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# Cloprostenol and eCG influence oestrus synchronisation and uterine development in mice

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**ABSTRACT**: The aim of this study was to investigate the efficacy of equine chorionic gonadotropin (eCG) and cloprostenol (CLO) administration on oestrus synchronisation, uterine development as well as serum LH and FSH concentrations in mice. One hundred and five KM mice were assigned into eCG-1, eCG-2, eCG-3, CLO-1, CLO-2, CLO-3 and control groups (CG, $n$ = 15). The eCG-1, eCG-2 and eCG-3 groups were intramuscularly injected with 10, 20 and 40 IU eCG. CLO-1, CLO-2 and CLO-3 were intramuscularly injected with 10, 15 and 20 μg cloprostenol acetate. The results showed that 93.33% and 66.67% of synchronised mice displayed oestrus within 18.68–37.59 h. Oestrus numbers, oestrus onset time (EOT) and oestrus rate in CLO and eCG groups were greater than in CG ($P < 0.05$). EOT in CLO and eCG groups were 19.88 ± 2.91 h and 34.84 ± 5.05 h. Uterine weights of treatment groups were larger than CG. Uterine weights of the eCG group were higher than those in the CLO group. Uterine horn longitudial diameters (ULD) in treatment groups were larger than CG during the experiment. ULD in eCG-2 and eCG-3 were significantly greater when compared to CG ($P < 0.05$ or $P < 0.01$). On days 14 and 21, uterine horn transverse diameters (UTD) in CLO-1, eCG-1, eCG-2 and eCG-3 subgroups were significantly larger than that of CG ($P < 0.05$ or $P < 0.01$). Serum LH concentrations in eCG and CLO increased. Increments in eCG and CLO groups were greater than that of CG. FSH concentrations in eCG mice were higher than those in CLO and CG mice ($P < 0.05$) on day 21. Thus, eCG and cloprostenol treatments in mice can improve uterine development and promote the secretion of LH and FSH.

**Keywords**: follicle stimulating hormone; equine chorionic gonadotropin; cloprostenol; oestrus synchronisation; mice

Synchronisation of oestrus allows for an increase in offspring per year, as it reduces the reproduction cycle and can be implemented regardless of the season. It also allows the time of delivery to be uniform throughout the animal production unit. The use of hormonal products permits the synchronisation of oestrus for natural mating or artificial insemination which can improve the reproductive performance of animals. Use of prostaglandins is an alternative method for controlling reproduction by eliminating the corpus luteum and inducing a subsequent follicular phase with ovulation (Abecia et al. 2011; Abecia et al. 2012). Progesterone or its analogues simulate the action of natural progesterone produced by the corpus luteum after the ovulation of animals (Abecia et al. 2012). Administration of progesterone or its analogues and prostaglandins modify the luteal phase of the cycle. Due to its ecbolic properties it also prevents uterine contractions and has an ovulatory effect (Arbeiter and Arbeiter 1985).

Cloprostenol is a potent prostaglandin (PGF) F2α analogue. It influences the oestrus of animals by lysis of the corpus luteum (Cuervo-Arango and Newcombe 2010). The intraperitoneal administration of cloprostenol three days apart could induce the appearance of oestrus in 80% of mice (Pallares and Gonzalez-Bulnes 2009).

Intramuscular injection of 175 μg of cloprostenol at artificial insemination had no effect on the litter sizes of sows. Injection of 525 μg of cloprostenol, however, was shown to increase the farrowing rate of sows. These findings indicate no consistent effect of cloprostenol administration on sow fertility

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(Kirkwood et al. 2007). However, cloprostenol was described to induce uteroplacental vasoconstriction in the rat probably via activation of the ETA receptor (Girsh et al. 2007). Such effects of cloprostenol on mice reproduction remain unknown.

