ATTEMPET TO PRODUCE COLD RESISTANCE RABBITS

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Abstract

The aim of this study was planned to evaluate the effect of cold exposure on diluted physical semen and pre mating does on fertility traits and tolerance offspring of NZW rabbit. A total of 420 mature NZW rabbits were used in this study. In vitrocold exposure to each of diluted physical semen, sperm enzymatic activities, during conservation to 37ºC (control), 15; 10 or 5 ºC (cold shock) to 30 minutes. Cold stress exposure of rabbit does were at 20 ºC (control), 15,10,5 ºC for 30 minutes pre-coitus. The obtained results revealed that, physical semen quality, spermatozoa storagability, decreased significantly (P≤0.01), while values of AST; ALT; ACP and ALP enzymes in diluted semen increased significantly (P≤ 0.01), due to temperatures of cold exposing at 15, 10 then 5 ºC, compared to control. Ovulation, kindling rates, litter size and weight at birth was highly significant (P≤ 0.01) and in descending order due to expose to cold stress pre artificial insemination for 30 minutes at 20, 15, 10 then 5 ºC, respectively. Re-mated rabbit does which exposed to different levels of cold stress pre previous mating did not record any significant in fertility traits. Mortality rate of growing rabbits produced from semen or rabbit does exposed to cold stress were significantly (P≤ 0.01) lower discerningly as compared with other cold treatments. In conclusion, exposing rabbit semen and does to very severe cold stress (Shock) produced offspring have more tolerance and adaptation to cold stress.

Key words: Rabbits; cold stress; semen quality; fertility traits; natural mating; AI

INTRODUCTION

Rabbits are very susceptible to heat or cold stress when the environmental temperature is high or cold (Marai et al., 2002 and Badawy et al., 2010).

Exposing animal or human live bodies to cold resulted in "Heat Shock" Proteins (HSP's). These heat shock proteins are stress proteins belong to multigene families that range in molecular size from 10 to 150 kDa and are found in all major cellular compartments (Hightower and Hendershot 1997), can reach 15% to 25% of total intracellular protein within minutes after physiological stress. (Lindquist and Craig 1988, Theodorakis and Morimoto1997).

There were many studies investigated the effects of early heat exposure on body temperature during a later period of exposure to high temperature (Badawy et al., 2010), whereas there are too lack information about exposing animals to cold stress. De Basilio et al. (2001) reported that body temperature significantly increased as a result of a 24h heat exposure at the age of 5days.

The in vitro studies, replaced in vivo studies which do not allow for effectively differentiating between maternal and direct effects of elevated temperature on the developmental competence of maturing oocytes. However, in vitro studies have proven beneficial to identify direct effects of heat stress on the oocyte. These direct effects of elevated temperature (41°C) alter constituents within the oocyte cytoplasm (Payton et al., 2003) and decrease blastocyst development after fertilization (Lawrence et al., 2004). These studies led us to study the in vitro effects of cold stress on rabbit spermatogenesis and semen quality and fertility.

The present study aimed to investigate fertilizing ability of semen and fertility traits

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of does exposed to cold stress. The study also aimed to provide a genetic model to examine potential interdependent relationships between stress inducible of Hsps and the mechanisms involved in cell survival and/or cell death pathways.

MATERIAL AND METHOD

The practical work of the present study was conducted in an Industrial Rabbitry, Agricultural Ahmed Oraby, Cairo Province, Egypt, during the period from February till November, 2014. The laboratory and analysis part was carried out in Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

A total number of 420 sexual mature New-Zealand White (NZW) rabbits at 5 months of age (360 nonparous does and 60 bucks) were used in the study. The study included two experiments.First experiment (in vitro part) was planned to evaluate effects of cold exposure (cold shock) on each of diluted physical semen quality, sperm enzymatic activities, during conservation at different low temperatures for up to 30 minutes. Cold exposure temperatures were 37 (control group), or 15; 10 or 5 ºC (cold shock groups) for up to 30 minutes. Second experiment (in vivo work) was designed to study effects of cold stress exposure (cold stress shock) pre mating of first parity on fertility traits of NZW rabbit does using artificial insemination. Cold stress exposure of rabbit does were at ambient temperature 20 ºC (control); 15; 10 or 5 ºC for up to 30 minutes pre-coitus. Rabbit does which exposed to cold stress pre artificial inseminations were tested for fertility traits during the next parity under normal conditions. Semen was collected artificially from rabbit bucks by means of an artificial vagina as described by Seleem (2003). Semen was pooled and evaluated immediately after collection. The pooled semen was then diluted with lactose-yolk citrate extender as described by Seleem (1996) to give the final extension rate (1:4). The diluted semen was kept in a water bath at 37 ºC to equilibrate the temperature.

