The Effect of Human Chorionic Gonadotropin on the Reproduction Performance in Lory Sheep Synchronized with Different Doses of Pregnant Mare Serum Gonadotrophin Outside the Breeding Season

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Abstract: Two experiments performed to determine the effects of different doses of PMSG and subsequent hCG treatment on the reproductive performance in estrus-induced mature Lory ewes. In first experiment 192 Lory anestrous ewes were divided into two groups and after synchronization with prostegestan sponge (Fluorogestone acetate, 40 mg FGA) the ewes in first group (T1) were injected 400 IU PMSG and in second group (T2) were injected 600 IU PMSG intramuscularly at sponge removal time. At insemination time (AI) time, ewes divided into 4 subgroups; T1 and T2 were injected 200 IU hCG and T1C and T2C were kept as the controls. In second experiment the effect of supplementing hCG at AI time or 12 days after AI were measured on the reproductive performance using 374 estrus-induced mature Lory ewes. After synchronization with prostegestan sponge, all ewes were injected 400 IU PMSG. The ewes then, were randomly divided into three groups: the ewes in (h0) were injected 200 IU hCG at AI time, (h1) were injected 200 IU hCG at day 12 after mating time and (C) were kept as the control group. Serum progesterone P4 concentrations were measured in days 12, 14 and 16 after AI in both experiments. The result of 1st experiment indicated that single lambs in T2 subgroup had higher weight compared with T1C subgroup at birth day (p<0.05). The prolificacy were higher in hCG treated groups compared with control (p<0.05). However, fertility did not differ significantly among subgroups. Mean weight of single lambs born was increased in T2 compared with T1C and T1h subgroup had higher P4 concentration compared with T1C subgroup (p<0.05). In Experiment 2, in comparison with control, the hCG increased prolificacy in h0 treatment (p<0.05). Mean weight of lambs born was significantly increased in h0 and h1 groups compared with control. The hCG increased P4 concentration in h0 and h1 group and the h1 had higher P4 concentration compare with other groups (p<0.05). It can be concluded that hCG injection at AI time increased progesterone concentrations and subsequent could improve reproductive performance in Lory ewes but there were no differences between the ewes treated with 400 or 600 IU PMSG.

Key words: Progestagen sponge, estrus synchronization, reproductive performance

INTRODUCTION

Treatment with intravaginal sponge impregnated with FGA for a period of 10-16 days and intramuscular injection of PMSG at intravaginal device removal, have been successfully used to improved the reproductive performance in ewes (Gomez-Brunet et al., 2006). Intramuscular administration of 400, 500 and 700 IU PMSG in sponge removal time increased the ratio of ovulation and twinning (Mehmet et al., 2006). In sheep, 30-40% of fertilized eggs are lost during the first 3 weeks of pregnancy. One of the major causes of embryonic loss is likely to be the inadequate luteal function (Ashworth et al., 1989).
Human Chorionic Gonadotropin (hCG), which is similar to LH in function, has been shown to increase luteal weight and endogenous synthesis of progesterone from the Corpus Luteum (CL) in sheep (Nephe et al., 1994). The increase in P4 concentrations after hCG treatment suggests that hCG by its LH like activity may provide luteotrophic stimulation to CL. This luteotrophic stimulation may either be in the form of conversion of small luteal cells to large luteal cells which then secrete higher concentrations of progesterone or may even be due to an increase in the size of large luteal cells (Khan et al., 2006). The beneficial effect of hCG administration on embryo survival may be through the stimulatory effect of hCG-induced progesterone on fetal growth, because it has been shown that progesterone supplementation increased subsequent fetal growth (Klæbaum et al., 1994).

The hCG has been administered to ewes at different times during the cycle after AI or breeding in an attempt to reduce embryonic mortality and improve reproductive performance, but the effectiveness of these treatments has not been consistent between studies and the timing of such hormonal treatments also may seem to be important. The administration of hCG on the day of mating, 4, 5 and 12 days post mating have been reported by Peters (1996), Thatcher et al. (2001), Carn et al. (2002) and Khan et al. (2003).