Although mice are laboratory animals widely used for research worldwide, studies on oestrus synchronisation in mice are scarce (Malakoff 2000; Pallares and Gonzalez-Bulnes 2009). The intraperitoneal administration of cloprostenol three days apart could induce the appearance of oestrus in 80% of mice within 72 to 96 h, but the degree of oestrus synchronisation was not reported (Pallares and Gonzalez-Bulnes 2009). Furthermore, there is no comparative information regarding the effects of eCG and cloprostenol on fertility and uterine development in mice (Ye et al. 2010; Cordova-Izquierdo et al. 2012). It is unknown whether oestrus synchronisation elicited by eCG and cloprostenol affects growth and daily gains (Zhang 2010; Cordova-Izquierdo et al. 2012). The present study was aimed at comparatively evaluating the effects of cloprostenol (CLO) and equine chorionic gonadotropin (eCG) administration at different doses on oestrus synchronisation and uterine development in mice. Further, the effects on LH and FSH secretion in treated mice were investigated in order to provide an empirical basis for the improved reproductive capacity of mice administered reproductive hormones.

## MATERIAL AND METHODS

**Animals**. The experiments were performed on a total of 105 Kunming mice (30 days old, body weight of 25.28 ± 2.26 g, bought from Experiment Animal Centre, Lanzhou University [License No. SCXK (Gansu) 2005-0007]). All mice were randomly assigned into three groups, namely, equine chorionic gonadotropin group (eCG), cloprostenol group (CLO, $n$ = 45 for each) and a control group (CG). Each experimental group was randomly divided into three subgroups, denoted as eCG-1, eCG-2, eCG-3, CLO-1, CLO-2 and CLO-3 ($n$ = 15), respectively.

All mice were housed in groups and kept in mouse cages equipped with automatic water dispensers. Mice received a commercial diet (Lanzhou Taihua Feed Co. Ltd, China), according to their physiological condition. Water was provided *ad libitum*. All procedures referring to animal treatment were approved by the Experiment Animal Care and Use Committee of Gansu province, the People's Republic of China.

**Experimental design**. For the eCG group, mice in eCG-1, eCG-2 and eCG-3 subgroups were intramuscularly injected with 10, 20 and 40 IU eCG (Ningbo Sansheng Pharmaceutical Co Ltd, Ningbo, Zhejiang, China) twice (on day 0 and 4), respectively. Mice in CLO-1, CLO-2 and CLO-3 subgroups were intramuscularly injected with 10, 15 and 20 μg cloprostenol acetate (C6116, Sigma, USA) twice (on day 0 and 4), respectively. Mice in the control group (CG) were injected with 0.5 mL saline solution twice (on day 0 and 4).

**Oestrus detection**. Oestrus detection was performed by direct observation of the mice after the start of the appearance of behavioural oestrus. Mice were observed twice daily following hormone administration. The oestrus onset time (EOT) was recorded. Oestrus behaviours were classified into four stages: prooestrus, oestrus, metoestrus and dioestrus. During the prooestrus the vulva appeared pink and the vagina was extended with a small amount of serous fluid. In oestrus, the vulva was wet and swollen, the appearance was pale and the vaginal orifice became enlarged. The vaginal orifice whitened and closed during the metoestrus and then closed tightly during the dioestrus (Fu et al. 2005).

**Sample collection**. The values of body weights and daily gains, oestrus onset time (EOT) and oestrus rates were recorded. After 5 mice from each subgroup were anaesthetised by injecting xylazine 0.1 mg/kg intramuscularly, they were sacrificed by decapitation on day 7, 14 and 21, respectively. The left and right uteri of each mouse were dissected aseptically and immediately weighed on an electronic scale, respectively. The longitudinal and transverse diameters of the left and right uterine horn were measured with a vernier caliper. The average value was calculated from the left and right uterine horn for each mouse. Then, collected uterine samples were fixed in 10% formaldehyde.

Blood samples were taken aseptically using vacutainers (Zhejiang Gongdong Medical Technology Co Ltd, Zhejiang, China) on day 7, 14 and 21, respectively. The samples were allowed to clot for 2 h at room temperature. After centrifugation (3000 × g, 20 min) the serum was stored at –20 °C until analysis.

