Journal of Mahanakorn Veterinary Medicine
Vol. 3 No.1 January - June 2008

งานวิจัย

PROGESTERONE PROFILES AND CONCEPTION OF DAIRY CATTLE ADMINISTERED WITH GONADOTROPIN RELEASING HORMONE IN LUTEAL PHASE POST INSEMINATIONS

Nussara Vadhanakul[1, #], Arun Chankrachang[1], Wanvipa Suthikrai[2] and Kriwon Hongyuntarachai[1]

Abstract

Administration of gonadotropin releasing hormone (GnRH) in luteal phase post insemination was studied in fertile crossbred dairy cattle for the purpose of assessing the effect of gonadotropin releasing hormone on endogenous concentration of progesterone and the potential impact on conception. Ninety-two crossbred dairy cattle were randomly divided into two groups (control and treatment groups) and inseminated when they were in estrus. Only the treatment group was administered with 10 mg of GnRH on day 12 post insemination. Serum of both groups were collected and analyzed for progesterone concentration by means of radioimmunoassay on day 0, 12, 18 and 25 post inseminations. All hormonal data and conception rate of both groups were statistically analyzed. The results showed that GnRH administered 12 days post insemination significantly increased serum progesterone on D18 post insemination ($P<0.05$). However, the conception rates obtained were not significantly different between control and treatment groups.

Keywords : Gonadotropin releasing hormone, Luteal phase, Post insemination, Progesterone, Conception, Dairy cattle

[1]Bureau of Biotechnology for Animal Production, Department of Livestock Development
[2]Faculty of Veterinary Science, Chulalongkorn University
[#]corresponding author / E-mail address: vnussara@gmail.com

Introduction

Early embryonic mortality is one of a major economic loss to dairy producers. Approximately 25% of bovine embryos are lost in the first 3 weeks of life (Peters, 1996) of which the greatest proportion seems to occur between 14 and 17 days after ovulation (Sreenan *et al*., 2000). A major contributing factor of early embryonic death in cattle is believed to be due to low progesterone production by the corpus luteum. Embryo development is related to concentrations of progesterone and the ability of the conceptus to secrete the antiluteolytic hormone, interferon-t (Mann *et al*., 1999). More exposure to progesterone by the embryo may increase its chance of secreting interferon-t and thus survive (Thatcher *et al*., 2001). By manipulating post-insemination concentrations of progesterone, conception rates may ultimately improve.

One avenue being pursued to increase progesterone concentration after insemination is the application of luteotropic hormones such as GnRH at various times after insemination in order to provide additional luteotropic stimuli to the corpus luteum or to interfere indirectly with luteolytic mechanisms. The mechanism involved derives from the physiological effect of GnRH in stimulating the release of LH and FSH from the anterior pituitary gland which results in inducing ovulation of dominant follicle at mid cycle, thereby possibly reducing estradiol secretion of ovarian follicles (Thatcher *et al*., 1989; Rettmer *et al*., 1992) as well as obtaining accessory corpus luteum formation. Lack of estradiol late in the estrous cycle probably prevents the changes in uterine receptor concentrations of oxytocin that are prerequisitefor luteolysis (Hughes *et al*., 1987) which increases the chances for embryo to establish maternal recognition of pregnancy before a new wave of estrogen – secreting follicles develops and initiates luteolysis.

The stage at which GnRH is given in luteal phase has been varied between days 1 and 3, 4 and 6, 7 and 10, 11 and 13 after insemination with variable results. The remarkable improvement in pregnancy rate was found mostly in the treatment between days 11 and 13 after insemination (Macmillan *et al*., 1986; Sheldon and Dobson, 1993; Drew and Peters, 1994; Peters *et al*., 2000). This might be due to the reason that it coincides approximately with maternal recognition of pregnancy as characterized by the embryonic secretion of the antiluteolytic factor interferon-t (Thatcher *et al*., 1996). However, the action of GnRH also depends on follicular status at the time of treatment. Cows with 3- wave cycles, the GnRH treatment between days 11 and 14 coincides with the rise or peak of the second follicular wave (Webb *et al*., 1992) and therefore high estradiol concentrations whereas estradiol concentrations are likely to be lower in two-wave animals at this time. This could be one possible reason for differences in study results.