The test tubes containing diluted semen were covered and twisted with dark plastic and were placed in water bathes at 37 ºC or at 15; 10 or 5 ºC (cold shock) then stored at these temperatures for up to 30 minutes. Percentages of each of advanced motility of spermatozoa, dead and abnormal spermatozoa and acrosomal damages were evaluated according to Salisbury et al., (1978). Storage ability of rabbit semen was performed according to Yassen and El-Kamash (1970)

The treated diluted semen samples to different cold shock temperatures were centrifuged at 6000 g for 20 minutes before removal of the supernatant and used for enzymatic assay. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes were determined according to Reitman and Frankel (1957). Acid phosphates (ACP) and alkaline phosphates (ALP) enzymes were determined calorimetrically according to Seleem and Rowida (2005).

Three rabbit does were randomly chosen from each group 14 hours after artificial insemination and scarified to record ovulation rate as described by Rowida and Seleem (2007) according to the following formula:

Ovulation rate = No. of ovulated follicles / Total No. of follicles

Artificial insemination (A.I) was applied by using semen diluted according to Adams (1981). The dose of insemination was 0.5ml contained >50 x106 motile sperm for each doe. Rabbit does used in artificial insemination were injected intramuscularly with 20 µg Gn-RH an hour before A.I.

Diluted semen was incubated at 37ºC and kept as a control group, or exposed to cold shock by using crash ice at temperatures 15; 10 or 5 ºC (cold shock groups) for up to 30 minutes. Rabbit does were exposed to cold stress 30 minutes pre artificial insemination in separated rooms using air conditions supplemented by thermostats. Temperatures were 20; 15; 10 or 5 ºC to represent absence cold stress (control); very severe; severe or moderate cold stress, respectively. Weaned rabbits after then were kept and housed in separated room inside the Rabbitry and were exposed to severe cold stress 6 hours daily.

Data were statistically analyzed using Least Squares Analysis of Variance according

to Snedecor and Cochran (1967). Percentage values were transformed to arcsin values before being statistically analyzed. Duncan's Multiple Range Test (Duncan, 1955) was used to compare the differences between significant means. Conception and Kindling rates were analyzed using the Contingency Tables according to Everitt (1977).

RESULTS AND DISCUSSIONS

1- Semen Quality: Table 1 showed the effects of cold exposure (cold shock) on NZW rabbit semen quality, during conservation at different temperatures up to 30 minutes. Sperm motility (%) means decreased significantly as the exposing period and temperature decreased. The decrease in ambient temperature adversely affected semen characteristics Nagwa et al (2006). The apparent decrease in sperm motility, during low ambient temperature might be due to a low sperm concentration of ejaculate and/or to the hypo-activity of the accessory glands, the testis and spermatogenesis processes (Abdel-Samee, 2004). The results of present study agreed with El-Bashary et al. (2005) and Nagwa et al. (2006). Storage ability decreased significantly as the exposing period increased. Percentages of each of dead spermatozoa, sperm abnormalities and acrosomal damages increased significantly ($P \leq 0.05$) as the exposing period increased and temperature decreased. This may be due to hydrogen ion (pH) value that is higher in low ambient temperature (Nagwa et al., 2006).

Increasing of dead sperm and sperm abnormalities during low ambient temperature may be related to spermatozoa characteristics are susceptible to oxidative stress which increased during adverse ambient temperature inducing damage of normal sperm (Badawy et al., 2010). However, plasma membrane of sperm contains large quantities of polyunsaturated fatty acids and their cytoplasm contains low concentrations of scavenging enzymes. In addition, the intracellular antioxidant enzymes cannot protect the plasma membrane that surrounds the acrosome and the tail (Aitken and Fisher, 1994) particularly during adverse ambient temperature.

Enzymatic activity: Table 2 showed the activity means of AST, ALT, ACP and ALP in the diluted New-Zealand White rabbit semen exposed to cold shock, during semen conservation at different temperatures for up to 10 minutes. Enzymatic activity of spermatozoa increased as the exposing temperature increased.

Exposure of growing and adult rabbits to cold stress, adversely affects their growth and reproductive traits. The drastic changes that occur in rabbits' biological functions are depression in feed intake and feed efficiency and utilization, disturbances in metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites. When exposure to THI 30 or more, rabbits can no longer regulate internal temperature (Marai et al., 2002)

Fertility traits and ovulation rate: Data presented in table 3 showed the fertility traits and ovulation rate means of New-Zealand White rabbit does inseminated artificially by using bucks exposed to cold shock 30 minutes before inseminating. Kindling rate (%) and litter size and weight decreased significantly ($P \leq 0.05$) as the exposing temperature decreased. While, there is no significant changes in ovulation rate (%) of does artificially inseminated due to heat exposing.

