



## RB-01

### Recombinant glycoprotein with eCG-like activity did not increase the incidence of multiple ovulation in beef cattle in a 5-day CoSynch protocol

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**Objectives:** The objective was to compare the effect of a glycoprotein with eCG-like activity on the incidence of multiple ovulation in *Bos taurus* beef cattle synchronized with a 5-d CoSynch plus progesterone protocol.

**Material & Methods:** Angus heifers of 22-month-old and suckled cows were submitted at random to either a 5-d progesterone CoSynch with reCG (reCG; n=140) or a 5-d progesterone CoSynch without reCG (Control; n=141). The body weight (BW; mean±SD) and body condition score (BCS, scale 1-9) of 22-month-old heifers of Herd 1 (n=95) and Herd 2 (n=97) were 377±21 Kg and 382±30 Kg, and 5.2±0.4 and 6.3±0.5 respectively, while suckled cows in Herd 3 (n=89) had 460±46 Kg and 5.2±0.5 units of BCS. The day of TAI was considered as Day 0 of the experiment. On Day -8, BCS was recorded and presence, number and location of CL (cyclicity) was determined based on ultrasonography. On the same day, all animals received 100 µg of GnRH analog (Gonadoreline acetate, 2 mL, im, Prolusyn®, Syntex) and a 1.0 g of progesterone intravaginal device (DIB®, Syntex). Ovulation in response to initial injection of GnRH was evaluated by the number and location of CL on Day -3 in relation to Day -8 based on ultrasonography. Also, on Day -3, all animals received 0.500 mg of sodium cloprostenol (2 mL, im, Ciclase®, Zoetis) at device removal and were assigned randomly to receive 300 IU (1.5 mL, im, reCG, Syntex) in heifers and 400 IU (2 mL, im, reCG, PCT/EP2019/073277, Syntex) in cows of reCG or remain as untreated controls. On Day -2 animals received a second dose of cloprostenol and finally on Day 0 (72 h after DIB removal), received 100 µg of gonadoreline acetate concurrent with TAI. On Day 7, the number of ovulations (single = 1; multiple ≥ 2) in response to synchronization of ovulation protocol was determined by ultrasonography. Data was analyzed using SAS (Statistical Analysis System). Optimal BCS was considered in heifers ≥6 and in cows ≥5. Baseline comparisons were established evaluating the distribution of cows in both treatment groups using a Chi-square test (Proc Freq, SAS system®). The effect of treatment group (reCG vs. control), herd (1, 2 and 3), BCS (<optimum vs. ≥optimum), cyclicity (anestrus vs. cyclic) and ovulation in response to initial injection of GnRH (yes vs. no) on incidence of multiple ovulation was evaluated by

univariate analysis with Chi-square test (Proc Freq, SAS system®). In addition, the effect of treatment adjusted by the rest of the variables and interactions on the incidence of multiple ovulation was evaluated by multiple logistic regression models (Proc Logistic, SAS system®) using the backward elimination procedure.

**Results:** There was no difference in the distribution of cows by herd (P=0.58) and cyclicity (P=0.96) between treatment groups. At the beginning of the experiment (Day -8), 85, 84 and 89 % of heifers and suckled cows from herd 1, 2 and 3, respectively, were cyclic. The percentage of heifers and suckled cows that ovulated in response to initial injection of GnRH was 38 and 55%, respectively. Two suckled cows did not ovulate in response to second injection of GnRH. A 7.2% (20/279) of animals had multiple ovulation in response to synchronization of ovulation protocol. In the univariate analysis, the incidence of multiple ovulation was not affected by treatment group [6.5% (9/139) and 7.9% (11/140) in reCG and control group, respectively; (P=0.65)]. There was also not affected by herd (P=0.90), BCS (P=0.33), cyclicity (P=0.59) and ovulation in response to initial injection of GnRH (P=0.76). In the multivariable analysis, the incidence of multiple ovulation was also not affected by treatment group (P=0.81).

**Conclusions:** In conclusion, the addition of reCG in a 5-d CoSynch plus progesterone protocol did not affect the incidence of multiple ovulation in *Bos taurus* beef cattle.

**Keywords:** reCG, 5-day CoSynch, Multiple ovulation.

## RB-02

### Prevalence and risk factors of purulent vaginal discharge and cytological endometritis in French beef cows

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**Objectives:** The primary objective of the study was to determine the prevalence of purulent vaginal discharge (PVD) and cytological endometritis (CE) in French beef cows and to find out cow-related risk factors associated with PVD and CE. A secondary objective was to evaluate the performance of leukocyte esterase test strips for the diagnosis of CE.