In Thailand, most studies were performed in limited number of dairy cattle of different status by using different days of GnRH administration and variable results were obtained (Pilachai *et al*., 2004; Sumransap *et al*., 2006). In this study, higher number of fertile dairy cattle raised under field condition of small-scale farms in Thailand were studied in order to obtain more accurate results in assessing the effectiveness of gonadotropin releasing hormone (GnRH) administrating during luteal phase

on endogenous concentration of progesterone and the potential impact on conception of fertile dairy cattle in the field condition of Thailand in order to understand progesterone profiles in different stages after GnRH treatment in fertile dairy cattle which will be very much useful for further application in improving reproductive performance of dairy cattle in Thailand.

Materials & Methods

Ninety-two fertile crossbred dairy cattle (>87.5% Holstein-Fresian crossbred), 3-6 years of age, good body condition score with regular estrous cycle and normal reproductive organs from rectal examination, were randomly selected for the study. They were all raised under similar condition of small scale farms in Saraburi Province with fair nutrition and management. They were then randomly allocated in two groups (control and treatment group). Both groups were inseminated when they were in estrus. Ten μg of gonadotropin releasing hormone (Buserelin, Recepta ®; Intervet Company) was intramuscularly injected on day 12 post insemination only in treatment group. Blood samples were collected via coccygeal venipuncture in both groups on day 0 (day of estrus), day 12, day 18 and day 25 post insemination. Serum was separated and stored at -20°C until assayed for progesterone. Concentrations of progesterone in serum were determined by radioimmunoassay (RIA). Sensitivity of the assay was 0.05 ng/ml Percentages of coefficient of variation (%CV) of intra-assay from three levels of control pools were 5.5, 6.7 and 10.9% whereas those of inter-assay were 16.0, 10.2 and 15.5 %. Pregnancy was verified by rectal palpation of uterus on day 60 after the insemination. All hormonal data were analyzed by means of general linear model whereas conception rates were compared by chi-square analysis and the categorical data procedures in order to determine the significant treatment effect. Ages of experimented animals in both groups were also adjusted using GLM procedures in order to reduce the error effect from different ages. The accuracy of pregnancy and non-pregnancy diagnosis by means of progesterone determination on day 25 post insemination were also studied by comparing with the results obtained from uterine palpation on day 60.

Results

Comparison of serum progesterone concentration on each day between control and treatment group showed no significant difference ($P>0.05$) whereas progesterone concentration on day 18 of treatment group was significantly higher than that of day 12 ($P<0.05$).

Discussion

From Table 1, it was found that progesterone level in each day were not significantly different between control and treatment groups ($P>0.05$). But progesterone level on day 18 of the treatment group seemed to be higher than that of control group which might be due to the effect of GnRH treatment that cause ovulation of dominant follicle of the second follicular wave and later formation of accessory corpus luteum. Thus progesterone level was higher on day 18 in treatment group. This was concurrent to the comparison of progesterone level between day 12 and day 18 in each group which showed that progesterone level on day 18 of treatment group was significantly higher than that of day 12 ($P<0.05$) whereas the progesterone level on day 12 and day

18 of the control group were not different significantly (P>0.05). This might indicate that in the treatment group of which GnRH was given enabled the increase of progesterone level in the luteal phase. This is concurrent to the study of Pilachai *et al*. (2004), Howard *et al*. (2006) and Peters (2005) which found higher progesterone concentration and accessory corpus luteum in cows after GnRH administration.

When conception of the animals was considered, regardless of treatment method (Table2), it was found that progesterone level on day 12 of conceived and non-conceived groups were not significantly different because they were all in luteal phase whereas progesterone level on day 18 of the conceived group was significantly higher than that of non-conceived group (P<0.0001). This indicated that increasing progesterone level in luteal phase post insemination might increase conception. The results were concurrent to the hypothesis that increasing progesterone concentration after artificial insemination would increase fertility (Stevenson *et al*., 2007) due to increase the chance of embryo development and its ability to secrete antiluteolytic hormone (Mann *et al*., 1999; Thatcher *et al*., 2001). Thatcher *et al*. (1994) also reported that manipulation of post-insemination concentration of progesterone by giving exogenous progesterone could stimulate embryo development and ultimately improved conception rates.

