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ANDROGEN SYNTHESIZING ACTIVITY OF CRYOPRESERVED TESTICULAR INTERSTITIAL CELLS UPON TRANSPLANTATION

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Background. Cells isolated from the testes of mammals and humans can be used for scientific purposes, maintaining certain animal lines and breeds, preserving biological material from endangered species, as well as in reproductive technologies. Most approaches for cryopreserving such cells utilize blood serum (or its derivatives) and dimethyl sulfoxide (DMSO). This can lead to unstable results, the spread of infections, altered expression of certain cell genes, and the manifestation of DMSO toxic effects. In our previous studies, serum-free media for testicular interstitial cells (ICs) were developed; the aim of this work was to investigate their ability to synthesize testosterone after cryopreservation.

Materials and Methods. ICs were obtained from mature rats via enzymatic digestion and cryopreserved in solutions containing 0.7 M DMSO and 100 mg/mL of one of the following polymers: dextran 40, hydroxyethyl starch, polyethylene glycol, or 1.4 M DMSO and 10% fetal bovine serum (FBS). The cooling rate was 1 °C/min. After cryopreservation, the cells were thawed in a water bath, the DMSO was removed, and their ability for basal and stimulated testosterone synthesis *in vitro* was assessed. Additionally, ICs were transplanted into castrated animals, and changes in free testosterone blood levels, seminal vesicle weight, and sexual behavior were examined.

Results. The capacity for stimulated testosterone synthesis was preserved only in cells cryopreserved in the solution containing dextran 40 (0.7DMSO + D40) and FBS (1.4DMSO + FBS). Cryopreserved ICs enhanced sexual behavior parameters

in castrated rats upon transplantation without removing the cryoprotective medium (0.7DMSO + D40), including mount and intromission latency, the mount and intromission frequency, ejaculation ability, and copulatory efficiency. Moreover, they helped maintain free testosterone blood levels and seminal vesicle weight in castrated animals after transplantation.

Conclusions. It was demonstrated that ICs cryopreserved in the serum-free medium (0.7DMSO + D40) retained their ability to synthesize and secrete testosterone. Furthermore, the use of 0.7DMSO + D40 allows the immediate use of cells after thawing, bypassing the step of cryoprotectant removal, which could facilitate the translation of experimental protocols into practice.

Keywords: DMSO, testicular cells, dextran, cryopreservation, transplantation, testosterone, sexual behavior

INTRODUCTION

Cryopreservation of testicular cells and tissue is essential for the establishment of germplasm banks, reproduction of endangered species and animal lines, and applications in reproductive technology (Lima & Silva, 2017; Patra *et al.*, 2021). Existing methods for the cryopreservation of testicular material often include blood serum (serum albumin) and dimethyl sulfoxide (DMSO) (Silva *et al.*, 2020; Patra *et al.*, 2021), including methods for separate interstitial cells – Leydig cells (Tai *et al.*, 1994; Chen *et al.*, 2007). However, the use of serum can lead to the propagation of infections and does not guarantee consistent medium compositions, which in turn affects the reproducibility of results (Lindroos *et al.*, 2009; Yamatoya *et al.*, 2022; Ghio *et al.*, 2023). Additionally, DMSO can be toxic (Awan *et al.*, 2020; Hassan & Ahmad, 2024). This is particularly critical when the material is intended for clinical use. Many cryopreservation protocols recommend the removal of DMSO before application (Riedhammer *et al.*, 2014; Awan *et al.*, 2022; Johnson *et al.*, 2025). However, this removal is typically accompanied by cell loss. Therefore, the development of cryopreservation media that are free of serum components and contain reduced concentrations of DMSO – or no DMSO at all – can greatly enhance the transition of cryobiological technologies from experimental research to clinical practice.

