Summary: The aim of this study was to investigate the effect of PGF<sub>2α</sub> analogues and GnRH in cows, which had no spontaneous oestrus at 55-65<sup>th</sup> days postpartum, on oestrus, serum estradiol 17 β and pregnancy rates. The study was conducted in the Trakia Region on a total of 88 Holstein cows aged between 3-8 years and had no spontaneous oestrus in 55 days postpartum. The cows were separated into two groups (I. group n=46, II. group n=42). 0.5 mg cloprostenol was administered twice with 11 days interval to the I. group by I.M route. After coming to heat, the cows (n=44) of the first group were divided into two sub-groups. One of these sub-groups received 2.5 ml GnRH (Receptal<sup>®</sup> Topkim) after the second cloprostenol and artificial insemination. Cows in the second group were treated with 25 mg Dinoprost by I.M route with 11 days interval. Like the first group these cows (n=39) also were divided into two sub-groups and one received a GnRH (Receptal<sup>®</sup> Topkim) injection following artificial insemination. All cows in the first and second groups were inseminated artificially on 72<sup>nd</sup> and 96<sup>th</sup> hours following the second prostaglandin injections. Daily blood samples were collected from cows in both groups from the PGF<sub>2α</sub> administration day to 96<sup>th</sup> hour after administration, in order to determine serum estrogen levels. Highest serum estrogen level was observed on the 3<sup>rd</sup> day after PGF<sub>2α</sub> administration in both (I. and II. groups) groups. Pregnancy was achieved in 32 of 46 cows (69.6%) in the first group and in 29 of 42 cows (64.1%) in the second group. In the first group, 11 (50%), 22 cows which had received GnRH after being inseminated conceived in the first cycle and other 8 conceived in the second cycle (36.4%). Eight of the cows which had not received GnRH conceived in the first cycle (n=22, 36.4%) and 5 (22.7%) cows conceived in the second cycle. In the second group 10 of 20 cows (n=20, 50%), which had received GnRH after being inseminated conceived in the first cycle and 6 (30%) conceived in the second cycle. Eight of the cows which had not received GnRH (n=19, 42.1%) conceived in the first cycle and 5 (26.3%) in the second cycle. Administration of PGF<sub>2α</sub> analogues introduced for induction of oestrus cycles and ovariun activities in postpartum anoestrus events, made no improvement in oestrus and pregnancy rates. However, administration of GnRH together with prostaglandins makes a positive effect on the number of inseminations per pregnancy and pregnancy rates.

Key Words: Postpartum cow, PGF<sub>2α</sub> analogues, Pregnancy rates, GnRH

Introduction

The period starting from parturition until the genital organs reach their normal dimensions and state suitable for pregnancy, is called the ‘postpartum period’. During this period, involution and atrophy of uterus, building up of deep tissues of the endometrium and uterus, re-starting of ovarian functions and cyclic activities and elimination of uterus lumen take place. As the result of these 4 fundamental activities, genital organs are returned to their normal pre-pregnancy status (2, 5, 14).

The postpartum period, which is also called the puerperal period, is one of the important periods in the reproductive life of a female. It is very important for the animal in order to continue its future reproductive life in normal limits to complete this period as soon as possible and without any problem (15).

A healthy puerperium is closely related with pregnancy and normal gestation (15). In dairies, reproductive efficiency is evaluated by high milk yield, health condition of animals and a good fertility. With poor fertility, not only the number of offsprings
decreases, but also keeping non-pregnant animals makes losses in milk yield and causes economic loss. A satisfactory fertility rate means getting a calf from each cow regularly every year. This can only be maintained with a healthy puerperium for the cows (12).

Prostaglandins are tissue hormones and are also known as ‘Autocids’. There are four prostaglandin groups, which are A, B, E, F according to their structure and function. Out of these, PGF$_{2\alpha}$ and Prostaglandin E are the most important ones which have direct effect on reproductive organs and fertility. Prostaglandins are synthesized form arachidonic acid. Purified PGF$_{2\alpha}$ was tried for the first time in 1944 for clinical use. The very first PGF$_{2\alpha}$ analogues were used in 1970 for treatment. These are Fluprostenol, Cloprostenol, Dinoprost, Tiaprost, Luprostiol (1, 6, 19, 20, 23).

