this study was to determine whether an experimental formula of a nonaqueous solution of cloprostenol sodium associated with fatty acids and solvents (PGF LA, BioRelease Technology) would induce complete luteolysis and increase oestrus manifestation and subsequent P/AI compared with the use of a commercial standard cloprostenol sodium product (PGF, Estron, Agener União Saúde Animal) in an oestradiol/progesterone-based TAI synchronisation protocol in multiparous and nulliparous beef females. Transrectal ultrasonography exams were performed on Day 0 to select females with detectable CL, on Day 9 to measure ovulatory follicle (OF) diameters, and 28–32 days after TAI for pregnancy diagnosis. 261 and 194 cross-bred cycling Angus cows and heifers with a body condition score (1–5 scale) of 2.9 ± 0.3 and 3.1 ± 0.2, respectively, received a 1 g P4 intravaginal device and 2 mg oestradiol benzoate, i.m. On Day 7, P4 devices were removed, and 1 mg oestradiol cypionate and either 500 mg PGF LA ($n = 112$ cows, $n = 100$ heifers) or PGF ($n = 149$ cows, $n = 94$ heifers) were given via i.m. Sacral and tail head regions were painted with marking paint to evaluate oestrus manifestation. Oestrus was determined by removal of 50% or more of tail paint between device removal and the moment of TAI, 48 h later. Blood samples ($n = 35$) were taken by coccygeal venipuncture before TAI to determine serum P4 concentrations by RIA. Continuous dependent variables were submitted to one-way ANOVA and predicted probability of pregnancy was analysed using a logistic regression model including parity, treatment, oestrus behaviour, and interactions. Intervals from luteolytic drug injection to oestrus (40.8 ± 8.6 h vs41.7 ± 7.8 h), oestrus manifestation (89% vs 85%), and OF diameter (11.1 ± 2 vs10.8 ± 1.9 mm) were similar ($P > 0.05$) between PGF LA and PGF treatment groups, respectively. Serum P4 concentrations at TAI were lower ($P < 0.05$) in the PGF LA (0.23 ± 0.20 ng/mL) compared with the PGF (0.65 ± 0.30 ng/mL) group. Pregnancy rate was higher ($P < 0.05$) in cows treated with PGF LA (63.3%) than in the PGF group (47.6%), being similar in heifers treated either with PGF LA (49%) or PGF (53.1%). Cows treated with PGF LA were 2.17 times (OR, $P = 0.04$) more likely to become pregnant after TAI than the PGF group, with oestrus manifestation and OF size not being influenced by the treatments ($P > 0.05$). In summary, PGF LA treatment was more effective to induce complete luteal regression, determining lower P4 concentrations at TAI and a higher pregnancy rate per AI in beef cows.

181 Effect of systemic administration of β-nerve growth factor during the periovulatory stage on corpus luteum development and function in dairy heifers

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There is evidence that the β-nerve growth factor (β-NGF) present in seminal plasma in llamas and alpacas exerts a luteotrophic effect in camelids and other species after systemic administration. The main goal of this study was to determine whether systemic administration of purified llama β-NGF before or after the preovulatory LH peak induced by oestradiol affects corpus luteum (CL) function in dairy heifers. Semen was collected from adult llamas using an ovine artificial vagina. Seminal plasma β-NGF was purified using a combination of hydroxylapatite and gel filtration chromatography. Holstein-Friesian heifers ($n = 24$) weighing between 320 and 330 kg received 2 mg of oestradiol benzoate (EB) plus an intravaginal progesterone device (DIB®, Zoetis, Day = 0). On Day 8, the device was removed along with an intramuscular (i.m) dose of 500 μg of cloprostenol (Boviprost®, Anasac), and 1 mg of EB was given i.m at Day 9 and then heifers ($n = 8$/per group) were randomly given an i.m. injection of either: (i) phosphate-buffered saline ([PBS] Control), (ii) 1 mg of purified β-NGF at the time of EB treatment (Pre-LH group), or (iii) 36 h after EB treatment (Post-LH group). Ovaries were examined daily by B-Mode ultrasonography from DIB removal to ovulation (disappearance of dominant follicles) and then by Power Doppler at days 2, 4, 6, 8, 10, 12, 14, 16, and 18 after ovulation to determine CL vascularisation. Blood samples were collected every 4 h from DIB removal until ovulation to determine plasma LH concentration and during the luteal phase for plasma progesterone concentration. Non-serial data were analysed using one-way ANOVA; CL profile development, vascularisation area, and hormone concentrations were analysed using PROC MIXED procedure in SAS. All females ovulated (100%, 24/24) after oestrus synchronisation. There was no significant difference in follicular diameter ($P = 0.65$) at the time of treatment nor in the maximum CL diameter ($P = 0.35$) after treatment. Plasma LH concentration was higher ($P < 0.001$) in females given β-NGF along with the dose of EB at Day 9 of the synchronisation protocol. Although the day-by-day profile CL diameter did not differ among groups ($P = 0.84$), heifers treated with purified β-NGF showed a significant increase in CL vascular area ($P = 0.04$) and plasma progesterone concentration ($P = 0.001$) at Day 12 and 14 after ovulation. Hence, administration of systemic heterologous β-NGF given before or after the LH peak induced by oestradiol benzoate enhances CL function.

