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RESEARCH ARTICLE

Cloprostenol and PMSG Administration Promote Estrus Synchronization, Uterine Development and FSHR Expression in Mice

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ABSTRACT

The present study aimed to investigate the effects of PMSG and cloprostenol (CLO) on the estrus synchronization, uterine development as well as follicle stimulating hormone receptor (FSHR) expression in animals. 105 female KM mice, 30 days old and body weight of 25.28±2.26g, were assigned into seven subgroups (n=15). Mice in PMSG-1 (pregnant mare serum gonadotrophin), PMSG-2 and PMSG-3 subgroups were injected with 10, 20 and 40IU PMSG. Mice in CLO-1, CLO-2 and CLO-3 subgroups were intramuscularly injected with 10, 15 and 20μg cloprostenol. Western-blotting was implemented to detect expression of uterine FSHR proteins. The results showed that 93.33% and 66.67% of synchronized mice of CLO and PMSG groups displayed estrus in 18.68-37.59h, respectively. Estrus numbers, estrus onset time (EOT) and estrus rates in CLO and PMSG groups were greater than in control group (CG) ($P<0.05$). EOT in CLO and PMSG groups were 19.88±2.91 hours and 34.84±5.05 hours. Uterine weights of PMSG group were higher than that of CLO and CG groups. Uterine wall thickness (UWT) of PMSG group was higher than that of CLO and CG groups. Uterine horn longitudial diameter (ULD) in experimental mice was greater than CG. ULD in PMSG-2 and PMSG-3 were significantly greater than CG. Uterine horn transverse diameter (UTD) of CLO-1, PMSG-1, PMSG-2 and PMSG-3 subgroups were significantly larger than CG ($P<0.05$). Expression levels of FSHR proteins were different among all subgroups. FSHR protein levels in CLO and PMSG increased lightly in comparison with CG. In conclusion, PMSG and CLO treatments could synchronize estrus of mice, enhance the uterine development of mice. Efficacy of CLO on estrus synchronization was greater than PMSG. The effects of PMSG on uterine development were stronger than cloprostenol. This is benefit for modulating the reproductive functions of animals.

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INTRODUCTION

The estrus synchronization permits for an increase of offspring per year, as it reduces the reproduction cycle and can be handled regardless of the season. It also allows the time of delivery be uniform throughout the animal production unit. The use of hormonal products permits the estrus synchronization for natural mating or artificial insemination, and improve the reproductive performance of animals. Administration of progesterone or its analogues and prostaglandins modified the luteal phase of the cycle and induced a subsequent ovulation (Abecia *et al.*, 2011; 2012). It also induced uterine contractions due to its ecbolic properties and has an ovulatory effect. Cloprostenol (CLO) is a potent prostaglandin (PGF) F2α analogue. It influences the estrus of animals by the lysis of corpus lutein (Cuervo-Arango and Newcombe, 2010). The intraperitoneal CLO administration could induce the estrus appearance of 80% mice (Pallares and Gonzalez-Bulnes, 2009). However, Marcelo *et al.* (2010) reported that there was no statistical difference in the pregnancy rates among cows that were injected with 150-500μg CLO. Such the actual effects of PMSG and cloprostenol administrations

in the estrus synchronization and uterine development of animals remain unclear.

Although mice are laboratory animals widely used for researches worldwide, management and researches of the estrus cycle in the mice are scarce (Crawford *et al.,* 2009; Zhang, 2010; Alejandro *et al.*, 2012). Usual methods for the induction of behavioral estrus and ovulation are by the introduction of a male, fertile or vasectomized. However, the degree of estrus synchronization was reported to be very limited. Thus, the application of a reliable method for estrus synchronization would be of interest for improving mice management and researches. CLO Administration induced the estrus appearance within 72 to 96h (Pallares and Gonzalez-Bulnes, 2009), but the degree of estrus synchronization was not reported. Furthermore, no information is available about the comparative researches about the uterine development after PMSH and cloprostenol treatment in mice (Chen, 2011). It is unknown whether PMSG and cloprostenol treatments impact the FSHR protein expression levels in mice uteri (Crawford *et al.,* 2009).

