22 –Improving sperm characteristics during in vitro storage of roosters semen by supplementing semen diluent with tomato juice

IMPROVING SPERM CHARACTERISTICS DURING *IN VITRO* STORAGE OF ROOSTER'S SEMEN BY SUPPLEMENTING SEMEN DILUENT WITH TOMATO JUICE

H. J. Al-Daraji I. A. Abdul-Hassan A. S. Ahmed R. H. Razuki

Depart. of Animal Resources, College of Agric., University of Baghdad. Ministry of Science and Technology

ABSTRACT

The goal of this experiment was to determine the effects of tomato juice inclusion in semen diluent on sperm characteristics during *in vitro* storage of roosters'semen. Sixty white layer roosters were used in this study. They were divided into six treatments; undiluted control, fresh semen (C1); diluted control (C2); and treatments T1, T2, T3 and T4 using diluted semen with inclusion of 1, 3, 5 and 7 ml of tomato juice per 100 ml of diluent, respectively. Mass activity, individual motility, dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities percentages were determined twice a week for 8 wks period after *in vitro* storage time of 0, 24, 48 and 72 hr of roosters'semen in refrigerator. Results showed that adding tomato juice up to 7 ml per 100 ml of semen diluent significantly ($P<0.05$) increased mass activity and individual motility percentages and decreased dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities percentages at all storage times when compared with control. No significant changes were observed in all parameters and storage times when tomato juice was added to the diluent up to 5 ml per 100 ml of diluent (T1, T2 and T3). Increasing the addition level of tomato juice from 5 to 7 ml per 100 ml to the diluent (T4) significantly led to additional improvement in all parameters studied *in vitro* storage times. Improvement sperm characteristics during storage of roosters'semen due to tomato juice addition to the diluent support published concepts about the positive effects of tomato juice as an antioxidant-rich product.

تحسين صفات النطف أثناء خزن السائل المنوي للديكة من خلال تجهيز المخفف بعصير الطماطة

حازم جبار الدراجي اسماعيل عبدالرضا عبدالحسن اياد شهاب احمد رعد حاتم رزوقي

قسم الثروة الحيوانية ، كلية الزراعة ، جامعة بغداد وزارة العلوم والتكنولوجيا

المستخلص

هدفت هذه التجربة تحديد تأثيرات إدخال عصير الطماطة في مخفف السائل المنوي في صفات النطف خلال خزن السائل المنوي للديكة في المختبر . استخدم في هذه الدراسة 60 من ديكة الدجاج البياض الأبيض وكانت هناك 6 معاملات هي : سيطرة بدون تخفيف ، سائل منوي طازج (C1) ؛ سيطرة مع التخفيف ، بدون إضافة عصير الطماطة (C2) والمعاملات T1 ، T2 ، T3 و T4 باستعمال سائل منوي مخفف مع إدخال 1 ، 3 ، 5 و 7 مل من عصير الطماطة لكل 100 مل من المخفف ، بالتتابع . تم تحديد نسب الحركة الجماعية ، الحركة الفردية ، النطف الميتة ، النطف المشوهة والتشوهات الاكروسومية مرتين بالأسبوع ولمدة 8 أسابيع وذلك بعد خزن السائل المنوي في المختبر لمدة صفر ، 24 ، 48 و72 ساعة . أظهرت النتائج بان إضافة عصير الطماطة إلى حد 7 مل لكل 100 مل من مخفف السائل المنوي أدت إلى زيادة معنوية في نسب الحركة الجماعية والحركة الفردية للنطف وانخفاض معنوي في نسب النطف الميتة والمشوهة والتشوهات الاكروسومية عند جميع فترات الخزن في المختبر . لم تلاحظ تغيرات معنوية في جميع الصفات المدروسة وعند جميع فترات الخزن عند إضافة عصير الطماطة إلى المخفف إلى حد 5 مل لكل 100 مل من المخفف (المعاملات T1 ، T2 و T3) . زيادة مستوى إضافة عصير الطماطة من 5 إلى 7 مل لكل 100 مل من المخفف(المعاملة T4) أدت إلى تحسن معنوي إضافي في جميع الصفات المدروسة وعند جميع فترات الخزن . تحسن صفات النطفة خلال الخزن المختبري للسائل المنوي للديكة نتيجة إضافة عصير الطماطة للمخفف يدعم المفاهيم المثبتة حول التأثيرات الإيجابية لعصير الطماطة كونه مادة غنية بمضادات الأكسدة .

