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Investigation of the Effect of Different Hormone Applications on Pregnancy Rates in Repeat Breeder Cows#

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Research Article

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ABSTRACT

The current study aimed to investigate the effects of GnRH during insemination and progesterone administration on conception on the fourth- and fifth days following insemination in Repeat Breeder (RB) cows. The study included 40 Holstein breed RB cows housed under identical care and feeding circumstances. The animals were divided into four groups: group I (GnRH administered immediately after insemination), group II (GnRH administered immediately after insemination + progesterone administered on days 4 and 5 after insemination), group III (progesterone administered on days 4 and 5 after insemination), and group IV (control). All animals in the study had their ovulation checked at the 24th and 36th hours after insemination, and the pregnancy was examined by USG on the 45th day. The initial estrus of non-pregnant animals and their days were established. The ovulation rates at the 24th hour was 70% in group I, 90% in group II, 50% in group III, and 50% in group IV, whereas in the 36th hour they were 30% in group I, 10% in group II, 50% in group III, and 50% in group IV, respectively. The pregnancy rates were 70% in group I, 80% in group II, 60% in group III, and 30% in group IV. The control group had lower pregnancy rates ($p < 0.05$) compared to the treatment groups. As a result, higher pregnancy rates were seen in the treatment groups compared to the control group, indicating that three distinct treatments can be used to increase pregnancy rates in RB cows.

Keywords: Cow, GnRH, Pregnancy rate, Progesterone, Repeated breeder

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Introduction

Although milk yields from dairy cows have increased globally in recent years, fertility has fallen and the number of inseminations per pregnancy has increased. One of the most crucial factors influencing cow fertility and the number of inseminations per pregnancy is the Repeat Breeder (RB) problem (Dochi, et al., 2008). Repeat Breeders are cows that do not conceive after at least three inseminations and have no clinically identifiable anomalies in the genital organs or estrous cycle (Yusuf, et al., 2010). The pathogenesis of Repeat Breeder Syndrome is complicated and not fully understood. However, two primary explanations are underlined. These include inability to fertilize due to delayed ovulation and early embryonic death (Gustafsson and Larsson, 1985; Diskin and Morris, 2008; Yusuf et al., 2010). The RB problem in high producing dairy cows generates significant economic losses. RB prevalence varies by country, ranging from 10% to 18% (Kimura, et al., 1987).

Eliminating the infertility present in RB cows, reusing these animals in breeding and reducing the operating losses can only be possible by applying effective treatment procedures. Hormone treatment can be applied to increase the fertilization success and reduce embryonic deaths in RB cows (Singh et al., 2017).

Gonadotrophin Releasing Hormone (GnRH) is essential for mammalian reproduction. The median eminence of the hypothalamus secretes GnRH, a neuropeptide. GnRH is delivered to gonadotrophs in the anterior pituitary lobe via portal veins. GnRH attaches to specific receptors in gonadotrophic cells, causing them to produce and release FSH and LH (D'Occhio, et al., 2000). It has been shown that GnRH supplied during insemination in Repeat Breeder cows increases pregnancy rates, while externally administered GnRH promotes embryonic life by increasing progesterone concentration (Parabaharan, et al., 2009; Dodamani, et al., 2010; Ergene, 2012).

Progesterone released by the corpus luteum is essential for the formation and maintenance of pregnancy. It regulates endometrial secretions, which are necessary to stimulate and mediate changes in conceptus growth and differentiation during early pregnancy in ruminants (Lonergan, et al., 2016). Exogenous progesterone administration during diestrus after insemination in RB cows has been shown to improve pregnancy rates (Ghasamzadeh, et al., 2010; Ferguson, et al., 2012).

Many studies have examined the effects of GnRH and progesterone treatment on the rates of conception in RB

cows; however, there is ongoing debate regarding the best time and method of administration. The current study, therefore, aimed to determine how progesterone and GnRH administration during insemination affected the rates of ovulation and pregnancy on days 4 and 5 following insemination.

