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EFFICACY OF hCG AND GnRH WITH RESPECT TO FOLLICULAR SIZE AND PRESENCE OF THE CORPUS LUTEUM IN COSYNCH PROTOCOL INTEGRATED WITH NORGESTOMET IN LACTATING COWS

ŞÜKRÜ METİN PANCARCI*, NECDET CANKAT LEHİMCİOĞLU, UMUT ÇAĞIN ARI, ÖRSAN GÜNGÖR[1], AND ÖZAY AKBULUT[2]

Faculty of Veterinary Medicine, Kafkas University, 36100 Kars, Turkey
[1]Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, 15100 Burdur, Turkey
[2]Akbulut Veterinary Clinic, 15040 Burdur, Turkey
smpancarci@hotmail.com
* Balikesir University, Faculty of Veterinary Medicine, 10460 Balikesir, Turkey

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Abstract

Efficacies of hCG and GnRH with respect to follicular diameter in Cosynch protocol were investigated. Lactating cows (n=119) received injections of GnRH and $PGF_{2\alpha}$ 7 d apart. Norgestomet ear implant was inserted at GnRH administration and removed prior to $PGF_{2\alpha}$ injections. Presence of the corpus luteum (CL) at GnRH and $PGF_{2\alpha}$ injections, and diameter of preovulatory follicle [small (SPF) <8 mm or large (LPF) ≥8 mm] at $PGF_{2\alpha}$ were determined with transrectal ultrasonography (USG). The animals were randomly treated with GnRH (0.01 mg; GPG, n=62) or hCG (1,500 IU; GPH, n=57) at timed artificial insemination (TAI) 56 h after injection of $PGF_{2\alpha}$. Pregnancies were diagnosed with USG 32-35 d after TAI. Pregnancies per TAI (P/TAI) were significantly ($P<0.05$) lower in GPH cows (28.1%; 16/57) compared to those in GPG animals (46.8%; 29/62). P/TAI with respect to follicular size did not differ between GPG and GPH cows, although P/TAI was numerically higher in LPF (30.0%; 15/50) than in SPF (14.3%; 1/7) in GPH cows. P/TAI in cows without CL at $PGF_{2\alpha}$ was 2.5 (1.0-6.7) times higher ($P<0.01$) in GPG (20.0%; 3/15) compared to GPH (10.5%; 2/19). In conclusion, use of hCG in milieu of the second GnRH in Cosynch+progesterone insert protocol did not have any advantage in lactating cows.

Key words: cow, GnRH, hCG, $PGF_{2\alpha}$, corpus luteum, follicle, insemination, pregnancy.

A timed artificial insemination (TAI) programme, designated as the Ovsynch protocol, has increased the efficiency of reproductive management in dairy cows because all cows are inseminated at the designated voluntary waiting period without the need for oestrus detection, and pregnancy rates are optimal (2, 15). The Ovsynch protocol involves injection of GnRH given 48 h after injection of $PGF_{2\alpha}$ to ovulate a newly recruited dominant follicle at 24 to 32 h that was previously induced with the injection of GnRH and $PGF_{2\alpha}$ given a 7 d interval (14). Cows can be artificially inseminated 16 h after the second GnRH injection owing to tight synchronisation of the ovulation (16). Although the 16 h interval from the second GnRH injection to TAI yielded the greatest fertility in dairy cows, it is often difficult to implement this scheme under field conditions (8). Therefore, many veterinarians under field conditions prefer to perform TAI concurrently with the second GnRH injection. This strategy is known as Cosynch because it eliminates one handling of cows and facilitates once-daily restraint of cows for administration of hormone injections and TAI (12).

Premature oestrus and ovulations prior to the second GnRH injections in Ovsynch protocol decrease the success of TAI (11, 23). Moreover, it was demonstrated that CIDR-based ovulation-synchronisation protocols suppressed uterine $PGF_{2\alpha}$ secretion in the following luteal phase and prevent premature luteolysis during Ovsynch protocol (18). Therefore, progestagens, such as intravaginal devices or ear implants, are widely used to decrease incidence of premature oestrus and ovulations during TAI protocols.

