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Effect of human chorionic gonadotrophin injection after artificial insemination on pregnancy establishment in dairy cattle

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Abstract

The objective of this study was to evaluate the effect of treating dairy cattle with exogenous human chorionic gonadotrophin (hCG), five (5) days post artificial insemination (AI) on serum progesterone (P4) concentration and pregnancy rate. In this experiment, five days after AI, cows were assigned randomly to two groups namely: a) treated group (67) which were administrered with 1500 IU hCG (Chorulon) and b) control group (61), which received no treatment. On day 5, 10, 15 and 20 after the artificial insemination, blood samples from a total of 8 cows (4 from each group) were collected and were analyzed for serum P4 concentration. Cows were detected for estrus according to standing heat by visual observation. Cows that were detected still in estrus after days 18-24 were re-inseminated and recorded as not pregnant (open). Pregnancy diagnosis was conducted by ultrasonographic examination and transrectal palpation of the uterus on approximately 60 days in cows that observed to be not in estrus. The conception rate in hCG treated and control groups were 52.5 and 36.1%, respectively. The results proved that there were no significant differences in conception rate between two groups ($p=0.0568$). However, pregnancy rates were reduced by hCG treatment. Average serum P4 concentrations did not differ between Hcg-treated and control groups on day 5 (0.377 versus 0.375 ng/ml). On day 20 serum P4 concentrations were greater in the treated group compared with the control group (3.085 versus 2.010 ng/ml). The treatment with hCG seemed to increase P4 level compared with the control. In conclusion, the results of this study showed that 1500 IU of hCG administered on 5 day post AI increased conception rate in dairy cows. This was supported by the results on serum P4 concentration which was greater in hCG treated group.

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INTRODUCTION

Reproductive performance in dairy cattle has reduced over the past years and is markedly lower than desired (Lucy, 2001; Washburn et al., 2002; de Vries and Risco, 2005). Several investigators have indicated a reduction in reproductive efficiency associated with an increase in genetic merit for milk production in dairy cows (Butler, 1998; Lucy, 2001; Wahburn et al., 2002). Dairy cattle with high milk production have low pregnancy rate, which cause major economic losses (Nevel and McGilliard, 1993). Declined reproductive performance is due to many elements including inaccuracy and inefficiency of estrus detection, missing out on the optimal time of insemination, anovulation or delayed ovulation, negative energy balance and nutrition, and inbreeding (Hermas et al., 1987; Nebel and Jobst, 1998; Butler, 2000). Some possible reasons for the decrease in reproductive efficiency of the modern dairy cattle may include decreased circulating concentrations of ovarian steroids (Vasconcelos et al., 1999), poor oocyte quality and early embryonic development (Sartori et al., 2002), and increased average body temperature (Berman et al., 1985; Kadzere et al., 2002). Another factor which causes low pregnancy rates is embryonic loss (Lamming et al., 1989; Chebel et al., 2004; Santos et al., 2004). Although fertilization rates in cows are announced to be higher than 90% (Diskin and Sreenan, 1980), the majority of embryonic mortality (70-80%) happens between 8 and 16 days post insemination (Sreenan et al., 2000; Dunne et al., 2000).

Circulating P4 controls LH pulsatility (Begfeld et al., 1996), follicular dynamics (Stock and Fortune, 1993), oviductal and uterine environments (Thatcher et al., 2001; Green et al., 2005), and embryonic development (Mann and Lamming, 2001). Maintenance of pregnancy during its early stage depends on secretion of P4. Reproductive performance in dairy cattle is decreased by 13% (Santos et al., 2004) and 11% (López-Gatius et al., 2002), due to late embryonic and early fetal mortality, respectively. In addition, luteal deficiency has been postulated as a cause of pregnancy failure during the first 3 weeks of pregnancy (Henricks et al., 1970; Butler et al., 1996; Mann and Lamming, 2001; Santos et al., 2004). A number of factors, including genetics (Lucy, 2001), nutrition (Gombe and Hansel, 1973), rate of steroid metabolism in high milk-producing cows (Wiltbank et al., 2000; Sartori et al., 2002; Sangsritavong et al., 2002), and heat stress (Wilson et al., 1998; Wolfenson et al., 2002) have an effect on P4 levels during early pregnancy. Reduced circulating concentration of P4 after fertilization is associated with increased early conception failure (Mann et al., 1999), poor embryo development (Walton et al., 1990), and reduced subsequent conception rates (Stronge et al., 2005; Mann et al., 2006; Demetrio et al., 2007). Suboptimal concentration of serum P4 in dairy cattle is a major reason for damaged embryo development and increased pregnancy losses (Moore et al., 2005).