**Materials and methods:** This prospective cohort study was conducted in 6 herds of Charolais and Blonde d'Aquitaine breeds from December 2020 to April 2021 in the region of New Aquitaine in France. The cows included underwent a thorough examination of the reproductive tract at 30 ± 15 days post-partum that consisted in chronological order of i) a visual observation of vaginal mucous secretion using the Metrichick device (Simcro Technology, Hamilton, New Zealand) ii) endometrial sample using a double-protected cytobrush, iii)

ultrasonographic examination in order to determine the diameter of the cervix, the presence of the uterine contents and to describe the ovarian structures.

Immediately after the endometrial sampling, the cytobrush was gently rolled onto a microscopic glass slide which was subsequently colored (May-Grünwald-Giemsa) in order to determine the percentage of polymorphonuclear (PMN) leukocytes. The cytobrush was cut and placed in a tube containing 1 mL isotonic sodium chloride (NaCl 0.9%) to perform a leukocyte esterase test (Multistix 10 SG; Siemens, Saint-Denis, France).

The cows were also blood sampled between 30 to 15 days before the expected date of calving and between 7 and 24 days after calving to determine the blood concentration of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate acid (BHBA) respectively. The body condition score (BCS) was determined before calving and at the time of reproductive examination. Some additional data regarding the calving (degree of assistance or caesarian section), post-calving diseases (uterine prolapse, retained fetal membranes, puerperal metritis) or the calves (twinning, birth weight, vitality after birth) were gathered. Risk factors associated with PVD or CE were identified using multivariable logistic regression.

**Results:** A total of 182 beef cows of Charolais (n=52) and Blonde d'Aquitaine (n=130) breed were enrolled in the study among which 66 were primiparous (36.2%) and 116 were multiparous (63.8%). The prevalence of PVD and CE defined as a percentage of PMNs superior to 6% were 7.1% and 45.0%, respectively. Herd prevalence of PVD and CE was highly variable. Risk factors positively associated with PVD and CE were the presence of a post-partum disease (*i.e.* retained fetal membrane or vaginal prolapse; OR = 5.93 (1.01-30.77) and OR = 7.0 (1.16-134.26) for PVD and CE respectively) and the birth weight of the calves (*i.e.*  $\geq 53$  kg, OR= 3.94 (1.09-15.99) or  $\geq 49$  kg, OR= 2.28 (1.19-4.43) for PVD and CE respectively). No association were found between occurrence of PVD or CE and the breed, the herd, cervix diameter, pre-partum blood NEFA concentration, post-partum BHBA, BCS at calving or BCS variation around calving or the presence of a corpus luteum at the time reproductive tract examination. The percentage of PMNs varied positively with the PVD score, but the correlation between PVD and CE was not significant. The leukocyte esterase test was significantly correlated with the % PMNs, and using a threshold of 6% PMNs for the definition CE, the sensibility, specificity, positive predictive value, negative predictive value of the leukocyte esterase test was 0.64, 0.66, 0.60 and 0.69 if the result is " $\geq 1+$ " and 0.31, 0.94, 0.81 and 0.63 if the result is " $\geq 2+$ ". Besides PVD or CE was not correlated with noticeable ultrasonographic changes of the uterine contents.

**Conclusion:** Prevalence of PVD was not previously reported in beef cows and appeared lower than observed in dairy cows. Cytological endometritis, which is not strongly correlated with PVD is much more prevalent in this study than previously reported. Beef cows that gave birth to heavy calves or suffered post-partum disease (uterine prolapse or retained fetal membrane in this study) might benefit a thorough evaluation of "uterine health" before the breeding period because they have higher risks to be affected by PVD or CE. In comparison with conventional cytology, the performance of reagent

leukocyte esterase strip as an alternative test was relatively moderate to good.

**Keywords:** Beef Reproduction Endometritis.

### RB-03

#### The influence of a natural *Neospora caninum* infection substantiated by serum antibody levels on the semen quality of Belgian Blue bulls

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**Objectives:** Worldwide, *Neospora caninum* (*N. caninum*), a cyst-forming protozoan parasite, is considered as one of the most important causes of bovine abortion/stillbirth. Even though studies have shown an association of *N. caninum* and the failure of reproductive performances in female animals, the importance of *N. caninum* on bull fertility has been neglected. The presence of *N. caninum* DNA in semen, however, indicates that *N. caninum* can as well infect the male reproductive system. Recent literature showed that sperm concentration, motility, and viability were significantly lower in testes tissue of slaughter bulls with natural neosporosis. To the best of our knowledge, no longitudinal studies are available in which the association between sperm quality parameters and peripheral *Neospora* antibody titers has been assessed. Our study aimed to assess the potential impact of a natural infection with *N. caninum* on the sperm quality of fresh and frozen/thawed semen of Belgian Blue Bulls located in Belgium, taking sperm samples harvested over a 1-year period into consideration.