On the basis of our previous studies (Gong *et al.*, 2011; Wei *et al*., 2011; Wei *et al*., 2013), the present study aimed to explore the effects of cloprostenol and PMSG administration at different doses on the uterine development and estrus synchronization of mice, also to investigate the correlation between the expression levels of FSHR proteins and development indexes of uteri of mice, so as to expound the mechanisms of PMSG and CLO in modulating the uterine development and estrus synchronization.

MATERIALS AND METHODS

Animals: The experiments were performed in 105 puberty KM mice (mus musculus), 30 days old and body weight of 25.28±2.26g, bought from Lanzhou University [License No. SCXK (Gansu) 2005-0007]. All mice were randomly assigned into PMSG, CLO and control group (CG). Mice of CLO and PMSG groups were randomly divided into three subgroups, marked as PMSG-1, PMSG-2, PMSG-3, CLO-1, CLO-2 and CLO-3 (n=15), respectively. All mice were housed in groups and kept in the room maintained at 22-24ºC. Water was provided *ad libitum*. Mice received a commercial diet. Body weight of each mouse was weighed. Animal treatments were approved by the Experiment Animal Care and Use Committee of Gansu province of China.

Experimental designs: Mice in PMSG-1, PMSG-2 and PMSG-3 subgroups were intramuscularly injected with 10, 20 and 40IU PMSG (Sansheng Pharmaceutical Co., Ningbo, China) twice (on day 0 and 4), respectively. Mice in CLO-1, CLO-2 and CLO-3 subgroups were injected with 10, 15 and 20μg cloprostenol acetate (Sigma, USA) twice (on day 0 and 4), respectively. Mice CG were injected with 0.5mL normal saline twice. All injections were performed at 8 to 9 AM in the morning.

Estrus detection: Estrus detection was done by direct observation of the mice after the start of appearance of behavioral estrus (Zhou, 1995; Zheng *et al*., 2013). Mice were observed twice daily. The estrus onset time (EOT) in each mouse was determined after the second injection continuously for 72h.

Sample collections: After 5 mice from each subgroup were anesthetized by injecting xylazine 0.1mg/kg intramuscularly, they were sacrificed by decapitation at day 7, 14 and 21, respectively. Left and right uteri of each mouse were dissected aseptically and weighed, respectively. The uterine horn longitudinal diameter (ULD) and transverse diameter (UTD) of left and right uteri were measured with a vernier caliper. Then collected uterine samples were fixed in 10% formaldehyde.

Blood samples were taken aseptically on day 7, 14 and 21. After centrifugation (3000×g, 20 min), the serum was stored at -20ºC until analysis.

Histological image measurement of uteri: Uterine samples were embedded with paraffin wax, sliced (5μm) and stained H&E. The sections were photographed under light microscope (Leica, Japan). Five sites of 4 sections in each section (totaling 140 sites for each subgroup) were measured. The data of uterine wall thickness (UWT), uterine endometrial thickness (UET), uterine perimetrium thickness (UPT) and longitudinal diameters of endometrial epithelial cells (LEEC) were measured using Image Pro Plus 2.0 (MOTIC Company, Hong Kong).

Western blotting analysis: To evaluate FSHR protein expression in uteri of mice, Western blotting was performed according to the described methods (Wei *et al*., 2013a, b). Relative contents of FSHR proteins were presented as the ratio between gray values of FSHR separated by that of β-actin. The experiments were replicated three times. The negative control was performed without primary antibody.

Statistical analyses: Statistical analysis was performed using SPSS v. 18.0 (SPSS Inc. Chicago, USA). Averages of all indexes described above were calculated based on data of 5 mice. Results were presented as the mean±SEM. After a square root transformation of the data, all variables complied with the assumptions for a one-way ANOVA. Pearson's model was utilized to analyze the correlations between indexes. When significant differences were identified, the pairwise differences were conducted using Tukey's post-hoc tests. $P<0.05$ was considered to be significant.

RESULTS

Estrus synchronization: Reproductive data on mice synchronized with PMSG and CLO are summarized in Table 1.

