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EVALUATION OF REPRODUCTIVE PERFORMENCE OF DOVES AFTER TREATMENT WITH INSEMINATION DOSE IMPLEMENTER HEPARIN

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ABSTRACT

The aim of the study was to evaluate the effect of implementer heparin on the interaction follicles - sperm, conceptual ratio and the number of pups born in inseminated doves inseminated The experimental group was inseminated with 0.5 ml insemination dose inseminated 15 min. incubation with heparin (25 000 IU / l ml) of concentrations of 0.06 ml / 0.5 ml / ID (0.06 ml = 10 mg). The control group was inseminated with 0.5 ml insemination dose without implementer. The addition of heparin implementer has led to an increase in the reproductive performance *in vitro* and *in vivo*. Number of zygotes with two pronucleus represented in the control group 74.5% of the total number of follicles stranded in the group. In the experimental group, the number of zygotes with two pronucleus accounted for 81.08% of the total number of follicles stranded in the group. *In vivo*, we found a conceptual ratio 89.28% with an average number of live pups per litter 10.64 ± 3.41 pc in experimental doves. In the control group the conceptual ratio was 72.00% with an average number of pups per litter 8.55 ± 3.22 pc. The conceptual relation between the control K and experimental GAG group of doves has show a statistically significant difference (χ24, 15 +). In the number of live born pups between control K and experimental GAG group a statistically significant difference (2.02 + T41) was observed too.

Keywords: Heparin, rabbit, reproduction, interaction sperm - follicules

INTRODUCTION

Implementers (extenders) can be characterized as substances added to the insemination dose to increase its capacity to fertilize or extend viable sperm.
Anchordogery *et al.* in **1987** described that certain additives added to the semen have the ability protect its individual components and affect the sperm vitality. These arguments were later confirmed in the studies of **James *et al.* (1992)**.
The positive impact of implementer on the quality indicators of semen of various species of livestock have already described several authors (**Champion *et al.* 1997**; caffeine - **Tathan *et al.* 2003, Matejašáková *et al.* 2005, Riha *et al.* 2006**, heparin and hyaluronan - **Januskauskas *et al.* 2001**, heparin - **Lapointe *et al.* 1996, Parrish *et al.* 1993, Fik *et al.* 2008abcd**, LHRH **Ondruška *et al.* 2008, Quintela *et al.* 2004, Slamečka 2009**).
The increase of conceptual relationship through the affecting of insemination doses with implementer have described several authors (**Ondruška *et al.* 2008, Vašíček 2009, Slamečka 2009, Marks and Ax, 1985; Parrish *et al.* 1988; Lapointe *et al.* 1996**).
Heparin is glycosaminoglycans (GAG), which is able to induce hypermotility, capacitation and thus binding of sperm acrosome. Many studies show that GAG plays a role in capacitation (**Handrow *et al.* 1982, Parrish *et al.* 1989, Chamberland 2001, Dapino *et al.* 2006**).
Chamberland *et al.* (2001) indicate that in mammals heparin affects motility parameters and is required for *in vitro* capacitation of bull sperm.
GAG has been found in the doves reproductive tract in several species of mammals (**Januskauskas *et al.* 2001**).
When incubated with GAG the sperm capacitation beings within a shorter time period than in the presence of lysophosphatidylcholin *in vivo* conditions (**Parrish *et al.* 1988**).
Rečková *et al.* (2006) studied 17 young bulls of Czech Pied breed and used a concentration of 250 million / ml (centrifuged sperm), the incubation lasted 3 and 6 hours. At untreated sperm with heparin, the average value of a share of sperm with unspoilt chromatin structure after 3 hours of capacitation of sperm decreased by 1.1% compared non-capacitating sperm. After 6 hours capacitation of sperm was the difference between the values of the proportion of sperm with unspoilt chromatin structure 2.0%. At sperm treated with heparin samples after 6 hours of incubation with heparin showed a smaller proportion of sperm with unspoilt chromatin structure. At 3 hours of incubation the difference between the untreated semen and with heparin was noticeable, but not statistically significant.
Parish *et al.* (1988) indicate that the incubation of sperm with heparin at a dose 10% increases the µg/10ml of fertilized oocytes, but these sperm had to be incubated for at least 4 hours before the fusion of gametes.
Fik *et al.* (2008a, b, c, d) tested the effect of heparin, at the concentration implementer 0.06 µl / ml of semen. Group of doves broiler rabbits, in which heparin was added to the insemination dose the conceptual ratio reached 70.06% compared to 55.94% in the control group. In the group of doves inseminated with the addition of heparin implementer the average number of live born pups per 1 inseminated dove was 6.28 pcs compared to 4.89 pcs in the control group, where the insemination dose was not affected with heparin.
Fik and Malíková (2013) tested the effect of heparin, at 0.06 µl / ml implementer on reproductive parameters (conceptual relationship, the number of live-born pups per litter, number of live-born pups per inseminated doe). They observed a higher conceptual proportion of 14.12 % in experimental group compared to the control group. These differences did not show a statistically significant difference.
Effects of heparin on sperm motility have described **Lapointe *et al.* (1996)**, on the extracellular calcium **Parrish *et al.* (1993)**.
Guang-Peng *et al.* (2004) found that heparin plays an important role not only in of sperm capacitation and acrosome response, but also affects the formation of pronucleus and early embryonic division. One possible reason could be that heparin binds to sperm and induces Ca^{2+} release and increases pH, which subsequently results in an increase in tyrosine phosphorylation of the protein (**Lane *et al.* 1999**) or in physiological and biochemical changes in relation to formation of pronucleus or the first mitotic division. The use of heparin in the system of IVF increases the success rate of fertilization, dividing and profit blastocyst.
Vašíček (2009) monitored through the affecting of insemination dose effect of heparin on interaction of sperm and follicles. The author noted an improvement of interactions in group, where the implementer were used, compared to the