(*)Received on 30/4/2005 – Accepted on 12/9/2006

22 –Improving sperm characteristics during in vitro storage of roosters semen by supplementing semen diluent with tomato juice

INTRODUCTION

Optimization of the management of roosters includes the need for efficient methods of semen storage. However, the current methods of semen storage are only effective for short periods of time (up to 12 hr) and need to be improved (32). Improvements in the methods of liquid storage of spermatozoa are limited by the lack of basic knowledge of the biochemical mechanisms regulating spermatozoa functions *in vivo* and *in vitro*. Lipids are known to have a major impact on the structure and function of spermatozoa both *in vivo* and *in vitro* (13, 22, 24, 28). In birds, lipids are believed to have a significant role in *in vitro* storage in the female uterovaginal glands(6,7). A decrease in the lipid content of chicken spermatozoa has been shown to occur after 48 hr of *in vitro* storage (8). It has been reported that a high consumption of tomato lowers plasma lipid peroxidation (27), and improves the antioxidant defense of low density lipoprotein (LDL) against attack by singlet oxygen (26). Also, epidemiological studies suggested that antioxidant capacity is improved by the consumption of tomato products, thereby decreasing the risk of the development of diseases relative to oxidative stress (11,19, 21,27). Tomatoes contain different compounds (e.g. carotenoids, vit. C, flavonoids) that may be responsible for the antioxidant properties. Although tomatoes contain an array of phytochemicals, most of the attention has been focused on lycopene, the main carotenoid in tomato products possesses the greatest quenching ability of singlet oxygen among the various carotenoids (14), and is effective in protecting blood lymphocytes from NO_2 radical damage (9).

Lycopene, the red pigment in tomatoes, is a natural pigment synthesized by plants and microorganisms (1). Antioxidants nutrients, including lycopene and other carotenoids, neutralize the adverse effects of free radicals. Hence the balance between free radicals and antioxidants is important for maintaining healthy body systems (18). Lycopene is one of the most potent antioxidants (14 and 25), with a singlet-oxygen-quenching ability ten times higher than that of α-tocopherol (14). Dietary supplementation with α-tocopherol , the major lipid-soluble antioxidant presents in cell membranes, has been demonstrated to reduce the susceptibility to lipid peroxidation and improved the characteristics and fertility of semen (10, 20, 23). The findings mentioned above may be led to expect that the use of lycopene sources may achieve at least the same results as related with semen quality characteristics. Thus, the objective of this work was to study the effect of the addition of tomato juice, as a lycopene-rich source , to semen diluents at different levels on some characteristics of roosters'semen stored for different times. To our knowledge, this is the first work describing the effects of the addition of different levels of tomato juice to semen diluents on semen quality traits in roosters.

MATERIALS AND METHODS

The experiment was carried out at the Poultry Farm of the College of Agriculture, University of Baghdad during the period from 1 /2 /2005 to 1 /4 /2005. Sixty white layer roosters, 24 wks old, were used. A study of the sperm was performed twice a week for a period of 8 weeks. Semen was collected by massage according to Burrows and Quinn (12). Care was taken to avoid any contamination of semen with the cloacal products and particularly with the transparent fluid excreted from the lymph folds of the cloaca during ejaculation. The semen extender referred to as Al-Daraji 2 diluent (AD_2D) (4), consist of potassium citrate, 0.64 g; sodium glutamate, 8.67 g ; sodium acetate, 4.3 g ; magnesium chloride , 0.34 g ; potassium diphosphate, 12.7 g ; potassium monophosphate , 12.7 g ; fructose , 5 g ; TES , 1.95 g ; vitamin A , 4 mg ; vitamin C , 16 mg and vitamin E , 8 mg .These ingredients are dissolved in 1 liter of distilled water. Both AD_2D and tomato juice (Tj) were used in this experiment. The experimental design was completely randomized for the experiment with six treatments : undiluted control , fresh semen (C1) ; diluted control, AD_2D-