As shown in Table 1, estrus numbers and estrus rate of CLO and PMSG groups were higher than that of control group ($P<0.05$ or $P<0.01$). EOT of CLO and PMSG groups were shorter than CG ($P<0.05$ or $P<0.01$). The estrus rate of CLO group was larger than that in PMSG group. Average EOT in CLO and PMSG groups was 19.88±2.91 hours and 34.84±5.05 hours, respectively. EOT of CLO group were less than that of PMSG group. EOT in CLO-3 and PMSG-3 was less than that in CLO-1 and CLO-2, PMSG-1 and PMSG-2 subgroups ($P<0.05$),

respectively. 66.670% to 93.33% of the synchronized mice displayed estrus within 18.68-37.59h after the cloprostenol and PMSG administrations. The estrus rates of CLO-3 and PMSG-3 was greater than that in CLO-1 and CLO-2, PMSG-1 and PMSG-2 subgroups (P<0.05), respectively.

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

As shown in Table 2, uterine horn transverse diameters (UTD) and longitudinal diameters (ULD) of PMSG group was higher than that of and CLO and CG groups (P<0.05 or P<0.01). During the experiment ULD of PMSG-3 subgroup was significantly higher when compared to CG (P<0.05 or P<0.01). In comparison with CG, UTD of PMSG and CLO treated mice increased (Table 2). On days 14 and 21, UTD of CLO-1, PMSG-1, PMSG-2 and PMSG-3 subgroups were significantly larger than that of CG (P<0.05 or P<0.01). The findings indicated that PMSG and cloprostenol treatment can improve the growth of mice.

In addition, uterine weight percentages (%) accounted for body weights in PMSG and CLO groups of mice were greater than that in CG group (Table 2). On day 14, the percentage of PMSG was significantly higher than CG (P<0.05 or P<0.01), especially in the PMSG-2 subgroup (P<0.01). This demonstrated that PMSG and CLO treatment could promote the uterine growth of mice.

Measurements of UWT, UET, UPT and LEEC in mice: The changes of uterine wall thickness (UWT), uterine endometrial thickness (UET), uterine perimetrium thickness (UPT) and longitudinal diameters of endometrial epithelial cells (LEEC) were shown as Fig. 1. As shown in Fig. 1 (Upper), UWT of PMSG group was higher than that of CLO and CG groups. In comparison with the control group, UWT of PMSG-2 and PMSG-3 subgroups significantly increased during the experiment (P<0.05).

UET of PMSG group was higher than that of CLO and CG groups (Fig. 1 Middle). UET of CLO-3 and PMSG-3 subgroups significantly thickened (P<0.05) when compared to CG and other subgroups. UPT of mice increased along with the doses of PMSG and cloprostenol administration (Fig. 1 Bottom). On day 21, UPT of CLO-3 subgroup and PMSG group were significantly larger than that of the CG group (P<0.05). However, there were no significant difference in LEEC between experimental groups and control group.

The findings of this study demonstrated that PMSG and cloprostenol could promote the development and growth of mouse uteri, especially for uterine perimetrium thickness (UPT). The effects were dose-dependent.

