INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–395/2014/16–2–427–430
http://www.fspublishers.org

Full Length Article

Influence of Different Doses of Equine Chorionic Gonadotropin on Follicular Population and Plasma Estradiol Concentration in Chinese Holstein Dairy Cows

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Abstract

The present study was designed to compare the effect of different doses of equine chorionic gonadotropin (eCG) on follicular population and plasma estradiol concentration in Chinese Holstein cows synchronized with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). One hundred cows were divided into five treatment groups. Each group (n=20) was administered with an injection of eCG at dose rate of 2, 2.5, 3, 3.5, or 4 IU/kg b.w., respectively, followed by administration of $PGF_{2\alpha}$ 48 h later. Ovarian scanning was performed by transrectal ultrasonography around estrus, while plasma estradiol concentration at administration of eCG, $PGF_{2\alpha}$, estrus and 12 h after estrus was measured by Radioimmunoassay (RIA). The results showed that medium follicles (5-10 mm) varied ($P<0.05$) among all treatment groups with an increasing trend. However, there was no significant difference ($P>0.05$) in the number of large follicles (>10 mm). Plasma estradiol concentration differed non-significantly ($P>0.05$) in all groups before estrus. At estrus, the concentration was at peak in high dose treatment group (4 IU/kg, $P<0.05$) than those of low dose treatment groups (2 and 2.5 IU/kg). The study concluded that eCG increases the recruitment and development of medium follicles in dose dependent manners however it has no subsequent effect on large follicles population.

Keywords: eCG; Estrus; Medium follicles; Large follicles; Estradiol

Introduction

Equine chorionic gonadotrophin (eCG) has been used as synchronizing agent for promoting follicular growth in cows. Numerous reports have been shown that treatment with eCG resumed follicular activity (Baruselli *et al.*, 2004; Rostami *et al.*, 2011; Jabeen *et al*., 2013) and exhibited a significant linear relationship with number of follicular population (Monniaux *et al.*, 1984) in heifers. In routine, eCG has been used to induce follicle maturation and to attain better oocyte and/or corpus letuem (CL) quality for obvious increase in pregnancy rate (Bó *et al.*, 2003). In heifers, treatment with eCG doubled follicular growth rate (Sá Filho *et al.*, 2010). Normally, an injection of eCG leads to an initial increase in the number of small follicles followed by their recruitment and selection into medium and large follicles (Driancourt *et al.*, 1991;).

High doses of eCG treatment may have a mixed response in regulating ovarian follicular population. In one way, it increases the developmental process of follicles and high doses of eCG may be helpful in recruiting more follicles into medium and large follicles, thus increasing the chances of multiple ovulation. Moreover, a relationship is already existed between eCG dose and multiple ovulations and/or births in dairy cattle (Andreu-Vázquez *et al.*, 2012). On the other hand, treatments with eCG can affect ovarian endocrine function and lead to an obvious increase in estradiol productions (Liu *et al.*, 2007). Some previous reports observed that eCG increased the amount of mRNA for cytochrome P45017α resulting in increased estradiol production (Soumano *et al.*, 1996; 1998). Many studies have revealed that a significant amount of estradiol is produced by the dominant follicles (Ireland *et al.*, 1984). The conception rates are most probably influenced by the overall estradiol concentration, mainly produced by the dominant follicles (Kiewisz *et al.*, 2011; Enginler *et al*., 2012).

Taken together, these observations imply that both number of follicles and estradiol concentrations might be affected by the treatment of different eCG doses and thus needed to be optimized in dairy animals. The present study was designed to investigate the effect of different doses of eCG on growing follicular populations and their association with estradiol concentration.

To cite this paper: Fu, S.B., H. Riaz, M.K. Khan, H.L. Zhang, J.G. Chen and L.G. Yang, 2014. Influence of different doses of equine chorionic gonadotropin on follicular population and plasma estradiol concentration in Chinese Holstein dairy cows. *Int. J. Agric. Biol*., 16: 427-430

Materials and Methods

Animals

All the experimental procedures were approved by the Animal Care and Use Committee (Approval ID: SCXK (Hubei) 2008-0005), College of Animal Science and Technology, Huazhong Agricultural University, PR China. eCG injection commercially available as Serum Gonadotrophin (1000 IU) and $PGF_{2\alpha}$ commercially sold as Cloprostenol Sodium Injection (0.2 mg) were purchased from Ningbo Sansheng Pharma chemicals, China. Briefly, 100 non-pregnant and healthy Chinese Holstein dairy cows were selected during the period of March and April. The cows were medium sized (Body condition score ranges from 3 to 3.5), weighing 450 to 550 kg and were housed in Jingdu dairy farm, Hubei, China. Before starting the experiment, all the cows were confirmed to be free from any reproductive problems. The animals were kept under same nutritional and management conditions while their cyclicity was monitored based on farm records. All the cows were randomly divided into five treatment groups of 20 animals each. Synchronization treatments were initiated on days 8-10 of the estrous cycle with a single intramuscular (IM) injection of respective doses of eCG (2, 2.5, 3, 3.5 or 4 IU/kg) in five different groups, followed by intramuscular administration of $PGF_{2\alpha}$ (0.4 mg/cow) 48 h later. Cows were observed for estrus by a teaser bull thrice a day.

