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Progesterone concentrations and pregnancy rates of repeat breeder cows following postinsemination PRID and GnRH treatments*

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Abstract: The objective of this study was to determine the effects of progesterone (PRID) application and gonadotropin-releasing hormone (GnRH) injection after artificial insemination on serum progesterone concentration and pregnancy rates in repeat breeder cows. In group 1, repeat breeder cows received an injection of GnRH on day 12 after artificial insemination. In group 2, repeat breeder cows received PRID from day 4 to day 11; group 3 received PRID from day 11 to day 18. Blood samples were obtained from all treated cows to assay serum progesterone concentrations on application days, 7 days after application, and 21 days after artificial insemination. In the control group repeat breeder cows did not receive any treatment. Blood samples were obtained from all control repeat breeder cows periodically after artificial insemination. Pregnancy rates were 20% in group 1, 26.6% in group 2, 40% in group 3, and 20% in the control group. Serum progesterone concentrations were significantly higher in group 1 and group 3 ($P < 0.05$) than the control group, 7 days after application. Application of PRID and GnRH after artificial insemination did not significantly improve pregnancy rates, despite the fact that serum progesterone concentrations were higher in treatment groups.

Key words: Cow, GnRH, PRID, repeat breeder

Tohumlamayı izleyen farklı günlerde PRID ve GnRH uygulanan repeat breeder ineklerde progesterone konsantrasyonları ve gebelik oranları

Özet: Bu çalışmada, repeat breeder ineklerde tohumlama sonrası progesteron uygulamalarının ve progesteron üretimini stimüle eden GnRH enjeksiyonunun serum progesteron konsantrasyonu ve gebelik oranları üzerine etkileri araştırıldı. Grup 1'deki hayvanlara tohumlamayı izleyen 12. günde GnRH enjeksiyonu yapıldı. Grup 2'deki hayvanlara tohumlama sonrası 4-11. günler arası PRID uygulandı. Grup 3'deki hayvanlara ise tohumlama sonrası 11-18. günler arası PRID uygulaması yapıldı. Tüm hayvanlardan uygulama günü, uygulama gününü izleyen 7. gün ve suni tohumlamayı izleyen 21. günde progesteron analizi için kan örnekleri toplandı. Kontrol grubundaki hayvanlara ise herhangi bir uygulama yapılmadı. Bu gruptaki ineklerden progesteron ölçümü için düzenli olarak kan örnekleri toplandı. Gebelik oranları; grup 1'de % 20, grup 2'de % 26,6, grup 3'de % 40 ve kontrol grubunda % 20 olarak hesaplandı. Uygulamayı izleyen 7. gündeki progesteron değerlendirmelerinde, grup 1 ve grup 3'deki progesteron konsantrasyon artışının kontrol grubuna oranla istatistiksel olarak önemli olduğu belirlendi ($P < 0,05$). Sonuç olarak, tedavi gruplarındaki uygulama girişimlerinin, materyal olarak alınan repeat breeder ineklerde endogen progesteron hormonu düzeylerini yükseltmesine rağmen, gebe kalma oranları üzerinde istatistiksel anlamda önemli bir etkisi olmadığı görüldü.

Anahtar sözcükler: İnek, GnRH, PRID, repeat breeder

* This study is summarized from a part of the author's PhD thesis.

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Introduction

In dairy cows, luteal insufficiency and lower progesterone concentrations are major causes of embryonic mortality and reduce pregnancy rates during early embryonic development (1,2). Delay in the normal rise in progesterone concentrations between days 4 and 5 postovulation and low systemic progesterone concentrations during the subsequent diestrus reduce pregnancy rates and result in lower conception rates (3).

Repeat breeding is a substantial problem in cattle breeding. It leads to large economic loss for the dairy producer due to additional inseminations, increased calving intervals, and higher culling rates. Repeat breeding has been defined as failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities (4).

