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THE IMPACT OF HEPARIN IMPLEMENTER (GAG) IN THE RABBIT INSEMINATION DOSE

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ABSTRACT

The aim of this work was to evaluate the impact of implementer heparin in insemination dose in rabbits selected on reproductive parameters.The experiment was monitored reproductive parameters (conceptual relationship, the number of live-born pups per litter, the number of dead-born pups per litter, the number of live-born pups per inseminated does) 156 does in the experimental group and 165 does in the control group. We used the ejaculate of synthetic broiler rabbit population with concentration of sperm 25-50 mil. / 0.5 ml / 1 ID. Heparin was added at a dose - 0.06 ml = 10 mg per 0.5 ml semen / 1 ID.
Assessing selected reproductive parameters in does inseminated with insemination dose with the addition of heparin, we observed a higher conceptual proportion of 14.12 % in the experimental group compared to the control group. These differences did not show a statistically significant difference (χ^2 3.56$^-$). The number of live-born pups per litter was 8.69 ± 4.10 pc in the experimental group and 8.41 ± 3.62 pc in the control group (P> 0.05). The number of dead-born pups was recorded 0.74 pc in the experimental group and 0.76 pc in the control group (P> 0.05). The number of live-born pups per litter per inseminated does we have seen improvement in favor of experimental group by 1.39 pc. This parameter was within each of experiments ranged from 0.91 to 1.66 pc live-born pups to inseminated does.

Keywords: Heparin, reproduction of rabbit, broiler rabbit, implementer

INTRODUCTION

Many studies have shown that heparin (GAG) plays a role in capacitating **(Handrow *et al.*, 1982; Parrish *et al.*, 1989; Chamberland, 2001; Dapino *et al.*, 2006).**
Chamberland *et al.* (2001) reports that in mammals, heparin affects motility characteristics, but also indicates the need for *in vitro* capacitating of bull sperm.
The incubation of sperm with GAG takes place capacitating in shorter time period than in the presence lysophosphatidylcholin *in vivo* **(Parrish *et al.*, 1988)**. GAG modulates protein binding capacitating in the membrane of sperm cells **(Miller *et al.*, 1990; Therien *et al.*, 1995).**
Mariňáková (2010) monitored the impact of heparin implementer of concentrations 0.06 ml/ID on selected reproductive indicators of broiler rabbit does. The author has seen improvement in the experimental group in the number of live-born pups in the litter and also the conceptual relation, but without statistically significant differences.
Reckova *et al.* (2006) studied 17 young bulls of Czech Spotted breed and use concentration of 250 million/ml (centrifuged sperm), incubation lasted 3 and 6 hours. In sperm untreated with heparin, mean proportion of sperm with intact chromatin structure after 3 hours of sperm capacitating decreased by 1.1 % compared incapacitating sperm. After 6 hours of sperm capacitating, the difference between the values of the proportion of sperm with intact chromatin structure was 2.0 %. In the heparin-treated sperm, samples after 6 h incubation with heparin showed a smaller proportion of sperm with intact chromatin structure. At 3 hours incubation, the difference between the untreated seed and heparin was noticeable, but not statistically significant.
The same authors suggest that the highly variable results of the *in vitro* system are not due to the negative impact of separation and incubation of sperm, because the proportion of sperm with intact chromatin structure was high enough for potential oocyte penetration after 6 hours of incubation.
Lane *et al.* (1999) re-evaluated 2 capacitation media, the heparin (GAGs) and the high density lipoprotein (HDL). Bull sperm were incubated in GAGs (12µg/ml) and HDL (10 - 160 micrograms/ml). The experiments found that heparin and HDL are suitable media for sperm capacitation, but with a different mechanism of action.
Seminal plasma proteins of bull support bull's sperm capacitation in presence of heparin **(Thérier *et al.*; 1995)** and high density lipoprotein **(Thérier *et al.*; 1997)**.
Parish *et al.* (1988) report that incubation of sperm with heparin at a dose of 10 µg/10 ml increase % of fertilized oocytes, but the sperm had to be incubated for at least 4 hours prior to the fusion of gametes.
Increasing the conceptual relationship in *in vitro* conditions described **Lapointe *et al.* (1996)**, who worked with bull semen. Sperm incubation with GAG also looked **Whitfield** and **Parkinson (1992)**, which also showed a positive effect on the fertility of GAG in *in vitro* conditions.
The results increases of fertility by influencing capacitation GAG describe **Marks** and **AX (1985)**.
Januskauskas *et al.* (2001) compared two capacitation fluid and this heparin and hyaluronan, and their impact on the acrosome reaction and itself capacitation. Heparin was labeled on the results as more appropriate capacitation medium. Their also reported significant results between a group ID with heparin and control group. In bull sperm incubation with hyaluronan not recorded significant changes in capacitation and acrosome reaction compared with the control group.
Chauhan *et al.* (1997) report that the capacitation of bull´s sperm by heparin requires 8 hours of exposure sperm to heparin 10 mg/ml for maximum acrosome semen response.
Januskauskas *et al.* (2001) did experiments with the addition of heparin implementer to the insemination dose bulls. They used a concentration of 5 mg/ml. Reported significant results ($P \leq 0.001$) in capicitation and acrosome reaction between the experimental group and the control group. The authors further suggest that increased acrosome reaction in the heparin group ID is not in correlation with the actual fertility.
Guang-Peng *et al.* (2004) found that heparin plays an important role not only in sperm and capacitation of acrosome response, but also affects early embryonic division. One possible reason could be that heparin binds to sperm, and induces the release of Ca^{2+} and an increase in intracellular free calcium and pH, which in turn result in an increase in tyrosine phosphorylation of the protein group **(Lane et al., 1999)** or the first mitotic dividing. The use of heparin in the IVF system increases the success rate of fertilization, dividing and blastocyst profit.
Vasicek (2009) followed the influence of insemination dose the effect of heparin on the interaction of sperm and egg. The author observed the improving