In our previous work, several serum-containing and serum-free compositions were developed for the cryopreservation of suspensions consisting of different types of cells – testicular interstitial cells (ICs) (Pakhomov *et al.*, 2022). One composition, which included 0.7 M DMSO and 100 mg/mL dextran 40 (D40), demonstrated exceptional protective ability for ICs. It is serum-free and contains a relatively low concentration of DMSO. Other compositions that used hydroxyethyl starch (HES) or polyethylene glycol (PEG) instead of D40 were less effective or entirely ineffective. The use of serum (fetal bovine serum) (10%) required a higher DMSO concentration (1.4 M), which increased its toxicity. Some mechanisms of cryoprotection provided by the media were described, and the metabolic activity and plasma membrane integrity of ICs were investigated. However, the functionality of the cryopreserved cells – for example, their ability to produce testosterone *in vitro* in response to stimulation, their *in vivo* effects on animal sexual behavior and the development of accessory sex glands upon transplantation – was not studied.

The aim of the research was to verify the functionality of cryopreserved testicular interstitial cells *in vitro* and *in vivo* after transplantation.

MATERIALS AND METHODS

Experimental animals. Male and female sexually mature rats (5–6 months old; weight ~ 400–450 g) obtained from the animal facility of the Institute for Problems of Cryobiology and Cryomedicine, NAS of Ukraine (Kharkiv), were used in the study (600animals in total). Animals had free access to standard laboratory chow and water. All manipulations with animals were approved by the Committee for Ethics in Animal Experimentation of the Institute for Problems of Cryobiology and Cryomedicine, NAS of Ukraine (Protocol No 1, 11.03.2020).

Isolation of testicular interstitial cells. Testicular tissue was isolated as previously described. Rats were euthanized by cervical dislocation, and their bodies were immersed in 75% ethanol for 5 min. The testes were excised, decapsulated, and cleared of blood vessels. Each testis was transferred into a 15-mL centrifuge tube containing 4 mL of DMEM F12 medium supplemented with 0.2 mg/mL collagenase (type I) and 0.1 mg/mL DNase I (both from Sigma-Aldrich, USA). Enzymatic digestion was performed at 34 °C in a thermostated shaking water bath set to 90 cycles/min for 10 minutes. Following this, 10 mL of collagenase-free DMEM F12 (Biowest, France) was added to each tube. The seminiferous tubule mass was separated by filtration through a double-layered 100 μm nylon mesh. The filtrates were centrifuged at 325 g for 3 min at room temperature. Supernatants were discarded, and the cell pellets were resuspended in 10 mL phosphate-buffered saline (PBS). The sedimentation step was repeated. The final cell concentration was adjusted to $4 \cdot 10^7$ cells/ml in PBS. These suspensions were then mixed 1:1 with concentrated cryoprotective media to achieve the desired final additive concentrations (see below), resulting in a final cell density of $2 \cdot 10^7$ cells/mL.

Cryopreservation. A 0.5 mL aliquot of the IC suspension ($4 \cdot 10^7$ cells/mL) was mixed 1:1 with pre-cooled (4 °C) concentrated cryoprotective media in 1.8 mL cryovials (Nunc, Denmark), yielding the final composition. The serum-containing medium consisted of 1.4 M DMSO and 10% FBS (1.4DMSO + FBS). Serum-free media contained 0.7 M DMSO combined with one of the following polymers at 100 mg/mL: dextran 40 (D40), hydroxyethyl starch (HES), or polyethylene glycol (PEG), denoted respectively as 0.7DMSO + D40, 0.7DMSO + HES, and 0.7DMSO + PEG. After nucleation was initiated, samples were cooled at a rate of 1 °C/min down to –80 °C. Once the target temperature was reached, samples were held at –80 °C for 7 minutes to stabilize before being plunged into liquid nitrogen. After storage, the samples were thawed in a 37 °C water bath until all visible ice crystals had melted. The cryoprotective media were gradually replaced with pure DMEM F12 through stepwise dilution and centrifugation. The ICs were then assessed for their *in vitro* secretory function. Alternatively, cells cryopreserved in 0.7DMSO + D40 were used directly for transplantation immediately after thawing.

