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REPRODUCTION PERFORMANCE OF RABBITS BY INCUBATED SEMEN WITH HEPARIN IN INDUSTRY RABBIT FARM

PERFORMANTELE REPRODUCTIVE OBŢINUTE ÎN FERME DE IEPURI CU MATERIAL SEMINAL INCUBAT CU HEPARINĂ

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The aim of the study was to evaluate the effect of incubation semen with heparin (25 000) in insemination dose on fertility rate of inseminated does, and on the number of all and liveborn young per litter. The experiment was realized in commercial farm. The farm was localized in areas of west Slovakia. The rabbit strain HYCOLE was used in experiment. Experimental does were inseminated with ID 0.5 cm^3 with the concentrations of spermatzozoa from 40 to 50 x 10^{-6} in cm^3. Experimental semen was incubated by heparin (0.06 microliter. per 1 inseminated dose) Control semen was incubated without heparin. In control part of experiment were used the same volume of ID, however without heparin. Incubation of semen dose with and without heparin was up to 1 hour. Rabbit does were inseminated after standard hormonal treatment. These studies demonstrate that heparin speed capacitation of rabbit sperm. The experimental group showed better result in conception rate (2.3 %), gravidity period and synchronisation of kindling.The number liveborn young rabbits per litter had statistically no significant ($p > 0.05$) effect. The data were evaluated by statistical method in statistic programme Microsoft Excel.

Keywords: rabbit, insemination, conception of rabbit, incubated by heparin, capacitation by heparin.

Introduction

The use of artificial insemination (AI) in rabbit farms has become a common practice in commercial rabbit production.

Ovulation in rabbits is normally induced as a result of mating and takes place after mating with 10 – 13 hours.

In mammals, freshly ejaculated sperm are incapable of fertilizing the egg. They gain this ability during their transit through the female reproductive tract, and this process is called capacitation (3).

Capacitation is an important process in rabbit sperm maturation and is an obligatory step prior to fertilization (3).

After ejaculation, mammalian spermatozoa undergo an obligatory maturational process called capacitation either in vivo during transit through the female genital tract (3)
or in vitro in defined media (12). Capacitation allows spermatozoa to undergo the zona pellucida-induced acrosome reaction (AR) and fertilize oocytes.
Capacitation of rabbit spermatozoa occurs in vivo in female genital tract. Capacitation period in vivo conditions is 10 – 13 hour.

The mechanism of capacitation is poorly understood, but it involves many biochemical changes. These include the removal of adsorbed components from the sperm surface, a change in membrane lipid composition, increased permeability to certain ions such as Ca^{2+}, a change in internal pH, and an increase in plasma membrane fluidity and in metabolism (12). There is also an increased hyperactivation that is believed to result from the redistribution of membrane components during capacitation (12). Apart from these changes, not much is known. In addition, several studies show that there is a decrease in the membrane cholesterol phospholipid ratio during capacitation (10).

All of these changes allow the spermatozoa to undergo the acrosome reaction (AR) following interaction with the zona pellucida, the egg's extracellular matrix (11). The rabbit acrosome reaction, which involves membrane breakdown and release of enzymes for penetration of egg investments, is induced by a specific stimulus in follicular fluid at the site of fertilization.

Many studies have shown that heparin-like glycosaminoglycans (GAG) found in follicular fluid play a role in capacitation of bovine sperm (14,16). Thus ejaculated sperm incubated with GAG capacitate in a shorter period and then undergo the AR in the presence of the zona pellucida in vivo or in the presence of lysophosphatidylcholine (lyso-PC) in vitro (8). Lyso-PC induces the AR in capacitated sperm only (8). It has been postulated that GAG modulate capacitation by binding to proteins of the sperm membrane (11). In cattle, heparin binds to sperm (12) and induces changes in the intracellular environment of the sperm. This results in Ca^{2+} uptake and an increase in intracellular free calcium and intracellular pH (12). Another change associated with heparin-induced capacitation in bovine sperm is an increase in protein phosphorylation (5). The changes in phosphorylation and cyclic nucleotide levels have also been observed in other sperm functions such as motility and in AR induction (8). Similar observations have been made in sperm of the pig, mouse, and hamster (5). Heparin sulfate has been shown to both stimulate sperm capacitation and cause decondensation of sperm chromatin. Recent studies have shown that sperm chromatin

decondensation following exposure to a low concentration of heparin sulfate is inversely correlated with penetration ability (2). Capacitation of bovine spermatozoa can occur in vitro in medium supplemented with a glycosaminoglycan, heparin (HEP) (14, 16). The putative mechanism is that HEP functions as a ligand for a receptor localized in the sperm plasma membrane, but such a receptor is as yet uncharacterized. This HEP, which is bound to the spermatozoa, appears to stimulate 1) the intracellular elevation of calcium, pH, and cAMP, which seem to be necessary to initiate the signalling pathway concomitant with capacitation (14, 16); and 2) the removal of seminal plasma proteins adsorbed to the plasma membrane, which are considered to be inhibitors of capacitation. Incubation of sperm with heparin (10 micrograms/ml) increased the percentage of oocytes fertilized, but this required exposing sperm to heparin for at least 4 h before adding them to oocytes (14). Incubation of rabbit sperm by heparin (0,06 µl / ID) increased conception rate (4).

