

Sexual maturity and reproductive phase of oocyte donor influence the developmental ability and apoptosis of cloned and parthenogenetic porcine embryos

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Abstract

This study investigated the influence of the sexual maturity and reproductive phase of oocyte donor on the developmental ability and quality of porcine embryos produced by somatic cell nuclear transfer (SCNT) or parthenogenesis (PA). Blastocyst quality was evaluated in terms of hatching ability, total nuclei number and types of apoptosis. Results revealed that maturation rate was not influenced by the reproductive status of the oocyte donor. However, when subjected to PA or SCNT, embryos derived from sexually mature sow oocytes developed to blastocysts at higher rates and had higher cell number than those derived from immature gilt oocytes ($p < 0.05$). Significant effect of reproductive phase, luteal versus follicular, was also noted with luteal stage oocytes yielding higher ($p < 0.05$) rate of blastocyst formation (PA: $54.3 \pm 1.3\%$ versus $44.8 \pm 0.3\%$; SCNT: $29.4 \pm 0.2\%$ versus $22.7 \pm 0.1\%$). Blastocysts derived from luteal phase oocytes also had higher ($p < 0.05$) hatching ability (PA: $44.2 \pm 1.1\%$; SCNT: $39.6 \pm 4.7\%$) and cell number (PA: 77.4 ± 4.9 ; SCNT: 54.9 ± 2.4) than those derived from follicular phase oocytes (PA: $34.9 \pm 0.9\%$, 67.2 ± 3.9 ; SCNT: $34.6 \pm 2.7\%$, 47.5 ± 2.9). TUNEL assay and Hoechst 33342 staining revealed that percentage of blastocysts showing total apoptosis did not differ among the groups. However, luteal phase oocyte-derived blastocysts had the highest incidence of nuclear fragmentation. Among cloned blastocysts that showed the signs of apoptosis, the highest index of total apoptosis was observed in prepubertal oocyte-derived blastocysts (5.2 ± 0.7). Blastocysts derived from luteal phase oocytes showed the lowest TUNEL index (2.0 ± 0.5). The present study therefore, indicates that the sexual maturity and reproductive phase of cytoplasm donor significantly influences the developmental ability, apoptosis and quality of blastocysts produced by SCNT

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or PA. Oocytes from sexually mature sows in luteal phase of their reproductive cycle may be better cytoplasm recipients for SCNT.

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1. Introduction

Abattoir derived ovaries constitute an economical source of oocytes for *in vitro* production of embryo for both commercial and research purposes. However, such oocytes are extremely heterogeneous in terms of quality and developmental competence (Gordon and Lu, 1990). A reduction in variability of the oocyte quality is very important when they are selected as recipients for somatic cell nuclear transfer (SCNT) or for research purposes (Chohan and Hunter, 2003).

Nearly all studies involving cloned embryo production in pigs have utilized prepubertal gilts as cytoplasm donor (Uhm et al., 2000a,b; Choi et al., 2006) although studies indicate that limited success was obtained when prepubertal oocytes were used as recipient cytoplasm for SCNT (Kuhholzer et al., 2001; Hyun et al., 2003), parthenogenetic activation (Du et al., 2005) or *in vitro* fertilization (Ikeda and Takahashi, 2003; Bagg et al., 2006) instead of sow-derived oocytes. Furthermore, interestingly, most study that showed the production of cloned piglets have utilized sow-derived oocytes obtained from abattoir (Lai et al., 2002) or purchased commercially (Kolber-Simonds et al., 2004; Lai et al., 2006; Li et al., 2006). These reports therefore, suggest the importance of the source of oocytes in determining the developmental competence of embryos and variation in experimental results due to ovary source.

Variable results have been described for *in vitro* maturation, fertilization and subsequent embryonic development for the oocytes collected from ovaries at different stages of reproductive cycle in bovine (Chian et al., 2002), human (Claman et al., 1992), cats (Karja et al., 2002) and dogs (Rodrigues et al., 2004). However, such studies are lacking for SCNT embryos in all species and for IVF, parthenogenetic as well as SCNT embryos in porcine. Bagg et al. (2004) reported that there were no differences between oocytes from CL (corpus luteum) and non-CL bearing ovaries, obtained from peri-pubertal pigs, for rates of meiotic maturation, cleavage and blastocyst formation following parthenogenetic activation.

Embryo quality is an important issue for *in vitro* produced porcine embryos: blastocysts derived from sow were of superior quality than those derived from prepubertal gilt (Marchal et al., 2001). Furthermore, *in vivo* blastocysts from older gilts showed no apoptosis (Kidson et al., 2004) while those from younger gilt showed 70.8% apoptotic nuclei (Long et al., 1998). In the light of this limited data in porcine, it is also likely that sexual maturity and reproductive phase of the oocyte donor might influence the embryo quality and apoptosis besides affecting their developmental ability. However, no data is available in the literature comparing the incidence and degree of apoptosis between sow and gilt-derived blastocysts. Nor does data is available comparing the quality and apoptosis of parthenogenetic and SCNT blastocysts produced from different reproductive phases of the oocyte donor.

This study therefore, investigated the influence of the sexual maturity and reproductive phase of cytoplasm donor on the developmental ability and quality of blastocysts produced by SCNT. Blastocyst quality was evaluated in terms of hatching ability, total nuclei number and nuclear fragmentation and apoptosis. As a control, these parameters were also evaluated for partheno-

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