Material and Methods

Animal Material

The study was conducted on 40 RB cows on a private farm in Istanbul's Silivri district. The cows were kept under the same care, feeding, and breeding management, and rectal and ultrasonographic examinations revealed that the cows had no gynecological issues despite having been inseminated at least three times without becoming pregnant.

All animals were fed Total Mixed Rations (TMR) ad libitum twice daily. They always had access to clean drinking water. The animals were randomly separated into four groups. Group I (n=10, received GnRH immediately after insemination); group II (n=10, GnRH administered immediately after insemination with progesterone administered on days 4 and 5 after insemination); group III ((n=10, progesterone administered on days 4 and 5 after insemination); and group IV ((n=10, control).

Experimental Design

Estrus was detected in the cows in the study by measuring the increase in uterine tone by rectal palpation as well as the existence of Graaf follicles in the ovaries. All animals were inseminated 12 hours after estrus onset with semen from the same bull with confirmed fertility, and the process was conducted by the same technician.

GnRH (Receptal, 0.0042 mg Buserelin acetate in 1 ml, Intervet, Türkiye) was administered by 2.5 ml IM injection to group I animals immediately after insemination. Animals in group II received 2.5 ml of GnRH by IM immediately after insemination and 250 mg of Medroxyprogesterone acetate (Farlutal Depo, Deva, Türkiye, contains 250 mg Medroxyprogesterone acetate in 1 ml) by IM injection once a day on days 4 and 5 after insemination. Animals in group III were administered 2.5 ml of saline (0.9% NaCl) immediately after insemination and 250 mg of Medroxyprogesterone acetate by IM injection once daily on days 4 and 5 after insemination. Animals in group IV were administered 2.5 ml saline by IM injection immediately after insemination and served as a control group. All animals in the study had ovulation control at the 24th and 36th hours, and pregnancy control was conducted by USG on the 45th day following insemination. The initial estrus of non-pregnant animals was observed and established in days.

Statistical Analysis

SPSS 17 package program was used to prepare statistical data. The results are expressed as mean ± standard deviation. The Chi-square test was used to compare the pregnancy rate, 24th hour ovulation rate, and 36th hour ovulation rate between the groups in the study. The average cycle days for RB were calculated using a one-way analysis of variance. Mean cycle days of RB were compared using the Duncan test. P-values <0.05 were considered statistically significant in the calculations.

Ethical Considerations

The presented study is summarized from the corresponding author's doctoral thesis, which was accepted by the Istanbul University Institute of Health Sciences on May 30, 2003. At that time, obtaining ethical approval was not mandatory and was not required by the authorized Institute. (The requirement to obtain a local ethical committee certificate for animal experiments was introduced by the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees, published in the Official Gazette No. 26220 by the Ministry of Environment and Forestry on July 6, 2006).

Results

The statistical comparison of pregnancy rates between the treatment and control groups revealed a statistically significant difference ($p < 0.05$) between the treatment group and the control group. Even though the GnRH + Progesterone group had a higher pregnancy rate than the other treatment groups, the difference was not statistically significant when the treatment groups were compared (Table 1). The second group had a higher ovulation rate at the 24-hour mark, but there was no statistically significant difference between the groups. The control group had the highest number of RB, whereas the second group had the lowest number. The progesterone-administered groups' cycle lengths in RB were longer ($p<0.05$) (Table 1).

Discussion

Repeat breeder animals in dairy cow breeding are a fundamental problem that decreases business profitability and must be addressed immediately. The current study sought to determine the impact of various treatment procedures on pregnancy in RB cows.

In research on RB cows (Amiridis et al., 2009; Parabaharan et al., 2009; Hailu et al., 2015), pregnancy rates in control groups without treatment were shown to be lower than in treatment groups. In this study, the control group had a lower pregnancy rate compared to the treatment groups. Many studies have used GnRH hormone in RB cows to increase pregnancy rates by preventing ovulation delays. While these researchers report pregnancy rates ranging from 50% to 87% with the administration of GnRH, pregnancy rates in the control group range from 33% to 48% (Behl et al., 2007; Kharchce and Srivastava, 2007; Dodomani et al., 2010; Pandey et al.,2016). Like these researchers, the current study attained a 70% pregnancy rate by GnRH administration.