Transplantation. The transplantation of ICs was performed as previously described. All surgical manipulations were conducted under general anesthesia: 7.5 mg/kg of animal mass tiletamine hydrochloride, 7.5 mg/kg of animal mass zolazepam hydrochloride (Zoletil, “Virbac”, France), 20 mg/kg of animal mass xylazine hydrochloride (Sedazine, Biowet, Poland). In orchiectomized animals (groups 2, 3, and 4), a ventral midline incision approximately 1 cm long was made to expose both testes. The vas deferens and associated blood vessels were ligated and severed, after which the testes were removed. The abdominal muscles and skin were sutured in separate layers. In control

animals (group 1), the testes were similarly exposed through a midline incision but were returned intact to the abdominal cavity.

One week after orchiectomy, animals in groups 3 and 4 received a single subcutaneous injection of 20 µL IC suspension into the scrotal area. Group 3 received native (non-cryopreserved) ICs, while group 4 received ICs cryopreserved in 0.7DMSO + D40 medium. Animals in group 2 were administered only the vehicle (DMEM F12). All experimental animals were pre-treated with a single dose of cyclosporine (20 mg/kg body weight) one day prior to IC transplantation; subsequent daily doses were reduced to 10 mg/kg. The minimal number of animals in a group was six (24 animals in total).

Sexual behavior. In this study, all female rats were ovariectomized prior to behavioral testing in order to induce a menopausal state (4 animals in total). The oviducts were ligated and excised, after which the abdominal wall and skin were sutured. Behavioral assessments were conducted starting two weeks post-ovariectomy and included four test sessions following transplantation. The tests were performed on days 7, 14, 21, and 28 after transplantation. Behavioral estrus was induced via subcutaneous injection of estrone (25 µg/rat; Zdorovye, Kharkiv, Ukraine) 48 hours before testing, followed by progesterone (500 µg/rat; Biofarma, Brovary, Ukraine) 4 hours before testing. During each session, male rats were given a 10-minute trial with a hormonally primed, sexually receptive female. Each male was placed into a mating cage 5 min prior to the introduction of the female.

Behavioral parameters were evaluated as previously described (Mokhtari & Zanboori, 2011): mount latency (ML) – the time from female introduction to the male's first mount attempt; intromission latency (IL) – the interval before the first intromission; ejaculatory latency (EL) – the time until the first ejaculation; mount frequency (MF) – the number of mounts without intromission before ejaculation; intromission frequency (IF) – the number of mounts with intromission preceding the first ejaculation. Copulatory efficacy (CE) was calculated as CE = IF/(MF + IF).

Measurements of free testosterone and mass of seminal vesicles. Four weeks after transplantation, animals were euthanized by cervical dislocation. Serum samples were collected and stored until analysis. Free testosterone (T) levels in serum were measured using a competitive enzyme-linked immunoassay without prior dilution (Xema, Ukraine). Seminal vesicles were excised and weighed. Additionally, the tissue was fixed in 10% buffered formalin, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections 5 µm thick were cut, stained with hematoxylin and eosin, and examined under a light microscope. The *in vitro* functional test for native and cryopreserved ICs was conducted as follows. Cells that were cryopreserved at an initial concentration of $2 \cdot 10^7$ cells/mL were resuspended in a final volume of 1 mL in DMEM F12 medium after removal of the cryoprotective solution. Each sample was divided into two 0.5 mL portions in plastic tubes: one for the study of basal secretion, and the other for secretion stimulated by hCG (Pregnyl 1500, Netherlands). For stimulation, an aliquot (10 µL) of hCG was added to achieve a final concentration of 1 IU. For the study of basal secretion, 10 µL of PBS was added instead of the hCG solution. The cells in the tubes were incubated for 3 h at 34 °C. After incubation, the cell suspension was mixed and centrifuged for 3 min at 325 g. The supernatant was collected and used to determine testosterone concentration by ELISA. For the functional test of native ICs, the concentration of cells obtained from rat testes was adjusted to $2 \cdot 10^7$ cells/mL and subjected to procedures analogous to the removal of the cryoprotective solution.

