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Porcine embryo collection using single subcutaneous administration of follicle-stimulating hormone in a large volume of saline

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Abstract. The effects of a single subcutaneous or intramuscular injection of follicle-stimulating hormone (FSH) on follicular growth and expression of estrous behavior and its single subcutaneous administration on the number of corpora lutea (CL) and embryos were investigated in pigs. All four sows that were subcutaneously administered 5 AU FSH expressed normal estrus and had no ovarian cysts. Two of the four sows that were administered 5 AU FSH intramuscularly did not exhibit estrus, and another sow had a short estrus period. All four sows had ovarian cysts. The mean numbers of CL, embryos, and blastocysts following the subcutaneous administration of 5 AU FSH (16.8, 16.0, and 13.8, respectively) did not differ significantly from those for the control animals treated intramuscularly with 1000 IU equine chorionic gonadotropin (18.5, 16.5, and 14.3, respectively). In conclusion, embryo recovery was possible using a single subcutaneous administration of FSH.
Key words: Embryos, Follicle-stimulating hormone, Gilts, Sows

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Many researchers have succeeded in producing viable piglets after the transfer of *in vivo*- or *in vitro*-derived embryos [1]. However, the number of embryos obtained from a valuable female pig is still limited. In pigs, the uterus is coiled in nature and 50–140 cm long in gilts and 100–170 cm in sows [2]. Thus, it is difficult to collect *in vivo*-derived embryos by uterine flushing via transrectal palpation of live animals, unlike in cattle and horses, and surgical procedures are required. In addition, embryos produced by *in vitro* production (IVP) have been derived from ovaries from slaughter houses, and piglets could be produced by IVP-embryos, although the ovaries from slaughter houses are often derived from growing-finishing gilts that have lower genetic value [3]. Nonetheless, the *in vivo* and *in vitro* techniques of porcine embryo production offer considerable promise for improved efficiency of embryo transfer in the pork industry and for biotechnological applications.

In pigs, a single administration of equine chorionic gonadotropin (eCG) is generally used to induce follicular growth for *in vivo* embryo recovery [4]. However, the long biological half-life of eCG causes follicle growth throughout the periovulatory period [5, 6] and induces the formation of large abnormal follicles after ovulation and before embryo recovery [7–9] in cattle. Therefore, instead of eCG, follicle-stimulating hormone (FSH), which has a short half-life, is usually administered twice daily over three [10, 11] to four [12, 13] days to maintain a sufficient blood concentration to induce an ovarian response in cattle. It has been reported that six serial intramuscular administrations of FSH for follicular growth, followed by human chorionic gonadotropin (hCG), increased the number of recovered embryos [14–16] and ovulation rate [17] compared with that of eCG treatment in pigs. Moreover, FSH treatment was able to increase corpora lutea (CL) [18] and the number of mature oocytes collected [19] during a superovulation procedure in prepubertal gilts. However, multiple FSH administrations are labor-intensive, expensive, and stressful to the donor animals.

To simplify FSH treatment for the induction of superovulation in cattle, polyvinylpyrrolidone and aluminum hydroxide gels have been used as a solvent for slow release of FSH after a single intramuscular administration [20–22]. However, these reagents do not have convenient handling properties. Recently, *in vivo* bovine embryos were obtained using superovulatory treatments with a single subcutaneous FSH administration with a large volume of saline [23, 24]. However, there has been no study on the subcutaneous administration of FSH in pigs. We preliminarily examined subcutaneous administration of 20 AU FSH dissolved in 20 ml saline to three cycling gilts at the follicular stage of a normal estrous cycle; however, none exhibited estrus (unpublished data). The administration of a high concentration of FSH might disturb the ovarian cycle in gilts. Iwamura *et al*. reported that abnormal estrus was induced in some prepubertal gilts when a total of 12 AU FSH was administrated for estrus synchronization [14]. Therefore, a larger volume of saline or a lower dose of FSH in a single subcutaneous administration may be suitable for estrus synchronization in pubertal gilts and sows. Here, we report on the response to single subcutaneous administration of a lower concentration of FSH instead of single intramuscular administration of eCG for porcine embryo collection.

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The present study was conducted according to the Institutional Guidelines for Animal Experiments at the Aichi Agricultural Research Center (approval No. 29-7). We examined the effects of a single subcutaneous administration of FSH on follicular growth and expression of estrous behavior. We also confirmed that porcine embryos could be obtained by the developed procedure.