**Detection of hormone levels**. Serum concentrations of FSH and LH were determined using a com-

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Table 1. Oestrus synchronisation efficacy in mice

| Group | Subgroup | Number of oestrus | Oestrus onset time (h) | Oestrus rate (%) |
|---|---|---|---|---|
| CLO | CLO-1 | 14 | 21.04 ± 2.75$^{c}$ | 93.33$^{c}$ |
| | CLO-2 | 15 | 19.52 ± 2.81$^{c}$ | 100.00$^{c}$ |
| | CLO-3 | 13 | 18.68 ± 3.16$^{c}$ | 86.67$^{b}$ |
| | Average | | 19.88 ± 2.91$^{**}$ | 93.33 |
| eCG | eCG-1 | 9 | 37.59 ± 5.62$^{b}$ | 60.00 |
| | eCG-2 | 11 | 34.51 ± 4.73$^{b}$ | 73.33 |
| | eCG-3 | 10 | 32.42 ± 4.81$^{b}$ | 66.67$^{a}$ |
| | Average | | 34.84 ± 5.05 | 66.67 |
| Control | CG | 7 | 53.43 ± 6.62$^{a}$ | 46.67$^{a}$ |

Different superscripts denote significant differences between groups
$^{*}P < 0.05$, $^{**}P < 0.05$

mercially available ELISA FSH detection kit and a LH detection kit for mice, respectively, according to the manufacturers' instructions (Shanghai Yanjing Biotechnology Co. Ltd, Shanghai, China). The analytical specificity was 100% for FSH and LH. The analytical sensitivities for FSH and LH were 0.03 IU/l and 0.02 ng/ml, respectively. The inter-assay CV was less than 4%. The correlation coefficient of the standard curve was 0.9995.

**Statistical analyses**. Statistical analysis was performed using SPSS v. 18.0 (SPSS Inc. Chicago, USA). Data are presented as means ± SEM. After a square root transformation of the data, all variables complied with the assumptions for a one-way ANOVA. When significant differences were identified, supplementary Tukey's post-hoc tests were performed to investigate pair-wise differences. $P < 0.05$ was considered significant.

## RESULTS

### Oestrus synchronisation efficacy

Reproductive data of mice synchronised with eCG and CLO are summarised in Table 1. Oestrus numbers, oestrus occurrence time (EOT) and oestrus rate in CLO and eCG groups were higher than those in the control group ($P < 0.05$ or $P < 0.01$). The values in CLO were larger than those in the eCG group. The oestrus numbers, EOT and oestrus rate of mice in CLO-2 were higher than in CLO-1 and CLO-3 subgroups ($P < 0.05$). Meanwhile, values in eCG-2 mice were greater than in eCG-1 and eCG-3 subgroups ($P < 0.05$). 93.33% and 66.67% of the synchronised mice displayed oestrus within 18.68–37.59h after the cloprostenol and eCG injections. Average EOT in CLO and eCG groups was 19.88 ± 2.91 hours and 34.84 ± 5.05 h, respectively. EOT in synchronised mice became shorter with increasing eCG and cloprostenol doses.

### Body weight and daily gain

As shown in Figure 1, body weight in the eCG-3 subgroup was highest over the course of the experiment. On day 7 and 18, body weight of the eCG-3 subgroup was larger than that of CG ($P < 0.05$). However, on day 21 body weight of the CLO-1 subgroup was lower than that of CG ($P < 0.05$). Other

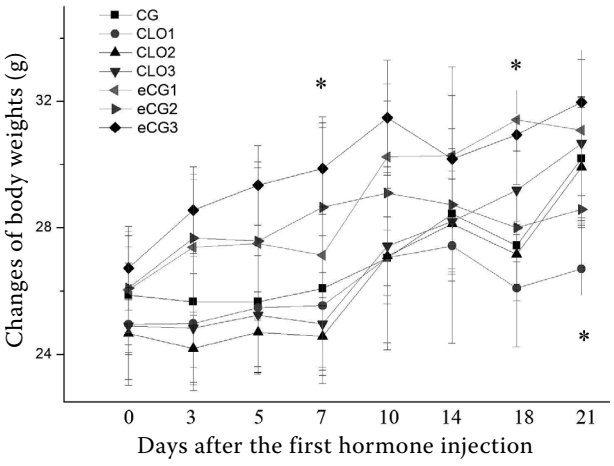


Figure 1. Body weights in mice after eCG and CLO treatment

$^{*}P < 0.05$ when compared to CG; $^{**}P < 0.01$ when compared to CG. This legend is also for the following figure