Contractions of the uterus after delivery continue for a while under the effect of PGF$_{2\alpha}$ and oxytocin. The majority of involution is completed during the first 5 days. Recent studies have revealed that it is PGF$_{2\alpha}$ which controls involution. Prostaglandin administrations for 10 days starting from the postpartum third day, were reported to complete involution in 6-13 days (9, 11).

PGF$_{2\alpha}$ administrations have the advantages of causing estrus synchronization, making artificial insemination easier, increasing the success of artificial insemination and shortening the estrus detection time. For estrus synchronization, 0.5 mg cloprostenol and 25 mg dinoprost is administered by I.M. route and estrus symptoms are expected to occur within 2-4 days. PGF$_{2\alpha}$ analogues are used in the infertilities and also to terminate unwanted pregnancies such as mummification, pyometra and metritis (4, 8, 19).

Kristula and Bartholomew (10) reported that PGF$_{2\alpha}$ analogues avoid retensio secundinarium during the postpartum period and achieve a 20% higher pregnancy rate. Pursley et al (17) reported a higher pregnancy rate in the PGF$_{2\alpha}$ + GnRH group when cows were treated with I.M. PGF$_{2\alpha}$ between the postpartum 36-280th days and inseminated during the heat occurring in the following 48-72 hours.

The goal of this study was to investigate the effects of PGF$_{2\alpha}$ analogues and GnRH on estrus, serum 17$\beta$ levels and pregnancy rates in cows not having spontaneous estrus during the 55-65th postpartum days.

**Material and Methods**

The study was conducted on a total of 88 Holstein cows between the ages of 3-8 years, which had no spontaneous estrus in postpartum 55 days. The cows were divided into two groups (I. group n=46, II. group n=42). The cows in the first group received a 0.5 mg dose of cloprostenol twice with 11 days interval. The cows which came to heat in this group were then divided into two sub-groups and one of these sub-groups was treated with 2.5 ml GnRH (Receptal$^\text{TM}$ Topkim) simultaneously with artificial insemination during the heat occurring at 96. hour following the second cloprostenol administration.
The 42 cows in the 2nd group received 25 mg dinoprost doses with 11 days interval. The 2nd group cows were divided also into two sub-groups. One of these sub-groups was treated with GnRH. The cows in the 1st and 2nd groups were inseminated artificially at 72, and 96 hours following the second prostaglandin treatment. At 35-45 days after the inseminations, pregnancies were diagnosed by rectal palpation, non-pregnant cows were treated again with prostaglandin, their cycles were induced and inseminated. Hormone evaluations were not done in cycles induced a second time.

Daily blood samples were collected for serum estrogen evaluation from 1st and 2nd group cows, starting from the second prostaglandin treatment until 96 hours after. Sera were separated and kept at -20°C until evaluation. Hormone was determined by commercial estrogen kits using the RIA technique.

Pregnancy rates and serum estrogen values among the groups were compared using Duncan tests.

Findings

Serum estrogen values of the groups I and II are presented in Figure 1. The highest estrogen levels were observed in both groups on the 3rd day after prostaglandin administration (Group I: 7.5 pg/ml, Group II: 7.3 pg/ml). When serum estrogen values of cloprostenol and dinoprost treated groups were compared, no significant difference was observed statistically (P > 0.05).

Figure 1. Serum estrogen values in Group I and II.
Estrus and pregnancy rates of Group I and II are presented in Table 1. There were 32 (69.6%) pregnancies out of 46 cows in group I, and 29 (69.1%) pregnancies out of 42 cows in group II. Eleven (50%) of the cows in the 1st group, which received a GnRH dose (n=22) following insemination, conceived at the first induced cycle and 8 (36.4%) conceived at the second.