interaction in the group which benefited the implementer compared to control group without implementer. However, the results were not significantly different.
The aim of studies of **Dapino *et al.* (2006)** was to evaluate the effect of heparin on *in vitro* boar sperm capacitation. The authors suggest that the optimal conditions for *in vitro* sperm capacitation were incubation in media with heparin for 120 minutes at 39 °C. These conditions increased the amount of capacitated sperm and acrosome response of boar sperm.
Many authors presented the works of increasing the reproductive performance by using implementer of heparin in cattle and other livestock, but such work almost entirely absent in broiler rabbits.
The aim of this work was to evaluate the impact implementer heparin in insemination dose in rabbits selected for reproductive parameters.

MATERIAL AND METHODS

Experiments were carried out in conditions of experimental breed of rabbit in Animal Production Research Centre, Nitra. Ejaculate was obtained by taking to an artificial vagina (50 °C) from 10 male synthetic lines P 91st. Sperm concentration was detected by Bürker counting cells. On the desired concentrations of the sperm (25 - 50 mil./0.5 ml/1 ID) was ejaculate diluted with commercial insemination diluents with antibiotic for rabbits.
In the female group was established experimental and control group. In the control and experimental group were not nulliparous female. ID for both groups was prepared together. According to the multiplicity of the experimental group was obtained required amount ID into another beaker and added with the required amount of heparin (GAG).
Heparin was added at a dose - 0.06 ml = 10 mg per 0.5 ml semen/1 ID. The dose of heparin was carried by using a micropipette. After dosing, the ID was stirred for at least 5 minutes to achieve maximum homogeneity and used implementer ID. The does line M 91 was inseminated with standard plastic pipettes. Monitored indicators: conceptual ratio, number of live pups per litter, the number of dead-born pups per litter and the number of live pups per inseminated female.

RESULTS AND DISCUSSION

Assessing selected reproductive parameters in does inseminated with insemination dose with the addition of heparin, we observed a higher conceptual proportion of 14.12 % in the experimental group compared to the control group. These differences did not show a statistically significant difference (χ^2 3.56^{-}).
In the repetition of experiments throughout the year, we have seen improvement in the ratio of conceptual experimental group used implementer heparin from 12 to 17.72 %. Within each experiment were inseminated in the experimental group minimum of 18 and maximum of 46 does.
Small improvement over the control group, we also recorded in the indicator of number of live-born pups per litter. In the number of live-born pups per litter per inseminated female we have seen improvement in favour experimental group by 1.39 pc. This parameter was within each of experiments ranged from 0.91 to 1.66 pc live-born pups to inseminated does.

Table 1 Summary results of the reproduction process affected by insemination implementer dose of heparin at a dose of 0.06 ml/0.5 ml in ID

Breeding	No. of females	Conceptual ratio in %			Number of live-born pups per litter			Number of dead-born pups per litter			Number live-born pups / inseminated female
		x	x_{max}	x_{min}	$x (x - x_{sd})$	x_{max}	x_{min}	x	x_{max}	x_{min}	
Exp.	156	70.06	63.04	77.8	8.69 ± 4.10	7.86	9.34	0.74	0.47	0.08	6.28
Accounts.	165	55.94	48.57	65.5	8.41 ± 3.62	7.9	8.86	0.76	0.07	1.2.	4.89
Statist. significant		$\chi^2 = 0.15^{(-)}$			P> 0.05			P> 0.05			$\chi^2 = 0.39^{(-)}$

(-) Statistically inconclusive (+) statistically significant

Based on the obtained results by using implementer heparin in insemination dose, we can conclude that the implementer can at the correct concentrations positively affect individual reproductive characteristics of broiler rabbits. Almost complete absence of scientific papers about using heparin as implementer in insemination dose in broiler rabbit, forces us to compare the obtained results with other types of livestock, for which are effects of implementer already partially explored and described.
The results of our experiments confirm some of the scientific work of **authors (Lane *et al*., 1999; Thérier *et al*., 1995; Parrish *et al.,* 1988; Chauhan *et al.,* 1990)**, but they studied the implementer in cattle, ox, respectively other types of farm and domestic animals. Implementer heparin at the concentration of 0.06 ml/ID at nulliparous broiler rabbits does studied also **Vasicek (2009)**. Author noted the positive impact of the implementer on the conceptual ratio and number of live-born pups per litter. **Mariňáková (2010)** also reported a positive impact of heparin at concentration 0.06 ml/ID on the female reproductive characteristics of broiler rabbits. In their scientific works mentioned authors have also shown that heparin as implementer of insemination dose has a positive effect on reproductive parameters.

CONCLUSION

Sperm-egg interaction is a key event in the process of fertilization. The successful fertilization prevent sperm capacitation and acrosome reaction, which can be effectively, stimulated by the addition of implementers to the insemination dose to rabbits. In this respect, such a restriction is in biology ejaculate very useful, in many cases can biotechnological interventions through simple and low financial investments effectively improve reproductive parameters of broiler rabbits.
The lowest level of the conceptual ratio was observed in the control group of females, where a heparin was not added to the insemination dose. The positive effect was reflected in the number of live-born pups per insemination. This indicator reflects the effective rate concept and number of live pups in this group.

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