The conception rate obtained from GnRH administration post inseminations in this study (Table 3) was not significantly different from that of control group which is consistent with the findings of Howard *et al*. (2006) and Peter *et al*. (1992) who observed no effect on conception rate in dairy cows, despite elevation of progesterone concentration. This contrasts with the study of Willard *et al*. (2003); Peters *et al*. (2000) and Kalaimani *et al*. (2007) who indicated the improvement of pregnancy rates in dairy cattle after GnRH administration in luteal phase post insemination. Lopez-Gatius *et al*. (2002) also reported that cows with an additional corpus luteum were eight times less likely to experience fetal loss than those with a single corpus luteum. Thatcher *et al*. (2006) found the similar results that induction of accessory corpus luteum and increased progesterone concentration reduced early embryonic mortality in cattle.

The conception rate after GnRH treatment post inseminations is varied depending on the injection time after AI and follicular status when treatment is initiated (Peters, 2005). Peter (2005) also reported that only treatment between day 11 and 13 resulted in improvement in pregnancy rate which was concurrent to some studies (Drew and Peters, 1994; Sheldon and Dobson, 1993) but contrasted to some studies (Schmitt *et al*., 1996; Willard *et al*., 2003; Kalaimani *et al*., 2007) which reported that GnRH administration on day 5-7 post insemination also gave good results. Considering follicular status at the treatment time, cows with 2 and 3 wave cycles showed different fertility responses to GnRH treatment post inseminations (Peter, 2005).

However, post insemination GnRH treatments have been reported to be particularly effective in conditions of heat stress, poorer body condition (Kaim *et al*., 2003; Willard et al., 2003; Pilachai *et al*., 2004) and in repeat breeding cows (Sumransup *et al*.,2006). Wolfenson *et al*. (1993) also suggested that progesterone concentration might be less in heat-stressed cows.

The accuracy of determining non-pregnancy was greater (90.91%) than that for determining pregnancy (83.93%) which is consistent with other studies which reported accuracies of 67 to 88% for pregnant and 87 to 100% for non-pregnant diagnoses (Gowan *et al*., 1982; Reimers *et al*., 1985; Wimpy *et al*., 1986). This could indicate the main advantage of the progesterone test in picking out the non-pregnant animals (Dobson and Kamonpatana, 1986).

Table 1 Serum concentration of progesterone on day 0, day12, day18 and day 25 post insemination in control and treatment groups

Day	Progesterone concentration (mean ± SE) ng./ml	
	Control group	Treatment group
0	0.26 ± 0.05	0.18 ± 0.05
12	4.29 ± 0.34	3.51 ± 0.35[a]
18	3.81 ± 0.66	4.78 ± 0.69[b]
25	2.92 ± 0.41	3.09 ± 0.40

[a,b]Figures within the same column with different superscripts differ significantly (P<0.05).

