The Effect of PMSG Treatment on Pregnancy Rates in Synchronized Heifers using Norgestomet Ear Implants

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ABSTRACT

This study was carried out in heifers, which were administered with norgestomet implants for estrus synchronization, with an aim to determine the effect of PMSG, administered to the animals on the day of the removal of the implants, on pregnancy rates. A hundred Holstein heifers, aged 15-16 months, constituted the material of the study. In this study all of the heifers were administered ear implants containing 3 mg norgestomet subcutaneously on the external surface of the ear. Following the insertion of the implants, on the same day, all of the heifers were intramuscularly administered 10 μg buserelin acetate. After a period of 10 days, the implants were removed. Two days prior to the removal of the implants, the heifers were injected with 500 μg of cloprostenol by intramuscular route. On the day of the removal of the implants, the animals were randomly assigned to two groups which comprised an equal number of heifers. The heifers included in Group I (n=50) were injected with 400 IU pregnant mare's serum gonadotropin (PMSG) by intramuscular route, whilst the heifers included in Group II (n=50) were not subjected to any treatment. At the 56th hour following the removal of the implants, all heifers were inseminated regardless of the presence of oestrus signs. The pregnancy diagnosis was carried out on 35th days after insemination. The pregnancy rates achieved in Groups I and II were determined as 86% (43/50) and 54% (27/50), respectively (P<0.001). It was concluded that, in heifers, which were synchronized with subcutaneous norgestomet implants, the addition of PMSG on the day of the removal of the implants increased the pregnancy rates.

Keywords: Heifer; Synchronization; Norgestomet; PMSG.

INTRODUCTION

The age at first insemination (AFI) and the age at first calving (AFC) are commonly used as fertility criteria in dairy production. Cyclic irregularities and the difficulty of oestrus detection in heifers may complicate their insemination and delayed pregnancy may cause economic losses (1, 2, 3). Today, the demand of the livestock sector for pregnant heifers, which are at a certain age and in the same stage of pregnancy has increased. It is intended that heifers, which have reached puberty, are inseminated as early as possible since this provides a major economic advantage (4, 5).

An effective estrus synchronization regimen is expected to synchronize the estrus of the treated animals within a 12 to 24-h period, stimulate high rates of oestrus and ovulatory response, and enable the achievement of a high pregnancy rate with artificial insemination (6). The most common methods used for the hormonal control of the oestrus cycle in cows include the establishment of an artificial corpus luteum (CL) function through the administration of progestogens for a certain time period, the stimulation of luteolysis by means of the administration of luteolytic agents, and the synchronization of ovulation by means of the combined administration of gonadotropin-releasing hormone (GnRH)

and prostaglandins for the regulation of the follicular and luteal functions (7, 8, 9).

Progesterone can be administered by parenteral injection, given by oral route as a feed additive (melengestrol acetate), be administered intravaginally in the form of natural progesterone using either a progesterone-releasing intravaginal device (PRID) or a controlled internal drug releasing (CIDR) device, or be administered as a subcutaneous implant (norgestomet) (10, 11, 12). In cows and heifers, norgestomet implants are inserted superficially, under the skin of the ear, using a specially designed implanted device. The implants are removed 9 days after being inserted, and oestrus is observed 2-3 days after the removal of the implants. Using a specially designed implanted device in the cow's ear inseminations to be performed without the detection of oestrus are either carried out twice at 48 h and 72 h or once at 56 h (10, 13, 14).

In order to ensure the success of progesterone-based estrus synchronization programs the development of the follicular wave needs to be controlled (15). This is required because the presence of dominant follicles in different developmental stages by the end of the synchronization period leads to variances in the duration of the proestrus period (15). The administration of GnRH at the beginning of the synchronization regimen causes either the luteinisation or the ovulation of the largest follicle in 60-80% of the animals. Thereby, the synchronization of the follicular waves is achieved (16, 17, 18, 19, 20).

During progesterone treatment, in some cases, the CL may show an effect for a longer period than progesterone. This adversely affects the synchronization process. For this reason, in order to ensure the regression of the physiological CL, it is suggested to administer a luteolytic hormone at the end of the treatment (8, 21, 22).

Either before or at the time of the removal of the progesterone source, pregnant mare serum gonadotropin (PMSG) can administered so as to stimulate follicular development, induce oestrus, enable the earlier onset of oestrus, regulate ovulations and possibly achieve a higher rate of ovulation. Furthermore, it is indicated that following the removal of the progesterone source, PMSG reduces the variances observed in the time to ovulation, and thereby, aids in the synchronization of the estruses and ovulations (21). In view of these data, it was suggested to combine progesterone-based estrus synchronization treatment with the administration of 400-700 IU of PMSG (7, 23, 24, 25, 26, 27).