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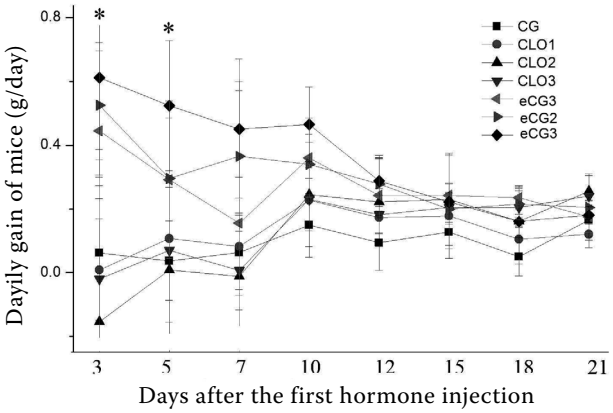


Figure 2. Daily gains in mice after eCG and CLO treatment

subgroups displayed no significant differences during the whole course of the experiment.

As shown in Figure 2, on days 3 and 5 daily gains in the eCG-3 subgroup were higher than in the CG and CLO-2 ($P < 0.05$). This demonstrated that the injection of 40 IU eCG could enhance the growth of mice more markedly than other eCG doses and CLO treatment.

## Uterine weights of mice

During the whole experiment, uterine weights of the six experimental subgroups were larger than those of the control subgroup (Figure 3). Uterine weight of the eCG group was higher than that of the CLO group with the maximum in the eCG-3 subgroup. On days 7, 14 and 21, the uterine weight of the eCG-3 subgroup was greater when compared to CG ($P < 0.05$ or $P < 0.01$). Meanwhile, on day 7 uterine weights of eCG-1 and eCG-2 were also greater in comparison with CG ($P < 0.05$). This illustrated that eCG and cloprostenol treatment can promote uterine development, and that eCG has a greater efficacy.

## Uterine horn transverse diameters (UTD) and longitudinal diameters (ULD)

Uterine horn longitudinal diameters (ULD) and transverse diameters (UTD) were measured using the vernier caliper, respectively. ULD values in experimental subgroups (excluding CLO-2) were larger than those of the control subgroups over the entire course of the experiment (Figure 4); meanwhile, ULD values in the eCG group were greater than those in the CLO group. ULDs in eCG-2 and eCG-3 were significantly higher when compared to CG ($P < 0.05$ or $P < 0.01$).

In comparison with CG, UTD of mice treated with eCG and CLO also increased (Figure 5). On days 14 and 21, UTDs of CLO-1, eCG-1, eCG-2 and eCG-3 subgroups were significantly greater than in the CG ($P < 0.05$ or $P < 0.01$). These findings indicate that eCG and cloprostenol treatment can improve the growth of mice.

## Serum hormone concentrations of mice

**Serum LH concentrations**. Serum LH concentrations in all mice increased following the cloprostenol and eCG injections (Figure 6). However, the incremental increase in eCG and CLO groups was greater than that of CG. On day 21, serum LH concentrations in CLO-2 and eCG-1 subgroups were higher in comparison with CG ($P < 0.05$).

