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# Effect of slow-release FSH on embryo recovery in dairy cows

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**Keywords**: bovine, superovulation, embryo flushing.

The objective was to study if a slow-release FSH superovulatory treatment (SLOW/2FSH) differs from the traditional 4-day FSH superovulatory treatment (TRAD/8FSH) in the numbers and grades of viable embryos and the numbers of degenerated embryos and unfertilized ova (UFO). Reduction of FSH treatments from eight to two diminishes restraining, discomfort and pain to the cows and labor and human errors in administration of treatments.Eight dairy cows (parity 1 to 4) were randomly designated into the TRAD/8FSH and SLOW/2FSH protocols in a cross-over study. First, the oestrous cycles of the cows were synchronized using 25 mg of prostaglandin (PG) (Dinolytic vet. 5 mg/ml, Zoetis Finland Oy, Finland) i.m., and 9 to 12 days after the synchronized oestrus either TRAD/8FSH or SLOW/2FSH treatment was initiated. TRAD/8FSH treatment consisted of eight declining i.m. doses (total 1000 IU) of FSH (Pluset vet, Laboratorios Calier, S.A., Spain) for four days at 6:00 h and 18:00 h. SLOW/2FSH treatment consisted of Pluset combined with hyaluronic acid (Hyonate®vet 10 mg/ml, Bayer Animal Health GmBH, Germany), 666 IU i.m. as 1st treatment at 6:00 h on the first treatment day and 334 IU i.m. as 2nd treatment 48 hours later. In the evenings of the 3rd and 4th day of each treatment, the cows were treated with 25 mg of PG and were inseminated after induced oestrus three times 12 hours apart, beginning 12 h after standing oestrus (= Day 0). Embryos were flushed non-surgically on Day 7. After flushing, CIDR devices (CIDR depot 1.38g, Zoetis Finland Oy, Finland) were inserted in the vaginae, removed after 12 days and a day before removal, 25 mg of PG was administered i.m. After this induced oestrus, the second cross-over run of the experiment was initiated 9 to 12 days later. The numbers of viable embryos, degenerated embryos and UFO were counted. All viable embryos were graded following IETS recommendations and cool-transported in straws to be analyzed in another study for their survival after 1, 3, 5 or 7 days in +4°C storage. Results are depicted as percentages of totals and averages ± SDs. Paired t-test was used to define difference between treatments. The number of viable embryos did not significantly vary between treatments (p=0.47). The TRAD/8FSH treatment yielded an average of 12.50±7.11 and SLOW/2FSH treatment an average of 10.13±4.67 viable embryos. In the TRAD/8FSH treatment, 82.0% (100/122) of the recovered structures were viable embryos, 6.5% (8/122) were degenerated embryos and 11.5% (14/122) were UFO. In the SLOW/2FSH treatment, 86.2% (81/94) were viable embryos, 8.5% (8/94) were degenerated embryos and 5.3% (5/94) were UFO. In the TRAD/8FSH and SLOW/2FSH treatments, 72.0% and 79.0% were grade I, 16.0% and 12.3% were grade II and 12.0% and 8.6% were grade III viable embryos, respectively. The results indicated no difference in the average number of viable embryos between treatments. However, slow-release FSH treatment yielded a higher percentage of viable embryos and less UFO than the traditional FSH treatment and therefore warrants further investigation. Olvi Foundation is acknowledged for funding the research.

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# Effects of docosahexaenoic acid on bovine granulosa cells in vitro: involvement of FFAR4 receptor

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**Keywords**: n-3 PUFA, lipid, signalling pathways.

