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Effect of intra-follicular luteinizing hormone and human chorionic gonadotropin administration in dairy heifers

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Abstract

The aim of this study was to evaluate the effect of intra-follicular treatment (IFT) with luteinizing hormone (LH) and human chorionic gonadotropin (hCG) in dairy heifers. Intra-follicular treatment with LH (2 μg, 0.2 ml, LH group), hCG (10 IU, 0.2 ml, hCG group) or saline (0.2 ml, S group) was done into the dominant follicle on the seventh day of a synchronized oestrous cycle. A response of the follicles subjected to IFT on D7 as well as plasma progesterone concentration on the day of IFT (D0) and seven days later (D7) were evaluated. Ovulation and development of a new corpus luteum occurred in 89.3%, 92.9%, and 3.7% of treated heifers in the LH, hCG, and S group, respectively. Plasma progesterone concentration was greater on D7 in comparison to D0 in the LH and hCG group (6.31 vs. 11.31 and 6.68 vs. 9.56, respectively). Intra-follicular treatment with LH and hCG had similar effects. The results can be used in research or in practice to treat persistent cases of ovarian dysfunction in cattle.

Ultrasound guided transvaginal ovarian injection, cattle, hCG, LH

Ultrasound-guided transvaginal follicular aspiration (TVFA) in cattle was first reported more than 30 years ago for oocyte aspiration in cattle (ovum pick up, OPU) (Pieterse et al. 1988). Since then, TVFA has been modified and improved to increase its feasibility and practical application. The collective term ovarian transvaginal ultrasonography (OTU) has been used to denote the entire system (Velazquez et al. 2014). Ovarian transvaginal ultrasonography is currently used to perform *in vivo* ovarian aspiration (the collection of oocytes, follicular fluid, granulosa cells), *in vivo* ovarian injections (injection into the ovarian stroma, intra-follicular and intra-luteal injection) and *in vivo* ovarian biopsies (biopsy of corpus luteum, collection of primordial ovarian follicles) (Velazquez et al. 2014). Intra-follicular injection (IFT) was first described in 1995 when IFT of hCG was reported (Kot et al. 1995). Since then, IFT of different substances such as phosphate-buffered saline and insulin-like growth factor-I (Ginther et al. 2004; Shahiduzzaman et al. 2010) as well as intra-follicular gamete transfer (Bergfelt et al. 1998; Lopez-Gatius and Hunter 2011; Kassens et al. 2015; Hoelker et al. 2017; Andrlikova et al. 2020) have been described in cattle.

Intra-follicular injection with luteinizing hormone (LH) was first reported in heifers pre-treated with deslorelin (Mala et al. 2013a), in which different LH doses were capable of inducing ovulation. Determination of minimum effective doses for IFT to induce ovulation in cycling dairy heifers resulted in doses of 0.1 μg of LH and 0.01 IU of hCG (Mala et al. 2013b). Their study had, however, only three heifers in one experimental group, so the results need verifying in a larger group of animals.

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The aim of the study was thus to determine and compare the effect of LH and hCG administered to the dominant follicle by evaluation of the ovarian response and plasma progesterone concentration in dairy heifers.

Materials and Methods

Animals, examination and treatment

Holstein heifers (n = 83) at the age of 14–15 months and 350–400 kg body weight were used for the experiment. Animals were kept at a commercial dairy farm under standard conditions in free barns and fed a total mixed ration containing corn silage, alfalfa haylage, cut straw, concentrates and a mineral supplement. Management of the animals was consistent with the policies of the Commission for Animal Welfare of the University of Veterinary and Pharmaceutical Sciences Brno (Protocol numbers: 12/2014, 7/2015), which complies with the ethical principles for conducting animal research.

Ovaries of the experimental heifers were scanned using a real-time B-mode ultrasound machine (SSD-500, Aloka, Japan) equipped with a linear ultrasound transducer (7.5 MHz UST 5561, Aloka). All ovarian structures present were measured and recorded. Heifers bearing a corpus luteum were synchronized by cloprostenol administration (500 μg i.m. *pro toto*, Oestrophan®, Bioveta, Czech Republic). Oestrus was detected by visual examination, and a further ultrasound examination was performed 7 days later (D0). Heifers bearing a corpus luteum and dominant follicle of a diameter larger than 10 mm on D0 were included in the IFT. Mostly one follicle was present; when two follicles were found, the larger one was treated.

The heifers were randomly divided into three groups according to the type of intra-follicular administration: heifers treated with LH (prof. Beckers, University of Liege, Belgium; 2 μg of LH diluted in 0.2 ml of saline, LH group, n = 28), heifers treated with hCG (Pregnyl®, Organon, Netherlands; 10 IU of hCG diluted in 0.2 ml of saline, hCG group, n = 28) and heifers treated with saline (0.2 ml, S group, n = 27).

All heifers were restrained in laying boxes using special fixation tubes limiting the movement of the animals in all directions. Intra-follicular treatment was performed as previously described (Cech et al. 2013). The mechanical procedure of IFT was considered successful when fluid turbulence inside the follicular cavity was visible on the scanner screen during IFT and the size of the treated follicle did not change after IFT. Further ultrasound examination was performed one week later. Ovulation of the follicle subjected to IFT and the development of a new corpus luteum was considered a positive physiological consequence of the intra-follicular treatments.