Table 1. Oestrus and pregnancy rates of Group I and II.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cows come to Heat (n)</th>
<th>Cows conceived</th>
<th>Conceived/come to Heat (%)</th>
<th>Conceived/Total animals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I n=46</td>
<td>44</td>
<td>32</td>
<td>72.7</td>
<td>69.6</td>
</tr>
<tr>
<td>(Cloprostenol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II n=42</td>
<td>39</td>
<td>29</td>
<td>74.4</td>
<td>69.1</td>
</tr>
<tr>
<td>(Dinoprost)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The pregnancy rate of the GnRH treated cows in Group I (n=22) was 86.4% and in those not treated (n=22) this was 59.1%. In Group II, 10 (50%) cows which received GnRH following insemination (n=22) conceived at the first cycle, and 6 (30%) at the second. Out of 19 cows, which did not receive GnRH in this group, 8 (42.1%) conceived at the first cycle and 5 (26.3%) at the second cycle. Total pregnancy rate of the GnRH treated cows in Group II (n=20) was 80% and in untreated cows this was 68.4% (Table 2).

Table 2. Pregnancy rates of Group I and II animals treated with GnRH and those not treated with GnRH.

<table>
<thead>
<tr>
<th>Number of Insemination</th>
<th>Group I n=46</th>
<th>Group II n=42</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment with GnRH (n=22)</td>
<td>No treatment with GnRH (n=22)</td>
</tr>
<tr>
<td>Pregnant at the first cycle (n)</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Pregnant at the second cycle (n)</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Pregnancy rates at the first cycle (%)</td>
<td>50</td>
<td>36.4</td>
</tr>
<tr>
<td>Pregnancy rates at the second cycle (%)</td>
<td>36.4</td>
<td>22.7</td>
</tr>
</tbody>
</table>
When the general pregnancy rates between the groups were evaluated statistically, no significant difference was observed (P>0.05), but the difference between GnRH treated and nontreated cows’ pregnancy rates was significant (P<0.05).

**Results and Discussion**

The postpartum period is a very important time especially in dairies, to maintain the fertility of the cows. Studies related to silent heat, speeding uterine involution and restarting ovarian activities in high yielding cows during postpartum period are of great value (18, 21, 22).

The highest estrogen values in the cloprostenol and dinoprost groups were observed on the 3rd day following treatment (I. group: 7.5 pg/ml, II. group: 7.3 pg/ml), suggesting the oestruses occur between 48-72 hours after prostaglandin treatment. Hariadi et al (7) reported achieving heat in cows synchronized with PGF$_{2\alpha}$ in an average 3.2 ± 0.1 days and Peters et al (16) reported this period as 72-96 hours. The highest estrogen levels on the 3rd day of cloprostenol and dinoprost treated cows and their symptoms in our study have been similar to those of other researchers.

In the first group, 50% of the post-insemination GnRH treated cows became pregnant at the first heat, 36.4% at the 2nd heat, 36.4% of those cows which were not treated with GnRH (n=22) became pregnant at the first cycle and 22.7% at the 2nd cycle. 50% of the GnRH treated cows in the second group (n=20) became pregnant at the first and 30% at the 2nd cycle, 42.1% of the ones which were not treated (n=19) became pregnant at the first and 26.3% at the second cycle. Xu and Burton (24) inseminated cows by using a GnRH + prostaglandin analogue or only a prostaglandin analogue and scored 85.6% and 81.2% pregnancy rates, respectively. De Rensis et al. (3) reported a 56% pregnancy rate in a GnRH + PGF$_{2\alpha}$ treated group. Momcilovic et al. (13) claimed that they have used GnRH following insemination in naturally oestrous showing cows and had higher pregnancy rates. Although there was no statistical difference between the cloprostenol and dinoprost groups’ pregnancy rates (p>0.05) in the present study, the total pregnancy rates of GnRH treated cows were 86.4% in group I and 80% in group II. These values were similar to Xu and Burton’s (24) and higher than De Rensis et al. (3). These rates were relatively higher than the rates of cows which were not treated with GnRH and these results were in accordance with the results of Momcilovic et al. (13). There was no statistical difference between the total pregnancy rates of Group I and II, showing that there is no difference between the prostaglandin analogues relating to pregnancy.

In conclusion, it can be said that treatment with PGF$_{2\alpha}$ analogues make no considerable difference on oestrous and pregnancy rate when administered during postpartum anoestrous to induce the oestrous cycle and ovarian activities in high yielding dairy cows. However, when GnRH is also administered with PGF$_{2\alpha}$ analogues, some beneficial effects on pregnancy rate and number of insemination per animal were observed.
References