In experiment 1, 12 Large White sows were used. They were divided into three groups of four animals each. The sows were administered 5 AU FSH (in 5 ml saline) intramuscularly, or 5 or 10 AU FSH (in 50 ml saline) subcutaneously (Fig. 1) for follicular growth stimulation (see Methods). Two of the four sows that were administered 5 AU FSH intramuscularly did not exhibit estrus, and another sow had a short estrus period (6 h) (Table 1). Moreover, all four sows developed ovarian cysts. Soeda *et al.* [25] reported that the period of estrus induced by gonadotropins (eCG followed by hCG) was shorter than that of natural estrus, but continued for 39.8 ± 19.3 h (mean ± SD). The four sows that were administered 5 AU FSH subcutaneously exhibited normal estrus periods (24–48 h) and had no ovarian cysts. In the group receiving a subcutaneous administration of 10 AU FSH, two of the four sows had ovarian cysts. These results suggested that a single intramuscular administration of 5 AU FSH prevented normal follicular growth and expression of estrus.

Although the exact cause and pathogeny of ovarian cysts was not completely determined, ovarian cysts have been induced by an injection of adrenocorticotropic hormones, which appear to cause luteinizing hormone (LH) release deficiencies [26]. In addition, Sesti and Britt [27] reported that ovarian cysts occurred in sows weaned during the phase of LH secretion inhibition (from the 3rd to 14th day after farrowing), which seemed to be induced by the suckling stimulus. In this study, a single intramuscular administration of 5 AU FSH might have caused a sudden increase and decrease in the blood concentration of FSH. This might have resulted in the excessively rapid growth of large follicles in ovaries and/or suppression of endogenous LH release, thereby causing transformation of these follicles into ovarian cysts. The subcutaneous administration of 5 AU FSH with 50 ml saline appeared to be adequate to induce normal follicle growth and estrus, whereas that of 10 AU FSH with 50 ml saline might have been excessive for this purpose.

In experiment 2, 16 Large White gilts (8–12 months old) were used to investigate the effects of a single FSH treatment on the number of CL and embryos. The gilts were equally divided into four groups that were administered 1000 IU eCG intramuscularly or 2.5, 5, or 10 AU FSH subcutaneously followed by hCG. In gilts treated subcutaneously with 5 AU FSH, the numbers of CL, embryos, and blastocysts at 7 days after hCG treatment (16.8, 16.0, and 13.8, respectively) were significantly higher ($P < 0.05$) than those in gilts subcutaneously treated with 10 AU FSH (3.0, 2.5, and 2.0, respectively; Table 2). There were no significant differences in the numbers of CL, embryos, and blastocysts among the groups receiving the 2.5 AU FSH, 5 AU FSH, and 1000 IU eCG treatments, although the number of embryos in the group receiving the 2.5 AU FSH treatment tended to be lower than that in the group receiving the 1000 IU eCG treatment ($P < 0.1$). Previous studies only reported greater numbers of embryos or oocytes recovered using serial intramuscular injections totaling over 10 AU FSH (Iwamura *et al*. 2001, 12 AU; Takahagi *et al*. 1998, 12 mg; Nottle *et al*. 1999, 25 mg; 1 mg is considered equivalent to 1 AU) than that using eCG. In the present study, the single subcutaneous administration of a smaller amount of FSH (5 AU) dissolved in 50 ml saline induced the development of ovarian follicles and increased the number of transferable embryos per gilt for embryo recovery, as well as the standard protocol using eCG. The single subcutaneous administration of 10 AU FSH was not apparently suitable for embryo recovery (Fig. 2). This might have occurred because the serial intramuscular injection of FSH in the previous study compared to the single subcutaneous administration of FSH in this study induced different dynamic blood concentrations of FSH in gilts. Thus, future investigation regarding injection methods and blood concentration dynamics of FSH are needed.

In conclusion, this study demonstrated that single subcutaneous administration of FSH could be used for superovulation treatment to recover *in vivo*-derived porcine embryos.