Table 1. Pregnancy rates, ovulation rates obtained from the animals included in the study and cycle times of returning animals

	Group 1 GnRH (n=10)	Group 2 GnRH + Progesteron (n=10)	Group 3 Progesteron (n=10)	Group 4 Control (n=10)
Pregnancy rates (%)	70^{a}	80^{a}	60^{a}	30^{b}
Ovulation rate at 24th hour (%)	70^{a}	90^{a}	50^{a}	50^{a}
Ovulation rate at 36th hour (%)	30^{c}	10^{b}	50^{a}	50^{a}
Cycle times for returning animals (days) (ort. ±SD)	21.0±1.0^{b}	37.5 ±10.6^{a}	39.0±12.0^{a}	22.85±3.18^{b}

** The difference between groups with different letters on the same line is significant (p<0.05)*

The corpus luteum secretes progesterone hormone, which is required for the beginning and maintenance of pregnancy in cows. Progesterone content in the early luteal stage (days 4–7) is critical for successful pregnancy in RB cows (Perez-Marin, 2012). Low progesterone concentrations in the diestrus period following estrus in RB cows have been linked to early embryonic death and reduced conception rates (Lopez-Gatius, et al., 2004; Parr et al., 2012). Based on this, many studies have attempted to increase the conception rate in RB cows by providing external progesterone throughout the luteal period (Amiridis, et al. 2009; Khoramian, et al. 2011; Ferguson, et al. 2012; Kumar, et al. 2012). Khoramian, et al. (2011) reported a pregnancy rate of 56% with progesterone application to RB cows for 7 days in the luteal phase, but Kumar, et al. (2012) reported a pregnancy rate of 48.48% with progesterone therapy for 5 days in the early luteal phase. The control groups had pregnancy rates of 29.6% and 31.57%, respectively. The current study found that administering a long-acting progesterone preparation on days 4 and 5 of the early luteal period resulted in a 60% pregnancy rate. The control group's pregnancy rate was determined to be 30%. Starbucks, et al. (2001) found that progesterone administration after insemination had little influence on conception rates. The differences between studies are related to variations in the progesterone analogues used, their dosages, and methods of delivery. Amiridis et al. (2009) observed that administering GnRH and progesterone combined resulted in a greater pregnancy rate in RB cows. Similarly, the current study found that using GnRH and progesterone combined resulted in a greater conception rate in RB cows, however this was not statistically significant when compared to other treatment groups. Behl, et al. (2007) found that GnRH treatment reduced ovulation time by 37.33±3.32 h compared to 53.33±2.66 h in the control group. Our study indicated that 24th hour ovulation rates were greater than other groups (70%-90%). This demonstrates how GnRH shortens the ovulation period.

In the present study, animals in the GnRH and control groups showed their second estrus in an average of 21.0±1.0 and 22.85±3.18 days, respectively, while the duration of estrus in the animals in the second and third groups administered progesterone was significantly prolonged (37.5±10.60, 39.0±12.0 days). However, Garveric, et al. (1988) and Selvaraju, et al. (2009) found that progesterone administration did not change the length of the estrous cycle in RB cows. Differences between studies could be attributed to the progesterone analogues used.

Conclusion

As a result, GnRH hormone can be administered immediately after insemination to keep RB cows in the herd, to achieve positive outcomes in artificial insemination experiments, to induce ovulation at the right time, and to boost pregnancy rates. It has been found that progesterone hormone can also be administered on the fourth and fifth days after insemination, and that combining GnRH and progesterone can result in a higher pregnancy rate. However, it is believed that additional research on the subject is needed.

Conflict of Interest

The authors report no actual or potential conflicts of interest.

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