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A tailored protocol for human ovarian follicle isolation for clinical application

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Context: In recent years different research teams have been working in the development of a transplantable artificial ovary with a view to restoring fertility in cancer patients at high risk of ovarian involvement who cannot benefit of any of the currently fertility preservation strategies. First essential requirement for developing an artificial ovary is a safe and efficient follicle isolation procedure.
Objective: The aim of this study was to improve our previously established human ovarian follicle isolation procedure and adapt it according to the unique tissue properties of each individual patient.
Methods: Thawed ovarian tissue from 22 patients was equally distributed between the previous (Vanacker et al., Fertil Steril 2011) and newly modified protocol, which involved fractionating enzymatic digestion over 3 time intervals in order to recover the first fully isolated follicles. Follicle yield and viability were compared soon after isolation. Follicles isolated using both protocols were then encapsulated in fibrin clots and their survival (caspase-3 and Ki67) and developmental stage (hematoxylin-eosin) were assessed. The efficacy of the modified protocol was further investigated by encapsulating and xenografting 100 human follicles together with 100,000 ovarian stromal cells to a SCID mouse for 7 days.
Results: More follicles (124 Â± 122) were isolated using the modified protocol than the previous procedure (88 Â± 85) ($p<0.01$). After follicle encapsulation, the modified protocol also yielded a higher percentage of primordial (15% vs 7%) ($p<0.05$) and primary (77% vs 66%) follicles ($p<0.05$). On the other hand, no difference was found in the percentage of caspase-3- and Ki67-positive follicles with either protocol. After one week of xenografting, a higher percentage of follicles (35%) was observed than in our previous study (Paulini et al., 2016 Reprod Biomed Online). They appeared to be healthy, with 72% and 28% at primordial and primary follicle stages respectively.
Conclusions: The modified protocol was found to maximize the number of isolated primordial and primary follicles without affecting their survival or development after isolation and xenografting. Such a protocol can also be specifically tailored to the tissue properties of each individual patient.

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