Table 1. Effects of follicle-stimulating hormone (FSH) administration on the expression of estrous behavior and occurrence of ovarian cysts in experiment 1

Amount of FSH (AU)	Route of administration	Sow ID	Estrus expression	Onset of estrus after hCG treatment (h)	Duration of estrus (h)	Ovarian cysts *
5	Intramuscular	1	no	–	–	yes
		2	no	–	–	yes
		3	yes	18	24	yes
		4	yes	18	6	yes
5	Subcutaneous	5	yes	24	24	no
		6	yes	0	42	no
		7	yes	0	48	no
		8	yes	18	30	no
10	Subcutaneous	9	yes	18	30	no
		10	yes	0	42	no
		11	yes	0	48	yes
		12	yes	18	6	yes

* Sows that had ovarian cysts (at least 1.5 cm in diameter) at 66 h after hCG injection.

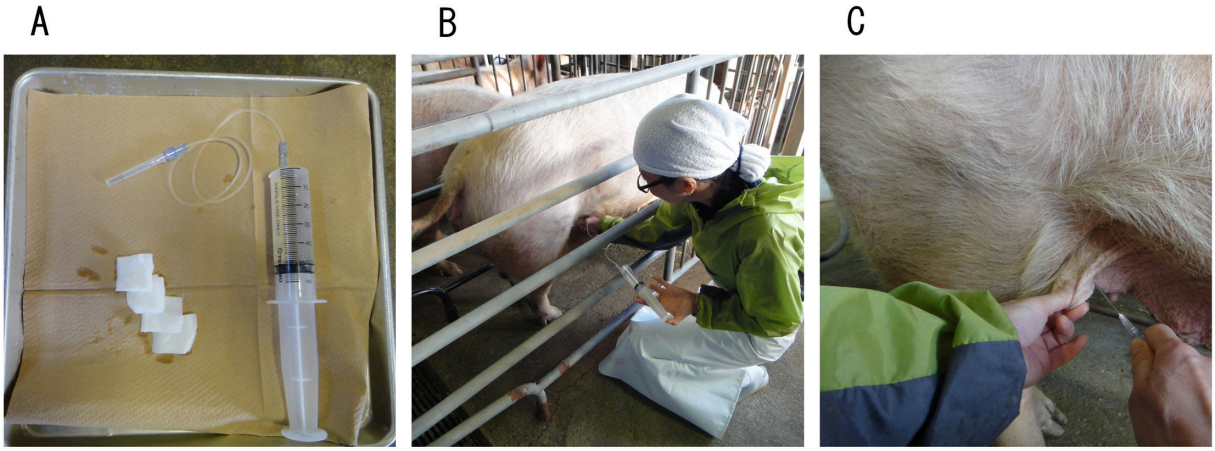

Fig. 1. Procedure for subcutaneous administration. (A) Fifty milliliter syringe, extension tube, and 18-gauge needle. (B) Loose roll of skin in one of the locations in the abdomen region (between mamma and back leg). (C) Point of the needle at the base of the roll of skin with the needle horizontal.

Table 2. Effects of gonadotropin administration on the number of corpora lutea (CL) and embryos in experiment 2

Group	N	The number of (mean ± SD)		
		CL of ovaries	Embryos recovered *	Blastocysts recovered †
FSH (2.5 AU)	4	8.8 ± 6.3 ^{ab}	7.5 ± 5.2 ^{ab}	7.3 ± 4.9 ^{ab}
FSH (5 AU)	4	16.8 ± 5.2 ^{b}	16.0 ± 5.6 ^{b}	13.8 ± 3.1 ^{b}
FSH (10 AU)	4	3.0 ± 4.8 ^{a}	2.5 ± 5.0 ^{a}	2.0 ± 4.0 ^{a}
eCG (1000 IU)	4	18.5 ± 5.5 ^{b}	16.5 ± 3.4 ^{b}	14.3 ± 6.1 ^{b}

* Including non-fertilized and degenerated oocytes. † Early blastocysts to hatched blastocysts. $^{a, b}$ Different letters indicate statically significant differences ($P < 0.05$). FSH, follicle-stimulating hormone; eCG, equine chorionic gonadotropin.

