# *TRIALS TO IMPROVE THE VIABILITY OF RABBIT SEMEN AT VARIABLE CONDITIONS OF PRESERVATION USING DIFFERENT DILUENTS*

Rowida M.R., Osman,R.H. and
Mona H.Shaker.
Department of artificial insemination and embryo transfer
Animal Reproduction Research Institute, Giza Egypt.

## ABSTRACT

Ten sexually mature rabbit bucks of (NZW) were used in this study. The present study aimed to investigate the effect of some diluents and differents regimes of dilution and preservation on the viability of rabbit semen .

Semen was collected by an artificial vagina and evaluated for volume (ml), individual motility (%); concentration of spermatozoa (ml × $10^6$); percentage of alive spermatozoa and total abnormalities by conventional technique. Pooled semen sample was divided into three parts, the first part was extended in Tris – egg yolk (pH 6.9), the second part was extended in Tris-egg yolk (pH 7.5) and the third part was extended in sodium chloride (pH 7.0). Each part again was subdivided into three portions; the first portion was incubated at 37˚C. for 3 hr.; The second portion was kept at 5 $^{o}$C. for 48 hr. and the third portion was frozen in French straws (0.25 ml.) and stored in liquid nitrogen ( -196˚C).

Our results revealed that the percentage of alive spermatozoa displayed a significant increase ($P<0.05$) at 37 $^{o}$C in sodium chloride extender than the other extenders, while there were no significant differences in individual sperm motility in the three extenders at 37 $^{o}$C. .On the other hand the individual sperm motility signed a significant increase ($p<0.05$) in Tris-egg yolk extender with pH 6.9 than the other extenders at 5˚C.
The post thawing motility was significantly higher ($P<0.05$) in Tris-egg yolk extended semen with pH 6.9 than the other extended semen. The extended semen with sodium chloride reported a highly significant percentage in acrosomal defects than the other extended semen with different pH.

# INTRODUCTION

Artificial insemination is a biotechnological tool used for genetic improvement. It is used in all animal species with many purpose including production, planning and control breeding which is associated to maximize enterprise profitability. Rabbits are not the exception where the artificial insemination is applied with the same objectives. A limitation factor for rabbit artificial insemination spread in commercial level is related to semen preservation. Fresh diluted semen has been used but its quality can be maintained only for a short period of time. Alvarino et.al. (1996) proved that semen storage for short time less than 48 hours at temperature between 5˚C-25˚C was commonly adopted in practice.

Hafez (1980) proved that the pH of rabbit semen extender must be alkaline which is comparable to the pH (6.74-7.59 ) of rabbit semen (Amin et.al.,1983; El Sherbiny 1987).Rowida et.al (2004) used Tris extender with two different pH (6.9 and 7.5) and proved that Tris at pH 7.5 gave the best result in fresh and chilled diluted semen than pH 6.9. Belloti (1986) reported that Tris- based extender had been successfully used for preservation of rabbit semen. Sodium chloride 0.9 % at pH 7.0 used successfully in A.I with fresh diluted semen (Rowida et.al. 2005 and Abdel Ghaffar 1992).

Artificial insemination is still practiced in a very limited scale in Egyptian rabbitry. One of the main constrain meet with the preservation of buck semen is the disability of this semen to be stored for long period of time without losses of its fertilizing capacity (Zidan et.al, 2002).

Frozen rabbit semen didn't show good results in intensive breeding therefore, the present study was done to investigate the effect of diluents (extenders) with variable pH under different temperatures and freezing processes to evaluate sperm motility, abnormalities, percentages of livability and acrosomal defects.

## MATERIALS AND METHODS

### Animals

Ten sexually mature (New Zealand White) rabbit bucks were used. Bucks were housed in individual cages under a constant photoperiod of 16 hr. light phase. Temperature and humidity of the building were recorded. Bucks were fed on a commercial diet and water with nipple drinkers was ad-libitum.