To synchronise and induce ovulation according to TAI protocol, hCG was used (3, 19). It has been reported that hCG and GnRH have similar effects on the ovary (7); however, half life of hCG is longer than natural LH, and hCG works independently from the hypothalamo-hypophyseal axis (10). Some studies demonstrated that the usage of hCG, instead of GnRH, improved ovulation rates and pregnancy rates (3, 19). Enhanced plasma progesterone concentrations and

increased pregnancy rates were reported following use of hCG in a TAI in dairy cows (4).

A longer follicular phase, ovulation of a smaller follicle, and lower progesterone concentrations during the early luteal phase have been observed following induction of luteolysis when a pre-ovulatory follicle was <10 mm (17). Follicle size may be an important factor to determine response to hCG, instead of the second GnRH in Cosynch strategy. It was hypothesised that the longer period of LH-like stimulation of the ovulatory follicle due to the extended half-life of hCG in blood (20, 21) should better stimulate the ovulatory follicle at concurrent ovulation induction and TAI in Cosynch strategy. Therefore, the aim of the study was to evaluate the efficacy of hCG instead of the second GnRH administration with respect to follicular size and presence of the corpus luteum (CL) in Cosynch protocol based on pregnancies per TAI in cattle.

Material and Methods

The experiment was conducted between May and June 2009 in five farms located in north-east (two farms) and south-west (three farms) part of Turkey. In the north-east farms, grazing Brown Swiss and in the south-west farms (free stall) Holstein cows were used. All cows received injections of 0.02 mg of GnRH (buserelin acetate, Receptal®, Intervet, Turkey; i.m.) and 7 d later 25 mg of $PGF_{2\alpha}$ (dinoprost trometamol,; Enzaprost®, CEVA DİF, Turkey). Concurrently, ear implant containing 3 mg of norgestomet (Crestar®, Intervet, Turkey) was inserted at GnRH injection and removed prior to $PGF_{2\alpha}$ injection in all animals. Then, the animals were assigned randomly to group I (GPG), which received at TAI, 56 h after injection of $PGF_{2\alpha}$, 0.01 mg of GnRH (buserelin acetate, Receptal®, Intervet, Turkey; i.m.; Cosynch, n=62) and group II (GPH), which received 1,500 IU of hCG (Chorulon®, Intervet, Turkey; i.m.; modified Cosynch, n=57). Body condition scores (BCS), using a 5-point scale with intervals of 0.25, were recorded during the Cosynch protocol (6). Cows were classified as having poor (BCS <2.5) or optimal (BCS >=2.5) conditions.

At the first GnRH and $PGF_{2\alpha}$ injections, the presence of visible CL was determined with transrectal ultrasonography (in north-east: Sonosite Vet 180 Plus equipped with 7.5 mHz (general mode) rectal linear probe, in south-west: Pie Medical, Esoate Aquila Pro). Afterwards, the animals were classified as dioestrous-prooestrous (premature luteolysis), metoestrous-dioestrous, dioestrous-dioestrous stages, and non-cycling during Cosynch protocol. At $PGF_{2\alpha}$ injection, diameter of the largest follicle of each cow was measured with transrectal ultrasonography. Afterwards, cows were classified as having a small (<8 mm) or large (≥8 mm) pre-ovulatory follicle, according to Sirois and Fortune (22). Pregnancies were diagnosed with trans-rectal ultrasonography 32-35 d after TAI.

Pregnancies per TAI (P/TAI) following the first service were analysed by the logistic regression-Stepwise Selection Procedure of SAS to determine independent variables among all variables and interactions. The mathematical model for P/TAI included location, replicate, breed, parity, BCS, presence of visible CL at $PGF_{2\alpha}$, size of follicle at $PGF_{2\alpha}$, treatment, sire, inseminator and cycling status during TAI protocol and interactions of treatment by parity, presence of visible CL, follicular size and cycling status, respectively. The Proc Freq procedure of SAS was utilised to obtain frequency values and for Chi-square analyses.

Results

Overall, P/TAI following TAI was significantly ($P<0.05$) lower in GPH group (28.1%; 16/57) compared to that in GPG group (46.8%; 29/62), based on separate Chi-square analyses (Fig.1). P/TAI did not differ between GPG and GPH groups with respect to follicular size, although P/TAI was numerically higher in cows with large preovulatory follicle (LPF) (30.0%; 15/50) than that in small preovulatory follicle (SPF) (14.3%; 1/7) in GPH group. Whereas, P/TAI were similar in cows with LPF (46.4%; 26/56) and those with SPF (50.0%; 3/6) in GPG group (Fig. 1).