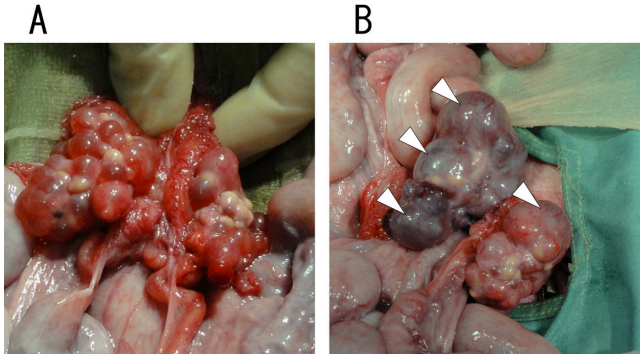

Fig. 2. Ovaries during the surgical recovery of embryos on day 7 after human chorionic gonadotropin (hCG) treatment in experiment 2. Almost all ovaries of the gilts had multiple corpora lutea (CL) and small follicles. (A) Ovaries of the gilt that was subcutaneously administered 5 AU follicle-stimulating hormone (FSH). (B) Few ovaries of the gilt that was subcutaneously administered 10 AU FSH had large follicles, approximately 3 cm in diameter (arrow heads).

Methods

In experiment 1, 12 Large White sows (one parity, 12 to 14 months old) were divided into three groups of four animals. On the day following weaning, sows of two groups were subcutaneously administered 5 or 10 AU FSH (Antrin 10, Kyoritsu Seiyaku, Tokyo, Japan) dissolved in 50 ml saline in the abdomen region using a 50 ml syringe, an extension tube, and 18-gauge needle (Fig. 1A). Briefly, the method of subcutaneous injection is as follows: 1) warm the physiological saline in the syringe, 2) connect the syringe to the tube and needle, 3) fill the fluid line with the FSH dilution from the syringe, 4) feed the sows and keep them in a standing position, 5) pick up a loose roll of skin in the abdomen region (between mamma and back leg; Figs. 1B and C), 6) disinfect with alcohol, 7) place the point of the needle at the base of the roll of skin with the needle horizontal and pointing toward the sow's head, 8) advance the needle slightly forward while pulling the roll of skin backward (this movement should be firm and steady), 9) release the roll of skin (the point of the needle should remain under the skin), and 10) begin the flow of fluids slowly by pushing the syringe pump. The sows showed little reaction to this treatment; however, some slightly raised their hind leg vertically. During subcutaneous administration, the gilts were fed general formula feed. The sows of the third group were intramuscularly administered with 5 AU FSH in 5 ml saline in the neck region. All sows in the three groups received 500 IU of hCG (Gestron1500, Kyoritsu Seiyaku, Tokyo, Japan) to induce ovulation in 72 h after FSH administration. Estrous detection was performed by a standing reflex at 0, 18, 24, 42, 48, 66, and 72 h after hCG administration. Ovaries were examined by transrectal palpation at 66 h after hCG administration. Both ovaries were sketched on graph paper. The follicles with a diameter of at least 1.5 cm were determined to be ovarian cysts.

In experiment 2, 16 Large White gilts with normal estrous cycles were equally divided into four groups. Pseudopregnancy was induced

in the gilts by administrating 20 mg of estradiol dipropionate (EDP; Ovahormone Depot; Aska Pharmaceutical, Tokyo, Japan) between 7 and 11 days after the end of estrus based on a previous report [28]. Between 20 and 35 days after EDP administration, the gilts were treated intramuscularly with a synthetic analog of prostaglandin $F_{2\alpha}$, cloprostenol (Planate, Intervet, Osaka, Japan), twice with a 24 h interval (first 0.26 mg and then 0.18 mg of cloprostenol). The gilts were subcutaneously administered 2.5, 5, or 10 AU FSH dissolved in a 50 ml saline with a second treatment of cloprostenol. They were then administered 500 IU of hCG at 72 h after the second cloprostenol treatment, followed by artificial insemination at 24 h after hCG injection. Seven days after hCG treatment, embryo recovery was performed surgically for gilts under general anesthesia, as described in a previous report [29]. The numbers of CL in the ovaries, embryos (including non-fertilized and degenerated oocytes), and blastocysts (from early blastocysts to hatched blastocysts) recovered were recorded. Gilts in the control group were intramuscularly administered 1000 IU eCG (Serarumon 1000, Kyoritsu Seiyaku) in 5 ml saline, which is used as a standard estrus synchronization treatment in pigs, instead of FSH. The numbers of CL, embryos, and blastocysts were compared among groups using a one-way ANOVA followed by the Tukey test. Differences were considered significant at a P-value < 0.05.

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