During the preimplantation phase of embryonic development, direct progesterone supplementations (3,5) and gonadotropin-releasing hormone (GnRH)/human chorionic gonadotropin (hCG) (6) injections are used to improve embryonic survival in repeat breeder cows. Progesterone-releasing intravaginal devices (PRID), controlled internal drug release (CIDR), and Synchro-Mate ear implants are some of the available progesterone preparations (3,7).

It has been hypothesized that increasing peripheral progesterone concentrations during the diestrus after insemination may improve embryo development and suppress luteolysis, resulting in reduced embryonic loss (3,6). In controlled studies where the relationship between early embryo development and maternal progesterone concentrations was examined, higher progesterone concentrations were associated with improved embryo development (8).

GnRH injection is another approach used to increase progesterone concentrations and enhance embryonic survival in repeat breeder cows after artificial insemination (6).

The objective of this study was to determine the effects of PRID and GnRH injection after artificial insemination on serum progesterone concentrations and pregnancy rates in repeat breeder cows.

Materials and methods

This study was conducted on 60 Holstein cows in 3 commercial dairy herds (20 cows from each commercial diary) in Nicosia and Famagusta provinces in the Turkish Republic of Northern Cyprus from May to September 2008. Cows included in the study were in their first to fifth lactation, produced an average of 9000 kg milk per lactation period, and had 3 to 6 unsuccessful inseminations within the current lactation (repeat breeders). Cows were inseminated at normal estrus by veterinarians. After artificial insemination, 45 repeat breeder Holstein cows were divided equally and randomly into 1 control and 3 treatment groups. In group 1, repeat breeder cows received an injection of GnRH (Receptal® inj., 0.0042 mg buserelin acetate/mL; İntervet Ltd., İstanbul, Turkey) on day 12 after artificial insemination. In group 2, repeat breeder cows received PRID without an estradiol capsule (PRID® 1.55 g progesterone; Sanofi Doğu Ilaç, Ankara, Turkey) from day 4 to day 11; in group 3, animals received PRID without an estradiol capsule (PRID) from day 11 to day 18. In group 1, blood samples were obtained on the day of GnRH injection, 7 days after GnRH injection, and 21 days after artificial insemination to assay serum progesterone concentrations. In groups 2 and 3, blood samples were obtained on PRID application and removal days and 21 days after artificial insemination to assay the serum progesterone concentrations. In the control group, 15 repeat breeder cows did not receive any treatment. After artificial insemination, blood samples were periodically (on days 4, 7, 11, 12, 15, 18, 19, and 21) obtained from all control repeat breeder cows to assay serum progesterone concentrations. Progesterone concentrations were measured by radioimmunoassay (RIA Progesterone®; Immunotech, France) in all blood samples. Cows that were detected in estrus after day 18 were reinseminated and recorded as nonpregnant from the previous artificial insemination. The uteri of cows not observed in estrus were palpated per rectum 45-50 days after insemination to determine pregnancy status. The differences in progesterone concentrations and pregnancy rates between treatment cows and control cows were analyzed by Mann Whitney test and SPSS 14.01.

Results

Of the 60 cows used in the study, 30 were allocated to the PRID-treated groups, 15 to the GnRH-treated group, and 15 to the control group. In the GnRH-treated group, 3 cows were pregnant and 12 nonpregnant at the rectal palpation on day 45 after the first insemination. In the PRID-treated group (between days 4 and 11) and PRID-treated group (between days 11and 18), 4 and 6 cows were pregnant at rectal palpation, respectively. In the control group, 3 cows palpated as pregnant (Table 1).

Serum progesterone concentrations of treated cows in group 1 and control cows were compared on day 12, on GnRH injection day, and on day 19 after artificial insemination. On day 12, serum concentrations of progesterone were not different between treated cows and control cows. On day 19 after treatment, the serum progesterone concentrations of treated cows were significantly higher than in control cows (Table 2).

Similarly, serum progesterone concentrations of cows in group 2 and control cows were compared, and no significant differences were observed between the groups on PRID application or removal day (Table 3). In group 3, the progesterone concentrations of treated cows on PRID removal day were significantly higher than the control cows ($P < 0.05$) (Table 4). Progesterone concentrations of pregnant cows in all groups on day 21 are shown in Table 5.