Ovarian Ultrasonography

Ovarian trans-rectal ultrasonography was undertaken at estrus period by a B-mode veterinary ultrasound scanner equipped with a 5.0 MHz linear array transducer (Zhang *et al.*, 2010). The ultrasonographic images of ovaries were collected to analyze the number of follicles (Fig. 1). Photographs of the ovaries were transmitted to a computer workstation and were analyzed to assess the extent of ovarian follicular activity during the experiment. The location and size of each follicle (≥5 mm in diameter) was recorded on an ovarian map.

Blood Collection and Estradiol Assay

Blood samples for estradiol concentrations were collected by tail vein puncture in heparinized, evacuated tubes at the time of administration of eCG, $PGF_{2\alpha}$, estrus and 12 h after estrus. All blood samples were kept at 4°C and centrifuged within 6 h of collection; plasma was removed and frozen at -20°C until assayed. Serum estradiol concentration was measured by RIA and was analyzed by a solid-phase radioimmunoassay kit (Beijing Chemclin Biotech Co. Ltd, Beijing, China). The intra- and inter assay coefficients of variation for estradiol calculated from the precision profiles were less than 10% and 15%, respectively. The sensitivities for the least detectable amount of estradiol were less than 3 pg/mL.

Statistical Analysis

All data were analyzed by ANOVA using SPSS (version 13.0, SPSS Inc., Chicago, IL) and have been presented as mean ± S.E.M. The probability of $P<0.05$ was considered statistically significant.

Results

Follicle Population

In this experiment, more than 70% cows showed estrus signs, including restlessness, vulvar discharge and standing heat. Among different groups, the number of medium follicles (5 to 10 mm) at estrus increased in a dose dependent manner. However, there was no significant difference in the number of medium follicles between the treatment groups ($P>0.05$) received 3 and 3.5 IU/kg of eCG. While the animal group treated with 4 IU/kg of eCG had higher number of medium follicles ($P<0.05$). Treatment with different eCG doses imparted a non-significant ($P>0.05$) effect on large follicle population (>10 mm). The ultrasonographic images of various medium and large follicles have been presented in Fig. 1.

Estradiol Concentration

Plasma estradiol concentration was similar among all groups at the time of eCG administration. After eCG treatment, serum estradiol concentrations started to increase ($P>0.05$) in all groups. Following $PGF_{2\alpha}$ administration, there was sharp increase ($P<0.01$) in estradiol concentration (Fig. 2).

Mean peak plasma estradiol concentration in all groups was attained at the time of estrus. Moreover, estradiol concentration was higher in the treatment group received high dose of eCG (4 IU/kg) ($P<0.05$) as compared to those received low doses of eCG (2 and 2.5 IU/kg). After estrus, serum estradiol concentration started to decrease in all treatment groups. The estradiol concentration was also prolonged in high dose treatment group (4 IU/kg) 12 h after estrus ($P<0.01$), while it was lowest in low dose treatment group (2 IU/kg).

Discussion

The present study confirmed that exogenous eCG administration has a positive effect on follicular development in Chinese Holstein cows. A similar discovery has been previously reported in Holstein cows with improved ovulatory stimulus after eCG treatment (Souza *et al.*, 2009). The eCG administration also appreciated the induction of new follicles in ruminants (Aba *et al.*, 2005). However, several reports indicated that eCG significantly increased the number of normal preantral follicles but without altering number of normal antral follicles (Warriach and Ahmad, 2009).

Table 1: Follicular population among five different doses of eCG (mean ± S.E.M)

eCG dose (IU/kg)	Cows in Estrus (n)	Number of Follicles (n)	
		Medium Follicles	Large Follicles
2	14	0.29±0.13[a]	1.07±0.07
2.5	18	2.56±0.27[b]	1.06±0.1
3	18	4.39±0.36[c]	1.17±0.12
3.5	16	4.63±0.46[c]	1.19±0.16
4	16	6.31±0.28[d]	1.28±0.18

a-d values within the same column with different superscripts differ significantly ($P < 0.05$)

