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A BACK-AND-FORTH WAY”**

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CALCIUM IONOPHORE A23187 EFFECT ON ACQUISITION OF FERTILIZING ABILITY IN EQUINE CRYOPRESERVED SPERMATOZOA

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Spermatozoa must undergo three events to fertilize an oocyte *in vivo*: capacitation, hyperactivation, and acrosome reaction. Therefore, it is necessary to induce these physiological events *in vitro* in the masculine gamete during assisted reproductive techniques. Even though the *in vitro* conditions under which many species acquire their fertilizing ability are well known, to this day, there is no standard protocol to induce these events in equine cryopreserved spermatozoa. Thus, there is no available protocol for embryo production through *in vitro* fertilization (IVF) with these samples. It has been proven that brief exposure of murine and bovine spermatozoa to calcium ionophore A23187 and the consequent calcium influx to the cell cytoplasm increases their fertilizing ability *in vitro* and embryo production due to the induction of capacitation and hyperactivation. Moreover, A23187 increases events related to capacitation without inducing the classical capacitation signaling pathway such as PKA-activation (pPKA) or protein tyrosine phosphorylation (pTyr). Given the current difficulties in equine embryo production through IVF with cryopreserved spermatozoa and the previously shown effects of the calcium ionophore in spermatozoa from other species, this work aimed to study the potential fertilizing ability of equine cryopreserved spermatozoa that were previously exposed to A23187. First, we studied the effect of A23187 (1 μ M) on spermatozoa. After 10 min of exposure, the ionophore decreased the spermatozoa motility (CASA, $P < 0.05$) without affecting their viability (HOS test, $P > 0.05$) or acrosome status (PSA-FITC, IF, $P > 0.05$). After removing the ionophore, the spermatozoa were incubated under non-capacitating conditions for 20 min, and motility was reassessed, and their fertilizing ability was studied. The previous incubation with A23187 increased the hyperactivated sperm population (CASA, $P < 0.05$) and the induction of capacitation-associated events such as progesterone-induced acrosome reaction (IF, $P < 0.05$) and the sperm ability to bind to bovine oocyte zona pellucida ($P < 0.001$) without activating the pTyr and pPKA pathways (IF, $P > 0.05$). Our results suggest that brief exposure of cryopreserved equine spermatozoa to calcium ionophore A23187 could be incorporated into the assisted reproductive techniques to increase spermatozoa fertilizing ability *in vitro* due to its effect on hyperactivation and capacitation in this species.

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EFFECT OF LOW-DOSE PHOSATE ON EMBRYO IMPLANTATION

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Although glyphosate-based herbicides are considered safe due to their low persistence, new evidence suggests that they could affect the correct embryo implantation and development even in low doses. The trophoblast surrounding the blastocyst plays a pivotal role in the invasion, migration, and spiral arteries remodeling from the decidua. This process is high-regulated, and its alterations could carry out preeclampsia, miscarriages, and other associated pathologies. Previous studies demonstrated that concentrations of 0.2 and 2 μ M of glyphosate (G) stimulated migration activity in a human endometrial carcinoma cell line (Ishikawa). This study aims to analyze *ex vivo* the effect of 2.5 μ M G in murine blastocyst development. The cellular migration was also assayed using the trophoblast cell line HTR-8/SVneo with 0.625, 1.25, 2.5, 5, and 10 μ M of G. E3.5 embryos, recovered from pregnant BALC/b mice, were placed on murine uterine epithelial cells monolayer with 2.5 μ M of G or vehicle (V). The implantation area and hatching/attachment time were registered for six consecutive days. The wound healing assay was performed to evaluate the migration activity. The monolayer was pretreated with G concentrations for 24 h, and the medium was renewed after scratching. Then, the uncovered areas were registered at 0 and 12 h. Cell viability was determined spectrophotometrically after 24 and 48 h of treatment using WST-1 reagent and by counting cells in a hemocytometer. All the assays were performed in triplicate. The blastocyst implantation area (G: 0.47 ± 0.03 mm²; V: 0.32 ± 0.14 mm²) and hatching/attachment time (G: 42.3 ± 10.5 h; V: 45.2 ± 19.9 h) were similar between groups. Cellular migration was stimulated at 0.625 μ M G compared to V ($P < 0.05$). These results suggest that even low concentrations of G could dysregulate some processes associated with implantation.

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MOLECULAR AND MORPHOLOGICAL ALTERATIONS IN THE UTERUS OF ADULT RATS FED WITH CAFETERIA DIET AND EXPOSED TO A GLYPHOSATE-BASED HERBICIDE

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The development of endometrial hyperplasia (EH), a preneoplastic lesion, is associated with environmental factors, including unhealthy diets and chemical exposure. In this sense, we have demonstrated that cafeteria diet (CAF) provoked EH in adult rats and that exposure to glyphosate-based herbicide (GBH) altered uterine development, leading to EH later in life. However, the potential interaction between these environmental factors which women are frequently co-exposed remains unclear. Thus, we evaluated whether the addition of a