Material and Methods

Studies were performed in industrial rabbit farm in west Slovakia. Experiment was performed during March and April of 2007.

In our experiment were used 268 females of strain HYCOLE. Breed houses had temperature between 18 – 22 ºC by using an air conditioned system. Light intensity was 70 lux. Artificial lighting programm was 14 hours of light, 10 hours of dark.

All does were individually housed in flat deck cages (0.3m) with external nest (0.12m).

Our does were first inseminated as 20 weeks old (3.7kg weight of body) and bucks were used for reproduction from the age of 23 weeks (4kg weight of body). Does were inseminated for 19 days post partum. Rabbit does were inseminated after standard hormonal treatment.

The fertility rate has been determined after kindling. The number of dead - born and live – born young per litter has been determined till 24 hours post partum.

Pregnant and lactating does were fed ad libitum and non pregnant and non lactating were restricted to 150 g per day. Two types of commercial diet were used: from 5 days before partum to 21 days post partum and from 21 days post partum to weaning (35 days). Feeding was provided by a commercial granulated concentrate that contained at least 15.5 % of crude protein and 15 % of crude fibre.

The females were divided into 2 groups. Experimental group and control group. The diluted fresh semen was divided to experimental and control. Then experimental and control sperm were incubated at a final concentration of 40 - 50 x 10^{-6} per ml under the same conditions: without or with heparin (0.06µl/ID) for capacitation. Ejaculated sperm were incubated with heparin (10µg/ml) and without heparin up to 1 hour.

Does were vaginally inseminated using glass pipettes in volume 0.5 ml per doe.

The females were divided into 2 groups. Experimental group (only multipara does) and control group (only multipara does). Gravidity period, conception rate (%), number of alive born pups, number of death born pups, average number of live - born per one inseminated doe were evaluated.

The statistical evaluation of obtained data was realized in computer programme Microsoft Excel using the method of t-test comparison.

Results and Discussion

These studies demonstrate that heparin speed capacitation of rabbit sperm.

The experimental group showed better result in conception rate above 2.3 %, number of liveborn pups above 0,6 pcs, gravidity period and synchronisation of kindling. The average number of live – born per one kindling doe was 11 in experimental group and 10.4 in control group. The number live – born young rabbits per litter had no statistically significant ($p > 0.5$) effect.The present study clearly demonstrates that the heparin can be added to insemination dose. Ejaculated sperm acquire fertilizing ability by interacting with capacitation factors present in the female genital tract. Several studies have shown that incubation with heparin or heparin-like GAG promotes capacitation in bovine sperm (3. 12).

For this study we incubated ejaculated sperm in the presence of heparin (10 µg/ml – 0.06µl/1 I.D.).Our experiment showed better result by fertilising rate in experimental group (2.3 % more).

Table 1

Reproduction peformance of experimental and control groups

Parameter	Experimental group	Control group
Inseminated does (pcs)	100	168
Non pregnant does (pcs)	8	19
Kindling does (pcs)	91	149
Live - born pups (pcs)	803	1164
Average number of live - born per one doe (pcs)	11	10.4
Dead - born pups (pcs)	28	51
Average number of dead - born per one doe (pcs)	0.28	0.34
Average number of live - born per one inseminated doe (pcs)	8.3	6.9
Conception rate after born (%)	91	88.7

Table 2

Kindling time of does

Days	Experimental group		Control group	
	Numbers of does	% of post partum does	Numbers of does	% of post partum does
1. day of kindling	0	0	3	1.8
2. day of kindling	7	7.7	11	6,5
3. day of kindling	40	43.9	51	30.4
4. day of kindling	26	29.4	46	27.4
Total	73	81	111	66.1
5. day of kindling	Hormonal stimulation of kindling			

Conclusion

The results obtained using fresh semen with heparin could be considered satisfactory and encouraging. However, the use of heparin must be about 0.06 µl/0.5ml semen.

We think that the better results of incubation by heparin and shorter gravidity period are probably connected with the shorter time of capacitation.

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