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Relationships between Ovulation and Fertilization Rate in Different Species of Pigs

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ABSTRACT

The aim of the present study was to investigate the ovulation rate and its relationship to fertilization ability in Landrace, Durock and Crossbred pigs. Gilts were natural mated at a body weight of at least 120 kg under the same hormone treatment. Embryos were surgically collected 1 day after natural mating (Day 0). Embryos derived from *in vivo*-fertilized oocytes were cultured in medium PZM-3. The ovaries were examined and the pathological findings were recorded. The number of corpus hemorrhagicum was counted, and was assumed to equal the ovulation rate. There was no difference in the number of corpus hemorrhagicum (20.4, 28.8 and 23.2) and ovulation (13.5, 26.8 and 17.2) in the Landrace, Durock and Crossbred pigs. The two pronucleus formation was 76.0, 80.0 and 86.9%. The Day-7 embryos had blastocyst rates of 68.0, 75.0 and 73.9%. There was no difference in the number of total cells and apoptotic cells. In the future, more studies require determining relationships between ovulation and fertilization rate in different species of pigs.

(Key words: Landrace, Durok, Crossbred, *in vivo* embryos, porcine)

INTRODUCTION

In swine industry, producers maintain a pool of replacement gilts (D'Alliare and Drolet, 1999) to ensure that a sufficient number of gilt will be readily available to meet their breeding target. Effective methods to induce superovulation and increase numbers of quality embryos have been elusive in swine. Litter size at birth is determined by ovulation rate, fertilization, embryo attachment and survival and uterine capacity. Variation in litter size born depends on the expression of pre-natal traits by dams and embryos (Bennet and Leymaster, 1990). They have succeeded when selection for higher ovulation rate was followed by direct selection for increased litter size (Lamberson *et al.*, 1991). They had the largest potential to improve reproductive efficiency and were followed by crossbred gilts (Irgang *et al.*, 1993). High prolificacy early in the life of sows is important to improve reproductive efficiency. Selection experiments aiming to improve the number of pigs born per litter have in resulted null or in very low genetic gains per generation (Bollet *et al.*, 1989; Neal *et al.*, 1989). Pig can ovulate in excess of ten per cycle. However, the mechanism that regulates the species-specific differences in the number of follicles that go onto ovulate during each reproductive cycle (ovulation-rate) is unknown. The aim of the present study was to investigate the ovulation rate and its relationship to fertilization ability in Landrace, Durock and Crossbred (Landrace × Large landrace × Yorkshire) pig.

MATERIALS AND METHODS

1. Animal Ethics

All animal experiments were approved and performed under the guidelines of the RDA Care and Experimentation Committee.

2. Collection of Embryos from Oviduct

The reproductive tracts of purebred Landrace, Durock and Crossbred (Landrace × Large White Landrace × Yorkshire) gilts were examined. Thirty one gilts were injected with PG 600 (Intervet International BV, Boxmeer, The Neverlands) at 200~300 days of age. Gilts that responded to the injection by exhibiting standing estrus continued to be observed. They were administered 1,500 IU of hCG (Intervet International BV, Boxmeer, The Netherlands) by intra muscular injection 72h after the PMSG injection. Embryos were recovered surgically

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at 30～32 h after mating with Landrace boar. Embryos were collected from the oviducts by flushing with 20 ml of sterile Dulbecco's phosphate buffered saline (Gibco BRL). The embryos were centrifuged at 15,000 × g for 5 to 10 min to visualize the pronuclei.

3. *In Vitro* Culture

In vivo embryos were cultured at 39℃, 5% CO_2, 5% O_2 at saturation humidity. After 7 days of incubation, embryos were examined the quality, blastocyst rate and total cell number.

4. Apoptosis Assays

The blastocysts on Day 7 were washed twice in PBS/PVP (PBS supplemented with 0.1% polyvinylpyrolidone) and fixed in 4% (v/v) paraformaldehyde solution for 24 hr at 4℃. Membranes were permeabilized in 0.5% Triton X-100 for 30 min at room temperature. A TUNEL assay was used to assess the presence of apoptotic cells (in situ cell death detection kit, TMR red; Roche, Mannheim), for 1 hr at 38.5℃ in the dark. The broken DNA ends of the embryonic cells were labeled with TDT and fluorescein-d UTP. After the reaction stopped, the embryos were washed and transferred into 10 μg/ml Hoechest 33342 for 30 min at room temperature in the dark. The embryos were washed three times and mounted on slides with Prolong antifade Ket (Cat. P-748, Molecular Probes, Eugene, OR). The slides were stored at −20℃. The numbers of apoptotic nuclei and total numbers of nuclei were determined from optical images of whole-mount embryos under an epifluorescent microscope (Nikon, Tokyo, Japan).