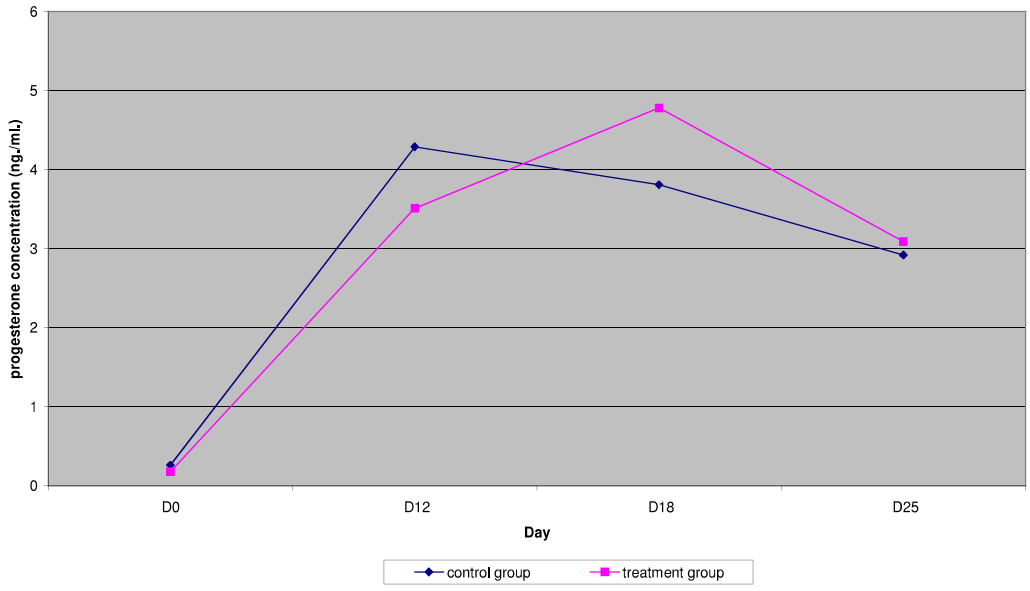

Figure 1 Progesterone profile in various days of control and treatment groups

When conception of the animals was considered, regardless of the treatment methods, serum concentration of progesterone between conceived and non-conceived groups were compared (Table 2).

Table 2 Serum concentrations of progesterone on day 0, day 12, day 18 and day 25 post inseminations in conceived and non-conceived groups

Day	Progesterone concentration (mean ± SE) ng./ml	
	Control group	Treatment group
0	0.18 ± 0.04	0.33 ± 0.06
12	3.81 ± 0.33	4.05 ± 0.38
18	5.31 ± 0.43[a]	1.76 ± 0.67[b]
25	4.42 ± 0.30[a]	1.19 ± 0.34[b]

[a,b]Figures within the same row with different superscripts differ highly significantly (P<0.0001).

It was found that progesterone concentration on day 18 of conceived group was significantly higher than that of non-conceived group (P<0.0001).

Conception rates between control and treatment groups were also compared (Table3). The results showed no significant difference between groups (P>0.05).

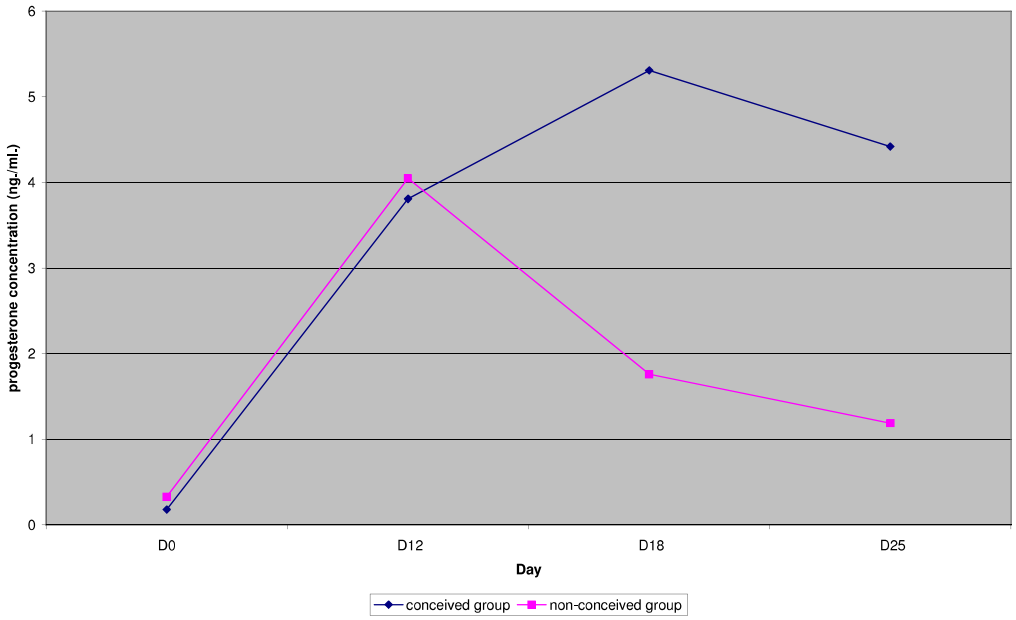

Figure 2 Progesterone profile in various days of conceived and non-conceived grounps

Table 3 Conception rate of control and treatment groups

Group	No. of pregnant cows / no. of inseminated cows	Conception rate (%)
Control	26 / 47	55.32
Treatment	25 / 45	55.56

The accuracy of early pregnancy diagnosis by means of progesterone determination on day 25 post inseminations compared to the results obtained from uterine palpation on day 60 post inseminations was 83.93 % whereas that of non-pregnancy diagnosis was 90.91 %.