Ishida et al. (1999) and Fukui et al. (2001) reported that the hCG treatment given at the early luteal phase increase the plasma P4 levels in hCG treated ewes, but this was not reflected in the pregnancy and lambing rates of the inseminated ewes. However, to improve the fertility, hCG would have to increase the fertilization rate and reduce the embryonic death rate or both.

There is a little information regarding synchronization efficiency and fertility induced by administration of hormones in Iranian Lory ewes during breeding and outside the breeding season. The purpose of this study was to investigate the effect of different doses of PMSG at sponge removal and the time of hCG administration on reproductive performance in Lory ewes outside of the breeding season.

**MATERIALS AND METHODS**

Two experiments were carried out in Iran, Lorestan, Pol dokhtar township, (latitude 43°-09°N), at 713.5 m above sea level during spring 2006. The ewes had access to the nearby stubble and low quality ranges for 8 h a day. Each ewe also received a diet on a daily basis consisting of 3 kg corn silage, 700 g chopped wheat straw, 200 g alfalfa hay and 150 g barley grain. The experiments started on 25 November, 2006. Estruses were induced by treating all ewes with an intravaginal sponge impregnated with synthetic progestagen (40 mg FGA, Intervet) for 13 days. Ewes were observed for estrus using teaser rams. Semen from 50 selected rams was collected using an artificial vagina and all of the ewes in estrous were inseminated civically 48 h after sponge removal with fresh semen which it was diluted with homogenized milk.

The number and weight of lambs born were recorded at lambing time. Blood samples were collected from jugular vein on days 12, 14 and 16 after AI from 20% of ewes chosen at random from each group. Blood samples were centrifuged to separate serum and were stored at -20°C until analyses for progesterone concentration.

**Experiment 1**

This experiment performed to determine the effects of PMSG treatments and subsequent hCG treatment on progestagen-synchronised Lory breed ewes. A total of 192 anestrous ewes, 4-5 years of age with a 35±4 kg body weight, were randomly divided into two groups and after synchronization with progestagen sponge, at sponge removal time, the ewes in first group (T1) were injected (i.m.,) with 400 IU PMSG and the ewes in second group (T2) were injected (i.m.,) with 600 IU PMSG. At insemination time (AI), each groups divided into 2 subgroups, T1h and T2h were injected 200 IU hCG and T1C and T2C were kept as the control groups.
Experiment 2
A total of 374 cyclic Lory ewes, 4-5 years of age with a 35±4 kg body weight, were used in this experiment. In this experiment the effect of supplementing hCG at AI or 12 days after AI were measured on the reproductive performance. After synchronization with progestagen sponge the ewes were randomly divided into three groups; the ewes in (h) were injected 200 IU hCG at AI time, ewes in (h_1) were injected 200 IU hCG, 12 days after mating time and the ewes in (C) were kept as the control group.

Statistical Analysis
The experimental design for first experiment was a 2×2 factorial. Data for serums P4 analyzed by use of a repeated measure procedure. The means were compared using Duncan’s multiple range tests when ANOVA indicated significant (p<0.05). Prolificacy (No. of lambs born alive per ewe lambing), fertility (% ewes lambing per ewes mated) were assessed by Chi-squared analysis. The data for mean birth weight were analyzed by ANOVA test.

RESULTS

Experiment 1
The dosage of PMSG and time of hCG injection did not increase fertility and prolificacy in the subgroups (Table 1).

Mean weight of lambs born was significantly increased in T_1h compared to T_1C in single lambs (p<0.05).

The mean serum P4 profiles of the ewes in the treatment groups are depicted in Table 2. The P4 concentration was decreased in days 14 and 16 in all groups, however in T_1C and T_1C the P4 concentrations were decreased significantly (p<0.05). In day 16, T_1h has higher P4 concentration compared with T_1C and T_1C subgroups but not statistically significant (p>0.05). Ewes in T_1h subgroup had higher P4 concentration in day 16 compare with controls (P<0.05) (Table 2).

Experiment 2
The fertility and prolificacy was higher in h1 compared with h2 and control. The hCG injection increased prolificacy in h1 group which was statistically significant (p<0.05) (Table 3). Mean weight of lambs born was significantly increased in h1 and h2 groups compare with control group in single lambs (Table 3).

