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In vitro development of somatic cell nuclear transfer (SCNT) pig embryos reconstructed from oocytes matured in different maturation culture¹**Eunsong Lee¹, Kilyoung Song²***Department of Veterinary Medicine, Kangwon National University**²College of Veterinary Medicine, Seoul National University*

This study investigated the effect of different duration of maturation culture and coculture with follicle cells during maturation on in vitro development of SCNT pig embryos. Immature oocytes were matured with or without follicular cell mass (Exp. 1) for 36, 39, or 42 hrs (Exp. 2) in M199 which was supplemented with 10% (v/v) pig follicular fluid, EGF, hormone, and cysteine. IVM oocytes were enucleated by aspirating polar body and metaphase chromosome. Skin fibroblasts with hDAF gene were injected into the perivitelline space of enucleated oocytes. Reconstructed oocytes were electrically fused and then activated 1 hr after fusion. Fused oocytes were cultured in a NCSU-23 medium for 6 days under a humidified atmosphere of 5% CO₂, 5% O₂ and 90% N₂.

Nuclear maturation was not improved by follicular cell coculture but SCNT embryos showed higher blastocyst formation when oocytes matured with follicular cells were used as recipient oocytes (Table 1). Nuclear maturation rate according to different duration of maturation did not show significant difference but fusion rate tended to decrease when oocytes were matured longer period. SCNT embryos reconstructed from oocytes matured for 42 hrs showed higher development potential to ≥ 2 -cell and blastocyst stage compared to those from oocytes matured 36 and 29 hrs (Table 2).



Table 1. Effect of follicular cell coculture during IVM on in vitro development of SCNT embryos

Coculture	MII rate (%)	Fused (%)	No. cultured	≥2-cell (%)	Blastocyst (%)	Cell No.
-	93.7	68.5	151	73.5	6.6	43.3
+	97.2	70.0	177	72.3	13.0	33.6

Table 2. In vitro development of SCNT embryos reconstructed with oocytes matured for 36, 39 and 42 hours in culture

IVM duration	MII rate (%)	Fused (%)	No. cultured	≥2-cell (%)	Blastocyst (%)	Cell No.
36	92.4	92.4	133	64.7	20.3	37.0
39	92.3	83.0	110	66.4	20.0	41.2
42	94.2	76.9	108	79.6	31.5	40.0

Keywords: *pig, oocyte maturation, nuclear transfer, embryo development, coculture*

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