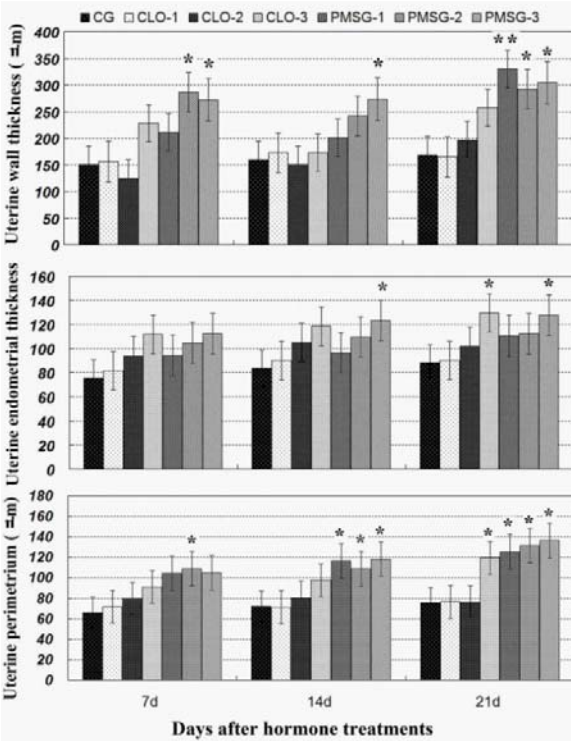

Fig. 1: Changes of uterine wall thickness (UWT) (upper), uterine endometrial thickness (UET) (middle) and uterine perimetrium thickness (UPT) (bottom) of mice after PMSG and CLO treatment. **Upper**: Uterine wall thickness (UWT) PMSG group was higher than that of CLO and CG groups. UWT of PMSG-2 and PMSG-3 subgroups significantly increased when compared to CG. **Middle**: Uterine endometrial thickness (UET) of PMSG group was higher than that of CLO and CG groups. UET of CLO-3 and PMSG-3 subgroups significantly thickened when compared to CG and other subgroups. **Bottom**: Uterine perimetrium thickness (UPT) mice increased along with the doses of PMSG and cloprostenol administration. On day 21, UPT of CLO-3 subgroup and PMSG group were significantly larger than that of the CG group (P<0.05).

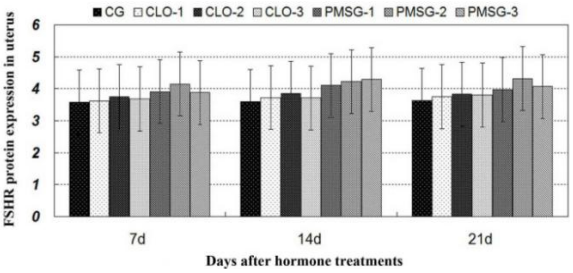

Fig. 2: FSHR protein expression in uteri of mice. Expression levels of FSHR proteins in CLO and PMSG groups increased along with the CLO and PMSG injection doses compared to CG.

Table 1: Estrus synchronization efficacy of mice

Group	Subgroup	Estrus number	EOT (h)	Estrus rate (%)
CLO	CLO-1	14	21.04±2.75**	93.33**
	CLO-2	15	19.52±2.81**	100.00**
	CLO-3	13	18.68±3.16**	86.67*
PMSG	PMSG-1	9	37.59±5.62	60
	PMSG-2	11	34.51±4.73*	73.33
	PMSG-3	10	32.42±4.81*	66.67
Control	CG	7	53.43±6.62	46.67

Note: EOT- Estrus onset time (h). * P<0.05 and ** P<0.01 when compared to CG.

Table 2: Measurements of uterine indexes and proportion (%) of uterine weights and body weights in mice

Subgroup	Uterine weights (g)			ULD (cm)			UTD (cm)			Uterus percentage (%)		
	7d	14d	21d	7d	14d	21d	7d	14d	21d	7d	14d	21d
CG	0.058	0.069	0.071	0.64	0.67	0.70	0.10	0.11	0.11	22.56	23.78	23.12
CLO-1	0.093	0.090	0.076	0.78	0.86	0.97	0.11	0.22*	0.13	25.35	28.22*	24.16
CLO-2	0.056	0.082	0.082	0.66	0.70	0.73	0.18	0.18	0.20*	24.35	28.28**	24.30
CLO-3	0.064	0.072	0.109	0.53	0.63	0.66	0.14	0.16	0.16	24.80	27.40*	25.47
PMSG-1	0.141*	0.122	0.107	0.92	1.03	1.12	0.13	0.19	0.21*	26.50	29.02**	27.26*
PMSG-2	0.137*	0.107	0.128	1.06	1.22*	1.34*	0.18	0.21*	0.25**	30.10**	30.57**	27.14*
PMSG-3	0.169*	0.142*	0.155*	1.16	1.25*	1.36*	0.16	0.18	0.20*	29.74**	28.84*	28.02*

Notes: ULD-Uterine horn longitudial diameters; UTD-uterine horn transverse diameters. Percentage: The uterus weight of each mouse accounted for the proportion of its body weight; Compared to CG, the difference was *significant (P<0.05) or **highly significant (P<0.01).