Low P4 concentration in blood would partly describe the low fertility in high producing dairy cattle, as P4 concentrations following AI are positively associated with embryo maturity and functionality (Garret et al., 1988), which is important for inhibiting luteolysis and maintaining pregnancy (Mann et al., 1995; Meyer et al., 1995). Therefore, it can be concluded that part of the embryonic losses in dairy cows are lost due to insufficient maternal luteal function (Lamming et al., 1989; Mann et al., 2001).

Several investigators have shown that cows which have lower P4 concentrations during subsequent diestrus have a lower pregnancy rate (Lamming et al., 1989; Mann and Lamming, 1999). In lactating dairy cows subjected to AI, pregnancy rate was positively influenced by the size of ovulatory follicle (Sartori et al., 2006) and serum concentrations of P4 on 7 day after ovulation (Demetrio et al., 2007). P4 administration during the luteal phase of the estrus cycle could elevate conception rate in cattle (Robinson et al., 1989; Macmillan et al., 1991; Sianangama and Rajamahendran, 1992). Inducing formation of an accessory CL (corpora lutea) during luteal phase of the estrus cycle with a gonadotropin exposure could be a way to elevate concentrations of P4 in serum during the luteal phase (Schmitt et al., 1996). Therefore, induction of additional corpora lutea (CL) that increases P4 concentrations in plasma (Thatcher et al., 2002) or administration of P4 (López-Gatius et al., 2004) reduced late embryonic and early fetal mortality.

The objective of the present study was to evaluate the effects of exogenous human chorionic gonadotrophin (hCG, 1500 IU) given on 5 day post AI on P4 concentration, and on pregnancy rate in dairy cattle.

MATERIALS AND METHODS

The selected animals and the experimental protocol were approved by institutional animal ethical committee of the National Institute of Animal Science (NIAS) in South Korea.

This study used 128 cows which animals were housed in a free stall building and fed a TMR (total mixed ration) formulated to meet or exceed NRC requirements of dairy cattle (NRC, 2001). For estrus detection, the cows were visually observed two times of day, one each in the morning and evening. The AI was conducted following the AM-PM rule. Cows were inseminated 12-16h after being observed to be in spontaneous estrus by a single experienced AI technician using semen from a commercial semen distribution center. Cattle with abnormal reproductive tracts, such as ovarian cysts, uterine or cervical abscesses, endometritis, or pyometra as discovered by palpation or ultrasonography were excluded from the experiment.

Figure 1 Schematic diagram of treatment protocols used in the experiment. hCG was injected in the morning of day 5 following AI, which was also day 5 of the estrus cycle (day 0 = estrus). Cows were randomly assigned to one of the two groups as follows: control group (n=61, no additional treatment) and treatment group (n=67, administered with an injection of 1500 IU, hCG (Chorulon, MSD Aminal Health, Seoul, Korea)). A total of eight animals (4 from each group) were randomly selected to obtain blood samples in the morning of days 5, 10, 15 and 20 after AI by puncturing the median coccygeal vessels with commercial evacuated tubes with no additives. The blood samples were centrifuged at 4°C (1200 x g for 15 minutes) for serum collection. Serum was frozen and stored at -20°C until P4 analysis. Serum concentrations of P4 were analyzed using the immunofluorometric assay (Immulite® 1000) using commercially-available kits (Siemens 06603261 Immulite® Progesterone Kit, Siemens Medical Diagnostics, Seoul, Korea).

Cows that were detected to be still in estrus after 18-24 days were re-inseminated and recorded as no pregnant (open) to the previous AI. Pregnancy was diagnosed by palpation per rectum of the uterine contents between days 50 and 60 after AI or ultrasonographic examination to determine pregnancy status. Ultrasonography was carried out using a B-Mode ultrasound scanner (MyLab™OneVET, esaote) equipped with a 5.0-MHz linear array probe. Percentages of pregnant cows were estimated by dividing the number of pregnant cows by the number of cows that were artificially inseminated.

Data were subjected to T test using the Statistical Analysis System (SAS Institute, Cary, NC, USA).

