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respectively). The maturation rate was higher for fresh tissue (94.1 ± 1.1%) than the two cryopreservation groups, while the slowly frozen group had better maturation rates than the vitrification group (80.1 ± 1.3 and 73.0 ± 1.9%, respectively). No statistical differences were observed in the cleavage and embryonic developmental rates between fresh tissue and cryopreservation groups. In conclusion, dissection method followed by puncture of bovine ovaries greatly maximizes the number of good quality oocytes recovered, as well as the embryo yield. Ovarian tissue can be successfully cryopreserved by slow freezing and vitrification.

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Determination of sex ratio in bovine semen by quantitative sybr green real time PCR

A Fani Maleki, H Moussavi, N Nassiri, A Vakili, T Pour and M Sekhavati

Department of Animal Science, Excellence Center for Animal Science, Ferdowsi University of Mashhad, Iran

At present, the only proven method for producing sexed semen in mammals is the cell sorting by flow cytometry, based on DNA difference. Cell sorting by flow cytometer provides a powerful tool for artificial insemination and production of predefined sexed embryos. Due to high costs and decrease in fertility rate, the extensive use of sexed semen in livestock depends extremely on sorting purity of sperm cells. Validating the accuracy of sperm sexing requires reliable procedures, therefore real time PCR assay can determinate sex ratio as reliable assay. In this study a SYBR Green real time PCR assay was used to determinate sex ratio in bovine sperm. Two primers were designed on specific X- and Y- chromosome genes. The Y-specific primers pair were designed on a conserved region of the bovine Y- chromosome-linked SRY gene that is responsible for male sex determination. the Y-product amplification length was 120 bp (GenBank accession no. HQ908797). Oligonucleotide X-specific primers were designed to amplify a 149 bp DNA fragment on the bovine proteolipid protein gene (PLP) (GenBank accession no.HQ875721). Two certified standard curves were obtained using of two plasmids containing the X- and Y- amplicons. The method was validated by a series of accuracy, repeatability, and reproducibility and by testing two sets of sorted and unsorted samples for X- and Y-chromosomes. The evolution of X-chromosome bearing sperm content in unsorted samples showed an average of 50.3 ± 0.97% for ejaculates and 51.60 ± 0.18% for the commercial semen. Accuracy (95.2%), repeatability (CV = 3.19%) and reproducibility (CV = 2.24%) was shown. This new method for quantification of the sexual chromosome content in spermatozoa might be reliable approach and providing a valid support to the sperm sexing technologies with low costs.

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Heterologous *in vitro* fertilization using bovine oocytes and stallion fresh and frozen semen

N Faria, A Chaveiro, M Faheem and F Moreira da Silva

University of the Azores, Angra do Heroismo, Protugal

The present experiment was designed to evaluate the interaction between fresh/frozen-thawed stallion spermatozoa and zona pellucida free bovine oocytes, to analyze the capacity of fertilization of fresh/frozen-thawed semen of stallion by heterologous fertilization and flow citometry. After semen collection from two stallions using an artificial vagina, sperm evaluation were immediately performed. Part of the semen sample was cryopreserved. Sperm viability and motility were reassessed immediately after thawing. The acrossome and plasma membrane integrity of the spermatozoa were evaluated on flow citometry, using propidium iodide (PI) and fluorescein isothiocyanate conjugated with Penut Agglutinin (FITC-PNA). *In vitro*-matured cow oocytes were inseminated with different percent live stallion sperm (hight (> 50%) or low (< 40%) viability stallion sperm). After 18 h of co-incubation, the oocytes were fixed, stained with 4′,6-diamidino-2-phenylindole (DAPI) and examined for the two polar bodies. The results of fertilization with fresh and frozen-thawed hight viability spermatozoa penetrated 51.9 ± 2.25% and 34.4 ± 2.7%. Lower rates of penetration were observed for fresh and frozen-thawed low viability spermatozoa 48.1 ± 6.95% and 13.7 ± 1.60% showing significant differences ($p < 0.05$). In flow citometry, we observed that the fresh and frozen-thawed hight viability have better results for the acrossome integrity 91.04 ± 0.7% and 76.4 ± 7.79%, than the semen with lower viability 27.44 ± 2.59% and 25.92 ± 2.97% and for the plasma membrane integrity the fresh and frozen-thawed hight viability semen have better results 83.6 ± 1.55% and 57.69 ± 9.01% than the lower viability 16.57 ± 3.17% and 6.87 ± 1.05%. These findings suggest that bovine oocytes provide a useful model for assessing the penetration potential of frozen-thawed stallion sperm.

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Analysis of IGF-I and IGF-II gene expression in goat oviduct cells using real-time PCR

J Fazeli[1], M Daliri[1], E Hashemi[1], T Harakinejad[2] and M Najari[3]

[1]National institute of genetic engineering and biotechnology (NIGEB), Tehran, Iran, [2]Department of Genetic and Animal Breeding, College of Agriculture, Zahjan University, Zanjan, Iran, [3]Department of Genetic and Animal Breeding, College of Agriculture, Qaemshar Azad University, Qaemshahr, Iran

Oviducts are dynamic organs that support gamete transport, maturation, fertilization and early embryonic growth and development. Oviducts are targets of estradiol and progesterone produced in response to Leutinizing hormone (LH) and Follicular Stimulation hormone (FSH) by stimulation of ovaries. In our previous study the expression of growth factors such as IGF-I & II ligand by oviduct cell under influence of hormones using RT-PCR method did not show any difference in stimulatory effect when compared with control group (without hormonal treatment). How ever in our new study we accurately analyzed the comparison of expression using real time-PCR.

In this study similar cell culture protocol were followed, we isolated the oviduct epithelial cells from goat oviduct slaughtered at local abattoir. Cells were cultured in TCM 199 medium supplemented with 10% FBS and various concentrations of estradiol, progesterone, LH & FSH. Total RNA were extracted from cell at 0, 24, 48 h culture and also from confluent monolayer stage, Quantitative real-time PCR was applied using two sets of IGF-I & IGF-II primers.

Quantitative real-time PCR showed significant differences in transcript levels between estradiol, progesterone, LH & FSH treatment for IGF-I and IGF-II genes, where as in previous result we visualized the expression of IGF-I (293 bp) and IGF-II (77 bp) ligand in all treated and non treated control goat oviduct epithelial cell.