

## P56 | Simultaneous assessment of plasma, acrosomal and mitochondrial membranes in rooster sperm

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Mito Tracker™ Green [MITO] is a probe capable to stain the mitochondrial membrane of different species; however, we have observed its ability to stain the acrosomal membrane of rooster sperm. The association of MITO and propidium iodide [PI] was used to simultaneously assay the mitochondrial, acrosomal and plasma membranes, of rooster sperm. MITO-PI staining was validated comparing the results with those obtained by SYBR-14/PI (plasma membrane integrity), and by blue aniline (acrosomal integrity in birds). Data were also correlated with motility variables (CASA) and DNA fragmentation (TUNEL). Two pools from 6 roosters Birchen Leonesa were refrigerated and evaluated for 9 days (18 evaluations). Aliquots of chilled sperm were diluted in 250 µL of HEPES, containing 0.4 µL of MITO (1 mM), to achieve a final concentration of  $5 \times 10^6$  sperm/mL. Diluted samples were incubated for 23 min at 5°C in the dark, at this point, 0.5 µL of PI were added and incubated for 2 min. The double staining showed 4 sperm categories of living sperm: IPIAIM, IPIADM, IPDAIM, IPDADM (I = intact, D = damaged, P = plasma membrane, A = acrosomal membrane, M = mitochondrial membrane). The same categories were identified in death sperm. A positive correlation (Spearman,  $p < 0.05$ ) was found between the % of sperm with IPIAIM and % viable sperm ( $R = 0.82$ ), % sperm with acrosome integrity ( $R = 0.83$ ) and % total motile sperm ( $R = 0.91$ ). Negative correlation ( $p < 0.05$ ,  $R = -0.83$ ) was seen between %IPIAIM and % DNA fragmentation. The association of MITO-PI fluorescent dyes demonstrated to be efficient in evaluating rooster sperm quality. Funded by European Union's Horizon 2020 Research and Innovation programme under grant agreement No. 677353.

## P57 | Morphometric description of a sow's large size ovaries

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In the development of a work 4 hybrid sows (Large White x Landrace) with an age between 14 and 16 months were used. The sows were underwent to an estrus synchronization treatment to

subsequently recover their genital tracts. In one of the sows, a normal genital tract was found, but both ovaries presented an unusual size, with great follicular growth. The synchronization treatment consisted in the oral administration of 20 mg altrenogest for 18 days followed by 1000 IU of serum gonadotropin intramuscularly at 9:00 h, on the 19th day of the beginning of the treatment. After that, 500 IU of chorionic gonadotropin were applied intramuscularly at 9:00 p.m. on day 21. Finally, sows were sacrificed on day 23 to extract the genital tracts. The morphometric characteristics of the large size ovaries and the normal ovaries ( $n = 3$ , mean  $\pm$  SEM) were: Weight (gr) of the ovaries, right 110/left 105 and right  $10.51 \pm 0.43$ /left  $12.21 \pm 0.18$ , respectively; Perimeter (cm), right 23.8 cm/left 24.3; right  $9.67 \pm 0.44$ /left  $11.40 \pm 0.81$ , respectively; Horizontal axis (cm), right 6.72/left 6.7 and right  $2.63 \pm 0.22$ /left  $2.97 \pm 0.13$ ; Vertical axis (cm), right 7.7/left 7.5 and right  $3.87 \pm 0.15$ /left  $3.93 \pm 3.94$ , respectively and Number follicles, right 148/left 127 and right  $12.00 \pm 1.53$ /left  $10.67 \pm 0.67$ , respectively. In a study published by Horsley et al. 2005 (*J. Anim. Sci.*, 83, 1690–1695) using a similar synchronization treatment in 25 nulliparous sows they established an average ovarian weight of  $9.06 \pm 0.45$ , appreciating 17.54 corpora lutea. Our results found in the studied ovaries were markedly higher than the normal ones in sows, with a considerably higher morphometry and a weight that exceeds in 10 times the normal ovaries.

## P58 | Genetic mosaicism rates following bovine oocyte microinjection with CRISPR components delivered as RNA of ribonucleoprotein

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Genetic mosaicism, i.e. the presence of more than two alleles in the same individual, is a common problem encountered following CRISPR microinjection in zygotes that occurs when genome edition takes place after DNA replication. This phenomenon weakens significantly the chances of direct KO generation in one-step, as all alleles generated must disrupt the open reading frame of the target gene. In previous experiments, we have found that early delivery of CRISPR components (oocyte vs. zygote injection) greatly reduces the percentage of mosaic embryos, but the form of delivery may also have an effect. CRISPR components can be delivered as RNA (mRNA encoding for Cas9 + sgRNA) or as ribonucleoprotein (RNP), the later acting faster as it does not require the translation of Cas9 protein and RNP assembly. The objective of this study has been to compare mosaicism rates between RNA or RNP delivery to bovine oocytes. In vitro matured bovine oocytes were microinjected with 300 ng/µl Cas9 mRNA and 100 ng/µl sgRNA (RNA group) or pre-assembled RNP (RNP group, 300 ng/µl Cas9 protein + 60 ng/µl sgRNA). Following microinjection, oocytes were fertilized in vitro