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182 Hormonal profile during resynchronisation using oestradiol benzoate and progesterone-based protocols associated or not with flunixin meglumine in cattle

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A potential strategy to reduce inter-insemination intervals is to identify nonpregnant females on D20 based on CL blood flow (colour-Doppler ultrasound) associated with a resynchronisation (RESYNCH) protocol beginning during diestrus, on D12 (D0 = oestrus), and artificial

insemination of open females on D22. Flunixin meglumine (FLU) is a nonsteroidal anti-inflammatory drug that inhibits prostaglandin synthase and antagonise oestradiol-related actions upon oxytocin endometrial receptors. The objectives were to: (1) determine whether oestradiol benzoate (EB) and a progesterone device (P4) given on D12 would change circulating oestradiol (E2) and disturb CL function (plasma P4); and (2) evaluate whether FLU treatment could prevent presumed detrimental effects of EB on CL. Lactating Holstein × Gir cows (n = 45) were submitted to an EB-P4-based ovulation synchronisation protocol (D10: 2 mg EB + P4 intravaginal device; D2: device removal + $PGF_{2\alpha}$; D1: 1 mg EB; D0: oestrus). On D12, cows were randomly allocated into four groups/treatments: (A) control (CTL), saline im; (B) 2 mg EB im, P4 device (EB-P4); (C) 2 mg EB im, P4 device, 1.1 mg/kg FLU im (EB-P4-FLU); and (D) P4 device on D12, dominant follicle ablation by OPU on D15 (OPU-P4). Blood samples were collected daily, and plasma was stored at −20°C. Solid-phase RIA was used to determine daily plasma P4 (D12 to D22; ImmuChem, ICN Pharmaceuticals Inc.) and plasma E2 at 48 h intervals (D13 to D21; Ultra-sensitive Oestradiol, Beckman Coulter Inc.). Data were analysed using PROC MIXED of SAS accounting for repeated measures. Main effects were group, day of cycle, and their interaction. Overall, we observed an effect of group (P = 0.0075 and P = 0.0068), day of cycle (P < 0.0001 and P < 0.001), and interaction group × day (P = 0.0001 and P = 0.0012) for both E2 and P4, respectively. On D13, EB-P4 and EB-P4-FLU had higher E2 compared with OPU-P4 and CTL (21.7 ± 2.7, 18.0 ± 3.4 vs 3.7 ± 0.9, and 8.76 ± 6.3 pg/mL, respectively; P < 0.001). Plasma E2 did not differ among groups or days from D15 onwards. Plasma P4 decreased from D12 to D22 in all groups; however, the rate of decrease differed. In groups receiving EB, P4 decreased from D12 to reach significant lower values on D17 (3.6 ± 0.3 vs 1.4 ± 0.2 ng/mL and 4.2 ± 0.4 vs 1.9 ± 0.4 ng/mL for EB-P4 and EB-P4-FLU, respectively; P < 0.0001), whereas plasma P4 decreased to lower values only on D20 in OPU-P4 (4.0 ± 0.3 vs 1.5 ± 0.3 ng/mL, D12 vs D20, respectively; P < 0.0001) and D19 in CTL (3.1 ± 0.4 vs 1.3 ± 0.4 ng/mL, D12 vs D19; P = 0.0045). In OPU-P4, plasma P4 peaked on D17 (4.0 ± 0.6 ng/mL) and remained high on D18 (3.2 ± 0.5), greater (P < 0.03) than for EB-P4 (1.4 ± 0.2 and 1.0 ± 0.2; D17 and D18) and EB-P4-FLU (1.9 ± 0.4 and 1.3 ± 0.4, respectively). In conclusion, RESYNCH using EB on D12 increased plasma E2 on D13 and may have anticipated functional luteolysis. Concomitant FLU treatment did not prevent this effect. Follicle ablation on D15 increased P4 48 h later, most likely by luteinisation of the aspirated follicle cells.