control group without implementer. However, the results were not significantly different.
Aim of studies of **Dapino *et al.* (2006)** was to evaluate the effect of heparin on *in vitro* boar sperm capacitation. The authors state that the optimal conditions for *in vitro* sperm capacitation were incubation in capacitation medium with heparin for 120 minutes at 39 ° C. These conditions increase the number of capacitated sperm and acrosome response of boar sperm.
The aim of this work was to evaluate the effect of implementer heparin on the conceptual ratio and the number of born pups in doves that were inseminated with the affected insemination dose.

MATERIAL AND METHODS

The experiments were performed on nulliparous females of synthetic maternal line M91, which live weight reached a min. 3500 g (at 4 month of age). The ejaculate was obtained from male synthetic meat line P91. Insemination dose (ID) has been adjusted by thinner on sperm concentration: 68 x 106 / 0.5 ml / ID.
The experimental group (labeled GAG) was inseminated with 0.5 ml of insemination dose influenced by 15 minutes incubation with heparin (25 000 I.U. / l ml) of concentrations of 0.06 µl / 0.5 ml / ID (0.06 µl = 10 µg).
The control group (labeled C) was inseminated with 0.5 ml of insemination dose - without implementer.
Subsequently to the both groups were administered intramuscularly Supergestran 0.1 ml = 2.5 mg GnRH / 1 ♀.

Procedure of the experiment

Tuesday: 15.00 am. - 48 hours before artificial insemination (A.I.) 25 I.U. PMSG (Sergon, Bioveta, Czech Republic) was applied to each dove for the synchronization of oestrus.
Thursday: 15.00 am. - Females were inseminated (A.I.) with fresh heterosperm doses. Immediately after A.I. each of control doves and every dove in the group GAG was affected intramuscularly with 2.5 ug synthetic GnRH (Supergestran, Ferring Pharmaceuticals, Czech Republic).
Friday: 9.00 am. - 11.00 pm., 18-20 hours after insemination we selected from the experimental group of doves (GAG) 4 doves and 6 doves from control group, from which we washed up follicles of doves oviduct (post mortem) to evaluate the interaction on sperm with eggs (before the formation of mucopolysaccharide package oocytes). After the humane kill of doves by electrical shock and bleeding, we prepared out genital tract-ovarian, oviducts and uterus (uterus bicornis we stopped in the first period) and placed in a Petri dishes (120 mm) with 2 ml of equilibrated Dulbecco's medium.
Flushing out of zygotes was performed using a ground green Luer-Lock needle (0,7 x35), equilibrated in Dulbecco +2.5% FCS, through the infundibulum of oviduct. Flushing out of zygotes was performed by equilibrated (37 ° C) Dulbecco medium. This was followed by preparation of microscopic specimens, photographs and evaluation of unfertilized oocytes and zygotes with two pronucleus as a result of successful sperm-follicles interactions. Zygotes were washed up in Petri dishes (60 mm). Of a binocular magnifier, at a magnification of 1.6 x we sought for washed up follicles. The
evaluation (presence of cumullar cells and sperm) was conducted under binocular magnifier with a magnification of 2.5 x 4 x under a light microscope 4x12 and 10x12.
On the zygote in Dulbecco drop we recorded spatially bound sperm on the zona pellucida. Zygote, once inside into the microvessel was rinsed in a drop of physiological saline and plated on labeled microscope slide. Zygotes were obtained directly from flushing out Dulbecco, using a modified glass microvessel with pusher of a binocular magnifying glass at a magnification of 1x.
The follicles were transferred on a dry and marked microscope slides with ground glass. The side of the glass was marked with a CentroFix indicating the site of the transferred zygotes. The zygote, once inside into the microvessel was rinsed in a drop of physiological saline and plated on labeled microscope slide.
On the drop with the zygote, we applied a drop of paraffin oil and immediately observed bound sperm under a light microscope with a reduced condenser. Then we took pictures of preparations of zygotes under immersion oil on Olympus CX41 light microscope with built-in digital camera, with lens 20 x without immersion oil with reduced condenser.
Other inseminated nulliparous doves were bred until birth, their *in vivo* results of the reproduction process (number of live and dead-born pups and the conceptual ratio) were prepared and were used to compare the results for observing an effect of selected implementer *in vivo* and *in vitro* conditions.