Discussion

The effects of GnRH injection on day 12 after artificial insemination and PRID application from days 4-11 and 11-18—in repeat breeder cows after insemination and non-treated repeat breeder control cows —were evaluated for pregnancy rates and blood serum progesterone levels; there were no significant differences among pregnancy rates in all groups.

Progesterone is an essential hormone in the maintenance of pregnancy in cows. Higher levels of progesterone concentration in early pregnancy are related to embryonic development and an increase in interferon-τ production and pregnancy rates (9). Low systemic progesterone concentrations on

Table 1. Pregnant and nonpregnant cows in treatment groups and control group.

	Cows	
	No. of pregnant (%)	**No. of nonpregnant (%)**
Group 1 (GnRH-treated)	3/15 (20%)	12/15 (80%)
Group 2 (PRID-treated, 4-11 days)	4/15 (26.6%)	11/15 (73.3%)
Group 3 (PRID-treated, 11-18 days)	6/15 (40%)	9/15 (60%)
Control group	3/15 (20%)	12/15 (80%)

Table 2. Progesterone concentrations on injection day and 7 days after injection in group 1 and control group.

	Progesterone (ng/mL)	
	Day 12	**Day 19**
Group 1	8.03 ± 1.92	8.25 ± 2.41
Control	5.58 ± 0.94	3.22 ± 0.53
P	P > 0.05	P < 0.05

*Treated cows received injections of GnRH on day 12 after artificial insemination.

Table 3. Progesterone concentrations on PRID application and removal days in group 2 and control group.

	Progesterone (ng/mL)	
	Day 4	**Day 11**
Group 2	1.36 ± 0.43	6.28 ± 1.27
Control	1.32 ± 0.27	4.81 ± 0.80
P	P > 0.05	P > 0.05

*Treated cows received intravaginal PRID from days 4-11 after artificial insemination.

Table 4. Progesterone concentrations on PRID application and removal days in group 3 and control group.

	Progesterone (ng/mL)	
	Day 11	**Day 18**
Group 3	5.76 ± 0.93	6.56 ± 0.71
Control	4.81 ± 0.80	3.38 ± 0.51
P	P > 0.05	P < 0.05

*Treated cows received intravaginal PRID from days 11-18 after artificial insemination.

day 5 postovulation or delays in the normal rise in progesterone between days 4 and 5 postovulation are related to reduced pregnancy rates (3,8,10,11).

Several researchers state that 7 days, between days 5 and 19 after insemination, are sufficient for progesterone application (3,5,8,11-15). In the above studies, intravaginal PRID or CIDR applications were the most common methods used; in the current study PRID was applied in 2 different groups of repeat breeder cows from days 4 to 11 and 11 to 18, for 7 days.

In this study repeat breeder cows (n = 15) received progesterone between days 4 and 11 after insemination. Application on these particular days was devised to maintain higher blood progesterone concentrations during blastogenesis and allow time for the corpus luteum to secrete progesterone after being formed. On application day, low progesterone rates (1.36 ± 0.43 ng/mL and 1.32 ± 0.27 ng/mL) in treatment and control groups, respectively, increased to 6.28 ± 1.27 ng/mL and 4.81 ± 0.80 ng/mL on the seventh day after treatment. However, pregnancy rates in treatment and control groups were 26.6% and 20%, respectively; there was no statistically significant difference between the 2 groups.

Another group of repeat breeder cows (n = 15) received progesterone application between days 11 and 18 after insemination. This application was timed to stop corpus luteum regression, support interferon-τ production, and recognize pregnancy by cow. On application day, blood progesterone rates in treatment and control groups were 5.76 ± 0.93 ng/mL and 4.81 ± 0.80 ng/mL, respectively. Higher progesterone levels in the 11-18 group than the 4-11 group reflected maturity of the corpus luteum and higher progesterone production on these days. After 7 days of application, progesterone levels were 6.56 ± 0.71 ng/mL in the treatment group and 3.38 ± 0.51 ng/mL in the control group. The averages of both values are sufficient to maintain a normal pregnancy. Pregnancy rates in treatment and control groups were 40% and 20%, respectively.