Table 3: Pearson correlation analyses of indexes in mice treated with PMSG

Items	BW	UW	ULD	UTD	UWT	UET	UPT	LEEC
UW	0.3856							
ULD	0.5351	0.7331**						
UTD	0.4636	0.4525	0.8900*					
UWT	0.5624	0.6704	0.8671*	0.8101**				
UET	0.6778*	0.7426**	0.9465**	0.7565**	0.8808**			
UPT	0.5675	0.6720*	0.9364**	0.8895**	0.8752**	0.8697**		
LEEC	0.3576	0.4179	0.2737	0.2574	0.3365	0.2441	0.2985	
FSHR	0.3003	0.5183	0.8810*	0.8751**	0.7079**	0.7722*	0.8293**	0.1395

Notes: BW- Body weight; UW- Uterus weight; ULD-Uterine horn longitudial diameters; UTD-uterine horn transverse diameters; UWT-uterine wall thickness; UET-uterine endometrium thickness; UPT-Uterine perimetrium thickness; FSHR-FSH receptor; LEEC-Longitudine diameters of endometrium epithelial cells. Compared to CG, the difference was *significant (P<0.05) or **highly significant (P<0.01).

Western blotting of FSHR protein in uteri: Uterine FSHR protein was detected by Western blotting in all samples of mice. Changes of FSHR protein expression levels were different among the all groups (Fig. 2). Expression levels of FSHR proteins in CLO and PMSG groups lightly increased along with the CLO and PMSG injection doses compared to CG. However, there were no significant differences in LEEC between experimental groups and control group. This illustrated that CLO and PMSG administration did not obviously affect the expression of FSHR proteins in the uteri of mice.

Correlations between protein expressions and uterine development: As shown in Table 3, Pearson's correlation analyses in PMSG group demonstrated that UW had a significant positive correlation with ULD, UET and UPT (r=0.7331, r=0.7426 and r=0.6720 respectively, P<0.05 or P<0.01). FSHR expression levels also had significant positive correlations with ULD, UTD, UPT (P<0.01), UWT and UET (P<0.05) respectively. Meanwhile, there were also highly significant positive correlations between UWT and UET as well as UPT (P<0.01). Namely, the increase of CLO and PMSG injection dose could promote the uterine development and FSHR expressions.

Pearson correlation analyses of cloprostenol group also showed that UWT had significant positive correlations with UW, UET and UPT respectively.

DISCUSSION

In this study, the estrus synchronization rates of CLO and PMSG treatments (93.33% and 66.67%, respectively. The estrus rates in CLO and PMSG were greater than that in CG. They were similar to early reports ewe (Alejandro *et al.,* 2012) and heifers (Richardson *et al*., 2002; Baris *et al*., 2012). However, estrus rates were greater than the early report (Zhou, 1995). Zhou (1995) demonstrated that 37.5%-62.16% mice showed estrus signs after treated using PMSG in combination with PGF2α and hCG. Alejandro *et al*. (2012) reported 70% of Dorper ewes had obvious signs of estrus following the intramuscular injection of 170mg cloprostenol twice with an interval of 10 days between doses. This indicated that the dose of cloprostenol and PMSG could influence the efficacy of synchronization estrus of animals. It is probably due to the promotion of LH secretion and increase of serum LH and FSH concentrations after the cloprostenol and PMSG injections. Our study also demonstrated that PMSG and cloprostenol treatments in mice could promote the secretions of LH and FSH (this will reported in another paper).

Cordova *et al*. (1999) reported that 25%, 20.8% and 50% of treated ewes showed estrus in 24 h, 48 h and 72 h after applying the cloprostenol treatment, respectively. EOT shortened slightly in the hormone-treated ewes (Baris *et al*., 2012). In our study, the synchronized mice displayed estrus within 18.68-37.59h. EOT of CLO and PMSG groups was shorter than that of normal control. Meanwhile, the shorter EOT was observed in CLO group when compared to PMSG group. Our results are consistent with those of heifers (Richardson *et al*., 2002; Ginther *et al*., 2007). The findings are lower than that of the previous report of ewes. The ewes displayed estrus within 72 to 96h following intraperitoneal administration of cloprostenol (Knight *et al*., 2000). This is likely that the absorption speed of intramuscular injection drugs is quicker than intraperitoneal administration (Pallares and Gonzalez-Bulnes, 2009).