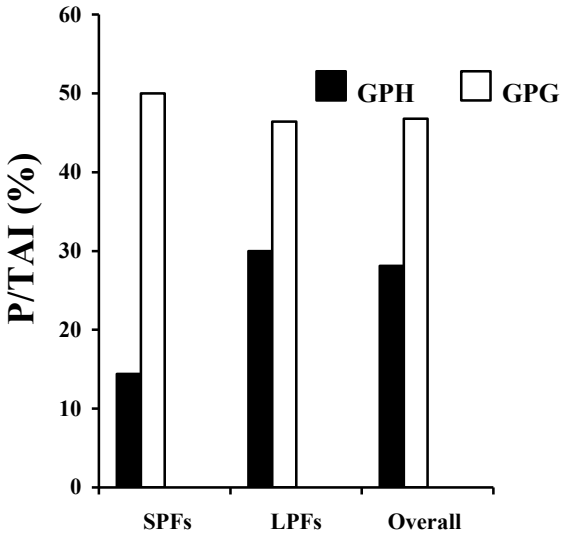


Fig. 1. P/TAI for cows with small preovulatory follicles (SPFs) and large preovulatory follicles (LPFs) in GnRH (GPG) and hCG (GPH) groups did not differ. Overall P/TAI differed ($P<0.05$) between GPG and GPH groups

Logistic regression analyses reveal that there was a significant ($P<0.01$) interaction effect of the presence of CL at $PGF_{2\alpha}$ injection and treatment on P/TAI (Fig. 2). In this regard, the odds ratio estimated for pregnancy in cows without CL was 2.5 (1.0-6.7) times higher in GPG group (20.0%; 3/15) compared to GPH group (10.5%; 2/19). Among cows with CL, P/TAI was higher in GPG group (55.3%; 26/47) than that in GPH group (36.8%; 14/38).

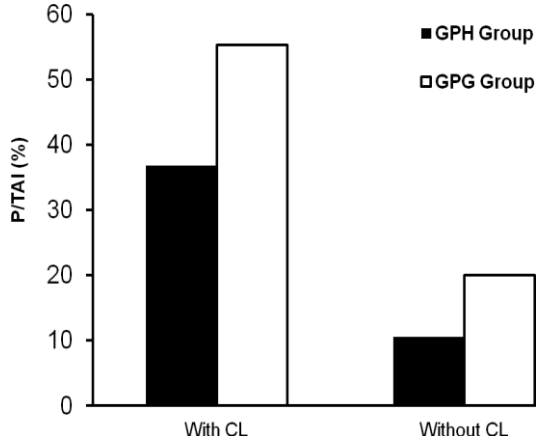


Fig. 2. Effect of the presence of visible CL at $PGF_{2\alpha}$ injection during Cosynch protocol on P/TAI

Discussion

In the study, P/TAI in GPH group was lower compared to that in GPG. It was demonstrated that hCG in lieu of GnRH could not be potent to achieve acceptable P/TAI in cows in Cosynch+progesterone protocol, which is in agreement with previous reports (5, 9). Although the effect of hCG is similar to LH induced by GnRH (7), concentration of hCG may be inadequate to induce ovulation of preovulatory follicles of different sizes. Moreover, it has been suggested that hCG can promote the maturation of bovine oocytes for fertilisation when used in concentrations greater than LH (1). In this regard, oocyte maturation, required for fertilisation, could not be completed in GPH group even if ovulation could be induced by hCG.

Follicle size could be an important factor affecting P/TAI in GPH group. Although there is no interaction effect of follicle size, numerically higher P/TAI was obtained in cows with LPFs compared to those with SPFs in GPH group. Whereas, P/TAI for cows with LPFs and for those with SPFs were similar in GPG group. This phenomenon could be attributed to LH receptors in follicles. In this matter, Nogueira *et al.* (13) determined that LH receptor mRNA is undetectable in granulosa cells of small follicles; whereas, LH receptor mRNA increased in accordance with follicular growth. Lower P/TAI in cows with small preovulatory follicles could be a reflection of impotence of hCG for either ovulation induction or oocyte maturation. Moreover, this inadequate effect of hCG in cows with SPFs could be due a to direct induction of ovulation without hypothalomo-hypophysial axis leading to lower amount of LH. Therefore, small preovulatory follicles could not be capable of ovulation following induction by hCG rather than induction by GnRH.