Statistical analysis. Data were processed with Statistica 10 (StatSoft, Inc., Tulsa, USA). Data are presented as medians along with 25th and 75th percentiles. For pairwise comparisons, the Mann–Whitney U test was applied. A p-value of ≤0.05 was considered statistically significant. To address the issue of multiple testing, we applied the Bonferroni correction, resulting in more stringent p-values of 0.01.

RESULTS AND DISCUSSION

In our previous research on the cryopreservation of ICs, one serum-free cryoprotective composition demonstrated the highest IC survival – 0.7DMSO + D40 (Pakhomov *et al.*, 2022). The viable cell recovery in IC samples and Leydig cell recovery after cryopreservation with 0.7DMSO + D40 (viability was assessed by trypan blue exclusion test, Leydig cells were detected by histochemical staining) were about 35 % and 60 % respectively, when the preservation of metabolic activity (assessed by MTT-test) of the samples reached 47 %. Other media were less effective (0.7DMSO + HES) or ineffective (0.7DMSO + PEG). The same indicators for 0.7DMSO + HES approached 25 %, 32 %, and 48 % while for 0.7DMSO+PEG – 6 %, 16 %, and 7 %. The serum-containing medium 1.4DMSO + FBS yielded satisfactory results in terms of cell survival but contained a higher DMSO concentration (1.4 M), which poses a risk of DMSO toxicity. Lower DMSO concentration resulted in a dissatisfactory outcome in terms of cell survival (Pakhomov *et al.*, 2022). The viable cell recovery in IC samples and Leydig cell recovery after cryopreservation with 1.4DMSO + FBS were about 21 % and 30 % respectively, when the preservation of metabolic activity of the samples approached 42 %. The secretory ability of the cell suspension was previously understudied. Herein, we show that cryopreservation did not affect the basal testosterone level but significantly reduced the ability to produce testosterone in response to hCG stimulation *in vitro* (**Table 1**). Cryopreservation with 0.7DMSO + D40 and 1.4DMSO + FBS partially preserved this ability. 0.7DMSO + PEG was nonfunctional (not shown).

Table 1. **Testosterone secretion of testicular interstitial cells *in vitro*, median (25th; 75th percentiles), pg/mL (n = 5)**

Media	Basal secretion	hCG stimulated secretion
Native ICs	82.1 (81.0; 83.5)	196.6 (110.2; 220.9)*
0.7DMSO + D40	80.0 (79.1; 82.9)	88.1 (87.2; 92.3)*
0.7DMSO + HES	85.8 (82.3; 86.6)	86.5 (82.7; 87.0)
1.4DMSO + FBS	79.5 (78.3; 82.2)	87.0 (85.8; 89.8)*

Note: * – the indicators were statistically different from basal secretion

Although the use of serum-containing media for the cryopreservation of testicular cells has been demonstrated in experimental research (Tai *et al.*, 1994; Izadyar *et al.*, 2002; Keros *et al.*, 2005; Chen *et al.*, 2007; Wyns *et al.*, 2010; Silva *et al.*, 2020; Patra *et al.*, 2021), the application of cryopreserved biological material requires the removal of both DMSO and serum prior to transplantation. Serum may transmit infections, and DMSO can exert toxic effects on the recipient (Yamatoya *et al.*, 2022; Ghio *et al.*, 2023). Therefore, the most promising serum-free medium, 0.7DMSO + D40, was used to investigate the possibility of transplanting cryopreserved ICs without removing

the cryoprotective medium. The ability of these cells to maintain sexual behavior and blood androgen levels after transplantation was evaluated.