In relative to the large animals (such as cows and ewes), the relatively high doses of PMSG were administered in this study based on our experiences and early studies (Zhang *et al*., 2007). The results showed that such high doses could be used to synchronize estrus of mice. The exact mechanisms are still to be explored.

Early studies indicated that GnRH agonist treatment could significantly reduce the uterine growth. After subcutaneous injection of GnRH in mice, the uterine volume was reduced by 36% at 12 weeks and 45% at 24 weeks, uterine weights decreased by 34.43% to 55.74% when compared with control. Uterine wall thickness reduced was by 8.64% to 14.03% (Wei *et al*., 2011).

Our results showed that the uterine weights of mice increased when compared to CG. Uterine weights of PMSG group were higher than that of CLO group, with a maximum in the PMSG-3 subgroup. ULD in experimental subgroups (excluding CLO-2) were larger than that of the control subgroup during the experiment, meanwhile ULD in PMSG group were greater than that in CLO group. In comparison with CG, UTD of mice treated with PMSG and CLO also increased. The findings demonstrated that PMSG and cloprostenol treatment could promote the uterine development and growth, especially for uterine perimetrium thickness (UPT). PMSG had a greater efficacy. Our results are different with previous reports (Chia *et al*., 2006; 2010; Wei *et al*., 2011). However, the mechanism of the effects needs to be further explored in the future.

The Pearson's correlation analyses in the present study demonstrated that there existed significant positive correlations between uterine weight and UWT, ULD, UET and UPT. Meanwhile, UWT had significant positive correlations with UET and UPT. There were significant positive correlations between FSHR protein expression levels and uterine indexes (such as ULD, UTD, UPT, UWT and UET). Namely, the increase of uterine development is due to the increment of UWT, UET and UPT. So far, similar reports have not been published (Fhulufhelo *et al*., 2012). Thus, the actual statistical correlations between these parameters need further researches. Our results laid a novel thought and method for studying quantitatively the effects of PMSG and cloprostenol on the reproductive functions in animals and human.

In conclusion, Cloprostenol and PMSG treatments could synchronize estrus of mice, enhance the uterine development and shorten EOT. The efficacy of CLO on estrus synchronization was greater than PMSG. However, the effects of PMSG on uterine development were stronger than CLO. CLO and PMSG did not significantly affect the expression levels of uterine FSHR proteins. This is benefit for modulating the reproductive functions and treating uterine diseases of animals.

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REFERENCES

Abecia JA, Forcada F and González-Bulnes A, 2011. Pharmaceutical control of reproduction in sheep and goats. Vet Clin Food Anim, 27: 67-79.

Abecia JA, Forcada F and González-Bulnes A, 2012. Hormonal control of reproduction in small ruminants. Anim Reprod Sci, 130: 173-179.

Alejandro CI, Manuel XCV, Gustavo RLC, Román EC, Alejandro CJC *et al*., 2012. Effect of cloprostenol and fluorogestone acetate more PMSG on synchronization and no return to estrus in seasonal anestrus Dorper sheep. J Appl Sci Res, 8: 1612-1614.

Baris AU, Ibrahim TFG, Sait S, Omer U, Sandra GP *et al*., 2012. Effects of oestrus synchronisation using melatonin and norgestomet implants followed by eCG injection upon reproductive traits of fat-tailed Morkaraman ewes during suckling, anoestrus season. Small Rumin Res, 108: 102-106.

Chenghu Z, 2010. Germplasm resources protection and development for Lanzhou fat-tailed sheep. Chin Anim Husb J, 46: 7-10. (In Chinese)

Chia CC, Huang SC and Chen SS, 2006. Ultrasonographic evaluation of the change in uterine fibroids induced by treatment with a GnRH analog. Taiwan J Obstet Gynecol, 45: 124-128.

