Additional file 1

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"Research Articles" category

Full title: Post-thaw ATP supplementation enhances cryoprotective effect of iodixanol in rat spermatozoa

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Additional Materials and Methods

Animals

Twenty one sexually mature female rats (8-10 weeks old) were used for artificial insemination (AI).

Surgical Artificial Insemination

Intra uterine insemination (IUI) was performed with sperm that was frozen using extender contained 2% OptiPrep^{TM,} which improved sperm evaluation parameters. In order to prepare pseudo pregnant females for artificial insemination (AI), 8-10 week-old SD rats were synchronized by using intraperitoneal injections of 40 μg Des-Gly^{10}, D-Ala^{6} LHRH ethylamide acetate salt hydrate and subsequently mated with vasectomized males 4 day after LHRH injection. For AI, the pseudo pregnant females were anesthetized by injection of Ketamine (4.17 mg/100 g body weight) and Xylazine (0.83 mg/ 100g body weight) mixture. The fat pad surrounding the ovary were gently grasped with a tissue forceps and retracted/extracted until exposing the utero-tubal junction using standard surgical procedure. Both frozen–thawed and fresh sperm samples were diluted in Hepes-buffered mKRB containing 0.4% (w/v) BSA until insemination. About 50 ul sperm suspension (containing 3-4×10^{6} spermatozoa) were loaded into a ~200 μm inner diameter glass transfer pipette, and the pipette was inserted into the uterine lumen and gently deposited. On the morning of day 21-22, the inseminated females were euthanized and were dissected to confirm pregnancy and counting numbers of live fetuses.

Table S1 Developmental competence of frozen-thaw rat spermatozoa in the presence of 2% Optiprep™ after surgical intra uterine insemination

Treatment	No. Recipients	No. Pregnant (%)	No. total live fetus	No. live fetus per recipient
Fresh	10	9 (90)[a]	83	9.2[a]
Frozen-thawed	11	4 (36)[b]	15	3.75[b]

Values within a column with different superscripts differ significantly ($P < 0.05$).