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Title:

***In Vitro* Activation of Thawed Frozen Iraqi Local Buck Spermatozoa and Fertilization of Goat Ova Using Some Additives to Certain Media**

Abstract:

Accidental death of valuable and highly genetic characters of male animals might affect its genetic factors distributions unless we create a new strategy to gain their germplasm contents (spermatozoa). The experiment was conducted from February 2016 to February 2017 and included the following:

1 – Semen collection from five adult local black bucks (3 - 4 years old) and the average weight of 55 kg by artificial vagina and evaluation of semen (pH, sperm concentration, percentage of massive and individual motility, viability and abnormalities as well as the level of AST and ALT in the seminal plasma).

Semen samples were divided in six aliquots and washed with PBS (1:9) by centrifuging at 1200 × g for 10 min at room temperature and seminal plasma was removed. After that, washing samples were diluted with six different extenders: egg yolk 10% (EY), tris buffer (TB), camel milk (CM), goat milk (GM), beat pulp juice (BJ) and green apple juice (GJ) extenders. Sperm diluents were divided into 2 parts, one with glycerol and the other without glycerol. The diluent without glycerol was added to spermatozoa before the sperm cells were taken into a refrigerator (5 to 6C°). The sperm-containing tubes were placed in a 250 mL beaker containing 150 mL of 25C° water. After 1.5 h in the refrigerator, when water temperature in the beaker reached 5 to 6C°, the diluent with glycerol was added to the sperm cells in three 10 -min intervals to reach a final concentration of 200×10^6 spermatozoa/mL. Diluted semen was evaluated as for fresh semen and loaded into 1 mL plastic eppendrof and frozen over nitrogen vapors for 10 min, 4 cm above the nitrogen level, plunged and stored in liquid nitrogen. After three months of semen freezing in liquid nitrogen, the samples were thawed at room temperature for 5 min and transferred to a 39 Cº incubator for 20 min and a small aliquot (0.1 mL) was removed from each specimen for the assessment of percent motility, progressive motility, live spermatozoa, normal spermatozoa and the level of AST and ALT.

EY 10% and TB treatments presented higher ($P < 0.05$) percentages of total motility and progressive motility compared with GM, CM, BPJ and GAJ treatments and the result showed that GM and CM treatments presented great similarities in all sperm parameters between them while BPJ and GAJ treatments were lower significantly in all parameters compared to all treatments.

2- Three media (DMEM, SOF and TCM-199) were used to determine the effect of media

on an activation of frozen thawed local buck semen, The Specimen of each straw was placed in centrifuge tubes (round bottom) and overlaid carefully with 1,3 ml of culture medium(swim-up method). The tubes must be put in the CO_2 incubator, at an angle around 45° at 39 C° for 60 min, After incubation, the top 1 ml from each tube was removed and pooled in a sterile centrifuge tube and centrifuged (300 g for 10 min). Simple layer technique(swim-up) relies on the ability of spermatozoa to swim out of seminal plasma into culture medium .The supernatant is removed again and the pellet is resuspended in the 1 ml of medium, after processing, sperm parameters were assessed. The result showed great decline in the sperm concentration after and TCM-199 media exhibited higher activation of sperm characteristic regardless of type of extender using in the dilution of specimen.

3- activation of frozen thawed local buck spermatozoa by several chemicals (Heparin, Caffeine, PX and G. glabra), frozen thawed spermatozoa of all experiment extenders incubated in TCM-199 media after treating with above chemicals and using of CO_2 incubator for the separation of spermatozoa by simple layer technique(swim-up). The result of EY10% diluted semen was shown that caffeine exhibited greater ($P<0.05$) sperm conc. and higher percentage of sperm motility (35.6±0.96, 78±2.54 respectively) than control (33.0±0.70 and 70±2.98 respectively) and G.glabra (33.2±0.65 and 70±2.82 respectively) and non-significant superiority than heparin (34.0±0.79 and 75±1.84 respectively) and PX (34.4±0.76 and 75±3.13 respectively). The treatment with caffeine resulted in a higher ($P<0.05$) percentage of progressive motility (58±2.23) compared to control, PX and G.glabra (46±2.12, 52±1.14 and 50±1.58 respectively) and non-significant difference with heparin (55±1.84), no significant difference among treatments in normal morphology. Nearly similar results obtained from activation of semen prepared from GM and CM extenders, while for TB , PX treatment was presented higher($P<0.05$) total motility compared to control and G.glabra(72±2.54 *vs*. 65±2.40 and 65±1.78 respectively) and non significant difference compared to heparin and caffeine(68±2.54 and 68±2.28 respectively), in addition px treatment exhibited higher ($P<0.05$) progressive motility compared to control(55±2.23 *vs*. 48±1.78) and non significant difference compared to heparin, caffeine and G. glabra(50±1.70, 52±2.30 and 50±2.40 respectively), no significant difference in the sperm concentration and normal morphology. No significant differences were found in sperm parameters after activation of semen diluted with BPJ and GAJ extenders

4- Oocytes were Collected from ovaries by two methods: aspiration and slicing, ovaries were brought from butchers markets in Basra. The overall number of oocytes, acceptable quality oocytes and mean oocytes recovered per ovary using the slicing technique was higher ($P<0.01$) during the period of the experiment, compared to the aspiration technique.

5- Oocyte maturation in three different media (SOF , DMEM and TCM-199) to determine the effect of media on the maturation rate of caprine oocyte .TCM-199 exhibited higher significant ($P<0.05$) difference compared to SOF and DMEM IVM media with regard to cumulus cell expansion when the COCs were matured *in vitro.*

6- In vitro fertilization of mature caprine oocytes with EY10%, TB and GM

cryopreserved spermatozoa, TCM-199 medium was used for incubation of samples in Co_2 incubator. The result showed that GM presented greater percentage of fertilized ova than EY10% and TB extenders (22.4% vs. 13% and 10.2%). The total fertilized oocytes are 40 from 270 cultured oocytes (14.8%).