Table 1: Reproductive performance of ewes in 1st experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>T_1h</th>
<th>T_1C</th>
<th>T_1h</th>
<th>T_1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ewes</td>
<td>48</td>
<td>50</td>
<td>48</td>
<td>46</td>
</tr>
<tr>
<td>Fertility (%)</td>
<td>45.8</td>
<td>36</td>
<td>37.5</td>
<td>39.1</td>
</tr>
<tr>
<td>Prolificacy (%)</td>
<td>145</td>
<td>100</td>
<td>144</td>
<td>111</td>
</tr>
<tr>
<td>Mean birth weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singles</td>
<td>4.2±0.13</td>
<td>3.5±0.21</td>
<td>3.8±0.15</td>
<td>3.8±0.09</td>
</tr>
<tr>
<td>Twins</td>
<td>2.3±0.33</td>
<td>-</td>
<td>2.7±0.2</td>
<td>2.2±0.12</td>
</tr>
<tr>
<td>Triplets</td>
<td>2.0±0.20</td>
<td>-</td>
<td>1.6±0.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Different superscripts in Rows differ significantly, a, b: p<0.05

Table 2: Mean serum P4 concentrations (ng/mL) of the ewes in treatment groups (Experiment 1)

<table>
<thead>
<tr>
<th>Days after AI</th>
<th>T_1h</th>
<th>T_1C</th>
<th>T_1h</th>
<th>T_1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>4.2</td>
<td>3.8</td>
<td>5.9</td>
<td>5.0</td>
</tr>
<tr>
<td>14</td>
<td>3.8</td>
<td>2.5</td>
<td>5.6</td>
<td>3.3</td>
</tr>
<tr>
<td>16</td>
<td>3.6</td>
<td>2.9</td>
<td>5.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Different superscripts in rows and columns differ significantly, a, b: p<0.05

Table 3: Reproductive performance of treatments ewes (Experiment 2)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>h1</th>
<th>h2</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ewes</td>
<td>128</td>
<td>121</td>
<td>125</td>
</tr>
<tr>
<td>Fertility (%)</td>
<td>50</td>
<td>47.9</td>
<td>35.2</td>
</tr>
<tr>
<td>Prolificity (%)</td>
<td>160º</td>
<td>129º</td>
<td>118º</td>
</tr>
<tr>
<td>Mean birth weight (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singles</td>
<td>3.75±0.14º</td>
<td>3.70±0.12º</td>
<td>3.20±0.12º</td>
</tr>
<tr>
<td>Twins</td>
<td>2.25±0.08</td>
<td>1.87±0.08</td>
<td>2.32±0.1</td>
</tr>
<tr>
<td>Triplets</td>
<td>2±0.09</td>
<td>2.22±0.16</td>
<td>1.94±0.10</td>
</tr>
</tbody>
</table>

Different superscripts in rows differ significantly; a, b: p<0.05

![Graph showing P4 concentration](image)

Fig. 1: Mean P4 concentration in treatment groups (Experiment 2)

The mean serum P4 concentration of the ewes in the hCG treated and control groups are depicted in Fig. 1. The hCG treatment increased P4 concentration in days 14 and 16 in treated groups compared with control and it was higher significantly in h1, (p<0.05).

**DISCUSSION**

It has been well-known for some time that treatment with PMSG increases both ovulation rate and proportionally, luteal progesterone production in FGA-synchronised ewes. Khan et al. (2006) reported that Gonadotrophin supplementation during early pregnancy can reduce embryonic losses. The injection of PMSG immediately after progestagen sponge removal may produce an increase in the rate of ovulation (Romano et al., 1997; Maxwell et al., 1993). The PMSG increased conception rate and litter size, but the response is highly variable and leads to embryonic losses. In earlier study we reported that there was no significant difference between ewes received 300, 450 and 600 IU of PMSG upon progestagen withdrawal (Hojabri et al., 2007).

In present study, there were no differences between the ewes treated with 400 or 600 IU PMSG (p>0.05) but the doses of 400 IU compared to 600 IU of PMSG were found to be less effective to induce multiple births (p>0.05). This might be because the dose used in this study was not sufficient to stimulate additional follicular development in native Lory breed used in this experiment. Intramuscular administration of 400, 500 and 700 IU PMSG in sponge removal time increased the ratio of ovulation and twinning (Mehmet et al., 2006).

