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905 Comparison of Different Techniques to Evaluate Membrane and Acrosome Integrity of Bovine Cryopreserved Sperm Cells

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During fertilization process, only spermatozoa with intact plasma membrane are able to go through capacitation, to bind to the zone pellucida, to undergo acrosome reaction and to fertilize an oocyte. Since membrane and acrosome integrity are essential for the initial steps of fertilization, their evaluation should be use as a routine tests. Stain exclusion methods had been used to assess those features. With the development of the staining technology the combination of fluorescent stains became widely used, however those techniques require fluorescence microscopy, which is not always available. The objective of the present study was to compare techniques to evaluate membrane and acrosome integrity of bovine sperm cells, using or not fluorescent probes. To evaluate membrane integrity eosin-nigrosin (E/N) and a combination of carboxyfluorescein diacetate and propidium iodide (CFDA) stains were used. For acrosome reaction, dual stain using trypan blue and giensa (TBG) and the combination of peanut agglutinin conjugated with fluorescein isothiocyanate (PNA-FITC) were performed. Frozen semen from four different bulls was used in a three replica experiment. In each replicate three straws were thawed and were pooled in a centrifuge tube. From that pool, samples were removed for evaluation of membrane and acrosome integrity using bright field (EN and TBG) and fluorescence (CFDA and PNA-FITC) microscopy, in which 200 cells per slide were evaluated. Data were compared using a t test. The mean percentage of spermatozoa with intact plasma membrane was similar (P>0.05) for the EN and CFDA technique, being 53.41 ± 14.57% and 45.39 ± 7.46%, respectively. However, a higher (P<0.05) percentage of cells with intact acrosome was detected by the TBG (54.93 ± 11.51) than by the PNA-FITC technique (42.86 ± 9.90). The results showed that simple analysis such as EN can be used to evaluate sperm plasma membrane integrity as efficiently as the CFDA. However, for acrosome integrity PNA-FITC detected a higher percentage of damaged cells. Therefore, the use of fluorescent probes seem to be more sensitive to detect alterations in the acrosome of cryopreserved bovine sperm cells then the TBG test.

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Key words: spermatozoa, acrosome, plasma membrane, bovine

906 The Effect of Uterine Biopsy on Reproductive Performance of Dairy Cattle: a Case-Control Study

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Uterine biopsy is a long existing technique used mainly for diagnostic and research purposes but is not used routinely in field practice due to previously claimed detrimental effect on reproductive performance of dairy cows. The objective of this study was to evaluate the effect of uterine biopsy on the reproductive performance of dairy cows in commercial herds. Fifty four cows in four Israeli Holstein dairy herds were randomly allocated for uterine biopsy before first service. The average time of biopsy from calving was 66.9 days (ranging from 44 to 104 days). One hundred and fifty seven control cows were paired-matched to the biopsy cows using the following criteria: farm, parity, twinning, postpartum uterine diseases and calving season. Reproductive performance of biopsy cows was not worse than that of the control cows. Biopsy cows did not differ significantly from the control cows in the first AI conception rates, 44.4% and 38.9%, respectively (P = 0.485), and did not differ significantly in pregnancy rate at 150 DIM, 55.6% and 45.2%, respectively. The OR of biopsy cows to be empty at 150 DIM was 0.6 (P=0.146). Using survival analysis to compare time to pregnancy, biopsy and control cows did not differ significantly in their pregnancy rates until 150 DIM (log-rank test, P = 0.246).

