

set of reagents "Progesterone - ELISA", from "Hema" Ltd. (Russia). Pregnancy in recipient cows was determined by rectal palpation on the 60th day after embryo transfer. A total of 171 blood samples from 57 recipients were tested by ELISA. According to the results of the rectal palpation of 57 recipient cows, 32 were pregnant (56.14% pregnancy rate). The concentration of progesterone in the barren recipients ( $n = 25$ ) was 1.547 on day 4, 2.485 Day 6, and on the 19th day 0.702 ng/ml, while in the pregnant recipient cows ( $n = 32$ ) the indexes relative to the barren ones were higher: on the 4th day 2.158, on the 6th day 4.185 and on the 19th day 12.100 ng/ml. The amount of progesterone in the barren recipients and in the pregnant cows ( $n = 57$ ) on the 4th and 6th days of the estrous cycle was 1.853 and 3.335 ng/ml, respectively. In conclusion, a high progesterone concentration in recipients has a positive effect on the engraftment of embryos. As a criterion for the evaluation of the physiological activity of the corpus luteum we recommend to use a method of determining the amount of progesterone in the blood plasma.

### P 259 | *Solea senegalensis* spermatozoa quality: are apoptotic cells and reactive oxygen species playing a role in F1 reproductive failure?

DG Valcarce<sup>1</sup>; O Chereguini<sup>2</sup>; M de la Hera<sup>2</sup>; I Martín<sup>2</sup>; MP Herráez<sup>1</sup>; I Rasines<sup>2</sup>; C Rodríguez<sup>2</sup>; V Robles<sup>2</sup>

<sup>1</sup>Institute for Animal Health and Livestock Development (INDEGSAL) and Department of Molecular Biology, University of León, León, Spain; <sup>2</sup>Spanish Institute of Oceanography, Santander, Spain

*Solea senegalensis* broodstock (F1 generation) presents a failure on spawning performance compared to wild-captured counterparts. It is known that the problem relies on males in the F1 generation. Courtship lack and low quality semen hinder the expansion of sole aquaculture. The absence of courtship in F1 individuals leads to the use of artificial fertilization protocols, which require sperm cryopreservation prior to fertility trials. Cryopreservation can generate reactive oxygen species (ROS) that could have a negative impact on spermatozoa function. The aims of this study were: (1) to evaluate ROS in males born in captivity (F1) and in wild individuals (2) to implement a selection method for optimal sperm subpopulation recovery prior to cryopreservation. The percentage of positive cells for dichlorofluorescein (DCF) and propidium iodide (PI) were determined by flow cytometry in both groups' males. The presence and distribution of  $H_2O_2$  within the cells was analyzed by confocal microscopy. In order to select non-apoptotic cell subpopulations magnetic activated cell sorting (MACS) was used and YOPRO and caspases were determined in the recovered population. Our results indicated a decrease in viability (55%) and DCF+ cells (66%) in slow F1 spermatozoa. Cryopreservation did not affect viability neither the presence of DCF+ cells in samples from wild individuals but decreased viability in F1 samples. Confocal studies demonstrated a colocalization of  $H_2O_2$  with active mitochondria but also with nuclear DNA. Finally, MACS significantly reduced the percentage of apoptotic cells (54%

and 75% removal in wild and F1, respectively) showing a potential future application on aquaculture. (Funding: AGL2015-68330-C2-1-R (MINECO-FEDER); AQUAGAMETE FA1205 COST Action; JCyL EDU1084/2012 and FSE)

### P 260 | Assessment of the effect of a new technique for laparoscopic partial closure of the inguinal canal on sperm production and testicular perfusion in stallions

FJ Vázquez; A Vitoria; L Barrachina; S Fuente; I de Blas; A Romero; L Gil

Faculty of Veterinary, University of Zaragoza, Zaragoza, Spain

In order to simplify other laparoscopic techniques, a new standing laparoscopic technique for partial closure of inguinal canal (PCIC) has been developed. This technique uses a new anchoring device and can be performed without advanced laparoscopic skills. The aim of this study was to develop a prior evaluation of the effects of this new technique on the stallion reproductive capacity, assessing the sperm production and testicular perfusion. Standing laparoscopic PCIC was performed unilaterally in 8 experimental stallions without evidence of inguinal hernia, using the contralateral canal and testicle as controls. Pre and postoperative testicular examinations and Doppler ultrasound scans were serially performed to assess the perfusion of both testicles. After 28 days, laparoscopic examination was performed in all horses. After that, they were castrated and seminal characteristics of epididymal sperm from both testicles were evaluated. Laparoscopic procedure was quickly and easily performed in all cases without any surgical complication. Significant differences in testicular perfusion and sperm characteristics were not found between both testicles, in studied variables: PSV, EDV, RI, PI, sperm concentration, percentage of live/dead and static/slow/medium/fast spermatozoa and hypo-osmotic stress test. The simplicity to implant the device, the absence of complications and the lack of adverse effects on sperm production and testicular perfusion, makes this new system a promising candidate for laparoscopic inguinal hernia repair. Further studies would be necessary to check the device performance in clinical cases of breeding stallions in a long-term period.

### P 261 | Immunohistochemical studies of the effect of ischemia on glucose transporter GLUT1 localization in porcine uterus

B Wąsowska; D Harasiuk; P Gilun; J Kwiatkowska

Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

Ischemia consistently causes a large increase in the extraction of glucose from the blood, reflecting a greater capacity for glucose