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EFFECT OF GONADOTROPIN RELASING HORMONE (GnRH) AND HUMAN CHORONIC GONADOTROPIN (hCG) SUMMER ADMINISTRATION ON PREGNANCY RATE AND SERUM PROGESTERONE IN DAIRY CATTLE

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ABSTRACT: *This study was conducted to determine the effect of treatment of dairy cows with GnRH just after insemination or with hCG on day 7 post-insemination during summer months on pregnancy rates and serum progesterone concentration. Twenty eight postpartum Holstein cows aged 3-7 years, were divided randomly into three groups. Group 1 included 10 cows injected with 2.5 ml of sterile saline just after insemination (control group). Group 2 involved 9 cows injected with 100ug of gonadotropin-relasing hormone (GnRH), just after insemination. Group 3 included 9 cows injected with 2500 IU of human chorionic gonadotropin (hCG), 7 days after insemination. Blood samples were collected from each cow on days: 0, 7, 14, 21, 28, 35 and 42 post-insemination day (day 0). Serum was obtained and stored at -20 °C until progesterone (P4) determination. Pregnancy was diagnosed by palpation approximately 50 days post-insemination.*

Results indicated that treatment cows with hCG on day 7 post-insemination improved the pregnancy rate by 26.7% over that in the control group. While, treatment cows with GnRH just after insemination decreased the pregnancy rate by -6.7% than that in the control untreated cows (from 40% to 33.3%). The differences in pregnancy rate among the experimental groups were insignificant .

Serum P4 concentration was insignificantly affected by treatment cows with GnRH or with hCG as compared to that untreated cows. In general, progesterone concentration tended to be higher in pregnant cows during the various periods after insemination than that found for nonpregnant cows. Serum P4 of the pregnant cows proved significant ($p<0.01$) progressive increase from day 0 until day 42 post-insemination. The lowest concentration (0.64 ±0.91 ng/ml) was recorded on day 0, and markedly ($p<0.01$) increased to 5.45±0.66 ng/ml on day 14 and 7.69±0.86 ng/ml on day 21 after insemination. Thereafter, it insignificantly increased until day 42. While, in nonpregnant cows, serum P4 level significantly increased ($p<0.01$) from 0.68±0.97 ng/ml on day 0 to 5.15±0.72 ng/ml on day 21 after insemination. Thereafter, it slightly decreased to be 3.06±0.47 ng/ml on day

42 after insemination. In conclusion, treatment cows in summer months with hCG on day 7 after insemination resulted in substantial improvement in pregnancy rate, while treatment cows with GnRH just after insemination slightly decreased it, without any significant affect on progesterone concentration.

Key words: ***Pregnancy rate, Progesterone, Dairy cattle.***

INTRODUCTION

Low conception rates are commonly observed in dairy cattle herds in summer months (Archbald *et al.* 1992). It resulted in significant productive and reproductive losses. High temperature and relative humidity during summer alter the hormonal and biochemical status of the animal. In this respect, Wolfenson *et al.* (2002); Jonsson *et al.* (1997) and Howell *et al.* (1994) reported that concentrations of progesterone decreased in heat-stressed cows as compared to that in either spring, winter, or with those cooled in summer by different methods.These finding suggested that the effects of heat stress during summer were resulted from an impaired follicle to an impaired CL. This insufficient progesterone secreted by CL during summer is a possible cause of early embryonic mortality and low fertility of cows (Hansen and Arechhiga. 1999). Previous researches have suggested that supplementation with exogenous sources of progesterone tended to increase progesterone secretion through promotion of accessory CL formation and seems to prolong life span and/or partially protect Cl against spontaneous luteolysis, which would improve pregnancy rate in cattle (Robinson *et al.* 1989; Breuel *et al.* 1990; Sianangama and Rajamahendran, 1992 and Schmitt *et al.* 1996). Some attempts have been made to minimize these adverse effects of heat stress and consequently improve pregnancy rate through the use of progesterone therapy.

Treatment with GnRH at the time of insemination of cows exhibiting estrus may elicit an early relase LH capable of inducing ovulation (Lucy and Stevenson, 1986; Stevenson *et al.* 1988) and significantly increased the pregnancy rate in dairy cows (Nakao *et al.* 1983). Ullah *et al.* (1996) reported that administration of GnRH to dairy cows at estrus improved pregnancy rates and increased serum progesterone. Opposite results were obtained with administration of hCG in cows (Schmitt *et al.*1996 and Santos *et al.* 2000) .