This study was carried out in heifers, which were administered with subcutaneous norgestomet implants for estrus synchronization, with an aim to determine the effect of PMSG, administered to the animals on the day of the removal of the implants, on pregnancy rates.

MATERIALS AND METHODS

Animals

A hundred Holstein heifers, aged 15-16 months and raised under the same management and feeding conditions at a private semi-outdoor commercial dairy farm, which was located in the Gaziantep province and known to keep regular records, constituted the material of the study. These heifers had been imported from the United States of America (USA) at an age of 10-11 months. The animals were given feed and water *ad libitum*. After being raised for a period of approximately 5 to 6 months, the animals were included in the study at an age of 15-16 months, at a body weight of 380-420 kg.

The study procedures were conducted under the experimental license (license number: 2013/34) of the Animal Experimental Local Ethics Committee of Mustafa Kemal University.

Management and Feeding of the Animals

The heifers were housed in free-stall barns and were fed on a total mixed ration (TMR) which were had 16.1% crude protein and 2746 kcal/kg ME. TMR was contained fifty-fifty concentrate feed and roughage. The roughage content consisted of alfalfa hay and corn silage. The animals had access to separate feeding and resting areas, as well as to open-air exercise areas.

Study Procedure

In this study all of the heifers were administered norgestomet implants (Crestar®, Intervet, Turkey) beneath the skin of the external surface of the ear. The implants were inserted using a specially designed applicator. Following the insertion of the implants, on the same day, all of the heifers were intramuscularly administered with 10 μg of buserelin acetate (Receptal®, Intervet, Turkey), which is an analogue of the GnRH.

After a period of 10 days, the implants were removed. Two days prior to the removal of the implants, all animals were injected with 500 μg of cloprostenol (Estrumate®, Intervet, Turkey) which is an analogue of $PGF_{2\alpha}$ by intra-

muscular route. On the day of the removal of the implants, the animals were randomly assigned to two groups, comprising an equal number of heifers. The heifers included in Group I (n=50) were injected with 400 IU PMSG (Folligon®, Intervet, Turkey) by intramuscular route (Figure 1), whilst the heifers included in Group II (n=50) were not subjected to any treatment (Figure 2). At the 56th hour following the removal of the implants, all heifers were inseminated regardless of the presence of oestrus signs.

The pregnancy diagnosis was carried out on 35th days after insemination by ultrasonography with a 6 to 8 MHz, linear-array transrectal real-time ultrasonography (Falco Pie Medical, Netherlands) (28).

Figure 1. Treatment of synchronization protocol in group I

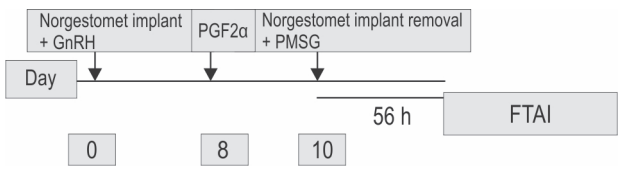
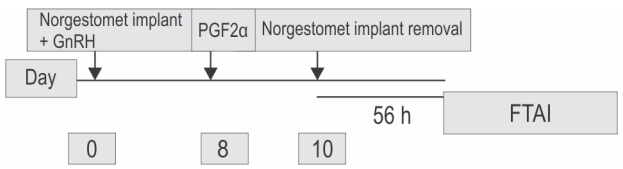


Figure 2. Treatment of synchronization protocol in group II



Statistical Evaluation

The intra-group and inter-group statistical analyses of the data obtained were performed with the chi-square test and by using the Statistical Program for Social Sciences (SPSS). The statistically significant level were regarded as $P<0.05$.

RESULTS

The pregnancy rates achieved in Groups I and II were determined as 86% (43/50) and 54% (27/50), respectively (Table 1). It was established that a higher pregnancy rate was achieved in the heifers which had also received PMSG in addition to the estrumate implant, and this difference was found to be highly statistically significant ($P<0.001$).

Table 1. Pregnancy rates of the treatment groups

	Group I	Group II	P
Pregnancy rate (%)	43/50 (86)	27/50 (54)	***

***: P <0.001

DISCUSSION AND CONCLUSIONS

The present study was carried out on 100 Holstein heifers, aged 15-16 months raised at a dairy farm located in the Gaziantep province, Turkey. The study was aimed at determining the impact of PMSG on the pregnancy rate of heifers, the estruses of which were synchronized with the use of subcutaneous norgestomet implants. PMSG was administered to the heifers on the day of the removal of the implants with an aim to increase the effectiveness of the fixed-time artificial inseminations (FTAI) that were performed without monitoring of oestrus signs.