**Materials and methods:** The present study took place from January 3<sup>rd</sup> till December 31<sup>st</sup> 2020. In total 92 Belgian Blue bulls kept for breeding purposes, spread over three locations of the Artificial Insemination Centre "Association Wallonne De L'Elevage" (AWE, Ciney, Belgium) were used in this study. Every two months, each bull was serologically screened for *N. caninum* antibodies. Only semen data of bulls that were six times negative or positive for *N. caninum* antibodies, were included in the study.

Semen ejaculates were collected with an artificial vagina twice weekly. In total 10.478 ejaculates of the 92 bulls were included, with 9.537 fresh samples originating from *Neospora* negative (n=84) and 941 from positive (n=8) bulls. Outcome variables assessed for the fresh samples included: concentration, volume, and subjective motility. Only semen samples with more than 60% of total sperm motility and concentrations higher than  $0.3 \times 10^9$  sperm cells/ml (n= 7.685 being 7.018 originating from *Neospora* negative bulls and 667 from positive bulls) were further extended and frozen. Immediately after thawing, total motility, progressive motility, and morphology were assessed.

Sperm parameters were used as the dependent variable of



interest. Generalized linear and binomial mixed models were used for statistical analysis of each outcome variable. Initially, individual explanatory variables age, herd, Temperature Humidity Index (THI) at the moment of sperm production as well as collection, and season of sperm production and collection, as well as the *Neospora caninum* antibody test results were tested in univariable models for each outcome variable. Akaike information criterion (AIC) values were used to select explanatory variables to build the multivariable model, where the *Neospora caninum* test result was forced in all models.

**Results:** Results revealed an overall apparent seroprevalence of *Neospora caninum* of 9,2% in our tested bull group.

For fresh semen: volume (negative *N. caninum* samples: 6,60 ml  $\pm$  2,55 ml; positive *N. caninum* samples: 6,28 ml  $\pm$  3,28 ml) and concentration (negative: 1.395,23x10<sup>6</sup> sperm cells/ml  $\pm$  493,77 x10<sup>6</sup> sperm cells/ml; positive: 1.471,53 x10<sup>6</sup> sperm cells/ml  $\pm$  532,41x10<sup>6</sup> sperm cells/ml) were normally distributed, where the subjective motility (negative: 64,29%  $\pm$  14,71%; positive: 61,21%  $\pm$  12,94%) was right skewed. After freezing: total motility (negative: 45,11%  $\pm$  11,60%; positive: 44,47%  $\pm$  12,49%), progressive motility (negative: 26,28%  $\pm$  8,17%; positive: 27,11%  $\pm$  8,81%) and normal morphology (negative: 96,03%  $\pm$  2,53%; positive: 95,90%  $\pm$  2,38%) were normally distributed.

Although there were numerical differences in the descriptive results for some outcome variables, no significant associations were detected between natural neosporosis, substantiated by ELISA-antibody levels, and any of our tested outcome variables on fresh and frozen/thawed semen samples.

**Conclusions:** Results suggest no significantly negative association between *N. caninum* and any of the tested outcome variables in the fresh nor in the frozen-thawed semen samples. Current evidence that *N. caninum* seropositive bulls have lower semen quality or semen production is therefore unlikely. Based on these results, there appears to be no reason to advise against the rearing and use for mating of bulls that are seropositive for *N. caninum*, but extensive field fertility studies should further confirm this.

**Keywords:** Belgian Blue Bulls; *Neospora caninum*; semen quality parameters.

#### RB-04

### Effects of single treatment with low doses of hCG in timed AI protocols on: follicle growth dynamics, ovulatory dispersion and ovulation rate on Nelore cows

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**Objectives:** Postpartum anoestrus is very prominent in Nelore cows and has a negative effect on reproductive results in the breeding session. The objective of this study was to evaluate follicle growth dynamics, ovulatory dispersion and ovulation rate in postpartum Nelore (*Bos taurus indicus*) cows

receiving low doses of hCG (Chorulon™ MSD- Brazil) vs eCG (Folligon™ MSD- Brazil) after progesterone device withdrawal on a timed AI protocol.