There are limited studies using hCG instead of the second GnRH in Cosynch protocol. Geary *et al*. (9) determined lower pregnancy rates in cows, which received hCG (34%) than in those treated with GnRH (49%). Similarly, the overall P/TAI was lower in hCG treated group (28.1%) than that in GnRH treated group (46.8%) in the study. A decrease in pregnancy rates in cows receiving hCG in Cosynch protocol could be due to a different reason in both studies. Moreover, De Rensis *et al*. (3) observed that season could be an important factor affecting success of hCG, as a replacement of the second GnRH in Ovsynch protocol. De Rensis *et al.* (5) pointed out that hCG increases pregnancy rates in warm climate; while, there is no effect on pregnancy rates in cool climate compared with GnRH in Ovsynch protocol. Geary *et al*. (9) reported that more hCG-treated cows exhibited short oestrous cycles following TAI. Perhaps, lower P/TAI in hCG group in the study could be owing to short oestrous cycles, which could impair maternal recognition of pregnancy or basically due to luteal deficiency following TAI. Furthermore, Geary *et al*. (9) reported lower pregnancy rate in multiparous cows than that in primiparous cows in Cosynch protocol. However, the same difference was not detected in the study. Probably, the addition of progesterone into Cosynch protocol could eliminate this problem.

Among cows without CL at $PGF_{2\alpha}$ injection in Cosynch + progesterone insert protocol, higher P/TAI in GPG group compared to GPH group could be due to different effects of GnRH and hCG among cows without CL. In this matter, cows without CL at $PGF_{2\alpha}$ injection could be either non-cycling or non-synchronised. It could be expected that GnRH could be more potent by inducing LH surge to induce ovulation in non-cycling cows. In spite of progesterone treatment in Cosynch protocol, hCG with its LH effect on ovarian level could not promote ovulation in cows with deep anoestrus because hCG did not have any effect on hypophysial level. A persistent dominant follicle (PDF) could be developed in non-synchronised animals because none of the cows was inseminated following oestrous detection during Cosynch + progesterone insert protocol in both groups. Probably, GnRH with endogenous LH surge could be better than hCG to induce ovulation among cows with PDF.

Similar pregnancy rates were reported in Ovsynch protocol with the use of hCG as the replacement of the second GnRH in dairy heifers 48 h after $PGF_{2\alpha}$ administration (19). However, pregnancy rates were lower in this study with ovulatory injection of hCG 56 h after $PGF_{2\alpha}$ in dairy cows and in a study with ovulatory injection of hCG in beef cows 48 h after $PGF_{2\alpha}$ (9) in Cosynch protocols. Lower pregnancy rates in Cosynch protocol with ovulatory injection of hCG could not be basically attributed to insemination time because half life of hCG is longer than GnRH. Although there was no direct comparison between Ovsynch *vs* Cosynch protocols with the use of hCG in the study, it can be considered that differences in pregnancy rates could not be due to timing of hCG injection. Perhaps, different ovulatory responses after hCG between cows and heifers could result in differences in pregnancy rates in Ovsynch and Cosynch protocols. Moreover, a similar dose of hCG was used by Schmit *et al.* (19) in Ovsynch protocol (3,000 IU) and by Geary *et al.* (9) in Cosynch protocol (2,500 IU) in a form of ovulatory injection.

Therefore, a lower pregnancy rate could not be caused by dosage of hCG in Cosynch protocol.

To conclude, hCG cannot be a good substitute for the second GnRH to increase P/TAI in Cosynch + progesterone insert protocol. The study warrants further investigations that will be carried out to determine the exact mechanism of deficiency of hCG in Cosynch strategies. Higher P/TAI in GPG group compared to GPH group among cows without CL at $PGF_{2\alpha}$ injection could indicate superior effect of GnRH in anoestrous cows.

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