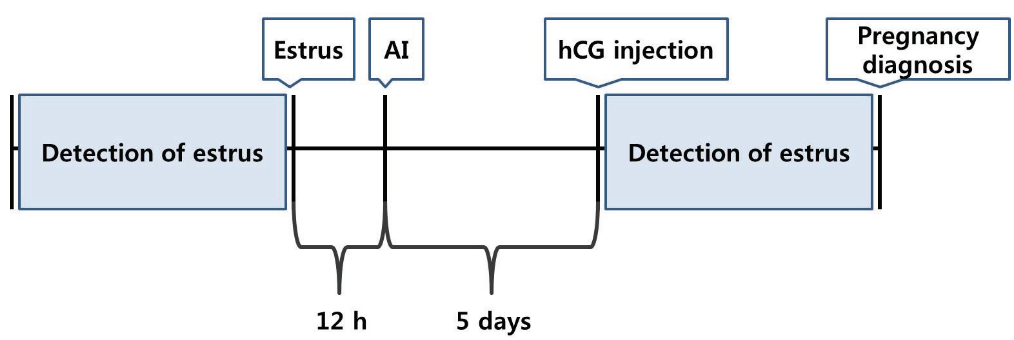

Figure 1. Treatment protocol for the experiment with hCG and no hCG given to dairy cattle 5 days after AI.

RESULTS

This experiment was conducted to evaluate whether the administration of hCG on 5 day post AI would enhance the pregnancy rate in dairy cows. A total of 128 cows were used for this study, 67 cows in the hCG group (treated) while 61 cows in control.

35 and 22 cows from the hCG and control groups, respectively were found as pregnant after ultrasonic examination or rectal examination. There was no significant difference in the pregnancy rate between hCG treated and control groups ($p > 0.05$). But a tendency for greater percentage of pregnant cows was observed in the treated group ($p=0.0568$, Table 1). The additional treatment

with hCG on day 5 increased the percentage of pregnant cows.

Concentrations of P4 in serum between days 0 and 5, days 5 and 10, days 10 and 15, and days 15 and 20 were calculated (Fig 2). Serum concentrations of P4 on day 5 were similar among postbreeding treatments. Elevations in P4 from day 5 to 10 were proportional for both control group and hCG group cows (Fig 2). On day 20, serum P4 concentrations were significantly greater than day 5. Average serum concentrations did not differ between hCG treatment and control groups on 5 day (Fig 2). However, the P4 concentrations of hCG treated cows were significantly greater on day 10, 15 and 20 (Fig 2).

Table 1. The effect of an injection of hCG on day 5 after AI on conception rate in dairy cattle

Treatment	No. of pregnant (%) ± SE	No. of open
Control group (n=61)	22 (36.1) * ± 0.062	39
Treatment group (n=67)	35 (52.2) * ± 0.061	32

* P = 0.0568

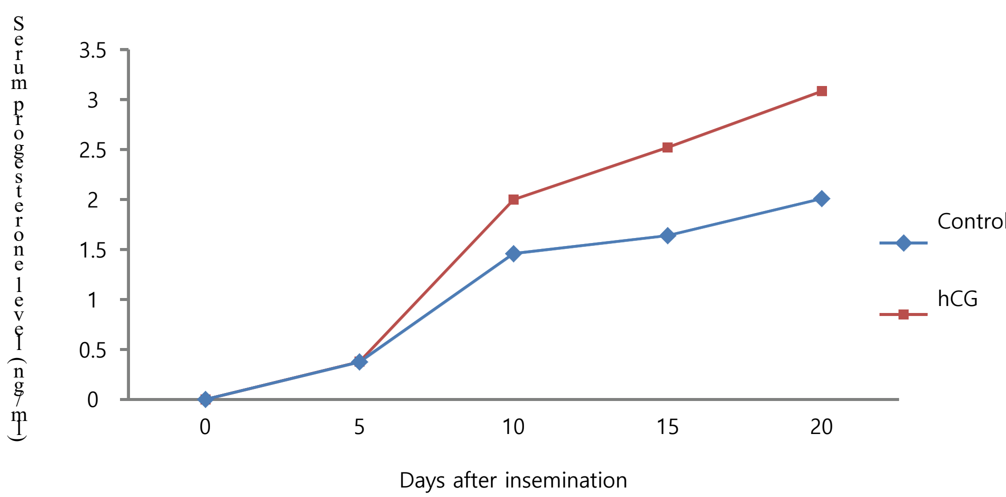

Figure. 2. In serum concentrations of progesterone (P4) between days 0 to 5, 5 to 10, 10 to 15 and 15 to 20 following AI in control and hCG groups.