Blood sampling and progesterone assay

Blood samples for progesterone assay were taken immediately before IFT (D0) and seven days later (D7). Blood was collected into heparinized tubes and centrifuged for 10 min at 2000 × *g*, within 30 min of collection. Plasma for the determination of progesterone was stored at −20 °C until analysis. The plasma progesterone concentration was determined in duplicates using commercially available kits according to the manufacturer's instructions (progesterone RIA IM 1188, Immunotech, Czech Republic). The inter- and intra-assay coefficients of variation for progesterone were 6.4% and 8.8%, respectively.

Statistical analysis

The significance of differences in D0 and D7 progesterone concentrations was assessed using paired *t*-test for each of the three treatments. Similarly, the differences in progesterone concentrations among the treatments were analysed using ANOVA. In the case of D0 (day of IFT) data, the differences between means were non-significant. For D7 data, significant differences were found using Tukey's multiple comparisons. The frequencies of particular outcomes of ovarian response for each of the three treatments were analysed using Pearson's $\chi 2$-test of independence. All tests were performed using R statistical software (R Core Team, 2020).

Results

The mechanical procedure of IFT was successful in all cases (n = 83). The ovulation rate of the follicles subjected to IFT was significantly greater in the LH and hCG groups ($P < 0.01$) than in the S group (89.3, 92.9 and 3.7%, respectively) (Table 1). Progesterone concentrations were significantly greater ($P < 0.01$) on D7 in comparison to D0 in the LH and hCG group. Progesterone concentrations in the LH group on D7 were significantly greater ($P < 0.05$) than progesterone concentrations in the S group (Table 2).

Table 1. Response to IFT using LH, hCG, and saline.

IFT	n	Response[#]	%
LH (2 μg)	28	25	89.3 [a]
hCG (10 IU)	28	26	92.9 [a]
Saline	27	1	3.7 [b]

IFT: intra-follicular treatment; LH: luteinizing hormone; hCG: human chorionic gonadotropin

[#] Presence of a new corpus luteum 7 days after IFT

[a,b] Values with different superscripts within the column are different, $P < 0.01$

Table 2. Plasma progesterone concentrations (ng/ml ± standard deviation) at the time of IFT (D0) and 7 days after IFT (D7).

IFT	D0	D7
LH (2 μg)	6.31 ± 2.91	11.31 ± 5.04[*a]
hCG (10 IU)	6.68 ± 1.76	9.56 ± 3.98[*a, b]
Saline	7.63 ± 3.96	7.72 ± 4.72[b]

IFT: intra-follicular treatment; LH: luteinizing hormone; hCG: human chorionic gonadotropin

[a,b] Values with different superscripts within the column are different, $P < 0.05$

[*] The value within the row is significantly different, $P < 0.01$

Discussion

In the present study, the intra-follicular administration of LH and hCG in dairy heifers had similar effects. There are only a few reports of intra-follicular hormone administration in cattle. The first study concerning ultrasound-guided IFT described the effects of different doses of hCG as well as the technical aspects of IFT (Kot et al. 1995). The precise calculations of an array of hCG doses for IFT have been described and ultrasound-guided IFT of substances has been reported as a useful research tool (Kot et al. 1995). The effect of IFT with LH in heifers previously treated with deslorelin has been described previously (Mala et al. 2013a). In that study, deslorelin implants were used to eliminate the possible activity of endogenous LH. The authors concluded that the ovulation of follicles subjected to IFT was the result of the local activity of intra-follicular injected LH (Mala et al. 2013a). The minimum effective doses of IFT using LH and hCG leading to ovulation in cycling dairy heifers were estimated in a subsequent study (Mala et al. 2013b). The minimum effective dose of 0.1 μg LH and 0.01 IU of hCG was established; however, doses of 1 μg LH and 10 IU hCG for IFT were recommended for practical use taking into account the plasma progesterone concentrations. Plasma progesterone concentrations 7 days after IFT showed a downward tendency with decreasing doses of hormones, although the heifers showed an ovulation of treated follicle. It was assumed that higher intrafollicular doses of hormones could be followed by the development of a corpus luteum and higher progesterone secretion. The total efficiency in positively responding groups was numerically lower after LH IFT (73.3%) compared to hCG IFT (100%). Although the difference was not significant due to the low number of experimental animals, the authors concluded that the results of their study should be confirmed in a larger number of treated animals (Mala et al. 2013b). Therefore we decided to use the doses of 2 μg LH and 10 IU hCG in the present study.

The results of the present study clearly demonstrated the similar ovulation rates of the follicles subjected to IFT in the LH and hCG groups, which was significantly higher than in the control group (89.3%, 92.9% and 3.7%, respectively). The ultrasound appearance of the corpus luteum after IFT was typical, as was the diameter, which varied between 15 and 22 mm. The numerically lower number of ovulations after IFT using LH described in the previous study (Mala et al. 2013b) was not confirmed. This difference could be a coincidence caused by the small number of experimental animals in that study. Plasma progesterone concentrations in the present study were significantly elevated in the LH and hCG group on D7. The increase in progesterone concentration (concentration of D7 – concentration of D0) was numerically higher in the LH group compared to the hCG

group (5 ng, 79% vs. 2.88 ng, 43%). Although these differences were not significant, the stimulatory effect of LH was shown by the significant difference in the P4 concentration between the LH and S group on D7.

Our study showed a similar positive ovarian response to IFT using 2 μg LH and 10 IU hCG as well as a significant elevation of plasma progesterone concentrations. Both hormones can be used for IFT with similar efficiency. It is important that one can be replaced by the other because the commercial availability of these two active substances changes over time. Intrafollicular hormone treatment can be used for research purposes and further clinical studies in stubborn cases of ovulatory failure in cattle.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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