### Semen collection and evaluation

Semen was collected twice weekly for Six weeks using an artificial vagina according to the method described by Evans and Maxwell, (1987).Semen ejaculates were individually evaluated microscopically and only ejaculates exhibiting active sperm motility (over 70%) were pooled and extended with different extenders having different pH (Tris-egg yolk pH 7.5, Tris-egg yolk pH 6.9 and Sodium chloride pH 7). The final extension rate was 1 semen: 4 extender.

Table (1) Showed the Composition of extenders /100ml distilled water

| Composition | Tris-egg yolk (pH 7.5) | Tris-egg yolk (pH 6.9) | Sodium Chloride (pH 7) |
|---|---|---|---|
| • Tris amino methan/gm | 3.028 | 3.028 | - |
| • Lactose/gm | 1.250 | - | - |
| • Glucose/gm | - | 1.25 | - |
| • Citric acid/gm | 1.520 | 1.53 | 0.09 |
| • Sod. Chloride/gm | - | - | - |
| • Egg yolk (ml) | 5.0 | 5.0 | 5.0 |
| • Penicillin – G – Sodium (IU) | 5000 | 5000 | 5000 |
| • Streptomycin sulphate (µgm) | 5000 | 5000 | 5000 |
| * Glycerol | 2% | 2% | 2% |

Each diluent was subdivided into three portions:-
The first portion was incubated at 37 $^{o}$C.for 3 hr and the second portion was preserved at 5 $^{o}$C. for 48hr, the percentages of sperm motility, dead and abnormal spermatozoa were estimated according to Salisbury et.al.; (1978). The third portion was processed for freezing. Extended semen was cooled gradually to 5 $^{o}$C. within 45min. and then equilibrated at 5$^{o}$C. for 2hr.The semen was then loaded in plastic French straws (0.25). The straws were exposed to liquid nitrogen vapor inside a foam box container ( 45×35 × 18 cm.) containing 10 liters of liquid nitrogen, above the liquid nitrogen surface 4cm. for 15 min. then plunged and stored in liquid nitrogen container (Hassan 1988).

The frozen straws were stored for at least 28 hr. before thawing and evaluation. They were thawed in a water bath at 37 $^{o}$C .for 30 sec.to examine post thawing motility . Semen smears stained by fast green stain FCF according to Wells and Awa ( 1970 ) were used to examin acrosomal defects.
These trials were replicated 4 times for evaluation and statistical analysis.

**Statistical analysis:-**

The data were recorded and analyzed statistically by using analysis of variance according to Snedecor and Cochran, (1982) using the general model program of SAS (1990).

## RESULTS

The obtained results are presented in table 2,3 and 4.
Table 2 showed that a high percentages of sperm motility and alive spermatozoa in different diluents with variable pH but there was significant differences between them ($p<0.05$) in which the sodium chloride revealed a high percentages of alive spermatozoa ( 88.0 ±1.7, 87.7 ±1.9 and 86.7 ±1.7 respectively).

The sperm abnormalities showed a high significant differences ( $p<0.05$) in Tris-egg yolk at pH 7.5 more than other extenders ( 5.3 ± 0.09 , 4.3 ± 0.5 and 3.8 ± 09 respectively ).

Table 3 revealed that the sperm motility in Tris-egg yolk at pH 7.5 was increased significantly ($p<0.05$) than other diluents ( 40.0 ± 4.6 , 26.3 ± 2.4 and 23.8 ± 3.8 respectively) during preservation at $5^0$c. after 48 hr.

Table 4 showed that acrosomal damage in Tris-egg yolk at pH 6.9 decreased significantly (p0.05) than the other extenders ( 23.3 ± 3.28 , 24.7 ± .88 and 29.7 ± 2.6 respectively ) . On the other hand the post thawing motility at zero hr increased significantly ($p<0.05$) with Tris –egg yolk at pH 6.9 than Tris at pH 7.5 and sodium chloride at ph 7.0 ( 38.5 ± 1.3 ,36.7 ± 1.5 and 35.0 ± 4.7 respectively ).