Oestrus synchronization protocols designed for cows are to a greater extent based on the performance of FTAI rather than on the detection of oestrus. Of such protocols, one which is found in common use, is the synchronization of estrus with progesterone, and is based on the inhibition of the secretion of the luteinizing hormone (LH), and thereby, on the prevention of ovulation and oestrus. It has been reported that an artificial serum progesterone level, close to that observed during the luteal phase (>1 ηg/ml), can be established with the intravaginal use of PRIDs and CIDR devices or by the use of subcutaneous progestogen implants. Initially, these protocols foresaw the use of the progesterone source throughout the oestrus cycle, yet subsequent research showed that the use of progesterone for more than 12 days decreased the resulting pregnancy rate (29, 30). In the majority of previous researches, progesterone implants have been reported to be maintained at the site of insertion for a period of 9-11 days (19, 29, 30, 31, 32). In view of the time period recommended in previous research (19, 29, 30, 31, 32), the implants were applied for a period of 10 days in the present study.

In progesterone-based estrus synchronization programs, the inadequate suppression of the initial dominant follicle brings about the delay of the start of a new follicular wave, and thereby, the absence of an adequately large ovulatory follicle at the time of insemination. Furthermore, literature reports also indicate that dominant follicles older than 8 days are considered aged and cause ovulation defects (29, 33). Therefore, it is suggested to administer either oestrogen or GnRH at the beginning of progesterone-based estrus synchronization programs (6, 32).

As estradiol esters are not allowed to be used in Europe, alternative estrus synchronization protocols, based on the injection of GnRH instead of estradiol, have been developed (34, 35). In the present study, in order to ensure the

control of the follicular waves, the animals in both groups were administered with GnRH at the time of the insertion of the implants. The injection of GnRH leads to the secretion of LH, and thereby, brings about the ovulation of the dominant follicle and the start of a new follicular wave (36). Therefore, the administration of GnRH at the beginning of progesterone-based estrus synchronization protocols has a positive effect on the results achieved (6, 36, 37). In their research on the comparison of different protocols based on the use of progesterone-releasing devices alone and such devices in combination with estradioland GnRH injections, Ryan *et al.* (30) determined that the fertility rate was high (58-60%) in the two groups, in which the follicular waves were induced with GnRH and estradiol, but low (50%) in the group, which received the progesterone-releasing device alone.

It has been reported that the preconditions for the achievement of an acceptable pregnancy rate with the performance of a single FTAI, are the presence of an adequately grown and mature preovulatory follicle and successful luteolysis synchronization (38). In the present study, 48 h prior to the removal of the implants, all of the animals received a prostaglandin injection.

Martinez *et al.* (39) attributed post-insemination low pregnancy levels in synchronized heifers to variances in the time of onset and duration of oestrus. It has been reported that while the delayed performance of FTAI in synchronized heifers decreases the resulting pregnancy rate, the fine tuning of the time of insemination increases the pregnancy rate (40, 41). Munro and Moore (42) reported that upon oestrus synchronization with the combined use of a PRID and PMSG, the highest pregnancy rate was achieved with inseminations performed at 56 h. In the present study, the pregnancy rates achieved with a single FTAI performed at 56 h, regardless of oestrus signs, was in agreement with the pregnancy rates and fertility levels reported in the previous studies referred to above. It is considered that the animals included in the present study being heifers, having been raised under favorable management and feeding conditions and displaying good body condition scores, also contributed to the pregnancy rates achieved.

Hixon *et al*. (43) reported that low pregnancy rates resulting from the use of progestogen-based estrous synchronization protocols could be related to insufficient LH secretion following the removal of the implants, and thus, to subsequent luteal dysfunction. Furthermore, Stock and Stolla (44) attributed low conception rates resulting from the use of PRID and CIDR-B devices to the prolongation of follicular development. These researchers also pointed out high estradiol concentrations in relation to the subluteal progesterone concentrations and the prevention of endometrial preparation for pregnancy as a result of the prolonged effect of estradiol. Therefore, when progesterone-based estrus synchronization protocols are used, in order to improve fertility, PMSG maybe injected either prior to or at the time of the removal of the progesterone source for the stimulation of follicular development, the induction of oestrus or the provision of an earlier onset of oestrus, regulation of ovulations, and the achievement of higher ovulation rates (24, 26, 27). In the present study, the conception rates achieved in Groups I and II were determined as 86% (43/50), and 54% (27/50), respectively ($p<0.001$). The results of the present study demonstrated that the pregnancy rates achieved in the heifers, which were administered with PMSG, were higher and this difference was found to be statistically significant.