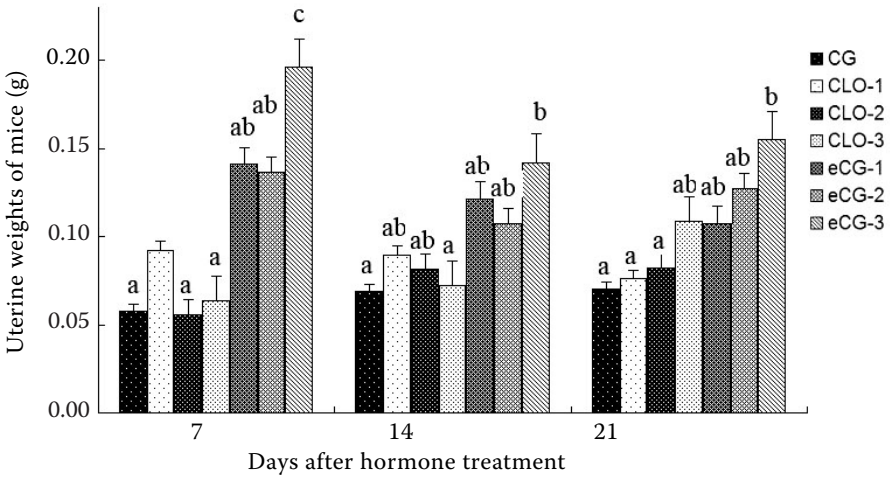


Figure 3. Uterine weights in mice after eCG and CLO treatment

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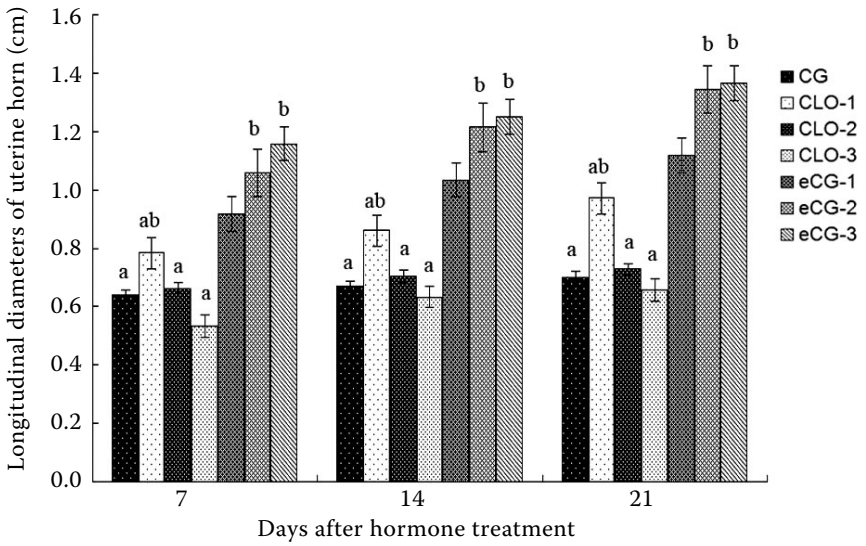


Figure 4. Longitudinal diameters of the uterine horns of mice. Data in the figure represent the average values of the left and right uterine horns

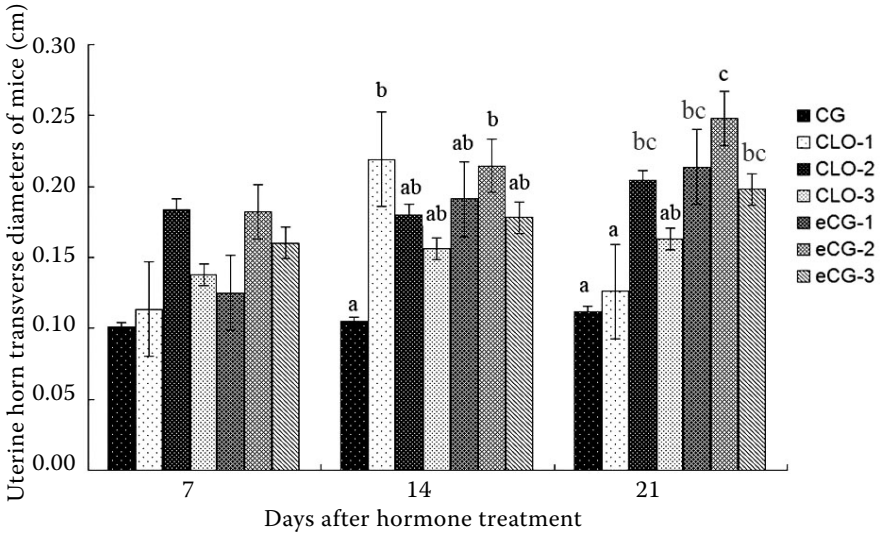


Figure 5. Transverse diameters of the uterine horns of mice. Data in the figure represent the average values of the left and right uterine horns

