Reprod Dev Biol 41(1) : 1-6 (2017)
http://dx.doi.org/10.12749/RDB.2017.41.1.1

ISSN : 1738-2432 (Print)
ISSN : 2288-0151 (Online)

Studies on Steroid Hormone Concentration during the Estrous Cycle in the MediKinetics Micropig®

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ABSTRACT

In all mammalian species, progesterone is essential to both the preparation for, and maintenance of, pregnancy. The 20 α-hydroxysteroid dehydrogenase (20 α-HSD) enzyme predominantly converts progesterone into its biologically inactive form 20 α-hydroxyprogesterone, thereby regulating its activity. Thus, to directly assess sexual maturation in the MediKinetics micropig®, we analyzed the concentration of the steroid hormones progesterone and estradiol during the estrous cycle.

Our results show that the progesterone level exhibited by the analyzed micorpig® was low at the beginning of the estrous cycle, and then abruptly increased to 30.32±10.0 ng/mL and 46.37±11.0 ng/mL by days 9 and 11 of the cycle, respectively. It reached the highest level 55.87±3.5 ng/mL on day 13 of the estrous cycle, before decreasing to 46.58± 13.1 ng/mL and 10.0±7.6 ng/mL by days 15 and 17 of the cycle, respectively. In contrast, the estradiol level was shown to be highest (27.13±11.2 ng/mL) at the initiation of the estrous cycle, after which point it decreased to 13.29±6.5 ng/mL and 10.94±5.9 ng/mL by days 4 and 5 of the estrous cycle, respectively. By day 17 of the estrous cycle, the estradiol level decreased to 4.13±7.6 ng/mL.

We anticipate that these results will provide useful information to enable the study of human ovulation and reproductive physiology using the MediKinetics micoripig® as a model system. We recommend further investigation to elucidate the functional mechanisms underlying the regulation of sexual maturation in the MediKinetics micropig®.

(Key words : Steroid hormone, Progesterone, Estradiol, Estrous cycle, MediKinetics micropig®)

INTRODUCTION

The development of each corpora luteum (CL) is tightly controlled by a balance of antagonistic effects exerted by luteotropic and luteolytic, as well as angiogenic and antiangiogenic, factors (Przygrodzka *et al*., 2016). In contrast to other domestic animals, the porcine CL is unique in that a single injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) does not induce luteolysis before day 12 of the estrous cycle (Guthrie and Polge 1976).

The enzyme 20 α-hydroxysteroid dehydrogenase (20 α-HSD) catalyzes the conversion of progesterone into its inactive form (20 α-hydroxyprogesterone), and thus plays a critical role in the regulation of luteal function in mammals by controlling progesterone activity (Naidansuren *et al*., 2011). In most species, ovarian CL, follicle, and interstitial cells undergo an HSD-catalyzed, stereo-specific transformation to adopt a 20 α-configuration (Seong *et al*., 2002). Thus, HSDs represent a major class of NADPH-dependent steroid hormone oxido reductases that are vital for the pre-receptor regulation of hormone action (Penning 1997, 2011). In all mammalian species, progesterone is essential to both prepare the body for pregnancy by regulating endometrium development to facilitate implantation, and to maintain pregnancy by inhibiting uterine contractions until birth (Seo *et al*., 2011). Aldo-keto reductases (AKRs) belong

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to a superfamily of NADPH-dependent reductases that act on diverse substrates, including simple carbohydrates, steroid hormones, and endogenous prostaglandins (Jez *et al*., 1997).

In previous studies, we evaluated 20α-HSD gene expression in the CL during the estrous cycle, and determined 20α-HSD to be highly expressed in the large luteal cells during the bovine estrous cycle (Naidansuren *et al*., 2011), bovine early pregnancy (Kim *et al*., 2014), and porcine estrous cycle (Seo *et al*., 2011). During early pregnancy, pig 20α-HSD known to be mainly localized in the trophoblast villus in the placenta, but also to be highly expressed in the glandular epithelial cells of the endometrium and the luminal epithelial cells of the uterus (Nanjidsuren *et al*., 2014). $PGF_{2\alpha}$ is known to induce abortion in several species, including rodents (Deis, 1971), and furthermore, administration of $PGF_{2\alpha}$ to pregnant rats increases luteal 20α-HSD activity (Telleria *et al*., 1999). We have also previously shown that pig 20α-HSD protein is most highly expressed in the ovary on day 0 of the estrous cycle, consistent with the observed 20α-HSD mRNA expression pattern at this point of the cycle (Seo *et al*., 2011).