It was suggested that the distribution of ovulations over a longer time period with the use of oestrus synchronization protocols based on the performance of FTAI, eventually causes lower conception rates (45). It has been indicated that the time of onset and duration of oestrus being highly variable in heifers could also result in low pregnancy rates when FTAI was performed (39, 40). Cavalieri *et al.* (21) reported that PMSG injection to cows synchronized with subcutaneous implants not only shortened the time from the removal of the implants to oestrus, the LH surge and ovulation, but also enabled the display of more evident oestrus signs and an increase in ovulation synchronization. Sa Filho *et al.* (46) determined that, of the cows they synchronized with the use of a Crestar preparation, those that were administered with PMSG at the time of the removal of the implants displayed an increase in the diameter of the dominant follicle, when compared to those that were not administered with PMSG. These researchers attributed this result to the effect of PMSG on FSH and indicated that a larger follicle diameter led to a higher ovulation capacity, a larger CL, and thus to an increase in the progesterone level. Duffy *et al.* (47) reported that PMSG administration at the time of the removal of the progesterone source enabled an increase in the ovulation rate.

Bo *et al.* (48) determined that the administration of 300-500 IU PMSG at the time of the removal of the progesterone source resulted in the display of more evident oestrus signs

and an increase in the pregnancy rate. Trı'bulo *et al.* (49) demonstrated that the administration of 400 IU PMSG increased both the plasma progesterone concentration and the conception rate. Roche *et al.* (7) suggested that the PMSG dose to be used in combination with progesterone should be 400-700 IU. In accordance with the dose recommendations made for PMSG in previous studies, the PMSG dose used in the present study was set as 400 IU. In agreement with the literature reports referred to above, it was ascertained that the pregnancy rate of the animals, which had received a PMSG injection, was higher and accordingly, it was considered that the administration of PMSG at the time of the removal of the progesterone source increased the pregnancy rate achieved.

In their research on two groups of heifers, both of which were applied implants for a period of 9 days and one which received 500 IU PMSG on the day of the removal of the implants, Chaudhari *et al.* (50) determined the pregnancy rates of the groups that received and did not receive PMSG as 66.67% and 33.33%, respectively. Furthermore, in their research on water buffaloes, Caesar *et al.* (51) detected pregnancy rates of 57.1% and 85.7% in the groups that were applied CIDR device alone and a CIDR device in combination with PMSG injection, respectively. In their research on progesterone-based estrous synchronization, the same researchers determined that PMSG administration had a positive effect on the ovulation and pregnancy rates. In their study on two groups of cows, both of which were applied a CIDR device for 8 days and injected with $PGF2_{\alpha}$ on the 8th day, and one of which was injected with 400 IU PMSG while the other was not on the 8th day, Breda *et al.* (52) determined pregnancy rates of 74.6% and 50.6% in the group that received PMSG and the group that did not receive PMSG, respectively. These researchers indicated that the difference between the two groups for the pregnancy rate was statistically significant (p<0.01). In another study they conducted in cows, Belloso *et al.* (53) used norgestomet implants alone for 9 days in one group, and a combination of an implant and an injection of 500 IU PMSG in the other. Following oestrus detection and the insemination of these animals, these researchers determined a pregnancy rate of 61.5% in the group that received an implant alone, and a pregnancy rate of 67.7% in the group that received a PMSG injection in association with an implant. Dodamani *et al.* (54) reported to have achieved a conception rate of 70% in two groups of water buffaloes, both of which were synchronized with Crestar (norgestomet) implants and one of which was injected with 500 IU PMSG while the other was not. Of two groups of heifers synchronized with PRIDs, Butler *et al.* (55) injected only one with 300 IU PMSG and detected a conception rate of 65.7% in the group that received PMSG and a conception rate of 63.7% in the group that did not receive PMSG. The researchers indicated no statistically significant differences existed between the groups for pregnancy rates.

In the present study, the conception rate of the group, which was administered with PMSG was found to be significantly higher (P<0.001), compared to the group that did not receive PMSG, a higher conception rate having been detected in the group that was administered with PMSG was found to be in agreement with the results previously reported by Chaudhari *et al.* (50), Caesar *et al.* (51), Breda *et al.* (52), Belloso *et al.* (53), Dodamani *et al.* (54) and Butler *et al.* (55).

The rate of fertilization was reported to be between the ranges of 75-100%. This rate is affected by embryonic and fetal mortality also by fertilization failure (56, 57). The reason of the higher pregnancy rates obtained in this study than the cited literatures (50, 52, 53, 55) may be originated from the both optimal nutritional status of the heifers and the control of the follicular wave development by GnRH.

It was concluded that, in heifers, which were synchronized with subcutaneous norgestomet implants, the injection of PMSG on the day the implants were removed with an aim to increase the effectiveness of FTAI was ascertained to increase the pregnancy rates achieved and to positively contribute to progesterone-based estrus synchronization protocols.

CONFLİCT OF INTEREST STATEMENT

Authors stated that there is no conflict of interest.

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