22 –Improving sperm characteristics during in vitro storage of roosters semen by supplementing semen diluent with tomato juice

diluted semen (C2); and treatments T1, T2, T3 and T4 using AD_2D-diluted semen with 1, 3 ,5 and 7 ml of tomato juice per 100 ml, respectively . All parameters studied were estimated after storage periods of 0, 24, 48 and 72 hours in refrigerator. The method of measuring the mass activity and individual motility tests has been described by Sexton (30). Percentages of dead spermatozoa were evaluated by using Fast green-eosin B stain (5). Abnormal spermatozoa were distinguished by using a Gentian violet-eosin stain (2). Acrosomal abnormalities test was carried out according to Al-Daraji (3).

The experiment was conducted using a completely random design. Data were analyzed using analysis of variance (ANOVA) (29). A significant difference was used at 0.05 probability level and differences among treatments were tested using the Duncan's procedure (17).

RESULTS AND DISCUSSION

Effects of tomato juice addition to the semen diluent on mass activity and individual motility percentages are presented in Tables 1 and 2, respectively. The use of AD_2D significantly increased mass activity and individual motility percentages at all *in vitro* storage times studied (C2 *versus* C1). Also, adding tomato juice up to 7 ml per 100 ml of the AD_2D led to a significant additional increase in mass activity and individual motility percentages at all *in vitro* storage times studied (T1, T2, T3 and T4 *versus* C2). No significant changes were observed in mass activity and individual motility percentages along with increasing tomato juice addition to the diluent from 1 to 5 ml per 100 ml of the diluent (T1, T2 and T3). While, increasing the addition level of tomato juice from 5 to 7 ml per 100 ml of AD_2D significantly led to an additional increase in mass activity and individual motility percentages at all *in vitro* storage times (T4 *versus* T1, T2 and T3).

Dead and abnormal spermatozoa, and acrosomal abnormalities percentages are shown in Tables 3, 4 and 5, respectively. Adding tomato juice to the diluent (AD_2D) even at low level in this experiment (1 ml / 100 ml of diluent,T1), resulted in a significant decreases in the percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities at all *in vitro* storage

Table 1 . Effect of tomato juice addition at different levels to the semen diluent on mass activity of rooster semen at different *in vitro* storage times (Mean ± SE).

Treatments		Storage times (hours)			
		0	24	48	72
C1	Fresh	79.6 ± 4.0 ^{d}	35.0 ± 2.7 ^{d}	15.2 ± 1.0 ^{d}	0.0 ± 0.0 ^{d}
C2	AD_2D	85.2 ± 2.6 ^{c}	80.7 ± 3.9 ^{c}	73.6 ± 2.8 ^{c}	60.9 ± 1.7 ^{c}
T1	AD_2D + Tj (1 ml / 100ml)	89.0 ± 1.7 ^{b}	84.1 ± 2.0 ^{b}	78.1 ± 3.0 ^{b}	67.2 ± 1.9 ^{b}
T2	AD_2D + Tj (3 ml / 100ml)	90.2 ± 3.6 ^{b}	85.0 ± 1.7 ^{b}	79.2 ± 1.8 ^{b}	68.1 ± 3.3 ^{b}
T3	AD_2D + Tj (5 ml / 100ml)	91.9 ± 2.9 ^{b}	85.9 ± 3.1 ^{b}	79.9 ± 2.2 ^{b}	68.9 ± 1.4 ^{b}
T4	AD_2D + Tj (7 ml / 100ml)	97.2 ± 3.0 ^{a}	91.2 ± 2.8 ^{a}	84.6 ± 1.7 ^{a}	80.0 ± 2.9 ^{a}

$^{a-d}$ Means in a column with no common superscript differ significantly ($P< 0.05$).
- AD_2D , Al-Daraji 2 diluent ; Tj , tomato juice.