Conclusion

This study indicates that the administration of exogenous GnRH in luteal phase post inseminations in fertile dairy cattle resulted in increased serum progesterone concentration. This might be beneficial in reducing early embryonic mortality especially in infertile or heat-stressed or repeat breeding cows which might have less progesterone concentration after insemination. However, the conception rate obtained from this study in treatment group was not significantly higher than that of control group which might be due to the variability of follicular status when treatment is initiated.

Acknowledgement

The authors would like to express their sincere thanks to Ms. Ratree Jintana for her cooperation in the laboratory throughout the study.

References

Dobson, H. and Kamonpatana, M. 1986. A review of female cattle reproduction with special reference to a comparison between buffaloes, cows and zebu. *J. Reprod. Fert*. 77: 1-36.

Drew, S.B. and Peters, A.R. 1994. Effect of buserelin on pregnancy rates in dairy cows. *Vet. Rec*. 134: 267-269.

Gowan, E.W., Etches, R.J., Bryden, C. and King, G.J. 1982. Factors affecting accuracy of pregnancy diagnosis in cattle. *J. Dairy Sci*. 65: 1294-1298.

Howard, J.M., Manzo, R., Dalton, J.C., Frago, F. and Ahmadzadeh, A. 2006. Conception rates and serum progesterone concentration in dairy cattle administered gonadotropin releasing hormone 5 days after artificial insemination. *Anim. Reprod. Sci.* 95: 224-233.

Hughes, T.L., Villa-Godoy, A., Kesner, J.S. and Fogwell, R.L. 1987. Destruction of bovine follicles: Effects on pulsatile release of luteinizing hormone and prostaglandinF2a – induced luteal regression. *Biol. Reprod*. 36: 523-529.

Kaim, M., Bloch, A., Wolfenson, D., Braw-Tal, R., Rosenberg, M., Voet, H. and Folman, Y. 2003. Effects of GnRH administered to cows at the onset of estrus on timing of ovulation, endocrine responses, and conception. *J. Dairy Sci*. 86: 2012-2021.

Kalaimani, S., Devanathan, T.G., Sridevi, P. and Thangavel, A. 2007. HCG and GnRH administration on 7th day post breeding in augmenting fertility in crossbred cows. Tamilnadu *J. Vet. Anim. Sci.* 3: 95-97.

Lopez-Gatius, F., Santolaria, P., Yaniz, J.L., Rutllant, J. and Lopez-Bejar, M. 2002. Factors affecting pregnancy loss from gestation day 38 to 90 in lactating dairy cows from a single herd. *Theriogenology*. 57: 1251-1261.

MacMillan, K.L., Taufa, V.K. and Day, A.M. 1986. Effects of an agonist of GnRH (buserelin) in cattle. III. Pregnancy rates after a post-insemination injection during metoestrus or dioestrus. *Anim. Reprod. Sci*. 11: 1-10.

Mann, G.E., Lamming, G.E., Robinson, R.S. and Wathes, D.C. 1999. The regulation of interferon-tau production and uterine hormone receptors during early pregnancy. *J. Reprod. Fertil. Suppl*. 54: 317-328.

Peters, A.R., Drew, S.B., Mann, G.E., Lamming, G.E. and Beck, N.F. 1992. Experimental and practical approaches to the establishment and maintenance of pregnancy. *J. Physiol. Pharmacol.* 43 (1): 143-152.

Peters, A.R. 1996. Embryo mortality in the cow. Animal Breedings Abstracts. 64: 587-598.