One of the main purposes of this study was to determine whether a single injection of hCG given to estrus-induced ewes at the time of insemination or 12 day after insemination could increase the secretion of P4 and subsequent fertility efficiency (p<0.05).

The results of this study showed that in synchronized and artificially inseminated Lory ewes, the injection of 200 IU hCG at the time of cervical AI or 12 days after AI can improve overall fertility
or prolificacy significantly and the fertility and prolificacy was higher in h4 compared with h12 (p<0.05). Therefore, the hCG injection at AI time was more effective to improve reproductive performance of Lory ewes compared with ewes were injected 200 hCG, 12 days after AI (p<0.05). The results of present study are in agreement with other reports i.e., Kittot et al. (1983) reported that hCG administration before the time of maternal recognition of pregnancy increased pregnancy rate in lactating, seasonal anestrous ewes. There are also studies reporting that hCG administration improved reproductive performance in sheep during the breeding season (MacMillan et al., 1986; Cami et al., 2002).

The h2 is more effective on P4 concentration in 2nd experiment and in day 16 was higher compared with control (p<0.05). Nephew et al. (1994) also reported that hCG administration on day 11 post mating increases pregnancy rate in sheep. They showed that hCG increased interferon secretion, luteal weights and conceptus length determined on day 13 of pregnancy. The effect of the hCG on pregnancy rate and fetal weights could be attributed to its effects on progesterone production and uterine secretions which were embryotrophic. This may result in a stronger signal for maternal recognition of pregnancy from embryos in hormonal treatment groups which would degenerate otherwise. The hCG administration has also been reported to increase the number of CL (Beck et al., 1998) and plasma progesterone concentration.

A trend toward higher fertility rates using hCG treatment at AI or 12 day after AI time has also been observed in cows on farms where reproductive efficiency was low (Nakao et al., 1983), as well as in heat-stressed dairy cows (Willard et al., 2003).

These results are in contrast with reports given by Khan et al. (2003), in which hCG given on day of mating not increased the pregnancy rate and litter size of ewe lambs. These results are similar to that previously reported by Gomez-Brunet et al. (2006). These results is also similar with earlier studies in cattle (Swanson and Young, 1990) and sheep (Zamiri and Hosseini, 1998), in which treatment of hCG on the day of AI or mating was not effective in improving fertility significantly.

As inadequate levels of progesterone during the early and mid-luteal phases of the estrous cycle has been related with decreased fertility due to abnormal development of embryos and early embryonic death (Ashworth et al., 1987). To the contrary Ishida et al. (1999) and Fuku et al. (2001) reported hCG treatment given at the early luteal phase also to increase the plasma P4 levels in hCG treated ewes, but this was not reflected in the pregnancy and lambing rates of the Lory inseminated ewes.

It is noteworthy that the birth weights of single lambs were higher in T2h group which is agree with Mehmet and Kuran (2003). It impossible that hormonal treatments used in the present study prevented the mortality of the twin embryos by stimulating the fetal growth.

When evaluating changes in P4 concentrations in control groups, it should be noted that the corpus luteum regressed between days 14 and 16 in these two control groups and plasma P4 levels on days 12, 14 and 16 were slightly higher in the hCG-treated ewes and no significant differences were found in prolificacy between hCG-treated ewes. These results were agreement with earlier studies in ewes (Zamiri and Hosseini, 1998; Khan et al., 2003), in which hCG treatment given at time of insemination caused an increase the number of lambs born per ewe lambing as a result of an increase in ovulation rate which it is disagree with Mehmet et al. (2006) and Gomez-Brunet et al. (2006). These differences could possibly be due to the different protocols used, but it is also probable that other factors such as breed, management systems, nutritional and physiological status, could also have affected the response of animals.

It can be concluded that the hCG injection increased progesterone concentration and subsequent improved reproductive performance in Lory ewes but there were no differences between the ewes treated with 400 or 600 IU PMSG. Likewise 200 IU hCG injection at AI time was more effective to improve reproductive performance of treated Lory ewes compare with ewes were injected 200 hCG, 12 days after AI.
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REFERENCES


