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Studies on Viability of Frozen Sperm and Pregnancy Rates after AI with Frozen-Thawed Canine Semen

B. K. Lee, Y. H. Chung and S. K. Kim[1†]

Dept. of Companion Animal & Animal Resource Science, Joong-Bu University

개 동결 정액의 생존성과 AI 후 임신율에 관한 연구

이봉구 · 정영호 · 김상근[1†]
중부대학교 애완동물자원학과

SUMMARY

본 연구는 개 채취 정액의 동결후의 생존성과 신선 및 동결 정자의 capacitation, acrosome reaction과 생존성을 조사하고, 아울러 신선 및 동결 정액을 자연 발정 또는 발정 유기 암캐에게 인공수정 후 임신율을 조사하였다.

개 채취 정액의 동결 융해 후의 생존성은 64.5±2.30%로서 신선 정액의 생존성에 비해 유의하게 낮게 나타났다($p<0.05$). 신선 및 동결 정액의 capacitation, acrosome reaction 및 생존성은 각각 52.5± 4.5%, 9.5±0.6%, 68.8±4.5% 및 16.2±3.2%, 3.2±0.5 %, 24.5±2.5%로 나타났다. 신선 및 동결 정액을 자연 발정 또는 발정을 유기한 암캐에 인공수정했을 때 임신율은 각각 50.0% 및 33.3%로서 동결 정액을 이용했을 때 임신율이 신선 정액에 비해 낮은 임신율을 나타냈다.

(Key words : canine oocytes, IVM/IVF, stages of reproductive cycle)

INTRODUCTION

In male dogs the number of sperm in ejaculate significantly decreased and female dogs have shown disturbances of reproductive cycle and reproductive diseases (Gunzel, 1986; Kim, 2001).

It was reported that the pregnancy rate was not decreased when artificial inseminated with fresh semen of the 2nd fraction of canine semen, but that the viability was decreased when the sperm was preserved *in vitro* (Maule, 1960; Arthur, 1975). Seager and Fletcher (1973) reported that when fertilized with semen preserved for 1~4 days, the pregnancy rate was 53%. Province *et al.* (1984), Davis *et al.* (1963) and Foote (1964) reported that the viability rate was maintained at 50% when the canine semen was cryopreserved with 20% egg yolk extender for 2~4 days or 4~8 days. Harrop (1962) reported that the survival rate of post-thaw sperm was 40~50%. Seager *et al.* (1975) reported the first success in pregnancy and parturition of canine by AI with frozen semen. Dog epididymal sperm collected after euthanasia has been frozen and successfully used after storage for 3.5 months, although the sperm

[1] 충남대학교 수의과대학 (College of Veterinary Medicine, Chungnam National University)
[†] Correspondence : E-mail : kskkim@cnu.ac.kr

recovery was poor, the study involved only one animal and the apparent fertility was low (Marks *et al.,* 1994). Harrop (1962) reported a 45~50% survival rate after thawing, which showed the possibility of long term freezing of canine semen. Seager and Fletcher (1973) reported pregnancy rate of 53% when inseminated with semen preserved for 1~4 days. Seager *et al.* (1975) reported the first success in pregnancy and parturition by using canine frozen-thawed semen.

The studies was carried out to investigate the viability of fresh and frozen sperm and the percentages of capacitated sperm and acrosome-reacted sperm and the pregnancy rates after artificial insemination with frozen-thawed semen.

MATERIALS AND METHODS

1. Collection and Freezing of Semen

Twenty male dogs and 60 female dogs were used in this study and they internal parasites and vaccinated prior to use. Semen was collected by hand massage method. The highest concentration of semen was used.

A 1.0 ml volume of saline was added to sperm suspension and then a 4.0 mL extender buffer modified with cryoprotectants was mixed with the sperm-saline suspension. The ejaculated semen were diluted with physiological saline and tris-buffer (1:3), centrifuged for 6 min with 700 g and the supernatant was removed. Semen was supplemented with 1.5 ml extender-1 at room temperature and the diluted solution was cooled for 45 min at 4℃. Following cooling for 25 min, 1.5 ml extender-2 and extender-2' were supplemented at 4℃ and then mixed. Freezing was carried out by the method of Kim (2001).

2. Viability of Frozen Sperm

Straw semen cryopreserved for 1 month was placed for 3 min at room temperature and then thawed in a water bath at 37℃. In order to remove cryoprotectants, frozen semen was shaken upside down and tris-buffer were removed and dissolved. The dissolved semen samples were moved to a slide glass and observed by microscope and the viability, survival rate, and morphological test was examined by using sperm analyzer imaging system (SAIS Si-100).

3. AI and Pregnancy Diagnosis

Non-estrus dogs were treated with 20 mg of $PGF_2\alpha$ and then with 2,000 IU of PMSG and 2,000 IU of HCG. Estrus, induced dogs and the dogs with natural estrous symptoms were inseminated artificially. Frozen straw semen (0.5 ml) was kept at room temperature for 30 sec thawed in water-bath at 37℃. Artificial insemination was carried out with transcervical catheter and thawed semen was deposited non-surgically into the uterine body or into a uterine horn at 1~4 days before and after ovulation.

