Proceedings of the 8th International Symposium on Canine and Feline Reproduction

ISCFR

June 22-25, 2016
Paris, France

In a joint meeting with the XIX EVSSAR Congress

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Fertility rates on bitches after artificial insemination with two different concentration of frozen semen
Carmen Cecilia Sicherle, Yamê Fabres, Gabriele B. Mothé, Camila de Paula Freitas - Dell’Aqua, Fabiana Ferreira de Souza, Nelmar Camargo, Frederico O. Papa, Maria Denise Lopes São Paulo State University Julio de Mesquita filho - UNESP Botucatu – Department of Reproduction and Veterinary Radiology. carmensicherle@terra.com.br

The first use of frozen dog semen date from 46 years ago [1], since then many improvements on frozen semen procedures and artificial insemination techniques have been developed. Research focus is on pregnancy rates which is still inconsistent when frozen semen is used. Also on this regard, the canine estrous cycle is complex and requires full understanding and monitoring to perform the AI at the right time since the life span of frozen/thawed semen is short and limited [2]. Freeze/thaw procedures cause sperm membrane injuries [3] and this could lead to a shortened life span and also a reduced capacity of the sperm to adhere to uterine epithelial cells [4]. Mason & Rous (2014), observed better pregnancy results when more than 100 x 10^6 live morphologically normal sperm were used on AI. Two different total AI sperm concentrations were tested: 160 x 10^6 sperm cells (N = 4) and 400 x 10^6 sperm cells (N = 5) where the mean progressive motility were 70% and a membrane integrity 40%. Sperm membrane integrity were checked every cryopreservation procedure using Propidium Iodide and Carboxifluorescein Diacetate fluorescent probes and only values above 40% of sperm integrity were accepted for the AIs. Semen was collected by digital manipulation from 4 different adult dogs. After centrifugation (800g/10 minutes) the pellet were diluted on Tris-egg-yolk medium with 8% of glycerol (one step), filled into 0.5 ml french straws and refrigerated at 50 C for 1 h. After this, straws were suspended 6 cm above liquid nitrogen for 20 min and plunged into liquid nitrogen. Semen was thawed at 400 C for 20 seconds. Nine bitches from different ages and sizes were inseminated 2 times, on consecutive days, with the same semen concentration for each AI. Inseminations were performed inside the uterus by transcervical catheterization, using an 43cm long and 9.5 Fr = 3.15 mm with a 5° viewing angle, an instrument port, rigid endoscope (TCI Endoscope – Minitub/Karl Stortz- Germany), on the days 5 and 6 after LH surge based on the measured of progesterone using RIA (for detection of LH surge, 2-3ng/ml, until the confirmation of ovulation, > 10ng/ml). All bitches were inseminated on standing position and no sedation was needed. Pregnancy was checked 30 days after AI by laparotomy at the same time of neutering the females. Two bitches were pregnant (40%) on the group inseminated with 400 x 10^6 sperm cells/AI (N = 5) and no pregnancy was observed on bitches inseminated with 160 x 10^6 sperm cells/AI (N = 4). Although 40% is not a good pregnancy, the results show that sperm concentration can directly influence the results of AI using frozen/thawed semen and confirm previous observation made by Mason & Rous (2014) were the use of more than 100 x 10^6 live and motile sperm cells lead to better AI results in the bitch. Financial support: FAPESP