*Austral J Vet Sci* **49, 119-122 (2017)**

SHORT COMMUNICATION

# Pregnancy rate in dairy cows treated with human chorionic gonadotropin five days after insemination

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**ABSTRACT.** This study tested whether the administration of hCG five days after insemination increased progesterone concentration and pregnancy rate (PR) in dairy cows. A total of 989 lactating Holstein cows with different parity and number of prior services were used. Cows were inseminated after overt estrus or at a fixed-time. Five days post-insemination, cows were randomly assigned to two treatment groups: hCG (n=482), which received 3500 IU of hCG by intramuscular injection; and the control group (n=507), which did not receive any treatment. Pregnancy was diagnosed by ultrasound on day 30 post-insemination, and gestation was confirmed on day 60 post-insemination by rectal palpation. In 15 cows from each treatment group, plasma progesterone concentration was determined on days 5, 11, and 15 post-insemination. Pregnancy rate was analysed using logistic regression. Variations in progesterone concentration between treatments were tested by ANOVA for repeated measurements. Progesterone concentration was higher on days 11 and 15 in cows treated with hCG, compared to the control group ($P<0.05$). Treatment with hCG increased pregnancy rate (47.5 vs. 37.4%. Odds ratio 1.3; $P<0.05$). No interaction was observed between treatment and body condition, prior services, milk production, parity, or insemination type (overt estrus or fixed time). Treatment with hCG did not reduce pregnancy losses between day 30 and 60 post-insemination ($P>0.1$). We conclude that injection of hCG five days after insemination increased progesterone concentration and pregnancy rate in dairy cows.

*Key words*: hCG, fertility, progesterone, dairy cows.

**RESUMEN.** Este estudio evaluó si la administración de hCG cinco días después de la inseminación incrementa la concentración de progesterona y la tasa de preñez (TP) en vacas lecheras. Se utilizaron 989 vacas Holstein de diferente número de parto y servicio. Las vacas se inseminaron después de la detección del estro o a tiempo fijo. El día cinco postinseminación las vacas se asignaron aleatoriamente a dos tratamientos: hCG (n=482), donde recibieron 3500 UI de hCG vía im; y control (n=507), donde no recibieron tratamiento. Se diagnosticó la gestación mediante ecografía el día 30 posinseminación y se confirmó el día 60 por palpación transrectal. En 15 vacas de cada tratamiento se determinó la concentración plasmática de progesterona los días 5, 11 y 15 postinseminación. Se analizó la TP mediante regresión logística y la concentración de progesterona mediante análisis de varianza. La concentración de progesterona fue más alta los días 11 y 15 en las vacas tratadas con hCG que en las control ($P<0,05$). El tratamiento con hCG incrementó la TP en comparación con sus controles (47,5 vs. 37,4%; odds ratio 1,3; $P<0,05$). No se observó interacción entre el tratamiento con la condición corporal, número de servicios, producción láctea, número de partos y el tipo de inseminación (detección de estro o tiempo fijo). El tratamiento con hCG no disminuyó la pérdida de gestaciones entre los días 30 y 60 postinseminación ($P>0.1$). Se concluye que la inyección de hCG cinco días después de la inseminación incrementa la concentración de progesterona y la tasa de preñez en vacas lecheras.

*Palabras clave*: hCG, fertilidad, progesterona, vacas lecheras.

## INTRODUCTION

The percentage of embryonic and fetal losses in dairy cows is close to 50%, and it is estimated that between 70 and 80% of these losses occur in the first 16 days post-insemination (Diskin *et al* 2011). One of the causes of embryonic loss is related to delayed embryo development, which reduces the embryo's ability to signal for the maternal recognition of pregnancy (Mann and Lamming 2001). This condition may be due to insufficient serum progesterone concentration in dairy cows (Mann and Lamming 2001, Wiltbank *et al* 2012). Progesterone stimulates the production of endometrial secretions vital to the development of the embryo and maintenance of pregnancy (Geisert *et al* 1992). Low progesterone concentration has been associated with abnormal early embryo development (Mann and Lamming 2001, Stronge *et al* 2005), while high progesterone concentration have favored embryo development and interferon-τ secretion (Mann *et al* 2006, Lonergan 2011).

In practice, various methods to supplement serum progesterone have been tested with the aim of improving pregnancy rates. In different studies, progesterone has been administered via implants or intra-vaginal devices, with inconsistent results (for review see Wiltbank *et al* 2014). Increases in blood progesterone concentration have also been achieved by inducing the formation of secondary *corpora lutea* (Santos *et al* 2001). Treatment with human chorionic gonadotropin (hCG) between days 4 and 7 post-insemination stimulates ovulation of the first wave dominant follicle and the formation of an additional *corpus luteum*, which results in increased progesterone level (Price and Webb 1989). The response to treatment

Accepted: 20.01.2017.

