OVARIAN TISSUE CRYOPRESERVATION BY VITRIFICATION VS SLOW FREEZING: NO DIFFERENCE IN EPIGENETIC AGE OR FOLLICLE DENSITY

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Background

Ovarian tissue cryopreservation involves removal and cryopreservation of ovarian cortex for future reproductive use; it is the only fertility preservation option for pre-pubertal girls undergoing gonadotoxic chemotherapy. Two cryopreservation methods, controlled-rate “slow” freezing and ultra-rapid vitrification, have been found to induce variable histologic changes (1); however, little data exist on the epigenetic impact of the cryopreservation technique on the tissue. Specifically, epigenetic age (using methylation markers to predict an individual’s age), has not been examined in ovarian tissue. Epigenetic age has been used as a surrogate for overall health and fitness, with accelerated age associated with stress-related insults to an individual’s cells (2). We hypothesized that cryopreservation of ovarian cortex may accelerate epigenetic aging of the whole tissue.

Objective

Our primary objective was to determine the epigenetic age of bovine ovarian cortex, either fresh or cryopreserved through vitrification or slow freezing. Our secondary objective was to compare the histologic results (follicle counts and follicle density) between the two cryopreservation methods.

Materials and Methods

Bovine ovaries were used, given similarity to human ovaries in pathology and follicular growth (3). Ovaries were harvested from nine female black angus heifers aged 28-30 months. The ovarian cortex was isolated in strips and processed in one of three ways: (a) fresh, (b) slow-freezing, and (c) vitrification. DNA was isolated from half of the strips while the other half were used for histologic analysis. The DNA was used to calculate the epigenetic ages using the Horvath mammalmethylchip array (4). The epigenetic ages between the three groups were compared using ANOVA.The histologic analysis was performed by a blinded pathologist, and the intact primordial follicle counts and follicle density were compared using a student’s t-test.

Results

Bovine epigenetic ages for all groups (fresh, slow frozen, vitrified) were similar and were predicted at 4-4.6 years of age (Table 1). Likewise, the primordial follicle counts and density did not differ between the two methods of cryopreservation, with % intact follicles of 24% (vitrification) vs 31% (slow freezing), $p = 0.96$.

Conclusions

Ovarian tissue cryopreservation using either vitrification or slow freezing did not alter the epigenetic age of the ovarian tissue. This provides reassurance that this method of fertility preservation does not prematurely age the cryopreserved and thawed tissue; however, further studies are needed to confirm these findings and to examine cell-specific epigenetic markers.

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Table 1: Epigenetic age by cryopreservation method

	Fresh n = 8	Vitrified n = 9	Slow Frozen n = 9	P value
Epigenetic age (pan-bovine predictor), yrs*	4.34 ± 1.42	4.33 ± 1.24	4.00 ± 1.60	NS
Epigenetic age (bovine oocyte predictor), yrs*	4.57 ± 1.46	4.10 ± 1.57	4.55 ± 1.88	NS

* mean ± standard deviation

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