The traits that declined significantly were the litter size and litter weight at weaning and the pre-weaning weight gain of pups for adult females. The conception rate declined considerably with cold stress. The traits that increased significantly ($P<0.01$) due to cold stress were pre-weaning mortality for adult females (Marai et al., 2001).

Kindling rates: It is interested to note that, kindling rates of New-Zealand White rabbit does re-mated after negative mating due to exposure to cold shock did not significantly affected (Table 4). Long-term cold conditioning leading to cold acclimation, of animals using HSP as a marker for heat acclimation, has had limited examination in vertebrates (Badawy et al., 2010).

Table1 Effects of cold exposure (cold shock) on NZW rabbit semen quality, during conservation at different temperatures for up to 30 minutes (Means ± SE)

Variable	Period of cold exposure (Minutes)	cold temperatures OC				Means ± SE
		37 (Control)	15	10	5	
Advanced sperm motility (%)	0.0	73.2 ± 2.3	44.7±1.9	36.9±1.1	12.4±1.0	41.8±1.3A
	10	72.9±3.2	26.1±1.6	11.3±0.8	6.3±0.2	29.2±0.8 B
	20	72.8±2.9	12.5±0.6	6.2±1.1	2.2±0.6	23.4±0.5C
	30	72.0±3.4	6.2±1.2	3.2±0.4	0.6±0.1	20.5±0.7 D
Means ± SE		72.7±1.6a	22.4±0.9b	14.4±0.6 c	5.4±0.3d	28.7±1.1
Storage ability		98.4±4.2 a	13.9±1.2 b	8.7±0.2 c	4.8±0.1 d	31.5±1.9
Dead spermatozoa (%)	0.0	14.9 ± 1.8	22.5 ± 2.5	26.1±2.9	32.6 ± 2.6	24.0±2.3 D
	10	16.2 ± 1.6	31.6 ± 2.9	39.3 ± 3.1	48.7 ± 3.6	34.0±2.2C
	20	17.4 ± 2.2	42.3 ± 3.8	54.9 ± 4.0	63.4 ± 5.2	44.5±2.6 B
	30	19.6 ± 2.4	54.0 ± 4.4	69.2 ± 6.2	77.3 ± 7.6	55.0±2.9 A
Means ± SE		17.0±1.4 d	37.6±2.8 c	47.4±2.6 b	55.5±2.9 a	39.4±2.2
Sperm abnormalities (%)	0.0	14.1 ± 1.4	17.6 ± 1.9	19.3 ± 1.5	20.1 ± 1.3	17.8±1.2 D
	10	14.8 ± 1.2	20.3 ± 1.3	21.2 ± 1.2	24.2 ± 1.8	20.1±1.4 C
	20	14.9 ± 1.6	22.5 ± 1.9	24.3 ± 1.8	29.3 ± 2.2	22.8±1.3 B
	30	15.4 ± 1.4	25.1 ± 2.0	27.2 ± 2.1	33.3 ± 2.9	25.3±1.5 A
Means ± SE		14.8±1.1 d	21.4±1.4 c	23.0 ± 0.9 b	26.7±1.5 a	21.5±1.0
Acrosomal damages (%)	0.0	10.8 ± 0.8	14.0± 1.1	16.2 ± 1.5	19.5±2.2	15.1±1.5D
	10	11.3 ± 1.0	21.3 ± 1.4	26.3 ± 1.4	33.2 ± 3.0	23.0±2.0 C
	20	11.9 ± 0.8	32.5 ± 1.2	39.3 ± 2.2	46.4 ± 3.1	32.5±1.7B
	30	12.8 ± 1.1	42.0 ± 1.7	51.2 ± 2.3	58.6 ± 3.0	41.1±2.1 A
Means ± SE		11.7±0.7 d	27.5±1.9c	33.3±1.9 b	39.34±2.3 a	28.0±1.7

Means within the same row (a, b, c& d) or the same column (A, B, C& D) bearing different letter superscripts are significantly different (P≤0.05)

Table 2 Activity of AST, ALT, ACP and ALP in the diluted NZW rabbit semen exposed to cold shock, during semen conservation at different temperatures for up to 10 minutes (Means ± SE)

Enzymes	Cold temperatures OC			
	37 (Control)	15	10	5
AST (U/ L)	22.3 ± 1.4d	27.5 ± 1.6c	34.5 ± 2.9b	42.8 ± 2.6a
ALT (U/ L)	15.3 ± 0.9d	21.0 ± 1.2c	26.9 ± 1.7 b	37.2 ± 2.4 a
ACP (U/ L)	16.8 ± 1.1 d	22.4 ± 1.8c	29.2 ± 2.9 b	41.1 ± 2.9a
ALP (U/ L)	31.2 ± 1.9 d	39.0 ± 2.8c	44.3 ± 3.6 b	52.4 ± 3.9 a

