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Studying glycerol toxicity on frozen chicken sperm and strategies to mitigate it

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Abstract ^{*}less than 2500 characters

Glycerol (GLY) is a common cryoprotectant for sperm cryopreservation in various species. However, its use has adverse effects, reducing sperm fertility. This is particularly evident in chickens, where the optimal sperm freezing protocol uses 11% GLY, while only 6% GLY in inseminated semen causes complete infertility. GLY toxic effects can manifest at two distinct stages: during freezing and thawing at 4°C, and in the oviducts at 41°C following insemination. Varying temperatures may contribute to different levels of GLY toxicity. Therefore, a better understanding of GLY cytotoxicity on sperm needs considering temperature variations. This study was first to investigate GLY adverse effects on the functional capacity of chicken sperm and their impact on sperm storage in the female genital tract. Sperm parameters, such as motility, membrane integrity, apoptosis, mitochondrial activity, ATP concentration, and perivitelline membrane penetration ability were examined. These evaluations were conducted after a 60-min incubation with various concentrations of GLY (0, 1, 2, 6, and 11%) at two temperatures (4 and 41°C). Furthermore, the ability of sperm to colonize sperm storage tubules (SST) was assessed through *in vivo* insemination and *in vitro* co-incubation in the presence of 0, 2, or 6% GLY. The results showed that only 6% and 11% GLY had an impact on sperm parameters at 4°C, while greater changes were observed at 41°C, especially regarding sperm motility. *In vivo*, 2% GLY reduced sperm arrival in SST by half, and a total absence of sperm was observed with 6% GLY. Conversely, 2% GLY during *in vitro* co-incubation did not decrease the number of SST filled with sperm, whereas a reduction was observed with 6% GLY. These findings highlight a greater impact of GLY at 41°C compared to 4°C and underscore the significant influence of GLY on sperm functionalities, particularly their motility and interaction with the female reproductive tract, including sperm storage in SST. In addition, to mitigate GLY negative impact during insemination, this study then aimed to design a new protocol for GLY removal after thawing by centrifugation on Percoll supplemented with sucrose (SP). This new technique was compared to the

conventional serial dilution (SD) method and found to be more effective in removing GLY. Indeed, it can enhance sperm storage in SST, improve fertility rates and duration, and simplify sample processing, making sperm cryopreservation practical in poultry farms.