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with hCG five days post-insemination has been inconsistent (Nascimento *et al* 2013). Santos *et al* (2001) treated first service cows with 3 300 IU i.m. of hCG resulting in an increase in blood progesterone concentration and higher pregnancy rate (PR). Moreover, in primiparous cows hCG injection increased PR, while it had no effect on multiparous cows (Nascimento *et al* 2013). In contrast, Kendall *et al* (2009) using the same treatment protocol showed increased pregnancy rate in multiparous cows, but not in primiparous cows. Nonetheless, other studies with first service cows (Schmitt *et al* 1996, Hanlon *et al* 2005) and with repeat breeders (Walton *et al* 1990) have failed to show the beneficial effect of hCG administration. This study tested whether treatment with hCG on day 5 post-insemination increased plasma progesterone concentration and pregnancy rate in dairy cows.

## MATERIAL AND METHODS

### ANIMAL HANDLING

This study was approved by the National Autonomous University of Mexico Institutional Animal Care and Use Committee. The study was undertaken in a dairy herd in northern Mexico (latitude 25°32'18'' N, longitude 103°27'55'' W, altitude above sea level of 1100 meters) and comprised of 6,000 lactating Holstein cows, milked twice daily and with an average milk production of 11,700 kg per lactation. Cows were fed a total mixed ration (TMR) formulated to meet the daily requirements for maintenance and production.

On day 25 postpartum, all cows began systematic treatment with three injections of PGF2α (Celosil, Intervet Schering-Plough Animal Health, Inc) at twelve-day intervals between injections. After the third PGF2α treatment, cows were mounted by another cow and with increased locomotor activity, as measured by pedometers, were inseminated 12h after the first visible signs of estrus. Cows that had not shown estrus, and therefore had not been inseminated by day 60 postpartum, were submitted to a fixed-time insemination protocol (FTAI). The voluntary waiting period in the herd was of 50 days.

### EXPERIMENTAL DESIGN

In this study, 989 cows varying in parity, number of prior services and type of estrus used for artificial insemination [natural estrus (n=516), estrus induced by PGF2α (n=378)) or FTAI (N=95)] were used. At day 5 post-insemination, cows were randomly assigned to two treatment groups: hCG (n=482), cows received 3500 UI of hCG[1] by intramuscular injection; the control cows (n=507), did not receive any treatment. At day 30 post-insemination pregnancy diagnosis was made by transrectal ultrasonography, and the number of *corpora lutea* was determined. A cow was considered pregnant upon detection of an amniotic vesicle and viable embryo (presence of heartbeat). At day 60 a second pregnancy diagnosis was done by transrectal palpation. In both groups, the body condition score (scale of 1-5) was registered on day 0 (day of artificial insemination (AI), and day 30 post-insemination. According to changes in BCS between day 0 and day 30, cows were divided in three categories: cows that gained body condition (≥1 point), cows that maintained body condition (gained or lost 0.5 points or less), and cows that lost body condition (≥ 1 point).

### SAMPLING AND HORMONE MEASUREMENT

Blood samples were taken on days 5, 11, and 15 post-insemination in 15 cows from each group. Plasma progesterone was measured using radioimmunoassay (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA, USA). Test sensitivity was 0.1 ng/mL, with an intra-assay variation coefficient of 8.1%.

### STATISTICAL ANALYSES

The relative contribution of independent variables to the probability of pregnancy at day 30 post-insemination, and the probability of losing a pregnancy between days 30 and 60 post-insemination was determined by logistic regression using SAS version 9.2 (SAS Institute Inc., Cary, NC). Independent variables were treatment (hCG or control), number of services (one, two, three, four or more), type of puerperium (physiological or pathological), production level (≤ 40 or > 40 kg), parity (primiparous or multiparous), change in body condition (loses, maintains, or gains), and type of estrus used for artificial insemination (natural estrus, estrus induced by PGF2α or FTAI). The probability of losing a pregnancy (embryonic death) between days 30 and 60 after AI was also analysed using a logistic regression model considering the same independent variables.

Progesterone concentration was compared between treatments using a variance analysis for a completely randomized design with repeated measurements over time. The model included treatment, time and their interaction. The percentage of cows that developed a secondary *corpus luteum* was compared using a chi-squared test. In all cases, statistical differences were declared at $P<0.05$.

## RESULTS AND DISCUSSION

Treatment with hCG on day 5 post-insemination increased in 10 points (45 vs. 35%) the proportion of inseminated cows that became pregnant ($P<0.05$; table 1). An effect of treatment and a treatment by time interaction on progesterone concentration was found ($P<0.05$). Progesterone concentration was higher at days 11 and 15

[1] Chorulon, Intervet Schering-Plough Animal Health, Inc.