5. Statistical Analysis

Data were subjected to a Generalized Linear Model procedure (PROC-GLM) of the Statistical Analysis System (SAS Institute, Cary, NC). Differences among treatment means were determined by using the Duncan's multiple rage tests. All data were expressed as Least Square (LS) mean±SEM (Standard Error of the sample Mean). A probablility of $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

1. The Number of Corpus Hemorrhagicum and Collected Embryos following Different Pig Species

After hormones treated gilts, the embryos were surgically collected 30～32 h after natural mating. We examined that relationship about corpus hemorrhagicum and number of ovulate oocytes (Fig. 1). There was no difference in the number of hemorrhagicum (20.4, 28.8 and 23.2, Table 1) and collected embryos were slightly higher in Durock than Landrace or Crossbred pigs (26.8 and 13.5, 17.2, Table 1). Crossbred gilts

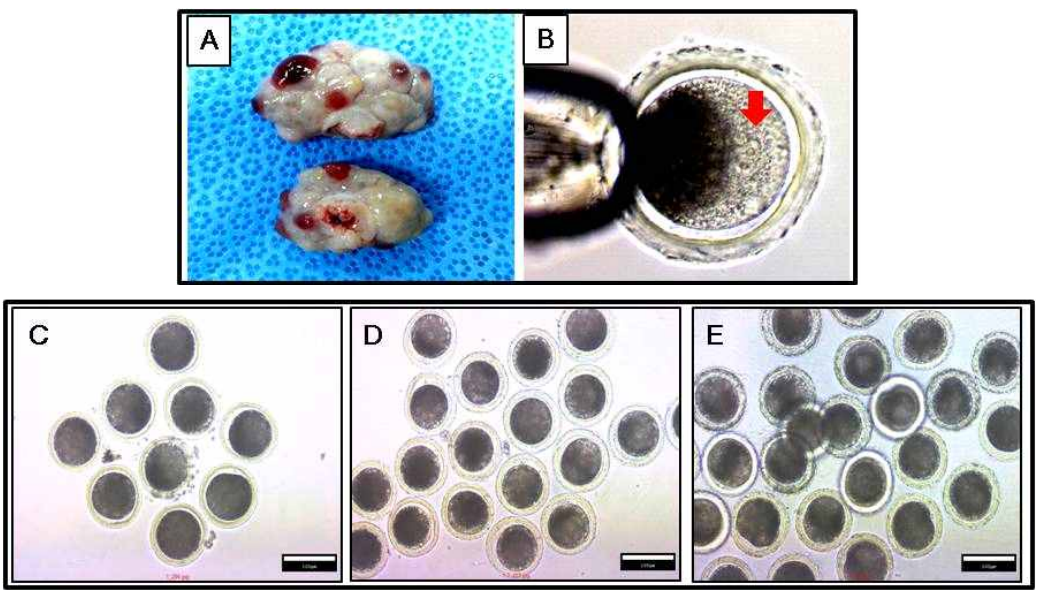

Fig. 1. Representative photographs of ovary and *in vivo* embryos. A: Corpus hemorrhagicum, B: Pronuclei, C-E: Landrace, Durock, and Crossbred *in vivo* embryos.

Table 1. The number of corpus hemorrhagicum and collected *in vivo* embryos following porcine species

Species (No. of head)	No. of corpus hemorrhagicum in ovary			No. of collection of embryos		
	Left	Right	Total	Left	Right	Total
Landrace (13)	11.3±3.1	9.1±2.5	20.4±2.1	7.0±3.1	6.5±1.1	13.5±1.9
Durock (10)	15.7±2.2	13.2±2.1	28.8±1.9	13.7±2.1	13.2±2.1	26.8±2.1
Crossbred (12)	11.6±2.1	11.6±2.1	23.2±2.1	8.2±1.1	9.0±2.1	17.2±1.8