**Materials and methods:** A total of 42 anestrous (absence of CL at the first day of the FTAI protocol) Nelore cows, with average BCS of 2.73 $\pm$ 0.05 (scale 0-5) and at 42 to 83 days postpartum were treated on day 0 with 2 mg of estradiol benzoate (Fertilcare Sincronização™, MSD-Brazil) and received an intravaginal progesterone (P4) device (Fertilcare 600®, MSD). On day 8, 12h before device withdrawal, an ultrasound evaluation was performed. Cows were homogeneously randomized to four treatment groups according to the highest follicle diameter. At the moment of the intravaginal P4-device removal all cows were treated with 0.5 mg Cloprostenol (Ciosin®, MSD), 1 mg of estradiol benzoate, and then randomized to receive either saline (Control Group, n = 8), 100 IU of hCG (Chorulon®, MSD, hCG-100 Group, n = 14), 150 IU of hCG (Chorulon®, MSD, hCG-150 Group, n = 11) or 300 IU of eCG (Folligon®, MSD, eCG Group, n = 9). Subsequently, ultrasound examinations were performed at 12h intervals until 96h post P4-device removal, in order to evaluate follicular growth and time of ovulation. Data for ovulatory dispersion and ovulation rate were analyzed by least-squares ANOVA with the PROC GLIMMIX procedure of SAS (v. 9.4; SAS Institute Inc., Cary, NC). Orthogonal contrasts were used to compare follicle growth means (C1=Control vs Gonadotropins; C2 = eCG vs hCG; C3=100 IU of hCG vs 150 IU of hCG).

**Results:** There were no differences in time to ovulation and distribution of ovulations across time among groups (51.6 $\pm$ 0.82h;  $P>0.95$ ). There were no differences in ovulation rate [83,3% (35/42);  $P=0.81$ ]. Dominant follicle growth within 24h periods was greater on cows treated with gonadotrophic hormones than the Control Group [gonadotropins = 2.28 mm d<sup>-1</sup>; Saline = 0.18 mm d<sup>-1</sup>;  $P=0.001$  (C1)], but hCG and eCG treatments had similar results [hCG = 0.96 mm d<sup>-1</sup>; eCG = 0.75 mm d<sup>-1</sup>;  $P=0.81$  (C2)] and different doses of hCG (100 IU vs 150 IU) had similar growth [150 IU of hCG = 1.05 mm d<sup>-1</sup>; 100 IU of hCG = 0.75 mm d<sup>-1</sup>;  $P=0.26$  (C3)].

**Conclusion:** In conclusion, different doses of hCG and eCG treatments resulted in similar dispersions between the first and last ovulations, and similar ovulation rates and follicular growth development rates after P4-device removal. Both gonadotrophic treatments (hCG and eCG) resulted in higher follicular growth rates than follicles in cows that did not receive a gonadotrophic stimulus.

**Keywords:** Anestrous, *Bos indicus*, Gonadotrophic, Postpartum, Ovarian Activity.

## RB-05

### Reproductive program in Avileña-Negra Iberica breed heifers (AECRANI) for an age programmed to the first birth of 2.5 years

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**Objectives:** To evaluate the reproductive efficiency of an assisted reproduction program based on estrus synchronization and fixed time artificial insemination (FTAI) in heifers of the Avileña-Negra Iberica breed (ANI) for an average age programmed at the first birth of 30 months. The reproductive model consisted of an estrus synchronization and through a Presynch + CIDR Cosynch and FTAI protocol with natural mating (NM) after 15 post-insemination.

**Materials and methods:** The program has been conducted with a sample of 94 ANI heifers from five livestock, located in the provinces of Salamanca, Ávila and Segovia (Castilla y León).

At the moment of insemination, all the heifers from the sample complied with the maturity and development index proposed by AECRANI (table 1).

Table 1. Recommendations AECRANI of the maturity and development index in heifers of ANI breed, for PPE of 30 months of age ( $PV_A = 650$  kg,  $A_A = 1.40$  m).

Age (months)	Maturity index (%)	Development index (%)	Phase
0	6,5	57	Birth
3	18,2	65	
7	33,6	74	Weaning
9	39,2	80	Recribed
12	47,6	85	Recribed
14	52,0	85	Recribed
16	56,4	88	Puberty
19	63,0	89	Coverage
21	67,4	90	Gestation
27	78,8	100	
30	85,0	100	1 postpartum week

$LW_A$  = Adult reproductive live weight;  $WH_A$  = Adult reproductive withers height

The average age of the heifers at insemination was  $18.4 \pm 2.9$  months, with an average live weight of  $395.7 \pm 56.5$  kg, withers height of  $127.4 \pm 4.1$  cm and an average corporal condition of  $3.0 \pm 0.4$  points. The average age, average live weight, withers height and corporal condition of the heifers of each livestock at the moment of artificial insemination (AI) are shown in table 2.