Means within the same row (a, b, c& d) bearing different letter superscripts are significantly different (P≤0.05)

Table 3 Fertility traits and ovulation rate of NZW rabbit does inseminated artificially by using semen exposed to cold shock 30 minutes before inseminating (Means ± SE)

Variable	37 (Control)	Cold exposure of diluted rabbit semen OC		
		15	10	5
Mated does (No.)	90.0	90.0	90.0	90.0
Conceived does (No.)	71.0	37.0	22.0	11.0
Kindling rate (%)	63.9a	33.3 b	19.8 c	9.9 d
Litter size at birth (No.)	7.92 ± 1.3 a	5.12 ± 1.1b	3.21± 0.9 c	2.0 ± 0.2 d
Litter weight at birth (gm.)	341.6 ± 21.9 a	223.6 ± 14.4 b	201.5 ± 11.5 c	179.5 ± 12.0 d
Ovulation rate (%)	79.6 ± 3.4	80.4 ± 2.9	82.1 ± 3.3	81.6 ± 2.7

Means within the same row (a, b, c& d) bearing different letter superscripts are significantly different (P≤0.05)

Table 4 Kindling rates of NZW rabbit does re-mated after negative mating due to exposure to heat stress (Means ± SE)

37 °C (Absence cold stress)	Cold stress exposure of rabbit does at temperatures (°C)		
	5 °C (Moderate cold stress)	10 °C (severe cold stress)	15 °C (very severe cold stress)
78.2	80.3	79.9	81.1

Offspring performance: The experiment evaluated the tolerance of offspring produced from cold shocked semen or cold stressed does and housed in Rabbitry at very severe cold stress conditions. Data presents in table 5 showed that mortality rate means of offspring produced from cold shocked semen was significantly higher than those of cold stressed does. The data were higher for control and decreased significantly and in descending order from the 1st group to the 3rd group. The idea that stress proteins can speed the physiological recovery is based on experimental evidence indicating that multiple proximate "signals" can activate the cold shock response and, thereby, evoke the endogenous protective mechanisms of Hsps. The next logical step would be to consider the possibility that related members of the multigene stress protein family confer similar or additional functional benefits (Lindquist and Craig 1988, Theodorakis and Morimoto1997).

In general the study concluded that, exposing rabbit semen and does to very severe cold stress produced offspring have more tolerance and adaptation to cold stress.

This study solves cold climate regions problems affecting animal and poultry production. Also, we wish contributing to solve protein deficiency at these provinces.

Table 5 Offspring performance of does inseminated artificially, the offspring were housed in Rabbitry at very severe cold stress (Means ± SE)

Variable	Offspring produced from	Cold-shocked semen, or cold-stressed does levels*				Means ± SE
		Control	1st	2nd	3rd	
Mortality rate (4-10 weeks)	Cold-shocked semen	25.1±2.9	17.1±2.2	8.8±1.4	2.9±0.7	13.5±1.2A
	Cold-stressed does	22.9±2.4	15.0±2.0	9.1±1.8	2.4±0.3	12.4±1.4 B
Means ± SE		24.0±2.3a	16.1±1.9 b	9.0±1.2c	2.7±0.5 d	12.9
Mortality rate (11-20 weeks)	Cold-shocked semen	13.1±1.9	6.2±1.1	4.0±0.9	1.8±0.6	6.3±0.7 A
	Cold-stressed does	14.7±2.2	5.5±1.4	3.1±0.6	1.5±0.7	6.2±0.9 B
Means ± SE		13.9±1.1 a	5.9±0.7 b	3.6±1.3 c	1.7±0.9 d	6.3
Kindling rate	Cold-shocked semen	22.2	34.6	52.3	59.8	42.2B
	Cold-stressed does	25.3	41.6	54.3	63.5	46.2 A
Means ± SE		23.8d	38.1 c	53.3b	61.7 a	44.2

Means within the same row (a, b, c& d) or the same column (A& B) bearing different letter superscripts are significantly different (P≤0.05)

*Cold-shocked semen at 37°C (control), or 15, 10 and 5 °C. Cold-stressed does at absence (control), or moderate, severe and very severe cold stress

CONCLUSIONS

It could be concluded that, exposing rabbit semen used in A.I. to cold shock deleteriously affected its quality, but produced a few number of offspring characterized by high tolerance to cold stress. Exposing rabbit does to cold stress to 30 minutes pre mating. More studies are still required in that trend.

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