Ovarian tissue cryopreservation, is a viable option for preserving female fertility. Since different options are available, we performed a 2x2 factorial design comparing viability of sheep ovarian tissue cryopreserved as fragments or whole organs using a conventional (CF) or directional freezing apparatus (DF).

Cortical fragments (10x5x1 mm) were immersed into Leibovitz L-15 medium, 10% FCS and 1,5 M DMSO, while whole ovaries were perfused with the same solution. CF was performed at 0.5°C/min in a Kryo 560M (Planer, UK). DF was performed at 0.01 mm/sec, resulting in cooling rates of 0.3°C/min with a Multi-Thermal-Gradient (IMT, Israel). In both cases freezing was arrested at -40°C before plunging the samples into liquid nitrogen. Immediately after thawing, the percentage of morphologically normal follicles found in DF whole ovaries (81%) was similar to that of fresh controls (90%). DF (72%) and CF (63%) cortical fragments showed both a significantly lower rate (P<0.05). Only 23% survived after CF of whole ovaries.

Whole ovaries and cortical fragments were cut in 2x2x1 mm pieces and cultured for 7 days in α-MEM medium supplemented with ITS, glutamine, pyruvate, hypoxantine, BSA, FSH and bFGF. After culture, the percentage of primordial follicles developed to the primary stage in DF whole ovaries, was similar to controls. A lower rate (P<0.05) of growing follicles was observed in all other groups, and CF whole ovaries were unable to support any development. We conclude that DF ensures better tissue viability than CF and that DF better preserve whole ovaries than cortical fragments.