Table 3 describes the synchronization protocol (Presynch + CIDR Cosynch) applied to the heifers of the different livestock in the study.

After 15 days of the AI, the stallions were introduced in the different livestock to review by means of natural mating (NM) the heifers that were not pregnant in the AI. The diagnosis of pregnancy was made by ultrasonography 70 days post-AI, thus confirming the pregnant heifers.

**Results:** Through the reproduction program used in this study, the average gestation rate in all livestock was 72.9%.

Table 2. Age, live weight, withers height and corporal condition of the ANI heifers of the different livestock studied at the time of AI.

	Livestock I	Livestock II	Livestock III	Livestock IV	Livestock V
Number of heifers	34	11	12	20	17
Age, months	18,2±1,1	18,0±1,5	19,4±4,2	15,62±0,3	21,6±3,4
Live weight, kg	395,5±43,6	420,1±26,5	346,8±41,8	354,6±29,6	463,2±52,7
Withers Height, cm	125,5±3,7	126,6±3,4	126,8±3,4	127,6±3,9	131,8±2,9
Corporal condition	2,8±0,4	3,1±0,2	3,1±0,3	2,9±0,2	3,3±0,3

Table 3. Estrous synchronization and protocol in ANI heifers.

Presynch + CIDR Cosynch	14 d	Day 0	5d	Day 5	1d	Day 6	2d	Day 8
		Aplicación CIDR				Retired CIDR		FTAI
	PGF <sub>2</sub> α	GnRH		PGF <sub>2</sub> α		PGF <sub>2</sub> α + eCG		GnRH

Table 4. Percentage of pregnant heifers after the application of the reproduction program with IATF.

	Livestock I	Livestock II	Livestock III	Livestock IV	Livestock V
Number of heifers	34	11	12	20	17
Age AI, months	18,2±1,1	18,0±1,5	19,4±4,2	15,62±0,3	21,6±3,4
Pregnant rate, %	91,2	63,6	91,7	65,0	52,9



Table 4 shows the gestation rate in the different livestock that have taken part in the study.

**Conclusions:** The use of a reproductive program based on the estrus synchronization and “Presynch + CIDR Cosynch” and IATF, in combination with the introduction of stallions for natural mating 15 days after insemination, is a recommended method in ANI heifers at the first birth of 30 months of age, given the excellent rate of pregnant heifers obtained in this study.

**Keywords:** Avileña-Negra, Synchronization, Insemination, Heifers, Birth.

to goiter and chondrodystrophy independently.

**Keywords:** Iodine, manganese, mineral, deficiency, hypothyroidism.

## RB-06

### An outbreak of congenital goiter and chondrodystrophy among calves born to spring-calving beef cows

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**Objectives:** An outbreak of concurrent congenital diffuse hyperplastic goiter and chondrodystrophy occurred at a commercial cow-calf operation in southern Alberta during spring 2018 (Homerosky et al., 2019). All affected calves were born to mature Angus cows and sired by one of two half-sibling mature Red Angus bulls. Matings during the previous four breeding seasons resulted in apparently normal calves. The objective was to investigate non-genetic etiologies of congenital goiter and chondrodystrophy.

**Materials and methods:** The herd was on pasture until November 2017 when they were transitioned to a ration comprised of wrapped high-moisture barley greenfeed bales and mixed grass hay bales. No supplemental trace minerals or vitamins were provided during the winter feeding period. Twenty of 35 calves born between April 5<sup>th</sup> and June 5<sup>th</sup>, 2018 were affected with goiter and generalized weakness in the presence or absence of varying degrees of chondrodystrophy. Incidence of stillbirths and neonatal deaths (N=7) and severity of clinical signs decreased as calving season progressed. All dams were in good body condition and appeared clinically healthy.

**Results:** Three deceased affected calves submitted for additional diagnostics tested negative for bovine herpesvirus, *Leptospira* spp., *Neospora caninum*, and bovine viral diarrhea. Liver concentration for iron was high, manganese was low, and cobalt was deficient in multiple calves. Cobalt may serve as a proxy for iodine as these two minerals must be supplemented continuously to cattle in Alberta. As such, iodine deficiency was determined to be the cause of goiter in these calves. The herd was treated by providing trace minerals with added ethylenediamine dihydroiodide.

**Conclusions:** Although congenital goiter and chondrodystrophy are observed globally in several species, there is a paucity of reports describing concurrent manifestations, especially in calves. In the current outbreak, it is unclear if hypothyroidism impaired normal fetal growth and development or if multiple concurrent mineral deficiencies predisposed these calves