After artificial insemination, the pregnant dogs did not show and had expanded abdomen symptom. At day 20 to 30 after AI. pregnancy was tested by ultra-sonography.

4. Statistical Analysis

For comparison of means, Duncan's multiple range test was performed using SAS package of General Linears Model (GLM) procedures (SAS Institute, 1996).

RESULTS AND DISCUSSION

1. Viability of Frozen Semen

The canine semen was diluted with tris-buffer solution and centrifuged to remove seminal plasma was suspended with extender-1, extender-2 solutions and cooled by vitrification.

Table 1 presents the viability of fresh and frozen semen thawed after freezing. The viability rates

of frozen semen by freezing were 64.5±2.30%. These rates were lower than that of the control group (84.2±2.55%). The above results of frozen-thawed semen were higher than that of Takeishi *et al.* (1975), who reported that the survival rate of whole semen after cryopreservation was 40~65%.

2. Sperm Category before and after Freezing

The acrosome status these capacitated, acrosome-reated and live sperm was is shown in Table 2.

The percentages of capacitated sperm and acrosome-reacted sperm was 52.5±4.5%, 9.5±0.6% fresh semen and 16.2±3.2%, 3.2±0.5% in frozen semen and live sperm of fresh and frozen semen were 68.8±4.5%, 24.5±2.5%, respectively. Province *et al.* (1984), Davis *et al.* (1963) and Foote (1964) who re-ported survival rate of 50% when the semen was cryopreserved with 20% egg yolk extender for 2 to 8 days. Harrop (1962) also reported the survival rate of 45~50% in freezing-thawed canine semen. The sperm capacitation rate *in vitro* in dog have been investigated by several researchers (Hewitt and England, 1997; Hewitt and England, 1998; Mahi and Yanagamachi, 1978: Nickson *et al.*, 1993; Shimazu *et al.*, 1992; Yamada *et al.*, 1993).

Table 1. Viability of frozen-thawed semen following slow and rapid freezing

Treatment	Motility of semen	
	Live (%)	Dead (%)
Fresh semen[a]	84.2±2.55	15.8±2.25
Frozen semen[b]	64.5±2.30	35.5±3.10

* [a,b] : Values with different letters within same columns differ significantly ($p<0.05$).

** Mean±S.D.

Table 2. Rate of capacitation and acrosome reaction prior to culture for fresh and frozen-thawed ejaculated sperm

Parameter	Fresh semen	Frozen semen
Capacitated sperm[a]	52.5±4.5	16.2±3.2
Acrosome-reacted sperm[b]	9.5±0.6	3.2±0.5
Live sperm[a]	68.8±4.5	24.5±2.5

* [a,b] : Values with different letters within same columns differ significantly ($p<0.05$).

** Mean±S.D.

3. Pregnancy Rate after AI

Table 3 presents the pregnancy rates after with fresh and frozen semen. The pregnancy rate of fresh semen and frozen semen were 65.0% and 40.0%. This results was significantly lower than that of fresh semen (65.0%).

The pregnancy rate was lower with result (81%) reported by Tsutsui and Shimizu (1973) in while AI was performed with fresh semen in the period of 54~108 hrs before and after ovulation. Generally, the pregnancy rate increased when artificial insemination was executed 1~2 days at the 1st fraction and 3~4 days at the 2nd fraction after estrus. There was no respective comparison here (Maule, 1960; Arthur, 1975). This result was similar a or a little lower than that of Seager and Fletcher (1973), who reported pregnancy rate of 53% when inseminated with semen preserved for 1~4 days.

Table 3. Pregnancy rates of frozen-thawed ejaculation and epididymal semen

Treatment	No. of dogs (%)*		
	Examined	AI	Pregnant
Fresh semen	20	20	13/20 (65.0)[a]
Frozen semen	30	25	10/25 (40.0)[a]

* [a,b] : Values with different letters within same columns differ significantly ($p<0.05$).

** Mean±S.D.

** Within 20~30 days after AI. Pregnancy was determined by ultrasound test.

CONCLUSION

The studies were carried out to investigate the viability of fresh and frozen sperm and the percentages of capacitated sperm and acrosome-reacted sperm and the pregnancy rates after artificial insemination with frozen-thawed semen.

1. The viability rates of frozen semen by freezing were 64.5±2.30%. These rates were lower than that of the control group (84.2± 2.55%).
2. The percentages of capacitated sperm and acrosome-reacted sperm was 52.5±4.5%, 9.5± 0.6% fresh semen and 16.2±3.2%, 3.2±0.5% in frozen semen and live sperm of fresh and frozen semen were 68.8±4.5%, 24.5±2.5%, respectively.
3. The pregnancy rate of fresh semen and frozen semen were 65.0% and 40.0%. This results was significantly lower than that of fresh semen (65.0%).

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