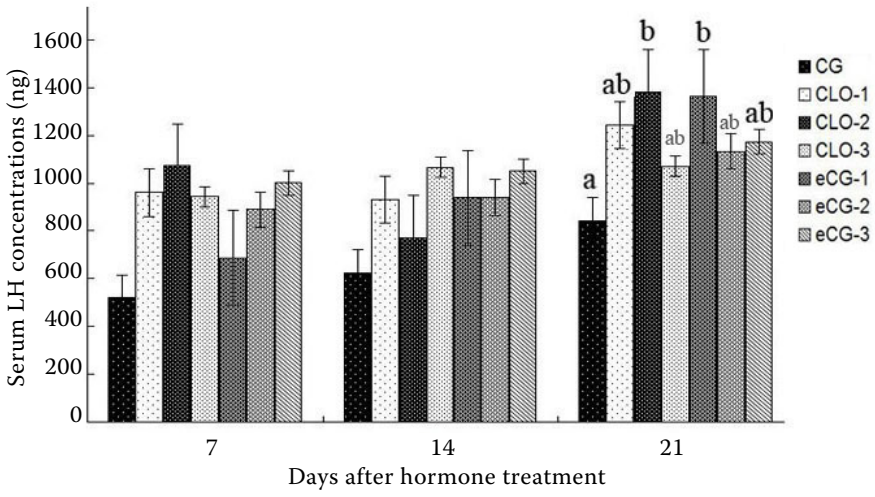


Figure 6. Serum LH concentrations after eCG and cloprostenol treatments. Serum LH concentrations in all mice increased after cloprostenol and eCG treatments with the maximum in the CLO-2 and eCG-1 subgroups on day 21

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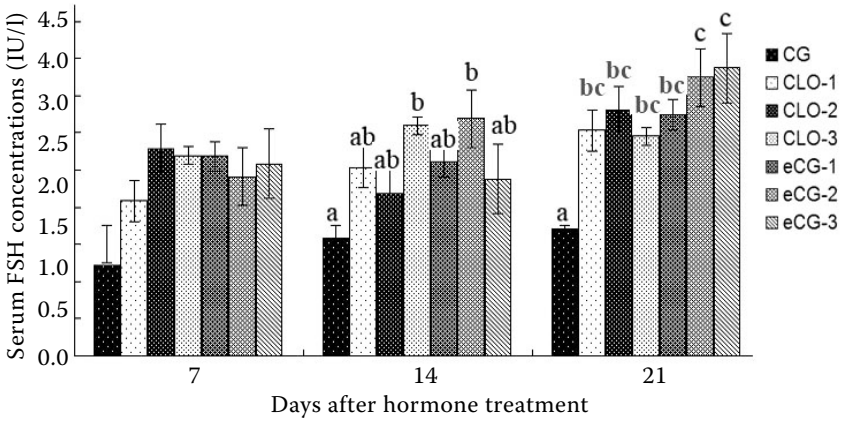


Figure 7. Serum FSH concentrations after eCG and cloprostenol treatments. Serum FSH concentrations in all mice also increased after the cloprostenol and eCG treatments

**Serum FSH concentrations**. As shown in Figure 7, serum FSH concentrations in the six experimental subgroups (EGs) rose along with increasing eCG and cloprostenol doses. On days 7 and 14, there were no significant differences between eCG and CLO groups. However, FSH concentrations in eCG mice were higher than those in CLO and CG mice ($P < 0.05$ or $P < 0.01$) on day 21, with a maximum increment in the eCG-3 subgroup.

The results indicated that eCG and cloprostenol treatment in mice could promote the synthesis and secretion of LH and FSH, while also enhancing its serum concentrations. The effects of eCG were stronger than those of cloprostenol.

## DISCUSSION

Induction and/or synchronisation of the oestrus cycle are routinely achieved, both in humans and other animal species, by administration of different combinations of hormones (Pallares and Gonzalez-Bulnes 2009). Research has shown that adult rats in different oestrus cycles exhibited different induction activities to hormones (Zhang et al. 2007). The synchronisation effect in mice decreased with successive generations within 12 generations (Schuler 1977). However, there is little information available about the comparative effects of different exogenous hormones on fertility and uterine development in mice (Zhu et al. 2012). The oestrus synchronization of mice was successful in this study. The typical behaviours of oestrus were observed in four stages, i.e. the prooestrus, oestrus, metoestrus and dioestrus stages. The results were consistent with previous reports (Fu et al. 2005; Zhu et al. 2012; Gravina et al. 2014).