Mount latency and intromission latency showed a tendency to decrease in groups 3 and 4 compared to group 2 (**Table 2**), indicating the involvement of transplanted ICs in maintaining sexual behavior. Groups 3 and 4 exhibited intromissions starting from the second week, while group 2 displayed them beginning in the third week (**Table 3**).

Table 2. **The effect of orchiedectomy and transplantation on mount latency and intromission latency, median (25th; 75th percentiles), seconds (n = 6)**

ML	Group 1	Group 2	Group 3	Group 4
Week 1	8 (4; 16)†	155 (97; 174)*	125 (90; 120)*	80 (65; 110)*
Week 2	1 (1; 3)	48 (36; 74)*	48 (32; 51)*	39 (32; 51)*
Week 3	1 (1; 1)	9 (8; 15)*	14 (11; 16)*	12 (10; 16)*
Week 4	1 (1; 1)	1 (1; 3)	1 (1; 1)	1 (1; 1)
IL				
Week 1	28 (11; 34)	-	-	
Week 2	10 (9; 17)	-	125 (35; 145)*	45 (33; 157)*
Week 3	4 (4; 5)	100 (65; 223)*	93 (45; 102)*	83 (23; 68)*
Week 4	2 (1; 6)	102 (51; 136)*	22 (11; 45)*,#	18 (10; 53)*,#

Note: * – the indicators were statistically different from group 1 (control), ($p \leq 0.05$); # – the indicators were statistically different from group 2 (orchiedectomized, without transplantation), ($p \leq 0.05$)

Mount frequency steadily increased over the observation period (**Table 3**). Orchiectomized animals with transplantation (groups 3 and 4) exhibited higher MF compared with group 2. In group 1, MF decreased by week 4 as the animals began intromissions earlier. This suggests that the animals became more experienced than the orchiectomized animals in groups 2, 3, and 4, and that the process of acquiring experience depends on the level of androgens in the blood.

Table 3. **The effect of orchiedectomy and transplantation on mount frequency and intromission frequency, median (25th; 75th percentiles), (n = 6)**

MF	Group 1	Group 2	Group 3	Group 4
Week 1	6 (1; 18)	1 (1; 4)*	2 (1; 5)*	4 (2; 5)*
Week 2	14 (3; 22)	5 (3; 10)*	7 (5; 11)*	6 (5; 11)*
Week 3	22 (11; 25)	6 (4; 11)*	9 (3; 9)*	12(3; 14)*
Week 4	13 (10; 19)	7 (2; 9)*	14 (11; 16)#	12 (10; 15)#
IF				
Week 1	4 (3; 6)	-	-	-
Week 2	11 (6; 19)	1 (1; 3)*	2 (1; 4)*	1 (1; 7)*
Week 3	17 (13; 28)	2 (2; 5)*	3 (3; 6)*	3 (1; 8)*
Week 4	18 (11; 27)	4 (1; 10)*	6 (5; 11)*	6 (5; 10)*

Note: * – the indicators were statistically different from group 1 (control), ($p \leq 0.05$); # – the indicators were statistically different from group 2 (orchiedectomized, without transplantation), ($p \leq 0.05$)

Intromission frequency increased over the observation period in all groups (**Table 3**). There were no differences between groups 3 and 4, which received transplants of native and cryopreserved ICs, respectively. The indicators of copulatory efficiency showed little change. However, there were also no differences between native and cryopreserved cells (**Table 4**).

Table 4. **The effect of orchiedectomy and transplantation on copulatory efficacy, median (25th; 75th percentiles), (n = 6)**

Copulatory efficacy	Group 1	Group 2	Group 3	Group 4
Week 1	0.50 (0.26; 0.75)	-	-	-
Week 2	0.47 (0.31; 0.67)	0.24 (0.15; 0.25)*	0.18 (0.08; 0.22)*	0.24 (0.20; 0.39)*
Week 3	0.53 (0.44; 0.55)	0.28 (0.25; 0.31)*	0.45 (0.36;0.50)*	0.41 (0.13; 0.75)*
Week 4	0.51 (0.47; 0.58)	0.33 (0.31; 0.50)*	0.35 (0.30; 0.42)*	0.29 (0.27; 0.48)*

Note: * – the indicators were statistically different from group 1 (control), ($p \leq 0.05$)

Two out of six animals with transplants in groups 3 and 4 had ejaculations by week 4. These data indicate no difference in the functionality of native and cryopreserved ICs.

