CE-CE than in H and CE-H cows.

Conclusions: At 45 dpp, cows with persistent CE show up-regulated gene transcription and protein expression of Adiponectin and Chemerin in the endometrium, compared with healthy cows and cows that recovered from CE. Results support a local production of these mediators, and a relationship with the inflammatory status of the postpartum uterus of dairy cows. The local production and signalling of these adipokines prompt for an autocrine and/or paracrine role in the inflammatory response exhibited by cows with subclinical endometritis. This turns these adipokines into potential biomarkers of endometrial inflammation, namely to identify cows at risk of persistent subclinical endometritis. However, further studies are warranted to clarify their role in the establishment of subclinical endometritis.

Keywords: Adiponectin, Chemerin, Endometritis, Cow.

RD-24

The Effect of Postpartum Metabolic Diseases on Fertility in Lactating Dairy Cows

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Postpartum (PP) metabolic disorders may become more important for the modern high producing lactating dairy cows. The aim of this study was to evaluate the effect of PP metabolic disorders on fertility in dairy cows. The study was conducted in a dairy farm with 5000 lactating dairy cows and milk yield was 11.148 kg for 305 d in 2019. Primiparous and multiparous cows were evaluated for metabolic disorders such as Retained Placenta (RP), Septic metritis (SM, Fever >39.4˚C

and reddish vaginal discharge with fetid odor after DIM 5), Milk fever (MF), Ketosis (BHBA higher than 1.2 ng/ml, DIM 3-7) and Displaced abomasum (DA). In multiparous cows, BCS (at the close up period) and its relation to PP metabolic disorders were also evaluated. All cows were timed inseminated (TAI at 75-81 DIM) with Double Ovsynch protocol for first service and second service were done by Resynch protocol (GnRH-7d-GnRH-7d-PGF-1d-PGF-56h-GnRH-16-20h TAI). Pregnancy diagnosis were done at the time of the first PGF (30-36 d after TAI) by ultrasonography. In multiparous cows mean BCS was higher (P<0.01) in the cows with PP metabolic disorders compared to the cows without metabolic disorders (BCS 3.42, BCS 3.37, respectively). Pregnancy rate after first (33.0%), and second (29.5%) service were higher (P<0.002) in multiparous cows without PP metabolic disorders compared to the multiparous cows with at least one metabolic disorders (21.7%, 19.0% respectively). Pregnancy rate of multiparous cows after first service was negatively effected (P<0.001) in the cows with RP, SM, and ketosis, tended to be effected (P<0.09) with MF, but not effected with DA. In addition, cows with ketosis had also lower (P< 0.03) pregnancy rates after second services. However in primiparous cows pregnancy rate was lower (P<0.03) only in cows with Ketosis. Thus, cows with higher BCS at close up period at higher risk of having PP metabolic disorders and cows with some of the metabolic disorders such as RP, SM and ketosis in early PP period having more effect on fertility at first service. The negative effect of ketosis on fertility may be more prominent compared to other PP metabolic disorders, because it's long lasting affect and in both primiparous and multiparous cows. Interestingly, primiparous cows less effected from metabolic disorders than multiparous cows except ketosis.

Keywords: Metabolic disorders, Fertility, lactating dairy cows.

Key performance indicators used by dairy consultants during the evaluation of reproductive performance in a first visit

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Objectives: The purpose of this study was to (1) describe the KPI that consultants specialized in dairy reproduction use to assess the reproductive status of a conventional dairy farm in a first visit, (2) categorize the different KPI according to their importance to the consultants, and (3) identify primary KPI that could be universally used in first visit to a conventional dairy farm.

Materials and methods: An online survey was sent worldwide and answered by consultants specialized in dairy reproduction (n=49) to find out the most suitable parameters in a first visit approach. The survey was comprised of 190 questions, 178 of them rated from 0 (irrelevant) to 10 (maximum importance) points. The questions were divided into 5 sections: 1) consultant and farm model, 2) general data of the farm, 3) cow reproduction, 4) postpartum and metabolic disease, and 5) heifer reproduction. The median, range, and 95% confidence interval were determined for each question. Afterwards, a multivariate analysis, using between-group linkage via Ward´s hierarchical clustering was conducted to generate clusters of consultants according to their response pattern. Lastly, a Chi-square test was conducted to assess the association between years of experience of the consultant and farm size within the clusters generated in each section of the questionnaire.

Results: The majority of the consultants considered 27 parameters to be highly important to analyze in a first visit.