22 –Improving sperm characteristics during in vitro storage of roosters semen by supplementing semen diluent with tomato juice

Table 2 . Effect of tomato juice addition at different levels to the semen diluent on individual motility of rooster semen at different *in vitro* storage times (Mean ± SE).

Treatments		Storage times (hours)			
		0	24	48	72
C1	Fresh	80.3 ± 1.7 [d]	36.9 ± 4.0 [d]	17.3 ± 1.0 [d]	0.0 ± 0.0 [d]
C2	AD_2D	87.6 ± 2.2 [c]	82.1 ± 1.7 [c]	75.0 ± 2.4 [c]	62.3 ± 1.8 [c]
T1	AD_2D + Tj (1 ml / 100ml)	91.0 ± 3.7 [b]	86.9 ± 2.8 [b]	80.1 ± 1.3 [b]	69.0 ± 3.3 [b]
T2	AD_2D + Tj (3 ml / 100ml)	92.7 ± 2.4 [b]	87.1 ± 3.0 [b]	81.8 ± 2.4 [b]	70.1 ± 1.7 [b]
T3	AD_2D + Tj (5 ml / 100ml)	93.8 ± 1.9 [b]	88.3 ± 1.7 [b]	82.9 ± 1.9 [b]	70.9 ± 2.6 [b]
T4	AD_2D + Tj (7 ml / 100ml)	98.9 ± 2.3 [a]	93.8 ± 4.1 [a]	87.0 ± 2.3 [a]	85.0 ± 1.3 [a]

[a-d] Means in a column with no common superscript differ significantly (P< 0.05).
- AD_2D , Al-Daraji 2 diluent ; Tj , tomato juice.

Table3. Effect of tomato juice addition at different levels to the semen diluent on dead spermatozoa of rooster semen at different *in vitro* storage times (Mean ± SE).

Treatments		Storage times (hours)			
		0	24	48	72
C1	Fresh	25.8 ± 1.0 [a]	64.8 ± 1.9 [a]	87.9 ± 3.3 [a]	100.0 ± 0.0 [a]
C2	AD_2D	20.0 ± 2.3 [b]	32.3 ± 2.7 [b]	40.1 ± 1.8 [b]	50.9 ± 1.3 [b]
T1	AD_2D + Tj (1 ml / 100ml)	14.0 ± 1.7 [c]	27.6 ± 1.8 [c]	30.0 ± 2.2 [c]	40.2 ± 2.8 [c]
T2	AD_2D + Tj (3 ml / 100ml)	13.2 ± 1.3 [c]	26.5 ± 2.0 [c]	28.1 ± 1.7 [c]	38.3 ± 1.7 [c]
T3	AD_2D + Tj (5 ml / 100ml)	12.1 ± 1.0 [c]	24.1 ± 3.1 [c]	27.3 ± 2.0 [c]	37.0 ± 2.1 [c]
T4	AD_2D + Tj (7 ml / 100ml)	4.2 ± 1.3 [d]	8.3 ± 2.4 [d]	15.0 ± 1.3 [d]	24.9 ± 1.3 [d]

[a-d] Means in a column with no common superscript differ significantly (P< 0.05).
- AD_2D , Al-Daraji 2 diluent ; Tj , tomato juice.

Table 4 . Effect of tomato juice addition at different levels to the semen diluent on abnormal spermatozoa of rooster semen at different *in vitro* storage times (Mean ± SE).