Testosterone levels decreased in all orchiectomized groups. However, in groups 3 and 4 with transplantation, the levels remained detectable (**Table 5**).

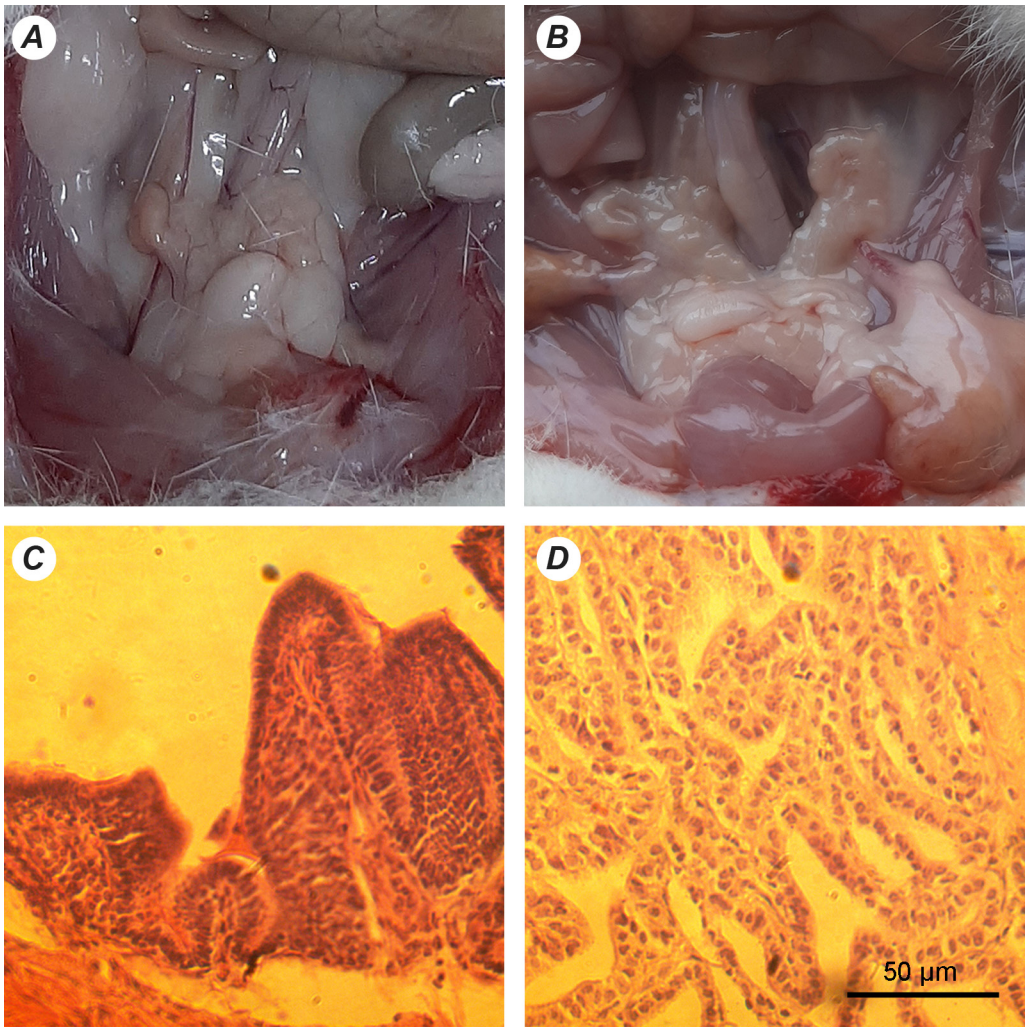
Table 5. **The effect of orchiedectomy and transplantation on the free testosterone level and the mass of the seminal vesicles, median (25th; 75th percentiles), (n = 6)**