The parameters that consultants specialized in dairy reproduction mostly consider as primary KPI are:

General data of the farm: Culling rate (%), pregnant cows (%), average DIM (d), cows culled for reproductive reason (%), 305-d yield (Kg), herd status for BVDV (present/absent) and % of 1st lactation cows in the herd.

Cows' reproduction: First service CR (%), overall pregnancy rate (%), voluntary waiting period (d), CR (%), heat detection rate (%), days open (d), cows not pregnant >200DIM (%), 21d. pregnancy rate (%), CR of the first service in 1st lactation cows, CR of the first service in multiparous cows (%), CR of the 1st lactation cows (%), CR of the multiparous cows (%), calving to first service interval (d), CR synchronized cows (%) and percent conceiving of served (%).

Heifers' reproduction: Heat detection rate (%), CR (%), CR of the first service (%), age at first calving (d) and heifers culled for reproductive reason (%).

Conclusion: Consultants never use only one single primary KPI to evaluate any of the presented sections, but they use several of them (in variable quantitative range). Despite the presence of a great number of parameters likely to be KPI to assess the reproductive performance of a farm, consultants clearly prefer parameters that provide information about heat detection, fertility and when the pregnancy is achieved regarding to the production cycle of a dairy cow.

Consultants also show high interest in getting a general overview of milk production, the farming efficiency and the heifer rearing process but the farm size and the years of experience of the consultant is other factor influencing in the type and numbers of parameters chosen to be KPI.

The parameters rated with the highest importance (rate 10) that could be considered for an easy, fast and universal use in a first visit to assess the reproductive status were: First service CR (%), overall pregnancy rate (%) and 21d pregnancy rate (%) for cows and age at first calving (d) for heifers.

Parameters that monitor postpartum and metabolic diseases are not considered necessary to evaluate the reproduction performance of a farm in a first visit.

Keywords: survey analysis, dairy consultant, reproductive performance, first visit, KPI.

Elevated tail: clinical sign for the early diagnosis of vaginal pathologies

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Elevated tail is a feature seen around defecation and urination. It is a clinical sign present in pathologies related to reproduction, urinary and digestive systems. In reproduction, this sign has been observed with calving contusions and hematomas of the vagina; with minor vaginal traumas after mating or vaginal explorations; and in cases of necrotic vaginitis, vestibulitis, and vulvitis postpartum. The sign occurs within 1–4 days post calving and it may persist for 2–4 weeks, until vaginitis resolves, except in cases of vaginal lacerations, pneumo, and urovagina in which the raised tail may stay in time. Vaginal disorders such as pneumovagina and urovagina reduce the fertility of the cow and they are conditions frequently underdiagnosed. In large farms with intensive reproduction management systems, it is uncommon to perform complete reproductive exploration of cows, including the vagina, of all cows, before first service. However, some cows can keep vaginal problems since calving and are diagnosed at the earliest as repeat breeder with >200 days in milk, as non-pregnant after≥3 services, when the correcting surgery may be not any more profitable.

Objectives: The present study assesses the usefulness of the sign of "elevated tail" as an indicator to detect in an easy and fast way, cows with vaginal pathologies. Our hypothesis was that selecting those post calving cows raising their tail, for a complete vaginal exploration during routine farm visits, will provide an early diagnosis of vaginal pathologies, allowing an efficient treatment of them, without notable extra work, reducing the culling rate due to reproduction.

Materials and methods: We worked in three Spanish dairy farms of 73, 263 and 250 cows in milk/farm, respectively, during one routine reproductive control. The cows that showed elevated tail at the visit (excluding those which were defecating, urinating, less than a day calvingor had recently been inseminated), were completely explored. This exam included rectal palpation, ultrasound exam and vaginal exploration, trying to identify the type of problem and vaginal content. The presence of air, urine, other vaginal content, and tears was recorded.

Results: A total of 586 cows in milk were present during these visits; 28 raised their tail (4.78%). In 7 cases out of 28 (25%) no pathologies were diagnosed, which would make a sensibility for the sign used as diagnoses method of 75% with 25% of false negatives. The remaining 21 animals (75%) showed the following reproductive problems: postpartum vaginitis in 6 cases (21.42%); pneumovagina 6 (21.42%); urovagina 4 (14.29%), pneumo and urovagina 3 (10.71%) and vaginal laceration 2 (7.14%).