Treatments		Storage times (hours)			
		0	24	48	72
C1	Fresh	26.7 ± 1.0 [a]	62.8 ± 3.0 [a]	95.9 ± 4.2 [a]	99.1 ± 4.8 [a]
C2	AD_2D	13.0 ± 1.7 [b]	25.1 ± 2.1 [b]	40.7 ± 2.0 [b]	57.0 ± 3.3 [b]
T1	AD_2D + Tj (1 ml / 100ml)	9.1 ± 1.1 [c]	20.2 ± 1.3 [c]	28.9 ± 1.3 [c]	43.0 ± 1.7 [c]
T2	AD_2D + Tj (3 ml / 100ml)	8.0 ± 1.3 [c]	19.8 ± 1.0 [c]	26.1 ± 0.9 [c]	40.1 ± 2.2 [c]
T3	AD_2D + Tj (5 ml / 100ml)	7.6 ± 1.0 [c]	18.3 ± 1.7 [c]	25.0 ± 1.3 [c]	40.8 ± 1.7 [c]
T4	AD_2D + Tj (7 ml / 100ml)	2.0 ± 0.9 [d]	7.9 ± 1.1 [d]	14.0 ± 2.6 [d]	23.0 ± 2.1 [d]

[a-d] Means in a column with no common superscript differ significantly (P< 0.05).
- AD_2D , Al-Daraji 2 diluent ; Tj , tomato juice.

22 –Improving sperm characteristics during in vitro storage of roosters semen by supplementing semen diluent with tomato juice

22 –Improving sperm characteristics during in vitro storage of roosters semen by supplementing semen diluent with tomato juice

Table 5. Effect of tomato juice addition at different levels to the semen diluent on acrosomal abnormalities of rooster semen at different *in vitro* storage times (Mean ± SE).

Treatments		Storage times (hours)			
		0	24	48	72
C1	Fresh	25.9 ± 1.0 [a]	75.2 ± 1.3 [a]	92.8 ± 3.0 [a]	99.3 ± 4.8 [a]
C2	AD_2D	18.0 ± 1.3 [b]	28.0 ± 1.2 [b]	45.2 ± 1.7 [b]	56.0 ± 1.3 [b]
T1	AD_2D + Tj (1 ml / 100ml)	13.1 ± 1.6 [c]	20.9 ± 2.0 [c]	31.3 ± 2.0 [c]	45.1 ± 1.1 [c]
T2	AD_2D + Tj (3 ml / 100ml)	12.3 ± 0.8 [c]	18.3 ± 1.7 [c]	30.0 ± 1.1 [c]	43.1 ± 0.8 [c]
T3	AD_2D + Tj (5 ml / 100ml)	11.1 ± 1.0 [c]	17.6 ± 1.2 [c]	29.5 ± 1.7 [c]	42.0 ± 1.7 [c]
T4	AD_2D + Tj (7 ml / 100ml)	3.9 ± 0.8 [d]	8.1 ± 1.7 [d]	15.0 ± 0.8 [d]	28.0 ± 1.1 [d]

[a-d] Means in a column with no common superscript differ significantly ($P< 0.05$).
- AD_2D , Al-Daraji 2 diluent ; Tj , tomato juice.

times (T1, T2, T3 and T4 *versus* C2). Also, no significant differences were found for percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities among AD_2D-diluted samples supplemented with 1, 3 and 5 ml of tomato juice per 100 ml of AD_2D (T1, T2 and T3, respectively) at all *in vitro* storage times studied. Furthermore, as tomato juice addition increased from 5 to 7 ml per 100 ml of diluent (T4), dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities percentages decreased significantly ($P<0.05$) when compared with other treatments in this study.

With increasing *in vitro* storage time, mass activity and individual motility percentages of fresh semen reached to 0 % accompanied with reaching of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities percentages to approximately 100 % after 72 hr of *in vitro* storage period. While, supplementation of the semen diluent with tomato juice can prolong the period required to reaching these percentages. These results are in agreement with previous reports by Sexton (31) who found that semen quality and fertility are generally decreased when semen is stored for 24 hr *in vitro*, and Douard *et al* (16) who found that the motility, viability and morphological integrity of spermatozoa decreased during storage.