Animals	Free testosterone level (pg/mL)	Mass of seminal vesicles (mg/g)
Group 1	14.08 (10.27; 17.32)	2.94 (2.52; 3.27)
Group 2	-*	0.18 (0.13; 0.19)*
Group 3	0.73 (0.69; 0.89)*,#	0.23 (0.21; 0.27)*,#
Group 4	0.72 (0.64; 0.72)*,#	0.28 (0.25; 0.35)*,#

Note: two out of six animals of group 2 had free testosterone level 0.11 and 0.21 pg/mL. Other animals had undetectable levels of free testosterone (≤0.06).
* – the indicators were statistically different from group 1 (control), ($p \leq 0.05$); # – the indicators were statistically different from group 2 (orchiedectomized, without transplantation), ($p \leq 0.05$)

Although the mass of seminal vesicles, which depends on androgen stimulation, decreased significantly, IC transplantation slowed down their degradation (see **Figure**). Desquamation of the secretory epithelium and a reduction in its surface area were observed. Residual secretions were present in the lumen of the seminal vesicles.

Native and cryopreserved with 0.7DMSO + D40 ICs were able to secrete testosterone *in vivo*. Since no differences were observed in the functionality of cryopreserved and native cells *in vivo*, the reduced capacity to produce testosterone in response to hCG observed *in vitro* was temporary. The use of 0.7DMSO+D40 allowed for simplified cryopreservation by omitting DMSO removal, thereby preventing the unnecessary loss of cells that typically accompanies this step.



The representative seminal vesicles of group 2 (***A***) and 3 (***B***).
Histology of the seminal vesicles of group 2 (***C***) and 3 (***D***)

The administration of DMSO during transplantation can cause vomiting, irritation, abdominal pain, and issues with kidney and heart function (Ikeda *et al.*, 2020; Ikeda *et al.*, 2022; Ehn *et al.*, 2023). It has been shown that during hematopoietic cell transplantation, approximately 20 to 60 mL of DMSO enters the body (300–800 mg/kg), while the maximum recommended dosage for humans is 1000 mg/kg (Júnior *et al.*, 2007). In our experiments, the maximum doses of DMSO and D40 introduced into the body with cryopreserved ICs were approximately 5 and 10 mg/kg, respectively. In our previous studies, we demonstrated that transplantation of tissue fragments cryopreserved with 1.5 M DMSO without DMSO removal led to the development of spermatogenic epithelium. This further supports the assumption regarding the safety of this type of transplantation.

Research prospects in the field of Leydig cell transplantation (as well as other testicular cell types, such as Sertoli cells, germ stem cells, etc.) open new avenues for the treatment of various forms of male hypogonadism and infertility. In our view, cell transplantation should not be considered a complete alternative to hormone androgen (testosterone) replacement therapy, but rather as part of a comprehensive approach to treating male infertility – particularly in cases following oncotherapy or chemotherapy during childhood – that may help reduce the risks associated with lifelong hormone therapy. The most relevant research directions include the optimization of cell sources for transplantation – specifically, the use of autologous, allogeneic, or induced pluripotent stem cells for differentiation into Leydig cells. This approach helps to minimize the risk of immune rejection and to preserve the patient's hormonal autonomy. The principles of cryopreservation of testicular cells may also be applied to the conservation of mammalian biological material for veterinary and ecological purposes.