Chunyan Z, Lijun L, Min D, Danfeng Z, Lihu Z *et al*., 2007. Effects of the PMSG dosages on superovulation and oocyte quality in immature rats. Chin J Comp Med, 17: 338-344. (In Chinese)

Cordova IA, Ruiz LG, Saltijeral OJ, Pérezy GJF and Degefa DT, 1999. Inducción y sincronización de celos en ovejas criollas anéstricas estaciónales con esponjas vaginales impregnadas en FGAy PMSG inyectable. Arch Zootec, 48: 437-40.

Crawford JL, Heath DA, Haydon LJ, Thomson BP and Eckery DC, 2009. Gene expression and secretion of LH and FSH in relation to gene expression of GnRH receptors in the brushtail possum (Trichosurus vulpecula) demonstrates highly conserved mechanisms. Reprod 137: 129-140.

Cuervo-Arango J and Newcombe JR, 2010. Cloprostenol in Equine Reproductive Practice: Something More Than a Luteolytic Drug. Reprod Dom Anim, 45: e8-e11.

Fhulufhelo VR, Tshimangadzo LN, Ben S, Johannes PCG and Khoboso CL, 2012. Oestrous synthronisation and pregnancy rate following artificial insemination (AI) in South African indigenous goats. J Appl Anim Res, 40: 292-296.

Ginther OJ, Gastal EL, Gastal MO and Beg MA, 2007. Effect of prostaglandin F2 on ovarian, adrenal, and pituitary hormones and on luteal blood flow in mares domestic. Anim Endocrinol, 32: 315-328.

Jisheng Z, 1995. Research on estrus synchronization and superovulation in KM mice. Chin J Exp Anim Sci, 5: 139-141. (In Chinese)

Knight M, Hoenh T, Lewis PE and Inskeep EK, 2000. Effectiveness of intravaginal progesterone inserts and FSH for inducing synchronized estrus and increasing lambing rate in anestrous ewes. J Anim Sci, 79: 1120-1131.

Marcelo GMC, Paulo TFA, Orivaldo SJ, Leandro I, Luis FS *et al*., 2010. Effects of lower doses of cloprostenol intramuscular or into vulvar submucosa on estrus induction and pregnancy rates in Nelore cows. Semina: Ciências Agrárias, Londrina, 31: 451-458.

Pallares P and Gonzalez-Bulnes A, 2009. A new method for induction and synchronization of oestrus and fertile ovulations in mice by using exogenous hormones. Lab Anim, 43: 295-299.

Peng Z, Yaguang T and He H, 2013. Observation and analysis of Kunming mice reproductive behaviour. Journal of Heilongjiang Anim Reprod, 21: 20-23. (In Chinese)

Richardson AM, Hensley BA, Marple TJ and Jonson SSK, 2002. Characteristic of estrus before and after first insemination and fertility of heifers after synchronized estrus using GnRH, PGF2a, and progesterone. J Anim Sci, 80: 2792-2800.

Suocheng W, Shien C, Zhuandi G, Xiahui O, Lifeng A *et al*., 2013a. Alarelin active immunization influences expression levels of GnRHR, FSHR and LHR proteins in the ovary and enhances follicular development in ewes. Anim Sci J, 84: 466-475.

Suocheng W, Xiahui O, Ayimuguli, Qiongyi L, Zhuandi G *et al*., 2013b. GnRHa active immunity regulates expression of LHR protein and development of uteri in ewes. J Appl Anim Res, 1-7. http://dx.doi.org/10.1080/09712119.2013. 783481

Suocheng W, Zhuandi G, Min W, Kun X and Jiuhai L, 2011. GnRH Agonist Active Immunization Influences Gonadotropin Receptor Expression in Pituitary Gland, Uterine Development and Secretion of Peripheral Reproduction Hormones in Female Mice. Clin Experim Med J, 5: 243-253.

Xiying C, 2011. Experiment of Promoting Synchronous Estrus in Sheep with Prostaglandin and Vaginal Sponge Plug. Chin Qinghai J Anim Vet Sci, 41: 23-24. (In Chinese)

Zhuandi G, Suocheng W and Min W, 2011. GnRH analogues enhanses LH and FSH secretion, ovarian development in female rabbits. Basic Clin Med, 31: 13-18.