Treatment of cows (Helmer and Britt 1986 and Rajamahendran and Sianangama 1992) and sheep (Farin *et al.* 1988) with hCG was associated with an increase in number of large luteal cells and a reduction in number of small luteal cells. Consequently, increased weight of CL and endogenous synthesis of progesterone and improved the pregnancy rates (Breuel *et al.* 1989). In this concern, Rajamahendran and Sianangama (1992) and Sianangama and Rajamahendran, 1992)reported that administration hCG on day 7 or day 14 post-insemination resulted in increasing progesterone and improved pregnancy rates with advance to the treatment on day 7.

Literatures about these effects under heat stress of summer months are not available. So, our study was carried out to determine if the administration of dairy cows with GnRH just after insemination or with hCG on day 7 post-insemination during summer months would affect the pregnancy rates and serum progesterone after insemination.

MTERIALS AND METHODS

The present study was carried out at the Dairy Facility Center, The Ohio State University, Columbus, Ohio, USA during summer (June to September). In this regard, 28 postpartum Holstein cows with no histories of reproductive disorders and free from internal and external parasites, aged 3-7 years and housed in free-stall pens . Cows were fed a total mixed diet consisting of alfalfa hay, corn silage, soybean meal, whole cotton seed, megalac and cargill to meet NRC (1987) recommendation and were milked twice daily.

Cows were observed for detection of estrus twice daily as normally occurs at the facility. As cow was observed in standing estrus she was inseminated after about 12 hours and allotted to one of the three subsequent treatment groups:

Group 1: Ten cows received a single intravenously injection of 2.5 ml of sterile saline just after insemination to assure the effect of the injection does not affect on the ovulation or development of the corpus luteum function (control group).

Group 2: Nine cows received a single intravenously injection of 100 ug of gonadotropin-relasing hormone (GnRh), just after insemination (GnRH group).

Group 3: Nine cows received a single intravenously injection of 2500 IU of human chorionic gonadotropin(hCG), 7 days after insemination (hCG group).

Blood samples were collected via jugular vein puncture from each cow on days: 0, 7, 14, 21, 28, 35 and 42 post-insemination. Blood samples were stored at 4 °C for 24 hrs to allow optimal coagulation and centrifuged at 3000 rpm for 15 minutes. Serum was obtained and stored at -20°C until progesterone determination. Serum samples were analyzed for progesterone using a double antibody radioimmunoassay (Anderson and Day, 1994). Pregnancy was diagnosed by palpation of the uterus per rectum, approximately 50 days post-insemination..

Data were statistically analysed by two way analysis of variance using the General Linear Models procedure of SPSS (1997). Pregnancy rates were compared using Chi-square analysis.

RESULTS AND DISCUSSION

Effect of GnRH and hCG administration on:

1- Pregnancy rate:

Data in Table 1 illustrated that cows treated with hCG on day 7 post-insemination expressed the highest pregnancy rate (66.6%). Thus it improved the pregnancy rate by 26.6% over that in the control group (40%). An opposite trend was recorded for cows treated with GnRH just after insemination, which decreased the pregnancy rate by -6.7% than that in the control cows. The differences in pregnancy rate were insignificant (Chi2= 1.93)

Table (1): Effect of administration of GnRH and hCG on the pregnancy rate in dairy cows.

	Groups		
	Control	GnRH	hCG
No.of cows	10	9	9
No. Of pregnant	4	3	6
Pregnancy rate%	40%	33.3%	66.6%
Difference		-6.7%	+26.6%

The increase in the pregnancy rate of treated cows with hCG was in quite agreement with the findings of McDermott *et al.* (1986); Breuel *et al.* (1989) and Sianangama and Rajamahendran (1992) and disagrees with the results of looney *et al.* (1984) and Breuel *et al.* (1990), who reported that there was no improvement obtained in pregnancy rate, when cows were treated with hCG. Similar to the negative effect of GnRH treatment on pregnancy rate (Table 1), Lewis *et al.* (1990) and Chenault, (1990) recorded a lower percentage by -2 to -7% than that observed for the untreated cows. Nakao *et al.* (1983) recorded a significant increase in pregnancy rate (from 3 to 7%) in response to administration of GnRH to cows characterized by low level of fertility. The same trend was also noted by Willard *et al.* (2003), when the pregnancy rate was improved from 19% for the untreated cows to 35% for the GnRH treated cows. These findings suggested positive effectiveness of GnRH treatment with the cows of low fertility.

