Influence of dose, repeated treatment and batch of hormone on ovarian response in heifers treated with PMSG

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Summary. The effect of two dose levels (1000 and 2000 i.u.) of three different commercially available batches of PMSG on the ovarian response (ovulations and follicles > 10 mm) of 42 heifers was examined in a randomized incomplete block experiment. Each animal was subjected to two consecutive but different treatments.

A significant effect of dose was observed and there were fewer ovulations, but no reduction in the number of follicles, after the second PMSG treatment. There was no evidence that the ovarian response was affected by the PMSG batch used.

Introduction

The ability of PMSG to induce multiple ovulations has led to the widespread use of this hormone for stimulating superovulation in cattle for the purpose of embryo transfer and preservation (see Gordon, 1975, for review). The larger the dose of hormone administered the greater is the ovarian response and the greater is the variability in response (Folley & Malpress, 1944; Gordon, Williams & Edwards, 1962; Lamond, 1970; Mauléon, Mariana, Benoît, Solari & Chupin, 1970; McGaugh & Olds, 1971; Gordon, 1975). Many authors have reported wide individual variations in ovarian response both in heifers and lactating cows when injected with a standard dose of PMSG (Rowson, 1951; Gordon et al., 1962; Hafez, Sugie & Hunt, 1963; Scanlon, Sreenan & Gordon, 1968; Mariana, Mauléon, Benoît & Chupin, 1970; Gordon, 1975; Newcomb, 1976). Many authors, including Baker (1973) and Polge & Rowson (1973), have suggested that at least part of this variability in response may be caused by variation between batches of PMSG in their ability to induce superovulation.

It is known that when heifers are treated with FSH, those preparations which contain substantial amounts of LH will stimulate follicular growth to a degree which is comparable with that obtained with higher doses of the more purified FSH preparations (Laster, 1972). PMSG has FSH and LH biological activities (Cole, 1936; Bangham & Woodward, 1966; Gospodarowicz, 1972) and this has led to the suggestion that the ratio of these components in different batches of PMSG may affect ovarian response (Lamond, 1970).

Stewart, Allen & Moor (1976), using rat testis radioreceptor assays to measure FSH and LH activity, found no significant difference in the FSH:LH ratios of 6 batches of commercially available PMSG (Folligon: Intervet Laboratories, UK). Schams et al. (1978), using similar radioreceptor assay methods, found similar FSH:LH ratios in 3 other batches of PMSG (Intervet Int. Ltd, Oss, The Netherlands). Although Stewart et al. (1976) reported no significant differences between groups in the mean ovulation rates recorded in a total of 99 heifers treated...
with one or other of 4 of the 6 batches of Folligon assayed in their study, these animals did not form part of a controlled experiment and other details of the ovarian response to the gonadotrophic stimulation were not recorded. In this paper we report the results of a planned and carefully controlled experiment to determine the influence of dose and batch of hormone in the heifer subjected to two consecutive PMSG treatments.

**Materials and Methods**

**PMSG**

The total gonadotrophic potencies of 3 distinct master batches of Folligon (Intervet Laboratories, UK) were measured by a rat ovarian weight bioassay (Fevold, 1937) using the second international standard of serum gonadotrophin (2nd IRF-PMSG) as the standard. The FSH-like and LH-like activities of the 3 batches were measured by rat testicular radioreceptor assays using highly purified human pituitary FSH and LH preparations as radiotracers and as standards (Stewart *et al*., 1976). Each batch of PMSG had been stored at $-20^\circ$C until used or assayed and each dose of PMSG for injection into animals was reconstituted in 10 ml physiological saline (9 g NaCl/l) immediately before injection into the gluteal region.

**Animals and treatment**

The 42 Hereford-cross heifers used were 15–18 months of age and had normal reproductive tracts, as determined by rectal palpation. Each animal was treated once with an anthelminthic (Nilverm: ICI, Macclesfield) and each had shown at least one normal oestrous cycle before the start of the experiment.