Conclusions: Our preliminary results seem to indicate that the clinical sign of an elevated tail may allow to efficiently select, with a high probability (≈75%), those cows with vaginal pathologies for a further and complete examination. This method will allow their prompt treatment, reducing the culling rate due to reproduction. Further studies will be implemented to describe the validity of this clinical sign as a diagnosis method of vaginal disorders in dairy cows.

Keywords: Tail, elevated, urovagina, pneumovagina, diagnosis.

Uterine contractility patterns of early puerperal hypocalcaemic dairy cows without and with fetal membrane retention

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Objectives: Characteristics of the early postpartum uterine contractility based on the measurement of intrauterine pressure (IUP) changes in hypocalcaemic cows without and with retained placenta during the first two days after calving has been compared in a field study.

Materials and methods: Myometrial contractions of altogether 31 early puerperal Holstein-Friesian cows at a large-scale Hungarian dairy farm were repeatedly measured using an open tip catheter system, which was suitable for digital intrauterine pressure (IUP) recording. Cows were divided into four groups according to their initial coccygeal venous blood Ca^{2+}-concentrations, which was measured on site between 14 and 17 hours after calving (ABL™ 77 portable blood gas and electrolyte analyser, Radiometer, Copenhagen, Denmark) and upon their status regarding placental expulsion. Cows that had lower than 1.06 mmol/l blood Ca^{2+} concentrations at this first blood withdrawal either belonged to Group HC-NRFM (hypocalcaemic, no retained fetal membranes, n=12) or to Group HC-RFM (hypocalcaemic, retained fetal membranes, n=6), while others that had at least 1.06 mmol/l blood Ca^{2+}, belonged either to Group NC-NRFM (normocalcaemic, no retained fetal membranes, n=8) or to Group NC-RFM (normocalcaemic, retained fetal membranes, n=5). Initially, a 4-hour IUP recording was performed, starting between 14 and 17 hours after calving, from which only the first hour was used for the recent analysis (labelled as pp12). In 12-hour intervals, three further recordings took place with a duration of 1 hour for each (labelled as pp24, pp36 and pp48, respectively). IUP data were collected from the previously pregnant uterine horn and were analysed using a semiautomatic, operator-made software (LabVIEW® 5.0, National Instruments, Austin, TX). Mean contraction frequency (FREQ), amplitude (AMP), duration (DUR), mean and total area under the pressure curves (AUC and TAUC) of the pressure cycles were calculated. At the end of each recording

session a further blood withdrawal has been made to determine Ca²⁺ concentrations.

Beside discovering possible associations among values of various IUP parameters and blood Ca^{2+} concentrations by the use of a regression analysis, repeated measures analysis of variance was used to calculate group- and time-related differences. In case of significance at $P<0.05$, Tukey post-hoc tests were performed to explore where significance between groups, recording times or their interactions appeared (STATISTICA v. 6.1., Statsoft, Inc., Tulsa, OK).

Results: All hypocalcaemic cows had a mild to moderate level of hypocalcaemia with the lowest initial Ca^{2+} concentration of 0.74 mmol/l in two cows. In all IUP parameters time dependent significant declines were found ($P<0.001$) when neither RFM nor Ca^{2+} status were distinguished. When both the effect of RFM and Ca^{2+} status were considered, also none of the parameters showed in their values an overall significant difference at $P<0.05$, which would have been attributed to either of these group differences. However, at pp12, hypocalcaemic cows with RFM had higher TAUC values, representing a more active uterine contractility than their NRFM alternatives ($P=0.0064$). At pp12 TAUC was significantly higher in cows with RFM ($P=0.0014$) and at pp24 AUC also in such cows ($P=0.0325$) without considering the initial levels of blood Ca^{2+}. However, without considering the time effect, despite consequently higher mean values in all IUP parameters in RFM cows, the differences were statistically not significant at $P<0.05$. When the grouping effect according to initial Ca^{2+} levels has not been taken into account, except DUR, all IUP parameters showed significantly higher overall means in cows with RFM than in cows without (FREQ: $P=0.0017$, AMP: $P=0.0079$, AUC: $P=0.0020$ and TAUC: $P=0.0001$), while the level of such hypocalcaemia did not influence uterine contractility. Blood Ca^{2+} has significantly changed by declining from pp12 to pp24 and gradually increasing thereafter ($P=0.012$), but among cows with and without RFM, no difference was found regarding this parameter.

Conclusions: Dairy cows with mild or moderate hypocalcaemia during the first two days postpartum showed rather similar uterine contractility patterns with a significant time related decline, however, cows with RFM had higher uterine mechanical activity, irrespective of their calcium status within the mentioned range.