907 Effect of the Induction of the Nursing on the Reproductive Parameters in Holstein Cows

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The drop fertility in the bovine milk is a reproductive problem of present time, since it has been observed that this decrease coincides with an increment in the production of milk; for what is convenient to generate an alternative that allows the reduction of lost, derived of the reproductive flaws through the hormonal induction of the nursing, since it has been observed under field conditions that these induced cows show reproductive similar parameters to the cows that it has a natural nursing. The objective of this study was to evaluate the reproductive efficiency after treatment of the cows Holstein induced hormonally to the nursing. The work was carried out in Tizayuca, Hidalgo, Mexico. They were formed two groups of animals, the group witness for 10 heifers and 20 cows of first and second parity and the treated group in a same way. The cows of the recently given birth to group witness were carried out rectal palpation to the 15 and 60 days after birth. To the cows of the treated group, they were applied a day 1 0.03 mg/Kg estradiol cypionate IM, 2 progesterone mg/Kg IM AND 500 mg of bovine somatotropine SC. Of the day 2 at the 7, 0.03 mg/Kg, estradiol cypionate IM, 2 progesterone mg /Kg IM. The day 8, 0.01 Mg/Kg estradiol cipionate IM, 500 progesterone mg/Kg IM. Of the day 9 at the 14 0.01 mg/Kg estradiol cypionate IM Day 15, 0.04 dinoprost mg/Kg IM and 500 mg of bovine somatotropine SC. Day 16 and 17 0.04 dinoprost mg/Kg IM Days 18 at the 20, 0.04 dexametazone mg/Kg IM Day 21, 500 mg of bovine somatotropine SC and 200 UI of oxtocine. Every 140 days 500 mg of bovine somatotropine SC. The obtained results went that the days to first heat in the treated groups it was of 84.27 ± 31.90 days and for the witness of 63.03 ± 21.33 days. The services for conception for the treated group were of 2.23 ± 1.13 and the witness of 1.63 ± 0.81. In open days the treated group was of 133.23 ± 55.02 days and the group witness of 84.87 ± 27.91 days. Interval among parities, for the treated group was of 415.17 ± 55.05 days and the group witness was 367.03 ± 27.96 days. The gestation percentage to 180 days was of 77%. It can be concluded that the cows induced hormonally to nursing have a reproductive smaller efficiency that the cows with natural nursing; however they allow that their reproductive and productive life return.

908 Progesterone Monitoring of Ewes before the Onset of the Breeding Season

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The aim of the study was to monitor the ovarian cyclic activity of ewes before the onset of the breeding season and after the oestrus induction using hormonal preparations by the milk progesterone determination. We followed Tsigai ewes from July 16 to September 14. We inserted vaginal sponges impregnated by 40 mg FGA for 12 days and 500 IU eCG were injected in the time of the sponge withdrawal. Five ewes were treated according to the OvSynch protocol (August 8 0.0125 mg leireline, August 12 0.125 mg PGF_{2α}, August 14 0.0125 mg leireline). Before the hormonal treatment ewes did not assign the cyclic activity of the ovaries (P4 concentrations 0.1 ± 0.7 nmol.l⁻¹). After the gestagen treatment we observed the increasing in milk P4 concentrations after mating (from August 24: 4.1 ± 15 nmol.l⁻¹, peak September 5: 14.3 ± 36.9 nmol.l⁻¹, decreasing from September 10: 4.1 ± 9.9 nmol.l⁻¹). Only one ewe from this group did not assign the cyclic activity of the ovaries after hormonal treatment. In group of ewes treated according to the OvSynch protocol there were three ewes recorded with increasing milk P4 concentrations in September 3 (3.2 ± 7.6 nmol.l⁻¹) and showed the peak in September 5 (5.7 ± 10.2 nmol.l⁻¹). Five days later the P4 concentrations decreased to level nearing the null (0.1 ± 0.7 nmol.l⁻¹). They slightly increased after this term (2.7 ± 4.7 nmol.l⁻¹). These preliminary results show at the fact that Tsigai ewes breed in foothills of Slovakia are strictly seasonally determined and their oestrous cycle in non-breeding season can be renewed only by the hormonal preparations in combination with breeder methods, e.g. incorporation of rams into the flock after 48 hrs from the last treatment.

Key words: ewe, progesterone, OvSynch, FGA