Peters, A.R. 2005. Veterinary clinical application of GnRH-questions of efficacy. *Anim. Reprod. Sci.* 88: 155-167.

Peters, A,R., Martinez, T.A. and Cook, A.J.C. 2000. A meta-analysis of studies of the effect of GnRH 11-14 days after insemination on pregnancy rates in cattle. *Theriogenology.* 54: 1317-1326.

Pilachai, R., Aiumlamai, S., Wongsrikaew, W. and Sirisatien, S. 2004. A study on day 5 post insemination administration of GnRH or hCG on serum progesterone concentrations and conception rates in dairy heifers during hot season. Abstracts of the 30th Veterinary Medicine and Livestock Development Annual Conference. The Thai Veterinary Medical Association Under the Royal Patronage. p. 61-62.

Reimers, T.J., Smith, R.D. and Newman, S.K. 1985. Management factors affecting reproductive performance of dairy cows in the northeastern United States. *J. Dairy Sci.* 68: 963-974.

Rettmer, I., Stevenson, J.S. and Corah, L.R. 1992. Pregnancy rates in beef cattle after administering a GnRH agonist 11 to 14 days after insemination. *J. Anim. Sci*. 70, 7-12.

Schmitt, E.J., Diaz, T., Barros, T.M., de la Sota, R.L., Drost, M., Fredriksson, E.W., Staples, C.R., Thorner, R. and Thatcher, W.W. 1996. Differential response of the luteal phase and fertility in cattle following ovulation of the first-wave follicle with human chorionic gonadotropin or an agonist of gonadotropin-releasing hormone. *J. Anim. Sci*. 74: 1074-1083.

Sheldon, I.M. and Dobson, H. 1993. Effects of GnRH administered 11 days after insemination on the pregnancy rates of cattle to the first and later services. *Vet. Rec*. 133: 160-163.

Sreenan, J.M., Diskin, M.G. and Morris, D.G. 2000. Embryo survival rate in cattle: a major limitation to the achievement of high fertility. *Occup. Publ. Br. Soc. Anim. Sci*. 26: 93-104.

Stevenson, J.S., Portaluppi, M.A., Tenhouse, D.E., Lloyd, A., Eborn, D.R., Kacuba, S. and DeJarnette, J.M. 2007. Interventions after artificial insemination: conception rates, pregnancy survival, and ovarian responses to Gonadotropin Releasing Hormone, Human Chorionic Gonadotropin, and progesterone. *J. Dairy Sci*. 90: 331-340.

Sumransup, P., Ngamkhum, S. and Vadhanakul, N. 2006. GnRH application for improving pregnancy rate in repeat breeding dairy cows. *J. Biotec. Liv. Prod*. 1: 29-39.

Thatcher, W.W., MacMillan, K.L., Hansen, P.J., and Drost, M. 1989. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. *Theriogenology.* 31: 149-164.

Thatcher, W.W., Staples, C.R., Danet-Desnyers, G., Oldick, B. and Schmitt, E.-P. 1994. Embryo health and mortality in sheep and cattle. *J. Anim. Sci*. 72 (3): 16.

Thatcher, W.W., De La Sota, R.L., Schmitt, E.J., Diaz, T., Badinga, L. and Simmen, F.A. 1996. Control and management of ovarian follicles in cattle to optimize fertility. *Reprod. Fertil. Dev.* 8: 203-217.

Thatcher, W.W., Guzeglu, A., Mattos, R., Binelli, M., Hansen, T.R. and Pru, J.K. 2001. Uterine conception interactions and reproductive failure in cattle. *Theriogenology.* 56: 1435-1450.

Thatcher, W.W., Bilby, T.R., Bartolome, J.A., Silvestre, F., Staples, C.R. and Santos, J.E.P. 2006. Strategies for improving fertility in the modern dairy cow. *Theriogenology*. 65: 30-44.

Webb, R., Gong, J.G., Law, A.S. and Rusbridge, S.M. 1992. Control of ovarian function in cattle. *J. Reprod. Fert. Supplement*. 45: 141-156.