Gonadotrophic hormones are known alternatives to progestogen application and they also increase endogen progesterone level (3,6).

GnRH hormone injections after insemination are used to increase the percentage of pregnancy in repeat breeder cows (16-18). GnRH injections are also used for stimulating ovulation during insemination. In addition, they increase the level of progesterone by providing luteal support in the early and medium luteal phases (19,20). However, there are different views about the effects of increasing endogenous progesterone by using luteotropic hormone (18,21,22). The different results obtained from GnRH applications between 11 and 14 days after insemination are related to follicular wave. In 3-follicular wave cycles, GnRH applications between days 11 and 14 of the cycle coincide with peak period

Table 5. Progesterone concentrations of pregnant and nonpregnant cows on day 21 in groups 1, 2, and 3, and control group.

	Progesterone (ng/mL) (Day 21)			
	Group 1	**Group 2**	**Group 3**	**Control group**
Pregnant cows	15.93 ± 5.63	10.84 ± 3.31	10.11 ± 1.44	7.44 ± 1.15
Nonpregnant cows	3.00 ± 1.08	0.66 ± 0.11	1.60 ± 0.63	1.78 ± 0.49

for the secondary follicular wave, and there is a high estradiol concentration during this period. The effect of applied GnRH is detected as an atresiation, luteinization, and ovulation of the developed follicle in the 2- and 3-wave cycles (23).

In this study, treated groups of repeat breeder cows (n = 15) received GnRH injections on day 12 after insemination. The objective of GnRH injection on day 12 is to decrease the estradiol concentration, which is high during this period, by luteinizing the dominant follicle and suppressing the rise of the oxytocin receptor and secretion of PGF_{2a} in the uterus. Many researchers also suggest days 11-13 for GnRH applications in the luteal phase (19,24-26).

In this study, blood progesterone concentrations of pregnant animals on the 19th day after insemination were numerically higher than pregnant animals in the control group; however, they were not statistically significant. GnRH injections increased blood progesterone concentration by forming accessory corpus luteum. Several researchers support the formation of accessory corpus luteum after GnRH injections (6,23,27).

Researchers have reported 59.6% pregnancy rates in a GnRH treated group; in the control group this rate was 59.1% on day 12 after insemination (28). At the end of GnRH application on day 12 after artificial insemination in the present study, a 20% pregnancy rate was obtained. Pregnancy rates in the control group were the same. Although the findings in the present research are numerically lower than the rates reported by Szenci et al. (28), both studies reached similar conclusions.

During this study, progesterone hormone concentrations were assayed on application day, 7 days after application, and 21 days after artificial insemination in treated groups. In the control group progesterone hormone concentrations were assayed periodically. The blood samples collected on application day and on day 7 after application were used to analyze the effects of treatment on blood progesterone hormone levels. The blood samples collected on day 21 were used to detect the pregnancy rates in all groups.

As shown in Table 5, progesterone concentrations of pregnant cows in the GnRH group on day 21 were 15.93 ± 5.63 ng/mL; the same rates were 3.00 ± 1.08 ng/mL in nonpregnant cows in the GnRH group. The same values in the second and third PRID groups were 10.84 ± 3.31 ng/mL and 10.11 ± 1.44 ng/mL for pregnant cows and 0.66 ± 0.11 ng/mL and 1.60 ± 0.63 ng/mL for nonpregnant cows, respectively. However, the blood progesterone concentrations of pregnant control group cows were 7.44 ± 1.15 ng/mL. According to these figures, endogenous progesterone hormone concentrations are higher for all pregnant cows in treatment groups than for pregnant control group cows.

In conclusion, application of PRID and GnRH injection after artificial insemination did not significantly improve pregnancy rates, despite the fact that serum progesterone concentrations were higher in the treatment groups.

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