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Ovarian secondary follicle showing oocyte

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agriculture. The urgency to enhance the current protocols and develop newer ones for various bee species and specifically the honey bees (Apis sp.) has escalated in recent years due. This is due to factors such as habitat loss, diseases of bees and the often-termed syndrome of Colony Collapse Disorder (CCD). Our approach hitherto has been to tackle issues with male and female germplasm storage by considering both cryopreservation and cold storage. In this presentation, we will summarize the strategies adopted to cryopreserve the honey bee embryos. We will compare the cryopreservation protocols for the honey bees versus the other insect species that have been cryopreserved at the USDA-ARS in Fargo, ND. We will define both the structural and physiological parameters that differentiates the bee embryos from other insect species. The studies indicate that the embryos' amenability to previously published vitrification techniques for dipterans are thwarted by the embryos' permeability characteristics and their differential rate of development versus consumption of internal lipid resources. With the stage-selection technique that we developed for the dipterans, we were able to revive 21.1 ± 15.4% of the honey bee that were vitrified. However, the proportion of embryos that were carried into the cryoprotectant loading phase was merely 34.5 ± 9.1%, indicating that most of the deleterious effects are from permeabilization rather than vitrification itself. In brief, our studies hoped for a singular template for insect embryonic cryopreservation. The feasibility of such a template now seems farfetched even among the subspecies. This presentation will elucidate further the reasoning for such a conclusion and also discuss how this project is being furthered.

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S33 RISK AND MECHANISM OF GLUCOSE METABOLISM DISORDER INVOLED IN THE OFFSPRINGS CONCEIVED BY FEMALE FERTILITY MAINTENANCE TECHNOLOGY

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Although female fertility preservation technology (FFPT) provides an effective option for preserving fertility in patients with cancer suffering from fertility loss due to cancer treatment, previous studies have shown that the technique has certain potential risks and requires an assessment of the health status of the offspring ,female fertility preservation technology (FFPT) may lead to growth restriction. The present animal study examined glucose metabolism of adult mice offspring born from ovarian tissue cryopreservation and orthotopic allotransplantation.The mice were divided into three groups: normal, fresh ovaries transplantation, and cryopreserved ovaries transplantation. We recorded the fasting blood glucose, glucose tolerance, and fasting serum insulin level were monitored for six months. Liver DNA, RNA, and proteins were extracted to detect the interaction between DNA methylation and Grb10 expression and insulin signaling pathway factors such as P-IGF1R、P-IRS2、P-AKT regulatated by Grb10.Compared with the normal group, the fasting blood glucose and fasting serum insulin levels were higher in the cryopreserved and fresh groups. The mRNA and protein expressions of Grb10 were higher in the fresh and cryopreserved groups. Compared with the normal group, the DNA methylation status of 4 of the 11 sites of the Grb10 promoter was lower in the cryopreserved group. Grb10 overexpression inhibited the downstream phosphorylation protein factor expression (p-IGF-1R, p-IRS2, and p-Akt) of the IGF-1R signaling pathway. Female fertility maintenance technology（FFMT）, including ovarian tissue cryopreservation（OTC）, and orthotopic allotransplantation techniques may tend to glucose metabolism disorder in offspring mice.

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S34 INFLUENCE OF CRYOPRESERVED PIGLETS SKIN FRAGMENTS OR AQUEOUS COLLOIDAL SOLUTION OF C60 FULLERENE ON DESTRUCTION AND INFLAMMATION SEVERITY AFTER SKIN CRYODESTRUCTION

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Skin cryodestruction is widely used in surgery and is accompanied by destruction and inflammation. Extracts of cryopreserved piglets skin fragments (CPSF) and aqueous colloidal solution of C60 fullerene (ACSF) are capable of improving repair processes. The effect of CPSF and ACSF on the destruction and inflammation rates in the serum of rats after skin cryodestruction was investigated. On day 7 of experiment the concentrations of diene conjugates (DCs) and thiobarbituric acid reactive substances (TBARS) in the placebo group were above normal, superoxide dismutase and catalase activity were below normal and did not differ from the CPSF or ACSF groups. The concentration of ceruloplasmin and C-reactive protein (CRP) in the placebo group was above normal. The introduction of CPSF or ACSF reduced the level of ceruloplasmin in 1.3 and 1.7 times; the CRP level was decreased in 1.4 or 1.3 times, respectively. On day 14 day of experiment increased levels of DC and TBARS were kept in the placebo and CPSF group. Following the introduction of the ACSF, both indices were normal. The activity of enzymes in the groups either CPSF or ACSF was higher than in the control, i.e. for superoxide dismutase this was in 1.5 or 1.6 times and for catalase in 1.4 times. The content of ceruloplasmin in the CPSF or ACSF groups was lower than in the placebo group in 1.4 and 1.3; the CRP level was lower in 1.6 or 1.5 times, respectively. On 21st day of experiment the level of DC and TBARS, activity of superoxide dismutase and catalase, as well as the content of ceruloplasmin and CRP in the groups CPSF or ACSF, unlike the placebo group, reached the norm. The results obtained indicate the potential of the ECF or PCFF use to reduce the destruction and inflammation severity after skin cryodestruction.

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S35 CRYOSURGERY CANCER FOCAL THERAPY AUGMENTS IMMUNOTHERAPY FOR TUMOR GROWTH CONTROL

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There is growing evidence that combining immunotherapy with focal therapy can serve as a potent in situ tumor vaccination strategy. These combinatory approaches provide a systemic anti-cancer immunity that leads to control of local recurrence and metastasis. Here, we exam the augmented effects of cryosurgery with anti-PD-1 immune checkpoint inhibitor (ICI) in a MC-38 murine colon carcinoma model. MC-38 cells were cultured and injected in the flank of C57BL/6 mice. Tumors grow to 4-6mm in the largest dimension and were treated (on day 0) by a customized Argon Joule-Thomson cryoablation needle (1.2mm in diameter). During cryosurgery, temperature on the surface of the tumor was monitored by an IR camera. Anti-PD-1 antibodies were given by IP injection at 100 μg on day 1, 3 and 5. For primary tumor growth delay, the tumors were treated with a sublethal dose by freezing (0 ˚C) to tumor edge. For secondary tumor challenge, primary tumors were ablated with a lethal dose by freezing the edge of the tumor to -20 ˚C. Secondary tumors were inoculated on the opposite side of the mice (N>20 for each group) on day 13. In both studies, tumor growth was monitored for 30 days after inoculation. In the primary tumor model, anti-PD-1 monotherapy did not provide statistically significant tumor growth delay, but did slow tumor growth after cryosurgery as compared to cryosurgery monotherapy. In the secondary tumor challenge model, anti-PD-1 increases the percentage of tumor-free mice from 52.2% with cryosurgery alone to 57.1% with combinatory therapy. The rate of long-term tumor protection is significantly higher than that of thermal therapy (heating to 60 ˚C tumor edge) with anti-PD-1 at 33.3% yet falls short of irreversible electroporation with anti-PD-1 at 94.4%. Our preliminary results suggest that cryosurgery combined with ICI improves both primary tumor treatment and secondary tumor rejection.

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S36 SELECTIVE BRAIN COOLING: A NOVEL APPROACH FOR THERAPEUTIC HYPOTHERMIA

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Stroke is a major cause for death and long-term disability. In the US, a stroke occurs every 40 seconds and there is a stroke-related death every 4

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