For the first PMSG treatment each animal was given a single i.m. injection of 1000 or 2000 i.u. PMSG (nominal potency) on Day 9, 10, 11 or 12 after oestrus. This was followed 48 h later with 1000 $\mu$g of a prostaglandin F-2α analogue, cloprostenol (Estrumate: ICI, Macclesfield), i.m. and the animals were inseminated once with fresh extended semen at the ensuing oestrus.

On Day 7 after oestrus and following fasting for 48 h the heifers were anaesthetized and the ovaries examined via a midventral laparotomy. The number of corpora lutea and the number of follicles $>10$ mm in diameter in the two ovaries were recorded. After the initial PMSG treatment all the heifers were allowed one oestrus which occurred either spontaneously or, when animals failed to exhibit oestrus by Day 24 of the treatment cycle, was induced with 500 $\mu$g cloprostenol. During this or the next cycle each heifer was given a second PMSG treatment between Days 9 and 12, followed by 1000 $\mu$g cloprostenol 2 days later in precisely the same manner as at the initial treatment. The mean ($\pm$ s.e.m.) interval between the two treatments was 51.0 $\pm$ 2.4 days.

**Experimental design**

There were 6 treatment combinations consisting of 3 batches of PMSG at each of 2 dose levels (D1 = 1000 i.u., D2 = 2000 i.u.), and since each heifer was treated on 2 occasions, this permitted the use of 2 of the treatment combinations on each animal, corresponding to an incomplete block design. However, since 42 heifers were used, there were 7 complete replicates of the treatment structure at each expected superovulation and individual analysis of the results of each PMSG treatment was quite valid.

Analysis of the results of the second PMSG treatment enabled an investigation of the residual effect of the first PMSG treatment. The incomplete block arrangement made possible a partition of the 'error' into a 'between-animal' and a 'within-animal' component in order to assess the usefulness of blocking in experiments of this type.
**Statistical analysis**

Since the variables of interest in this experiment were either integers or percentages, and so unlikely to satisfy the assumptions of normality and homoscedasticity (equality of variances), various transformations were applied before analysis and the results were compared with the analyses of the raw data. Also, when appropriate, some randomization tests were used to confirm the findings. The results of applying these various techniques were virtually identical so that for ease of interpretation the summarized data in Table 2, which were obtained from analyses of variance, are presented in the original units.

**Results**

As shown in Table 1, the total gonadotrophic potency of Batch 3 was lower than those of Batches 1 and 2, but the FSH:LH ratios of the three batches were not significantly different.

The results in Table 2 show that the higher dose of PMSG gave a substantially increased response in the number of ovulations and follicles, but there was no clear evidence of a dose effect in the proportion of follicles present in the ovaries.

**Table 1.** The total gonadotrophic potency per ampoule (nominal potency 1000 i.u.) and FSH:LH ratio (μg:μg) of the three batches of PMSG used (values in parentheses are the 95% confidence limits)

<table>
<thead>
<tr>
<th>Batch</th>
<th>Total gonadotrophins (i.u.)*</th>
<th>FSH : LH ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code</td>
<td>Number</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1817</td>
<td>1010 (800–1250)</td>
</tr>
<tr>
<td>2</td>
<td>1853</td>
<td>1020 (800–1250)</td>
</tr>
<tr>
<td>3</td>
<td>2101</td>
<td>850 (740–950)</td>
</tr>
</tbody>
</table>

* Relative to the International Standard for PMSG.
† Relative to highly purified standards (Stewart et al., 1976).