Commonly used model animal species, (for examples, mice, rats, rabbits, and dogs), are not always appropriate to study the potential pharmacological and toxicological effects of a given drug candidate for subsequent clinical use in humans (Peter *et al*., 2016). We propose that the minipig may be a good alternative model organism in this case because many aspects of its anatomy and physiology are very similar to that of humans (Jorgensen 1998; Bode *et al*., 2010; McAnulty *et al*, 2012).

Numerous studies demonstrate the applicability of nuclear-transfer cloning in pigs (Vajta *et al*., 2007; Kues *et al*., 2006; Deppenmeier *et al*., 2006). The Gottingen minipig is a useful and unique resource that was originally, developed by the Institute of Animal Breeding and Genetics, Georg August University of Gottingen. It is characterized by a light skin color resulting from a "dominant white" genetic status rather than albinisms, as shown by the presence of melanin in the skin, and pigment in the retina (Foster *et al*., 2010; Marklund *et al*., 1998). While the minipig is established as an effective model for various human systems (for example, the skin, digestive tract, and cardiovascular system), its use as a model for human reproductive biology requires verification.

In the present study, we investigated steroid hormone levels produced during the estrous cycle in the SPF MediKinetics micropig®. We anticipate that our results will provide useful information regarding ovulation and reproductive physiology in this model animal.

MATERIALS AND METHODS

Materials

Progesterone and estradiol kits were obtained from DRG (Germany). Micropigs (7~8 month-old; 25~27 kg) were obtained from MediKinetics (PyeongTaek, Korea). All other chemicals were obtained from local suppliers. All animal experiments, (including also the maintenance of animal housing), were performed in accordance with the Hankyong National University Animal Care and Use Committee Guidelines (approval number: HKNU 2014-04).

Collection of Blood Samples during the Estrous Cycle

The blood samples of four MediKinetics micropigs® were collected from the jugular vein, every 2 days during the estrous cycle. Each sample was stored at 4°C, and the serum for hormone analysis collected via centrifugation of the blood sample (3,000 rpm, 15 min).

Steroid Hormone Analysis

The concentration of progesterone and estradiol in each serum sample was measured by specific ELISA (DRG, Germany). Aliquots (25 μL) of serum, control, and standard curve (Progesterone: 0~400 ng/mL; Estradiol: 0, 25, 100, 250, 500, 1,000, 2,000 pg/mL), samples were dispensed into appropriate wells of a 96-well plate. The enzyme conjugate was then dispensed into each well, and thoroughly mixed 10 s, before incubation of the plate (120 min, room temperature). After incubation, the plate was briskly shaken to remove the contents of each well, and then rinsed three times with diluted wash solution (400 μL/well). Residual droplets were removed by stroking the plate sharply on absorbent paper. The substrate solution (100 μL) was added to each well and incubated (15 min, room temperature). Finally, the enzymatic reaction was stopped by adding 50 μL of stop solution to each well, and the absorbance (OD) of each well at 450 nm was determined using microtiter plate reader.

RESULTS

Progesterone Concentration during Estrous Cycle

First, we examined the progesterone concentration exhibited by the MediKinetics micropig® during the estrous cycle. One (No. 30) of the four micropigs did not exhibit normal pattern of hormone production during

this process; thus, it was eliminated from further analysis. As shown in Fig. 1, the progesterone levels of the three remaining micropigs followed a similar pattern. The progesterone level observed at the initiation of the estrous cycle was 0.28±0.1 ng/mL. This gradually increased to 11.78±6.5 ng/mL by day seven of the estrous cycle, and then abruptly increased to 30.32±10.0 ng/mL and 46.37±11.0 ng/mL on days nine and 11 of the estrous cycle, respectively.

The progesterone level reached its highest point at 55.87±3.5 ng/mL on day 13 of the cycle. After this point, the progesterone level progressively decreased to 46.58±13.1 ng/mL and 10.0±7.6 ng/mL by days 15 and 17, respectively. Finally, it returned to its initial level (1.47±1.5 ng/mL) on day 17 of the estrous cycle. Thus, progesterone levels in the MediKinetics micropig® were observed to be high between days 11 to 15 of the estrous cycle.