The discrepancy in pregnancy rates in response to treatment of cows with either hCG or GnRH could be due to differences in dose, type of hormone, potency, timing (before or/and after insemination) and/or season of the treatment. Lucy and Stevenson (1986) reported that the use of GnRH at or near the time of AI elicits an LH release capable of inducing ovulation, meanwhile, Mee *et al.* (1990) listed that administration of GnRH to dairy cows failed to improve pregnancy rates regardless time of GnRH administration (either being before or after AI). According to interaction of season with treatment effect, BonDurant *et al.* (1991) and Archbald et al. (1992) postulated that pregnancy rates in cows treated with GnRH during spring appeared to be significantly higher compared to those treated during summer months. This finding suggested seasonal effect on ovarian activity in cattle (McNatty *et al.* 1984 and Savio *et al.* 1990), especially the development of the preovulatory follicle and the subsequent CL or due to seasonal differences in LH secretion. This may explain the negative effect of GnRH treatment following insemination on pregnancy rate obtained during summer months in the present study.

2- Progesterone concentration (ng/ml) in blood serum:

Data listed in Table 2 and Fig. 1 indicated that the overall average of serum progesterone of the pregnant animals proved significant ($p<0.01$) progressive increase from the day of insemination (day 0) until day 42 post-insemination. In other words, the lowest concentration (0.64 ±0.91 ng/ml) was recorded on day 0, then it insignificantly increased to 2.12± 0.35 ng/ml on day 7 after insemination and markedly ($p<0.01$) increased to 5.45±0.66 ng/ml on day 14 and 7.69±0.86 ng/ml on day 21 after insemination. Thereafter, the progesterone concentration insignificantly increased being 8.04±0.82, 9.61±0.89 and 8.17±0.59 ng/ml on days 28, 35 and 42, respectively.

Table 2: Changes in progesterone concentration (ng/ml) in blood serum of pregnant cows after insemination as affected by GnRH and hCG treatments.

Days after insemination	Pregnant			
	Control (G1) n=4	GnRH (G2) n=3	hCG (G3) n=6	Overall average ±S.E
0	$0.44^{c}\pm0.17$	$0.75^{c}\pm0.22$	$0.72^{c}\pm0.12$	$0.64^{c}\pm0.91$
7	$1.92^{bc}\pm0.16$	$2.28^{bc}\pm1.19$	$2.17^{c}\pm0.58$	$2.12^{c}\pm0.35$
14	$5.52^{ab}\pm1.34$	$4.98^{ab}\pm0.84$	$5.65^{b}\pm1.16$	$5.45^{b}\pm0.66$
21	$8.59^{a}\pm1.85$	$6.05^{ab}\pm1.74$	$7.91^{ab}\pm1.20$	$7.69^{a}\pm0.86$
28	$8.41^{a}\pm1.84$	$7.47^{a}\pm1.05$	$8.08^{ab}\pm1.35$	$8.04^{a}\pm0.82$
35	$9.55^{a}\pm2.43$	$8.47^{a}\pm1.59$	$10.22^{a}\pm1.04$	$9.61^{a}\pm0.89$
42	$7.16^{a}\pm0.97$	$7.59^{a}\pm1.24$	$9.13^{a}\pm0.89$	$8.17^{a}\pm0.59$

Means in columns with different letters are significantly differed ($p<0.01$).