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183 Ovulatory follicle size, time of ovulation, and pregnancy rates to AI in lactating dairy cows treated with a new gonadotrophin-releasing-hormone-based protocol with lengthened proestrus

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Two experiments evaluated ovulatory follicle size, time of ovulation, and pregnancy rates to AI (P/AI) in lactating Holstein cows synchronised with an oestradiol-based protocol or with a new gonadotrophin-releasing hormone (GnRH)-based protocol with lengthened proestrus named Web-Synch. Lactating Holstein cows (n = 39 in Experiment 1 and 256 in Experiment 2) 160.0 ± 7.1 days in milk, producing 35.6 ± 0.8 kg of milk per day, 2.8 ± 0.3 lactations, body condition score 3.1 ± 0.1 (1–5 scale; mean ± s.e.m.) and managed in a confinement system, were randomly allocated into one of two treatment groups. On Day 0, cows in the Control group received 2 mg oestradiol benzoate (Oestradiol, Over) and a vaginal device containing 1 g of progesterone (P4, Sincrover, Over). On Day 6, cows received 150 μg D (+) cloprostenol (PGF, Prostal, Over). On Day 7, P4 devices were removed, and cows received a second PGF, 140 IU recombinant equine chorionic gonadotrophin (reCG, FoliRec, Zoovet) and 1 mg oestradiol cypionate (Estrosinc, Over). Cows in the Web-Synch group were treated with PGF and a P4 device on Day 5, and 10 μg buserelin (GnRH, Gestar, Over) on Day 0; P4 device removal, PGF, and reCG was done on Day 6, and a second dose of PGF was administered on Day 7. Cows in both groups were tail painted for oestrus detection. In Experiment 1, cows were scanned ultrasonographically (Mindray M7) twice daily from P4 device removal to ovulation. In Experiment 2, all cows with >30% of the tail paint rubbed off on Day 9 (48 h after P4 device removal in the Control group and 72 h after P4 device removal in the Web-Synch group) were AI at that time, and cows without the tail paint rubbed off in both groups received 10 μg GnRH and were AI 12 h later. Cows in Experiment 2 were examined for pregnancy 30 days after AI. Data were analysed using the GLM mixed procedure for continuous data in Experiment 1 and for binary data with a logit link in Experiment 2 (InfoStat, 2021). In Experiment 1, the mean (± s.e.m.) interval from P4 device removal to ovulation was longer (P < 0.05) in the Web-synch group (101.6 ± 2.9 h) than in the Control group (78.3 ± 3.1 h), but the diameter of the ovulatory follicle did not differ (P = 0.3; 19.7 ± 0.8 and 18.5 ± 0.8 mm for the Web-Synch and Control groups, respectively). In Experiment 2, expression of oestrus did not differ between groups (Web-Synch: 74.0%, 94/127 and Control: 74.4%, 96/129, P = 0.9), but P/AI was greater (P = 0.01) in the Web-Synch group (54.3%, 69/127) than in the Control group (39.5%, 51/129). In summary, the GnRH-based synchronisation protocol (Web-Synch) resulted in a longer proestrus period and greater P/AI than the conventional oestradiol-based protocol in lactating dairy cows.

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