Estradiol Concentration during Estrous Cycle

We next analyzed the estradiol concentration exhibited by the MediKinetics micropigs® during the estrous cycle. The estradiol levels of three of the four analyzed micropigs were observed to follow similar patterns (Fig. 2), whereas the estradiol concentration in the other micropig (No. 84) was detected at an unexpectedly high level up to day 17 of the estrous cycle. At this point, the level of estradiol dramatically decreased, plateauing at a low level in a similar manner to that observed in the three concordant micropigs.

The estradiol level in the three concordant micropigs was highest (27.13±11.2 ng/mL) at the initiation of the estrous cycle, and then decreased to 13.29±6.5 ng/mL and 10.94±5.9 ng/mL by days 4 and 5 of the cycle, respectively. The estradiol level gradually decreased to 8.06±6.5 ng/mL and 8.02±6.7 ng/mL on days 15 and 17, respectively. It decreased to 4.13±7.6 ng/mL on day 17 of the estrous cycle, before reaching its lowest level (1.47±0.8 ng/mL) on day 21. Thus, the observed high level of estradiol production immediately decreased after the initiation of the estrous cycle in the MediKinetics micropig®, and remained low for the rest of the cycle.

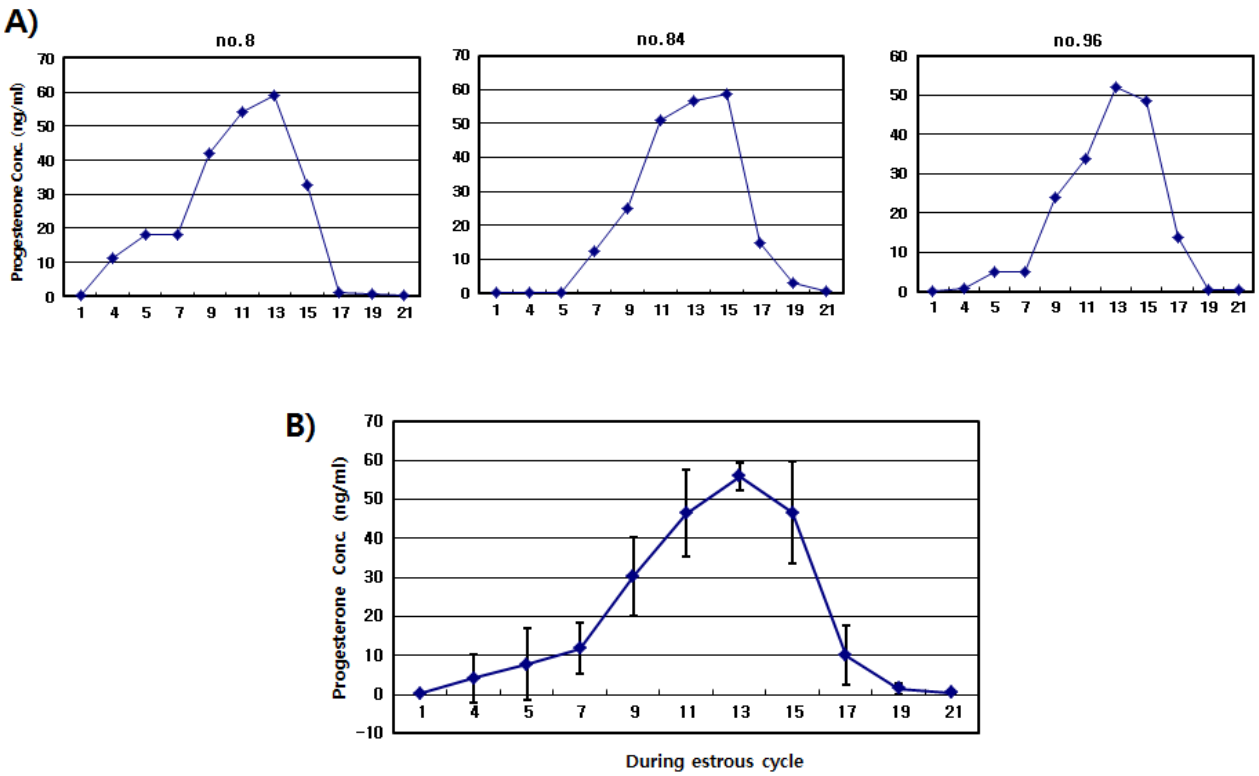