RESULTS AND DISCUSSION

Intravaginal application of GAG pointed out on a tendency to increase the relative number of zygotes with 2 pronucleus in experimental doves (81.8%), relative to control group of doves (74.5%). These differences were not statistically significant (χ2 0.15 to 0.72 -). Similar tendency of results indicates also **Vašíček (2009)**. The increase % of fertilized oocytes using the heparin in insemination dose states also **Parrish *et al.* (1988)**.
In vitro analysis of the results of oocytes fertilization relatively equally replicate *in vivo* results obtained in the average number of pups in litters. In the group of GAG doves this represented a value of 10.64 ± 3.41 pups, while in the control doves the average number of pups per litter reached a value below 8.55 ± 3.22 pc.
Overview of the results of *in vitro* analysis of the experiments with intravaginal application of heparin is given in Table 1.

Table 1 *In vitro* analysis of experiments with intravaginal application of heparin

Studied indicators	Control group of doves (K)	Doves with intravaginal applied GAG-heparin 0.06 ml / 1 I.D. (GAG)	
n	6	4	χ^2
Number of ovulations	83	69	
Number of flushed up oocytes and zygotes	55 (100%)	33 (100%)	
Number of zygotes with 2 pronucleus	41 (74.5%)	27 (81.8 %)	K:GAG$^{(-)}$
Number of unfertilized oocytes	14 (25.5 %)	6 (18.2 %)	K:GAG$^{(-)}$

(-) statistically non-significant
(+) statistically significant

Number of nulliparous doves inseminated with intravaginal application GAG was 28 The conceptual ratio of experimental doves was 89.28%. Give litter the 25 doves with an average number of pups per litter 10.64 ± 3.41 pcs, with the total number 266 pcs of live born pups and with the number of dead born pups 4 pcs.
Number of inseminated nulliparous doves in the control group was 25 Conceptual ratio in the control group was 72.00%. Give litter the 18 doves with an average number of pups per litter 8.55 ± 3.22, with a total of 154 pieces of live born pups and the number of dead born pups 4 pcs.
In the conceptual relation between the control group K and experimental group of doves GAG was observed a statistically significant difference (χ24, 15 +).
In the number of live born pups between control group K and experimental GAG was also a statistically significant difference (2.02 + T41). The increase of the conceptual ratio using the implementer heparin in insemination dose in rabbits describes Fik et al. (2008 a, b, c, d).

CONCLUSION

Based on the results, we can say that influencing on insemination dose by implementer heparin has led to an increase in reproductive performance *in vitro* and also *in vivo*. Number of zygotes with two pronucleus represented in the control group 74.5% of the total number of follicles washed up in the group. In the experimental group, the number of zygotes with two pronucleus accounted for 81.08% of the total number of follicles washed up in the group.
In pursuing of reproductive characteristics in *in vivo* conditions was observed in doves from experimental group a conceptual ratio 89.28% with an average number of live born pups per litter 10.64 ± 3.41 pcs. In the control group the

conceptual ratio was 72.00% with an average number of pups per litter 8.55 ± 3.22 pcs. In the conceptual relation between the control group K and experimental group GAG of doves was observed a statistically significant difference (χ24, 15 +).
In the number of live born pups between control group K and experimental group GAG was also a statistically significant difference (2.02 + T41).

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