**Table 1.** Pregnancy rate (PR) at day 30 post-insemination and Odds ratio for hCG treatment, number of prior services and change in body condition score.

| Variables | Class | n | PR | Odds ratio | IC 95% | *P*-value |
|---|---|---|---|---|---|---|
| Treatment | Control | 507 | 37.4 | Reference | | |
| | hCG | 482 | 47.5 | 1.3 | 1.04-1.79 | 0.02 |
| Prior Services | First | 357 | 48.4 | Reference | | |
| | Second | 304 | 40.7 | 0.7 | 0.50-0.98 | 0.04 |
| | Third | 194 | 39.1 | 0.6 | 0.44-0.94 | 0.02 |
| | Fourth | 134 | 34.3 | 0.5 | 0.31-0.77 | 0.001 |
| Change in Body Condition | Loses | 583 | 28.1 | Reference | | |
| | Maintains | 112 | 60.7 | 3.7 | 2.43-5.70 | <0.001 |
| | Gains | 294 | 63.6 | 4.5 | 3.30-6.20 | <0.001 |

in cows treated with hCG, when compared to control cows ($P<0.05$). Eighty three percent (191/229) of cows treated with hCG and diagnosed as pregnant by ultrasound on day 30, had two *corpora lutea*, compared to 9.4% (18/190) of the control cows ($P<0.05$). Therefore, the rise in pregnancy rate achieved in the present study could be explained by an increased plasma progesterone concentration. Lactating dairy cows have subnormal progesterone concentration, as a result of higher liver blood flow that increases the rate of progesterone catabolism (Sangsritavong *et al* 2002, Stronge *et al* 2005). Reduced progesterone concentration in the blood has been associated with delayed embryonic development (Mann and Lamming 2001, Stronge *et al* 2005). Mann *et al* (2006), noted that augmented progesterone concentration in blood on days 5 to 9 post-insemination increased trophoblast size and interferon-τ concentrations. It is therefore plausible to suggest that in the present study, the higher progesterone concentration observed in cows treated with hCG could have favor embryonic development, which increased embryonic competence to establish the maternal pregnancy recognition.

In this study, changes in body condition were shown to influence pregnancy rate, and no interaction with treatment was observed. Cows that gained or maintained body condition score in the 30 days following insemination had an increased probability of gestation when compared to cows that lost body condition (table 1). The relationship between loss of body condition and pregnancy rate detected in this investigation is in agreement with other effects related to changes in body condition. In this way, the loss in body condition score postpartum seems to be linked to an increase in the interval from parturition to first ovulation (Butler 2000) and to an increase in days open (Patton *et al* 2007). On the other hand, loss of body condition score after 50 days postpartum could be associated to diseases, such as lameness or mastitis that can affect early embryonic development (Chebel *et al* 2004).

The probability of gestation was affected by the number of prior services; pregnancy rate was higher in cows at first service and decreased with the number of services (table 1). This outcome may be due to the unintended selection of subfertile cows with subsequent inseminations (repeat breeders) (Morales-Roura *et al* 2001). This coincides with observations by Roque *et al* (2016) who also found that pregnancy rate was higher in first service cows than in cows serviced after 150 days in milk. There was no interaction between hCG treatment and the number of prior services, nor between treatment and the changes in body condition ($P>0.1$). Parity, milk production and type of artificial insemination procedure did not affect the probability of pregnancy ($P>0.1$).

Contrary to expectations, pregnancy rate was similar between primiparous and multiparous cows (table 1). In several studies, primiparous cows have been found to be more fertile than multiparous cows (Nascimento *et al* 2013, Orozco *et al* 2016). No interaction between treatment and parity was found. Pregnancy rate on day 60, was higher in cows treated with hCG (41%; 198/482) than in control

**Table 2.** Percentage of cows that lost their pregnancy between days 30 and 60 post-insemination and Odds ratio according to the number of prior services.

| Number of service | n | Pregnancy losses (%) | Odds ratio | IC 95% | *P*-value |
|---|---|---|---|---|---|
| First | 33/173 | 19.0 | 5.18 | 1.9-22.4 | 0.028 |
| Second | 13/124 | 10.4 | 2.57 | 0.55-11.9 | 0.22 |
| Third | 13/76 | 17.1 | 4.54 | 0.97-21.1 | 0.054 |
| Fourth | 2/46 | 4.3 | Reference | | |

cows (31%; 160/507); hCG did not reduce pregnancy losses between days 30 and 60 post-insemination (table 2). In cows at first service, a higher proportion of pregnancy losses was noted in comparison to cows with 4 or more services. It is difficult to discuss causes of this outcome with the data obtained in the present study.

We conclude that hCG injection five days after insemination increases progesterone concentration and pregnancy rate in dairy cows.

## ACKNOWLEDGEMENTS

This study was financed in part by project IN219811-3 and PASPA-DGAPA of the National Autonomous University of Mexico. The authors would like to thank the owner of Empresa Lechera Beta San Gabriel S.A. de C.V., as well as MSD Salud Animal, México.

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