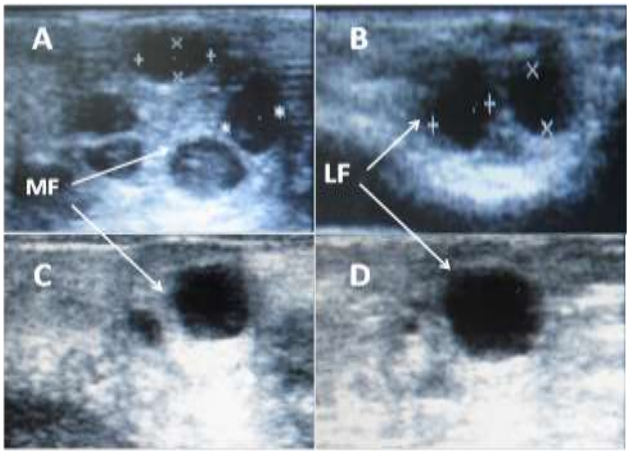

Fig. 1: Ultrasonograms of medium follicles (MF) (A), large follicles (LF) (B), a small and medium follicle (C) and a large follicle on the ovary (D)

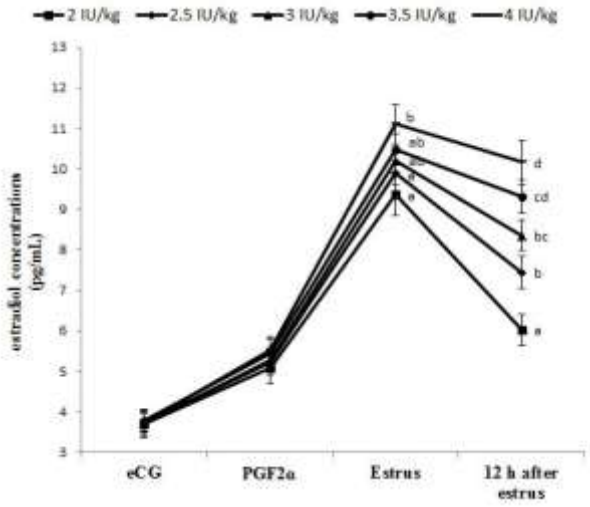

Fig. 2: Plasma estradiol concentrations at different events in Chinese Holstein cows

A positive influence of different doses of eCG on follicular population in Chinese Holstein cows was demonstrated in this study, which is an agreement with previous report in Pelibuey ewes (González-Reyna *et al.*, 1999). The findings that the number of medium follicles increased at the highest dose of eCG are in agreement with a previous report in Holstein cows (Gonzalez *et al.*, 1994). However, the number of large follicles remained unaffected among all treatment groups. This conclusion may be supported by many previous results, which indicated that large follicles might inhibit the development of other growing follicles and reduced the superovulatory response to eCG (Guilbault *et al.*, 1991; Huhtinen *et al.*, 1992).

Results from this study demonstrated that a sudden increase in estradiol concentrations was observed after administration of $PGF_{2\alpha}$ up to estrus in all treatment groups. It is generally believed that plasma estradiol concentration increased significantly after follicles reach 8-9 mm diameter (Sheldon and Dobson, 2000). Similar increase is also reported in beef cows where estradiol began to rise about 48 h before estrus in all groups and reached highest at the time of estrus (Henricks *et al.*, 1973). Moreover, the plasma estradiol concentration in the highest eCG dose group (4 IU/kg) was higher than the other groups (2 and 2.5 IU/kg) at estrus. This finding might be due to the reason that estradiol is mainly produced by the dominant follicle (Evans *et al.*, 1997; Yimer *et al.*, 2012) and an increasing tendency of total number of large follicles was observed as the dose of eCG increased in this study.

The plasma estradiol concentrations started to decline after estrus, and the estradiol concentration in the higher dose groups was prolonged than in the lower dose groups. High doses of eCG lead to excessive follicular development which often resulted in incomplete ovulations, and these un-ovulated follicles secretes abnormally high levels of estradiol, which might be a reason of high plasma estradiol concentration in the group administered with high dose of eCG in the present study. However, the high estradiol concentration may have adverse effects on embryo development (Ziecik *et al.*, 2005). Some reports revealed that large eCG doses caused reproductive disorders during final follicular development and oocyte maturation which are known to proceed according to a chronologically fixed hormonal sequence in normally cyclic cows (Gonzalez *et al.*, 1994) and lead to lower pregnancy rate (Fu *et al.*, 2012).

In conclusion, this study showed that the higher dose of eCG for synchronization increased the number of medium follicles and affected the longevity of estradiol concentrations.

Acknowledgments

This work was supported by Special Fund for Agro scientific Research in the Public Interest (201003060) and the earmarked fund for Modern Agro-industry Technology Research System (CARS-37-04B).

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(Received 25 March 2013; Accepted 10 April 2013)