Table (2) Effect of different extenders with variable pH on the percentages of alive spermatozoa, Sperm motility and sperm abnormalities during incubation at $37^0$C. for 3 hr.

| Parameter\Extender | Tris egg-yolk (pH 7.5) | Tris egg-yolk (pH 6.9) | Sodium Chloride (pH 7) |
|---|---|---|---|
| Alive sperm | 87.7 ± 1.9 ab | 86.7 ± 1.7 bc | 88.0 ± 1.7 a |
| Sperm motility | 82.5 ± 1.7 a | 83.3 ± 1.7 a | 83.3 ± 1.1 a |
| Sperm abnormality | 5.3 ± 0.09 a | 4.3 ± 0.5 a | 3.8 ± 0.9 b |

- Means with different subscripts a,b,….within raws are significantly different at least $p<0.05$

Table (3) Effect of different extenders with variable pH on the percentages of sperm motility during preservation at $5^0$C. for 48 hr.

| Parameter\ Extender | Tris egg-yolk (pH 7.5) | Tris egg-yolk (pH 6.9) | Sodium Chloride (pH 7) |
|---|---|---|---|
| 0 hr. | 57.5 ± 3.2 a | 45.0 ± 2.9 c | 47.5 ± 2.9 b |
| 24 hr. | 51.3 ± 3.2 a | 38.8 ± 1.3 b | 37.5 ±1.4 c |
| 48 hr. | 40.0 ± 4.6 a | 26.3 ± 2.4 b | 23.8 ± 3.8 c |

- Means with different subscripts a,b,….within raws are significantly different at least $p<0.05$

Table (4) Effect of different extenders with variable pH on the percentages of acrosome defects and post thawing motility.

| Parameter\ Extender | Tris egg-yolk (pH 7.5) | Tris egg-yolk (pH 6.9) | Sodium Chloride (pH 7.0) |
|---|---|---|---|
| Acrosome defects | 24.7 ± 0.88 b | 23.3 ± 3.28 c | 29.7 ± 2.6 a |
| Post thawing motility at 0hr | 36.5 ± 1.5 b | 38.5 ± 1.3 a | 35.0 ± 4.7 c |
| Post thawing motility after1 hr | 19.16 ± 1.53 a | 16.66 ± 2.11 c | 18.57 ± 0.92 b |
| Post thawing motility after 2 hr | 11.66 ± 2.11 a | 7.5 ± 1.12 c | 8.57 ± 0.92 b |

- Means with different subscripts a,b,….within raws are significantly different at least $p<0.05$

## DISCUSSION

The sperm motility and alive spermatozoa showed a high percentages in different diluents with variable pH (Table 2) but there was a significant difference between them in which the sodium chloride revealed a high percentages of alive sperm and sperm motility (88.0 ± 1.7 &83.3 ± 1.1) respectively.

So high motility and alive spermatozoa percentages while decrease in the percentage of sperm abnormalities means that the three extenders ( Tris pH 6.9, Tris pH 7.5 and Sodium chloride pH 7) was provided a suitable environment for spermatozoa at 37 $^0$C.

In Table 3 : The effective use of chilled semen for A.I. depends on the ability of extender to protect the spermatozoa during storage . One basic component of semen is biologically buffered to minimize pH substance due to metabolic by-products of sperm (Watson, 1990). Castellini,et.al.,( 1992) proved that inorganic buffer have a limited buffering capacity, whereas Tris extender are more appropriate for the storage of rabbit semen at low temperature.

Bautuchai and Tanpipat (1989), Zeidan et.al. (2002) and Rowida et.al. (2004) showed that high percentage of sperm motility, alive spermatozoa and low percentage of acrosome abnormality during preservation at 5$^0$c.Also sperm motility was decreased in the progression of incubation time up to 2 days. This phenomenon may be due to increase lactic acid accumulation as a

result of sperm anaerobic metabolism in both the osmotic pressure and pH of the media which exerts a toxic effect on sperm cell ( Abdel- Salam,2002 and Rowida,2003).