DISCUSSION

Several studies have evaluated strategic treatments with gonadotropins to ameliorate conception rates in dairy cattle after AI (Ambrose et al., 1999; Santos et al., 2001; Peters et al., 2002; Willard et al., 2003; Bartolome et al., 2005). Treatment with gonadotropins such as hCG and GnRH (Gonadotropin-Releasing Hormone), 5 to 7 days after ovulation has the potential to stimulate luteal function (Hoyer and Niswender, 1985), to lead ovulation of the dominant follicle and formation of additional CL (Santos et al., 2001), and to elevate the occurrence of cows experiencing 3-wave follicular cycles (Diaz et al., 1998), which was associated with enhanced conception rates (Ahmad et al., 1997; Santos et al., 2001). Because, the alteration in the number of follicular waves during the estrus cycle has been found to benefit the likelihood of conception (Ahmad et al., 1997), perhaps cows with 3-wave cycles have a delay in developing potential estrogenic follicles and a longer luteal lifespan than those with 2-wave cycles (Diaz et al., 1998; Araújo et al., 2009), which is helpful to early embryo survival.

The results of the present study showed that the injection of hCG 5 days following AI had little influence on pregnancy rates in dairy cattle, and it also increased P4 concentration. This was consistent with the theory that hCG given on day 5 of the estrus cycle induced ovulation of the first-wave dominant follicle resulting in formation of an additional CL thereby increasing endogenous P4 concentrations on day 20. The mechanism of action of hCG is via the derivation of an additional CL by ovulating the dominant follicle of the first follicular wave, as well as having a provocative effect on the production of P4 by the original CL (Schmitt et al., 1996). The cows in the hCG treated group had higher P4 concentrations between days 5 and 20 after AI compared with cows in the control group. This is in agreement with a prior study (Schmitt et al., 1996) that reported elevated P4 concentrations between days 11 and 16 in Holstein heifers injected with hCG 5 days following estrus. This result was also consistent with the findings of Beltran and Vasoncelos (2008) which indicated that injection of hCG 5 days following AI tended to ameliorate conception rates in dairy cows. Kerbler et al. (1997) found luteotropic effects as established by elevation in P4 concentrations when cows were administrated with 1500 IU hCG 5 days post AI. They used heifer but we used lactating dairy cow of low reproductive efficiency.

Several studies have showed low P4 concentrations in serum of cows that failed to conceive and this was apparent as early as day 6 following insemination (Thatcher et al., 2001). It has earlier been proposed that low P4 concentrations during early embryonic development may create pregnancy failure and thereby decrease the conception rate (Lucy, 2001). Elevating endogenous P4 concentrations at an early stage (day 5) and late stage (day 15) of the estrus cycle may help decline embryonic death in cows and pregnancy maintenance. These findings lead to the study of the effects of addition of diestrus P4 levels following insemination. It has been postulated that high P4 following insemination may strengthen embryo development and may suppress luteolysis, ultimately resulting in declined embryonic loss (Peters et al., 1992; Mann et al., 1999). Luteal deficiency during the very early stages of pregnancy has been postulated as a factor of pregnancy failures (Shelton et al., 1990). Garrett et al. (1998) observed that bovine embryos isolated from P4-treated cows on day 14 post insemination were more progressive in development compared to those of control animals. Moreover, Mann and Lamming (1999) observed that P4 administration following insemination produced an overall advance in pregnancy rate of 5%. Past researches (Johnson et al., 1958; Robinson et al., 1989; Macmillan et al., 1991) indicated elevated P4 concentrations during diestrus ameliorated embryonic development. P4 is necessary for orchestrating the histotrophic environment for nourishment of the conceptus (Santos et al., 2004). P4 also inhibits luteolysis by reducing sensitivity to oxytocin by binding to oxytocin receptors (Grazzini et al., 1998) and strengthening conceptus development which, promotes secretion of interferon-τ(Mann and Lamming, 2001). Mann et al. (2006) indicated that early P4 supplementation resulted in a fourfold elevation in trophoblast length and a six fold elevation in uterine concentration of interferon-τ The increase in serum P4 estimated in the present study perhaps is due to combined effects on the primary and accessory CL.

In summary, the injection of exogenous hCG 5 days post insemination resulted in elevated serum concentrations of P4, and did result in higher pregnancy rates. Heat stress in dairy cattle has been associated with decreased reproductive performances. Administration of hCG following AI may be beneficial in ameliorating conception rate only in certain situations such as heat stress. In addition, this is one of the recommended methods in treating and/or managing dairy cattle in order to ameliorate their reproductive efficiency.

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