It was observed that the phospholipids content of turkey spermatozoa is severely affected by *in vitro* storage and the evolution of phospholipids is parallel to the decrease in semen quality (16). This could originate from the endogenous metabolism of the fatty acids of the membrane phospholipids and induce membrane destabilization (16). The semen of birds showed a tendency to form high concentrations of the products of lipid peroxidation during *in vitro* storage and this was associated with a partial or complete loss of fertilizing ability (33).

Addition of antioxidants to the semen diluent increase semen quality (15). In the present work, the addition of tomato juice to the semen diluent led to increase quality of semen stored *in vitro* and this may be attributed to lycopene found in tomato juice. Lycopene possess a singlet-oxygen-quenching ability 10 times higher than that of α-tocopherol (14) and α-tocopherol has been demonstrated to reduce the susceptibility to lipid peroxidation and improved semen quality traits (10,20,23), therefore, the use of tomato juice as a lycopene-rich source in the semen diluent in this study may be the probable reason for the amelioration occur in all semen characteristics studied.

In conclusion, enrichment of semen diluent by adding tomato juice increases semen quality and can prolong the *in vitro* storage time of roosters'semen.

22 –Improving sperm characteristics during in vitro storage of roosters semen by supplementing semen diluent with tomato juice

REFERENCES

1.Agarwal, S. and A. V. Rao. 2000. Tomato lycopene and its role in human health and chronic diseases. Can. Med. Assoc. J. 163(6):739-744.

2.Al-Daraji , H.J. 1998 . Effect of ascorbic acid supplement-ation on physiological and productive traits of Fawbro broiler breeder flocks reared under hot climate . Ph.D. dissertation , Dept. of Animal Producton , College of Agriculture , University of Baghdad .

3.Al-Daraji , H.J. 2001 . Effects of holding temperature and time on acrosomal abnormalities of fowl sperms . Indian J. Anim. Sci. 71(1):32-34.

4.Al-Daraji , . H.J. 2004 . Diluent supplementation with vitamins A, C and E for improving fertilizing ability of indigenous roosters'semen. Patent No. 3195, issued from C.O.S.Q.C., Iraq.

5.Al-Daraji , H.J., B.T.O Al-Tikriti, K.H.Hassan and A.A.Al-Rawi.2002. New techniques for determination of avian spermatozoa abnormalities . Res. J. Bio. Tech. 4(1):47-64.

6.Bakst, M. 1993. Changes in sperm surfaces associated with epididymal transit. Oviducal sperm storage in poultry. Reprod. Fert. Dev. 5 : 595-599.

7.Bakst, M., G. Wishart and J. P. Brillard . 1994. Oviducal sperm selection, transport and storage in poultry. Poult. Sci. Rev. 5 : 117-143.

8.Blesbois, E., I. Grasseau and D. Hermier. 1999. Changes in lipid content of fowl spermatozoa after liquid storage at 2 to 5 C. Theriogenology 52 : 325-334.

9.Bohm, F., J. H. Tinkler and T. G. Truscott. 1995. Carotenoids protect against cell membrane damage by nitrogen dioxide radical. Nat. Med. 1: 98-99.

10.Brezezinska-Slebozinska, E. A., B. Slebodzinki, B. Pietras and G. Wieczorek. 1995. Antioxidant effects of vitamin E and glutathione on lipid peroxidation in boar semen plasma. Bio. Tra. Ele. Res. 47: 69-74.

11.Burney, P. G. J., G. W. Comstock and J. S. Morris. 1989. Serologic precursors of cancer : serum micronutrients and the subsequent risk of pancreatic cancer. Am. J. Clin. Nutr. 49 : 895-900.

12.Burrows, W. H. and J. R. Quinn. 1937. The collection of spermatozoa from the domestic fowl and turkey. Poultry Sci. 16: 19-24.

13.Cross, N. 1998. Role of cholesterol in sperm capacitation. Biol. Reprod. 59: 7-11.

14.DiMascio, P., S. Kaiser and H. Sies. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. Biophys. 274: 532-538.