Willard, S., Gandy, S., Bowers, S., Graves, K., Elias, A. and Whisnant, C. 2003. The effects of GnRH administration post insemination on serum concentrations of progesterone and pregnancy rates in dairy cattle exposed to mild summer heat stress. *Theriogenology*. 59: 1799-1810.

Wimpy, T.H., Chang, C.F., Estergreen, V.L. and Hillers, J.K. 1986. Milk progesterone enzyme immunoassay: modifications and a field trial for pregnancy detection in dairy cattle. *J. Dairy Sci*. 69: 1115-1121.

Wolfenson, D., Luft, O., Berman, A. and Meidan, R. 1993. Effect of season, incubation temperature and cell age on progesterone and prostaglandin F 2α production in bovine luteal cells. *Anim. Reprod. Sci*. 32: 27-34.

ระดับฮอร์โมนโปรเจสเตอโรนและการผสมติดในโคนมที่ได้รับฮอร์โมนโกนาโดโทรปินรีลิซซิ่งในระยะลูเตียลหลังการผสมเทียม

นุสสรา วัฒนกุล[1#] อรุณ จันทร์กระจ่าง[1] วรรณวิภา สุทธิไกร[2] และไกรวรรณ หงษ์ยันตรชัย[1]

บทคัดย่อ

ศึกษาการให้ฮอร์โมนโกนาโดโทรปินรีลิซซิ่ง ในระยะลูเตียล หลังการผสมเทียมในโคนมที่มีความสมบูรณ์พันธุ์ปกติ เพื่อให้ทราบถึงการเปลี่ยนแปลงของระดับฮอร์โมนโปรเจสเตอโรนในระยะต่างๆ หลังการผสมเทียม และผลต่อการผสมติด โดยศึกษาในโคนมลูกผสมจำนวน 92 ตัวที่มีความสมบูรณ์พันธุ์ปกติ สุ่มตัวอย่างเป็น 2 กลุ่ม คือ กลุ่มควบคุม และกลุ่มทดลอง โคทั้งหมดได้รับการผสมเทียม เมื่อแสดงอาการเป็นสัด ในขณะที่โคกลุ่มทดลองได้รับการฉีดฮอร์โมนโกนาโดโทรปินรีลิซซิ่ง จำนวน 10 ไมโครกรัม เข้ากล้ามเนื้อ ในวันที่ 12 หลังการผสมเทียม เก็บซีรัมจากโคทั้ง 2 กลุ่ม ในวันที่ 0, 12, 18 และ 25 หลังผสมเทียม เพื่อตรวจวิเคราะห์หาระดับของฮอร์โมนโปรเจสเตอโรนในซีรัมโดยเทคนิคเรดิโออิมมิวโนเอสเสย์ ตรวจการตั้งท้องโดยการล้วงคลำผ่านทางทวารหนักในวันที่ 60 หลังการผสมเทียม เปรียบเทียบระดับของฮอร์โมนโปรเจสเตอโรนในวันต่างๆ และอัตราการผสมติด ระหว่างกลุ่มควบคุม และกลุ่มทดลอง โดยวิธีการทางสถิติ ผลการศึกษาพบว่า การให้ฮอร์โมนโกนาโดโทรปินรีลิซซิ่ง ในวันที่ 12 หลังการผสมเทียม จะเพิ่มระดับของฮอร์โมนโปรเจสเตอโรนในวันที่ 18 หลังการผสมเทียมอย่างมีนัยสำคัญ ($P<0.05$) ในขณะที่อัตราการผสมติดระหว่างกลุ่มควบคุม และกลุ่มทดลอง ไม่แตกต่างกันอย่างมีนัยสำคัญ

คำสำคัญ : ฮอร์โมนโกนาโดโทรปินรีลิซซิ่ง, ระยะลูเตียล, หลังการผสมเทียม, โปรเจสเตอโรน, การผสมติด, โคนม

[1]สำนักเทคโนโลยีชีวภาพการผลิตปศุสัตว์ กรมปศุสัตว์
[2]คณะสัตวแพทย์ศาสตร์ จุฬาลงกรณมหาวิทยาลัย
[#]ผู้เขียนผู้รับผิดชอบ/ อีเมล์: vnussara@gmail.com