Table 4 showed that acrosomal damage in Tris buffer extender with pH 6.9 decreased significantly ($P<0.05$) than Tris buffer with pH 7.5 and sodium Chloride with pH 7. This agrees with those of Kestin and Tekin (1994) and Zeidan et.al. (2002) .While post thawing motility increased significantly ($P<0.05$) with Tris buffer pH6.9 than Tris buffer pH 7.5 and sodium chloride pH 7. In addition, Post thawing motility after 2 hrs (Table 4) decreased significantly ($P<0.005$) with the advancement of incubation at $37^0$c up to 2 hr.. This in agreement with El-Gaafary et.al. (1993) , Chen et,al. (1989) and Hassan (1988). The effect of hydrogen ion concentration on freezability of rabbit semen extended in Tris glucose buffer at pH 6.9 was higher than at pH 7.5 and sodium chloride at pH 7.The present data indicates that pH higher than 6.9 was more harmful to rabbit spermatozoa. The changes in pH harm the spermatozoa would be produced by the drop on temperature (Salisbury et.al.,1978).

**Conclusion**:
The present results indicated that sodium chloride is effective as a diluent to obtain good results at 37 $^0$C. The Tris extender pH 7.5 is of great value for preservation of rabbit semen at $5^0$C. The Tris extender at pH 6.9 is more efficient for maintaining a good quality frozen thawed rabbit semen.

## REFERENCES

**Abdel Ghaffar, A. El-sayed (1992):** Some studies on the artificial insemination rabbits.PHD.thesis, Zagazig Uneversty.

**Abdel-Salam, A.M. (2002):** Studied on freezing of rabbit semen. MSc. Thesis, Faculty of Agriculture, zagazig UN, zagazig, Egypt.

**Alvarino,J.M.R.,Lopez,F.J.; Delarco,J.A. and Delgado,F. ( 1996 ):** Artificial insemination of rabbits with diluted semen stored of 24 hr.In; World Rabbit Cong., 6,1996. Tolouse,Anais.Tolouse ,2: 37 – 40.

**Amin,S.O., Anwar,A. and El-Zeiny,M. ( 1983 ):** Semen characteristics of three breeds of rabbits and the use of two types of diluents. Egypt Poltry Sci., 3: 27 – 34.

**Bantuchai,S. and Tanpipat,S. ( 1989) :** Motility and viability of rabbit spermatozoa diluted in Tris-egg yolk and egg yolk citrate. Kaen Agricutural Journal, 18, 97 – 106.

**Bellotti,G.F.(1986):**Artificial insemination in rabbit .Praxis Veterinarian ,Italy,7:15-16.

**Castellini,C.; Battaglini,M. and Lattaioli,P. (1992 ):** effects of cryoprotectants and freezing on rabbit semen quality. J. Appl. Rabbit Res., 15: 431 – 438.

**Chen, y, yang, x.and Foot,R.H.(1989):**Timed breeding of rabbits with fresh and frozen thawed semen and evidence of acrosome alteration following freezing and thawing.Anin.Reprod.sci.,18:35-41.

**El Gaafary, M.N., Rashwan,A.A. and Zenat,A.Ibrahim (1993) :** Investigation on the deep freezing of rabbit semen in straws. Egyptian Ameican Conference on Physiology of animal production El-fayoum, Egypt, PP: 17 – 25.

**El-Sherbiny,A.M. ( 1987 ):** Seasonal variations in seminal characteristics of rabbits. M.Sci., Thesis, Fac.Agr.,Ain Shams Univ.,Egypt.

**Evans,G. and Maxwell,W.M.C. (1987):** Salmon's artificial insemination of sheep and goats. Butterwarths pty limited, Australia.

**Hafez,E.S.E. ( 1980 ):** Reproduction of farm animals. 4th Edition, L A A +Febiger,Pheladlephia, U.S.A.

**Hassan, A. M. A. (1988)**: Studies on freezing of Rabbit semen. B. Sc. Agric. (Animal Production), Al-Azhar University.

**Kestin,O. and Tekin,N. ( 1994 ) :** The effect of acrosomal defects on fertility of frozen rabbit semen.Turk Veterinerlikve Hayvancilik Dergis, 18, 299 – 305.