**Table 2.** The effect of batch (B) and dose (D) of PMSG on the ovarian response of heifers (no. of animals = 42, total no. of treatments = 84)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean response in relation to batch of PMSG</th>
<th>Mean response in relation to dose of PMSG</th>
<th>Differences between Treatments 1 and 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ovulations</td>
<td></td>
<td></td>
<td>3-12 ± 1·36 (P &lt; 0·03)</td>
</tr>
<tr>
<td>B1</td>
<td>7-95</td>
<td>D1</td>
<td>3-60</td>
</tr>
<tr>
<td>B2</td>
<td>9-41</td>
<td>D2</td>
<td>12-16</td>
</tr>
<tr>
<td>B3</td>
<td>6-28</td>
<td>Diff. = 8·56 ± 1·73 (P &lt; 0·01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>s.e. = 1·43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of follicles ( &gt;10 mm diam.)</td>
<td></td>
<td></td>
<td>N.S.</td>
</tr>
<tr>
<td>B1</td>
<td>3-15</td>
<td>D1</td>
<td>0-83</td>
</tr>
<tr>
<td>B2</td>
<td>3-27</td>
<td>D2</td>
<td>5-50</td>
</tr>
<tr>
<td>B3</td>
<td>3-08</td>
<td>Diff. = 4·67 ± 0·78 (P &lt; 0·01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>s.e. = 0·64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ovarian response (ovulations + follicles &gt; 10 mm diam.)</td>
<td></td>
<td></td>
<td>3·21 ± 1·61 (P &lt; 0·05)</td>
</tr>
<tr>
<td>B1</td>
<td>11-10</td>
<td>D1</td>
<td>4-43</td>
</tr>
<tr>
<td>B2</td>
<td>12-68</td>
<td>D2</td>
<td>17-66</td>
</tr>
<tr>
<td>B3</td>
<td>9-36</td>
<td>Diff. = 13·23 ± 2·05 (P &lt; 0·01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>s.e. = 1·69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of follicles &gt; 10 mm diam.*</td>
<td></td>
<td></td>
<td>12·14 ± 4·92 (P &lt; 0·02)</td>
</tr>
<tr>
<td>B1</td>
<td>29·8</td>
<td>D1</td>
<td>29·49</td>
</tr>
<tr>
<td>B2</td>
<td>22·6</td>
<td>D2</td>
<td>24-43</td>
</tr>
<tr>
<td>B3</td>
<td>28·6</td>
<td>Diff. = 5·06 ± 6·71 (N.S.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>s.e. = 5·4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* (No. of follicles/total response) × 100; results of weighted analyses.
While the number of ovulations occurring after the second PMSG treatment was significantly less than that after the first treatment, there was no similar decrease in the number of follicles, and the proportion of follicles was therefore significantly higher after the second treatment.

There was no evidence that the actual dose of PMSG (1000 or 2000 i.u.) administered initially affected the response to the subsequent treatment. The residual dose effect (total ovarian response (TOR) after the second PMSG treatment (T2) when D2 was administered at the first treatment (T1), minus TOR at T2 when D1 was administered at T1) was estimated as 0.91 ± 2x71 on the total ovarian response. The main dose effect (TOR at D2 – TOR at D1) on the total response was 14.5 ± 3.0 after the first PMSG treatment and 12.26 ± 2.0 after the second.

Although the day of the cycle on which the PMSG was injected varied from 9 to 12 in both halves of the experiment, inclusion of this variable as a covariant in the analysis indicated that it did not exert any systematic effect on the response.

There was no evidence of a differential effect of batch on response nor was the batch × dose effect significant. However, the total ovarian response for Batches 1 and 2 (ovulations + follicles > 10 mm) was higher than that for Batch 3 which showed the lower gonadotrophin potency in the rat ovarian weight test. An examination of the within-batch variability in response did not reveal any differences in the homogeneity of the batches.

In heifers receiving 1000 i.u. PMSG, those with single ovulations had a longer interval between cloprostenol treatment and oestrus (2 days in 5 heifers, >2 days in 11 heifers) than did those with multiple ovulations (2 days in 24 heifers, >2 days in 2 heifers (χ² = 14.5, d.f. = 1, P < 0.001).

Surprisingly, the error variance between animals was not significantly greater than the variation within animals, perhaps reflecting the erratic response of the animals in the two treatments. This suggests that blocking in this type of experiment is not really advantageous.

