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**Sterile Filtered Paraffin Oil Supports In Vitro
Developmental Competence in Bovine Embryos
Comparable to Co-culture**

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The most commonly used culture environment has been the micro drop culture method using oil rather than large scale medium, with the exception of certain specific culture methods. This study was to investigate whether sterile filtered light paraffin oil (SPO) overlaying supports in vitro developmental competence of bovine follicular oocytes better than washed light mineral oil (WMO) overlaying. In addition, the effects of the two types of oil overlaying were compared with oil overlaying plus co-culture (CC) on bovine embryo development in vitro. Bovine follicular oocytes were retrieved from a slaughtered ovary, matured in vitro, and then fertilized and cultured in a 50 ul drop overlayed with WMO or SPO. The in vitro development rates were then examined and compared. Also, day 2 embryos grown in vitro under WMO overlaying were cultured in the two types of oil (WMO and SPO) or in the oil plus co-culture (WMO+CC and SPO+CC) using adult ear skin fibroblasts for 6 days. Cell number and hatching development rates were examined in developed blastocysts in each treatment group during subsequent culture for 48 hr. WMO or SPO overlaying resulted in significantly different bovine follicular oocyte development from day 6 embryo development after IVF (morula:30.6 vs. 44.8%, blastocyst: 21.7 vs 32.8%, respectively) ($p < 0.05$). Also, treatment of the day 2 embryo cultures with SPO overlaying or oil plus CC (WMO+CC or SPO+CC groups) reached significantly higher development rates from the

morula stage compared to embryo cultures treated with the WMO overlaying ($p < 0.05$). However, the development rates of the SPO treatment group (morula: 72.7, blastocyst: 53.1%) were slightly high compared to development of the culture treated with WMO+CC (69.6%, 50.4%, respectively). This similar developmental competence pattern was also observed in cell number and embryo hatching rate. Therefore, SPO overlaying alone can support similar developmental competence as WMO overlaying plus co-culture for bovine embryo development in vitro. Thus, the oil selection for culture significantly influences pre-implantation embryo development in vitro.

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