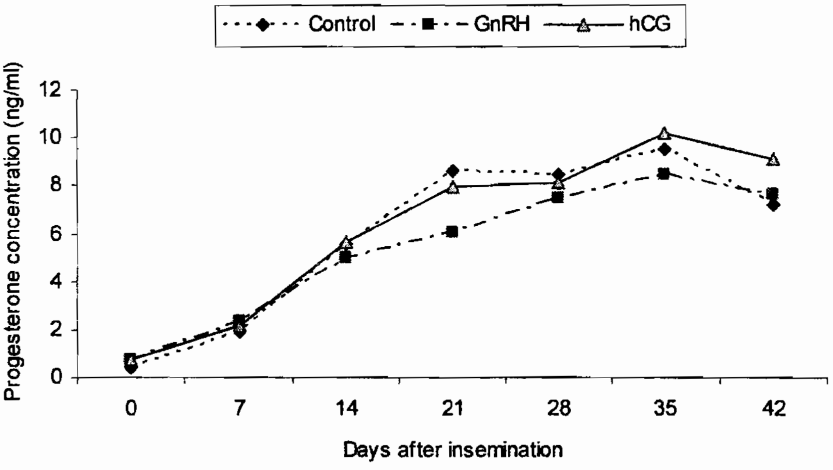

Fig 1: Changes in progesterone concentration (ng/ml) in blood serum of pregnant cows after insemination as affected by GnRH and hCG treatments.

The tabulated data further illustrated that the average of serum progesterone concentration was not significant affected by the treatment with either GnRH or hCG as compared to that in the untreated cows at any of the experimental period. These results are in agreement with that obtained by Hansen and Arechhiga (1999) . The increase in progesterone production could be attributed from hypertrophy of luteal cells in the spontaneous CL and from accessory CL formation following ovulation (Helmer *et al.* 1986). However, the low progesterone concentration in the treated cows with GnRH just after insemination as observed in the present study from day 14 until day 42, could be due to interference of the treatment with normal ovulation or CL formation, as well as the changes in the size and/or number of small and large luteal cells (Echternkamp and Maurer, 1983 and Farin *et al.* 1988). It also may be resulted from a reduction in LH receptors (Mee *et al.* 1990).

Pattern of changes in concentration of serum progesterone in the nonpregnant cows remarkably differed than that in the pregnant cows (Tables 2,3 and Figures 1,2). It clearly appears that the overall average of serum progesterone concentration significantly increased ($p<0.01$) from 0.68±0.97 ng/ml on day 0 to 5.15±0.72 ng/ml on day 21 after insemination (Table 3). Thereafter, it slightly decreased to be 3.06±0.47 ng/m on day 42 after insemination. No significant differences were detected in response to the hormone treatments. On the meantime, the interaction between the post-insemination period and the hormone treatment was not significant. However, the progesterone concentration was slightly higher in the GnRH treated group compared to the other groups starting from day 21 after insemination (Table 3 and Fig. 2). It is worthy mentioning that none of the nonpregnant cows exhibited symptoms of estrus behavior either on day 21 or later, regardless the hormone treatments. On the other hand the progesterone concentration during this period was significantly higher ($p<0.01$) than that detected on day 0 (Table 3). This may suggest ovarian disorders, and/or late embryonic mortality after day 21, since the progesterone concentration on day 21 was 6.05 and 6.01 ng/ml in the GnRH and hCG treated groups. Multiple ovulation in the next expected cycle may be expected and explaining the higher progesterone level after day 21 in comparing with that on day zero.

Table 3: Changes in progesterone concentration (ng/ml) in blood serum of nonpregnant cows after insemination as affected by GnRH and hCG treatments.

Days After insemination	Nonpregnant			
	Control (G_1) n = 6	GnRH (G_2) n = 6	hCG (G_3) n = 3	Overall average±SE
0	$0.89^{b}\pm0.15$	$0.55^{c}\pm0.15$	$0.51^{a}\pm0.18$	$0.68^{d}\pm0.97$
7	$2.45^{ab}\pm0.58$	$1.57^{cd}\pm0.31$	$1.18^{a}\pm0.43$	$1.84^{cd}\pm0.29$
14	$4.50^{a}\pm0.46$	$3.71^{abc}\pm0.56$	$6.57^{a}\pm3.77$	$4.59^{ab}\pm0.75$
21	$3.81^{a}\pm1.22$	$6.05^{abc}\pm0.91$	$6.01^{a}\pm1.89$	$5.15^{a}\pm0.72$
28	$3.67^{a}\pm0.93$	$3.88^{ab}\pm1.08$	$2.65^{a}\pm2.34$	$3.55^{abc}\pm0.68$
35	$2.81^{ab}\pm0.77$	$4.33^{a}\pm1.29$	$1.190^{a}\pm0.87$	$3.09^{bc}\pm0.66$
42	$2.99^{ab}\pm0.77$	$3.23^{b}\pm0.56$	$2.89^{a}\pm1.74$	$3.06^{bcd}\pm0.47$

Means in columns with different letters are significantly differed (p<0.01).

