INFLUENCE OF TEMPERATURE, SERUM AND GONADOTROPIN SUPPLEMENTATION IN SHORT AND LONG-TERM ORGANOTYPIC CULTURE OF HUMAN PREPUBERTAL TESTICULAR TISSUE

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Abstract Body

Cryopreservation of immature testicular tissue (ITT) is an experimental strategy for the preservation of fertility in prepubertal boys exposed to a gonadotoxic onset, as is the case of oncologic patients. In order to restore their fertility, in vitro organotypic culture of ITT has been proven in mouse as a feasible strategy to produce sperm. With the aim of screening factors affecting human ITT in vitro, samples from 5 prepubertal cancer patients (aged 7 to 14) were subjected to in vitro organotypic culture in gas-liquid interphase for up to 70 days, exposed to combinations of different temperatures (37ºC versus 34ºC), serum (fetal bovine serum (FBS) versus KnockOut Serum replacement (KOS)) and gonadotropin supplementation (addition of FSH and LH). Compared with 34ºC cultures, samples cultured at 37ºC showed accelerated fibrosis, significantly higher intratubular apoptosis (p<0.05), and significant downregulation for germ cell markers DAZL, VASA, UTF1, FGFR3, SYCP3 and ACR (p<0.05). Moreover, supplementation with FSH/LH triggered the number of spermatogonia entering meiosis as shown by the significantly higher percentage of VASA+/SYCP3+ cells in 34ºC conditions (p<0.05). Nevertheless, long term culture resulted in an almost complete germ cell loss in all experimental conditions, indicating that organotypic culture was not able to support in vitro spermatogenesis. This was correlated with a fail in maturation of Sertoli cells shown by the lack of AR expression, and a disorganization of the blood-testicular barrier within tubules shown by the ZO-1 staining. Supporting these results, AMH secretion significantly increased at 34ºC during the first 14 days of culture in FBS conditions (p<0.05), but disappeared by the end of culture in all conditions. In contrast, Testosterone secretion was triggered in 37ºC conditions reaching it’s peak at day 14 (p<0.05), whereas 34ºC conditions displayed a peak at day 28 in KOS cultures (p<0.05) that remained constant by the end of culture.