Fig. 1. Progesterone levels in the MediKinetics micropig® during the estrous cycle. The day upon which the first estrous symptom was observed was designated as day 1 of the estrous cycle. Blood samples were collected from four micropigs throughout the cycle, and the progesterone level in each sample then analyzed using an ELISA system. Both separate data (A), and the average progesterone levels (B), are shown for three analyzed micropigs. Data in (B) are shown as the mean±SEM. (NB: one micropig was excluded from analysis).

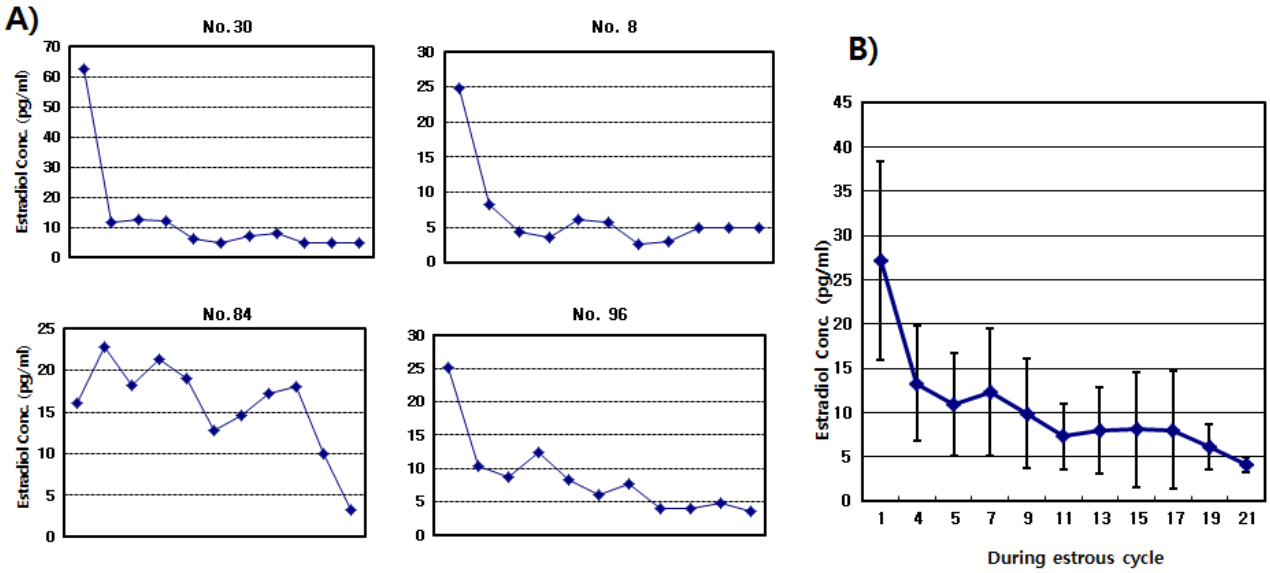

Fig. 2. Estradiol levels in the MediKinetics micropig® during the estrous cycle. The day upon which the first estrous symptom was observed was designated day 1 of the estrous cycle. Blood samples were collected from four micropigs throughout the cycle, and the estradiol level in each sample then analyzed using an ELISA system. Both separate data (A), and the average estradiol levels (B), are shown for the four analyzed micropigs. Data in (B) are shown as the mean±SEM.

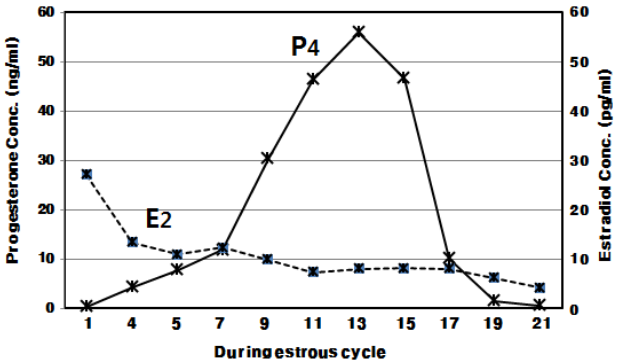

Fig. 3. Progesterone and estradiol levels in the MediKinetics micropig® during the estrous cycle. The observed progesterone and estradiol levels during the estrous cycle are shown. The average data from Figures 1 and 2 are overlaid to allow a direct comparison of hormone concentration data during the progression of the estrous cycle.