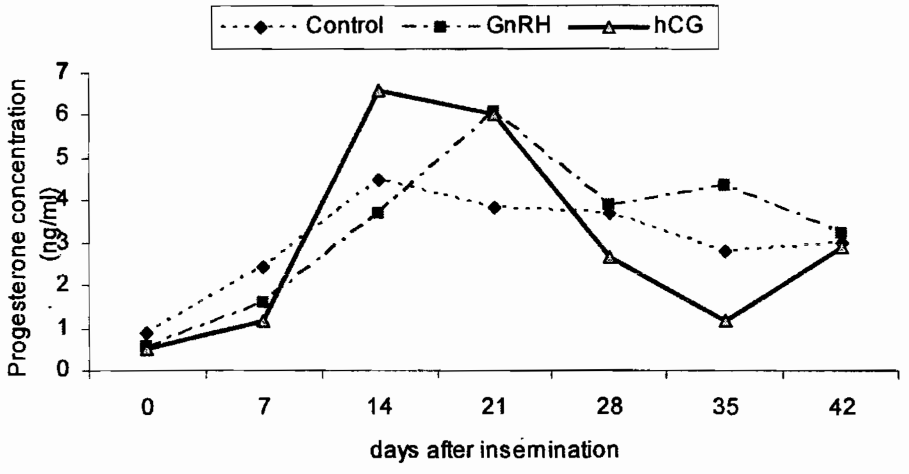

Fig 2: Changes in progesterone concentration (ng/ml) in blood serum of nonpregnant cows after insemination as affected by GnRH and hCG treatments

As shown in Tables 2 , 3 and Fig. 3 it clearly appears that, the incidence of pregnancy in the experimental animals was associated with higher progesterone concentration in the pregnant cows than that recorded for nonpregnant cows. In this concept, the difference in the overall average of serum progesterone between the pregnant and nonpregnant animals was evident from samples of day 7 after breeding until day 42 (Fig. 3). These results are similar to that obtained by Lewis *et al.* (1990) and Willard *et al.* (2003) in dairy cattle and El-Moghazy *et al.* (2006) and El-Sobayil *et al.* (2007) in buffaloes. In conclusion, treatment cows in summer months with hCG on day 7 after insemination resulted in substantial improvement in pregnancy rate, while treatment cows with GnRH just after insemination slightly decreased it, without any significant affect on progesterone concentration.

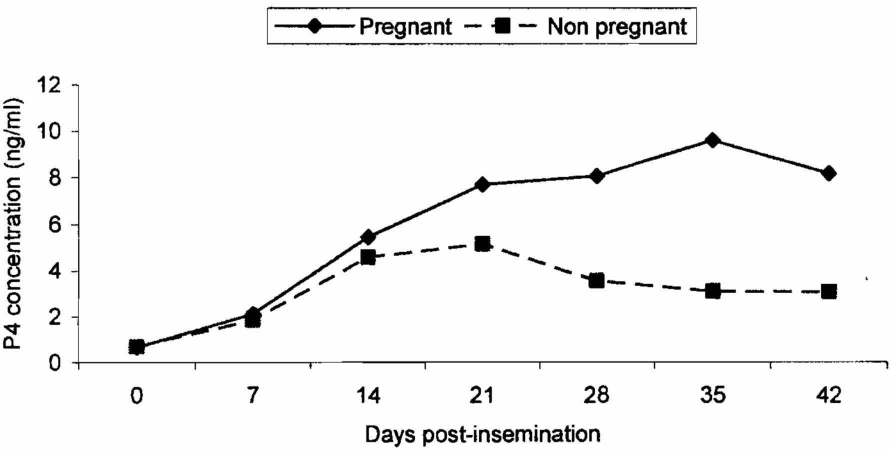

Fig 3: Changes in the overall average of progesterone concentration (ng/ml) in blood serum of pregnant and nonpregnant cows.