had the highest ovulation rate and the largest number of live embryos (Irgang *et al.*, 1993). We suggested that the different of number hemorrhagicum and ovulation embryos was lost under collected from oviduct. Marked genetic differences in ovulation rates have been found in different breeds of sheep and provide a powerful tool to elucidate the mechanisms which control ovulation rate (Webb *et al.*, 1998). However, there is no difference between breed gilts. Ovulation rate can be a major determinant of reproductive efficiency. The processes of recruitment and selection lead to the development of a number of ovulatory follicles that is specific for particular species and breeds. Large differences between breeds have reported. Besides these differences, considerable variation exists within a given breed (Despres *et al.*, 1992; Bidanel *et al.*, 1996; Evans and O'Doherty, 2001). Several factors interfere with ovulation rate at following breed, age, level of nutrition etc (Paterson, 1982; Burnett *et al.*, 1988; Tilton *et al.*, 1995; Bidanel *et al.*, 1996). Large white gilts used naturally displays high ovulation rate (Bolet *et al.*, 1986). The duration of folliculogenesis from primordial follicle to ovulation is shorter in the pig (Campbell *et al.*, 2003b; Hunter *et al.*, 2004). Estrous cycle length does not vary greatly, although the follicular phase is longer in the pig and this is associated with increased numbers of follicles selected for ovulation (Hunter *et al.*, 2004).

2. Ability of Fertilization and Development between Landrace, Durock and Crossbred Pig Embryos

As shown in Table 2, the average rate of embryos recovered from the gilts was 76.0%, 80.0% and 86.9% of embryos were at the 2 pronuclei. These rates are similar to values reported for domestic pigs (Baker RD and Coggins EG, 1968; Hunter RHF 1964, 1966; Longenecker DE and Day BN, 1968). Most oocytes were synchronously recovered at the 1-cell stage. The frequency of polyspermy was 8.0% (2/25), 10.0% (2/20) and 4.4% (1/23) in different species, respectively. Baker and Coggins (1968) induced superovulation in prepubertal domestic gilts with hormons and found that 6% of the recovered and uncleaved oocytes were polypermic, suggesting that an increased dosage of eCG results in polyspermy. There were no significant differences among groups in the number of total cell (49.5, 52.8 and 49.1) and apoptotic cell (0.31, 0.32 and 0.2) (Table 3, Fig. 2).

The results of the present study suggest that the different species pigs were no difference in ovulation, fertilization and embryo quality. These results also suggest that pigs, especially

Table 3. The number of total cell and apoptotic cell in different species

Species	No. of blastocysts	No. of total cell	No. of % apoptotic cell
Landrace	13	49.5(643)±3.3	2(8.0)±2.0
Durock	12	52.8(634)±5.6	2(10.0)±2.4
Crossbred	10	49.1(491)±2.0	1(4.4)±1.9

Table 2. Ability of fertilization and development between Landrace, Durock and Crossbred pigs

Species	No. of IVC	No. of (%) 2 PN	No. of (%) 3 PN	No. of (%) non-fertilization	No. of (%) BL
Landrace	25	19 (76.0)	2 (8.0)	4 (16.0)	17 (68.0)
Durock	20	16 (80.0)	2 (10.0)	2 (10.0)	15 (75.0)
Crossbred	23	20 (86.9)	1 (4.4)	2 (8.7)	17 (73.9)

* PN : pronucleus, BL : 7D blastocysts.

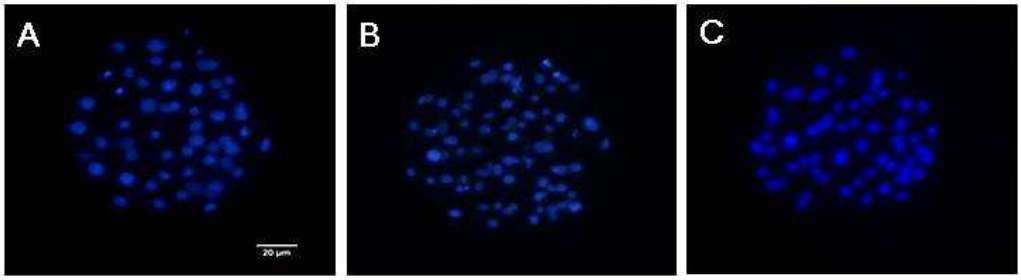

Fig. 2. Representative photographs of *in vivo* blastocysts. The embryos labeled for Hoechest 33342 (blue). A, B and C: Landrace, Durock and Crossbred.

transgenic pigs could be useful for biomedical science as a model for human genetic disorders and as donors for xeno-transplantation.

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