Introduction

A recent study conducted upon the reproductive status of dairy cattle in Kars province showed that the widespread abnormal vaginal discharge (84.4%) and infertility (40%) were among the most common clinical problems encountered at both farm and small family breeding levels during the postpartum period (1). This is not surprising since the cows are housed mostly under...
rather primitive conditions (poor nutrition and management) (2) due mainly to the harsh climate (long winter) and high altitude (limited pasturelands) in this area. Hence, these severe conditions force researchers to design new strategies using multidisciplinary approaches to combat reproductive problems such as infertility.

Attempts to improve pregnancy rates following AI have been reviewed recently (3). Many factors (hormonal imbalance, micro-organisms, etc.) play crucial roles in the aetiology of infertility. Studies using various exogenous hormones (e.g., $\text{PGF}_2$) and/or intrauterine antimicrobials (e.g., gentamicin) have been carried out to overcome infertility in cows reared in Kars (4-6). As compared to $\text{PGF}_2$ injection only, $\text{PGF}_2$ supplemented with gentamicin sulphate can yield better calving rates in repeat breeding cows, especially during winter (6). Additionally, it was noted that higher levels of fertility can be achieved by progestagen analogues as compared to $\text{PGF}_2$ injections only, particularly in cows with inactive ovaries (7). Likewise, the injection of $\text{PGF}_2$ before the removal of progestagen sponges or the PRID increases the rates of oestrus synchronisation (8). However, there appears to be no literature available on the use of $\text{PGF}_2$ and gentamicin or their combination in a PRID regime in infertile cows in Kars province.

Therefore, the efficacy of $\text{PGF}_2$ and intrauterine gentamicin or their combination in a PRID regime was investigated for the treatment of infertility in cows.

Material and Methods

Animals: A total of 46 infertile (suboestrous, repeat breeder or anoestrous, determined by anamnesis as well as by rectal palpation), lactating cows of various ages (3 to 10) and breeds (mostly cross-bred) were used during winter (January and February). All the animals were privately owned (less than 5 cows for each breeder) and were kept in rather primitive indoor shelters. They were fed mainly barley straw or grass hay (poor or medium quality) as well as bran and/or ground barley and salt, together with water ad libitum.

Experimental protocols: Of the 46 infertile cows, body condition scores (BCSs, 1-5 scale: 1 = emaciated to 5 = obese, with 0.25 unit increments) as assessed by the method described by Lowman et al. (9) were recorded prior to the experiment. The BCSs of cows were up to 3.00 units, ranging from 2.00 to 3.00 units. Animals were then divided into 4 balanced (by BCS) experimental groups as follows: i) PRID (1.55 g of progesterone plus 10 mg of oestradiol benzoate) (Sanofi DIF Doğu İlaç AŞ, İstanbul) for 12 days and followed by AI at 48 and 72 h (group 1, n = 8), ii) PRID for 12 days plus an i.m. injection of 0.150 mg of cloprostenol (Dalmazin®, VETAŞ, İstanbul) on day 10 and AIs as described in group 1 (group 2, n = 10), iii) PRID for 12 days and AIs followed by intrauterine administration of 250 mg of gentamicin sulphate (Gentasol® inj., ECZACIBAŞI, İstanbul) in 50 cc of 0.9% NaCl solution 30 min after the last insemination (group 3, n = 18), and iv) PRID for 12 days plus an injection of cloprostenol on day 10 and AIs followed by gentamicin administration after the last insemination (group 4, n = 10).

Rectal palpation: The ovarian (inactivity as well as the presence of any follicles or corpus luteum, the feeling of small, dry and hard-surfaced ovaries etc.) and uterine status (uterine tonicity related to oestrus as well as the presence of possible infection, etc.) of the cows were determined both before the experiment and at the time of each AI.

Parameters measured: The oestrus signs (as determined mainly by observation of standing heat and vaginal discharge, rectal palpation of follicular development and uterine tonicity, etc.) and the 60-120 days NRR % following the first service AIs were recorded.

Statistical analysis: Data represented as means (± SEM) were analysed by correlation and regression analysis (of the initial reproductive status of infertile cows) along with chi-square analysis within the Minitab statistical software (Minitab for Windows) (10). Differences between the experimental groups were considered significant when P < 0.05 (11).