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تأثير معاملة الأبقار بـــهرمون hCG أو هرمون GnRH صيفا على معدل الحمل وتركيز البروجسترون فى الأبقار

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الملخص العربى

أجريت هذه التجربة لدراسة تأثير معاملة أبقار اللبن بهرمون GnRH بعد التلقيح مباشرة أو بهرمون hCG بعد التلقيح بسبعة أيام علـــى معـــدل الحمـــل وتركيـــز هرمـــون البروجسترون فى سيرم الدم . تم إستخدام ٢٨ بقرة هولستين يتـــراوح أعمارهـــا مـــن ٣-٧ أعوام قسمت عشوائيا إلى ثلاث مجاميع : اشتملت المجموعة الأولى على عـــدد ١٠ بقـــرات حقنت بعد التلقيح مباشرة بـــ ٢,٥ مل محلول فسيولوجى ، واشتملت المجموعة الثانية علـــى عدد ٩ بقرات حقنت بـــ ١٠٠ ميكروجرام GnRH بعد التلقـــيح مباشـــرة ، كمـــا اشـــتملت المجموعة الثالثة على عدد ٩ بقرات تم حقنها فى اليوم السابع بعد التلقيح بـــ ٢٥٠٠ وحـــدة دولية من هرمون hCG . تم جمع عينات الدم من كل بقرة فى الأيام : صـــفر , ٧ ، ١٤ ، ٢١ ، ٢٨ ، ٣٥ ، ٤٢ بعد التلقيح ، وتم الحصول على السيرم وحفظه تحت -٢٠°م لتقـــدير البروجسترون . كما تم تشخيص الحمل بطريقة الجس بعد ٥٠ يوما من التلقيح تقريبا.

وقد أوضحت النتائج أن معاملة الأبقار بهرمون hCG فى اليوم السابع بعد التلقـــيح أدت إلى تحسين معدل الحمل فى الأبقار (٦٦,٦%) بمعدل يزيد عن الأبقار الغير معاملة بـــ ٢٦,٧% , بينما معاملة الأبقار بهرمون GnRH بعد التلقيح مباشرة أدى الى انخفاض معدل الحمل عن الأبقار الغير معاملة بمعدل -٦,٧% (من ٤٠% إلى ٣٣,٣%) . وكانت الاختلافات الإحصائية في معدلات الحمل بين المجاميع الثلاثة غير معنوية .

كما أوضحت النتائج أن معاملة الأبقار بهرمون GnRH أو بهرمون hCG لم تؤثر بدرجة معنوية على تركيز هرمون البروجسترون مقارنة بالأبقار الغير معاملة . أرتفع مستوى

تركيز البروجسترون فى سيرم الأبقار الحامل فى الفترات المختلفة بصفة عامة عن مستواه فى الأبقار الغير حامل ، وكان ارتفاع مستوى هرمون البروجسترون فى الحيوانات الحامل ارتفاعامعنويا (١%) من يوم التلقيح وحتى اليوم ٤٢ بعد التلقيح . حيث ارتفع مستوى الهرمون من ٠,٦٤ ± ٠,٩١ نانو جرام/مل فى يوم التلقيح وبدرجة معنوية (١%) الى ٥,٤٥ ± ٠,٦٦ نانو جرام/مل فى اليوم ١٤ والى ٧,٦٩ ± ٠,٨٦ نانو جرام/مل فى اليوم ٢١ بعد التلقيح وكانت الزيادة بعد ذلك بدرجة غير معنوية . أما بالنسبة للحيوانات الغير حامل فقد ارتفع مستوى هرمون البروجسترون بدرجة معنوية ٠,٠١% من ٦٨,٠± ٠,٩٧ نانو جرام/مل) يوم التلقيح الى ٥,١٥ ± ٠,٧٢ نانو جرام/مل فى اليوم ٢١ ثم بدأ بعد ذلك فى الانخفاض حتى وصل الى ٣,٠٦ ± ٠,٤٧ نانو جرام/مل فى اليوم ٤٢ بعد التقليح . يستخلص من هذه الدراسة أن معاملة الأبقار فى شهور الصيف بهرمون hCG فى اليوم السابع بعد التلقيح أدت الى تحسين جوهرى فى معدل الحمل ، بينما معاملة الأبقار بهرمون GnRH بعد التلقيح مباشرة أدى الى إنخفاض معدل الحمل وبدون تأثير معنوي على تركيز هرمون البروجسترون في سيرم الدم.