The Effect of Different Freezing Rates on Fertilizing Ability of Frozen-Thawed Chicken Spermatozoa

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Summary. The effect of two different freezing velocities ($1^{\circ}\text{C}/\text{min}$ and $6^{\circ}\text{C}/\text{min}$) on fertilizing ability of spermatozoa from the roosters selected for high fertility with frozen-thawed spermatozoa and the randomly selected control males was determined. Significantly ($P < 0.05$) higher fertility was obtained when semen of the selected male was frozen at a rate of $1^{\circ}\text{C}/\text{min}$ to -70°C . In contrast, semen frozen at $6^{\circ}\text{C}/\text{min}$ until -70°C showed no significant difference in fertility between two male lines. The results of the present study indicate the possibility of male selection for higher freezability of spermatozoa. Better viability and fertilizing capacity of frozen-thawed fowl spermatozoa were suggested to be obtained with a freezing rate of $1^{\circ}\text{C}/\text{min}$. **KEY WORDS;** CHICKEN, SPERMATOZOA, FREEZING, FERTILITY.

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Efforts to freeze chicken semen have been relatively successful, but the fertility of frozen-thawed semen after single insemination remains lower (Watanabe, 1967; Mitchel *et al.*, 1977; Lake and Stewart, 1978) as compared with that of bull semen. Recently, Lake *et al.* (1981) and Lake and Ravie (1984) has obtained considerably higher fertility with freeze-thaw rooster sperm, inseminating at a 3-day interval or three consecutive days. Lake and Stewart (1978) suggested the possibility of genetic difference between male chicken with regard to freezability of spermatozoa. Scott *et al.* (1978) reported a significant difference in fertility of spermatozoa between males of fourth generation selected for duration of fertility with frozen semen and randomly selected males. Sexton *et al.* (1978) also obtained the results showing the highest fertility with semen from the male with higher sperm freezability. These results indicate that genetic parameters associated with viability and fertility of frozen-thawed rooster semen may be different.

On the other hand, cooling velocity of semen at a rate of $1^{\circ}\text{C}/\text{min}$ to -35°C was successful for better viability of freeze-thaw turkey spermatozoa (Marquez and Ogasawara, 1977; Sharp *et al.*, 1982). However, no fertility study has yet been conducted to establish optimal rate of freezing for spermatozoa from different male birds.

This experiment was carried out to determine the effect of different freezing velocity on fertilizing capacity of spermatozoa of the cockerels of high fertility with freeze-thaw spermatozoa and the randomly selected males.

Materials and Methods

A total of 20 males of a line (generation 4) of chicken selected for duration of fertility of frozen-thawed semen and 20 males of the randomly selected control line were used in this study (Scott *et al.*, 1978). Pooled semen samples were collected from each male group, diluted 1 to 3 with Lake's solution containing 13.64%

Table 1. Experimental procedures

Group	Male line		Apparatus		Freezing rate	
	Selected	Control	Cryocool-100	Macdonald Apparatus	1°C/min	6°C/min
1		○	○		○	
2	○		○		○	
3		○		○	○	
4	○			○	○	
5		○		○		○
6	○			○		○

of glycerol (Lake, 1968) and packaged in a 0.5 ml plastic straw. Semen samples were kept in an iced-water bath (0–3°C) until frozen. Two different apparatuses and two methods of freezing were employed for each semen group (Table 1). Same samples were frozen with either Cryocool-100 (Cryocool, Ontario, Canada) or Macdonald College Freeze Thaw Apparatus (Mitchell *et al.*, 1975) at freezing speed of 1°C or 6°C/min until –70°C, followed by plunging the straws directly into liquid nitrogen (–196°C). When semen temperature reached –196°C, the straws were taken out, placed in an iced-water bath and deglycerolized by the method of Mitchell *et al.* (1977). Following deglycerolization, spermatozoa were resuspended in Lake's deglycerolizing fluid (Lake, 1968) to produce approximately $1-3 \times 10^9$ cells/ml of semen. Sperm motility was scored immediately after thawing of frozen semen according to the method of Wheeler and Andrews (1943).

Ninety-six White Leghorn hens were divided into six groups and inseminated with semen of each experimental group. All hens were inseminated intravaginally by depositing semen into the posterior site of the vagina. Eggs were saved for 7 days, starting 2 days after insemination, incubated, and candled at 5 days of incubation. Percent fertilizity up to day 7 after insemination was measured. Percent data were transformed to arcsin and all data were subjected to statistical analysis using analysis of variance followed by the *t*-test for comparing difference between the groups (Steel and Torrie, 1980).