Discussion

As was expected from earlier studies the higher the dose of PMSG administered the greater was the ovarian response. However, the dosage did not affect the proportion of follicles present in the ovaries and the reduction in the numbers of ovulations at the second treatment could not therefore be attributed simply to an increase in numbers of non-ovulating follicles because although there was a significant increase in the proportion of follicles present at the second treatment there was no absolute increase. The reduction in total ovarian response at the second treatment was therefore attributable to a decrease in the number rather than the proportion of ovulations.

Reduction in the ovarian response of cattle to repeated superovulation treatments has been recorded by several authors (Willett, Buckner & McShan, 1953; Dziuk, Donker, Nichols & Petersen 1958; Hafez, Jainudeen & Lindsay, 1965; Saumande & Chupin, 1977). Ovulation rate also declines in sheep with consecutive PMSG treatments, an effect which appears to be cumulative but which is partly overcome by treating ewes at alternate cycles (Clarke, 1973). The interval between the two PMSG treatments may therefore influence the response at the second treatment although Willett et al. (1953) did not find that prolongation of the intervals between treatments increased number of ovulations. It has been suggested that the injection of exogenous gonadotrophin may, in the rat, result in the loss of specific hormone receptor sites (Conti, Harwood, Hsueh, Dufau & Catt, 1976) although the inhibition of response is temporary, lasting only 5–7 days (Hsueh, Dufau & Catt, 1977), i.e. a much shorter time than the 51-day interval between treatments in our experiment.

An explanation of the reduction in ovarian response to repeat PMSG treatments in the present, and other, reports is difficult in view of findings which indicate that antibodies to PMSG are unlikely to be formed when normal levels of PMSG are administered to cattle (Nakahara, Yamauchi, Kataoka & Kaneda, 1964; Schams et al., 1978) or sheep (Clarke, 1973). The
possibility that the reduced response to repeat treatment might be attributable to immunity to either the FSH or to the LH moieties of PMSG individually seems most unlikely because both activities are located in the β sub-unit of the PMSG molecule (Papkoff, 1978). Examination of the effect of anti-PMSG on the FSH: LH ratio of PMSG (using rat testis radioreceptor assays for the measurement of FSH and LH) has not revealed any alteration in the ratio despite a 60% reduction in total activity (F. Stewart, personal communication).

The fact that the initial dose of PMSG administered did not affect the response to the subsequent treatment indicates that it is improbable that a relative depletion of the antral follicle population in the ovaries after the first treatment could explain a reduced response at the second. It is also unlikely that high steroid levels after the first PMSG treatment (Booth, Newcomb, Strange, Rowson & Sacher, 1975) impaired follicle development because the two doses of PMSG at the first treatment would have been expected to exert different effects on steroid production. The reason for the reduction in response after the second PMSG treatment remains obscure.

The observation that there was no difference in response according to the day on which PMSG was injected, when treatment was initiated between Days 9 and 12 after oestrus, supports previous findings (Newcomb & Rowson, 1976; Greve, 1976) that when PMSG is injected in the mid-luteal phase, after Day 8, there is no significant effect of day of treatment on response.

Although in the present experiment the lowest ovarian response was obtained with the batch of PMSG having the lowest total gonadotrophin potency, the differences were not significant (see Table 2). The finding of non-significant differences in FSH: LH ratio between batches supports the finding of Stewart et al. (1976) and Schams et al. (1978) and also the suggestion that it is unlikely that the variation in response which occurs between animals treated with PMSG is due to differences in the FSH: LH ratio of the PMSG preparations used (Stewart et al., 1976).

The results of this experiment indicate that treatment of heifers with the three different batches of standard commercial preparations of PMSG did not appear to be the cause of the variation in ovarian response.

We are indebted to Intervet Laboratories Limited for the supply of PMSG and 30 heifers. Mrs Francesca Stewart kindly assayed the FSH and LH activities of the PMSG batches used and Organon B.V., Oss, assayed the total gonadotrophin potency. We are grateful to Mr Peter Jackson, I.C.I., Macclesfield, for the gift of cloprostenol and to Dr W. R. Allen for reading and discussing this manuscript. One of us (R.N.) is secondment from the Milk Marketing Board (England and Wales).

References

Gospodarowicz, D. (1972) Purification and physico-


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