DISCUSSION

This is the first report describing progesterone and estradiol levels in the MediKinetics micropigs® during the estrous cycle. In the present study, progesterone level was very low at the initiation of the estrous cycle and reached the highest level on day 13 of the cycle. After then it decreased dramatically to its initial level. However, the estradiol level was the highest at the initiation of the estrous cycle, and then decreased to 13 ng/mL by day 4 of the cycle. The estradiol level gradually decreased to 4 ng/mL on day 17 of the estrous cycle.

Administration of $PGF_2\alpha$ topregnantrats (Olofsson *et al.*, 1972) and pseudo-pregnant rats has been shown to increase luteal 20α-HSD activity (Deis, 1971), and similarly, $PGF_2\alpha$ markedly stimulates activity of 20α-HSD in mice (Stocco*et. al.*, 2000, 2002). In addition, 20α-HSD is itself responsible for $PGF_{2\alpha}$ production in cattle(Madore *et. al.*, 2003). We have previously shown that 20α-HSD in the pig is mainly expressed in the ovary and placenta during the estrous cycle and pregnancy (Seo *et. al.*, 2011; Nanjidsuren *et. al.*, 2014). The activity of 20α-HSD has been investigated in a variety of mammalian tissues, including the ovary (Seo *et. al.*, 2011; Seong *et. al.*, 1998, 2003; Yoshida *et. al.*, 1999; Zetser *et. al.*, 2001), and placenta (Naidansuren *et. al.*, 2012; Nanjidsuren *et. al.*, 2011, 2014; Shiota *et. al.*, 1993).

In the human, The metabolic clearance rate appears to be relatively constant throughout the cycle. Progesterone production rate based on known plasma concentrations of 0.75~2.5 mg/day in the follicular phase and 15~50 mg/day in the luteal phase (Lin *et al.*, 1972). Human placental 20α-HSD was first demonstrated and progesterone and 20α-OHP are interconverted in the placenta (Billiar *et al.*, 1973). The equilibrium of the reaction in human placental mitochondria favors the formation of progesterone (Rabe *et al.*, 1982). Progesterone also plays a role in ovum transport, endometrial cell proliferation and differentiation, decidualization, and the

process of implantation (Albrecht and Pepe 1990). Thus, progesterone hormone is essential to several important events in the establishment and maintenance of human pregnancy.

In bovines, 20α-HSD protein expression has been detected in the ovary during the estrous cycle, and similarly robust 20α-HSD protein expression has been identified on the cotyledon side of the placenta immediately before parturition (Naidansuren *et. al.,* 2011). 20α-HSD mRNA and protein are co-localized in large luteal cells, the placenta, and the endometrium during early pregnancy (Kim *et. al.,* 2014). In the monkey and deer, 20α-HSD protein is expressed in the ovary at the pre-ovulation, and in the placenta at the pre-parturition stages, respectively (Nanjidsuren *et. al.,* 2011; Naidansuren *et. al.,* 2012). Thus, 20α-HSD expression in both the ovary and the placenta is essential for the normal progression of the estrous cycle, pregnancy, and parturition.

In the mouse, strong 20α-HSD protein expression has been detected in the placenta on days 16 and 18 of pregnancy (manuscript in preparation). This observation supports previous studies that also report the presence of 20α-HSD protein in the mouse placenta (Seong *et. al.,* 2002; Shiota *et. al.,* 1993). Nevertheless, the functional significance of 20α-HSD during pregnancy and parturition requires further study.

To the best of our knowledge, this is the first study to analyze steroid hormone production in the MediKinetics micropig® duringtheestrouscycle.Further investigation is required to elucidate the functional significance of 20α-HSD for sexual maturation in the MediKinetics micropig®.

ETHICS

All described experiments were conducted in accordance with the Hankyong National University Animal Care and Use Committee Guidelines (approval number: HKNU 2014-04).

FUNDING

This study was supported by the KRIBB Research Initiative Program (KGM4251622), Republic of Korea.

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(Received: January 16 2017/
Revised February 14 2017/
Accepted February 15 2017)