Results

Details of the oestrus synchronisation protocols are presented in Table 1. For each experimental group, the mean BCS and initial reproductive status of cows are also given. The condition scores of cows in groups 1 to 4 were $2.63 ± 0.07$, $2.58 ± 0.04$, $2.65 ± 0.09$ and $2.48 ± 0.08$ units, respectively (P > 0.05).
The oestrus rates in groups 1 to 4 were 75.0 – 16.4%, 90.0 – 10.0%, 72.2 – 10.9% and 100.0 – 0.0% with no significant differences between them. Likewise, there were no significant differences in the NRRs between the groups (1-4): 37.5 – 18.3%, 30.0 – 15.3%, 27.8 – 10.9% and 60.0 – 16.3%, respectively. However, oestrus rates and NRR % were numerically higher in cows in group 4 (PRID + PGF$_2$a + gentamicin) than those in the other groups. The results are summarised in Tables 2 and 3.

Discussion

Oestrus synchronisation is a very useful technique in cattle practice as it allows fixed-time AI or improved heat detection efficiency. Interest in synchronising oestrus has further increased with the introduction of improved techniques using combinations of GnRH or prostaglandin in AI programmes. The PRID (mainly progesterone) has also been widely employed with success by specialists of reproduction across European countries. It is also becoming increasingly popular in Turkey but with rather limited awareness still among local farmers/breeders. From this standpoint, the present study is the first scientific attempt to introduce PRID into veterinary practice in our region (north-eastern Turkey), where geographical and climatic conditions are rather severe.

In the present study, it appeared that the NRR (%) in “PRID + PGF$_2$a” (30%) and “PRID + gentamicin” group (27.8%) was similar to those reported previously (12-14). Lourens (15) reported that the pregnancy rate after the

<table>
<thead>
<tr>
<th>Group</th>
<th>Protocols used</th>
<th>n</th>
<th>Initial reproductive status of infertile cows $^\dagger$</th>
<th>BCS (1-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SO(n)</td>
<td>RB(n)</td>
</tr>
<tr>
<td>1</td>
<td>PRID 12 days + AIs</td>
<td>8</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>PRID + PGF$_2$a day 10 + AIs</td>
<td>10</td>
<td>2*</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>PRID + AIs + gentamicin</td>
<td>18</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>PRID + PGF$_2$a + AIs + genta.</td>
<td>10</td>
<td>5</td>
<td>3</td>
</tr>
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|               | SO: Suboestrus; RB: Repeat Breeder; AO: Anoestrus; $^\dagger$ According to rectal palpation findings; |
|               | * According to the anamnestic information. |

Table 1. The animal profiles in different oestrus synchronisation protocols using PGF$_2$a and gentamicin or their combination in a PRID regime.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Oestrus (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>75.0$^\dagger$ ± 16.4</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>90.0$^\dagger$ ± 10.0</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>72.2$^\dagger$ ± 10.9</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>100.0$^\dagger$ ± 0.0</td>
</tr>
</tbody>
</table>

$^\dagger$ Means having the same superscript within the same column are not significantly different (P > 0.05).

Table 2. The effects of PGF$_2$a and gentamicin or their combination in PRID regime on the oestrus in infertile cows.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>NRR % (60-120 day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>37.5$^\dagger$ ± 18.3</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>30.0$^\dagger$ ± 15.3</td>
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<tr>
<td>3</td>
<td>18</td>
<td>27.8$^\dagger$ ± 10.9</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>60.0$^\dagger$ ± 16.3</td>
</tr>
</tbody>
</table>