15.Donoghue, A. and D. Donoghue. 1997. Effects of water- and lipid-soluble antioxidants on turkey sperm viability, membrane integrity and motility during liquid storage. Poultry Sci. 76: 1440-1445.

16.Douard, V., D. Hermier and E. Blesbois. 2000. Changes in turkey semen lipids during liquid *in vitro* storage. Biol. Reprod. 63: 1450-1456.

17.Duncan, B. D. 1955. Multiple range and multiple F test. Biometrics 11: 1-42.

18.Fawzi, W. W., M. G.Herrera and P. Nestel. 2000. Tomato intake in relation to mortality and morbidity among Sudanese children. J. Nutr. 130:2537-2542.

19.Franceshi, S., E. Bidoli, C. La Vecchia, R. Talamini, B. D'Avanzo and E. Negri. 1994. Tomatoes and risk of digestive-tract cancers. Int. J. Cancer. 59: 181-184.

20.Geva, E., J. B. Lessing, B. Bartoov, L. Lenergeva, N. Zabludovsky and A. Amit. 1996. The effect of antioxidant treatment on human spermatozoa and fertilization rate in an *in vitro* fertilization program. Fert. Ster. 66:430-434.

21.Giovannucci, E., A. Ascherio, E. B. Rimm, M. J. Stampfer, G. A. Colditz and W. C. Willett. 1995. Intake of carotenoids and retinol in relation to risk of prostate cancer. J. Natl. Cancer Inst. 87:1767-1776.

22.Jones, R. 1998. Plasma membrane structure and remodeling during sperm

22 –Improving sperm characteristics during in vitro storage of roosters semen by supplementing semen diluent with tomato juice

maturation in the epididymis. J. Reprod. Fert. Suppl. 53: 73-84.

23. Kessopoulou, E., H. J. Powers, K. K. Sharma, M. J. Russel, I. D. Cooke and C. L. R. Barratt. 1995. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat oxygen species-associated male infertility. Fert. Ster. 64: 825-831.
24. Mann, T. and C. Lukwak-Mann. 1981. Male Reproductive Function and Semen. Berlin: Springer-Verlag : 495.
25. Miller, N. J., J. Sampson, L. P. Candeias, P. M. Bramley and C. A. Rice-Evans. 1996. Antioxidant activities of carotenes and xanthophylls. FEBS Lett. 384 : 240-246.
26. Oshima, S., F. Ojima, H. Sakamoto, Y. Ishiguro and J. Terao. 1996. Supplementation with carotenoids inhibits singlet oxygen-mediated oxidation of human plasma low density lipoprotein. J. Agric. Food Chem. 44: 2306-2309.
27. Parfitt, V. J., P. Rubba, C. Bolton, G. Marotta, M. Hartog and M. Mancini. 1994. A comparison of antioxidant status and free radical peroxidation of plasma lipoproteins in healthy young persons from Naples and Bristol. Eur. Heart J. 15:871-876.
28. Parks, J. and D. V. Lynch. 1992. Lipid composition and thermotropic phase behavior of boar, bull, stallion and rooster sperm membrane. Cryobiology 29: 255-266.
29. SAS, Institute. 2001. SAS User's Guide: Statistics version 6.12 ed. SAS Institute, Inc., Cary, NC., USA.
30. Sexton, T. J. 1976. Studies on the dilution of turkey semen. Brit. Poult. Sci. 17: 179-186.
31. Sexton, T. J. 1986. Comparison of commercial diluents for holding turkey semen 24 hours at 4 C. Poultry Sci. 67: 131-134.
32. Thurston, R. 1995. Storage of poultry semen above freezing for twenty-four to forty-eight hours. In: Proceedings of First International Symposium on Artificial Insemination of Poultry : 107-122.
33. Wishart, G. J. 1984. Effects of lipid peroxide formation in fowl semen on sperm motility, ATP content and fertilizing ability. J. Reprod. Fert. 71:113-118.