CONCLUSION

Conclusions can be drawn as follows: (a) native and cryopreserved ICs preserved with 0.7DMSO + D40 retained the ability for both basal and stimulated testosterone secretion *in vitro*; (b) the reduced ability for stimulated secretion observed in cryopreserved cells is a temporary phenomenon, as the cells were capable of testosterone secretion *in vivo*; (c) the loss of gonads significantly impaired sexual behavior and the acquisition of sexual experience; (d) IC transplantation had a positive effect on sexual behavior and the ability to acquire sexual experience; (e) no functional differences between native and cryopreserved interstitial cells were observed *in vivo* following transplantation.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

Animal studies: all international, national, and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [P.O.V.]; methodology, [P.O.V.; T.N.O.]; validation, [P.O.V.]; formal analysis, [P.O.V.; T.N.O., P.O.S.; R.N.O.; M.V.V.].; investigation, [P.O.V.; T.N.O., P.O.S.; R.N.O.]; writing – original draft preparation, [P.O.V; R.N.O.]; visualization, [P.O.S.; R.N.O.] supervision, [P.O.V.].

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АНДРОГЕНСИНТЕЗУЮЧА АКТИВНІСТЬ КРІОКОНСЕРВОВАНИХ КЛІТИН ІНТЕРСТИЦІЮ СІМ'ЯНИКІВ У РАЗІ ТРАНСПЛАНТАЦІЇ

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Обґрунтування. Клітини, ізольовані зі сім'яників ссавців і людини, можуть бути використані для наукових цілей, підтримки деяких ліній і порід тварин, збереження біологічного матеріалу тварин, що перебувають на межі зникнення, а також у репродуктивних технологіях. У більшості підходів для кріоконсервування таких клітин використовують сироватку крові (або її похідні) та диметилсульфоксид (ДМСО). Це може призводити до нестабільних результатів і поширення інфекцій, змінювати експресію певних генів клітин, а також до прояву токсичних ефектів ДМСО. У наших попередніх дослідженнях розроблено безсироваткові середовища для клітин інтерстицію (КІ) сім'яника, а метою представленої роботи є дослідити їхню здатність щодо синтезу тестостерону після кріоконсервування.

Матеріали та методи. КІ були отримані зі статевозрілих щурів за допомогою ферментної обробки та кріоконсервовані у розчинах, які містили 0,7 М ДМСО та 100 мг/мл одного з полімерів: декстран 40, гідроксіетилкрохмаль, поліетиленгліколь; або 1,4 М ДМСО та 10% фетальну бичачу сироватку. Швидкість охолодження 1 °С/хв. Клітини після кріоконсервування нагрівали на водяній бані, видаляли ДМСО та досліджували на предмет здатності до базального і стимульованого синтезу тестостерону *in vitro*. Крім того, КІ трансплантували кастрованим тваринам і досліджували зміни концентрації вільного тестостерону в крові, маси сім'яних пухирців і статевої поведінки.

Результати. Здатність до стимульованого синтезу тестостерону зберігалася тільки у тварин кріоконсервованих у розчині, що містив декстран 40 (0,7ДМСО + Д40) та ФБС (1,4ДМСО + ФБС). Кріоконсервовані КІ мали здатність підвищувати показники статевої поведінки кастрованих тварин у разі трансплантації без видалення кріопротекторного середовища 0,7ДМСО + Д40: латентних періодів садок та інтромісій, кількості садок та інтромісій, здатності до еякуляції та копуляторну ефективність. Крім того, вони сприяли підтриманню рівня вільного тестостерону крові та маси сім'яних пухирців у разі трансплантації кастрованим тваринам.

Висновки. З'ясовано, що КІ, кріоконсервовані у безсироватковому середовищі 0,7ДМСО + Д40, зберігали здатність до синтезу і секреції тестостерону. Крім того, застосування 0,7ДМСО + Д40 дає змогу використовувати клітини одразу після нагрівання, минаючи етап видалення кріопротекторних сполук, що може сприяти впровадженню експериментальних напрацювань у практику.

Ключові слова: ДМСО, клітини сім'яників, декстран, кріоконсервація, трансплантація, тестостерон, статева поведінка

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