Keywords: Cattle, Uterine contractility, Hypocalcaemia, Placenta retention.

Novel methods to analyse mucus. Use of FTIR-spectroscopy to examine bovine cervical vaginal mucus

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Objective: Cervical vaginal mucus (CVM) has important functions related to reproduction and reproductive disease in cattle. To facilitate further study of CVM, an appropriate method of storing and processing CVM samples needs to be developed. The aim of this study was to compare the effects of different methods of storage and processing of CVM on the spectra obtained by Fourier Transform Infrared (FTIR) spectroscopy.

Material and Method: 16 CVM samples were taken from 15 Norwegian Red dairy cows housed at Livestock Production Research Centre at the Norwegian University of Life Sciences. The cows were selected based on convenience of the likelihood that they would be able to provide 2ml of CVM when sampled.

The cows were secured, and their vulvas cleaned with a chlorhexidine solution, before being dried with paper towel. A Metricheck™ device was used to sample CVM. The samples were transferred to Eppendorf® tubes. All samples underwent light microscopy to characterise ferning patterns before freezing and after thawing.

The samples were separated into two equal groups (1 and 2) and processed as follows: 1) Samples were further split into two groups and frozen (FRO) or frozen and freeze-dried (FD). 2) Samples were split into two groups and frozen (FRO) or kept fresh (F).

FRO: Samples were frozen in a freezer at -20°C.

FD: samples were frozen in a freezer at -20°C before being freeze-dried in a Labconco freeze-drier using high vacuum at -52°C. The samples were mixed with approximately 100μl water/g dried sample and 70g of acid washed glass beads (710-1, 180μm) /g sample before disrupting.

F: Fresh samples were treated directly after sampling.

Once processing was complete, the FRO and FD were thawed at room temperature. The F and FRO groups underwent one of five treatments. These were: no treatment, three durations of ultrasound treatment (5s, 10, s and 15s), and disruptor treatment. The FRO and F disruptor-treatment involved 0.5ml of mucus applied to 250mg of acid washed glass beads. All disruptor samples were run on a work program (5500 rpm, 6 cycles à 20s, 20s pause) in a Precellys Evolution disrupter-machine. For the ultrasound treatment, 0.5ml of mucus was placed on ice and homogenized with an ultrasonic homogenizer a 2mm tip was inserted 1cm into the mucus with amplitude of 40%. Five second exposures were applied once, twice or three times, giving samples exposed 5, 10 and 15 seconds.

After treatment, all samples were transferred to IR-light-transparent silicon 384-well microplates (Bruker Optik, Germany), approximately 10μl/ well, with 3 replications, and dried at room temperature. FTIR spectroscopy was performed using a high-throughput screening eXTension (HTS-XT) unit coupled to a Vertex 70 FTIR spectrometer (both Bruker Optik, Germany) allowing performance of high-throughput screening (HTS) transmission mode measurements. The spectra were recorded in the region between 4000 and 500 cm−1 with a spectral resolution of 6 cm−1 and an aperture of 5.0 mm.

A general analysis of the whole spectra was performed before the different informative regions were analysed. Spectra in the 1654 cm−1-region <0.2 or >2.5, were removed from the analysis. The data were pre-processed using a second de-

rivative calculated with the Savitzky-Golay algorithm, window size 11, second polynomial order. Three informative regions were split as follows: lipids (3050–2800 cm−1 and 1800–1700 cm−1), proteins (1700–1500 cm−1) and polysaccharides (1200–700 cm−1). The whole spectra and the informative regions were all pre-processed using Extended Multiplicative Signal Correction (EMSC) and analysed with principal component analysis.

Results*:* Light-microscopy showed little, or no, change in ferning patterns before and after freezing. All the preparation methods improved handling and homogenisation compared to the F group. Scatterplots show that the variable individual cow had a greater effect on the spectra (general and informative groups) than the preparation method. However, variation exists between preparation methods within cow. The F samples show most inter-cow variation, followed by the ultrasound treated samples. Whilst the disruptor samples show least variation.

Conclusions: Provisional results reveal that a homogenising technique may be useful in measuring changes in CVM. Further work needs to be performed to identify the optimal dilution for sample analysis. Treatment with disruptors appears to provide a better result than ultrasonographic treatments, although this needs further study. Freezing does not seem to alter the results.

Keywords: Cervical vaginal mucus, bovine, Fourier Transform Infrared spectroscopy.