**Rowida,M.R. (2003):** A comparative study of different addetives of extended rabbit semen .J.Egypt.Vet.Med.Assoc.,63 (4);351-361.

**Rowida.M.R.,El-Serief,R.H. and Seleem.T.S.T.(2005)**: Effect of incubation time of diluted rabbit semen and time of Gn-RH treatment of does on fertility trait. The 4th inter. Con. on Rabbit prods. In Hot clin, sharm El-Sheikh, Egypt, 191-196.

**Rowida,M.R. , Tayseer, I.M. and El-Sheltawi,M.A.F. ( 2004 ):** Rabbit semen quality and fertilizing ability as affected by the pH of extender. J.Egypt.Vet.med.Assoc. 64 No.4: 137 – 147.

**Salisbury,G.W.;Van Demark,N.L.and Lodge, J.R. (1978 ):**Physiology of reproduction and artificial insemination of cattle. 2nd ed.W.H. Freeman and Company San Francisco.

**SAS (1990):** SAS/STAT User's Guide (Version 6, 4th ed.) SAS Inst.Inc.,Cary,NC.

**Snedecor,G.W. and W.G.Cochran (1982):** Statistical Methods. 2nd Ed. Iowa Univ.Press.Ames,Iowa.

**Watson, P.F. (1990):** Artificial insemination and preservation of semen. In: Lamming G.E. 9 Ed.) Marchall's Physiology of reproduction, Vol 2. Reproduction in Male.Churchill Livingstone editional, Edinburgh.

**Wells,M.E. and Awa,O.A. (1970):** New technique for assessing acrosomal characteristics of spermatozoa.J.Dairy Sci.,53 : 227.

**Zeidan,A.E.B.,Abdel-Kariem,M.A.,Mohamed,M.M. and Bahgat, L.B. ( 2002):** 3rd Sci.Con. on rabbit production in Hot climates, 8 -11 Oct. : 317 – 328.

## محاولات لتحسين حيوية حيامن الأرانب تحت ظروف حفظ مختلفة

أجريت هذه الدراسة علي عدد ١٠ ذكور أرانب نيوزيلندي بالغ لمعرفة تأثير بعض المخففات المختلفة ذات قيم PH مختلفة ونظم التخفيف والحفظ علي حيوية السائل المنوي للأرانب.

تم جمع السائل المنوي وتقسيمه إلي ثلاثة أجزاء كل جزء تم تخفيفه بالمخففات الثلاثة التالية :

ترس PH 9.6 ، ترس PH 7.5 و كلوريد الصوديوم PH 7 ، وكل سائل منوي تم تخفيفه قسم إلي ثلاثة أجزاء. الجزء الأول تم تحضينه عند درجة حرارة 37 درجة مئوية لمدة ثلاث ساعات . الجزء الثاني تم حفظه عند درجة حرارة 5 درجة مئوية لمدة 48 ساعة .أما الجزء الثالث تم تجميده في قصيبات و حفظه في النيتروجين السائل عند 196- درجة مئوية .

و أوضحت النتائج أن مخفف كلوريد الصوديوم أفضل معنويا (علي مستوي 5% ) علي نسبة الحركة الأمامية عند درجة حرارة التحضين 37 درجة مئوية عن باقي المخففات . بينما لا يوجد اختلاف معنوي بين المخففات الثلاثة علي الحركة الأمامية عند درجة حرارة التحضين 37 درجة مئوية . وكذلك كانت الحركة الأمامية أفضل معنويا ( علي مستوي 5%) مع المخفف ترسPH 7.5 عند درجة حرارة 5 درجة مئوية .

أما المخفف ترس PH 6.9 أفضل معنويا عند درجة حرارة 5 درجة مئوية للحركة الأمامية بعد الإسالة . كما لوحظ أن نسبة التشوه الأكروسومى أعطي أعلي نسبة معنوية (علي مستوي 5%) في مخفف كلوريد الصوديوم.