We calculated rates of oestrus synchronisation (93.33% and 66.67% for the cloprostenol and eCG treatment, respectively) in the present work. The intraperitoneal administration of cloprostenol three days apart was shown to induce oestrus in 80% of mice within 72 to 96 h (Pallares and Gonzalez-Bulnes 2009). Our results are similar to this earlier report.

The oestrus rates in CLO and eCG were greater than those in CG with the maximum oestrus rate in CLO-2. This indicated that doses of cloprostenol and eCG could influence the efficacy of oestrus synchronisation in mice. The mice used in this study were 30 days old, i.e. in the stage of puberty. The effects of cloprostenol on mice in puberty were stronger than those of eCG. This is probably due to the promotion of LH secretion and increase of serum LH and FSH concentrations after the cloprostenol and eCG injections (Zhou 1995). Cordova-Izquierdo (1999) reported that progestogens are ineffective in anoestrus animals. However, the efficiency was increased when used in combination with other hormones such as eCG (Uslu et al. 2012).

The synchronised mice displayed oestrus within 18.68–37.59 h after the cloprostenol and eCG injections. The EOT of CLO and eCG groups was shorter than that of CG. Moreover, a shorter EOT was observed in the CLO group when compared to the eCG group. These findings are consistent with earlier studies (Pallares and Gonzalez-Bulnes 2009; Bunger et al. 1982), which reported oestrus of mice within 72 to 96 h following intraperitoneal administration of cloprostenol. It is likely that the absorption speed of drugs following intramuscular injection is higher than after intraperitoneal administration (Pallares and Gonzalez-Bulnes 2009). The exact mechanisms are still to be explored.

Reproductive hormones may significantly impact uterine growth and development (Chia et al. 2006). eCG treatment affected the endometrial and myometrial thickness. However, eCG was described

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to have no significant effect on uterine glands in postnatal day 15 to day 21 mice (Yuan et al. 2008). In our results the uterine weights of the eCG group were higher than those of the CLO and CG groups with the maximum in the eCG-3 subgroup. ULD values in experimental subgroups (excluding CLO-2) were larger than in the CG during the course of the experiment; further, the ULD values in the eCG group were greater than those in the CLO group. In comparison with CG, the UTD of mice treated with eCG and CLO also increased. The findings illustrated that eCG and cloprostenol treatment could promote uterine development, and that eCG has a greater efficacy. It is possible that the mice administered eCG treatment developed larger follicles and higher oestrogen concentrations (Dezhkam and Sadrkhanlou 2009). The eCG shows high LH- and FSH-like activities and has a high affinity for both FSH and LH receptors in the ovaries, which stimulate oestradiol and progesterone secretion (De Rensis and Lopez-Gatius 2014).

A previous report indicated that eCG may promote uterine growth of postnatal day 21 mice, and stated that the effects on uterine development increased gradually along with the age of the mice (Yuan et al. 2008). To date, there is little information available regarding the changes in body weight and daily gains after oestrus synchronisation treatment (Cordova-Izquierdo et al. 2012; Zhang 2010). In the present study, the body weight of the eCG-3 subgroup was higher than that of the remaining subgroups over the course of the experiment. On day 21, the body weight of the CLO-1 subgroups was less than that of CG. Daily gains in eCG-3 subgroups were higher than those in CG and CLO-2 subgroups on days 3 and 5. Detailed analyses of the results demonstrated that the injection of 40 IU eCG could enhance the growth of mice more markedly than other eCG doses and CLO treatment. Our results will need to be verified in future studies.

FSH and LH are synthesised and stored in gonadotrophin cells under the regulation of multiple mechanisms (Crawford et al. 2009). In this study, serum LH and FSH concentrations in eCG and CLO mice increased following the cloprostenol and eCG injections. However, FSH concentrations in eCG mice were higher than those in CLO and CG mice on day 21, with a maximum increment in the eCG-3 subgroup. The findings indicate that eCG and cloprostenol could promote the synthesis and secretion of LH and FSH in mice.

## CONCLUSION

The eCG and cloprostenol treatments in mice can synchronise oestrus, improve uterine development and growth, promote the synthesis and secretion of LH and FSH, and enhance serum concentrations. The effects of eCG are stronger than those of cloprostenol. This knowledge can be used to improve mice management and will also be of benefit to scientific research.

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