Results and Discussion

As shown in Table 1, fertility measured in this study was slightly lower than that previously reported (Mitchell *et al.*, 1977; Lake and Stewart, 1978; Sexton *et al.*, 1978). Lower fertility obtained here may be due partly to the amount of glycerol that remained after processing or the depth of insemination site. However, significant differences were observed between the male lines and freezing velocities (Table 2). The highest fertility was obtained when semen of the selected male was frozen at 1°C/min to –70°C using Cryocool-100 apparatus, the result being statistically significant ($P < 0.05$). No significant difference in fertility was found between Cryocool-100 and Macdonald Apparatus when semen from males in the high group was cooled at a rate of 1°C/min. When semen from the selected males was frozen at 6°C/min with Macdonald Apparatus, fertility was significantly ($P < 0.05$) poor as compared with that of obtained using Cryocool-100 at 1°C/min.

Semen from the control males showed lower fertility in any experimental condition (Table 2). Semen cooled at 6°C/min with Macdonald Apparatus, fertility was significantly ($P < 0.05$) between two male lines. Scott *et al.* (1978) reported a significant difference in fertility duration between the selected and control males when semen was frozen at 6°C/min to –196°C. Such a difference in fertility rate with the same method as described by Mitchell *et al.* (1977) is due probably to the difference of the remaining amount of glycerol after processing and/or

Table 2. Viability and fertilizing capacity of spermatozoa from males selected for high fertility with frozen-thawed sperm and randomly selected males

Group	Ne. of hens used	Sperm motility score	No. of sperm inseminated ($\times 10^6$ cells)	Egg production (%)	Fertility (%)
1	21	$4.01 \pm 0.31^{ab*}$	100	69.69 ± 11.95	$3.69 \pm 4.90^{b*}$
2	23	4.28 ± 0.32^a	100	71.21 ± 12.25	17.70 ± 10.28^a
3	11	3.75 ± 0.33^{bc}	100	67.51 ± 14.74	1.47 ± 1.98^b
4	12	4.25 ± 0.31^a	100	67.74 ± 16.10	6.75 ± 6.92^{ab}
5	12	3.43 ± 0.35^c	100	69.70 ± 11.60	1.68 ± 2.22^b
6	12	3.63 ± 0.31^{bc}	100	71.80 ± 12.30	2.46 ± 2.38^b

* Mean $\pm$ standard deviation. Means with different superscripts are significantly different ($P < 0.05$).

the depth of insemination site. With poultry semen, best results can be achieved when chicken semen is cooled through the transition phase (-5 to -15°C) at a slow cooling rate (1 to $8^\circ\text{C}/\text{min}$) to some temperature below -15°C before storage at -196°C (Sexton, 1979). On the other hand, thawing temperature also affects post-thaw motility and fertilizing capacity of frozen poultry semen (Sexton, 1979; Watanabe *et al.*, 1968).

Recently, data have been shown with bull semen to indicate that factors such as freeze rate, thaw rate, and level of cryoprotectant interact, and an optimal combination of these variables is needed for satisfactory results (Robbins *et al.*, 1972). However, rate of freezing can exert marked effects on sperm survival (Pickett *et al.*, 1976), and slower cooling has also been associated with greater post-thaw motility for spermatozoa frozen either in ampules or in straws (Mcfee and Swanson, 1960; Martin, 1965; Ennen *et al.*, 1976). These valuable parameters, which have been established in bull semen, remain to be solved for freezing fowl semen.

Sperm activity after freeze-thaw process was inclined to be higher when semen was cooled at $1^\circ\text{C}/\text{min}$ using both equipments though the results were not significant. These results postulate the slower cooling rate ($1^\circ\text{C}/\text{min}$) for better viability of freeze-thaw spermatozoa. In the present study, better fertility was directly proportional to higher sperm motility ($r=0.755$).

The results of the present experiment show that the male can be selected for higher freezability of sperm and that freezing velocity at

$1^\circ\text{C}/\text{min}$ may be successful for better viability and fertilizing capacity of frozen-thawed fowl spermatozoa.

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鶏精液の凍結速度が精子の受精能力に及ぼす影響

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精子の耐凍性を指標に選抜した雄鶏および通常の雄鶏から採取した精液を, -70°Cまで1°C/分ならびに6°C/分の冷却速度で凍結した場合の精子の受精能力を比較検討した。選抜した雄鶏の精液を1°C/分の速度で-70°Cまで凍結した場合に, 最も高い受精率が得られた。一

方, 6°C/分の速度で冷却した場合には, 二つの雄鶏精液間に受精率の差は認められなかった。

これらの結果から, 精子の耐凍性を指標にして雄鶏を選抜することができること, また鶏精液の凍結の際は, 冷却速度は1°C/分が適正であることが推定された。