Previous studies suggest a beneficial effect of dietary fish oil supplementation (enriched in n-3 polyunsaturated fatty acid (PUFA) on reproductive variables in dairy cows. These PUFA influence female reproduction by acting at the uterine and ovarian levels (Leroy JL, et al. Reprod Domest Anim. 49:353-61; 2014). Recently, we showed that docosahexaenoic acid (DHA, the most active n-3 PUFA) was able to affect oocyte quality by increasing blastocyste rate after *in vitro* maturation and fecundation (Oseikria M, et al. Theriogenology. 85:1625-1634.e2; 2016), but no data is available on its potential effects on ovarian somatic follicular cells. Our objectives were to assess the effect of DHA on proliferation, steroidogenesis and signalling pathways in bovine granulosa cells (GC). The potential involvement of the receptor FFAR4 in the effects of DHA was investigated in bovine GC through FFAR4 expression and FFAR4 agonist (TUG-891) assessment in functional studies. Primary GC cultures were performed after dissection of ovarian small follicles (3-6 mm) collected from slaughterhouses. Recovered GC were cultured in serum-free McCoy's 5A medium with insulin (10 µg/L) in absence or presence of DHA (1, 10, 20 or 50 µM) or TUG-891 (1, 10 or 50 µM) for the appropriate times. Fatty acid composition of total lipids in GC after 24h DHA treatment was assessed by gaz chromatography. Cell proliferation after 24h and steroidogenesis after 48h were measured by tritiated thymidine incorporation in cells and by ELISA of secreted progesterone and estradiol in culture medium, respectively. Phosphorylation of MAPK14, AMPK, MAP1/3 and AKT signalling pathways were assessed by western Blotting in GC treated with DHA for 5 to 60 min. These parameters were statistically analysed using either Kruskal-Wallis test or non parametric permutational ANOVA. We showed that FFAR4 mRNA and protein were expressed in bovine GC. GC proliferation was stimulated after 10 and 50 µM DHA treatment and a similar increase was observed with TUG-891 at 1 and 50 µM. Progesterone secretion was enhanced after 20 and 50 µM DHA supplementation, whereas a slight decrease was observed with TUG-891 at 1µM. Estradiol secretion was increased after DHA 1, 10 and 20 µM treatment, whereas no effect of TUG-891 was reported. The DHA content in total lipids was increased in GC supplemented with 10 and 50 µM DHA for 24h compared to control GC. DHA had no effect on MAPK1/3, AKT and AMPK phosphorylation, whereas it stimulated transiently MAPK14 phosphorylation after 30 min DHA treatment at 10 µM and 50 µM similarly to TUG-891. In conclusion, this work showed that DHA is able to highly incorporate the GC total lipids after 24h supplementation. Moreover, both DHA and TUG-891 stimulated similarly GC proliferation and MAPK14 phosphorylation, whereas only DHA increased steroid secretion from GC, suggesting that DHA could influence female fertility by acting on GC partly through FFAR4 and MAPK14 pathway for GC proliferation and through other mechanisms on steroidogenesis.
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# Polymers used to reduce a number of fsh-injections during superstimulation treatement for superovulation induction in cows

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**Keywords**: bovine, FSH, prolongators.

Over 50% of more than 1 million bovine embryos presently produced in the world are in vivo derived. Induction of superovulation has been one of the major methods for embryo production for the past 40 years. However, there were and still are many attempts to develop protocols allowing fewer injections and to find substances prolonging FSH effects during superovulation induction in cows. In our work we examined the ability of polyvinyl alcohol (PVA) and polyethylene glycol (PEG) to affect the release of FSH after injection and to act as prolongators during hormonal treatment. To achieve this goal, two experimental groups of cows were treated with FSH-Super (LLC Agrobiomed, Russia) plus either PVA or PEG. Animals were assigned semi-randomly to two experimental groups. Each of the two working compositions was prepared directly before injection. To inject a single animal the necessary amount of polymer (either 0.9 g of PVA or 2.5 g PEG) was mixed with 1000 IU of FSH and dissolved in 7.5 ml of 0.9% NaCl solution. Animals of the first group (group I; n=98) were injected once with FSH plus PVA. Animals of the second group (group II; n=96) were injected once with FSH plus PEG. Subcutaneous injection of FSH plus either PEG or PVA was conducted once at the 10th day of the cycle, if there was a well-defined corpus luteum on one of the ovaries. The injection was located in the shoulder blade area. Injection of the PGF2a (0.5 mg) was conducted intramuscularly 48 hours post FSH injection. Artificial insemination was conducted 48 hours after PGF2a injection and was repeated two more times at 12 h intervals. On day 7 after the first insemination, corpora lutea were determined by trans-rectal palpation and embryos were collected by a standard non-surgical flushing procedure. Embryo quality was assessed according to IETS Manual. Portion of cows with a reaction to treatment was significantly higher ($P<0.05$) in the second group (86.4%) than in the first group (74.5%). Total number of ovulations and collected embryos were 1080 and 811 (14.2±8.2 and 10.7±8.1 per donor, respectively) in group I and 1181 and 922 (14.2±7.4 and 11.1±6.6 per donor, respectively) in group II. Relative portion of grade 1-2 embryos in each group differed significantly (64.2% in group I and 79.0% in group II; $P<0.001$). Total number of the collected embryos of grade 3 and lower was 114 in group I and 89 in group II. Their relative number was also significantly larger in group I compared to group II ($P<0.05$). There was also a significantly larger ($P<0.001$) portion of oocytes collected in group I (21.7%) than in group II (11.3%). The results of our study indicate that treatment of cows with a single injection of FSH plus PEG results in higher flushing outcomes after superstimulation compared to single treatment with FSH plus PVA. Thus PEG may be a more effective agent for optimization of the superovulation induction procedure using FSH in cows.