$^\dagger$ Means having the same superscript within the same column are not significantly different (P > 0.05).
first service AI was 77.8% following a PRID regime, but the animals used in that trial were 18-month-old, well-managed heifers in a commercial dairy herd. In contrast, the present study was carried out in mature, poorly conditioned infertile cows that had been sheltered in rather primitive conditions. There are many other factors such as early embryonic death, infectious agents and poor nutrition that can decrease the success of synchronisation programmes and pregnancy rates (16). Of course, the ultimate goal of all oestrus synchronisation programmes in cows, as with other livestock species, is to achieve an optimum success rate, a live calf. Hence, these undesirable conditions given should always be considered (and optimised where possible) for comparisons of different studies conducted by other researchers. Furthermore, the present results of “PRID + PGF$_{2\alpha}$ + gentamicin” are higher than those reported by Mateus et al. (17) using “PRID + PGF$_{2\alpha}$ + eCG”: the oestrus rates were 100% vs. 93% while the NRRs were 60% vs. 36%, respectively. These differences between the studies may also be due to some other co-factors such as BCS (13), physiological status or using some combined hormones, e.g., eCG and PMSG (14,18), that can all affect the outcome of reproductive manipulations in a broad sense.

Although there were no significant differences in any of the parameters studied between the experimental groups, NRRs appeared to be higher in the “PRID + PGF$_{2\alpha}$ + gentamicin” group (60%), as compared to others (ranging from 27.8% to 37.5%). It was also observed that the NRR in the “PRID + gentamicin” group was rather low (27.8%). Therefore, these results may indicate that PGF$_{2\alpha}$ could increase the likelihood of successful conception. Indeed, it has been demonstrated that the use of PGF$_{2\alpha}$ along with PRID application improved the pregnancy rates (13,14,19). Likewise, Munro and Moore (20) found that the poorer calving performance of cows was associated with relatively high peripheral levels of plasma progesterone at the time of PRID removal, implying the presence of residual secretory luteal tissue that needs to be removed. Indeed, progesterone administration is considered an ‘effective’ method in timed AI when it is combined with a luteolytic agent (21). In this context, PRID is an intravaginal device that has a gelatine capsule containing oestradiol attached to act as a luteolytic. Oestradiol is not fully effective as a luteolytic in cattle; therefore, slightly improved synchrony can be expected if prostaglandin is given prior to PRID withdrawal. This will remove any luteal tissue that has failed to regress due to initial oestradiol administration (21). Indeed, the oestrus rate (100%), as with NRR (60%), appeared to be highest in the “PRID + PGF$_{2\alpha}$ + gentamicin” group in this study. Hence, it is considered that these results may be due to the use of PGF$_{2\alpha}$ injection plus gentamicin administration (6). Indeed, Öztürkler et al. (6), using “PGF$_{2\alpha}$ + gentamicin + hCG”, achieved a higher treatment/calving rate, as compared to controls with PGF$_{2\alpha}$ only (38.9% vs. 15%, respectively). Furthermore, Öztürkler and Uçar (3) concluded from the literature that the bacteria isolated from cultures of the genital tract of repeat breeder animals were claimed to have the highest sensitivity against gentamicin, as compared to many other antibiotics. Hence, we did not undertake any such tests in the present study. However, it was likely that the cows used herein might have had mild (subclinical) endometritis, but this could not have been observed in our routine examinations (by rectal palpation) or inspections, as reported previously (16). Therefore, we further considered that it would be logical to include an antimicrobial, namely gentamicin, to combat possible genital infections (with no apparent signs). Moreover, a number of previous studies (22-24) showed that PGF$_{2\alpha}$ injections prior to AI could be used not only for synchronisation but also for treatment of endometritis. Therefore, it is considered that the combination of PGF$_{2\alpha}$ and gentamicin in a PRID regime may be beneficial to solve the infertility problem in cows, particularly for those having poor or medium BCS and being sheltered in rather primitive conditions as studied herein. For confirmation, further studies are needed in future.

Finally, there was no significant correlation or relationship between the initial reproductive status (SO, RB and AO) of infertile cows either with oestrus or with NRR% in the PRID regime used. Hence, the present findings imply that the nature of initial infertility may have a limited effect on the rates of oestrus and NRR in the PRID regime used in cows.

In conclusion, the overall results suggest that oestrus synchronisation by PRID combined with PGF$_{2\alpha}$ and intrauterine gentamicin administration after AI may increase the oestrus and pregnancy rates in infertile (suboestrous, repeat breeder or anoestrous) cows. However, further studies with a higher number of cows, to be sheltered in similar nutritional and managemental conditions, are warranted to achieve more reliable oestrus synchronisation and fertility data.
References


